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**14. ABSTRACT**

Prostate cancer is the second leading cause of cancer death among American men in 2019. The majority of the death is due to the development of castration resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT). Despite the development and use of next generation anti-AR signaling inhibitors (ASI) such as abiraterone and enzalutamide, resistance to ASI remains the major clinical challenge. The proposed research is based on the finding that protein arginine methyltransferase 5 (PRMT5) is a novel epigenetic activator of AR transcription. If PRMT5 targeting can inhibit or eliminate AR transcription, combining PRMT5 targeting with androgen synthesis inhibition should exhibit a better treatment effect for CRPC. During the past grant period, we have successfully demonstrated that PRMT5 cooperates with pICln to epigenetically activates AR transcription to promote prostate cancer cell growths in vitro and in vivo. Further, we have provided evidence that co-targeting PRMT5 with Abiraterone or Enzalutamide may exhibit better cell killing effects.

**15. SUBJECT TERMS**

CRPC, PRMT5, AR, AR-V7, epigenetics, HNPC, ADT, ASI, transcription, abiraterone, enzalutamide

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**Table of Contents**

**Page**

**1. Introduction……………………………………………………….4**

**2. Keywords……………………………………………………….4**

**3. Accomplishments………..……………………………………..4-13**

**4. Impact………………………..……………………………………13-14**

**5. Changes/Problems...….…………………………………………14**

**6. Products…………………………………….………..……………14-16**

**7. Participants & Other Collaborating Organizations……………16-18**

**8. Special Reporting Requirements……………………………………18**

**9. References……………………………………………………..18-19**

**10. Appendices……………………………………………………19-151**

## 1. Introduction

Prostate cancer is the second leading cause of cancer death among American men in 2019 (1), and the vast majority of these patients die of the progression of hormone naïve prostate cancer (HNPC) to castration resistant prostate cancer (CRPC), a lethal status of the disease (2-4). The major mechanism underlying the development of CRPC is reactivation of the androgen receptor (AR), the driver of prostate cancer development and progression. AR reactivation mechanisms include AR overexpression (with or without *AR* gene amplification), AR mutations, AR splice variants, and androgen-independent activation of AR by AR modulators as well as de novo androgen synthesis in prostate cancer cells (3, 4). In fact, abiraterone was approved by the FDA in 2011 for CRPC treatment because of its ability to inhibit CYP17A1, a critical enzyme involved in the de novo androgen synthesis in prostate cancer cells (5). We have recently discovered that protein arginine methyltransferase 5 (PRMT5), an emerging epigenetic enzyme involved in epigenetic control of target gene expression (6-8), is overexpressed in prostate cancer tissues, and its expression positively correlates with the expression of AR (9). Preliminary data strongly suggest that PRMT5 regulates prostate cancer cell growth through epigenetic control of AR expression. Based on these novel findings, *we hypothesize that co-targeting androgen synthesis and AR expression simultaneously will overcome the mechanisms of AR reactivation and provide an effective treatment for CRPC.* The goal of proposed research is to provide preclinical evidence that inhibiting androgen synthesis by abiraterone in combination with inhibiting or eliminating AR expression by PRMT5 targeting is an effective and novel therapeutic approach for CRPC treatment. We used CRPC cells and xenograft mouse models to test our hypothesis. Our results have demonstrated that PRMT5 is a critical epigenetic regulator of AR transcription in CRPC cells and that targeting PRMT5 by genetic knockdown or pharmacological inhibitors effectively suppresses CRPC cell growth *in vitro* and *in vivo*. Future preclinical evaluation of PRMT5 inhibitors in advanced preclinical mouse models will provide preclinical evidence for future clinical trials.

## 2. Keywords

PRMT5, epigenetics, AR, CRPC, HNPC, ADT, ASI, transcription, abiraterone, enzalutamide

## 3. Accomplishments

**3A. What were the major goals of the project?** There are three major goals in this project as defined by three Specific Aims in the approved SOW.

Major Goal 1: To determine whether and how PRMT5 regulates the expression of full-length AR and AR splice variants in CRPC cell lines

Major Goal 2. To test whether PRMT5 targeting in combination with abiraterone shows a better killing effect in CRPC cells

Major Goal 3. To evaluate whether PRMT5 targeting plus abiraterone as a combination therapy shows a better treatment effect for CRPC xenograft tumors and patients derived xenografts in mice

**3B. What was accomplished under these goals?**

**Major Goal 1**: **To determine whether and how PRMT5 regulates the expression of full-length AR and AR splice variants in CRPC cell lines (Months 1-12) *__Completed.__***

**Goals 1:** There are three subtasks in this Major Goal. *__Subtask 1__* is to examine the expression of PRMT5, AR and major AR variants in CRPC cell lines by qPCR and Western blotting, *__Subtask 2__* is to establish PRMT5 knockdown cell lines and examine the effect of PRMT5 knockdown or inhibition on the expression of AR and/or AR variants, and *__Subtask 3__* is to determine if the regulation of AR or AR variant expression is through epigenetic regulation.

We proposed to use three CRPC cell lines (C4-2 cells, 22Rv1 cells and LNCaP95 cells) to perform these experiments. During the grant period, we have successfully completed all of these proposed experiments and added additional experiments as wells. The following are a summary of our accomplishments which have been published in *Cancer Research* (Beketova et al, *Cancer Res* 2020, 80:4904-4917) (10).

(1). We established PRMT5 knockdown cell lines in C4-2 and demonstrated that knockdown of PRMT5 can down-regulate expression of AR in C4-2 cells and suppressed the growth of C4-2 cells. This was published in the 2017 Oncogene paper (Figure 1C, 1E) (9).

(2). We examined the expression of AR and AR variants and confirmed that both full-length AR and AR-V7 are expressed in 22Rv1 cells but not in LNCaP95 cells.

(3). We established PRMT5 knockdown cell lines in 22Rv1 and examined the effect of PRMT5 knockdown on the expression of AR and AR-V7 in 22Rv1 cells. Also, we examined effect of PRMT5 inhibition by two inhibitors, BLL3.3 (9, 11) and JNJ-64619178 (12, 13). We confirmed that knockdown of PRMT5 expression or pharmacological inhibition of PRMT5 by BLL3.3. or JNJ-6461978 down-regulated the expression of AR and AR-V7 at the protein and mRNA levels. Consistent with these, PRMT5 knockdown or inhibition also suppressed cell proliferation in 22Rv1 cells. These results are published in Figure 1A-P and Supplementary Figure S1A-S1E (Beketova et al., *Cancer Res 2020*) (10).

(4). We extended the above findings from C4-2 and 22Rv1 cells to two more CRPC cell lines VCaP and LNCaP 95 and observed that inhibition of PRMT5 by BLL3.3 or JNJ-64619178 similarly down-regulated the expression of AR at the protein and mRNA levels and suppressed cell proliferation. These results are published in Supplementary Figure S1F-S1M (Beketova et al., *Cancer Res 2020*) (10).

(5). We confirmed that the regulation of AR expression by PRMT5 is through epigenetic regulation. PRMT5 binds to the promoter region of *AR* and catalyzes H4R3me2s and H3R2me2s. Interestingly, both Sp1 and Brg1 also bind to the promoter region of *AR* in 22Rv1 cells. This is similar to the binding of Sp1 and Brg1 to the same region in LNCaP cells (Deng et al., *Oncogene* 2017, Figure 3) (9). Thus, PRMT5 utilizes the same mechanism to epigenetically regulates transcription of *AR* in HNPC and CRPC cells. The results for the epigenetic regulation of AR by PRMT5 are presented in Figure 1R-1S and Supplementary Figure S1O-S1P (Beketova et al., *Cancer Res 2020*) (10).

(6). To determine if PRMT5-mediated regulation of AR expression is the major mechanism, we performed AR rescue experiments in the background of PRMT5 knockdown and observed that exogenously expressed AR completely abolished the growth inhibition induced by PRMT5 knockdown and restored cell proliferation to the SC control. This result is presented in Supplementary Figure S4 (Beketova et al., *Cancer Res 2020*) (10).

(7). We also confirmed that the regulation of cell proliferation by PRMT5 is mainly through G1 cell cycle progression without any significant effect on cell death. These results are presented in Figure 1Q and Supplementary Figure S1N (Beketova et al., *Cancer Res 2020*) (10).

**Major Goal 2. To test whether PRMT5 targeting in combination with Abiraterone shows a better killing effect in CRPC cells *in vitro* (Months 13-24). *Completed***

There are two subtasks in this major goal. *Subtask 1* is to examine if abiraterone may affect the expression PRMT5 and AR-V7 expression, and *Subtask 2* is to examine the effect of targeting PRMT5 in combination with abiraterone can exhibit a better cell killing or growth inhibitory effect. We have completed these two subtasks and added additional experiments to evaluate if co-targeting PRMT5 and AR shows better cell killing or growth inhibitory effect in 22Rv1 cells. The following are major findings.

(1). We examined the expression of PRMT5, AR and AR-V7 in LNCaP cells treated with Abiraterone and did not see any effect. We also did not see any effect of BLL3.3 or JNJ-64619178 on the expression of PMRT5 in LNCaP, VCaP and LNCaP95 cells. This is consistent with the literature. The results are presented in Figure 1B-1D, Supplementary Figure S1F-S1M in LNCaP, VCaP and LNCaP95 cells, Supplementary Figure S8 in 22Rv1 xenograft tumors (Beketova et al., *Cancer Res 2020*) (10).

(2). We examined the effect of co-targeting PRMT5 in combination with abiraterone or enzalutamide on cell growth. Using BLL3.3 or JNJ-64619178 as a PRMT5 targeting approach, we observed increased cell killing when combined with either Abiraterone or Enzalutamide. Calculation of the combination index indicates that BLL3.3 or JNJ-64619178 can synergistically inhibit 22Rv1 cell growth when combined with Abiraterone or Enzalutamide. These results are presented in Figure 7A-7B and Supplementary Figure S7 (Beketova et al., *Cancer Res 2020*) (10).

**Major Goal 3. To evaluate whether PRMT5 targeting plus abiraterone as a combination therapy shows a better treatment effect for CRPC xenograft tumors and patients derived xenografts in mice (Months 1-6 and 19-36) *Completed***

Since our collaborator Dr. Chenglong Li is still working on the development of his PRMT5 inhibitor BLL3.3 derivatives, we do not have any inhibitor now for *in vivo* evaluation. To this end, we took an alternative approach to use our Dox-induced PRMT5 knockdown cell lines to evaluate if PRMT5 knockdown in combination with Abiraterone or Enzalutamide shows better tumor killing effect. The following are two major accomplishments.

(1). We previously demonstrated that Dox-induced knockdown of PRMT5 in LNCaP xenograft tumors significantly suppressed xenograft tumor growth in mice. This result is presented in Figure 5 (Deng et al., *Oncogene* 2017) (9). To evaluate if PRMT5 knockdown also has tumor suppressive effect in CRPC tumors, we first performed *in vivo* inducible knockdown of PRMT5 to evaluate the effect of PRMT5 knockdown on 22Rv1 xenograft tumor growth in mice. Compared to SC control xenograft tumors, Dox-induced knockdown of PRMT5 significantly suppressed the growth of 22Rv1 xenograft tumors. Consistent with our *in vitro* observations, knockdown of PRMT5 only inhibited cell proliferation rather than induced cell death. These results are presented in Figure 6A, 6C, 6D and 6F (Beketova et al., *Cancer Res 2020*) (10).

(2). To determine the effect of co-targeting PRMT5 with AR signaling inhibitors Abiraterone or Enzalutamide on tumor growth *in vivo*, we used 22Rv1-shPRMT5 and 22Rv1-shSC cell lines to establish xenograft tumors in NRG mice and then treated mice with Doxycycline (Dox) to induce PRMT5 knockdown in combination with Abiraterone or Enzalutamide. We observed that knockdown alone can achieve the same effect as the combination of PRMT5 knockdown with Abiraterone or Enzalutamide. However, examination of Ki-67 staining in tissues suggest that PRMT5 knockdown in combination with Abiraterone or Enzalutamide dose show better inhibitory effect than PRMT5 knockdown alone. Interestingly, Abiraterone or Enzalutamide alone did not show significant growth inhibitory effect nor induction of apoptosis. Thus, it is likely that co-targeting PRMT5 and the AR signaling could be synergistic. This requires further validation using potent PRMT5 inhibitors for *in vivo* evaluation. These results are presented in Figure 7B-7F and Supplementary Figure S8 (Beketova et al., *Cancer Res 2020*).

As the PRMT5 inhibitor BLL3.3 derivatives in my collaborator lab are still under development, we are currently evaluating the effect of JNJ-64619178 on xenograft tumor growth in mice. If successful, we will evaluate if co-targeting PRMT5 in combination with Abiraterone or Enzalutamide shows a better tumor killing effect using CRPC cells and PDX lines in the future.

**Other Achievements**

**PRMT5 is a master epigenetic activator of DNA damage response.** The identification of PRMT5 as an epigenetic activator is very exciting and novel. This is contrary to the prevailing view in the field that PRMT5 mainly functions as an epigenetic repressor (6, 8, 14). To extend this finding, we also performed an RNA-seq analysis to identify target genes of PRMT5 in radiation-induced DNA damage response (DDR) and discovered that PRMT5 in fact activates transcription of many target genes that are critical for DNA damage repair, particularly for DNA double-strand break repair. Furthermore, we also confirmed that the regulation of these DDR genes by PRMT5 is also dependent on pICln, but independent of MEP50. These novel findings together with the finding that PRMT5 regulation of AR transcription is also pICln-dependent but MEP50-independent collectively suggest that pICln is a novel cofactor of PRMT5 to epigenetically activate transcription of their target genes. These novel findings are published in *iScience* (Owens et al, *iScience* 2020) (15).

**MEP50 is not required for epigenetic activation of PRMT5 target genes.** The identification of PRMT5 as an epigenetic activator of *AR* transcription is very exciting. As MEP50 was considered an obligate cofactor for PRMT5 enzymatic activity, we next examined the role of MEP50 in *AR* transcription. We established Dox-inducible MEP50 knockdown cell lines in LNCaP and 22Rv1. Contrary to our expectation, we surprisingly found that knockdown of MEP50 did not have any effect on AR expression. Also, MEP50 does not bind to the promoter region of *AR*. These expected findings are presented in Figure 2 and Supplementary Figure S2 (Beketova et al., *Cancer Research* 2020) (10).

**Identification of pICln as a cofactor of PRMT5 to epigenetically activate transcription of target genes.** The lack of effect of MEP50 on regulation of AR expression prompted us to search

for other PRMT5 interacting proteins or cofactors that may cooperate with PRMT5 (6, 8, 14). We performed ChIP-qPCR and determined that pICln, but not MEP50, COPR5 and Riok1, bound to the promoter region of *AR*, where PRMT5 binds. This finding suggests that pICln may function as a cofactor of PRMT5 to regulate *AR* transcription. We then established Dox-inducible pICln knockdown cell lines in 22Rv1 and LNCaP cells and confirmed that pICln indeed regulates *AR* transcription. Additionally, knockdown of pICln in 22Rv1 xenograft tumors also significantly suppressed tumor growth in mice through induction of apoptosis and inhibition of cell proliferation. These results are presented in Figure 3, Figure 6B, 6C, 6E and 6G as well as Supplementary Figure S4 (Beketova et al., *Cancer Research* 2020) (10). As discussed above, we also confirmed that pICln also cooperates with PRMT5 to activate transcription of DDR genes (Owens et al., *iScience* 2020) (15).

***Differential regulatory roles of PRMT5 and pICln in cell cycle and cell survival.*** Since we have identified pICln as a cofactor to cooperate with PRMT5 to epigenetically regulate expression of AR and suppress cell growth in CRPC cells, we wanted to determine how cell growth was suppressed. We performed flow cytometry analysis in 22Rv1-shPRMT5#1 cell line and observed that knockdown of PRMT5 by doxycycline mainly increased G1 population, suggesting that PRMT5 mainly regulates G1 progression. However, knockdown of PRMT5 did not appear to induce cell death. We also performed similar flow cytometry analysis in 22Rv1-shpICln cell line and observed that knockdown of pICln mainly increased G2 population, suggesting that pICln may promote G2 progression. Interestingly, knockdown of pICln appeared to decrease the number of viable cells and increased the number of dead cells, suggesting that pICln may be involved in regulation of cell survival. The differential effect of PRMT5 and pICln on cell cycle progression and cell survival suggests that PRMT5 and pICln may also have distinct cellular roles even they cooperate to regulate AR expression. There results are presented in Figure 1Q and Figure 3M (Beketova et al., *Cancer Res 2020*) (10).

**RNA-seq analysis confirmed the regulation of AR signaling by PRMT5 and pICn, but not MEP50.** Our novel discovery of pICln as a cofactor of PRMT5 to epigenetically regulate transcription of *AR* let us further explore their role at the genome-wide level. To this end, we performed RNA-seq in PRMT5 knockdown cells, MEP50 knockdown cells, and pICln knockdown cells to specifically identify genes that are regulated by PRMT5 and MEP50 or pICln. We found that PRMT5 cooperates with MEP50 as well as pICln to regulate different sets of genes. Importantly, we confirmed that PRMT5/pICln, but not PRMT5/MEP50, regulates the expression of many genes in the AR signaling. We also performed qPCR to confirm these sequencing results. Collectively, our results provide strong evidence that PRMT5 and pICln regulate the expression of AR and AR signaling in HNPC and CRPC to promote prostate cancer cell growth in an AR-dependent manner. These results are presented in Figure 4 (Beketova et al., *Cancer Res 2020*) (10).

**Clinical significance of PRMT5/pICln-dependent regulation of AR expression in prostate cancer tissues.** To determine the clinical significance of our findings, we collaborated with Dr. Jiaoti Huang at Duke University and analyzed the expression of AR and AR-V7 and the expression of PRMT5, pICln and MEP50 as well as their subcellular localization using a CRPC TMA (20 cases) and a HNPC TMA (32 BPH, 20 HNPC with Gleason Score 6, and 20 cases with Gleason

Score >7). We observed a significantly higher positive correlation between the expression of PRMT5 or pICln and AR or AR-V7 when compared with that between the expression of MEP50 and the expression of AR or AR-V7 in HNPC and CRPC tissues. In particular, the nuclear-localized PRMT5 and pICln showed even higher correlation with the expression of AR. Further, the expression between PRMT5 and pICln is also highly correlated. We also retrieved mRNA expression data from 4,624 patients from cBioPortal and established similar correlations at the mRNA level. Significantly, higher expression of PRMT5 or pICln showed poor survival when comparing with patients with lower expression of PRMT5 or pICln. Thus, the regulation of AR or AR-V7 expression by PRMT5 and pICln is clinically relevant and significant. These results are presented in Figure 5. Supplementary Figures S5 and S6 (Beketova et al., *Cancer Res 2020*) (10).

**PRMT5: a putative oncogene and therapeutic target in prostate cancer**. Our results generated during the grant period have provided several new insights into the epigenetic role of PRMT5 in DDR response and AR signaling in prostate cancers (Deng et al., *Oncogene* 2017, Owens et al., *iScience* 2020, Beketova et al., *Cancer Research* 2020). These novel findings let ***Cancer Gene Therapy*** invite us to write a review titled "PRMT5: A putative oncogene and therapeutic target in prostate cancer" (Beketova et al., *Cancer Gene* Ther 2021) (16).

**3C. What opportunities for training and professional development has the project provided?**

***3C-1. Research Trainings.*** During the grant period, the following four people have been involved in the project and received training (one-on-one research training).

***Elena Beketova***, a graduate student from PULSe (Purdue University Life Science Umbrella) Program, was recruited to the lab in May 2016 after she completed one-year rotations. It was a perfect time for her to join the lab and work on the project. Soon after she joined the lab, she generated some preliminary data to confirm our hypothesis that PRMT5 may also epigenetically regulate the expression of AR and AR variant in CRPC to promote CRPC cell growth. During her tenure in the lab (05/2016 – 12/2020), she received training through weekly meetings with me, attending journal club presentations and seminars, and presenting research work in multiple local and national conferences and meetings. She made 12 presentations in total including her oral presentation titled "Protein arginine methyltransferase 5 promotes prostate cancer growth via interaction with pICln to epigenetically activate androgen receptor expression" at the 2019 SBUR annual conference (11/2019). Notably, she also received Jenkins-Knevel Award for Outstanding Graduate Research from Purdue University College of Pharmacy and gave an oral presentation titled "Role of PRMT5 in regulation of AR expression and prostate cancer cell growth." In addition, she received multiple fellowships, honors, and awards. These include Purdue university Jenkins-Knevel Award for Outstanding Graduate Research, 2019 SBUR Travel Award, Purdue University Center for Cancer Research SIRG Graduate Assistantship, Purdue University Center for Cancer Research Travel Award, Award for Graduate Student Poster Competition at the Health and Disease Research Poster Session, Purdue University Interdisciplinary Life Science Program Fellowship, and Purdue University Graduate School Bilsland Dissertation Fellowship. She is the first author of the 2021 *Cancer Research* paper (10) and the 2021 *Cancer Gene Therapy* review article (16). She is also the second author of the 2020 *iScience* paper (15). Elena successfully

dissertation defense and graduated in December 2020, and she is now working in a pharmaceutical company to conduct clinical trials.

***Jake Owens***, a graduate student of the MCMP (Medicinal Chemistry and Molecular Pharmacology) program, joined my lab in January, 2015 after he completed his rotations. He worked closely with Elena and participated partially in this DoD project. Particularly, he collaborated with Elena to identify pICln as a cofactor of PRMT5 to epigenetically activate transcription of DDR genes and *AR* in prostate cancer cells. He received training through weekly meetings with me, attending lab meetings, journal clubs, and conferences locally and nationally. He also attended several conferences through his CTSI Graduate Research Fellowship. Jake also attended the 2018 and 2019 SBUR (Society of Basic Urological Research) conferences and presented a poster on the role of PRMT5 in epigenetic regulation of DNA damage response genes. Jake gave 24 presentations during his tenure in the lab. Jake also received multiple fellowships and awards including the 2018 Jenkins-Knevel Award for Outstanding Graduate Research in the College of Pharmacy, 1st Place for Oral Presentation Award at Indiana CTSI 2017 Annual Meeting, 2nd Place for Oral Presentation Award at the 2017 Indiana Basic Urological Research (SBUR) Symposium. Jake is the first author of the 2020 *iScience* paper (15) and the second author of the 2020 *Cancer Research* paper (10) and the 2021 *Cancer Gene Therapy* (16). He is also the first author of another manuscript under review. Currently, Jake is working as a Medical Writer at Avant Healthcare.

***Xuehong Deng***, a senior lab technician who has been working on the project, worked closely with graduate students and postdoc on the project and provided training and technical support to Elena Beketova, Jake Owens and other lab members. She developed and maintained multiple Dox-inducible cell lines for Jake and Elena, conducted IHC analysis for human prostate cancer tissues, xenograft tissues, and many other molecular experiments presented in her 2017 Oncogene paper (9).

***Jogendra Pawar, Ph.D.*** Dr. Pawar was recruited to the lab as a postdoc in February, 2020. Unfortunately, the lab lockdown due to COVID-19 pandemic was announced soon after he just completed paperwork. However, he was reviewing literature in the field and presented two talks during our virtual journal club presentations. In addition, he attended the virtual lab meetings on a weekly basis. I also met with him once a week to discuss experimental design and planning. Since the lab reopening in the end of June, 2020, he has been involved in evaluating the effect of BLL3.3 derivatives from our collaborator Dr. Chenglong Li. Recently, he has started to evaluate the effect of JNJ-64619178 as a PRMT5 inhibitor in regulation of PRMT5 target genes including AR and DNA damage response genes. If successful, we will evaluate the effect of JNJ-64619178 as a PRMT5 inhibitor in mice.

***Jonathan Malola***, a pharmacy student in the Purdue University College of Pharmacy, was working on the project under the supervision of Elena Beketova and Xuehong Deng. He learned molecular biological techniques and helped with some molecular cloning. In addition, he also used bimolecular fluorescence complementation (BiFC) to investigate the interactions of PRMT5 with MEP50 and several other cofactors (pICln, Riok1 and WDR5). He successfully demonstrated that pICln and MEP50 similarly bind to the N-terminal TIM barrel domain of PRMT5. This result was

presented in Supplementary Figure S3 (Beketova et al., *Cancer Research* 2020) (10), and he is a coauthor this article.

***3C-2. Conference presentations*** *(most relevant presentations to this project)*

Beketaova E. Role and function of protein arginine methyltransferase 5 in prostate cancer cell growth. PULSe Graduate Program Seminar Series (1/2020)

Beketaova E., Owens, J.L., and Hu, C.D. Protein arginine methyltransferase 5 promotes prostate cancer growth via interaction with pICln to epigenetically activate androgen receptor expression. 2019 SBUR (Nov, 2019)

Beketaova E. Role of PRMT5 in regulation of AR expression and prostate cancer cell growth. Jenkins-Knevel Award for Outstanding Graduate Research Symposium (Purdue University College of Pharmacy, Nov, 2019)

Owens, J.L., Deng, X., Beketova, E., Tinsley, S.L., Asberry, A. and Hu, C.D. (2019) PRMT5 acts as a master epigenetic regulator to promote repair of DNA damage and is a novel therapeutic target to improve cancer radiation therapy – Poster presentation at 2019 SBUR Annual Conference (Nov 2019)

Beketova, E., Deng, X., Owens, J.L., Hu, C.D., Targeting PRMT5 as a novel approach for the treatment of castration-resistant prostate cancer. Midwest Chromatins and Epigenetics Meeting, Purdue University, June 10-12, 2018

Owens, J.L. and Hu, C.D. (March 2018) PRMT5: An emerging epigenetic regulator of the DNA damage response and novel therapeutic target for prostate cancer radiosensitization – Oral presentation at Purdue University, STAT598 bioinformatics seminar

Owens, J.L. and Hu, C.D. (March 2018) Basic and translational research update: PRMT5's connection to radiation therapy – Oral presentation, Indiana CTSI pre-doctoral fellowship annual meeting.

Beketova E., Deng X., Hu C.D. (2018) Protein Arginine Methyltransferase 5 as a Potential Target for Treatment of Castration-Resistant Prostate Cancer. Poster presentation at the Society of Basic Urologic Research annual meeting.

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (2018) PRMT5 is a master epigenetic regulator of the DNA damage response and is a novel therapeutic target for prostate cancer radiosensitization – Poster presentation at Purdue University, Indiana Basic Urological Research (IBUR) Symposium

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (2018) PRMT5 as a putative therapeutic target for prostate cancer treatment – Poster presentation at Turkey Run, Medicinal Chemistry and Molecular Pharmacology retreat

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (2018) PRMT5 acts as a master epigenetic regulator to promote repair of DNA damage and is a novel therapeutic target to improve cancer radiation therapy – Poster presentation, Indiana CTSI 2018 annual meeting September

Beketova E., Owens J.L., Deng X., Hu C.D. (Oct 9th, 2017) Protein arginine methyltransferase 5 as an epigenetic activator of androgen receptor expression in castration resistant prostate cancer. Poster presentation at Turkey Run, Medicinal Chemistry and Molecular Pharmacology retreat

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (May 2017) PRMT5 acts as a master epigenetic regulator to promote repair of DNA damage and is a novel therapeutic target to improve cancer radiation therapy – Poster presentation, Indiana CTSI 2017 annual meeting

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (October 2017) PRMT5 functions as a master epigenetic activator of DNA damage response – Poster presentation at Turkey Run, Medicinal Chemistry and Molecular Pharmacology retreat

Owens, J.L., Beketova, E., Deng, X., and Hu, C.D. PRMT5 as a novel therapeutic target for prostate cancer treatment – Poster Presentation at Purdue University, Medicinal Chemistry and Molecular Pharmacology Research Day

Owens, J.L., Beketova, E., Deng, X., and Hu, C.D. PRMT5 as a putative therapeutic target for prostate cancer treatment – Oral Presentation. Purdue University Cancer Center Cell Identity and Signaling (CIS) Research Presentation

Elena Beketova, Xuehong Deng, Jake Owens, Chang-Deng Hu. Protein Arginine Methyltransferase 5 as an Epigenetic Activator of Androgen Receptor Expression in Castration-Resistant Prostate Cancer. The Health and Disease: Science, Culture and Policy Research Poster Session, Purdue University, March 23, 2017. Third place award received for poster presentation.

Elena Beketova, Xuehong Deng, Jake Owens, Chang-Deng Hu. Protein Arginine Methyltransferase 5 as an Epigenetic Activator of Androgen Receptor Expression in Castration-Resistant Prostate Cancer. Indiana Basic Urological Research (IBUR) Symposium, June 9, 2017. Oral Presentation

Elena Beketova, Xuehong Deng, Jake Owens, and Chang-Deng Hu. Protein Arginine Methyltransferase 5 as an Epigenetic Activator of Androgen Receptor Expression in Castration-Resistant Prostate Cancer. 2017 Annual Retreat of Purdue University Center for Cancer Research. Purdue University, October 12, 2016

**3D. How were the results disseminated to communities of interest?**

The Health and Disease Symposium (2017): Science, Culture and Policy Research Poster Session was designed to disseminate the discoveries on campus to the entire Purdue community. Elena Beketova presented her major findings on the role of PRMT5 in epigenetic regulation of

CPRC in this symposium. The purpose of this symposium is to stimulate the interest in health science and to promote collaborations across the campus.

Dr. Chang-Deng Hu was the 2018 recipient of the Lafayette Lions Club Award for outstanding achievements in cancer research at Purdue. He presented his research to the Club, a local cancer research support community, on May 16, 2018.

**3E. What do you plan to do during the next reporting period to accomplish the goals?**

Not Applicable.

## 4. Impact

***4A. What was the impact on the development of the principal discipline(s) of the project?***

AR is the driver of prostate cancer development and progression and is the valid therapeutic target for prostate cancer treatment. ADT by suppressing androgen levels or inhibiting the activity of AR is the primary treatment option for metastatic disease. Unfortunately, AR reactivation via increased expression (gene amplification), mutation or expression of splice variants that are not responsive to conventional ADT is the underlying mechanisms of resistance to ADT. As such, patients inevitably develop into CRPC. The next generation anti-AR signaling inhibitors (ASI) Abiraterone and Enzalutamide remain ineffective. The findings from this support provide evidence that co-targeting of AR expression via PRMT5 knockdown and androgen synthesis via Abiraterone or AR inhibition via Enzalutamide is more effective in killing CRPC cells *in vitro* and potentially *in vivo*. As AR reactivation is the major mechanism underlying CRPC development, targeting PRMT5 could potentially overcome AR reactivation by eliminating AR transcription, particularly in combination with androgen synthesis inhibition or AR inhibition. Importantly, we have also identified pICln as a novel cofactor of PRMT5 to activate AR transcription in prostate cancer cells. This raises a very interesting possibility that developing inhibitors specifically targeting the PRMT5/pICln interaction may offer a specific and unique approach to treat HNPC and CRPC.

***4B. What was the impact on other disciplines?***

Although it is generally thought that PRMT5 functions as an epigenetic repressor in multiple human cancers, our findings provide evidence that PRMT5 also functions as an epigenetic activator to activate transcription of AR and DNA damage response genes by symmetrically dimethylating H4R3 not only in HNPC but also in CRPC cells. This further confirm that AR reactivation is the mechanism of CRPC. As epigenetic regulation is a tissue-specific and complex process that involves formation of multiple protein complexes, identification of pICln as a novel cofactor of PRMT5 raises an interesting possibility that PRMT5/pICln may cooperate to epigenetically activate gene transcription whereas PRMT5/MEP50 may epigenetically repress gene transcription. This will offer a unique opportunity to understand basic mechanisms of epigenetic regulation in general. This is also supported by the finding that MEP50, an obligate PRMT5 cofactor, did not participate in epigenetic regulation of AR transcription. Consistent with this, we also demonstrated that pICln cooperates with PRMT5 to epigenetically activate

transcription of genes in DNA damage response (15). Future identification of additional PRMT5/MEP50/pICln targets will strengthen this hypothesis and warrant additional in-depth studies. Furthermore, biochemical and structural studies will reveal how they may function as an activator vs a repressor. We are currently working to solve the structure of PRMT5 in complex with pICln to understand how they work together to activate transcription of AR and DNA damage response gens and to help future development of novel inhibitors targeting PRMT5/pICln interaction.

***4C. What was the impact on technology transfer?***

Nothing to Report.

***4D. What was the impact on society beyond science and technology?***

Nothing to Report.

## 5. Changes/Problems

Nothing to Report.

## 6. Products

***6A. Publications, conference papers, and presentations***

*Journal Publications: A manuscript is under preparation.*

Beketova, E., Owens, J.L. and Hu, C.D. Protein arginine methyltransferase 5 (PRMT5): A putative oncogene and therapeutic target in prostate cancer. *Cancer Gene Therapy* 2021, doi: 10.1038/s41417-021-00327-3. Online ahead of print.

Beketova, E., Fang, S., Owens, J.L., Liu, S., Chen, X., Zhang, Q., Asberry, A.M., Deng, X., Maloa, J., Huang, J., Li, C., Pili, R., Elzey, B.D., Ratliff, T.L., Wan, J. and Hu, C.D. Protein arginine methyltransferase 5 promotes pICln-dependent androgen receptor transcription in castration-resistant prostate cancer. *Cancer Res* (2020), 80:4904-4917

Owens, J.L., Beketova, E., Tinsley, S.L., Asberry A.M., Deng, X., Huang, J., Li, C., Wan, J. and Hu, C.D. PRMT5 cooperates with pICln to function as a master epigenetic activator of DNA double-strand break repair genes. *iScience* (2020), 23:100750

Deng, X., Shao, G., Zhang, H.T., Li, C., Zhang, D., Cheng, L., Elzey, B.D., Pili, R., Ratliff, T.L., Huang, J., Hu, C.D. Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. *Oncogene*, 36:1223-1231 (2017)

*Presentations by Chang-Deng Hu (PI) not reported above:*

07/27/21 Place: Brooke Army Medical Center, Department of Clinical Investigation (San Antonio)

Title: Neuroendocrine differentiation: An emerging mechanism of therapy resistance and tumor recurrence

03/18/21 Place: 2021 6th International Conference on Pharmacy and Pharmaceutical Science (Tokyo)
Title: Protein arginine methyltransferase 5 (PRMT5): An emerging oncogene and therapeutic target in prostate cancer
**Invited Keynote Speaker** (http://www.icpps.org/keynote.html)

09/15/20 Place: 2020 5th International Conference on Pharmacy and Pharmaceutical Science (Tokyo)
Title: Treatment-induced neuroendocrine differentiation in prostate cancer: Therapeutic challenges and opportunities
**Invited Keynote Speaker** (http://www.icpps.org/keynote.html)

07/08/19 Place: Purdue-SEU Biotechnology and Data Science Symposium
Title: Bimolecular fluorescence complementation (BiFC): From single molecular visualization to genome-wide investigation

06/07/18 Place: Department of Radiation Oncology, Chinese University of Sciences and Technology First Affiliated Hospital
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation

05/31/18 Place: Jinan University School of Medicine
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation

05/30/18 Place: Sun Yat-sen University Cancer Center
Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance

05/24/18 Place: Department of Urology, Wannan Medical College Yiji Shan Hospital
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation

03/28/18 Place: Utsunomiya University Center for Biosciences Research and Education
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation

03/19/18 Place: Xuhui Hostpital of Fudan University Zhongshan Hospital
Title: Neuroendocrine differentiation of prostate cancer: From basic research to drug discovery

03/12/18 Place: Bengbu College of Medicine
Title: Neuroendocrine differentiation of prostate cancer: Translational medicine research and training of physician scientists

09/14/17 Place: University of Colorado Denver Cancer Center
Title: Neuroendocrine differentiation: Am emerging mechanism of therapy resistance and tumor recurrence

06/12/17 Place: Jinan University School of Medicine
Title: Protein arginine methyltransferase 5 (PRMT5): An emerging oncogene and therapeutic target in prostate cancer

05/15/2017 Place: Northwesten University School of Medicine, Department of Pathology
Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance

## 7. Participants & Other Collaborating Organizations

### *7A. What individuals have worked on the project?*

*The following are the nearest person months worked during the entire grant period (08/01/16-07/31/21)*

| **Name:** | Chang-Deng Hu |
|---|---|
| **Project Role:** | Hu |
| **Perner ID:** | 90024721 |
| **Nearest person month worked:** | 7.8 |
| **Contribution to Project** | Dr. Hu has supervised students, postdoc and the technician to conduct the proposed research. |
| **Funding Support** | Purdue University, R01 CA212403 and PC150697 |

| **Name:** | Jogendra Pawar |
|---|---|
| **Project Role:** | Postdoc |
| **Perner ID:** | 10020769 |
| **Nearest person month worked:** | 6 |
| **Contribution to Project** | Dr. Pawar has been involved in evaluation of JNJ-64619178 as a novel PRMT5 inhibitor |
| **Funding Support** | PC150697 and R01 CA212403 |

| **Name:** | Jake Owens |
|---|---|
| **Project Role:** | Graduate Student |
| **Perner ID:** | 00147536 |

| Nearest person month worked: | 16.5 |
|---|---|
| **Contribution to Project** | Dr. Owens helped with qRT-PCR, ChIP analysis and RNA-seq data analysis |
| **Funding Support** | PC150697 and other graduate fellowships |

| **Name:** | Xuehong Deng |
|---|---|
| **Project Role:** | Technician |
| **Perner ID:** | 90025073 |
| **Nearest person month worked:** | 23 |
| **Contribution to Project** | Ms. Deng generated stable cell lines, provided technical assistance, performed IHC studies and many other experiments |
| **Funding Support** | PC150697 and R01 CA212403 |

| **Name:** | Elena Beketova |
|---|---|
| **Project Role:** | Graduate Student |
| **Perner ID:** | 00119730 |
| **Nearest person month worked:** | 35 |
| **Contribution to Project** | Elena was mainly working on the project to elucidate the role of PRMT5 in regulation of AR expression |
| **Funding Support** | PC150697 and other graduate fellowships |

| **Name:** | Jonathan Malola |
|---|---|
| **Project Role:** | Pharmacy Student |
| **Perner ID:** | 79715 |
| **Nearest person month worked:** | 9 |

| Contribution to Project | Mr. Malola worked on plasmid constructions and BiFC experiments |
| --- | --- |
| **Funding Support** | Purdue College of Pharmacy Summer Undergraduate Research Fellowship and PC150697 |

***7B. Has there be a change in the active other support of the PI/PI(s) or senior/key personnel since the last reporting period?***

***<u>Current Active Grants</u>***
Title: Role and targeting of PRMT5 in prostate cancer
Source: NCI RO1
Role: Contact PI (**Multi-PI** with Chenglong Li and Jiaoti Huang)
Total Cost Requested:
Grant Period: 06/09/2017-05/31/2022
Goal: The goal of this proposal is to elucidate the molecular mechanisms by which PRMT5 promotes prostate cancer cell growth, improve the potency of BLL3.3, and conduct a preclinical evaluation of PRMT5 inhibition for castration resistant prostate cancer treatment.

Title: Development and preclinical evaluation of a novel targeted radionuclide therapy for metastatic castration-resistant prostate cancer
Source: DoD (2020 PCRP)
Role: PI
Grant Period: 08/01/21-07/30/24
Total Cost:
Goal: The goal of this project is to develop a targeted radionuclide delivery system of a novel PRMT5 inhibitor as a radiosensitizer.

***7C. What other organizations were involved as partners?***

Nothing to report.

## 8. Special Reporting Requirements

N/A

## 9. References


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10. E. Beketova *et al.*, Protein Arginine Methyltransferase 5 Promotes pICln-Dependent Androgen Receptor Transcription in Castration-Resistant Prostate Cancer. *Cancer Res* 80, 4904-4917 (2020).
11. L. Alinari *et al.*, Selective inhibition of protein arginine methyltransferase 5 blocks initiation and maintenance of B-cell transformation. *Blood* 125, 2530-2543 (2015).
12. D. Brehmer *et al.*, Discovery and Pharmacological Characterization of JNJ-64619178, a Novel Small Molecule Inhibitor of PRMT5 with Potent Anti-Tumor Activity. *Mol Cancer Ther* 10.1158/1535-7163.MCT-21-0367 (2021).
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15. J. L. Owens *et al.*, PRMT5 Cooperates with pICln to Function as a Master Epigenetic Activator of DNA Double-Strand Break Repair Genes. *iScience* 23, 100750 (2020).
16. E. Beketova, J. L. Owens, A. M. Asberry, C. D. Hu, PRMT5 in prostate cancer: A putative oncogene and therapeutic target. *Cancer Gene Ther* PMID: 33854218 DOI: 10.1038/s41417-021-00327-3 (2021).

## 10. Appendices

### *10A: Four manuscripts*

Deng et al., *Oncogene* 2017

Owens et al., *iScience* 2020

Beketova et al., *Cancer Res* 2020

Beketova et al., *Cancer Gene Therapy* 2021

### *10B: Meeting abstracts*

2018 SBUR abstracts from Jake Owens and Elena Beketova
2019 SBUR abstracts from Jake Owens and Elena Beketova

### *10C: PI's CV*

OPEN

Oncogene (2017) 36, 1223–1231

www.nature.com/onc

ORIGINAL ARTICLE

# Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth

X Deng[1], G Shao[1,2], H-T Zhang[1,3], C Li[4], D Zhang[5], L Cheng[6], BD Elzey[7], R Pili[8], TL Ratliff[7,9], J Huang[10] and C-D Hu[1,9]

Protein arginine methyltransferase 5 (PRMT5) is an emerging epigenetic enzyme that mainly represses transcription of target genes via symmetric dimethylation of arginine residues on histones H4R3, H3R8 and H2AR3. Accumulating evidence suggests that PRMT5 may function as an oncogene to drive cancer cell growth by epigenetic inactivation of several tumor suppressors. Here, we provide evidence that PRMT5 promotes prostate cancer cell growth by epigenetically activating transcription of the androgen receptor (AR) in prostate cancer cells. Knockdown of PRMT5 or inhibition of PRMT5 by a specific inhibitor reduces the expression of AR and suppresses the growth of multiple AR-positive, but not AR-negative, prostate cancer cells. Significantly, knockdown of PRMT5 in AR-positive LNCaP cells completely suppresses the growth of xenograft tumors in mice. Molecular analysis reveals that PRMT5 binds to the proximal promoter region of the AR gene and contributes mainly to the enriched symmetric dimethylation of H4R3 in the same region. Mechanistically, PRMT5 is recruited to the AR promoter by its interaction with Sp1, the major transcription factor responsible for AR transcription, and forms a complex with Brg1, an ATP-dependent chromatin remodeler, on the proximal promoter region of the AR gene. Furthermore, PRMT5 expression in prostate cancer tissues is significantly higher than that in benign prostatic hyperplasia tissues, and PRMT5 expression correlates positively with AR expression at both the protein and mRNA levels. Taken together, our results identify PRMT5 as a novel epigenetic activator of AR in prostate cancer. Given that inhibiting AR transcriptional activity or androgen synthesis remains the major mechanism of action for most existing anti-androgen agents, our findings also raise an interesting possibility that targeting PRMT5 may represent a novel approach for prostate cancer treatment by eliminating AR expression.

*Oncogene* (2017) **36,** 1223–1231; doi:10.1038/onc.2016.287; published online 22 August 2016

## INTRODUCTION

Protein arginine methyltransferase 5 (PRMT5) is a type II arginine methyltransferase that epigenetically regulates gene transcription by symmetrically dimethylating histone H4 arginine 3 (H4R3me2s), histone H3 arginine 8 (H3R8me2s) or histone H2A arginine 3 (H2AR3me2s).[1,2] PRMT5 also modulates the function of non-histone protein substrates by dimethylating arginine residues on the proteins. By regulating transcription of target genes or post-translational modifications of signaling proteins, PRMT5 is implicated in the regulation of many cellular processes such as cell cycle progression, apoptosis and DNA-damage response. Accumulating evidence shows that PRMT5 is overexpressed in several human cancers, and its expression positively correlates with disease progression and poor outcomes.[3–8] Mechanistic studies have suggested that PRMT5 may function as an oncogene by epigenetic repression of several tumor suppressor genes or by post-translational modification of signaling molecules.[9,10]

Prostate cancer remains the most common non-cutaneous cancer among American men.[11] Although many molecules and signaling pathways that regulate prostate cancer development and progression have been identified and characterized, androgen receptor (AR) signaling is the most important factor that drives prostate cancer development and progression.[12–14] Thus, targeting AR signaling, such as androgen deprivation therapy (ADT), is a standard treatment for patients with locally advanced and metastatic disease. Despite the initial response to ADT, the majority of prostate cancers progress to a lethal status known as castration resistant prostate cancer (CRPC) owing to AR reactivation, which includes AR gene amplification, AR mutations, AR splice variants, androgen-independent activation of AR by AR modulators and intratumoral *de novo* androgen synthesis in prostate cancer cells.[13,15,16] Recent evidence further shows that AR reactivation is also the major mechanism of resistance to the two next-generation anti-androgen agents abiraterone and enzalutamide.[17,18] Therefore, the expression of wild-type or mutant AR is absolutely required in both hormone naive prostate cancer and CRPC. However, compared with extensive studies of AR co-activators and co-repressors including epigenetic regulators,[19–24] how AR expression is regulated, particularly at the epigenetic level, remains largely unknown.

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E-mail: hu1@purdue.edu
Received 27 February 2016; revised 10 June 2016; accepted 5 July 2016; published online 22 August 2016

Here, we report that PRMT5 is highly expressed in prostate cancer tissues and that its expression positively correlates with the expression of AR. Molecular analysis reveals that PRMT5 epigenetically activates the transcription of AR via symmetric dimethylation of H4R3 and promotes prostate cancer cell growth *in vitro* and xenograft tumor growth in mice. Given that current AR-targeting strategies, which are largely based on the inhibition of AR transcriptional activity or inhibition of androgen synthesis, are ultimately ineffective, our findings raise an interesting possibility that targeting PRMT5 may be explored as a novel therapeutic approach to inhibit or eliminate AR expression for prostate cancer treatment.

## RESULTS

### PRMT5 expression is required for prostate cancer cell growth in an AR-dependent manner

We and others previously reported that knockdown of PRMT5 inhibited cell growth in LNCaP cells.[25,26] To further investigate this, we examined the role of PRMT5 in DU145 and PC-3 cells by transiently knocking down PRMT5, and did not observe any significant effect on cell growth when compared with scrambled control (SC; Supplementary Figure S1a–d). Knockdown of PRMT5 in LNCaP cells also exhibited a pronounced inhibitory effect on colony formation in soft agar (Supplementary Figure S1e). Next, we established stable cell lines using LNCaP and DU145 that can be induced by doxycycline (Dox) to express short-hairpin RNA (shRNA), and confirmed that inducible knockdown of PRMT5 indeed showed significant growth inhibition in LNCaP cells (Figure 1a), but not in DU145 cells (Figure 1b). Because DU145 and PC-3 cells do not express detectable level of AR,[27] these results suggest that PRMT5 may regulate prostate cancer cell growth in an AR-dependent manner. To confirm this, we established Dox-inducible stable cell lines using LNCaP-derived CRPC cell line C4-2 cells that express a higher level of PRMT5 and AR (Supplementary Figure S2), and normal prostate epithelial RWPE-1 cells that do not express detectable AR in the absence of androgen stimulation.[28,29] Again, knockdown of PRMT5 significantly inhibited cell growth in C4-2 cells, but had no effect on cell growth in RWPE-1 cells (Figures 1c and d). Consistent with the growth inhibition in LNCaP and C4-2 cells, PRMT5 knockdown also downregulated AR expression (Figure 1e). As a result, the mRNA level of AR target genes *PSA*, *KLK2* and *TMPRSS2* was decreased by PRMT5 knockdown[30] (Figure 1f). To further confirm that AR mediates the effect of PRMT5 on the regulation of cell growth, we performed a rescue experiment by expressing FLAG-AR under the control of a CMV promoter, and observed that overexpressed FLAG-AR completely abolished the growth inhibition induced by PRMT5 knockdown (Figures 1g and h). Similar results were obtained when the LNCaP stable cell line was used and the target gene expression was partially rescued (Supplementary Figure S3). Thus, AR downregulation is likely responsible for the growth inhibition induced by PRMT5 knockdown.

Recently, a PRMT5-specific small molecule inhibitor Compound 5 (named here as BLL3.3) has been identified.[31] To determine whether inhibition of PRMT5 by BLL3.3 can recapitulate the effect of PRMT5 knockdown in prostate cancer cells, we treated LNCaP cells with BLL3.3, and observed that the growth of LNCaP cells and the expression of AR were significantly inhibited (Supplementary Figures S4a and b). No inhibitory effect was observed when DU145 and RWPE-1 cells were similarly treated with BLL3.3 (Supplementary Figures S4c and d). These results provide additional evidence that the enzymatic activity of PRMT5 is required for AR expression and cell growth in prostate cancer cells.

### AR is an epigenetic target of PRMT5 in prostate cancer cells

To determine how PRMT5 regulates AR expression, we examined the effect of PRMT5 knockdown on AR transcription by performing quantitative real-time PCR (qRT-PCR), and observed that transient knockdown of PRMT5 decreased the mRNA level of AR by ~50% (Figure 2a). As PRMT5 may regulate AR transcription epigenetically or indirectly via the regulation of AR transcriptional regulators, we examined the effect of PRMT5 knockdown on the AR-Luciferase reporter gene (AR-Luc) activity, and observed that PRMT5 knockdown had no impact on the AR-Luc activity (Figure 2b). This result suggests that a native chromatin status is required for the downregulation of AR by PRMT5 knockdown. Thus it is likely through epigenetic control of AR transcription. Indeed, the symmetric dimethylation status of H4R3 was significantly enriched on the proximal promoter region of the AR gene when compared with H3R8 and H2AR3 (Figure 2c), despite that all three antibodies can efficiently immunoprecipitate histones H4, H3 and H2A (Supplementary Figure 5). Knockdown of PRMT5 exhibited a greater inhibitory effect on the methylation status of H4R3 (Figure 2d), but a lesser effect on H3R8 and H2AR3 (Supplementary Figure S6). Consistent with this, knockdown of PRMT5 reduced the binding of PRMT5 to the proximal promoter region of the AR gene (Figure 2e), and decreased the level of H4R3me2s on the AR promoter region (Figure 2f). Further, treatment of LNCaP cells with the PRMT5 inhibitor BLL3.3 also decreased the level of AR and H4R3me2s (Supplementary Figure S4b). Taken together, these results demonstrate that PRMT5 epigenetically activates AR transcription by symmetrically dimethylating H4R3.

### PRMT5 interacts with Sp1 and Brg1 on the AR promoter

To determine how PRMT5 is recruited to the AR promoter, we examined whether PRMT5 interacts with Sp1, the major and only well-characterized transcription factor that positively regulates AR transcription in prostate cancer cells.[32,33] Indeed, Sp1 was co-immunoprecipitated with PRMT5 from LNCaP cells (Figure 3a). Because both H3R8me2s and H4R3me2s are associated with the activation of target gene expression when PRMT5 is associated with the ATP-dependent chromatin-remodeling enzyme Brg1,[34,35] we performed co-immunoprecipitation and found that Brg1 was also co-immunoprecipitated with PRMT5 from LNCaP cells (Figure 3b). To substantiate this finding, we established a Dox-inducible Sp1 knockdown cell line (LNCaP-shSp1) and confirmed that knockdown of Sp1 indeed repressed AR expression (Figure 3d). Significantly, knockdown of Sp1 in this cell line not only abolished the binding of Sp1 to the proximal promoter region of the AR gene (Figure 3d), but also abolished the binding of PRMT5 (Figure 3e) as well as reduced the binding of Brg1 to the same region (Figure 3f). These results together suggest that Sp1, PRMT5 and Brg1 form a complex on the AR proximal promoter region to activate AR transcription.

### PRMT5 is overexpressed in human prostate cancer tissues and correlates with AR expression

Next, we examined the expression level of PRMT5 in a human prostate cancer tissue microarray (TMA) consisting of 32 benign prostatic hyperplasia (BPH) tissues and 40 prostate cancer tissues (20 with Gleason score 6 and 20 with Gleason score $\geqslant 7$), and found that PRMT5 expression was significantly higher in prostate cancer tissues than BPH tissues (Figure 4a). Although there is no statistically significant difference in the expression scores between prostate cancer tissues with Gleason score 6 and those with Gleason score 7 and above, 60% of prostate cancer tissues with Gleason score 7 and above showed moderate to high expression (total expression score 40–60) of PRMT5 whereas 40% of prostate cancer tissues Gleason score 6 had similar expression of PRMT5.

Because PRMT5 subcellular localization appears to be an important determinant of cell fate,[36,37] we compared the expression level of PRMT5 in both the cytoplasm and the nucleus and observed that some cells showed more nuclear or cytoplasmic localization of PRMT5. However, there was no significant difference in PRMT5 subcellular localization in either BPH tissues

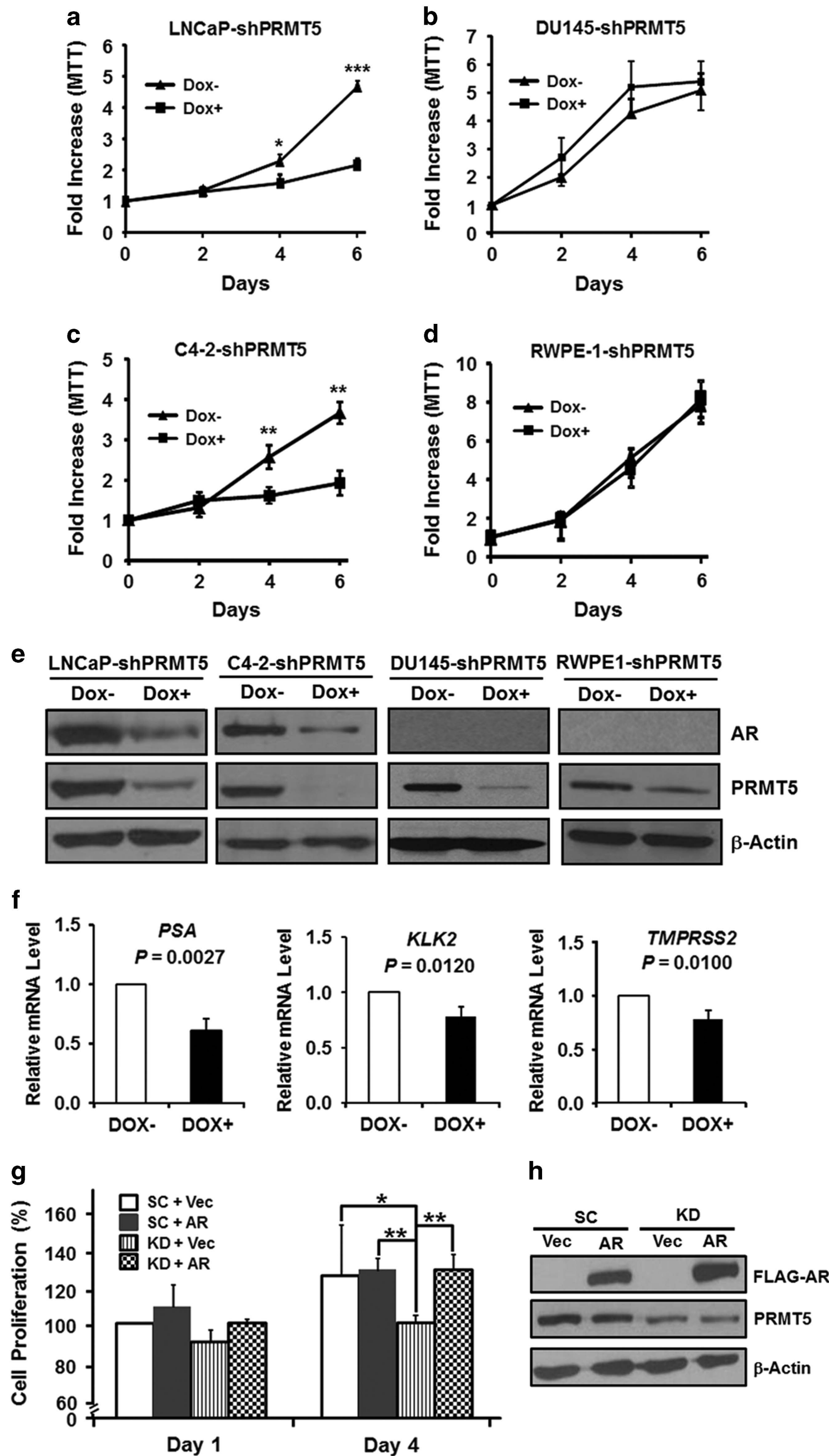


**Figure 1.** PRMT5 regulates prostate cancer cell growth in an AR-dependent manner. (**a**–**d**) Induction of PRMT5 knockdown by doxycycline (Dox+) inhibited cell proliferation in AR-expressing LNCaP and C4-2 cells but not in DU145 and RWPE-1 cells that do not express AR. (**e**) PRMT5 knockdown induced by Dox decreased AR expression in LNCaP and C4-2 stable cell lines. (**f**) Knockdown of PRMT5 in LNCaP-shPRMT5 cells reduced the mRNA level of the indicated AR target genes measured by qRT-PCR. (**g**) Restored cell growth by exogenous expression of FLAG-AR in LNCaP cells transiently co-transfected with SC, or pLKO-Tet-On-shPRMT5 (KD) in combination with pFLAG-CMV (Vec) or pFLAG-CMV-AR (AR). (**h**) Representative Western blots from **g** to verify the expression of FLAG-AR and the knockdown of PRMT5. *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.

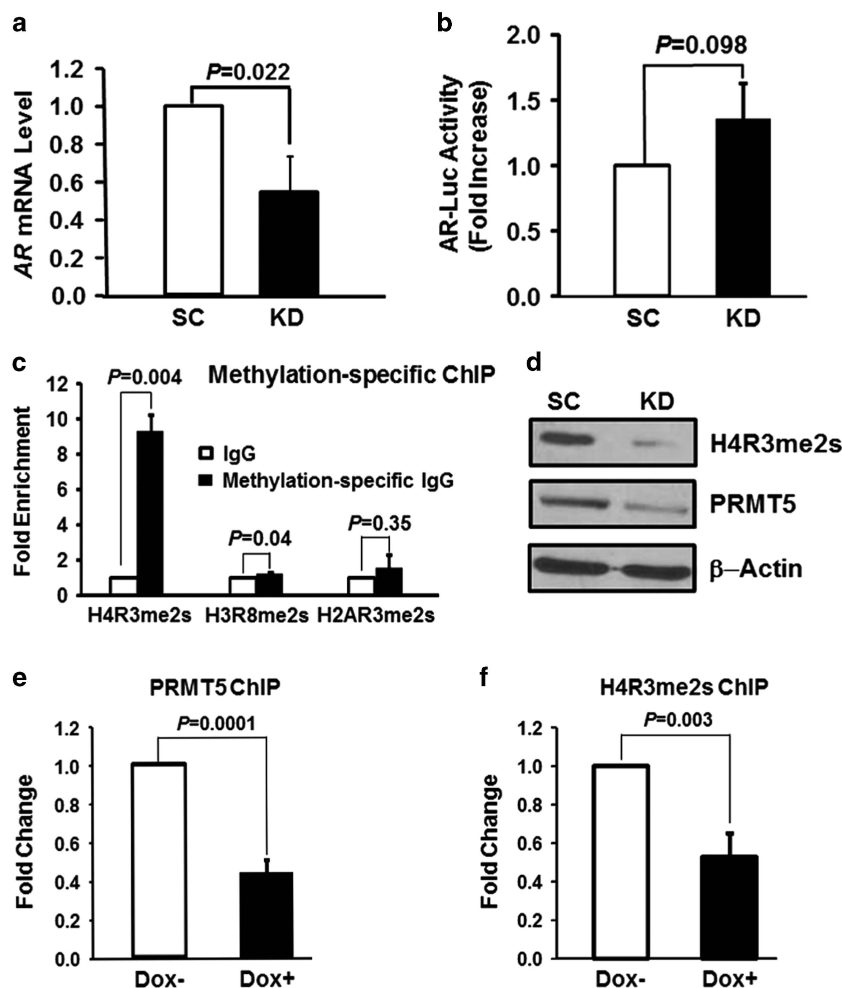


**Figure 2.** Epigenetic activation of AR transcription by PRMT5 in LNCaP cells. (**a**) Transient knockdown of PRMT5 (KD) reduced AR mRNA level when compared with SC. (**b**) Transient knockdown of PRMT5 had no effect on the AR-luciferase reporter gene (AR-Luc) activity. (**c**) Enrichment of H4R3me2s, but not H3R8me2s and H2AR3me2s, on the proximal promoter region of the AR gene in LNCaP cells. (**d**) Transient knockdown of PRMT5 reduced symmetric dimethylation of H4R3 (H4R3me2s). (**e**) Knockdown of PRMT5 induced by doxycycline (Dox+) reduced PRMT5 binding to the proximal promoter region of the AR gene when compared with cells without Dox (Dox – ). (**f**) Knockdown of PRMT5 induced by doxycycline (Dox+) reduced the enrichment of H4R3me2s on the proximal promoter region of the AR gene when compared with cells without Dox (Dox – ).

or prostate cancer tissues (Supplementary Figure S7). To analyze the correlation between AR and PRMT5 expression, we examined the expression of AR from the same TMA. In fact, PRMT5 expression in the nucleus correlated positively with AR expression in prostate tissues (Figures 4b and c). We also retrieved data from Oncomine that have >60 cases in each study, and found that PRMT5 expression correlated with AR at the transcript level in prostate cancer tissues (Figure 4d). Thus, it is likely that nuclear-localized PRMT5 may activate AR transcription in prostate tissues.

### PRMT5 knockdown inhibits AR expression and suppresses the growth of xenograft tumors in mice

To determine whether PRMT5 expression is necessary for the growth of xenograft tumors in mice, we used Dox-inducible stable cell lines expressing PRMT5 shRNA (LNCaP-shPRMT5) or SC (LNCaP-SC) to establish xenograft tumors in nude mice. As shown in Figure 5a, knockdown of PRMT5 completely suppressed the growth of LNCaP xenograft tumors. In fact, tumor growth in 8 out of 10 Dox-treated mice were completely suppressed. There was no significant difference in the growth of tumors derived from LNCaP-SC regardless of the Dox status (Figure 5b). The expression level of PRMT5 and AR was also downregulated in Dox-treated residual tumor nodules derived from LNCaP-shPRMT5 when compared with Dox-untreated (Figure 5c). Similar expression of PRMT5 and AR was observed in SC control tumors regardless of the Dox status (Figure 5d). These results demonstrate that PRMT5 is required for the growth of xenograft tumors in mice.

## DISCUSSION

AR signaling is a critical determinant of prostate cancer development and progression. Many studies have characterized how AR transcriptional activity is modulated by its co-activators and co-repressors.[19,21,24] However, how the transcription of AR itself is regulated, particularly at the epigenetic level, remains poorly understood. Here, we provide evidence showing that PRMT5 is a novel epigenetic activator of AR transcription in prostate cancer. First, knockdown of PRMT5 or inhibition of PRMT5 by a small molecule inhibitor specifically inhibited the growth of prostate cancer cells in an AR-dependent manner. Second, knockdown of PRMT5 specifically inhibited AR transcription. Third, PRMT5 binds to the proximal promoter region of the AR gene along with Sp1

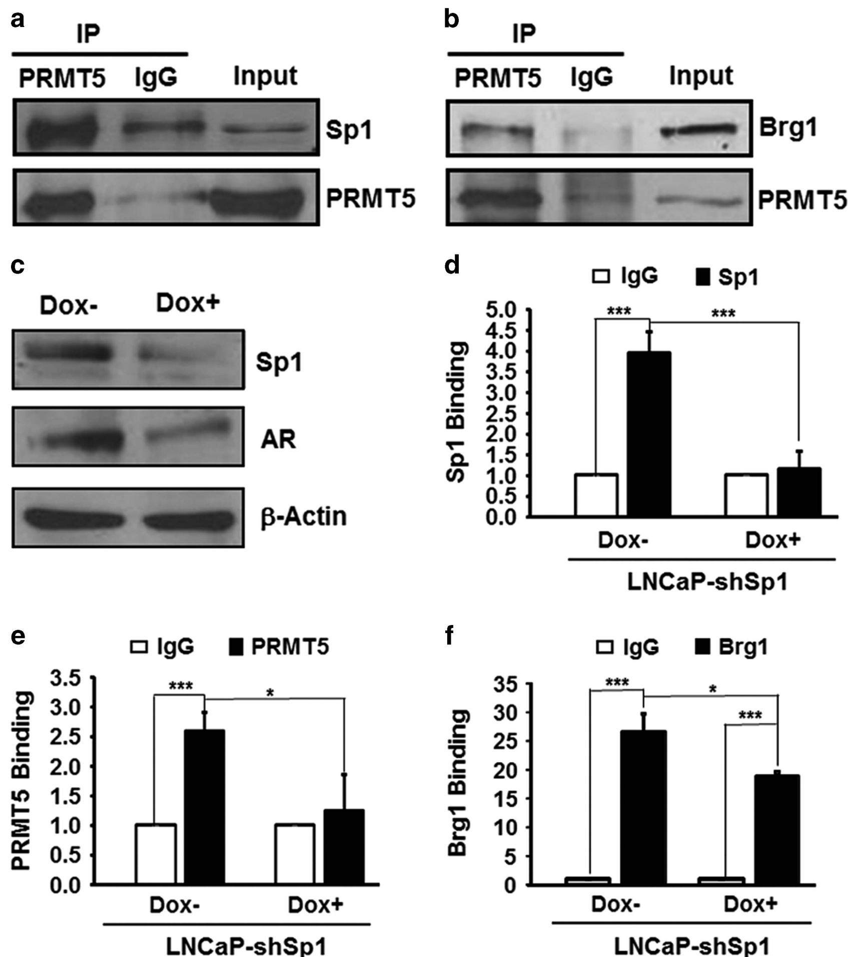


**Figure 3.** PRMT5 interacts with Sp1 and Brg1 on the proximal promoter region of the AR gene in LNCaP cells. (**a**) Co-immunoprecipitation of Sp1 with PRMT5. (**b**) Co-immunoprecipitation of Brg1 with PRMT5. (**c**) Knockdown of Sp1 induced by doxycycline (Dox+) reduced AR expression in Dox-inducible stable cell line LNCaP-shSp1. (**d**–**f**) Dox-induced knockdown of Sp1 reduced the binding of Sp1, PRMT5 and Brg1 to the same proximal promoter region of the AR gene. $*P < 0.05$ and $***P < 0.001$.

and Brg1. Fourth, H4R3me2s is highly enriched on the proximal promoter region of the AR gene. Fifth, PRMT5 is highly expressed in prostate cancer tissues and its expression correlates positively with AR expression at both mRNA and protein levels. Finally, depletion of PRMT5 expression completely suppressed the growth of LNCaP xenograft tumors in mice by downregulating AR expression.

Transcriptional regulation of gene expression is a tightly regulated process that involves the participation of multiple transcriptional regulatory proteins such as transcription factors, co-activators and co-repressors as well as chromatin-remodeling enzymes. Consistent with the fact that Sp1 is the major and well-characterized transcription factor that activates AR transcription in prostate cancer cells,[33,38] we indeed confirmed that Sp1 binds to the AR promoter and regulates AR expression in LNCaP cells. Because PRMT5 interacts with Sp1 and Brg1 and because Sp1 knockdown also reduces the binding of PRMT5 to the AR promoter, we suggest that Sp1 may recruit PRMT5 to the AR promoter. Interestingly, Brg1, an ATP-dependent chromatin remodeler,[39] was also recruited to the AR promoter through its interaction with PRMT5. This finding suggests that PRMT5-mediated H4R3 dimethylation could also activate transcription of target genes such as AR when Brg1 is recruited to the promoters (Figure 6), though PRMT5 generally represses transcription of target genes. Interestingly, PRMT5-mediated H3R8 dimethylation is also involved in transcriptional activation of target genes when Brg1 is recruited to the target gene promoters.[34,35] Although this manuscript was in preparation, a recent report showed that PRMT5 can dimethylate H4R3 and H3R8 to regulate the expression of the protein kinase FLT3 in acute myeloid leukemia cells via two distinct pathways.[40] Thus, dimethylation of either H3R8 or H4R3 by PRMT5 may permit ATP-dependent chromatin remodeling, leading to activation or repression of target gene transcription. Given that PRMT5 and Brg1 also cooperate to repress transcription of target genes[41–43] and that AR transcription is subjected to the regulation of DNA methylation and histone lysine methylation,[44] it is likely that AR transcription is subjected to a high order of epigenetic regulation. Future studies to gain insight into the epigenetic regulation of AR may offer new opportunities to develop novel targeting strategies to inhibit or even eliminate AR expression. Because PRMT5 may exhibit an opposite role in the cytoplasm and nucleus in cells,[9,25] it remains to be determined whether cytoplasmic- and nuclear-localized PRMT5 may have distinct effects on the transcription of AR.

The present finding has significant clinical implications due to the central role of AR in prostate cancer development and progression. Our findings here, together with a previous study showing that PRMT5 may form a complex with MEP50 and AR to modulate the transcriptional activity of AR,[45] raise an interesting

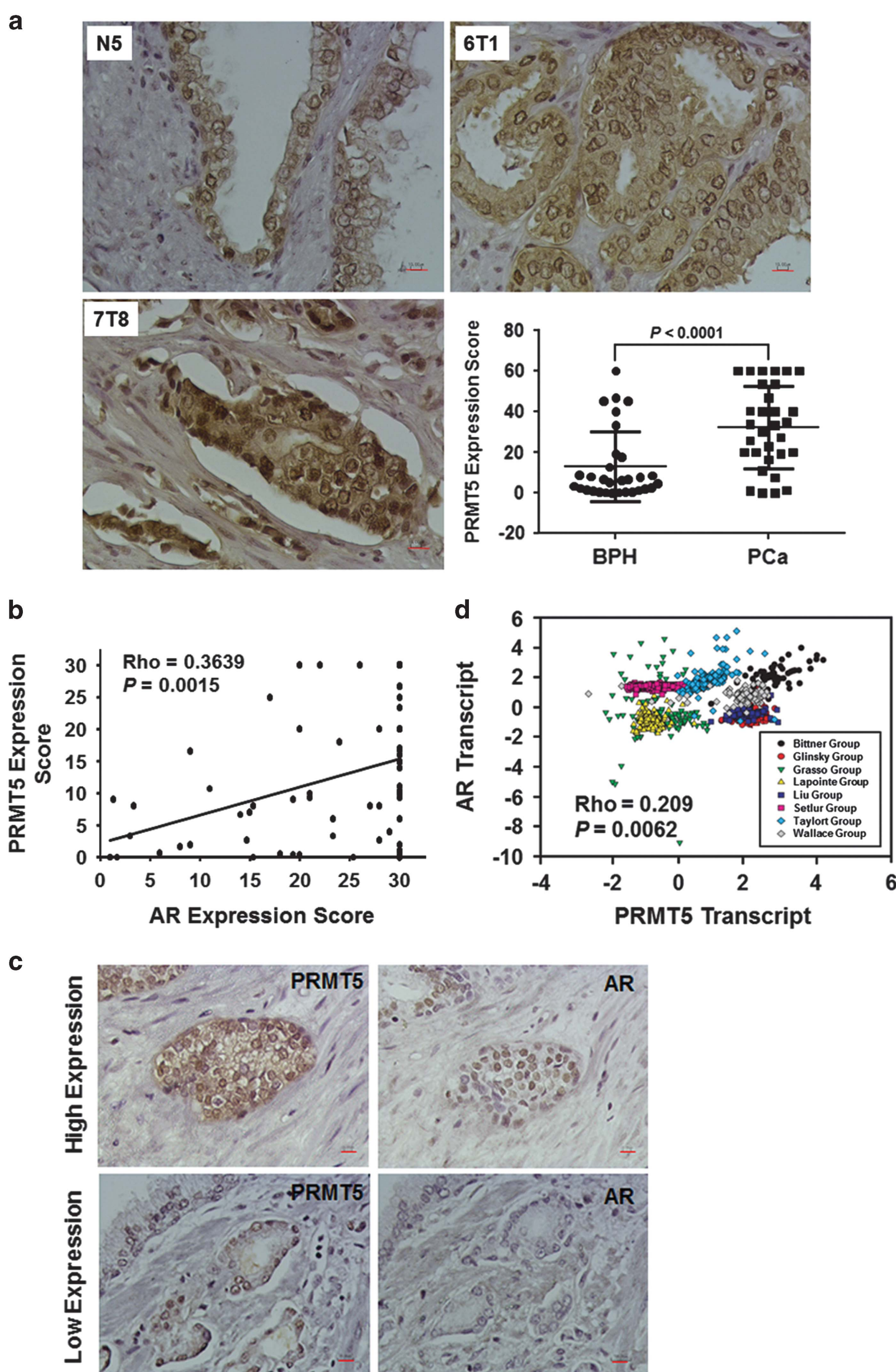


**Figure 4.** PRMT5 expression correlates positively with AR expression in prostate cancer. (**a**) Shown are representative immunohistochemistry staining images (magnification ×400) of PRMT5 in benign tissue (N5), Gleason 6 prostate cancer tissue (6T1) and Gleason 7 prostate cancer tissue (7T8). The total expression score of PRMT5 is significantly higher in prostate cancer tissues (PCa) when compared with BPH. Scale bar, 30 μm. (**b**) PRMT5 expression correlates positively with AR expression at the protein level in the same TMA from **a**. (**c**) Representative images of PRMT5 and AR expression from serial sections of prostate cancer tissues. The upper panels show higher expression of both PRMT5 and AR in the nucleus and the lower panels show weaker expression of both PRMT5 and AR in the nucleus. Scale bar, 30 μm. (**d**) PRMT5 expression correlates positively with AR expression at the transcript level. The data were retrieved from Oncomine database.

possibility that targeting PRMT5 may have a dual effect on both the expression and activity of AR. Thus, PRMT5 may be an ideal target for development of novel therapeutics. As radiotherapy in combination with adjuvant ADT is the current standard treatment for locally advanced prostate cancer, combining radiotherapy with PRMT5 targeting may be an alternative approach. Perhaps targeting AR expression by inhibiting PRMT5 may avoid some adverse effects often seen with ADT. It is worth noting that PRMT5 also regulates the expression of AR in the CRPC line C4-2. As AR reactivation is the major mechanism underlying the development of CRPC[13,14] and the resistance to the next-generation anti-androgen therapy,[17,18] targeting PRMT5 alone or in combination

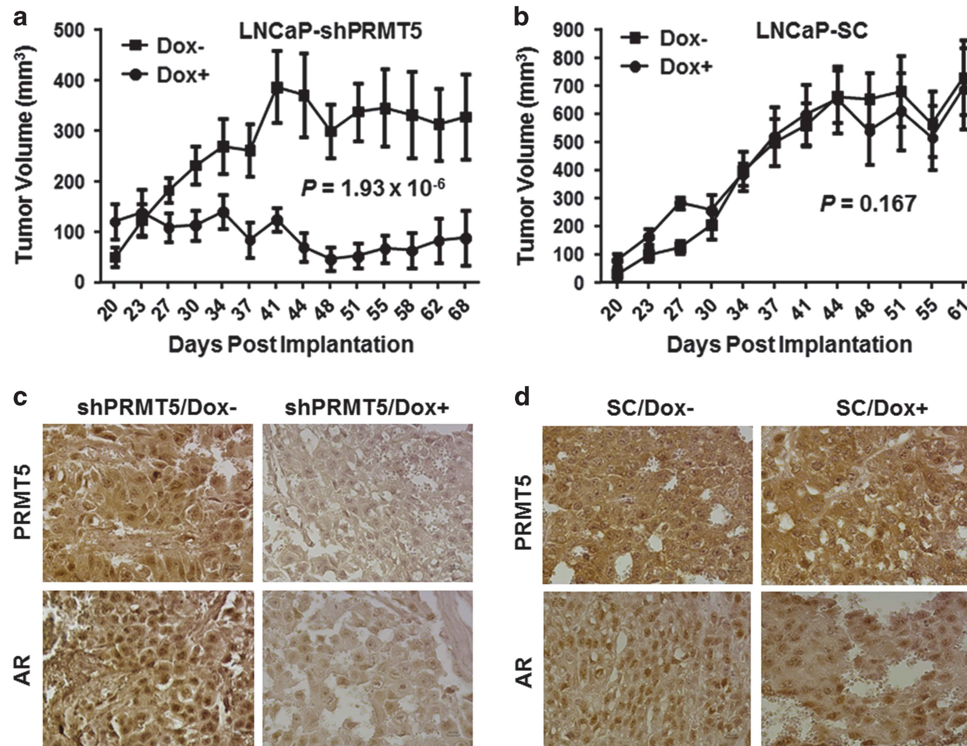


**Figure 5.** Knockdown of PRMT5 suppresses the growth of xenograft tumors in mice. (**a**) LNCaP-shPRMT5 cells were implanted subcutaneously into the right lower flanks of 10 nude mice per group, and the tumor growth was monitored twice weekly in Dox-treated (Dox+) and untreated (Dox −) mice. (**b**) Similar experiment was performed as described in **a** for LNCaP-SC cell line. (**c** and **d**) Representative images showing inhibition of PRMT5 and AR expression in Dox-treated tumor nodules. No effect on PRMT5 and AR expression in xenograft tumors derived from LNCaP-SC was observed. Scale bar: 10 μm.

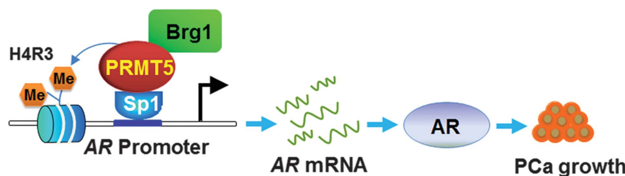


**Figure 6.** Proposed model for epigenetic activation of AR transcription by PRMT5.

with other AR-targeting agents may exhibit a better treatment efficacy than the existing treatments. Given that two small molecule inhibitors of PRMT5 have been developed,[31,40,46] preclinical evaluation of these inhibitors alone or in combination with radiotherapy or other AR-targeting agents may lead to the development of novel therapeutic approaches for prostate cancer treatment.

## MATERIALS AND METHODS

### Cell lines and culture

Prostate cancer cell lines LNCaP, DU145, and PC-3 as well as RWPE-1 cells were purchased from ATCC (Manassas, VA, USA) and C4-2 cells were purchased from M.D. Anderson Cancer Center (Houston, TX, USA). All frozen stock received were immediately expanded and aliquots were prepared and stored in liquid nitrogen for future use, and cells were maintained for no longer than 3 months as described previously.[30,47] Cell line authentication was performed by IDEXX BioResearch (IMPACT I). The establishment of stable cell lines was described previously.[26,30]

### Plasmid construction

The pLKO-Tet-On plasmid for expressing shRNA was obtained from Addgene (Cambridge, MA, USA),[48] and the two shRNA sequences that target 5′-GCCCAGTTTGAGATGCCTTAT-3′ (#1577) and 5′-CCCATCCTCTTCCCTATTAAG-3′ (#1832) for PRMT5 knockdown and that target 5′-CCACTCCTTCAGCCCTTATTA-3′ (#2310) for Sp1 knockdown were selected for constructing pLKO-Tet-On-shPRMT5 and pLKO-Tet-On-shSp1 as described previously.[30] The pLKO-Tet-On-SC and pFLAG-CMV-AR were constructed before.[30] The *AR* promoter luciferase reporter gene construct and the *PSA* promoter luciferase reporter gene construct were kindly provided by Dr Donald Tindall. pFLAG-CMV-AR was made by subcloning the *AR* cDNA into pFLAG-CMV vector. All plasmids were confirmed by DNA sequencing.

### Cell proliferation assay

The cell proliferation assay was performed using MTT reagent (Sigma, St Louis, MO, USA). For transient transfection experiments, LNCaP, DU145 or PC-3 cells ($4 \times 10^3$) were seeded in 48-well plates for 24 h, and then transiently transfected with pLKO-Tet-On-shPRMT5 (#1577) or the SC control using FuGENE HD or FuGENE 6 (Promega, Madison, WI, USA) for 96 h after the transfection. For MTT analysis, cell medium was removed and 70 μl of MTT solution (0.5 mg/ml) was added into each well and incubated at 37 °C for 4 h. At the end of incubation, MTT solution was removed and 130 μl of DMSO was added into each well and incubated at 37 °C for another 10 min. The plates were then read at 560 nm with TECAN Microplate Reader (TECAN, Mannedorf, Switzerland). For LNCaP, DU145, C4-2 and RWPE-1 stable cell lines, similar procedure was followed except that Dox was added at 1 μg/ml to induce PRMT5 knockdown during culture. At least three independent experiments were performed and the mean ± s.d. was presented. Student's *t*-test was performed to determine the statistical significance. The effect of PRMT5 inhibitor BLL3.3 on the growth of LNCaP, DU145 and RWPE-1 cells was similarly determined by MTT.

### Soft-agar growth assay

The soft-agar growth assay to measure anchorage-independent proliferation of LNCaP cells was performed by using the 96-well plate format as described previously.[49] Briefly, LNCaP cells were transfected with pLKO-Tet-On-shPRMT5 (#1577) or pLKO-Tet-On-SC for 24 h, and then $2.5 \times 10^3$ cells were added into the middle layer agar. Dox was added into each layer

of soft agar at 1 µg/ml to induce the expression of shRNAs. The plates were incubated at 37 °C, 5% $CO_2$ for 7 days. To quantify the colony-formation efficiency, 16 µl of AlamerBlue Cell Viability Reagent (Invitrogen, Carlsbad, CA, USA) was added into each well and incubated at 37 °C for another 4 h. Fluorescence intensity was measured at 570EX nm/600EM nm using Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA). Experiments were performed in triplicate, and results from three independent experiments were analyzed and presented as mean ± s.d. Student's *t*-test was used to determine the statistical significance.

### qRT-PCR and western blotting

To determine the effect of PRMT5 knockdown on AR expression, PRMT5 were transiently or stably knocked down in LNCaP cells for 96 h, and total RNA was isolated using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA). One microgram of total RNA was used for reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Promega) according to manufacturer's instruction. The qRT-PCR analysis of *AR* or AR target genes (*PSA*, *KLK2*, *TMPRSS2*) was performed as described previously.[30] Antibodies against AR (SC-816, Santa Cruz, CA, USA), PRMT5 (07-405, Millipore, Billerica, MA, USA), PSA (1984-1, Epitomics, Burlingame, CA, USA), FLAG (Sigma, F-1804), Sp1 (ab13370, Abcam, Cambridge, MA, USA), H4R3me2s (Abcam, ab5823), H3R8me2s (Abcam, ab130740), H2AR3me2s (Abcam, ab22397), and Brg1 (Abcam, ab110641) were used for western blotting analysis.

### Chromatin immunoprecipitation assay

The LNCaP stable cell line or parental cells were cultured in the presence or absence of Dox (1 µg/ml) for 96 h. At the end of induction, 270 µl of 37% formaldehyde was added into each dish and incubated at room temperature for 10 min. Then 1 ml of 1.25 M glycine was added to stop the cross-linking reaction. Cells were then harvested, resuspended in 1 ml of immunoprecipitation buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% TritonX-100, 0.5% NP-40, 0.5 mM DTT, and protease and phosphatase inhibitors), and finally sonicated (Branson Sonifier250set, Wilmington, NC, USA) to prepare sheared chromatin. Antibodies against PRMT5 (Millipore, 07-405), Sp1 (Santa Cruz, SC7824), Brg1 (Abcam, ab110641), H4R3me2s (Abcam, ab5823), H3R8me2s (Abcam, ab130740), H2AR3me2s (Abcam, ab22397) and IgG (Santa Cruz, SC2027) were used to immunoprecipitate protein-DNA complexes for isolation of PCR-ready DNA using the Fast ChIP protocol described previously.[50] The co-immunoprecipitated proximal promoter region of *AR* (−493 to − 226) was quantified by qRT-PCR. Results were normalized to the IgG control and are presented as mean ± s.d. from three independent experiments. Student's *t*-test was used to determine the statistical significance.

### Co-immunoprecipitation of PRMT5 with Sp1 and Brg1

Total cell lysate of LNCaP cells was prepared in immunoprecipitation buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% TritonX-100, 0.5% NP-40, 0.5 mM DTT, and protease and phosphatase inhibitors) for co-immunoprecipitation. Anti-PRMT5 antibody or IgG was used to immunoprecipitate PRMT5 from 500 µg of total lysate, and co-immunoprecipitated Sp1 and Brg1 was detected with Sp1 and Brg1 antibodies.

### Expression of PRMT5 and AR and the analysis of their correlation in prostate cancer tissues

A TMA consisting of 32 BPH tissues and 40 prostate cancer tissues (20 with Gleason score 6 and 20 with Gleason score ⩾ 7) was used for immunohistochemistry analysis of PRMT5 and AR expression. Briefly, paraffin section of the TMA was deparaffinized in xylene and rehydrated in graded ethanol, followed by inactivation of endogenous peroxidase activity in 3% hydrogen peroxide for 10 min. Antigen retrieval was performed by heating slides in 10 mM Tris-HCl (pH 10) for 30 min in microwave. After three washes with phosphate-buffered saline containing 0.1% Tween 20 (PBST), slides were blocked in 5% non-fat milk in PBST at room temperature for 1 h. The primary antibodies against PRMT5 or AR was incubated at 4 °C overnight, followed by three washes with PBST and incubation with HRP-conjugated anti-rabbit secondary antibodies (Amersham, Pittsburgh, PA, USA) at room temperature for 1 h. The signal was developed with diaminobenzidine for 10 min, and sections were counterstained with hematoxyline. The semi-quantification of PRMT5 and AR expression was performed as described previously with slight modifications.[51] The intensity was scored as 0 (no expression), 1 (low expression), 2 (moderate expression) and 3 (high expression), and the percentage of cells showing the expression was scored ranging from 0 to 10 with 10 as the highest percentage (100%). The expression score for cytoplasmic- and nuclear-localized PRMT5 was respectively determined by the intensity score times the percentage (0–30), and the total expression score is the sum of the cytoplasmic and nuclear expression scores (0–60). The unpaired *t*-test was used to determine the statistical significance of the total mean expression score between BPH and prostate cancer tissues, and paired *t*-test was used to determine the difference in expression scores between cytoplasmic-localized PRMT5 and nuclear-localized PRMT5. The same semi-quantification method was used for AR expression in the nucleus.

To determine the correlation between the expression of PRMT5 and AR in the nucleus in prostate tissues, their nuclear expression scores were used for Pearson's analysis. To determine the correlation of PRMT5 and AR expression at the transcript level, we retrieved their expression data from 8 studies that have $>60$ tissues from Oncomine. The statistic Q was calculated to test the homogeneity of effect sizes across studies for each of the three methods (Pearson's, Spearman's and Kendall's),[52] and it was found that the effect sizes across studies were not homogeneous (all with *P*-value $< 1e-12$). Therefore, we used a random-effects model for the meta-analysis of each method.[53]

### Xenograft tumor growth in nude mice

Animal experiments were approved by the Purdue University Animal Care and Use Committee. Male athymic nude mice (5–7 week old) were purchased from Harlan Laboratories (Indianapolis, IN, USA), and $3\times10^6$ cells of established stable cell lines that inducibly express PRMT5 shRNA or SC were co-injected subcutaneously into the right lower flank of 20 mice with Matrigel (1:1 in volume). Assuming that PRMT5 knockdown can reduce tumor volume by 30% and that standard deviation within each group is about 25% of the mean tumor volume, a sample size of 10 male mice per group will have over 80% power to detect a 30% difference between the two groups at alpha level 0.05. Mice were randomly divided into two groups (10 mice/group) for each stable cell line by using Excel-based randomization method, and treated with Dox (1 mg/ml in drinking water) or without Dox (drinking water only). Tumor growth was monitored twice weekly, and tumor volume was calculated using ½ × L × W × H without using blinding method. At the end of experiments, tumors were resected and formalin fixed, and paraffin embedded. Immunohistochemistry analysis of PRMT5 and AR expression was similarly performed as described above. We used the following linear mixed model to model the j-th observed xenograft tumor volume of i-th mouse, that is, yij, assuming cubic polynomial growth of tumors over time,

$$y_{ij} = \gamma_{i0} + \gamma_{i1}t_j + \gamma_{i2}t_j^2 + \gamma_{i3}t_j^3 + \varepsilon_{ij}, \;\; \varepsilon_{ij} \sim N(0,\sigma^2), \;\; \gamma_{ik} \sim N(\beta_k + \delta_k D_i, \sigma_k^2),$$

Where, $t_j$ is the number of days after implantation for the j-th observation, $D_i$ indicates whether the i-th subject is under Dox. The random-effects are independent, and the errors of the same subject are assumed to follow a first-order continuous autoregressive model.

To evaluate the effects of Dox on the tumor growth, we are subject to test the $H_0$: $\delta_0 = \delta_1 = \delta_2 = \delta_3 = 0$ against $H_a$: at least one of $\delta_0$, $\delta_1$, $\delta_2$, $\delta_3$ is not zero.

We used the likelihood ratio test ($\chi^2$-test) to conduct the hypothesis tests.

For PRMT5 knockdown, the *P*-value is $1.9305\times10^{-6}$. For SC, the *P*-value is 0.1670. Error bar, s.e.m.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

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## Legends to Supplementary Figures

**Figure S1. Transient knockdown of PRMT5 inhibits prostate cancer cell growth in LNCaP cells but not DU145 and PC-3 cells.** (**a**) Knockdown of PRMT5 in the indicated prostate cancer cells transiently transfected with scrambled control (SC) or pLKO-Tet-On-shPRMT5(#1577). (**b-d**) Transient knockdown of PRMT5 inhibited cell proliferation in LNCaP, but not in DU145 and PC-3 cells assayed by MTT. (**e**) Transient knockdown of PRMT5 by pLKO-Tet-On-shPRMT5(#1577) significantly inhibited colony formation in soft agar assay when compared with SC.

**Figure S2. Western blotting analysis of PRMT5 and AR expression in LNCaP and C4-2 cells.** Higher expression level of PRMT5 and AR was observed in C4-2 cells.

**Figure S3. Rescue of PRMT5 knockdown-induced growth inhibition by AR overexpression in LNCaP cells.** (**a**) LNCaP-shRNA stable cell line were transfected with pFLAG (Vector) or pFLAG-AR (AR) and cultured for 7 days in the presence of doxycycline (Dox+) or absence of doxycycline (Dox-). Cell growth was determined by Trypan blue staining, and the fold increase of cell growth was determined by dividing the total number of cells at day 7 by the number of cells seeded initially. (**b**) Cell lysate from (**a**) was used to determine the total expression of AR using an anti-AR antibody and the expression of PRMT5 using an anti-PRMT5 antibody. (**c-e**) Similar experiments were performed as described in (**a**), and total RNA was isolated for qPCR quantification of *PSA*, *TMPRSS2* and *KLK2*. Three independent experiments were performed and mean+SD was presented. The Student's *t*-test was used for *P* value calculation between the indicated two groups.

**Figure S4**. **Inhibition of PRMT5 by a small molecule inhibitor attenuates cell proliferation and reduces AR expression in LNCaP cells**. (**a**) LNCaP cells were treated with 10 μM of BLL3.3, a selective small molecule inhibitor of PRMT5, and cell growth was determined by MTT. (**b**) LNCaP cells were incubated with BLL3.3 (10 μM) for 6 days, and the down-regulation of AR expression and the inhibition of symmetric dimethylation of H4R3 (H4R3me2) by the inhibitor were confirmed by Western blotting. Note that BLL3.3 had no effect on the expression level of PRMT5. (**c** and **d**) Similar cell growth experiments were performed for DU145 and RWPE-1 as LNCaP and no inhibitory effect was observed.

**Figure S5**. **Immunoprecipitation of histones H4R3, H3R8 and H2A by methylation-specific antibodies**. LNCaP cells were crosslinked and chromatins were fragmented as did for ChIP analysis except that proteins were not digested with protease K. Antibodies that recognize H4R3me2s, H3R8me2s and H2AR3me2s were used to immunoprecipitate H4R3, H3R8 and H2A, respectively. All three histones were efficiently immunoprecipitated when compared with the IgG control.

**Figure S6**. **Effect of PRMT5 knockdown on the methylation status of histones.** The established doxycycline (Dox)-inducible PRMT5 knockdown cell line LNCaP-shPRMT5 was induced by Dox (1 μg/ml) for 96 h (Dox+) or without Dox induction (Dox-), and total cell lysate was prepared for Western blotting analysis of H4R3me2s, H3R8me2s, and H2AR3me2s.

**Figure S7**. **Expression of PRMT5 in the cytoplasm and nucleus in prostate tissues.** The expression score of both cytoplasmic and nuclear expression of PRMT5 in a prostate cancer TMA was semi-quantified, and the paired *t*-test was used to determine the statistical significance in the subcellular localization of PRMT5 in both BPH (32 cases), prostate cancer tissues with Gleason score 6 (20 cases), and prostate cancer tissues with Gleason score 7 and above (20 cases).

## Figure S1

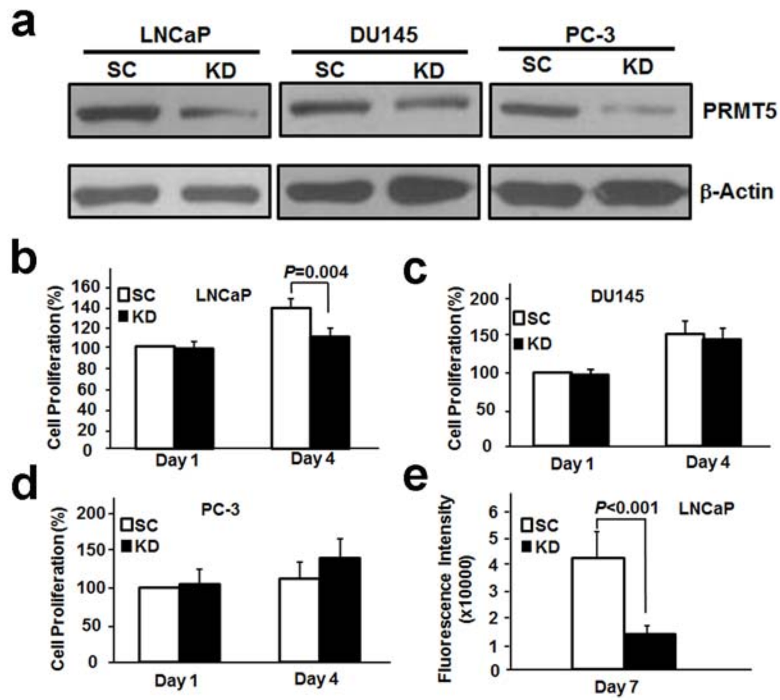
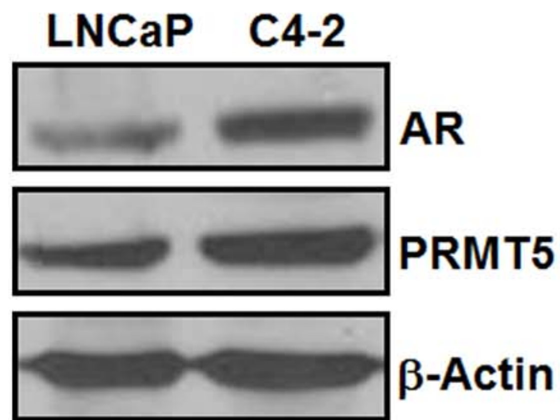


Figure S2



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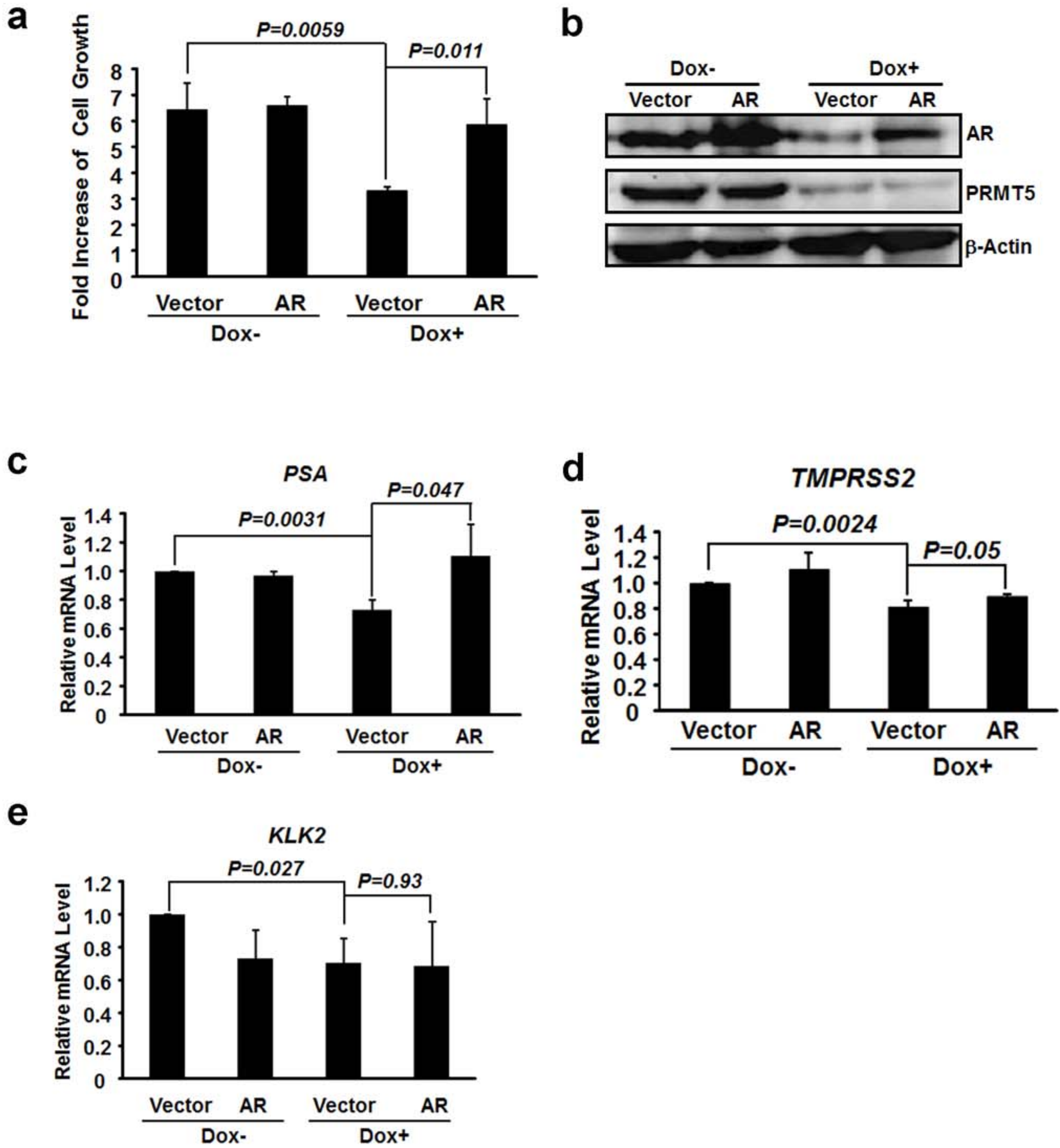
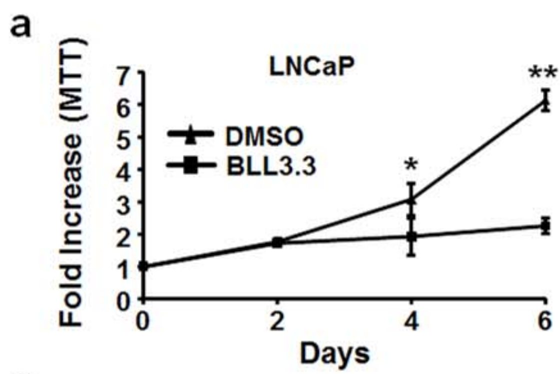
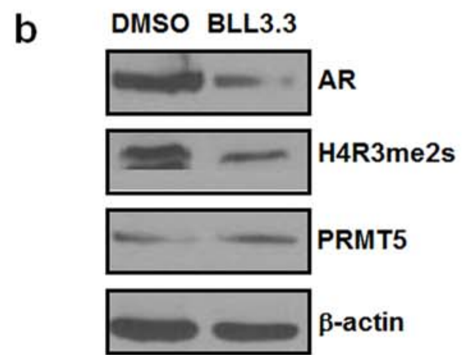
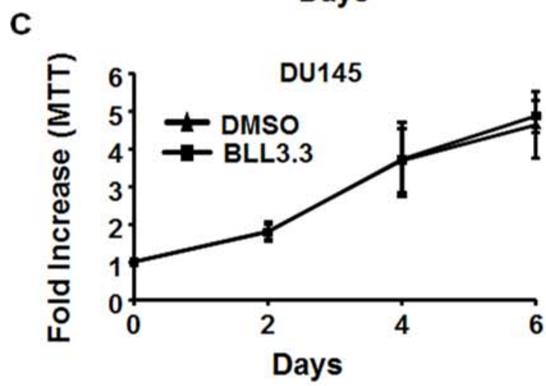


## Figure S4



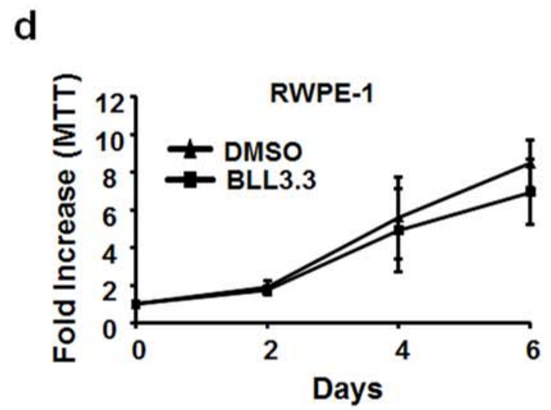



**Figure S5**

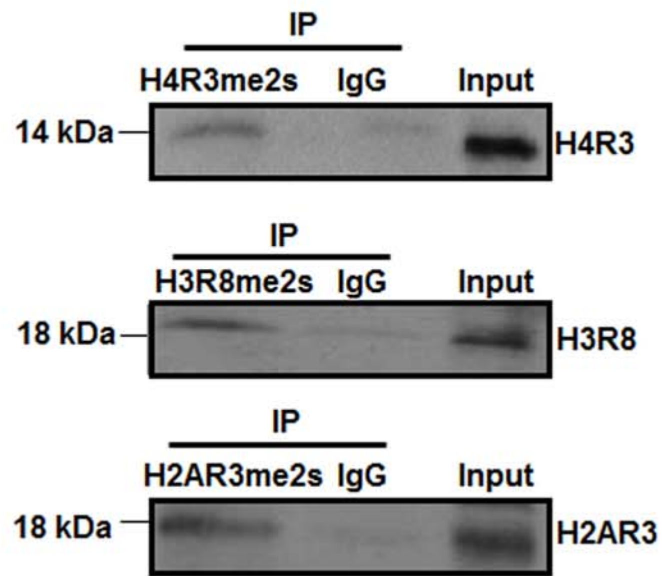


Figure S6

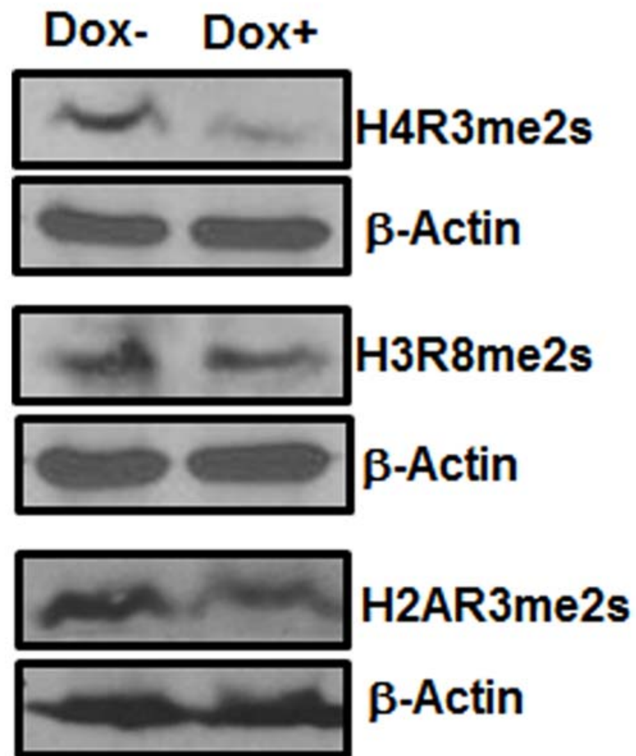
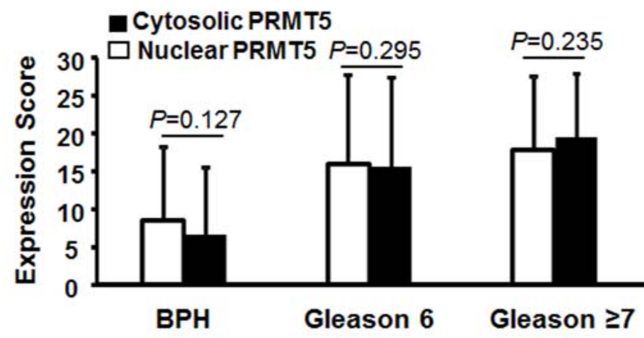


Figure S7



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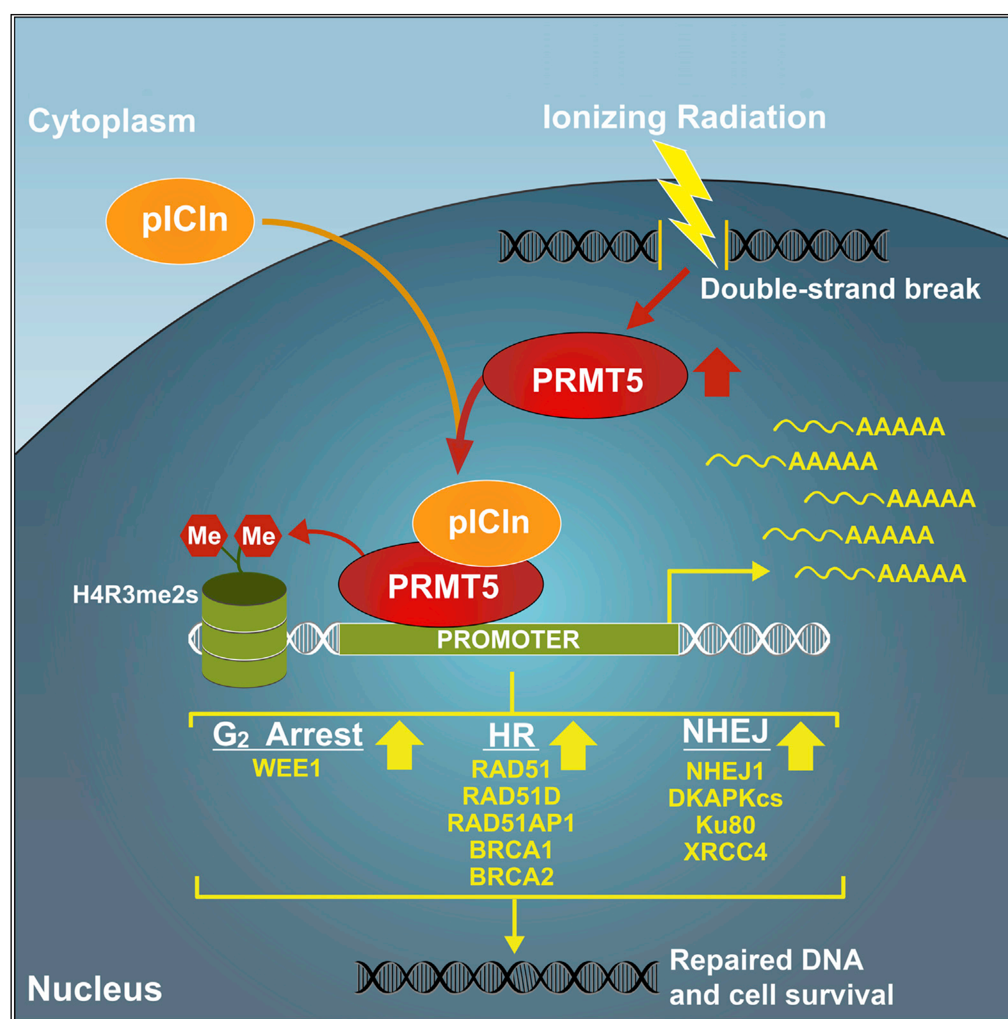
# PRMT5 Cooperates with pICln to Function as a Master Epigenetic Activator of DNA Double-Strand Break Repair Genes

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## HIGHLIGHTS

PRMT5 activates transcription of DSB repair genes upon DNA damage

pICln cooperates with PRMT5 to activate transcription of DSB repair genes

Targeting PRMT5 is effective to sensitize multiple cancer types to radiation

PRMT5 expression positively correlates with DSB repair genes in cancer tissues

## DATA AND CODE AVAILABILITY

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# PRMT5 Cooperates with pICln to Function as a Master Epigenetic Activator of DNA Double-Strand Break Repair Genes

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## SUMMARY

**DNA double-strand break (DSB) repair is critical for cell survival and genome integrity. Upon recognition of DSBs, repair proteins are transiently upregulated to facilitate repair through homologous recombination (HR) or non-homologous end joining (NHEJ). We present evidence that PRMT5 cooperates with pICln to function as a master epigenetic activator of DNA damage response (DDR) genes involved in HR, NHEJ, and $G_2$ arrest (including RAD51, BRCA1, and BRCA2) to upregulate gene expression upon DNA damage. Contrary to the predominant role of PRMT5 as an epigenetic repressor, our results demonstrate that PRMT5 and pICln can activate gene expression, potentially independent of PRMT5's obligate cofactor MEP50. Targeting PRMT5 or pICln hinders repair of DSBs in multiple cancer cell lines, and both PRMT5 and pICln expression positively correlates with DDR genes across 32 clinical cancer datasets. Thus, targeting PRMT5 or pICln may be explored in combination with radiation or chemotherapy for cancer treatment.**

## INTRODUCTION

Repair of DNA double-strand breaks (DSBs), the most lethal DNA damage, is critical for cell survival and maintenance of genome integrity (Khanna and Jackson, 2001). DSBs can be induced both endogenously as well as exogenously through DNA damaging agents or ionizing radiation (IR). Upon recognition of extensive DSBs, repair proteins are upregulated (Khalil et al., 2012; Rieger, 2004; Russell et al., 2003) and recruited to the sites of damage to facilitate repair through either homologous recombination (HR) or non-homologous end joining (NHEJ) (Thompson, 2012). Although the highly regulated recruitment and action of repair proteins are well characterized, little is known about how their expression is induced upon DNA damage.

Protein arginine methyltransferase 5 (PRMT5) is an emerging epigenetic enzyme that regulates cellular processes including cell proliferation, differentiation, and cell cycle progression (Karkhanis et al., 2011; Stopa et al., 2015). PRMT5 regulates these cellular processes through changes in gene expression via symmetrical dimethylation of arginine residues in histones H4R3 (H4R3me2s), H3R2 (H3R2me2s), H3R8 (H3R8me2s), and H2AR3 (H2AR3me2s) and post-translational regulation of non-histone substrates (Stopa et al., 2015). PRMT5 activity is modulated by several interacting proteins including MEP50, which is believed to be the obligate cofactor of PRMT5 and required for PRMT5 methyltransferase activity (Burgos et al., 2015; Chen et al., 2017; Karkhanis et al., 2011; Stopa et al., 2015). Accumulating evidence suggests that PRMT5 may act as an oncogene to promote cancer cell growth (Karkhanis et al., 2011; Stopa et al., 2015). Consistent with this, PRMT5 is overexpressed in several cancers and its elevated expression correlates with disease progression and poor prognosis (Karkhanis et al., 2011; Stopa et al., 2015; Yang and Bedford, 2013). Thus, PRMT5 has been proposed as a potential therapeutic target for cancer treatment (Richters, 2017).

We have recently reported that PRMT5 is overexpressed in ∼60% of intermediate- and high-risk prostate cancer cases and that PRMT5 expression in prostate cancer tissues positively correlates with androgen receptor (AR) expression (Deng et al., 2017). Mechanistically, PRMT5 is recruited to the AR promoter to activate AR transcription through H4R3me2s in prostate cancer cells (Deng et al., 2017). Because AR drives prostate cancer development and progression, targeting AR signaling through androgen deprivation therapy (ADT) is a standard of care to treat metastatic prostate cancer (Heidenreich et al., 2014). ADT is also

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used as a radiosensitization approach to enhance radiation therapy (RT) for localized prostate cancer patients (Golabek et al., 2016). Our finding that PRMT5 activates AR transcription raised the possibility that PRMT5 may be a therapeutic target for prostate cancer radiosensitization.

Contrary to our expectation, we observed that targeting PRMT5 sensitized prostate cancer cells to IR independently of AR expression. Here, we present evidence that PRMT5 cooperates with pICln, independently of its canonical cofactor MEP50, to function as a master epigenetic activator of DNA damage response (DDR) genes in various cell types. Upon DNA damage, PRMT5 and pICln upregulate target genes that encode proteins involved in HR (RAD51, RAD51D, RAD51AP1, BRCA1, and BRCA2), NHEJ (NHEJ1/XLF and DNAPKcs), and $G_2$ arrest (WEE1). Targeting PRMT5 or pICln decreases expression of these DDR genes and hinders repair of DSBs in multiple cancer cell lines suggesting that PRMT5 may play a conserved role in DDR. Thus, targeting PRMT5 or pICln may be explored as a monotherapy or in combination with radiation or chemotherapy for cancer treatment. Significantly, both PRMT5 and pICln expression positively correlates with the expression of these target genes across most of the 32 clinical cancer datasets analyzed. Although PRMT5, along with its obligate cofactor MEP50, primarily functions as an epigenetic repressor, our results demonstrate that PRMT5 together with pICln can activate gene expression and provide a potential mechanism for the transient upregulation of repair proteins upon DNA damage.

## RESULTS

### Targeting PRMT5 Sensitizes Prostate Cancer Cells to IR in an AR-Independent Manner

ADT is the only approved clinical radiosensitization approach for prostate cancer treatment (Golabek et al., 2016). Because we recently identified PRMT5 as a novel epigenetic activator of AR (Deng et al., 2017), we tested whether targeting PRMT5 can mimic ADT to sensitize prostate cancer cells to IR. To this end, we established lentivirally infected stable pools with doxycycline (Dox)-inducible PRMT5 knockdown and observed that knockdown of PRMT5 sensitized AR-expressing LNCaP prostate cancer cells (LNCaP-shPRMT5 pool) to IR when compared with scramble control cell lines (LNCaP-shSC) (Figure 1A). Likewise, inhibition of PRMT5 by our inhibitor BLL3.3 (Alinari et al., 2015; Deng et al., 2017) also sensitized LNCaP cells to IR (Figure 1B). Consistent with previous findings that AR regulates several target genes involved in NHEJ (Goodwin et al., 2013; Polkinghorn et al., 2013; Tarish et al., 2015), pharmacological inhibition of PRMT5 with BLL3.3 in irradiated LNCaP cells indeed caused a decrease in AR expression and a concomitant decrease in the expression of Ku80/XRCC5, XRCC4, and DNAPKcs/PRKDC at the mRNA level (Figure 1C). Contrary to our expectation, knockdown of PRMT5 also sensitized AR-negative prostate cancer cell lines PC3 and DU145 to IR when similar Dox-inducible knockdown stable cell lines (PC3-shPRMT5 pool and DU145-shPRMT5 pool) were used (Figures 1D and 1E). However, BLL3.3 treatment had little to no effect on the expression of AR-target genes involved in NHEJ in irradiated AR-negative DU145 cells (Figure 1F). Given these results, we isolated single-cell-derived clones to develop Dox-inducible knockdown stable cell lines (LNCaP-shPRMT5 and LNCaP-shPRMT5 #2) for all subsequent studies. Dox-induced PRMT5 knockdown is shown in Figures S3B–S3F and is reported previously (Deng et al., 2017). These results suggest that the radiosensitization effect of PRMT5 targeting in prostate cancer cells is likely mediated through both AR-dependent and -independent mechanisms.

### PRMT5 Regulates the Repair of DNA Double-Strand Breaks in Prostate Cancer Cells Independently of AR Expression

Next, we determined if the radiosensitization effect of PRMT5 targeting was due to defects in the repair of IR-induced DSBs. We first treated LNCaP cells with IR and quantified DSBs via γH2AX foci analysis to assess the formation and repair of IR-induced DSBs. The majority of DSBs were repaired within 2–6 h following IR treatment (Figures 2A and 2B). To assess if PRMT5 is required for efficient repair of IR-induced DSBs, we analyzed γH2AX foci 6 h following IR in more detail. Cells with PRMT5 knockdown retained significantly more DSBs 6 h following IR treatment than cells without knockdown, indicating a defect in DSB repair (Figures 2C and 2D). Nearly identical results were obtained using a different PRMT5-targeting shRNA (LNCaP-shPRMT5 #2) (Figures 2E and 2F). Treatment of LNCaP cells with BLL3.3 conferred the same effect as PRMT5 knockdown (Figures 2G and 2H), whereas Dox-induced expression of scramble control (SC) shRNA in LNCaP-shSC cells had no effect (Figures 2I and 2J). Cells with PRMT5 knockdown retained significantly more γH2AX foci even 24 h following IR treatment than cells without knockdown (Figures 2K and 2L), indicating a prolonged defect in DSB repair. The defects in DSB repair upon PRMT5 knockdown were unlikely an artifact of crosstalk between histone posttranslational modifications, as we observed similar results when quantifying DNA damage directly via comet assay (Figures S1A–S1D). Furthermore, knockdown of

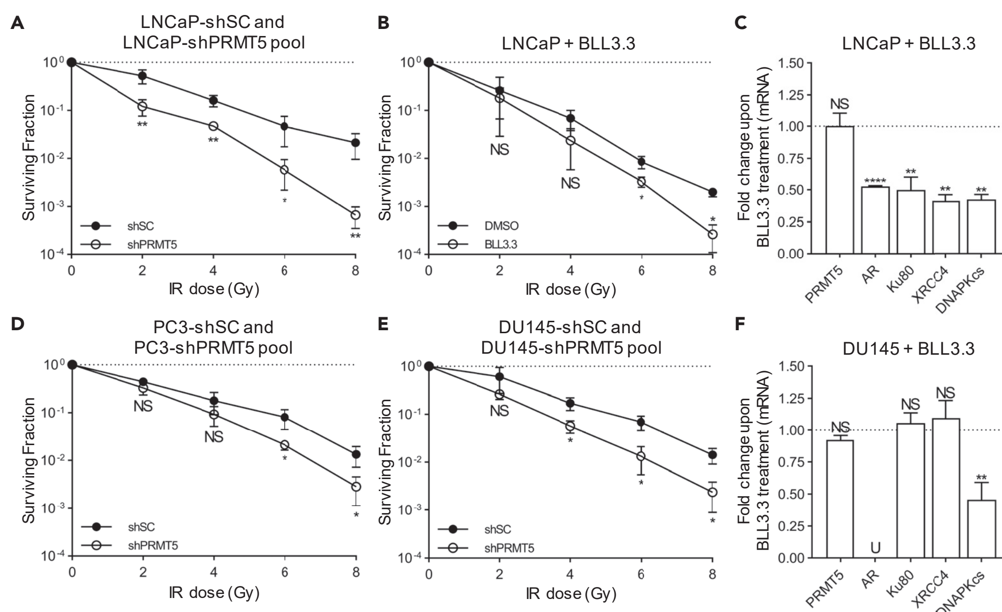


**Figure 1. Targeting PRMT5 Sensitizes Prostate Cancer Cells to IR in an AR-Independent Manner**

(A, B, D, and E) Quantification of the surviving fraction via clonogenic assay immediately following the indicated dose of IR in the indicated cell lines. Dox treatment was used to express PRMT5-targeting shRNA (shPRMT5) or scramble control-targeting shRNA (shSC). BLL3.3 treatment was used to inhibit PRMT5 activity (A: LNCaP-shPRMT5/shSC, B: LNCaP + DMSO/BLL3.3, D: PC3-shPRMT5/shSC, E: DU145-shPRMT5/shSC).

(C and F) Quantification of mRNA via RT-qPCR 24 h post 2 Gy IR in LNCaP (C) and DU145 (F) cells. For each biological replicate, the value for BLL3.3 was normalized to the value for DMSO to calculate the fold change in mRNA expression upon PRMT5 inhibition.

Points in A, B, D, and E are the mean ± s.d. of three independent experiments. Bars in C and F are the mean ± s.d. of three independent experiments. Statistical analysis for A, B, D, and E comparing experimental with the control (''shSC'' or ''DMSO'') was performed using Welch's *t* test of log-transformed data, whereas statistical analysis for C and F comparing experimental with the control (''DMSO'') was performed using Welch's *t* test (*p ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, NS p > 0.05, U = undetected).

PRMT5 also hinders repair of etoposide-induced DSBs (Figures S2A and S2B), which differ in their mechanism of DSB generation and are replication dependent (Furuta et al., 2003; Montecucco and Biamonti, 2007; Treszezamsky et al., 2007), suggesting that PRMT5 may be required for repair of DSBs independently of how they are formed. Thus, the radiosensitization effect of PRMT5 targeting in prostate cancer cells is likely due to defects in the repair of IR-induced DSBs.

To further confirm that PRMT5 also regulates the repair of IR-induced DSBs independently of AR, we performed rescue experiments. Although exogenously expressed AR in Dox-treated LNCaP-shPRMT5 cells fully rescued AR protein levels (Figures 2M and 2N), the repair of IR-induced DSBs was only partially rescued (Figures 2O and 2P). Thus, PRMT5 can also regulate repair of IR-induced DSBs through an AR-independent mechanism.

## PRMT5 Regulates NHEJ, HR, and $G_2$ Arrest in Response to IR

The ability to repair DSBs is mainly dependent on NHEJ and HR as well as $G_1$ and $G_2$ cell-cycle arrest. We next analyzed IR-induced Ku70 and RAD51 foci formation to examine if PRMT5 knockdown would affect NHEJ or HR repair, respectively. Consistent with the finding that targeting PRMT5 causes a decrease in AR-target genes involved in NHEJ, PRMT5 knockdown decreased IR-induced Ku70 foci formation (Figures 3A and 3B), indicating that PRMT5 regulates NHEJ. Interestingly, PRMT5 knockdown also decreased IR-induced RAD51 foci formation (Figures 3C and 3D), confirming that PRMT5 regulates HR repair of IR-induced DSBs as well.

We next investigated whether PRMT5 knockdown has any effect on cell cycle. Consistent with previous findings (Lim et al., 2014; Scoumanne et al., 2009; Wei et al., 2012; Yang et al., 2016), cells with PRMT5

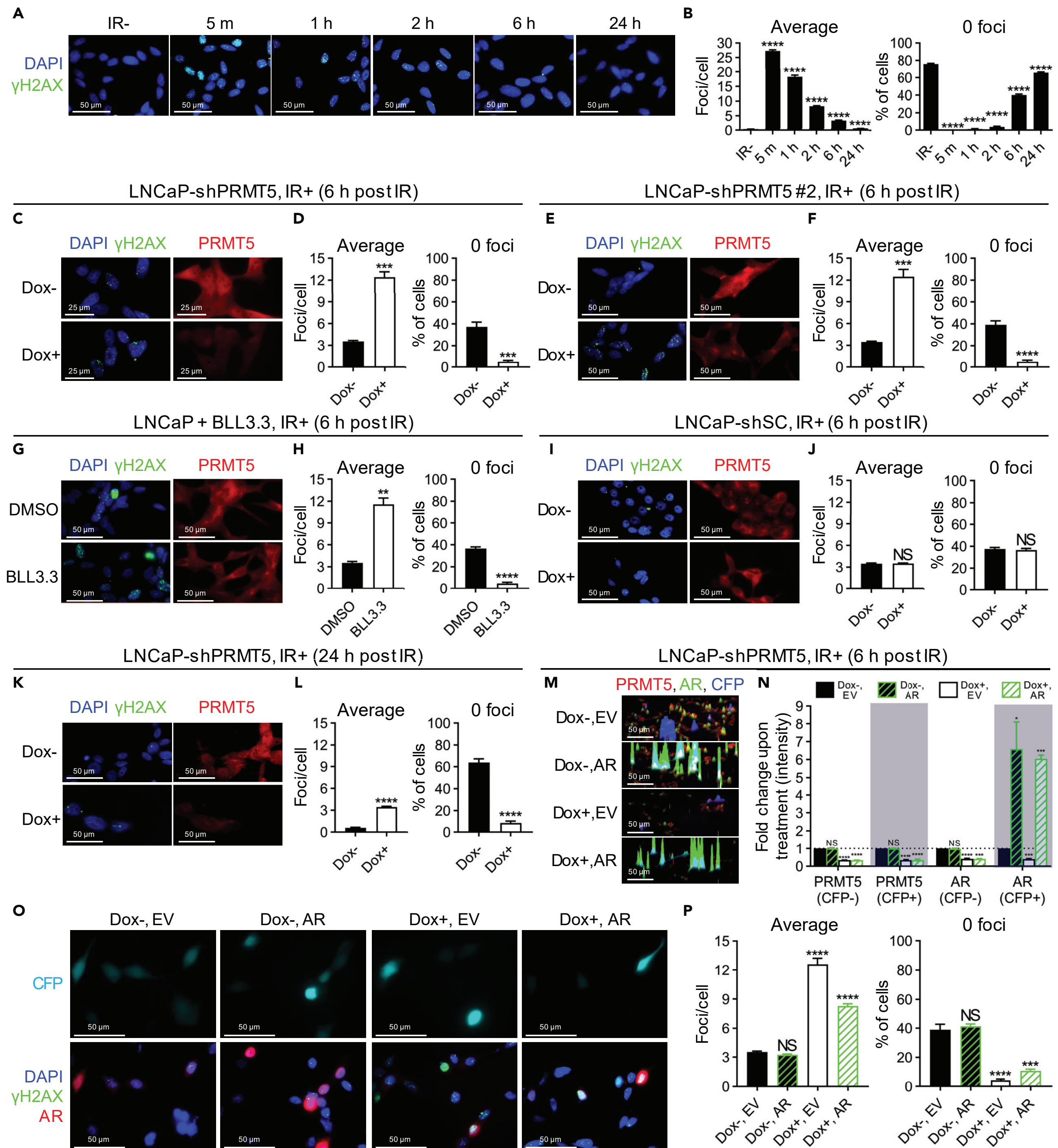

**Figure 2. PRMT5 Regulates the Repair of DNA Double-Strand Breaks in Prostate Cancer Cells Independently of AR Expression**

(A) Time course of the formation and repair of DSBs (γH2AX foci) at the indicated minutes (m) or hours (h) post 2 Gy IR in LNCaP cells.

(B) Quantification of DSBs in each individual cell from A: "average" indicates the average number of DSBs in each cell and "0 foci" indicates the percentage of cells that do not contain any DSBs.

(C, E, G, I, and K) DSBs 6 h or 24 h post 2 Gy IR in the indicated cells (C: LNCaP-shPRMT5, E: LNCaP-shPRMT5 #2, G: LNCaP, I: LNCaP-shSC, K: LNCaP-shPRMT5) with (Dox+) and without (Dox−) PRMT5 knockdown/scramble control (SC) knockdown or with (BLL3.3) and without (DMSO) PRMT5 inhibition.

(D, F, H, J, and L) Quantification of DSBs from C, E, G, I, and K as described above in Figure 2B (C: LNCaP-shPRMT5, E: LNCaP-shPRMT5 #2, G: LNCaP, I: LNCaP-shSC, K: LNCaP-shPRMT5).

**Figure 2. *Continued***

(M) LNCaP-shPRMT5 cells were co-transfected with plasmids encoding Flag-AR (AR) or empty vector (EV) and a plasmid encoding cerulean fluorescent protein (CFP). Fluorescence images acquired 6 h post 2 Gy IR are representative immunocytochemistry images in 3D where each peak is a cell and the height of each peak is the intensity of signal. Blue peaks represent transfected CFP-expressing cells (CFP+). Colors are indicated as follows: endogenous PRMT5 (red), endogenous and exogenous AR (green), and exogenous CFP (cerulean).

(N) Quantification of protein intensity from M in untransfected (CFP−) and transfected (CFP+) cells. For each biological replicate, values were normalized to the value for "Dox−/EV" to calculate the fold change in protein expression upon treatment.

(O) DSBs at 6 h post 2 Gy IR in LNCaP-shPRMT5 cells where AR expression was rescued via co-transfection with plasmids encoding Flag-AR (AR) or empty vector (EV) and a plasmid encoding Cerulean fluorescent protein (CFP) as a transfection control.

(P) Quantification of DSBs in transfected cells (defined as CFP+) from O as described above.

Fluorescence images in A, C, E, G, I, and K are representative immunocytochemistry images (blue = DAPI, green = γH2AX, red = PRMT5). Fluorescence images in M are representative immunocytochemistry images (red = PRMT5, green = AR, and blue = CFP). Fluorescence images in O are representative immunocytochemistry images (blue = DAPI, green = γH2AX, red = AR, Cerulean = CFP). All bars are the mean ± s.d. of four independent experiments. Statistical analysis for B, N, and P comparing experimental with the control ("IR−", "Dox−", "DMSO", or "Dox−/EV") was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test, whereas statistical analysis for D, F, H, J, and L comparing experimental with the control ("Dox−" or "DMSO") was performed using Welch's *t* test (*p $\leq$ 0.05; **p $\leq$ 0.01, ***p $\leq$ 0.001, ****p $\leq$ 0.0001, NS p > 0.05).

knockdown had an increase in the $G_1$ population and a concomitant decrease in the S population in the absence of IR (Figure 3E) indicative of $G_1$ arrest. Upon IR treatment, cells with PRMT5 knockdown retained the ability to undergo IR-induced $G_1$ arrest (or already arrested in $G_1$ phase due to PRMT5 knockdown prior to IR) yet failed to arrest at $G_2$ (Figure 3F). This result suggests that PRMT5 regulates IR-induced $G_2$ arrest but may not be required for IR-induced $G_1$ arrest. Overall, our findings that PRMT5 can regulate repair of IR-induced DSBs through an AR-independent mechanism and that targeting PRMT5 sensitizes prostate cancer cells to IR independently of AR expression are likely due to the regulation of multiple DDR pathways by PRMT5.

Because PRMT5 is an emerging epigenetic regulator (Karkhanis et al., 2011; Stopa et al., 2015), we reasoned that PRMT5 may regulate the expression of genes involved in the repair of DSBs. We performed RNA-seq analysis of both non-irradiated (IR-) and irradiated (IR+) LNCaP-shPRMT5 cells with PRMT5 knockdown (Dox+) and without PRMT5 knockdown (Dox-). We identified 2,036 differentially expressed genes (DEGs) upon PRMT5 knockdown in IR−cells and 1,710 DEGs in IR+ cells (Figure 3G and Tables S1 and S2). Comparing the IR− and IR+ datasets, we determined that 886 genes were differentially regulated only in IR+ cells, of which 563 were downregulated (Figure 3H and Table S3). Consistent with our functional studies, multiple genes encoding repair proteins in HR and NHEJ and genes involved in $G_2$ arrest were identified as DEGs. Using Gene Ontology (GO) analysis, we identified several GO functions and KEGG pathways associated with DDR such as "DNA damage repair" and "cell-cycle regulation" that were significantly over-represented in IR+ only DEGs (Figure 3I and Table S4). Ingenuity pathway analysis (IPA) of the IR+ only DEGs conferred similar outcomes as GO analysis and further revealed that PRMT5 likely regulates genes involved in $G_2$ arrest as well as repair proteins such as BRCA1 and BRCA2 (Figure 3J and Table S5). Results from our RNA-seq analysis suggest PRMT5 regulates expression of DDR genes in response to IR.

### PRMT5 Activates Transcription of Genes that Encode Proteins Involved in the Repair of DSBs

Next, we sought to validate a potential role for PRMT5 in regulating the transcription of genes required for DSB repair. Notably, we identified six DEGs that encode repair proteins (RAD51, RAD51D, RAD51AP1, BRCA1, BRCA2, and NHEJ1/XLF), and the regulation of these genes by PRMT5 in both irradiated and non-irradiated cells was verified by reverse transcriptase quantitative real-time PCR (RT-qPCR) (Figures 4A and S3A) and Western blot (Figures S3B–S3F) (additional genes were also individually verified at the mRNA level in Figure S3G). IR induces the expression of these genes at both the mRNA (Figure 4B) and protein level (Figures S3B–S3F) on a timescale consistent with the repair of IR-induced DSBs, suggesting that PRMT5-mediated upregulation of these genes upon IR is critical for DSB repair. The regulation of positive control genes by PRMT5 was also confirmed: knockdown of PRMT5 prevented repression of IVL expression (Saha et al., 2016) and prevented activation of AR expression (Deng et al., 2017) (Figures 4A and S3G). As expected, IR did not affect expression of IVL or AR at the mRNA level (data not shown).

Chromatin immunoprecipitation (ChIP)-qPCR assays using LNCaP-shSC and LNCaP-shPRMT5 cell lines confirmed that PRMT5 indeed bound to the proximal promoter regions of these six genes that encode

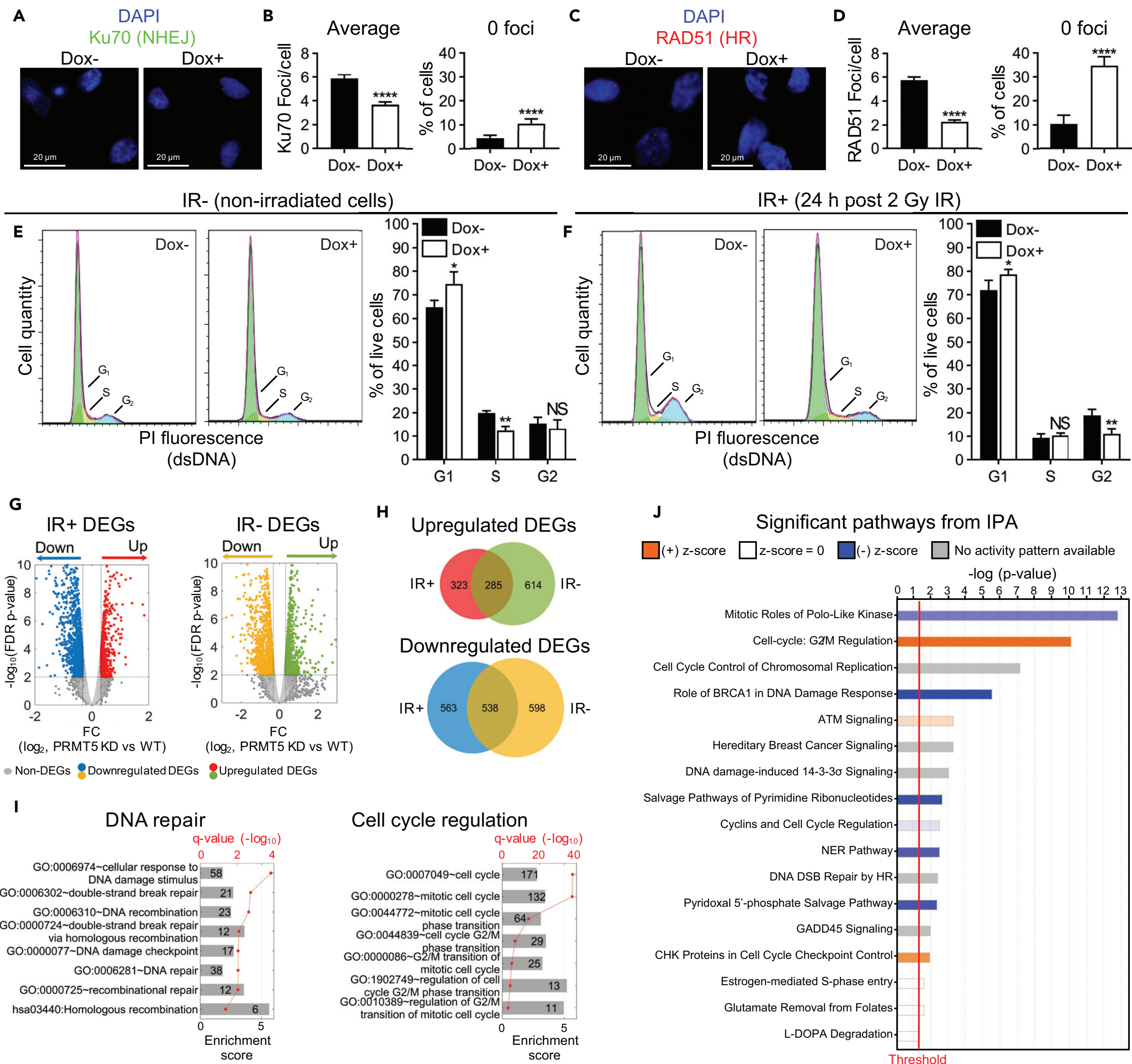


**Figure 3. PRMT5 Regulates NHEJ, HR, and $G_2$ Arrest in Response to IR**

(A) NHEJ repair foci (Ku70) 1 h post 2 Gy IR in LNCaP-shPRMT5 cells with (Dox+) and without (Dox−) PRMT5 knockdown.

(B) Quantification of Ku70 foci from A as described in Figure 2B.

(C) HR repair foci (RAD51) 1 h post 2 Gy IR in LNCaP-shPRMT5 cells with (Dox+) and without (Dox−) PRMT5 knockdown.

(D) Quantification of RAD51 foci from C as described in Figure 2B.

(E) Cell-cycle analysis via flow cytometry of propidium iodide (PI) stained LNCaP-shPRMT5 cells with (Dox+) and without (Dox−) PRMT5 knockdown.

(F) Cell cycle analysis via flow cytometry of PI-stained LNCaP-shPRMT5 cells 24 h post 2 Gy IR, with (Dox+) and without (Dox−) PRMT5 knockdown.

(G) RNA-seq analysis 1 h post 2 Gy IR in irradiated (IR+) and non-irradiated (IR−) LNCaP-shPRMT5 cells with (Dox+) and without (Dox−) PRMT5 knockdown. Volcano plot shows statistical significance (false discovery rate, FDR-corrected p values) vs fold change (FC, in logarithm scale with base 2) between PRMT5 knockdown and WT in IR+ and IR− cells, respectively. Upregulated DEGs (red or green) and downregulated DEGs (blue or yellow) are indicated in color.

(H) Venn diagram indicating the overlap of DEGs between IR+ (red or blue) and IR− (green or yellow) samples.

(I) Gene ontology (GO) analysis of IR+ only DEGs that were downregulated upon PRMT5 knockdown. Groups of GO terms related to DNA repair and cell-cycle regulation were identified to be significantly enriched in the DEG set. The height of each bar represents the enrichment score for the GO term, whereas the q-value (FDR-corrected p value) in red indicates the significance of enrichment. The number in the bar indicates the number of DEGs associated with the corresponding GO annotation.

**Figure 3. *Continued***

(J) Differentially regulated pathways of IR+ only DEGs that were downregulated upon PRMT5 knockdown identified by IPA. The pathways with the highest –log (p value), represented by the bars, are shown. Pathways shown in blue (negative *Z* score) are inhibited upon PRMT5 knockdown, whereas pathways in orange (positive *Z* score) are activated upon PRMT5 knockdown.

Fluorescence images in A and C are representative immunocytochemistry images (blue = DAPI, green = Ku70, red = RAD51). Bars in B and D are the mean ± s.d. of three independent experiments, whereas bars in E and F are the mean ± s.d. of four independent experiments. Graphs in E and F are representative flow traces of cells in various cell-cycle stages (green = $G_1$, orange = S, blue = $G_2$). Statistical analysis comparing experimental with the control ("Dox−") was performed using Welch's *t* test (*p ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, NS p > 0.05).

for repair proteins (Figures 4C and S4A). IR treatment further increased the binding of PRMT5 at a time point prior to the upregulation of these genes (Figures 4C and S4A). Consistent with potential epigenetic activation of these genes by PRMT5, the promoter regions of these genes were selectively enriched with PRMT5-catalyzed H4R3me2s (but not H3R2me2s, H3R8me2s, or H2AR3me2s) as well as the activating histone modification H3K9ac, both of which were induced by IR (Figures 4D, 4E, and S4B–S4F). Consistent with our previous finding (Deng et al., 2017), PRMT5 binding and the enrichment of H4R3me2s and H3K9ac at the proximal promoter region, but not a distal region, of the AR gene were also confirmed (Figures 4C–4E and S4A–S4F). To confirm the specificity of our ChIP experiments, we repeated experiments after knocking down PRMT5. PRMT5 knockdown decreased both PRMT5 binding and enrichment of H4R3me2s and H3K9ac at the proximal promoter regions, further supporting the specificity of our ChIP assays and suggesting that PRMT5 contributes to transcriptional activation of these genes via methylation of H4R3 (Figures S4A–S4F). Collectively, these data demonstrate that PRMT5 is required to maintain basal expression of DDR genes, and PRMT5 facilitates the IR-induced transient upregulation of DDR genes by activating their transcription.

Given that PRMT5 was required for IR-induced $G_2$ arrest, we also sought to validate putative PRMT5 target genes involved in the regulation of $G_2$/M transition (DEGs: CCNB2, CDC20, CDC25C, CDK1, and WEE1). RT-qPCR analysis demonstrated that PRMT5 knockdown decreased their expression in both non-irradiated and irradiated cells (Figures 4F and S3G). Interestingly, ChIP-qPCR assay results suggest that out of the five putative target genes, WEE1 may be the only direct target gene of PRMT5 (Figures 4G–4I and S4A–S4F). Since CCNB2, CDC20, CDC25C, and CDK1 are typically activators of $G_2$ progression and WEE1 is an activator of $G_2$ arrest, it is possible that PRMT5 actively regulated WEE1 expression while the changes in CCNB2, CDC20, CDC25C, and CDK1 expression were a secondary effect. Therefore, although positive and negative regulators of $G_2$ arrest were downregulated at the mRNA level upon PRMT5 knockdown, the net phenotypic effect is impaired IR-induced $G_2$ arrest.

### pICln Is Also Required for Transcriptional Activation of DDR Genes and for Efficient Repair of DSBs

As MEP50 is believed to be the obligate cofactor of PRMT5 and required for PRMT5 methyltransferase activity (Stopa et al., 2015; Wilczek et al., 2011; Chen et al., 2017; Burgos et al., 2015), we determined if MEP50 plays a role in regulating the expression of DDR genes. Using Dox-inducible MEP50 knockdown stable cell lines isolated from single-cell-derived clones (LNCaP-shMEP50), we unexpectedly observed that knockdown of MEP50 did not affect the expression of PRMT5 target genes involved in DDR at the mRNA (Figure 5A) or protein (Figures S5A and S5B) level in untreated or irradiated LNCaP-shMEP50 cells. However, consistent with the previous finding that PRMT5 and MEP50 represses IVL expression (Saha et al., 2016), knockdown of MEP50 caused an increase in IVL expression (Figure 5A). Furthermore, MEP50 knockdown did not affect the repair of IR-induced DSBs (Figures 5B and 5C). These results suggest that PRMT5 may not rely on MEP50 to regulate transcription of genes involved in DDR.

We previously performed mass spectrometry analysis of PRMT5-immunoprecipitated lysate to identify interacting proteins of PRMT5 in LNCaP cells (Zhang et al., 2016). We predictably identified MEP50 (peptide fragment *ILLWDTR*), but we also identified pICln (Chari et al., 2008; Friesen et al., 2001, 2002; Guderian et al., 2011; Meister et al., 2001) (peptide fragment *GLGTGTLYIAESR*) as an interacting protein of PRMT5. We then developed Dox-inducible pICln knockdown stable cell lines isolated from single-cell-derived clones (LNCaP-shpICln) and surprisingly observed that knockdown of pICln caused a decrease in PRMT5 target gene expression at the mRNA (Figure 5D) and protein (Figures S5C and S5D) level in untreated and irradiated LNCaP-shpICln cells. Furthermore, knockdown of pICln impaired repair of IR-induced DSBs (Figures 5E and 5F) to an extent comparable to PRMT5 knockdown. Although MEP50 was present at the promoter of the control gene IVL, MEP50 was not present at the promoter of PRMT5 target

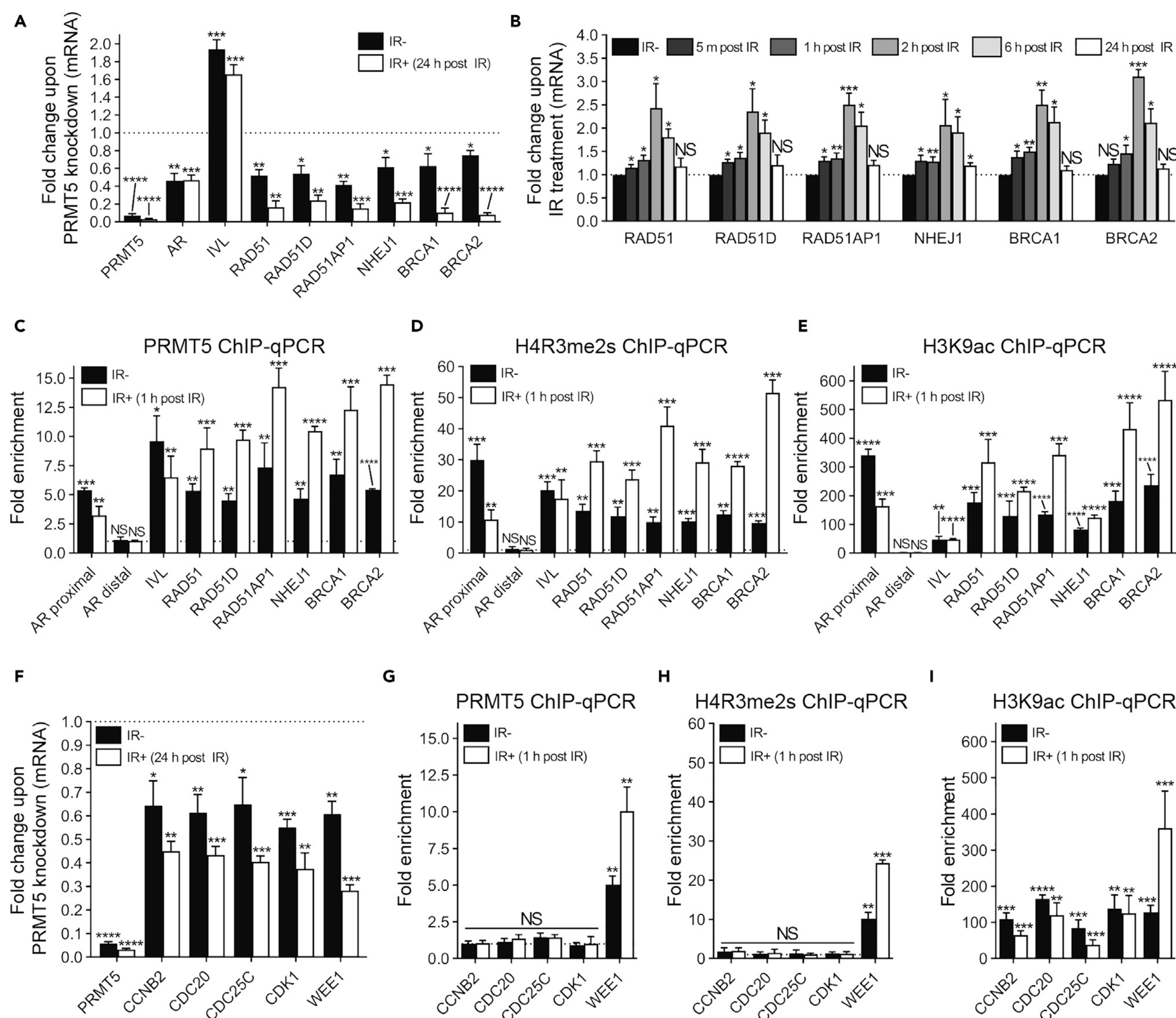


**Figure 4. PRMT5 Activates Transcription of Genes that Encode Proteins Involved in the Repair of DSBs**

(A and F) Quantification of mRNA via RT-qPCR 24 h post 2 Gy IR in LNCaP-shPRMT5 cells with (Dox+) and without (Dox−) PRMT5 knockdown. For each biological replicate, the value for Dox+ was normalized to the value for Dox− to calculate the fold change in mRNA expression upon PRMT5 knockdown in both irradiated (IR+) and non-irradiated cells (IR−) (See also Figure S3G).

(B) Quantification of mRNA via RT-qPCR at the indicated time points post IR in irradiated (IR+) and non-irradiated (IR−) LNCaP-shPRMT5 cells without PRMT5 KD. For each biological replicate, the value for IR+ was normalized to the value for IR− to calculate the fold change in mRNA expression upon IR treatment (see also Figure S3A for experiments with PRMT5 KD).

(C–E and G–I) Quantification of enrichment (C and G: PRMT5, D and H: H4R3me2s, and E and I: H3K9ac) at the promoter region of the indicated genes 1 h post 2 Gy IR via ChIP-qPCR in irradiated (IR+) and non-irradiated (IR−) LNCaP-shSC cells via ChIP-qPCR. For each biological replicate, the value for IP was normalized to the value for IgG to calculate the fold enrichment (See also Figure S4).

All bars are the mean ± s.d. of three independent experiments. Statistical analysis for A, B, and F comparing experimental with the control ("Dox−" or "IR−") was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test, whereas statistical analysis for C–E and G–I comparing experimental with the control ("IgG") was performed using Welch's *t* test (*p ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, NS p > 0.05).

genes involved in DDR while pICln was present (Figures 5G, 5H, S5E, and S5F). Upon IR, binding of pICln to the promoter of DDR genes increased (Figure 5H) and knockdown of PRMT5 almost completely abrogated enrichment of pICln (Figure S5F), suggesting that PRMT5 recruits pICln to targeted promoter regions. Additionally, other previously identified PRMT5 interacting proteins, RioK1 (Guderian et al., 2011) and COPR5 (Lacroix et al., 2008; Paul et al., 2012, 2015), were not found at the promoter of DDR genes in

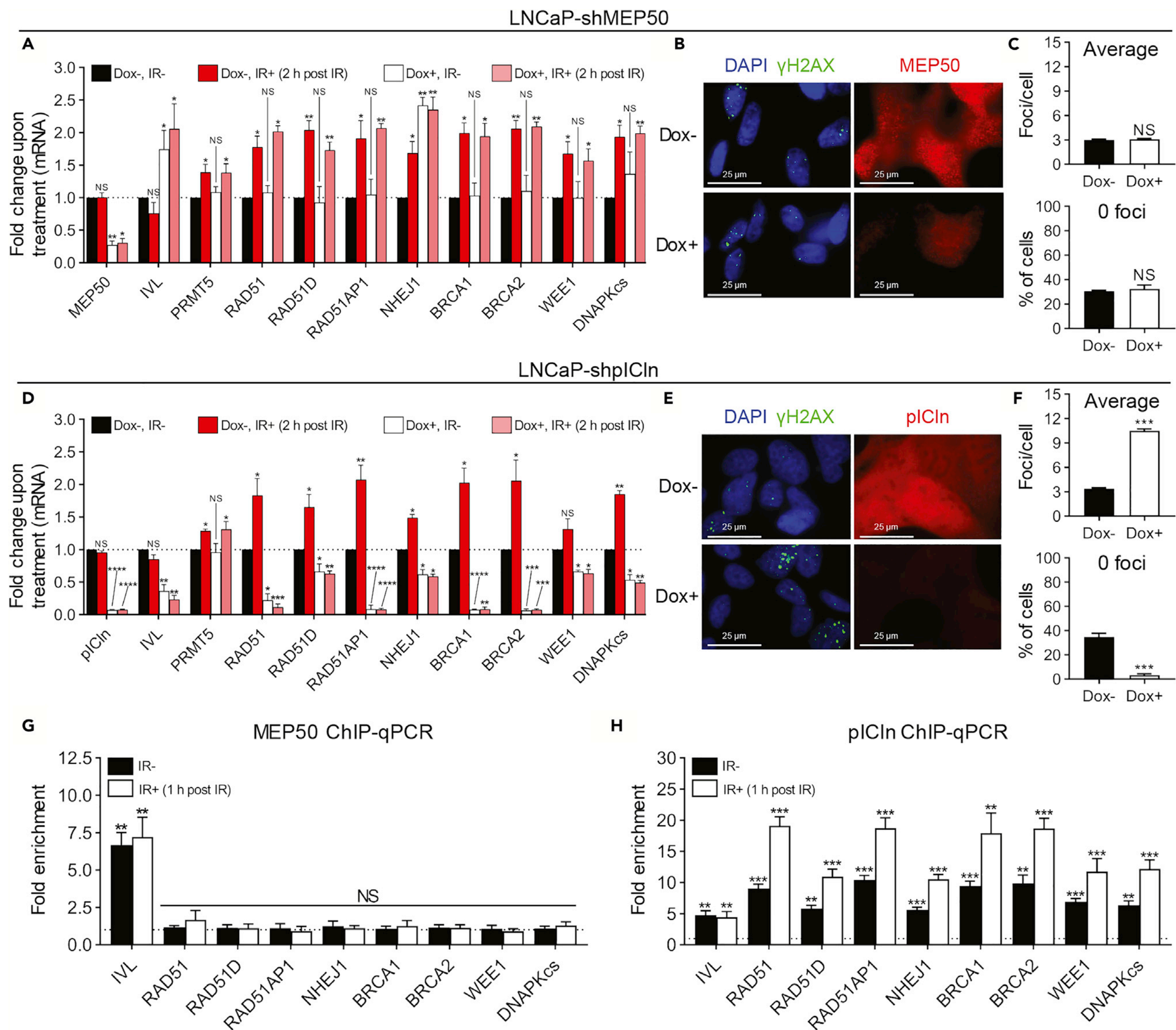


**Figure 5. pICln is Also Required for Transcriptional Activation of DDR Genes and for Efficient Repair of DSBs**

(A) Quantification of mRNA via RT-qPCR 2 h post 2 Gy IR in LNCaP-shMEP50 cells with (Dox+) and without (Dox−) MEP50 knockdown. For each biological replicate, values were normalized to the value for "'Dox−, IR−" (untreated) to calculate the fold change in mRNA expression upon treatment.

(B) DSBs 6 h post 2 Gy IR in LNCaP-shMEP50 cells with (Dox+) and without (Dox−) MEP50 knockdown.

(C) Quantification of DSBs in each individual cell from B as described in Figure 2B.

(D) Quantification of mRNA via RT-qPCR 2 h post 2 Gy IR in LNCaP-shpICln cells with (Dox+) and without (Dox−) pICln knockdown. For each biological replicate, values were normalized to the value for "Dox−, IR−" (untreated) to calculate the fold change in mRNA expression upon treatment.

(E) DSBs 6 h post 2 Gy IR in LNCaP-shpICln cells with (Dox+) and without (Dox−) pICln knockdown.

(F) Quantification of DSBs in each individual cell from E as described in Figure 2B.

(G and H) Quantification of enrichment (G: MEP50 and H: pICln) at the promoter region of the indicated genes 1 h post 2 Gy IR via ChIP-qPCR in irradiated (IR+) and non-irradiated (IR−) LNCaP-shSC cells via ChIP-qPCR. For each biological replicate, the value for IP was normalized to the value for IgG to calculate the fold enrichment (see also Figures S5E and S5F).

Fluorescence images in B and E are representative immunocytochemistry images (blue = DAPI, green = γH2AX, red = MEP50 or pICln). All bars are the mean ± s.d. of three independent experiments. Statistical analysis for A and D comparing experimental with the control ("Dox−, IR−") was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test, whereas statistical analysis for C, F, G, and H comparing experimental with the control ("Dox−" or "IgG") was performed using Welch's *t* test (*p ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, NS p > 0.05).

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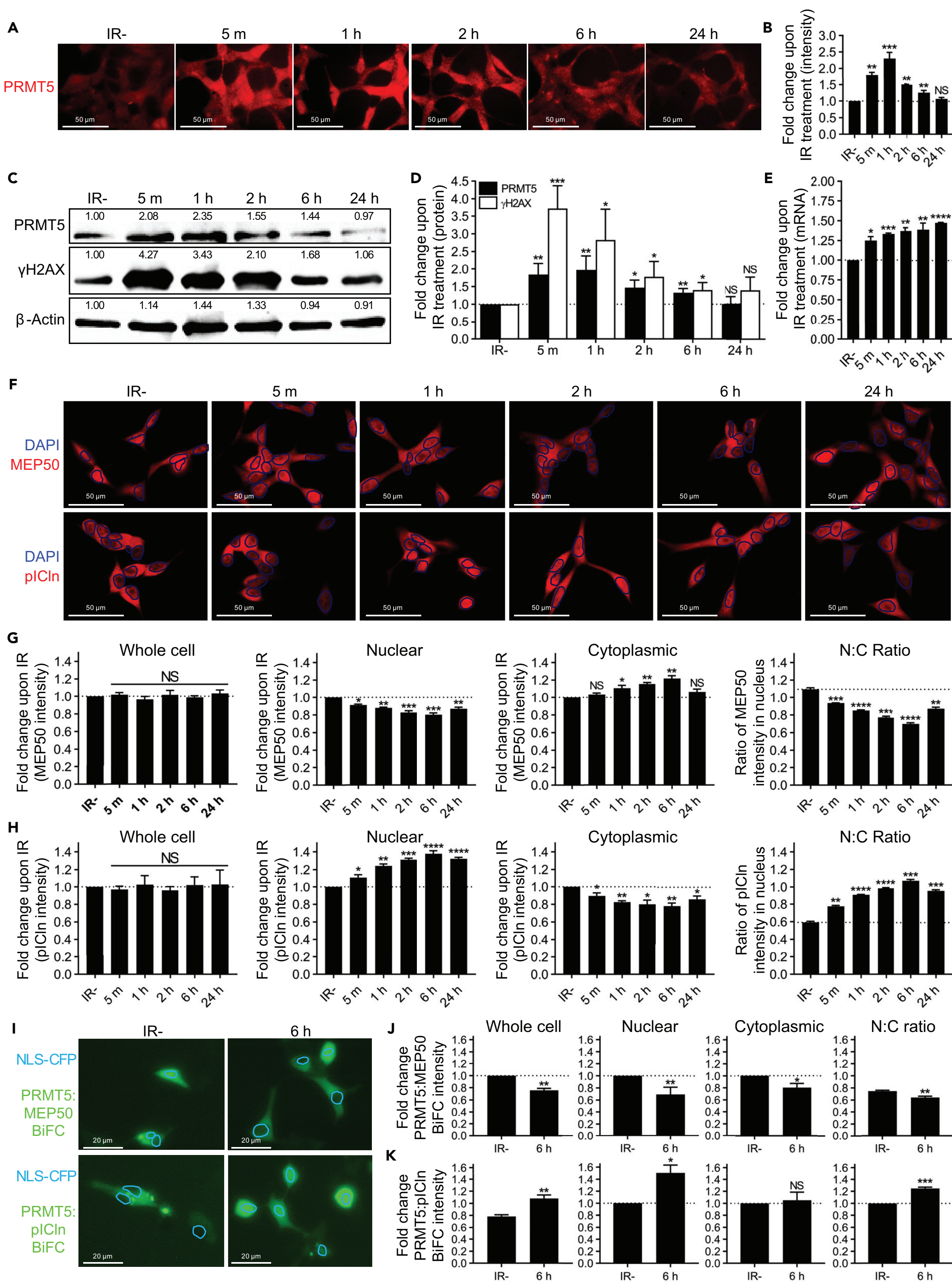

**Figure 6. IR Induces PRMT5 Expression, pICln Nuclear Localization, and the PRMT5:pICln Interaction in the Nucleus**

(A) Time course of PRMT5 expression at the indicated minutes (m) or hours (h) post 2 Gy IR in LNCaP cells.

(B) Quantification of PRMT5 expression in images from A. For each biological replicate, values were normalized to the value for "IR−" to calculate the fold change in protein expression upon IR.

(C) Representative Western blot showing the time course of protein expression at the indicated minutes (m) or hours (h) post 2 Gy IR in LNCaP cells. Values shown indicate the intensity relative to IR− for the biological replicate used as the representative Western blot.

(D) Quantification of protein expression via Western blotting from C. For each biological replicate, values were normalized to the value for "IR−" to calculate the fold change in protein expression upon IR.

(E) Time course of PRMT5 expression at the mRNA level at the indicated minutes (m) or hours (h) post 2 Gy IR in LNCaP cells via RT-qPCR. For each biological replicate, values were normalized to the value for "IR−" to calculate the fold change in mRNA expression upon IR.

(F) Time course of MEP50/pICln expression/localization at the indicated minutes (m) or hours (h) post 2 Gy IR in LNCaP cells.

(G) Quantification of MEP50 expression/localization in images from F: "Whole cell" indicates MEP50 expression in the entire cell, "Nuclear" indicates MEP50 expression in the nucleus, which was defined by DAPI staining, "Cytoplasmic" indicates MEP50 expression in the cytoplasm that was defined as staining outside DAPI, and "N:C ratio" was calculated by dividing the value for nucleus by the value for cytoplasmic for each cell individually such that an N:C ratio of 1 indicates equal expression in both the nucleus and cytoplasm.

(H) Quantification of pICln expression/localization in images from F as described above.

(I) PRMT5:MEP50 and PRMT5:pICln interaction 6 h post 2 Gy IR in irradiated (6 h) and non-irradiated (IR−) LNCaP cells via BiFC assay.

(J) Quantification of PRMT5:MEP50 BiFC intensity in images from I: "Whole cell" indicates BiFC intensity in the entire cell, "Nuclear" indicates BiFC intensity in the nucleus that was defined by NLS-CFP signal, "Cytoplasmic" indicates BiFC intensity in the cytoplasm that was defined as staining outside NLS-CFP signal, and "N:C ratio" was calculated by dividing the value for nucleus by the value for cytoplasmic for each cell individually such that an N:C ratio of 1 indicates equal interaction in both the nucleus and cytoplasm. NLS-CFP was used as a transfection control and a marker of the nucleus.

(K) Quantification of PRMT5:pICln BiFC intensity in images from I as described above.

Fluorescence images in A are representative immunocytochemistry images (red = PRMT5). Fluorescence images in F are representative immunocytochemistry images (blue = DAPI and red = MEP50 or pICln). Blue circles outline DAPI staining to allow for better visibility of expression in the nucleus. Fluorescence images in I are representative images from BiFC assay (green = PRMT5:MEP50 and PRMT5:pICln, cerulean = NLS-CFP). Blue circles outline NLS-CFP signal to allow for better visibility of expression in the nucleus. All bars are the mean ± s.d. of three independent experiments. Statistical analysis for B, D, E, G, and H comparing experimental with the control ("IR−") was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test, whereas statistical analysis for J and K comparing experimental with the control ("IR−") was performed using Welch's *t* test (*$p \leq 0.05$; **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, NS $p > 0.05$).

untreated or irradiated LNCaP cells (Figures S5G and S5H). These results suggest that PRMT5 may cooperate with pICln to regulate transcription of DDR genes.

## IR Induces PRMT5 Expression, pICln Nuclear Localization, and the PRMT5:pICln Interaction in the Nucleus

Given the importance of PRMT5 in DSB repair and activation of target gene expression upon IR, we hypothesized that IR may induce PRMT5 expression. To test this, we analyzed PRMT5 protein expression via both immunocytochemistry and Western blotting at various time points following IR of LNCaP cells. Indeed, IR induced PRMT5 expression as quickly as 5 m, and the induction lasted for nearly 24 h (Figures 6A–6D). Importantly, the peak of PRMT5 protein expression (between 5 m and 1 h) coincided with the increased recruitment of PRMT5 to the promoters of DDR genes (1 h). Similarly, changes in PRMT5 expression closely mirrored the time course of DSB repair. The rapid induction of protein expression likely suggests a post-transcriptional or post-translational regulation. However, RT-qPCR analysis confirmed a small, but significant, sustained induction of PRMT5 expression at the mRNA level (Figure 6E). This result suggests that transcriptional activation of PRMT5 also contributes to prolonged elevation of PRMT5 expression. Furthermore, PRMT5 was upregulated at the protein level by etoposide treatment (Figures S2C–S2F), suggesting that DNA damage, in general, can signal the induction of PRMT5. Collectively, these results indicate that PRMT5 is upregulated upon IR to promote repair of IR-induced DSBs, and that the upregulation of DDR genes is likely facilitated by IR-induced upregulation of PRMT5.

To gain insight into how pICln functions with PRMT5 to regulate the expression of genes involved in DDR, we first determined if IR affects expression of MEP50 or pICln. However, neither MEP50 nor pICln protein expression was affected by IR (Figures 6F–6H). We next analyzed the subcellular localization of MEP50 and pICln upon IR. Upon IR, the nuclear:cytoplasmic (N:C) ratio of MEP50 decreased, whereas the N:C ratio of pICln increased (Figures 6F–6H). This suggests that upon IR there is more pICln and less MEP50 in the nucleus to interact with PRMT5. IR-induced nuclear localization of pICln thus likely contributes to IR-induced pICln binding to the promoters of genes involved in DDR. To assess the protein-protein interaction (PPI) directly, we utilized bimolecular fluorescence complementation (BiFC) assay (Hu et al., 2002; Kodama and Hu, 2010, 2012; Shyu and Hu, 2008), which is uniquely applicable in assessing the subcellular

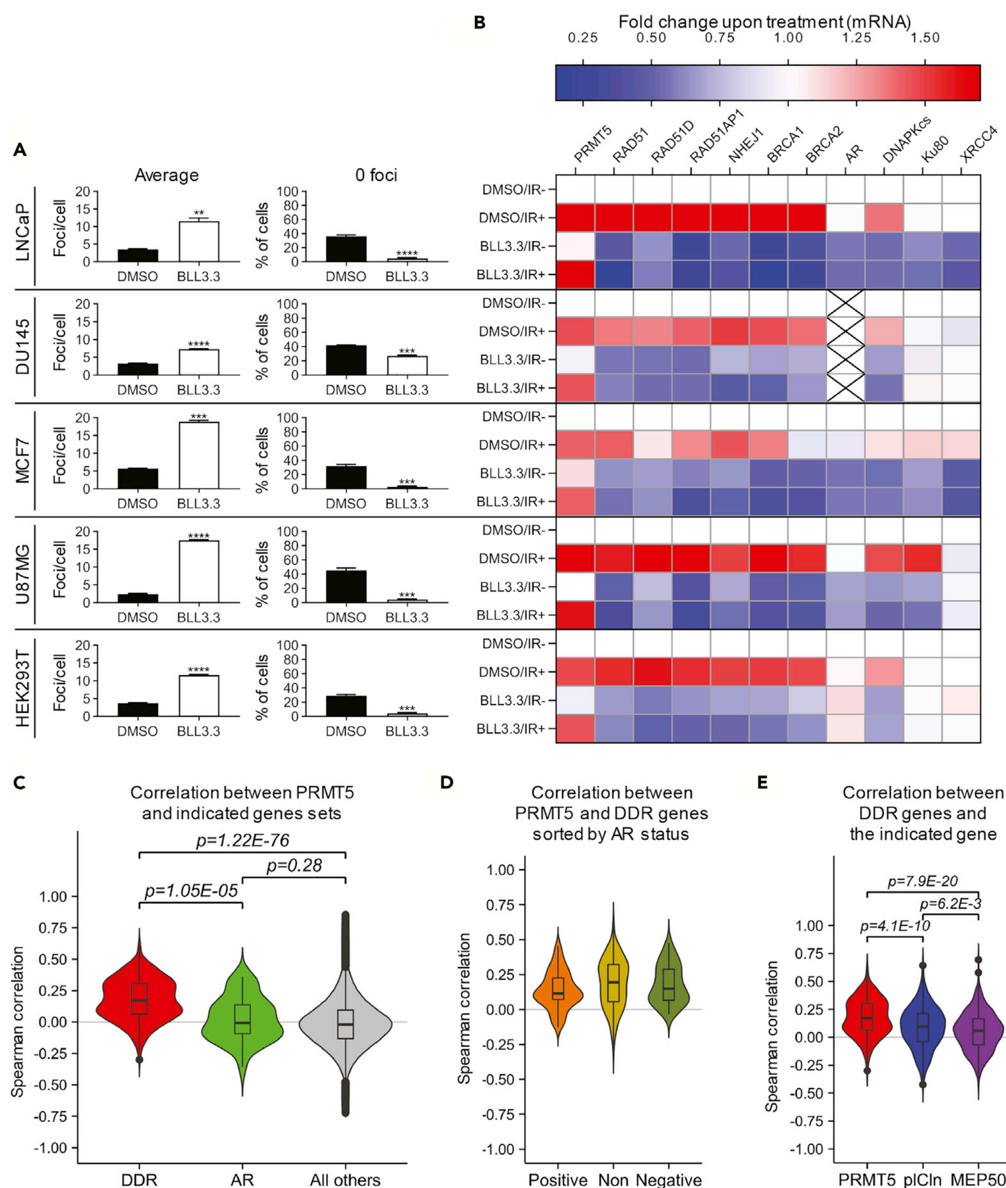


**Figure 7. PRMT5 Regulates the Expression of DDR Genes in Multiple Cancer Cell Lines, and PRMT5 Expression Positively Correlates with DDR Genes in Human Cancer Tissues**

(A) Quantification of DSBs 6 h post 2 Gy IR in the indicated cell lines with (BLL3.3) and without (DMSO) PRMT5 inhibition as described in Figure 2B (see also Figures S7A–S7E for representative images).

(B) Quantification of mRNA via RT-qPCR 6 h post 2 Gy IR in the indicated cell lines with (BLL3.3) and without (DMSO) PRMT5 inhibition. For each biological replicate, values were normalized to the value for "DMSO/IR−" (untreated) to calculate the fold change in mRNA expression upon treatment (see also Figures S7F–S7J for statistical analysis).

(C) Violin plots representing Spearman correlations comparing the mRNA expression level between PRMT5 and DDR genes (DDR), PRMT5 and AR (AR), or PRMT5 and all other genes (All other) across 32 clinical cancer datasets from TCGA. The gene set for DDR genes was defined as RAD51, RAD51D, RAD51AP1, NHEJ1, BRCA1, BRCA2, WEE1, DNAPKcs, Ku70, Ku80, an XRCC4 (see also Figure S8).

(D) Violin plots representing Spearman correlations comparing the mRNA expression level between DDR genes and PRMT5. Cancer types were stratified by the correlation coefficient (c.c.) between PRMT5 and AR: positively correlated (c.c. > 0 & p < 0.01) (Positive), negatively correlated (c.c. < 0 & p < 0.01) (Negative), or not correlated (p > 0.01) (Non).

(E) Violin plot representation of Spearman correlation values between DDR genes and either PRMT5, CLNS1A (pICln), or WDR77 (MEP50).

**Figure 7. *Continued***

Bars in A and values used in the heatmap in B are the mean of three independent experiments. Box-and-whiskers plots in C–E show the median value (line) and interquartile range between the first and third quartiles (box). The upper whisker extended to the largest value no further than "1.5 x interquartile range" and the lower whisker extended to the smallest value at most "1.5 x interquartile range". Outliers beyond the whiskers are shown as dots. Statistical analysis in A comparing experimental to the control ("DMSO") was performed using Welch's *t* test (**p $\leq$ 0.01, ***p $\leq$ 0.001, ****p $\leq$ 0.0001). Statistical analysis in C–E was performed using Wilcoxon test, and the p values are displayed.

localization of PPIs in live cells (Pratt et al., 2016). BiFC confirmed an IR-induced PPI increase between PRMT5 and pICln and a decrease in the PRMT5:MEP50 PPI, particularly in the nucleus (Figures 6I–6K). Overall, these results suggest that the regulation of genes involved in DDR by PRMT5 is facilitated by the IR-induced PRMT5:pICln interaction in the nucleus.

### The Transcriptional Regulation of DSB Repair Genes by PRMT5 Is Not Dependent on RuvBL1 or Tip60

While this study was ongoing, *Clarke et al* reported that PRMT5 participates in the DSB repair choice process and promotes HR through methylation of RuvBL1: Methylation of RuvBL1 by PRMT5 alters the RuvBL1:Tip60 complex, promotes Tip60-mediated acetylation of histone H4K16, demotes 53BP1 binding to DSBs, and initiates DSB repair via HR (Clarke et al., 2017). We sought to confirm that the mechanism we describe here is independent of RuvBL1 and Tip60. We determined that knockdown of RuvBL1 did not affect expression of PRMT5, RAD51, RAD51D, RAD51AP1, NHEJ1, and Tip60 at the protein level (Figures S6A and S6B), nor did it affect the efficiency of repair of IR-induced DSBs in LNCaP cells (Figures S6C and S6D). This is consistent with another study reporting that knockdown of RuvBL1 had no effect on IR-induced 53BP1 foci in hematopoietic cells (Hamard et al., 2018). Therefore, methylation of RuvBL1 by PRMT5 likely affects DSB repair choice (favoring HR over NHEJ) but not the overall efficiency of repair, whereas the transcriptional activation of DDR genes by PRMT5 is likely required for repair of DSBs.

Similarly, while this study was ongoing, *Hamard* et al. also reported that PRMT5 regulates DSB repair choice via splicing of Tip60 in hematopoietic cells: PRMT5 is required for appropriate splicing of Tip60, which in turn allows for normal Tip60 acetyltransferase activity, demotes 53BP1 binding to DSBs, and initiates DSB repair via HR (Hamard et al., 2018). Knockdown of Tip60 did not affect the expression of PRMT5 or the putative PRMT5 target genes we characterized (Figures S6E and S6F), suggesting that PRMT5-assoicated splicing of Tip60 does not affect the regulation of DDR genes by PRMT5. Given the role of Tip60 in DDR, PRMT5-assoicated splicing of Tip60 likely regulates DSB repair choice but not DSB repair efficiency or at least does not affect PRMT5-associated transcriptional regulation of genes involved in DDR.

### PRMT5 Regulates the Expression of DDR Genes in Multiple Cancer Cell Lines, and PRMT5 Expression Positively Correlates with DDR Genes in Human Cancer Tissues

To extend our findings and determine if the role of PRMT5 in DSB repair is conserved across multiple cell types, we performed similar experiments in AR-negative prostate cancer cells (DU145), luminal breast cancer cells (MCF7), glioblastoma cells (U87MG), and non-cancerous HEK293T cells. Inhibition of PRMT5 by BLL3.3 hindered repair of IR-induced DSBs as the cells retained significantly more $\gamma$H2AX foci 6 h following IR treatment (Figures 7A and S7A–S7E). Although DDR genes were generally upregulated upon IR, targeting PRMT5 also caused a decrease in the expression of PRMT5 target genes involved in DSB repair in both irradiated and non-irradiated cells (Figures 7B and S7F–S7J). These results suggest that PRMT5 may function as a key regulator of DSB repair in multiple tissue types.

To assess potential clinical significance of our findings, we analyzed mRNA expression in 32 clinical cancer datasets achieved from The Cancer Genome Atlas (TCGA) Pan-Cancer analysis (The Cancer Genome Atlas Research Network et al., 2013). We focused on PRMT5, pICln, MEP50, AR, and DDR genes, which were identified as primary target genes of both PRMT5 and AR. The expression of PRMT5 correlated positively with DDR genes in almost all cancers (Figures 7C and S8A). As a control, we assessed if PRMT5 generally correlated with the expression of all genes in the transcriptome. As expected, there was no correlation across the cancer datasets (Figure 7C). PRMT5 expression generally did not correlate with AR and varied significantly for individual cancer types (Figures 7C and S8A). However, the strength of correlations between PRMT5 and several AR target genes involved in DDR seemed independent of the correlation between PRMT5 and AR (Figure S8A). To assess this further, we sorted the cancer types into three groups based on the correlation

coefficient between PRMT5 and AR: positively correlated, negatively correlated, and not correlated. We observed no difference in the correlation between PRMT5 and DDR genes when the cancer types were stratified (Figure 7D), indicating that PRMT5 correlates positively with DDR genes independently of any correlation with AR. This leaves the possibility that AR target genes involved in DDR may also be primary target genes of PRMT5. However, similar RT-qPCR and ChIP-qPCR experiments revealed that only DNAPKcs is likely a target gene of PRMT5 (Figures S4A–S4F). DNAPKcs also had the highest correlation with PRMT5 across the 32 clinical cancer datasets (Figure S8A), further implicating DNAPKcs as a PRMT5 target gene.

As our data suggested that pICln, but not MEP50, cooperates with PRMT5 to regulate DDR genes, we also analyzed the correlations between MEP50 or pICln and the same DDR genes. Although MEP50 expression positively correlated with some DDR genes in some cancers (Figures 7E and S8A), pICln exhibited significantly stronger correlations with these DDR genes than MEP50 in almost all cancers (Figures 7E and S8A). Furthermore, PRMT5 correlated more significantly with pICln than MEP50 (Figure S8B). Collectively, our results suggest that PRMT5 and pICln play a conserved role in activating expression of genes required for the repair of IR-induced DSBs.

## DISCUSSION

### PRMT5 Functions as an Epigenetic Activator to Regulate the Repair of DSBs

Upon recognition of DNA DSBs, repair proteins (such as RAD51, BRCA1, and BRCA2) are transiently upregulated to facilitate repair through HR or NHEJ (Khalil et al., 2012; Rieger, 2004; Russell et al., 2003). Although this transient upregulation is required for cell survival following genotoxic stresses, there is a long-standing question of how proteins are quickly upregulated to promote repair of DNA damage. Here, we present evidence that PRMT5 functions as a master epigenetic activator of DDR genes to facilitate the repair of DSBs.

In this study, we determined that PRMT5 activated transcription of multiple genes that encode well-characterized repair proteins involved in HR (RAD51, RAD51AP1, RAD51D, BRCA1, and BRCA2) and NHEJ (NHEJ1 and DNAPKcs). DSB repair occurs in three phases: (1) recognition of DSBs via sensor proteins, (2) initiation of repair by repair proteins, and (3) resolution of repair (Thompson, 2012). Our studies suggest that PRMT5 primarily regulates the expression of repair proteins as opposed to DNA damage sensors or proteins involved in the resolution of repair. Since we also confirmed that PRMT5 regulated the expression of several other genes involved in various phases of DDR by RT-qPCR (Figure S3G), it remains to be determined whether these genes are also target genes of PRMT5.

The epigenetic regulation of genes is cell type, temporal, and context dependent. Our data suggest that PRMT5 likely activates transcription of DDR genes in a variety of cell types. However, we cannot rule out the possibility that PRMT5 regulates different DDR genes in different cells. For example, although we determined that PRMT5 activated transcription of RAD51, *Clarke* et al. demonstrated that depletion of PRMT5 impaired HR (reduced IR-induced RAD51 and BRCA1 foci) without affecting the expression of RAD51 or changes in cell cycle (Clarke et al., 2017). There are a few possible explanations: (1) their experiment was performed in HeLa-shPRMT5 stable cell lines and were not performed in inducible knockdown lines. It is possible that these cells compensated for depleted PRMT5 although all experiments were conducted on low passage cells to minimize effects of chronic PRMT5 depletion. (2) PRMT5 may not regulate RAD51 expression in HeLa cells. (3) HeLa cells may respond differently to depleted PRMT5 as evidenced by a lack of cell-cycle changes in HeLa cells that we and others have observed in other cell lines (Lim et al., 2014; Scoumanne et al., 2009; Wei et al., 2012; Yang et al., 2016). As an epigenetic regulator, it is possible that PRMT5 may not regulate the same cohort of DDR genes in every tissue but rather regulates the same pathways such as HR, NHEJ, and $G_2$ arrest.

During preparation of this manuscript, *Braun et al* reported that PRMT5 post-transcriptionally regulates the splicing out of detained introns (DIs) of genes to modulate gene expression (Braun et al., 2017). However, our analysis of their data showed that the majority of DEGs we identified either do not contain DIs or DI splicing of our DEGs was not affected by PRMT5 targeting. Additionally, *Tan* et al. reported that PRMT5 is required for appropriate splicing in hematopoietic stem cells and that targeting PRMT5 causes increased intron retention and exon skipping events (Tan et al., 2019). In their study, they performed functional enrichment analysis on alternative splicing events upon PRMT5 knockdown and they identified that genes associated with "DNA repair" were enriched. However, there was little overlap between the genes we validated

as PRMT5 target genes (RAD51, RAD51AP1, RAD51D, BRCA1, BRCA2, NHEJ1, DNAPKcs, and WEE1) and genes they identified as splicing targets. Interestingly, they functionally validated five splicing targets (FANCA, FANCG, MUTYH, RTEL1, and RAD52), and we identified both FANDCA and FANCG as ''IR+ only'' downregulated DEGs in our RNA-seq analysis. However, we did not pursue further validation of these genes. Overall, our findings are likely independent of potential splicing changes upon PRMT5 knockdown, and the role of PRMT5 in transcriptional regulation of DDR genes likely mediates the transient upregulation of repair proteins upon DNA damage.

### PRMT5 Is Required for Efficient Repair of DSBs

We demonstrate that PRMT5 is required for efficient repair of DSBs. Interestingly, knockdown of PRMT5 alone caused an increase in spontaneous DSBs independent of external DNA damage inducers (Figures S2A and S2B), indicating that PRMT5 is required to repair endogenous DSBs. Two recent studies have also demonstrated that PRMT5 is required for efficient repair of DSBs in additional cell lines (Clarke et al., 2017; Hamard et al., 2018). We provide evidence that the activation of gene expression by PRMT5 is essential to DSB repair efficiency, whereas the regulation of RuvBL1 and Tip60 by PRMT5 likely only affects DSB repair choice. Specifically, PRMT5-catalyzed methylation of RuvBL1 and PRMT5-associated splicing of Tip60 may promote HR over NHEJ, yet the DSB can be repaired regardless of pathway choice. Therefore, observation that PRMT5 is required for efficient repair of IR-induced DSBs is most likely explained by our finding that PRMT5 activates transcription of DDR genes. Because Tip60 is required for ATM activation (Bhoumik et al., 2008; Sun et al., 2005) and ATM phosphorylates H2AX to form γH2AX foci and signals the initiation of DSB repair (Burma et al., 2001), we did not perform γH2AX foci analysis to assess repair of IR-induced DSBs. Because Tip60 has been shown to be essential for γH2AX foci formation (Ikura et al., 2000; Murr et al., 2006), we would have observed a decrease in γH2AX regardless if knockdown of Tip60 would affect repair of IR-induced DSBs.

### PRMT5 Regulates DSB Repair Independently of AR

We recently reported that PRMT5 is an epigenetic activator of AR (Deng et al., 2017). In prostate cancer cells, AR has been reported to regulate DSB repair via HR and NHEJ (Asim et al., 2017; Goodwin et al., 2013; Li et al., 2017; Polkinghorn et al., 2013; Spratt et al., 2015; Tarish et al., 2015). Upon IR, AR is recruited to the promoter of DDR genes to activate their expression (Goodwin et al., 2013) (∼8–24 h post IR), albeit at a much later time point than the recruitment of PRMT5 to the promoter of DDR genes (∼1 h post IR). Knockdown or inhibition of AR signaling has also been shown to directly impair HR (Asim et al., 2017; Polkinghorn et al., 2013), and recent studies suggest that AR may be essential for HR particularly in castration-resistant prostate cancer (CRPC) (Asim et al., 2017; Goodwin et al., 2013; Li et al., 2017). However, AR's role in regulating NHEJ is more established in that AR transcriptionally activates genes involved in NHEJ (Goodwin et al., 2013; Polkinghorn et al., 2013; Spratt et al., 2015; Tarish et al., 2015).

As we reported that targeting PRMT5 decreases AR expression (Deng et al., 2017), the requirement of PRMT5 for efficient repair of DSBs could be mediated through the regulation of AR. Indeed, we demonstrated that targeting PRMT5 caused a decrease in AR expression and concomitant decrease in the expression of AR target genes involved in NHEJ (Ku80, XRCC4, and DNAPKcs). However, several pieces of evidence in our study suggest that PRMT5 also regulates repair of DSBs independently of AR: (1) PRMT5 targeting sensitized both AR-positive and AR-negative prostate cancer cells to IR; (2) exogenous expression of AR only partially rescued the impairment of IR-induced DSB repair by PRMT5 knockdown; (3) targeting PRMT5 hindered the repair of IR-induced DSBs in AR-negative DU145 cells and several other cancer cell lines with varying AR expression level; and (4) in clinical cancer datasets, PRMT5 expression was positively correlated with the expression of DDR target genes regardless of its correlation with AR. Collectively, these data strongly suggest that although targeting PRMT5 may mimic targeting AR to sensitize prostate cancer cells to IR, PRMT5 can regulate DSB repair independently of AR expression.

### PRMT5 Likely Regulates the Repair of IR-Induced DSBs via Multiple Mechanisms

In this study, we determined that PRMT5 is required for efficient repair of DSBs via activation of DDR genes. Additionally, there are several reports suggesting that PRMT5 may regulate the repair of DNA damage via multiple mechanisms. As detailed above, recent reports show that PRMT5 regulates the DSB repair choice process and promotes HR through methylation of RuvBL1 (Clarke et al., 2017) and altered splicing of Tip60 (Hamard et al., 2018). Indeed, RuvBL1 was identified in our mass-spec analysis (peptide fragment *TISHVIIGLK*) as a potential interacting protein of PRMT5 in LNCaP cells. Therefore, our observation that

PRMT5 knockdown decreased HR-associated RAD51 foci may be partially explained by these previous results.

PRMT5 can also regulate protein expression via splicing (Braun et al., 2017; Tan et al., 2019). These two studies identified some PRMT5 splicing targets involved in DDR. For example, PRMT5 is required to maintain appropriate expression of functional RAD52 (Tan et al., 2019), and recent reports demonstrate the importance of RAD52 to HR (Hanamshet et al., 2016; Mahajan et al., 2019; Manthey et al., 2017). Future studies may determine if PRMT5-associated splicing directly affects the repair of IR-induced DSBs.

PRMT5 has also been shown to methylate and regulate several proteins associated with DDR: p53 (Du et al., 2016; Durant et al., 2009; Jansson et al., 2008; Scoumanne et al., 2009), E2F1 (Cho et al., 2012; Wu et al., 2015; Zheng et al., 2013), FEN1 (Guo et al., 2010, 2012), RAD9 (He et al., 2011), KLF4 (Hu et al., 2015), and TDP1 (Rehman et al., 2018). As detailed in these studies, DNA damage via etoposide, hydroxyurea, doxorubicin, and UV can induce PRMT5-catalyzed methylation of these non-histone substrates, which alters the cellular response to DNA damage. For example, PRMT5-catlyzed methylation of p53 altered binding to p53 target genes, which promoted cell-cycle arrest and inhibited apoptosis (Jansson et al., 2008). However, these studies did not identify a direct role for PRMT5 in the repair of DSBs or in the response to IR. For example, PRMT5-catalyzed methylation of RAD9 was essential to the cellular response to hydroxyurea but did not play a significant role in the cellular response to IR. It is likely that there are unknown PRMT5 substrates involved in DDR, and future studies may determine if PRMT5-catalyzed methylation of these proteins directly affects DSB repair efficiency.

In various yeast species, the PRMT5 homologue Hsl7 was shown to interact with and promote degradation of WEE1 homologues to promote $G_2$ progression independently of its methyltransferase activity (Cid et al., 2001; Theesfeld et al., 2003; Yamada et al., 2004). Although it is unknown if PRMT5 interacts with WEE1 protein in human tissues, the studies in yeast contrast with our study in which PRMT5 activated transcription of WEE1 and promoted DNA damage-induced $G_2$ arrest. The opposing post-translational and transcriptional regulation of WEE1 by PRMT5 may be modulated by PRMT5 methyltransferase activity and/or the absence or presence of DNA damage. In fact, the PRMT5-mediated changes in cell cycle in the Xenopus egg are independent of transcription because Xenopus egg extracts can cycle without *de novo* mRNA transcription (Yamada et al., 2004). Therefore, PRMT5 likely modulates WEE1-mediated cell-cycle changes in multiple ways.

### pICln May Function as a Cofactor of PRMT5 to Epigenetically Regulate Gene Expression Independently of MEP50

There is a long-standing view in the field that the cofactor MEP50 is required for PRMT5 methyltransferase activity and epigenetic function (Burgos et al., 2015; Chen et al., 2017; Karkhanis et al., 2011; Stopa et al., 2015). In solution, PRMT5 can exist as a homodimer or homotetramer. With MEP50, PRMT5 forms a hetero-octomeric complex ($PRMT5_4$:$MEP50_4$) (Antonysamy et al., 2012). Consistent with these structural studies, biochemical studies have provided evidence that purified PRMT5:MEP50 complex can catalyze dimethylation of various histone substrates including H4R3 (Burgos et al., 2015; Wang et al., 2014). However, our data suggest that PRMT5 works with pICln for the transcriptional activation of DDR genes via H4R3me2s. This is inconsistent with a previous report where *Pesiridis et al* showed that titration of pICln decreased H3 and H4 methylation by PRMT5 in an *in vitro* methylation assay (Pesiridis et al., 2009). However, as PRMT5 functions in a larger complex, the *in vitro* assay using proteins from a bacterial expression system might not recapitulate the biochemical and cellular conditions required for H4R3me2s *in vivo*. In our study, knockdown of MEP50 did not affect the expression of PRMT5 target genes involved in DDR, and MEP50 was not present at any of the target gene promoters characterized. Instead, pICln was present at the promoter regions of PRMT5 target genes along with H4R3me2s, and knockdown of pICln caused a decrease in PRMT5 target gene expression and impaired IR-induced DSB repair. Thus, it is likely that pICln may function as a cofactor of PRMT5 to activate transcription of DDR genes. As the epigenetic regulation of gene expression likely involves formation of a larger protein complex in a gene-specific manner, future characterization of PRMT5 and its cofactors or interacting proteins *in vivo* will provide mechanistic insight into the regulation of expression of PRMT5 target genes.

Although PRMT5-catalyzed histone methylation is predominantly repressive (Stopa et al., 2015), recent studies show PRMT5 can function as an activator of gene expression (Deng et al., 2017; Chen et al.,

2017; Tarighat et al., 2016). Activation or repression is not likely dependent solely on PRMT5-catalyzed histone methylation, as H4R3me2s has been shown to be both a repressive (Chen et al., 2017) and active chromatin mark (Deng et al., 2017). Therefore, additional factors are required to mediate the positive or negative epigenetic regulation by PRMT5. Because PRMT5 does not contain a DNA binding domain, additional proteins that recruit PRMT5 to sites on the genome may play a role in mediating the epigenetic function of PRMT5. Future studies will elucidate the full structure and interactome of PRMT5 on DNA and will determine differences between its active and repressive complexes. This will also provide an answer for how the same PRMT5-catalyzed histone modifications can mediate gene repression and activation. As we reported that PRMT5 functions as an epigenetic activator of AR expression (Deng et al., 2017), future work will determine if this is dependent on pICln. It is possible that pICln promotes activation by PRMT5, whereas other cofactors, such as MEP50, may promote repression by PRMT5.

### PRMT5 Targeting May Be Explored for Cancer Treatment

According to the American Cancer Society, over half of all cancer patients receive RT. RT induces DSBs in DNA, which are lethal to cells if not repaired. Although potentially curative, tumors can still regrow following RT. For example, 10% of prostate cancer patients with low-risk disease and 30-50% of patients with high-risk disease treated with RT still experience tumor recurrence (Boorjian et al., 2011; D'Amico et al., 2008). Thus, identification of novel therapeutic targets to enhance RT will likely reduce cancer mortality.

PRMT5 is overexpressed in many cancers, and its overexpression correlates with poor prognosis (Karkhanis et al., 2011; Stopa et al., 2015; Yang and Bedford, 2013). Our findings suggest that PRMT5 overexpression may increase the efficiency of DSB repair and confer survival advantages particularly following DNA-damaging treatments. For example, upregulation of RAD51, a putative PRMT5 target gene, has been shown to promote resistance to DNA damaging agents (Petermann et al., 2010; Schild and Wiese, 2010) and decreasing RAD51 expression sensitizes cancer cells to IR (Hayman et al., 2012). Because targeting DSB repair is a validated therapeutic approach for cancer treatment (Gavande et al., 2016), our findings that PRMT5 expression positively correlates with multiple DDR genes across clinical cancer datasets strongly suggests that PRMT5 targeting may be explored as a monotherapy or in combination with RT or chemotherapy for cancer treatment. PRMT5 may also be a particularly attractive therapeutic target for prostate cancer patients because targeting PRMT5 decreases AR expression (Deng et al., 2017) and targeting AR signaling via ADT enhances RT for prostate cancer patients (Golabek et al., 2016).

One criticism of PRMT5 targeting is potential systemic side effects as epigenetic regulators typically have essential roles in various tissues. Although targeting PRMT5 does not affect the growth of AR-negative DU145 and PC3 cells as well as normal prostate RWPE-1 cells (Deng et al., 2017), we do find that targeting PRMT5 inhibits repair of IR-induced DSBs in non-cancerous HEK293T cells. It is reasonable to suspect that targeting PRMT5 may also sensitize adjacent normal tissue to RT. Given advances in RT, the amount of adjacent normal tissue that is irradiated is minimalized. Thus, it is likely that the combination of PRMT5 targeting and RT will allow for either a lower dose of drug or IR to limit side effects. Alternatively, targeted delivery of PRMT5 inhibitors as radiosensitizers will circumvent systemic toxicity. This can be effectively achieved through prostate specific membrane antigen-based delivery (Rowe et al., 2016). Nevertheless, our findings here provide convincing evidence that PRMT5 functions as a master epigenetic regulator to activate transcription of DNA damage repair genes and is a potential therapeutic target to enhance RT or chemotherapy for cancer treatment.

### Limitations of the Study

We present evidence that PRMT5 cooperates with pICln to function as a master epigenetic activator of DDR genes in various cell types. Although this potentially explains the long-standing question of how repair proteins are quickly upregulated to promote the repair of DNA damage, the precise molecular mechanisms on how PRMT5 and pICln function together to activate gene expression remain to be determined. Our findings also suggest that the regulatory role of PRMT5 in the activation of DDR genes is independent of its canonical cofactor MEP50: MEP50 was not present at the promoter of DDR genes, and knockdown of MEP50 did not affect expression of DDR genes nor did it affect repair of IR-induced DSBs. Although we provide several lines of evidence suggesting that pICln, but not MEP50, may participate in transcriptional regulation of DDR genes by PRMT5, it is possible that our knockdown of MEP50 was not sufficient to prevent the regulation of DDR gene expression. Thus, future studies with CRISPR-based knockout or PRMT5:MEP50 protein-protein interaction inhibitors may be needed to further evaluate a potential role

for MEP50 in the regulation of PRMT5 target genes involved in DDR. Given that previous biochemical assays demonstrate that PRMT5 requires MEP50 for methyltransferase activity, further biochemical assays of PRMT5 with its cofactors in the presence of nucleosomes may provide mechanistic insight into the modulation of PRMT5 catalytic activity by its cofactors in the context of transcriptional regulation. These studies combined with both structural analysis of the PRMT5 complex at the promoter of target genes involved in DDR and genome-wide analyses such as ChIP-seq and ATAC-seq will likely provide mechanistic evidence for how PRMT5 functions with pICln and/or other cofactors to regulate transcription of DDR target genes.

## METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

## DATA AND CODE AVAILABILITY

RNA-seq datasets generated in this study are available at the Gene Expression Omnibus (GEO) under accession number GSE111620.

## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.100750.

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## AUTHOR CONTRIBUTIONS

Conceptualization: J.L.O., J.H. (consultations for clinical aspects), and C.D.H.; Methodology: J.L.O., X.D., and E.B.; Validation: J.L.O., E.B., S.L.T., A.M.A., and X.D.; Formal Analysis: J.L.O., S.L., and J.W.; Investigation: J.L.O., E.B., S.L.T., A.M.A., S.L., and X.D.; Resources: X.D.; Data Curation: J.L.O.; Writing—Original Draft: J.L.O.; Writing—Review & Editing: J.L.O., E.B., S.L.T., A.M.A., J.W., and C.D.H.; Visualization: J.L.O., S.L., and J.W.; Supervision: J.W. and C.D.H.; Project Administration: J.L.O. and C.D.H.; Funding Acquisition: J.H., C.L., J.W., and C.D.H.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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iScience, Volume *23*

## Supplemental Information

# PRMT5 Cooperates with pICln to Function as a Master Epigenetic Activator of DNA Double-Strand Break Repair Genes

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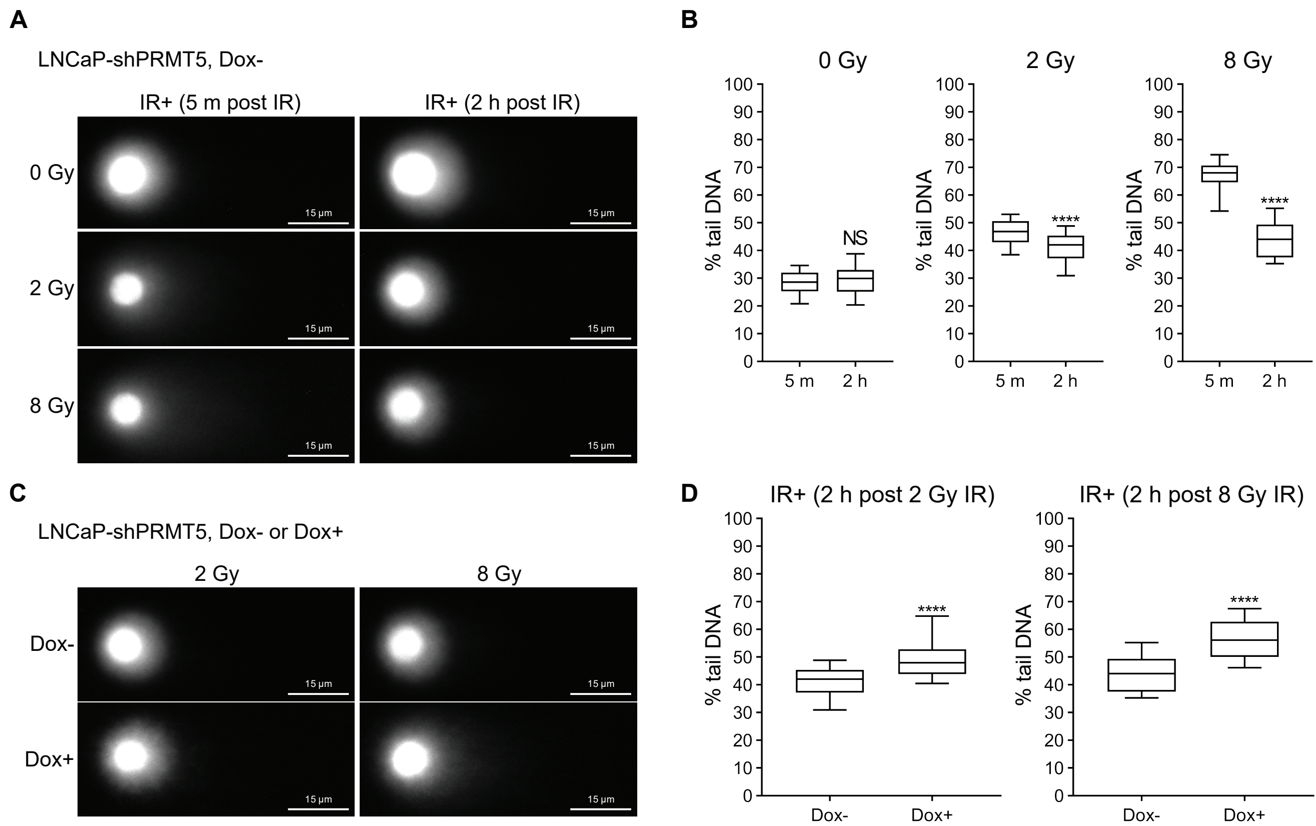



**Figure S1. PRMT5 regulates the repair of IR-induced DSBs in prostate cancer cells. Related to Figure 2.**

(A) Migration of DNA via neutral comet assay at the indicated time points post the indicated dose of IR in LNCaP- shPRMT5 cells without (Dox-) PRMT5 knockdown. The 5 m time point indicates how much DNA damage is induced by the indicated dose of IR. The 2 h time point indicates how much DNA damage is repaired within 2 h post the indicated dose of IR.
(B) Quantification of DNA damage in each individual cell via calculating the relative amount of DNA in the tail vs. head of the comet ('% tail DNA') from A.
(C) Migration of DNA via neutral comet assay 2 h post indicated dose of IR in LNCaP-shPRMT5 cells with (Dox+) and without (Dox-) PRMT5 knockdown.
(D) Quantification of DNA damage in each individual cell via calculating the relative amount of DNA in the tail vs. head of the comet ('% tail DNA') from C.

Box and whiskers plot in B and D show the median value (line), interquartile range (box), and 10-90 percentile (whiskers) of pooled '% tail DNA' values from 3 independent experiments. Statistical analysis comparing experimental to the control ('Dox-') was performed using Mann-Whitney U-test (**** $P \leq 0.0001$ and NS $P > 0.05$).

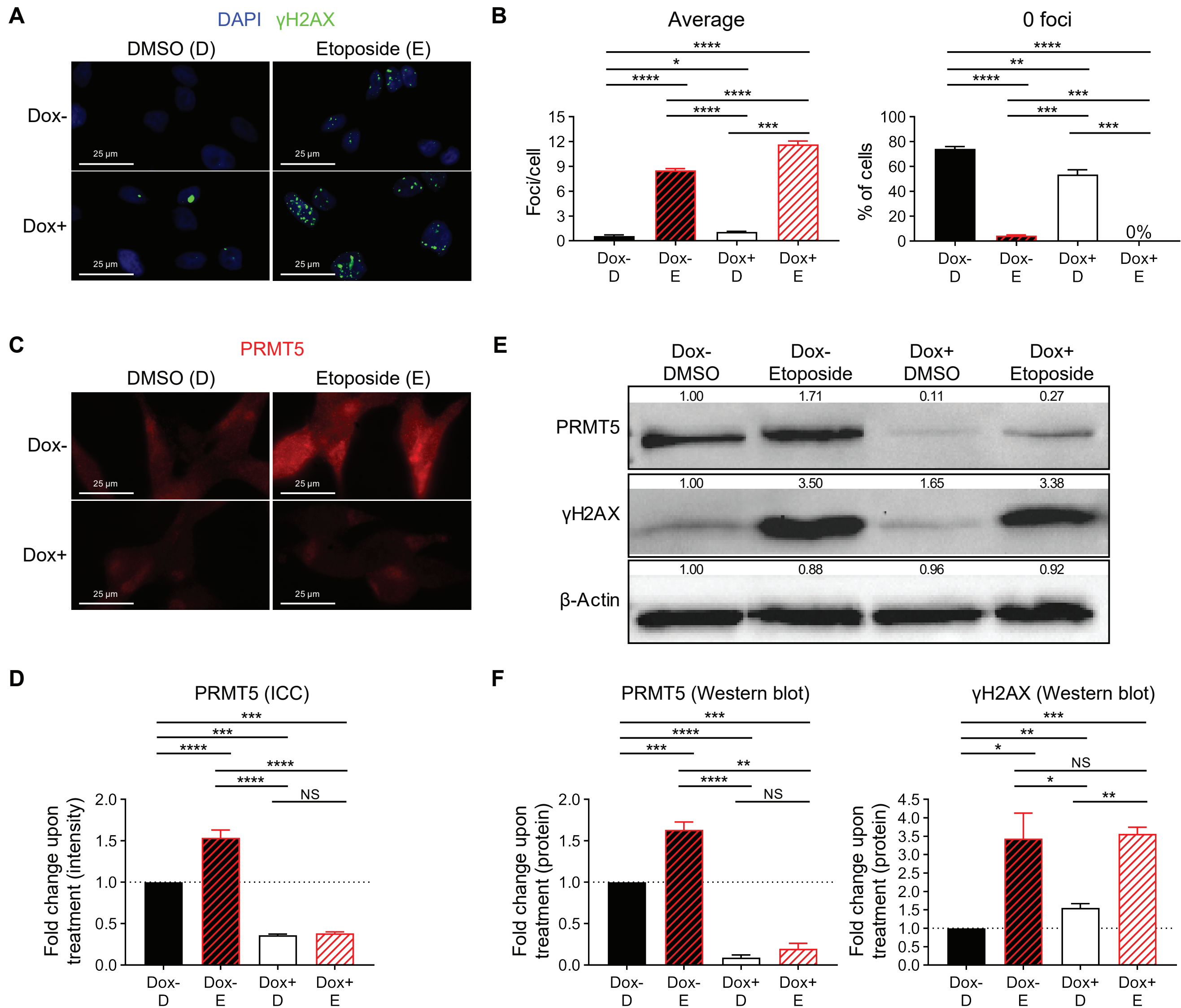



**Figure S2. PRMT5 regulates the repair of etoposide-induced DSBs in prostate cancer cells. Related to Figure 2.**

(A) DSBs after 48 h of etoposide (E) or DMSO (D) treatment in LNCaP-shPRMT5 cells with (Dox+) and without (Dox-) PRMT5 knockdown.
(B) Quantification of DSBs in each individual cell from A as described in Figure 2B.
(C) PRMT5 expression in cells from A.
(D) Quantification of PRMT5 expression in images from C. For each biological replicate, values were normalized to the value for 'Dox-,DMSO' to calculate the fold change in protein expression upon treatment.
(E) Representative western blot showing changes in protein expression in cells from A. Values shown indicate the intensity relative to 'Dox-,DMSO' for the biological replicate used as the representative western blot.
(F) Quantification of protein expression via western blotting from E. For each biological replicate, values were normalized to the value for 'Dox-,DMSO' to calculate the fold change in protein expression upon treatment.

Fluorescence images in A and C are representative immunocytochemistry images (blue = DAPI, green = γH2AX, and red = PRMT5). Bars in B are the mean ± s.d. of 4 independent experiments and bars in D and F are the mean ± s.d. of 3 independent experiments. Statistical analysis was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test (* P ≤ 0.05; ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001, NS P > 0.05).

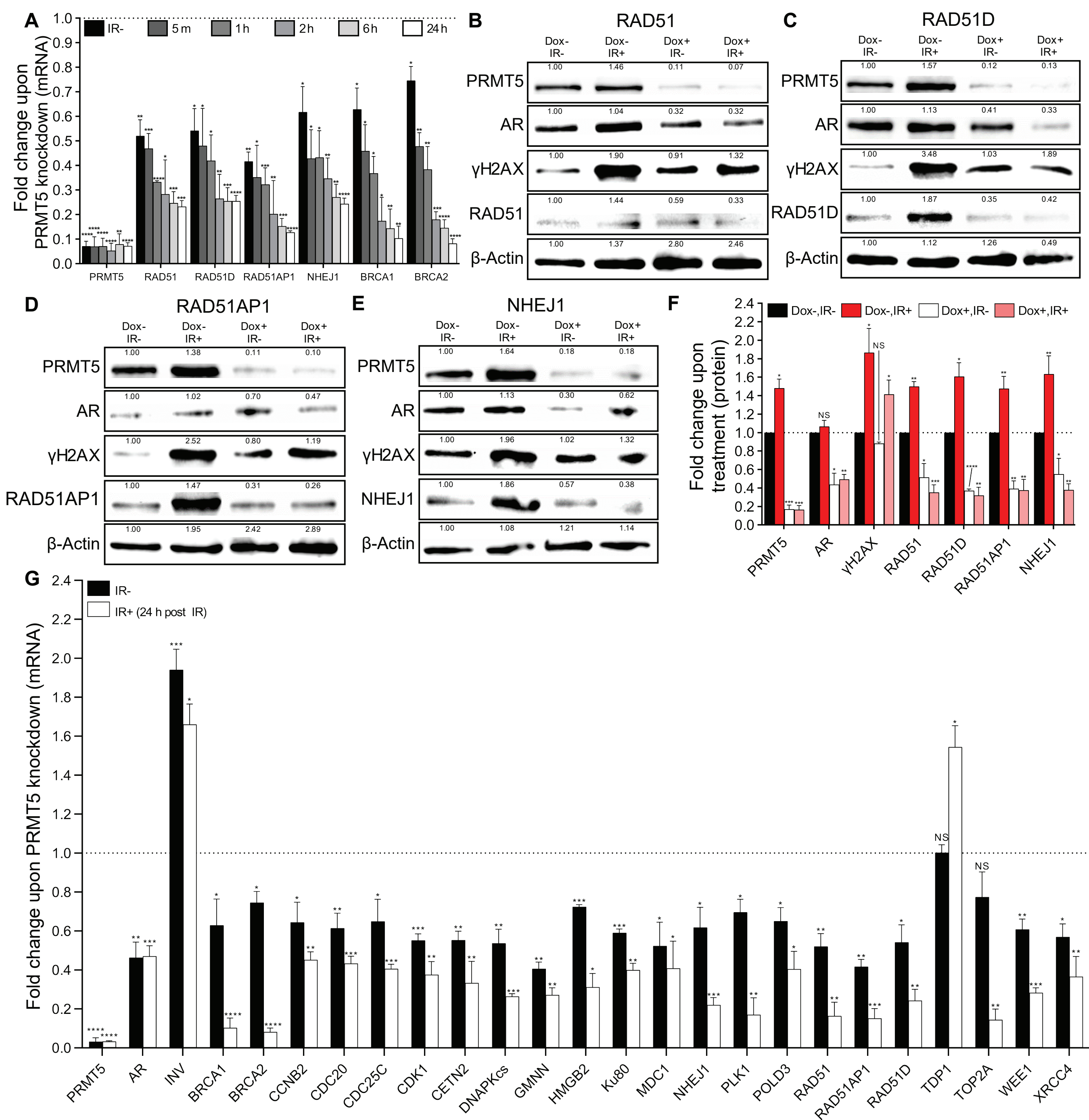



**Figure S3. PRMT5 activates transcription of genes that encode proteins involved in the repair of DSBs. Related to Figure 4.**

(A) Quantification of mRNA via RT-qPCR at the indicated time point post 2 Gy IR in irradiated (IR+) and non-irradiated (IR-) LNCaP-shPRMT5 cells with (Dox+) and without (Dox-) PRMT5 knockdown. For each biological replicate, the value for Dox+ was normalized to the value for Dox- to calculate the fold change in mRNA expression upon PRMT5 knockdown (See also Figure 4B).
(B)-(E) Representative western blots showing protein expression at 6 h post 2 Gy IR in irradiated (IR+) and non-irradiated (IR-) LNCaP-shPRMT5 cells with (Dox+) and without (Dox-) PRMT5 knockdown. Values shown indicate the intensity relative to IR- for the biological replicate used as the representative western blot.
(F) Quantification of protein expression via western blotting from B-E. For each biological replicate, values were normalized to the value for 'Dox-/IR-' to calculate the fold change in protein expression upon treatment.
(G) Quantification of mRNA via RT-qPCR 24 h post 2 Gy IR in irradiated (IR+) and non-irradiated (IR-) LNCaP- shPRMT5 cells with (Dox+) and without (Dox-) PRMT5 knockdown. For each biological replicate, the value for Dox+ was normalized to the value for Dox- to calculate the fold change in gene expression upon PRMT5 knockdown (See also Figure 4A and 4F).

Bars in A, F, and G are the mean ± s.d. of 3 independent experiments. Statistical analysis comparing experimental to the control ('Dox-' or 'Dox- / IR-') was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test (* P ≤ 0.05; ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001, NS P > 0.05).

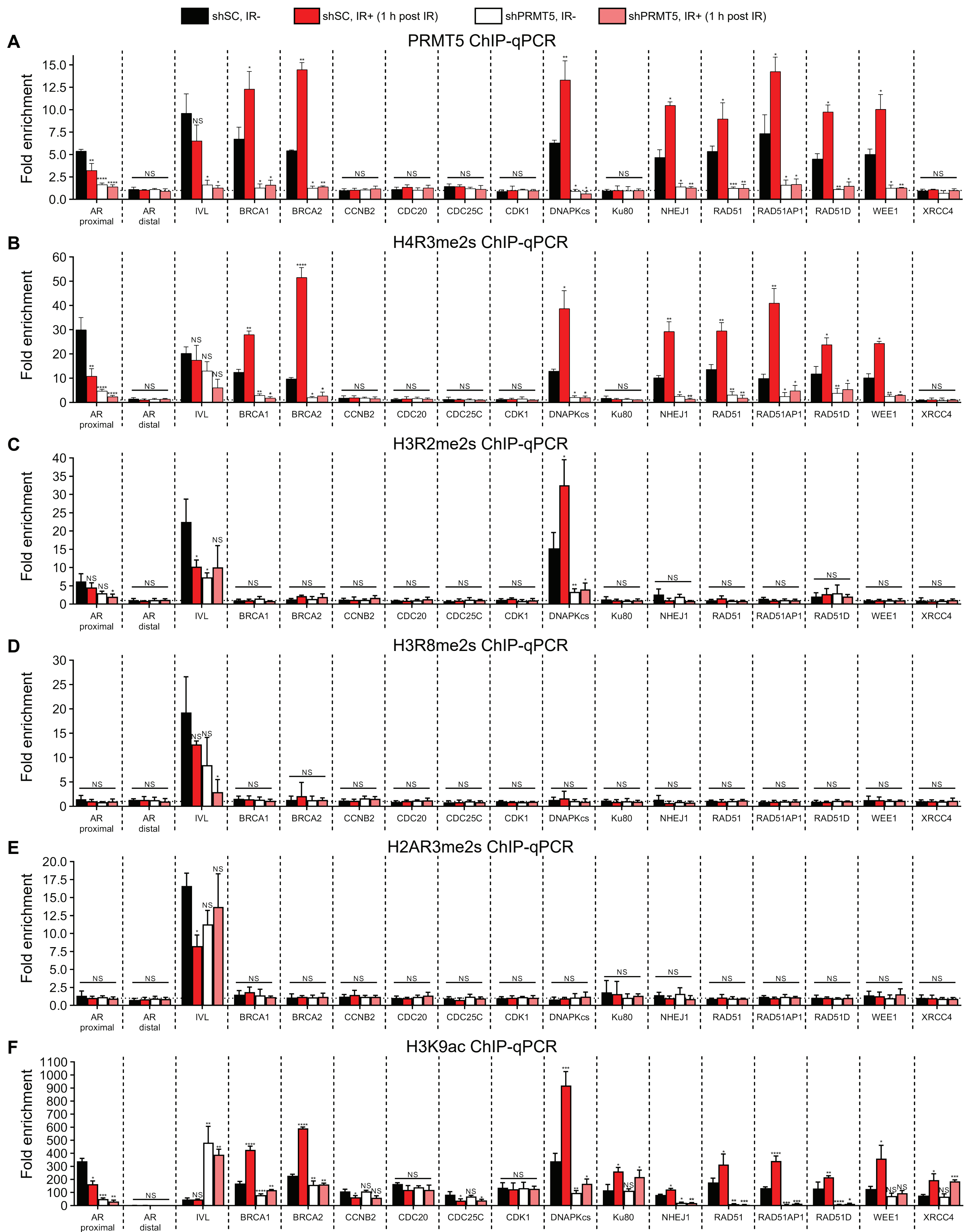
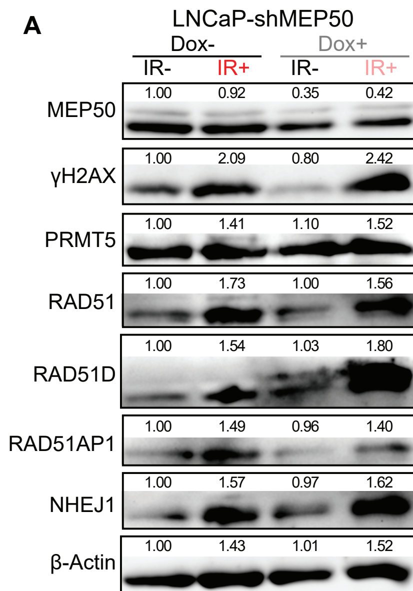
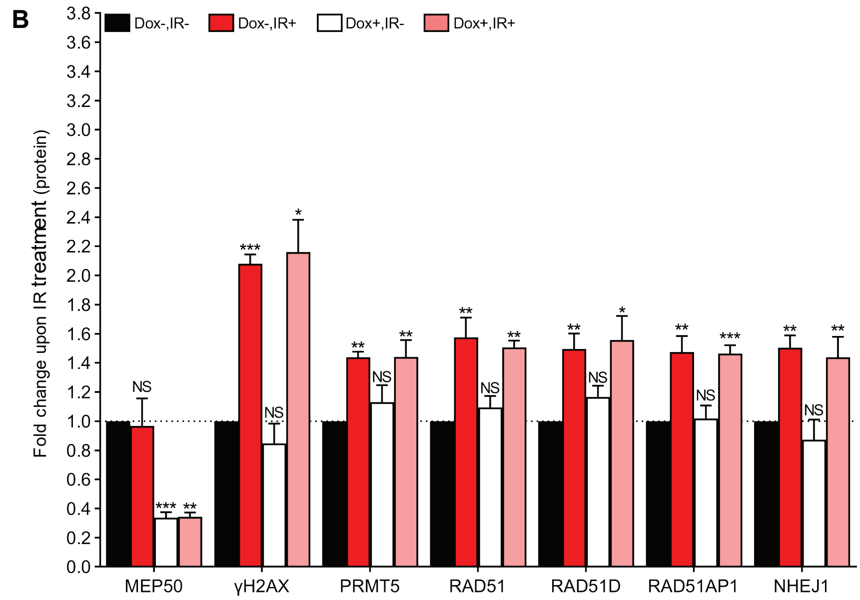


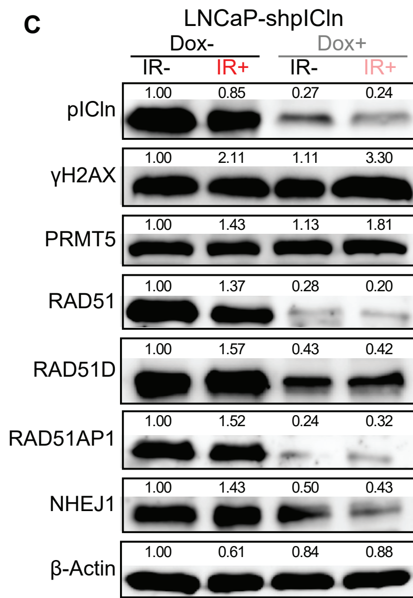

**Figure S4. PRMT5 likely functions as an epigenetic activator of DDR genes. Related to figure 4.**

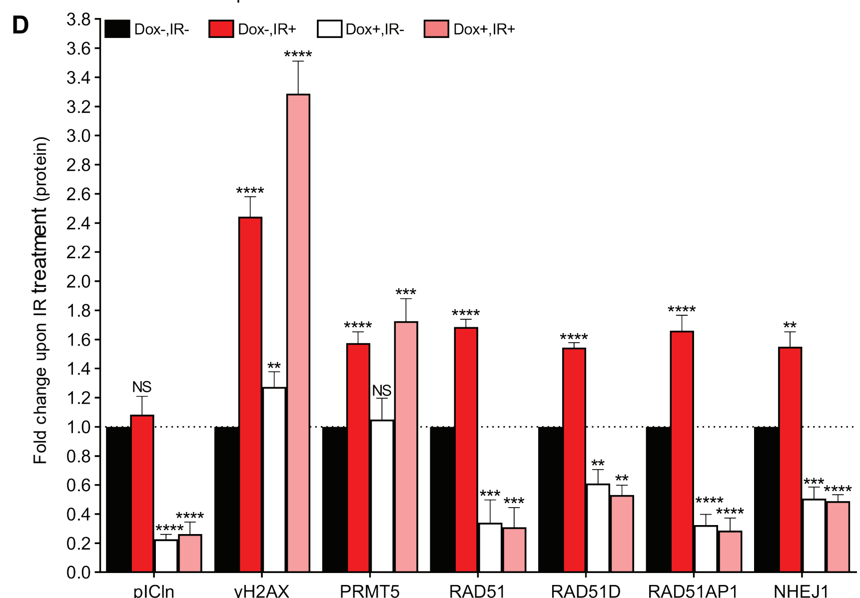
(A)-(F) Quantification of enrichment (A: PRMT5, B: H4R3me2s, C: H3R2me2s, D: H3R8me2s, E: H2AR3me2s and F: H3K9ac) at the promoter region of the indicated genes 1 h post 2 Gy IR in irradiated (IR+) and non-irradiated (IR-) LNCaP-shSC or shPRMT5 cells via ChIP-qPCR. For each biological replicate, the value for ChIP was normalized to the value for IgG to calculate the fold enrichment (See also Figures 4C-E and G-I).

All bars are the mean ± s.d. of 3 independent experiments. Statistical analysis was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test and the comparison to the control (‘shSC, IR-’) is shown (* P ≤ 0.05; ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001, NS P > 0.05).

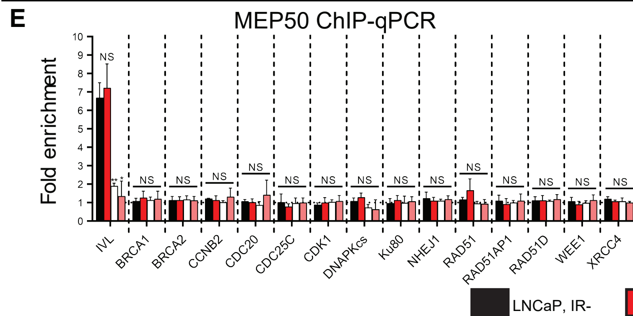


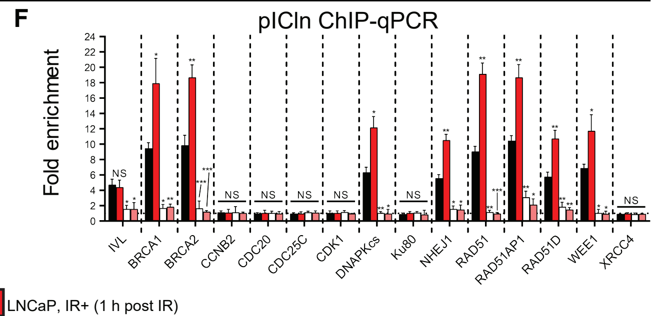



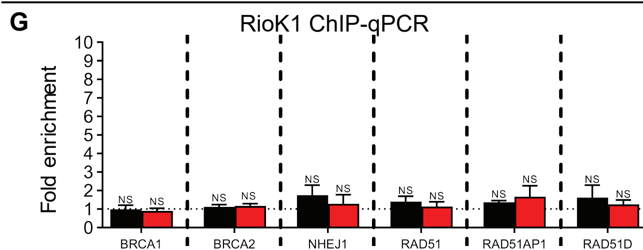




shSC, IR- shSC, IR+ (1 h post IR) shPRMT5, IR- shPRMT5, IR+ (1 h post IR)




LNCaP, IR- LNCaP, IR+ (1 h post IR)



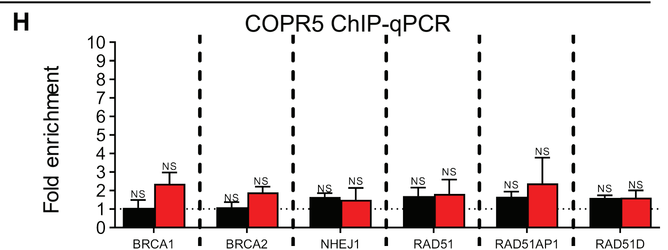



**Figure S5. pICln is also required for transcriptional activation of DDR genes and for efficient repair of DSBs. Related to Figure 5.**

(A) Representative western blots showing protein expression at 2 h post 2 Gy IR in irradiated (IR+) and non-irradiated (IR-) LNCaP-shMEP50 cells with (Dox+) and without (Dox-) MEP50 knockdown. Values shown indicate the intensity relative to 'Dox-,IR-' for the biological replicate used as the representative western blot.
(B) Quantification of protein expression via western blotting from A. For each biological replicate, values were normalized to the value for 'Dox-,IR-' to calculate the fold change in protein expression upon treatment.
(C) Representative western blots showing protein expression at 2 h post 2 Gy IR in irradiated (IR+) and non-irradiated (IR-) LNCaP-shpICln cells with (Dox+) and without (Dox-) pICln knockdown. Values shown indicate the intensity relative to 'Dox-,IR-' for the biological replicate used as the representative western blot.
(D) Quantification of protein expression via western blotting from C. For each biological replicate, values were normalized to the value for 'Dox-,IR-' to calculate the fold change in protein expression upon treatment.
(E)-(F) Quantification of enrichment (E: MEP50 and F: pICln) at the promoter region of the indicated genes 1 h post 2 Gy IR in irradiated (IR+) and non-irradiated (IR-) LNCaP-shSC or shPRMT5 cells via ChIP-qPCR. Dox was applied to establish and maintain PRMT5 knockdown (shPRMT5) or express scramble control shRNA (shSC). For each biological replicate, the value for IP was normalized to the value for IgG to calculate the fold enrichment (See also Figure 5G and 5H).
(G)-(H) Quantification of enrichment (G: RioK1 and H: COPR5) at the promoter region of the indicated genes 1 h post 2 Gy IR via ChIP-qPCR in irradiated (IR+) and non-irradiated (IR-) LNCaP cells via ChIP-qPCR. For each biological replicate, the value for IP was normalized to the value for IgG to calculate the fold enrichment.

Bars in B and E-H are the mean ± s.d. of 3 independent experiments. Bars in D are the mean ± s.d. of 6 independent experiments. Statistical analysis for B, D, E, and F was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test and the comparison to the control ('shSC, IR-') is shown while for G and H, statistical analysis comparing experimental to the control ('IR-') was performed using Welch's t-test (* $P \leq 0.05$; ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, NS $P > 0.05$).

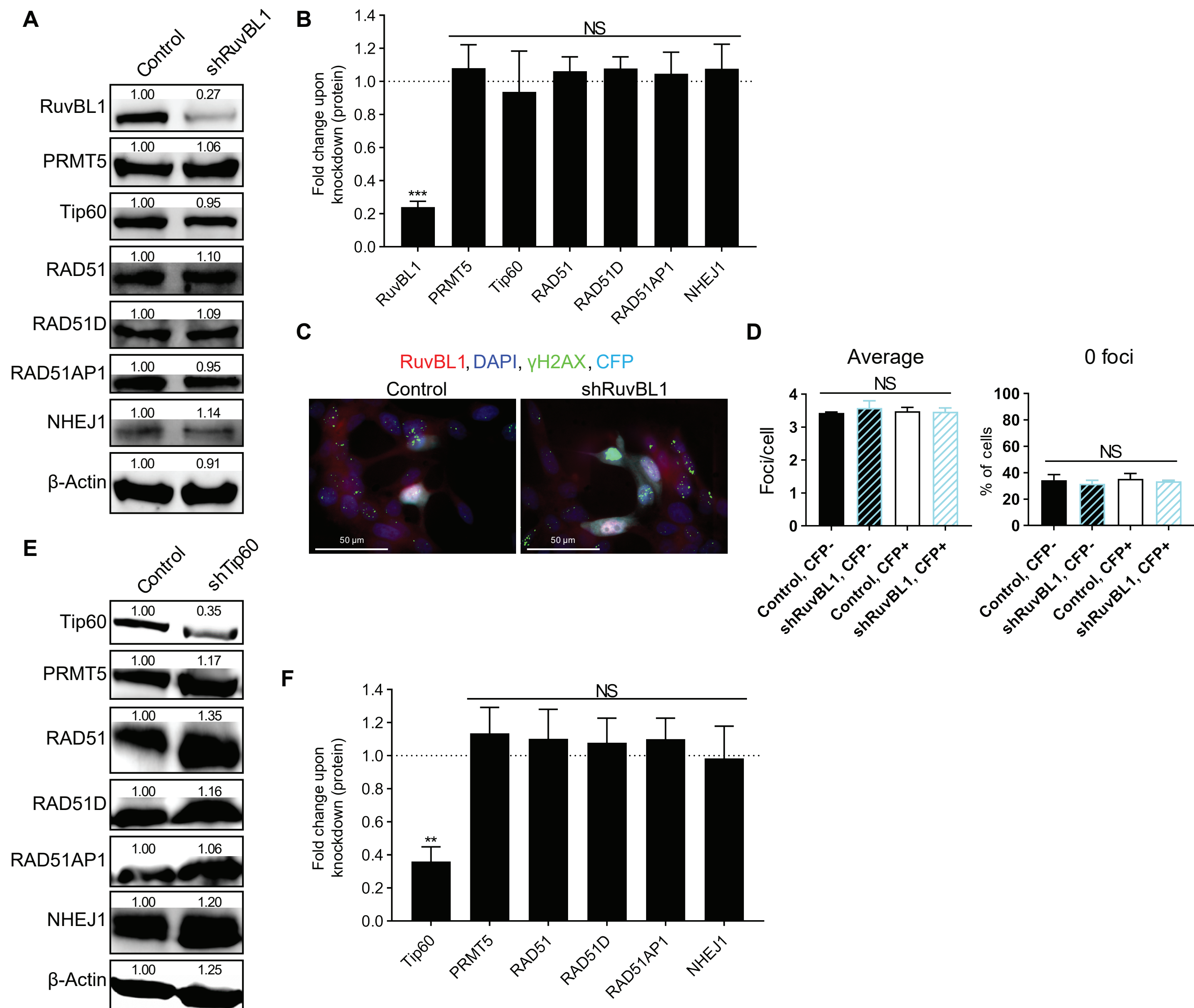



**Figure S6. The transcriptional regulation of DSB repair genes by PRMT5 is not dependent on RuvBL1 or Tip60. Related to Figure 4.**

(A) Representative western blot showing the protein expression in LNCaP cells with (shRuvBL1) and without (control) RuvBL1 knockdown. Values shown indicate the intensity relative to control for the biological replicate used as the representative western blot.
(B) Quantification of protein expression via western blotting from A. For each biological replicate, values were normalized to the value for 'control' to calculate the fold change in protein expression upon RuvBL1 knockdown.
(C) DSBs 6 h post 2 Gy IR in LNCaP cells with (shRuvBL1) and without (control) RuvBL1 knockdown.
(D) Quantification of DSBs in each individual cell from C as described in Figure 2B. CFP was used as a transfection control such that RuvBL1 was knocked down solely in CFP+ cells
(E) Representative western blot showing the protein expression in LNCaP cells with (shTip60) and without (control) Tip60 knockdown. Values shown indicate the intensity relative to control for the biological replicate used as the representative western blot.
(F) Quantification of protein expression via western blotting from E. For each biological replicate, values were normalized to the value for 'control' to calculate the fold change in protein expression upon Tip60 knockdown.

Fluorescence images in C are representative immunocytochemistry images (blue = DAPI, red = RuvBL1, and cerulean = CFP). Bars in B, D, and F are the mean ± s.d. of 3 independent experiments. Statistical analysis for B and F comparing experimental to the control ('control') was performed using Welch's t-test while statistical analysis for D was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test (* $P \leq 0.05$; ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, NS $P > 0.05$).

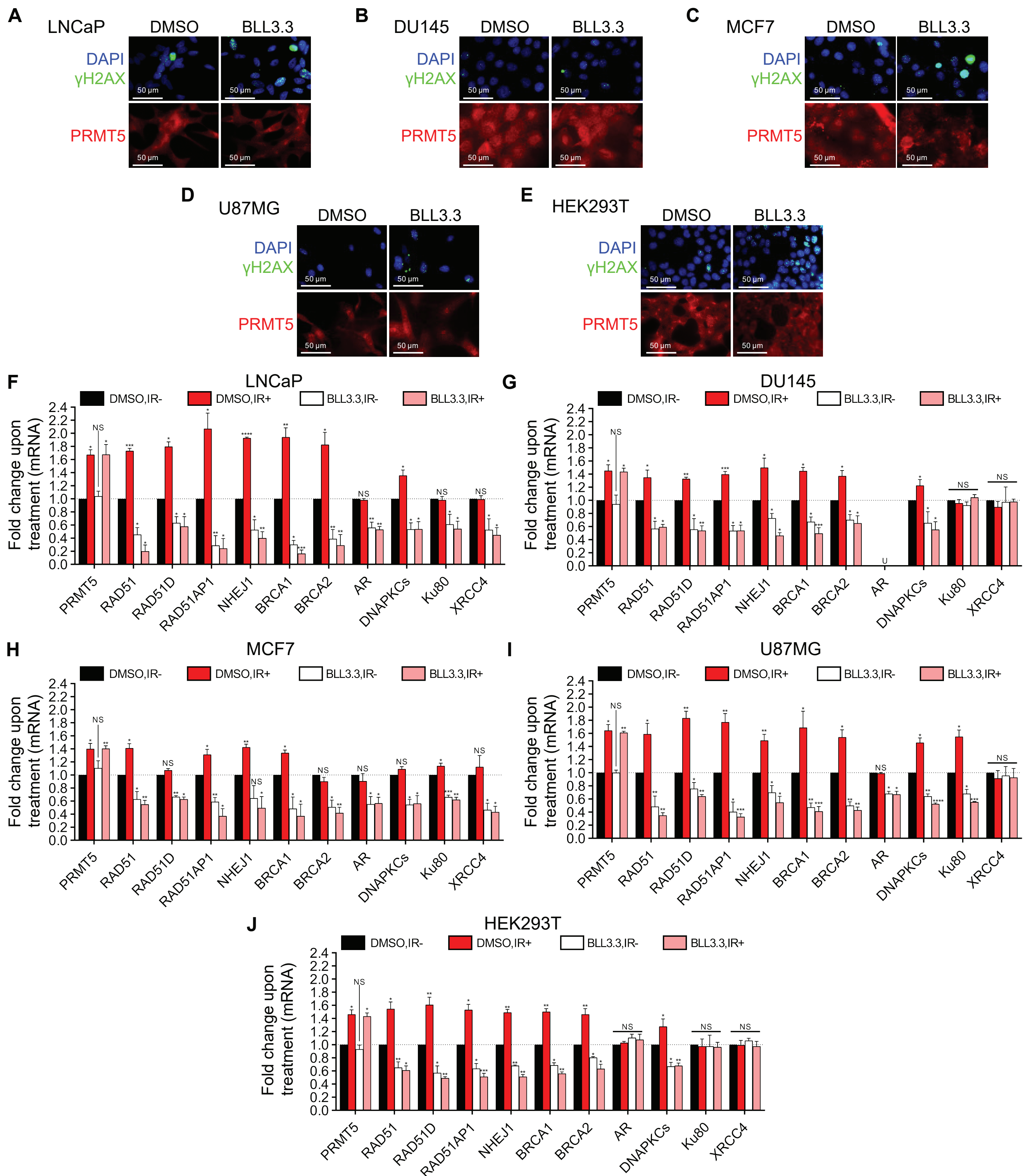



**Figure S7. The role of PRMT5 in the repair of DSBs is conserved in multiple cancer cell lines. Related to Figure 7.**

(A)-(E) DSBs 6 h post 2 Gy IR in the indicated cell lines (A: LNCaP, B: DU145, C: MCF7, D: U87MG, E: HEK293T) with PRMT5 inhibition (BLL3.3) or without PRMT5 inhibition (DMSO) as described in Figure 2B. Fluorescence images in A-E are representative immunocytochemistry images (blue = DAPI, green = γH2AX, and red = PRMT5) (see also Figure 7A for statistical analysis).

(F)-(J) Quantification of mRNA via RT-qPCR 6 h post 2 Gy IR in the indicated irradiated (IR+) and non-irradiated (IR-) cell lines (F: LNCaP, G: DU145, H: MCF7, I: U87MG, J: HEK293T) with PRMT5 inhibition (BLL3.3) or without PRMT5 inhibition (DMSO). For each biological replicate, values were normalized to the value for 'DMSO,IR-' (untreated) to calculate the fold change in mRNA expression upon treatment. (See also Figure 7B).

Bars are the mean ± s.d. of 3 independent experiments. Statistical analysis comparing experimental to the control ('DMSO,IR-') was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test (* $P \leq 0.05$; ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, NS $P > 0.05$).

**Figure S8. The expression of PRMT5 and pICln correlates with the expression of DDR genes in most human cancer tissues. Related to Figure 7.**

(A) Heatmap of Spearman correlation values between the indicated DDR genes and either PRMT5, CLNS1A (pICln), or WDR77 (MEP50) at the mRNA level across 32 clinical cancer data sets. The right section shows the correlation values between AR and PRMT5, pICln, or MEP50, respectively. Red indicates a positive correlation while blue indicates a negative correlation. The cancer types (y-axis, not listed) were sorted by the mean value of correlations between PRMT5 and DDR genes. (See also Figures 7C-7E).
(B) Violin plot representation of Spearman correlation values between CLNS1A (pICln) or WDR77 (MEP50) and PRMT5.

Box-and-whiskers plots in B show the median value (line) and interquartile range between the first and third quartiles (box). The upper whisker extended to the largest value no further than ‘1.5 x interquartile range’ and the lower whisker extended to the smallest value at most ‘1.5 x interquartile range’. Statistical analysis was performed using Wilcoxon test and the p-values are displayed.

## LIST OF SUPPLEMENTAL FILES

Table S1 - RNA-seq in IR- cells, Related to Figure 3G
Table S2 - RNA-seq in IR+ cells, Related to Figure 3G
Table S3 - DEGs in IR+ cells, Related to Figure 3H
Table S4 - GO analysis, Related to Figure 3I
Table S5 - IPA in IR+ cells, Related to Figure 3J
Table S6 - Antibodies list, Related to Methods
Table S7 - Primers list, Related to Methods

## TRANSPARENT METHODS

**Cell lines and cell culture.** LNCaP, DU145, PC3, and HEK293T were purchased from ATCC (Manassas, VA, USA) and cultured as described previously (Deng et al., 2017; Zhang et al., 2014). MCF7 cells were a gift from the Chun-Ju (Alice) Chang lab, and U87MG cells were a gift from the Emily Dykhuizen lab. Upon arrival, all cell lines were immediately expanded and aliquots were prepared and stored in liquid nitrogen. Cells were maintained for no longer than 30 passages or no longer than three months as described previously (Deng et al., 2011; Hsu and Hu, 2013). Cell line authentication for LNCaP cells was performed by IDEXX BioResearch (IMPACT I) and the absence of mycoplasma contamination for all cell lines was verified using LookOut® PCR Mycoplasma Detection Kit (Sigma, St. Louis, MO, USA). Knockdown cell lines were generated using the pLKO-Tet-On system. The pLKO-Tet-On plasmid for shRNA expression was obtained from Addgene (Cambridge, MA, USA) (Wiederschain et al., 2009), and shRNA sequences that target PRMT5 #1 (5'-CCCATCCTCTTCCCTATTAAG-3': referring to #1832) (Deng et al., 2017), PRMT5 #2 (5'-GCCCAGTTTGAGATGCCTTAT-3': referring to #1577) (Deng et al., 2017), SC (5'- CAACAAGATGAAGAGCACCAA-3'), MEP50 (5'- CCTCACAAGGACTCTGTGTTT-3'), and pICln (5'-CCAACAGTTGCTGGACAGTTT-3') were used for the construction of plasmids for stable cell line generation as described previously (Deng et al., 2017; Hsu and Hu, 2013). Lentiviral stably infected pools with Dox-inducible expression of PRMT5-targeting shRNA (shPRMT5 #2: referring to #1577 (Deng et al., 2017)) (LNCaP-shPRMT5 pool, PC3-shPRMT5 pool, and DU145-shRNA pool) were established and used for clonogenic assays. Stable cell lines with Dox-inducible expression of PRMT5-targeting shRNA (LNCaP-shPRMT5: referring to #1832 (Deng et al., 2017), LNCaP-shPRMT5 #2: referring to #1577 (Deng et al., 2017)) or scramble control-targeting shRNA (shSC) (LNCaP-shSC, PC3-shSC, and DU145-shSC) were established from individual clones and characterized previously (Deng et al., 2017). Stable cell lines with Dox-inducible expression of MEP50-targeting shRNA or pICln-targeting shRNA (LNCaP-shMEP50 and LNCaP-shpICln) were established from individual clones and characterized in this study.

**Dox-induced knockdown and inhibitor treatment conditions.** For Dox-inducible cell lines, Dox was applied at the final concentration of 1 µg/mL every 48 h to establish and maintain PRMT5 knockdown (shPRMT5), MEP50 knockdown (shMEP50), pICln knockdown (shpICln), or express scramble control shRNA (shSC). The number of days of Dox treatment was optimized: shPRMT5 and shSC cells were grown for 4 days and had 4 days of Dox treatment, shMEP50 were grown for 4 days and had 2 days of growth followed by 2 days of Dox treatment, and shpICln cells were grown for 5 days and had 5 days of Dox treatment. For parental cell lines, cells were treated with the PRMT5 inhibitor BLL3.3 (10 µM) or an equal volume of DMSO (control) every 48 h beginning 24 h after plating to inhibit PRMT5 activity. For IR experiments, cells were subjected to IR following the knockdown or inhibitor treatment described above.

**Ionizing radiation conditions.** For clonogenic assays, cells were irradiated using the GC-220 device (Atomic Energy of Canada, Ottawa, Canada) with a Co-60 radiation source as described previously (Deng et al., 2008, 2011). For all other experiments, cells were irradiated using the X-RAD 320 biological irradiator device (PXi Precision X-Ray, North Branford, CT, USA) with an x-ray tube radiation source at an average dose rate of ~1 Gy/25 sec. All IR treatments were carried out in normal air at room temperature, and cells spent minimal time outside incubators during treatment. Non-irradiated controls were 'mock-irradiated' by being taken out of the incubator for the same time period as irradiated counterparts.

**Clonogenic assays.** Clonogenic assays to quantify the surviving fraction following IR was performed similar to previously reported (Deng et al., 2008, 2011). For Dox-inducible cell lines, Dox was applied at the final concentration of 1 µg/mL every 48 h to establish and maintain PRMT5 knockdown (shPRMT5) or express scramble control shRNA (shSC). Additionally, LNCaP cells were treated with the PRMT5 inhibitor BLL3.3 (10 µM) or an equal volume of dimethyl sulfoxide (DMSO) (control) every 48 hours beginning 24 hours after plating to inhibit PRMT5 activity. After 4 days, when cells reached ~80% confluency, cells were subjected to the indicated dose of IR and immediately harvested, collected, counted, and reseeded on fresh 6 well plates for clonogenic assay. After 14 days of growth, the number of colonies were counted to calculate the surviving fraction. The number of cells for reseeding was optimized based upon how much cell death was observed: (LNCaP: 0 Gy-500 cells, 2 Gy-700 cells, 4 Gy-1000 cells, 6 Gy-5000 cells, and 8 Gy-10000 cells), (PC3: 0 Gy-50 cells, 2 Gy-100 cells, 4 Gy-200 cells, 6 Gy-600 cells, and 8 Gy-1000 cells), (DU145: 0 Gy-50 cells, 2 Gy-100 cells, 4 Gy-200 cells, 6 Gy-400 cells, and 8 Gy-800 cells).

**Immunocytochemistry (ICC) for quantification of IR-induced DSBs** (Kuo and Yang, 2008)**, NHEJ-associated foci** (Rapp, 2004; Slupianek et al., 2006)**, and HR-associated foci** (Rapp, 2004; Slupianek et al., 2006). Cells were seeded on 6 cm dishes containing glass coverslips and treated as described elsewhere. When cells reached ~80% confluency, cells were treated with 2 Gy IR and then fixed with 3.7% formaldehyde in phosphate buffered saline (PBS) at room temperature for 20 minutes at the indicated time points: First, we assessed the formation and repair of DSBs by analyzing γH2AX foci in a time course following radiation (5 m and 1, 2, 6, and 24 h). Given that the majority of DSBs are repaired within 2-6 h following IR, we assessed the effect of knockdown or inhibitor on the repair of DSBs at the 6 and 24 h timepoints. To assess potential impact on HR or NHEJ, we assessed RAD51 and Ku70 foci, respectively, at 1 h following IR treatment which is when the majority of repair occurred. After fixation at the indicated timepoints, cells were permeabilized with 0.2% Triton X-100 in PBS at room temperature for 5 m. Cells were then blocked with 5% milk blocking solution in PBS, stained with the indicated primary antibodies diluted in 5% milk blocking solution in PBS, and stained with 4,6-diamidino-2-phenylindole (DAPI final 10 μg/mL) and indicated secondary antibodies diluted in 5% milk blocking solution in PBS. Cells on coverslips were mounted on glass slides using the ProLong® Antifade Kit (Invitrogen Molecular Probes, Eugene, OR, USA) and sealed with clear nail polish. Cells were then imaged via the Nikon TE2000 inverted fluorescence microscope under oil immersion (60x objective) (Nikon Instruments Melville, NY, USA). The number of foci was manually recorded for each cell (defined via nuclear DAPI staining). At least 60 cells were counted for each biological replicate. The arrays of foci counts for each biological replicate were subjected to further analysis separately to determine the average number of foci per cell and percentage of cells with zero foci. The primary antibodies used were anti-PRMT5-rabbit (1:1000), -γH2AX-mouse (1:1000), -γH2AX-rabbit (1:200), -AR-mouse (1:1000), -AR-rabbit (1:100), -RAD51-rabbit (1:1000), -Ku70-mouse (1:500), -MEP50-rabbit (1:100), -pICln-rabbit (1:1000), and -RuvBL1-rabbit (1:100). Secondary antibodies used were anti-mouse-FITC (1:100) and anti-rabbit-rhodamine red (1:1000). All antibodies are described in **Table S6**.

**Immunocytochemistry (ICC) for quantification of protein expression or subcellular localization.** Immunocytochemistry was performed as described above. Images were analyzed via ImageJ (Schneider et al., 2012). First, the background was subtracted from the image using the rolling ball method (http://imagej.net/plugins/rolling-ball.html). For PRMT5 expression, regions of interest (ROI) were outlined for each individual cell. For AR expression, ROI were outlined for each nucleus (as defined by DAPI staining). For MEP50 and pICln expression and subcellular localization, ROI were outlined for each individual cell, nucleus only (as defined by DAPI staining), and cytoplasm only (as defined by signal outside of DAPI staining). The average intensity for each ROI was measured and at least 60 cells were counted for each biological replicate. The arrays of intensity counts for each biological replicate were subjected to further analysis separately and were analyzed via both "D'Agostino & Pearson" and "Shapiro-Wilk" normality tests to evaluate distribution. Because not all samples were normally distributed, the median value was used for each biological replicate. To determine the nuclear:cytoplasmic ratio (N:C) the value for nucleus was divided by the value for cytoplasmic for each cell individually such that an N:C ratio of 1 indicates equal expression in both the nucleus and cytoplasm.

**Comet assay.** To determine if PRMT5 regulates the repair of IR-induced DSBs, we used comet assay to quantify DNA damage directly. LNCaP-shPRMT5 cells were seeded on 6 cm dishes and Dox was applied at the final concentration of 1 μg/mL every 48 h to establish and maintain PRMT5 knockdown. After 4 days, when cells reached ~80% confluency, cells were treated with the indicated dose of IR and then harvested and counted after either 5 m or 2 h. The 5 m timepoint indicates how much total DNA damage is induced by radiation. Comparing the 2 h timepoint to the 5 m timepoint indicates how much DNA damage is repaired. Twenty thousand cells per group were diluted in 100 μL of 0.5% Low Melting Agarose in PBS at 45°C and 50 μL of diluted cells were immobilized onto pretreated VWR Superfrost Plus slides (previously dipped in 1% Agarose in nanopure water and allowed to dry overnight). Glass coverslips were placed on top of the cell dilution and the slides were placed in 4°C for 10 minutes to solidify the agarose. Slides were moved to room temperature for 5 minutes, the coverslips removed, and immobilized cells were lysed in 4°C neutral lysis buffer (10mM Tris HCl pH 8.0, 100 mM EDTA, 2.5 M NaCl, 1% Sarkosyl, 0.5% Triton X-100) for 60 minutes at 4°C. Slides were removed from lysis buffer and equilibrated in Neutral Comet Electrophoresis Buffer (90 mM Tris HCl pH 8.0, 90 mM Boric Acid, 2 mM EDTA) for 20 minutes. Electrophoresis was performed at 14V, 27mA for 60 minutes. After electrophoresis, slides were equilibrated in 0.4 M Tris-HCl pH 7.4 for 5 minutes at room temperature. The equilibration buffer was replaced with fresh buffer, and the slides were incubated for an additional 5 minutes. This wash was repeated one additional time for a total of three washes. Sixty μL of DAPI (0.5 μg/mL in $H_2O$) was applied dropwise to the agarose pad, and slides were incubated at 4°C for 15 minutes. Comets were then imaged via the Nikon TE2000 inverted fluorescence microscope (20x objective) (Nikon Instruments Melville, NY, USA) and analyzed with ImageJ (Schneider et al., 2012). To quantify the '% tail DNA' in each cell from the images, we utilized the comet assay plugin created by Robert Bagnell (2011) based on the NIH Image comet assay by Herbert M. Miller (1997). At least 65 cells were analyzed across 3 biological replicates and the '% tail DNA' values were pooled for statistical analysis via Mann-Whitney *U*-test. Although several reports using comet assay have used various data representation and statistical analysis (Higo et al., 2017; Lee et al., 2017; Mo et al., 2018; Nassour et al., 2016), because of the high variance within each biological replicate and lack of normal distribution we used the Mann-Whitney *U*-test (Dungrawala et al., 2017; Liu et al., 2018; Xiao et al., 2018).

**Etoposide treatment.** To assess if PRMT5 is required for repair of DSBs in general, we used etoposide to induce replication-dependent DSBs. LNCaP-shPRMT5 cells were seeded on 6 cm dishes containing glass coverslips and Dox was applied at the final concentration of 1 µg/mL every 48 h to establish and maintain PRMT5 knockdown. When cells reached ~60% confluency, cells were treated with either etoposide (10 µM) or an equal volume of DMSO. Forty-eight h after initiation of treatment, coverslips were transferred to a new dish and subjected to γH2AX-foci analysis described above while the remaining cells were harvested and subjected to western blot analysis. Although not shown, experiments with short etoposide treatments (2 h, 6 h) were unsuccessful and the 48 h etoposide treatment time was likely optimal because cells could undergo DNA replication which induced DSBs.

**Transient transfection for rescue of AR expression.** To evaluate if the role of PRMT5 in the repair of IR-induced DSBs is independent of AR, LNCaP-shPRMT5 cells were seeded on 6 cm dishes containing glass coverslips and Dox was applied at the final concentration of 1 µg/mL every 48 h to establish and maintain PRMT5 knockdown. Forty-eight h following seeding, cells were transfected with pCMV-Flag2-AR, as described previously (Deng et al., 2017; Hsu and Hu, 2013), or pCMV-Empty Vector plasmid using FuGENE HD (Promega, Madison, Wisconsin, USA). pCMV-HA-CFP was used as a transfection control. Upon reaching ~80% confluency (48 h following transfection), cells were treated with 2 Gy IR and subjected to immunocytochemistry analysis. Only transfected cells (CFP+) were subjected to γH2AX foci analysis, while both transfected and non-transfected cells were subjected to protein expression analysis as described above. For the microscope images, we used 3D representation to show the expression of multiple proteins in a single cell at the same time. Each peak is a cell and the height of each peak is the intensity of signal.

**RNA-seq for identification of PRMT5 target genes in response to IR.** LNCaP-shPRMT5 cells were seeded on 6 cm dishes and Dox was applied at the final concentration of 1 µg/mL every 48 h to establish and maintain PRMT5 knockdown for 4 days. Cells were harvested 1 h following a 2 Gy IR treatment and total RNA was isolated using Trizol Reagent (Ambion, Carlsbad, CA, USA). PolyA+ RNA libraries were generated according to the Illumina "TruSeq Stranded mRNA Sample Preparation Guide" (15031047E) with the following considerations: (1) an Agilent Bioanalyzer RNA-Nano kit was used to assess RNA concentration and rule out sample degradation. (2) Heat and divalent cation fragmentation of the polyA+ RNA was undertaken for 4 m rather than the default of 8 m. (3) The number of PCR cycles for library amplification was determined by the yield of cDNA. For both RNA-seq analyses, we ran 8 cycles of PCR instead of the 15 cycles mentioned in the manual. (4) Final cleanup was performed using a 0.8:1 bead:sample ratio with AmPure XP beads instead of the 1:1 mentioned in the manual. IR+ group was run on an Illumina HiSeq 2500 using High Output flowcell to produce paired-end 101 base reads. IR- group was run on an Illumina NovaSeq 6000 S4 flowcell that generated paired-end 151 base reads. Additionally, IR- samples were prepared and run with unique dual indexes to mitigate potential "index-hopping" associated with Illumina instruments using "exclusion amplification" clustering on patterned flowcells.

RNA-seq quality was assessed by FastQC, and STAR RNA-seq aligner (Dobin et al., 2013) was used to map all high-quality sequences to the human genome (GENCODE GRCh38). Read counts were evaluated using Subread featureCounts (Liao et al., 2014) to summarize uniquely mapped reads to the gene level according to the GENCODE M25 annotation file. Data was normalized by trimmed mean of M value method to obtain the final profile of gene expression (base-2 log scale). EdgeR (Robinson et al., 2010) was used to perform differential expression analysis by comparing Dox+ (PRMT5 KD) and Dox- (no KD) for IR+ and IR- groups. After removing low-expressed genes (average expression levels lower than 1 for both conditions), we defined genes as differentially expressed genes (DEGs) if their FDR-adjusted p-values were less than 0.01, and the magnitudes of fold-changes (FCs) were larger than log2 (1.25).

Gene Ontology (GO) and pathway analysis were performed on the 'IR+ only' DEGs. GO analysis was performed using the web-based tool DAVID functional annotation analysis (http://david.abcc.ncifcrf.gov/home.jsp v6.8) (Huang et al., 2007, 2009). Only GO annotations with FDR-adjusted p-values less than 0.05 and the fold enrichment score larger than 1.5 were selected as significantly over-represented GO terms. Pathway analysis on IR+ only DEGs was performed using Ingenuity Pathway Analysis (IPA) (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) to identify differentially regulated pathways upon PRMT5 knockdown in irradiated cells.

**RNA isolation, reverse transcription, and RT-qPCR**. Cells were seeded on either 6 cm or 10 cm dishes and treated as described elsewhere. Total RNA was isolated using Trizol Reagent (Ambion, Carlsbad, CA, USA). RNA concentration and integrity were verified by agarose gel electrophoresis. cDNA synthesis was done using High Capacity cDNA Reverse Transcription Kit (Promega, Madison, WI, USA) as described previously (Deng et al., 2017; Hsu and Hu, 2013; Zhang et al., 2014). qPCR was performed using FastStart Universal SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) on the QuantStudio 6 Flex System and QuantStudio™ Real-Time PCR Software (ThermoFisher, Waltham, MA, USA). Forty cycles were run and samples without $C_T$ values were deemed undetected. Technical duplicates were run for each sample and the $C_T$ values used for further analysis were the average of the technical duplicates. Samples where $C_T$ values for technical duplicates were >0.5 apart were re-run. Non-template controls (NTC)s with autoclaved double-distilled $H_2O$ were also run for each primer set and primer sets where $C_T$ values for NTC were lower than 37 (indicating high background) were re-run. Amplicon size and specificity were verified for each primer set via agarose gel electrophoresis. PRMT5, AR, and GAPDH primers were used previously (Deng et al., 2017; Zhang et al., 2014). IVL primers were used previously (Chew et al., 2013; Saha et al., 2016). All primers used are described in **Table S7**.

**Chromatin immunoprecipitation (ChIP)-qPCR assay.** LNCaP-shPRMT5 or LNCaP-shSC cells were seeded on multiple 10 cm dishes and Dox was applied at the final concentration of 1 μg/mL every 48 h to establish and maintain PRMT5 knockdown (shPRMT5) or express scramble control shRNA (shSC). After 4 days, when cells reached ~80% confluency, cells were treated with 2 Gy IR. One hour following IR (prior to the repair of the majority of DSBs and at the same time as the peak of IR-induced PRMT5 protein expression), cells were fixed/crosslinked and chromatin was prepared for ChIP-qPCR as described previously (Deng et al., 2017). Chromatin fragments were verified to be ~500 base pairs by agarose gel electrophoresis. Antibodies used for immunoprecipitation were anti-PRMT5-rabbit, -H4R3me2s-rabbit, -H3K9ac-rabbit, -H3R2me2s-rabbit, -H3R8me2s-rabbit, -H2AR3me2s-rabbit, -MEP50-rabbit, -pICln-rabbit, and IgG-rabbit. All antibodies are described in **Table S6**. Primers used for ChIP-qPCR are described in **Table S7**.

**Flow cytometry cell-cycle analysis.** LNCaP-shPRMT5 cells were seeded on 6 cm dishes and Dox was applied at the final concentration of 1 μg/mL every 48 h to establish and maintain PRMT5 knockdown. After 4 days, when cells reached ~80% confluency, IR+ cells were treated with 2 Gy IR. Cells were harvested 24 h following IR, resuspended in PBS, and filtered through a 70 μm nylon cell strainer to remove all cell aggregates. A single cell suspension was prepared and verified via microscopy. Cells were then fixed in 70% ethanol, stained with a Propidium Iodide (PI) containing solution (20 μg/mL PI and RNaseA diluted in PBS) and subjected to flow cytometry analysis via the Guava EasyCyte Flow Cytometer (Guava Technologies, Hayward, CA, USA). At least 20,000 live cells were counted for each biological replicate. Flow cytometry data was analyzed via FlowJo (FlowJo, LLC, Ashland, Oregon, USA). Live cells were gated for analysis to remove any sub-$G_1$ cells and then were subjected to cell cycle analysis via Dean-Jett-Fox modeling (Fox, 1980).

**Western blot.** Cells were seeded on either 6 cm or 10 cm dishes and treated as described elsewhere. Cells were harvested in lysis buffer (100 mM Tris-HCl pH 8.0, 15 mM $MgCl_2$, 100 mM KCl, 5 μg/mL of each Chymostatin, Leupeptin, Pepstatin A, and antipan in DMSO, 1% Triton X-100, 1 mM PMSF in ethanol, and 1 mM DTT) or RIPA buffer (10 mM Tris-HCl pH 8.0, 5 mM EDTA, 1% Triton X-100, 0.1% Sodium Deoxycholate, 0.1% SDS, 140 mM NaCl, 5 μg/mL of each Chymostatin, Leupeptin, Pepstatin A, and antipan in DMSO, and 1 mM PMSF in ethanol) and total protein concentration was measured using Bradford method. Approximately 20-30 μg total protein was run on a 10-15% SDS-PAGE and western blotting was performed as described previously (Deng et al., 2017; Hsu and Hu, 2013; Zhang et al., 2014). Band/protein intensity was quantified using Image Lab™ (Bio-rad, Hercules, CA, USA). Antibodies used for western blot were anti-PRMT5-rabbit (1:1000), -AR-rabbit (1:2000), -γH2AX-rabbit (1:1000), -RAD51-rabbit (1:2000), -RAD51D-rabbit (1:2000), -RAD51AP1-rabbit (1:1000), -NHEJ1-rabbit (1:2000), -β-Actin-mouse (1:2000), -MEP50-rabbit (1:500), -pICln-rabbit (1:2000), -RuvBL1-rabbit (1:1000), -Tip60-rabbit (1:500), -rabbit-HRP (horseradish peroxidase) (1:1000), -mouse-HRP (1:1000). All antibodies are described in **Table S6**.

**Bimolecular Fluorescence Complementation (BiFC) assay.** LNCaP cells were grown to ~60% confluency and transfected with plasmids to visualize the PRMT5:MEP50 interaction (pMYC-VN155-PRMT5, pHA-VC-MEP50, and pFlag-NLS-CFP) and PRMT5:pICln interaction (pMYC-VN155-PRMT5, pHA-VC-pICln, and pFlag-NLS-CFP). Forty-eight hours following transfection, cells were treated with 2 Gy IR. Immediately prior to IR and 6 h following IR (the time point with the largest changes in MEP50 and pICln subcellular localization), cells were imaged via the Nikon TE2000 inverted fluorescence microscope (20x objective) (Nikon Instruments Melville, NY, USA). Images were then analyzed with ImageJ (Schneider et al., 2012). First, the background was subtracted from the image using the rolling ball method (http://imagej.net/plugins/rolling-ball.html). ROI were outlined for each individual cell, nucleus only (as defined by NLS-CFP staining), and cytoplasm only (as defined by staining outside NLS-CFP signal). The average intensity for each ROI was measured and at least 50 cells were counted for each biological replicate. The arrays of intensity counts for each biological replicate were subjected to further analysis separately and were analyzed via both “D’Agostino & Pearson” and “Shapiro-Wilk” normality tests to evaluate distribution. Because not all samples were normally distributed, the median value was used for each biological replicate. To determine the nuclear:cytoplasmic ratio (N:C) the value for nucleus was divided by the value for cytoplasmic for each cell individually such that an N:C ratio of 1 indicates equal protein-protein interaction in both the nucleus and cytoplasm.

**Transient knockdown of RuvBL1 and Tip60.** To confirm that the mechanism we describe here is independent of PRMT5-mediated regulation of RuvBL1 and Tip60, we performed similar assays with knockdown of RuvBL1 or Tip60. First, we obtained MISSION® shRNA bacterial glycerol stocks containing shRNA expression plasmids (RuvBL1: TRCN0000018911, TRCN0000018912, TRCN0000018913, TRCN0000018914, and TRCN0000319216. Tip60: TRCN0000020314, TRCN0000020315, TRCN0000020317, TRCN0000020318, and TRCN0000298504) (Sigma-Aldrich/Millipore Sigma, St. Louis, Missouri, USA). Using maxiprep, we isolated the shRNA expression plasmids and generated viral particles in HEK293T cells as described previously (Hsu and Hu, 2013) via co-transfection of all 5 shRuvBL1 or all 5 shTip60 expression plasmids along with pCMV-HA-CFP as a control. Although we could have used transient transfection of individual shRNA expression plasmids, we used viral particle transduction with all 5 shRNA expression vectors at once to ensure sufficient knockdown. FuGENE HD (Promega, Madison, Wisconsin, USA) was used as the transfection reagent, pHR'-CMV-8.2ΔVPR was used as the packaging plasmid, and pHR'-CMV-VSV-G was used as the envelope plasmid. Forty-eight hours following transfection, media from the HEK293T cells was collected, passed through a 0.45 μm filter, and applied to the LNCaP cells for viral particle transduction. Viral particles were applied to the LNCaP cells both 24 hours and 72 hours after plating.

LNCaP cells were transduced with either shRuvBL1 or shTip60 viral particles once and again after 48 h to establish RuvBL1 or Tip60 knockdown. Cells were then treated with 2 Gy IR and subjected to γH2AX-foci analysis and western blot analysis described above.

**Correlation analysis of TCGA clinical cancer data sets**. Gene expression profiles of 32 clinical cancer data sets from TCGA Pan-Cancer analysis (The Cancer Genome Atlas Research Network et al., 2013) were retrieved from cBioPortal (Cerami et al., 2012; Gao et al., 2013). Using the mRNA expression of PRMT5, pICln, MEP50, AR, and DDR genes which are primary target genes of both PRMT5 and AR, we calculated the Spearman correlations between gene pairs for each cancer type. The gene set for DDR genes was defined as RAD51, RAD51D, RAD51AP1, NHEJ1, BRCA1, BRCA2, WEE1, DNAPKcs, Ku70, Ku80, an XRCC4. Although we did not perform additional studies on Ku70, Ku70 was included as it is another well-studied, key regulator of NHEJ. In **Figure 7D**, a cutoff of $p < 0.01$ was used to determine the significance of correlation between PRMT5 and AR as either positive, negative, or no correlation if $p > 0.01$, in order to stratify the cancers into the different types.

**Statistical analysis.** No statistical methods were used to predetermine sample size. For the correlation analysis of TCGA clinical cancer data sets, statistical analysis was performed using Wilcoxon rank sum test in R 3.5.3. (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/). All other statistical analyses were performed using Graphpad Prism 7.00 and 8.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). Statistical analysis for comet assay and RNA-seq analysis are described above. For all other experiments, statistical analysis was performed on raw data with assumed normal distribution. For all qPCR experiments, statistical analysis was performed on $\Delta C_T$ values ($C_T$ value of gene normalized to $C_T$ value of GAPDH control). For all ChIP-qPCR experiments, statistical analysis was performed on $\Delta C_T$ values ($C_T$ value of gene normalized to $C_T$ value of IgG control). For all western blot experiments, statistical analysis was performed on normalized raw intensity values (intensity value of protein divided by the intensity value of β-Actin). When comparing two sample groups, we used unpaired, two-tailed *t*-tests with Welch's correction (Welch's *t*-test) because standard deviations were not always equal for all groups. When comparing multiple sample groups, in order to compare the means or medians among all the samples and incorporate the standard deviation of each of the samples, Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test was used. For **Figures 1A**, **1B**, **1D**, and **1E**, as the variance in the mean among samples were small and the dose-response occurred on a log scale, statistical analysis was performed using Welch's *t*-test of log-transformed data. All relevant statistics are reported in the corresponding legends.

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# Protein Arginine Methyltransferase 5 Promotes pICln-Dependent Androgen Receptor Transcription in Castration-Resistant Prostate Cancer

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## ABSTRACT

The majority of advanced prostate cancer therapies aim to inhibit androgen receptor (AR) signaling. However, AR reactivation inevitably drives disease progression to castration-resistant prostate cancer (CRPC). Here we demonstrate that protein arginine methyltransferase 5 (PRMT5) functions as an epigenetic activator of *AR* transcription in CRPC, requiring cooperation with a methylosome subunit pICln. *In vitro* and in xenograft tumors in mice, targeting PRMT5 or pICln suppressed growth of CRPC cells. Full-length AR and AR-V7 transcription activation required both PRMT5 and pICln but not MEP50. This activation of transcription was accompanied by PRMT5-mediated symmetric dimethylation of H4R3 at the proximal *AR* promoter. Further, knockdown of PRMT5 abolished the binding of pICln (but not vice versa) to the *AR* proximal promoter region, suggesting that PRMT5 recruits pICln to the *AR* promoter to activate *AR* transcription. Differential gene expression analysis in 22Rv1 cells confirmed that PRMT5 and pICln both regulate the androgen signaling pathway. In addition, PRMT5 and pICln protein expression positively correlated with AR and AR-V7 protein expression in CRPC tissues and their expression was highly correlated at the mRNA level across multiple publicly available CRPC datasets. Our results suggest that targeting PRMT5 or pICln may be explored as a novel therapy for CRPC treatment by suppressing expression of AR and AR splice variants to circumvent AR reactivation.

**Significance:** This study provides evidence that targeting PRMT5 can eliminate expression of AR and can be explored as a novel therapeutic approach to treat metastatic hormone-naïve and castration-resistant prostate cancer.

## Introduction

Prostate cancer remains the second leading cause of cancer death in American men (1). The primary cause of prostate cancer mortality is the development of metastasis (2). Currently, androgen deprivation therapy (ADT), in combination with either docetaxel or abiraterone acetate, is the first-line treatment for metastatic prostate cancer (3). Because the growth of prostate cancer cells is dependent on androgen receptor (AR) signaling, suppressing AR signaling via ADT inhibits tumor growth. Despite initial positive response in the majority of patients, ADT eventually fails, leading to the development of castration-resistant prostate cancer (CRPC; ref. 4).

AR reactivation drives CRPC progression and occurs via multiple mechanisms (*AR* gene amplification, expression of ligand-independent splice variants, or mutations of AR, and others; ref. 4). For example, AR splice variant 7 (AR-V7) presents in 18% to 28% of CRPC tissues (5). AR-V7 expression correlates with poor patients' prognosis (5). Because AR-V7 lacks the ligand-binding domain, it is constitutively active and can regulate transcription of AR target genes despite castrate levels of androgens (6). Inhibitors that target AR signaling, such as enzalutamide, demonstrate poor outcome towards CRPC that express AR-V7. Moreover, targeting full-length AR (AR-FL) can increase AR-V7 expression, exacerbating the condition (6). Thus, there is an urgent need to develop therapeutic approaches to overcoming AR reactivation. Because we have recently shown that protein arginine methyltransferase 5 (PRMT5) activates AR transcription in hormone-naïve prostate cancer (HNPC; ref. 7), we investigated whether PRMT5 also regulates the transcription of AR and AR variants in CRPC.

PRMT5 is a methyltransferase, which symmetrically dimethylates arginine residues in histones (H4R3, H3R8, H3R2, and H2AR3) to regulate transcription of target genes (8–11). Although PRMT5 is generally considered an epigenetic repressor (8–11), PRMT5 also functions as an epigenetic activator (7, 12, 13). Although *in vitro* studies suggest that PRMT5 interacting proteins methylosome protein

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50 (MEP50) and methylosome subunit pICln enhance PRMT5 enzymatic activity (14, 15), how these proteins cooperate with PRMT5 to regulate gene transcription *in vivo* remains unknown. MEP50 functions as a critical PRMT5 cofactor facilitating substrate recognition and positioning via interaction with the N-terminal region of PRMT5 to form a heterooctameric complex (14, 16). Because both pICln and MEP50 can enhance PRMT5 activity towards SmD3 protein *in vitro* (17, 18), pICln may interact with PRMT5 similarly as MEP50 does to activate PRMT5 methyltransferase activity or alter substrate specificity. Indeed, we recently demonstrated that pICln, but not MEP50, cooperates with PRMT5 to activate transcription of DNA damage response genes (12). Here we provide evidence that PRMT5 promotes the growth of CRPC cells via epigenetic activation of transcription of both AR-FL and AR-V7 in a pICln-dependent, but MEP50-independent, manner. Results from our *in vitro* and *in vivo* studies suggest that targeting PRMT5 may present a promising approach for CRPC treatment.

## Materials and Methods

### Cell lines and reagents

LNCaP, 22Rv1, VCaP, COS-1, and 293T cells were purchased from ATCC. LN95 cells were a kind gift from Dr. Jun Luo of Johns Hopkins University. Frozen cultures were recovered and expanded in complete media: LNCaP and 22Rv1 in RPMI1640 (Corning), LN95 was cultured in RPMI1640 without phenol red (Corning), 293T, COS-1, and VCaP in DMEM (Corning) supplemented with 10% FBS (Atlanta Biologicals) or for LN95 charcoal-stripped FBS (Corning), 2 mmol/L L-glutamine (Corning), and 100 units/mL penicillin and 100 μg/mL streptomycin (Gibco). Cells were not passaged more than 30 times. Long-term storage, cell authentication and *Mycoplasma* contamination check were described previously (12). Methocel A4M was purchased from Sigma, abiraterone acetate and enzalutamide were purchased from MedChemExpress.

### Bimolecular fluorescence complementation

Bimolecular fluorescence complementation (BiFC) plasmids (250 ng each) encoding a protein of interest fused to the N- or C-terminal fragment of the Venus fluorescent protein (VN155 or VC155) and 100 ng of the plasmid encoding the Cerulean fluorescent protein (CFP, as a positive control for transfection) were cotransfected into COS-1 cells and BiFC efficiency (YFP/CFP) was analyzed essentially as described previously (19). For BiFC competition assay, 500 ng of the plasmid encoding a PRMT5 interacting protein (MEP50 or pICln) or empty vector control were cotransfected to analyze the inhibition of PRMT5:MEP50 interaction. Results are presented as median $\pm$ SD from three independent biological replicates.

### Xenograft tumor growth

Animal experiments were performed in the Biological Evaluation Facility of the Purdue University Center for Cancer Research approved by the Purdue University Animal Care and Use Committee. Six to eight weeks old male nonobese diabetic-Rag1(null)-γ chain(null) (NRG) mice were castrated, and 14 days later $2 \times 10^5$ cells of 22Rv1-shPRMT5, or 22Rv1-shpICln, or 22Rv1-shSC in 100 μL of RPMI1640 media were mixed with 100 μL of Matrigel (200 μL total) and injected subcutaneously into the right lower flank (10 mice/group). After tumor volumes reached $\sim$100 mm$^3$, mice were treated with Dox (1 mg/mL in drinking water) to induce the expression of shRNA or treated 5 days/week with ASI in 0.5% Methocel orally (abiraterone acetate 200 mg/kg/day, enzalutamide 25 mg/kg/day), vehicle, or in combination. Tumor growth was measured every 2 to 3 days, and tumor volume was calculated using $^1/_2 \times L \times W \times H$ without blinding method. When control tumors reached nearly 2,000 mm$^3$, tumors were resected for IHC analysis.

### Clinical data analysis

Gene expression profiles of 34 PC data sets were obtained from Gene Expression Omnibus (GEO; ref. 20), cBioportal (21, 22), and Oncomine (Supplementary Table S1; ref. 23) with total of 4624 samples. Gene expression levels were $\log_2$ transformed and median centered. Gene expression profiles from cBioportal were downloaded with annotation of “mRNA_median_Zscores.” If one gene had multiple gene expression files in the same dataset, the sum of all corresponding mRNA levels was used. Spearman's rank correlation coefficients were calculated to evaluate the correlations of specific gene pairs. Wilcoxon rank sum test was used to compare the differences between groups for all 34 datasets.

The clinical information and gene expression data for PC (24) were downloaded from cBioPortal (21, 22) for the survival analysis. Patients were divided into two groups based on the top and bottom 50% quantile of expression levels for selected genes. Survival probability was computed in R using the survfit function in the R package survival. Kaplan–Meier plots were generated using the ggsurvplot function of package survminer.

Construction of CRPC tissue microarray (TMA) containing samples from 20 patients and HNPC TMA containing samples from 72 patients (32 with BPH, 20 with prostate cancer Gleason score 6 and 20 with prostate cancer Gleason score ≥7) was described previously (7, 25).

### Additional methods

Stable cell line generation, cell proliferation assay, cell-cycle analysis, chromatin immunoprecipitation (ChIP)-qPCR, reverse transcription and qPCR, IHC staining and scoring, RNA sequencing (RNA-seq) analysis (deposited in GEO, accession number GSE154951), Western blot, and statistical analysis were performed as described previously (7, 12). Detailed procedures for these methods are provided in Supplementary Materials and Methods.

## Results

### PRMT5 promotes growth of CRPC cells via epigenetic activation of AR expression

To determine the role of PRMT5 in CRPC, we analyzed effect of PRMT5 inhibition on the growth of CRPC cell line 22Rv1, which expresses both AR-FL and AR-V7. Treatment of 22Rv1 cells with our PRMT5 inhibitor BLL3.3, also called CMP5 (7, 26), reduced cell proliferation compared with DMSO control (**Fig. 1A**) and downregulated AR-FL and AR-V7 at protein and mRNA levels (**Fig. 1B–D**). Another PRMT5 inhibitor JNJ-64619178 (JNJ), which is currently in phase I clinical trial for non-Hodgkin lymphoma and solid tumors (27), similarly reduced cell growth and downregulated AR-FL and AR-V7 expression (**Fig. 1E–H**). To corroborate these findings, we used lentivirus-based shRNA constructs (two separate shRNA constructs per gene) to establish doxycycline (Dox)-inducible PRMT5 knockdown cell lines in 22Rv1 (22Rv1-shPRMT5 and 22Rv1-shPRMT5#2). PRMT5 knockdown inhibited cell growth (**Fig. 1I**; Supplementary Fig. S1A) and decreased expression of both AR-FL and AR-V7 (**Fig. 1J–L**; Supplementary Figs. S1B–S1D) whereas expression of scramble control (22Rv1-shSC) did not affect cell growth or protein expression (**Fig. 1M–P**). Moreover, expression of several AR target



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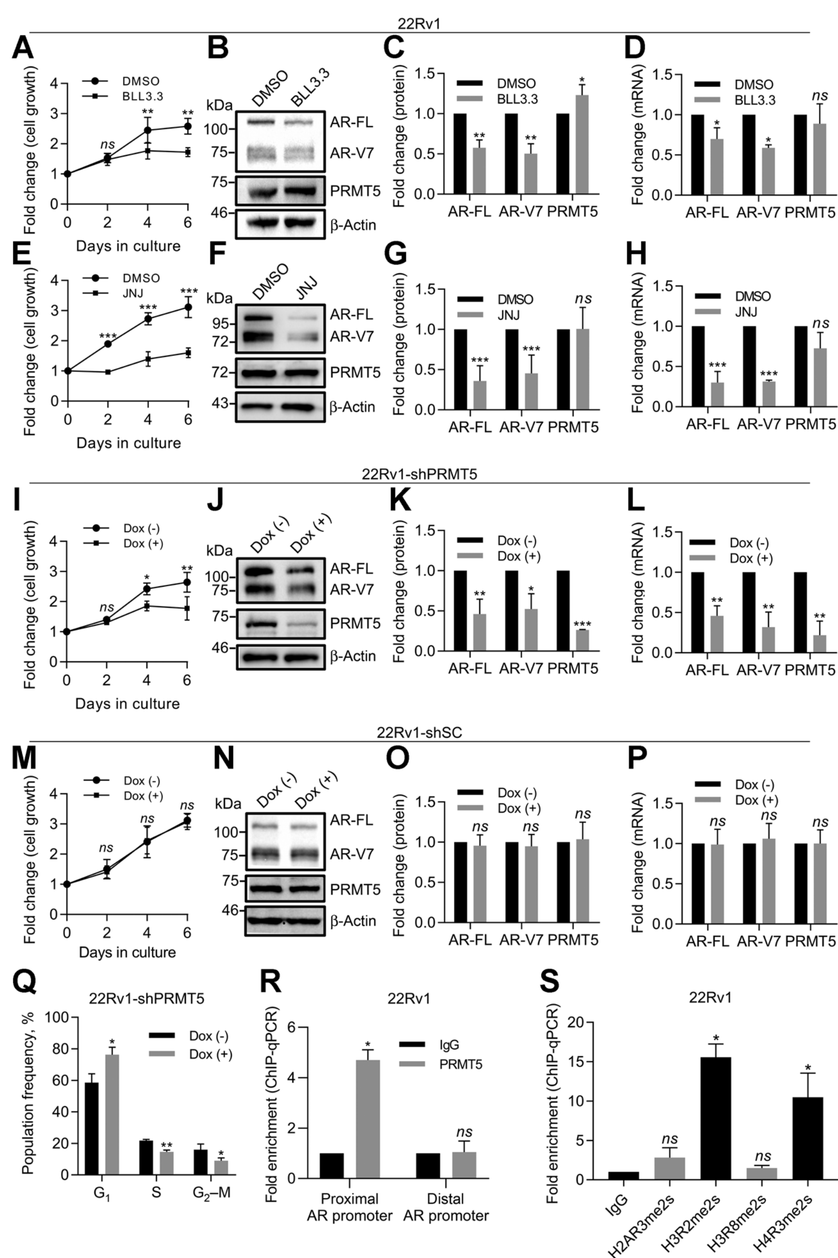


**Figure 1.**

PRMT5 promotes growth of CRPC cells via epigenetic activation of AR expression. **A,** Growth curve (MTT assay) of 22Rv1 cells incubated with 10 μmol/L PRMT5 inhibitor (BLL3.3) or equal volume of vehicle (DMSO) for 6 days. **B** and **C,** Representative Western blot images (**B**) and quantification (**C**) of protein expression in cell lysates from Day 6 of **A**. **D,** qPCR analysis of gene expression in cells from Day 6 of **A**. **E,** Growth curve (MTT assay) of 22Rv1 cells incubated with 10 μmol/L PRMT5 inhibitor (JNJ-64619178, referred to as JNJ) or equal volume of vehicle (DMSO) for 6 days. **F** and **G,** Representative Western blot images (**F**) and quantification (**G**) of protein expression in cell lysates from Day 6 of **E**. **H,** qPCR analysis of gene expression in cells from Day 6 of **E**. **I,** Growth curve (MTT assay) of 22Rv1 cells with doxycycline-inducible PRMT5 knockdown (22Rv1-shPRMT5) incubated in the presence [Dox (+)] or absence [Dox (−)] of doxycycline for 6 days. **J** and **K,** Representative Western blot images (**J**) and quantification (**K**) of protein expression in cell lysates from Day 6 of **I**. **L,** qPCR analysis of gene expression in cells from Day 6 of **I**. **M,** Growth curve (MTT assay) of 22Rv1 cells with doxycycline-inducible scramble control expression (22Rv1-shSC) incubated in the presence [Dox (+)] or absence [Dox (−)] of doxycycline for 6 days. **N** and **O,** Representative Western blot images (**N**) and quantification (**O**) of protein expression in cell lysates from Day 6 of **M**. **P,** qPCR analysis of gene expression in cells from Day 6 of **M**. **Q,** Flow cytometry analysis of cells following propidium iodide staining at Day 6 of **I** (sub-$G_1$ cells were gated out). **R,** ChIP-qPCR for PRMT5 binding to the proximal or distal AR promoter. **S,** ChIP-qPCR for the enrichment of the indicated histone methylations on the proximal promote region of AR. For MTT, Western blotting, cell cycle, and qPCR analysis, statistical significance of group difference was determined for "DMSO vs. BLL3.3," "DMSO vs. JNJ," or "Dox (−) vs. Dox (+)." For ChIP-qPCR, values were normalized to the corresponding IgG control, and indicated statistical significance of group difference was determined for "specific IP vs. IgG IP." For all experiments, results are mean ± SD from three independent experiments. For Western blotting of AR, the AR N-20 antibody (sc-816; Santa Cruz Biotechnology) was used. Student *t* test with Welch's correction was performed to determine statistical significance of group difference. ns, nonsignificant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



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genes (28) was suppressed upon PRMT5 knockdown (Supplementary Fig. S1E), consistent with the decreased AR expression. These results suggest that PRMT5 regulates cell growth, AR expression, and AR signaling in 22Rv1.

Because AR reactivation occurs via several mechanisms, we next determined whether PRMT5 regulates AR expression in other CRPC models. We reported previously that PRMT5 targeting downregulates AR expression and inhibits growth in C4–2 cells, which model CRPC via AR overexpression (7). We further evaluated the effect of PRMT5 inhibition in VCaP cells, which bear AR gene amplification (29), and LN95 cells, which express AR splice variants (30), and observed that both PRMT5 inhibitors BLL3.3 and JNJ suppressed cell growth and downregulated AR expression (Supplementary Figs. S1F–S1M). Cell-cycle analysis confirmed that PRMT5 knockdown caused $G_1$ arrest in 22Rv1 (**Fig. 1Q**), consistent with previous observations (31, 32). No significant induction of cell death was observed (Supplementary Fig. S1N). Collectively, our results suggest that PRMT5 promotes cell growth via activation of *AR* transcription in CRPC cells with AR overexpression, *AR* gene amplification, or expression of AR splice variants.

To investigate whether PRMT5 regulates AR expression via histone methylation at the *AR* promoter, we performed ChIP assays in 22Rv1 using PRMT5-specific antibody. PRMT5 bound to the proximal region of the promoter [−493 to −226 bp from transcription start site (TSS)] but not to the distal region (−4481 to −4308 bp from TSS; **Fig. 1R**). ChIP-qPCR analysis revealed that H4R3me2s and H3R2me2s were highly enriched at the *AR* proximal promoter (**Fig. 1S**). Further, PRMT5 knockdown decreased these enrichments and PRMT5 binding, confirming that PRMT5 methylates H4R3 and H3R2 at the *AR* promoter (Supplementary Fig. S1O). As observed in LNCaP cells (7), Brg1 and Sp1 also bound to the same region (Supplementary Fig. S1P). Taken together, our findings demonstrate that PRMT5 activates AR transcription in CRPC cells by binding to the proximal region of the *AR* promoter to methylate histones H4 and H3 in a similar manner as in HNPC cells (7).

### MEP50 is not required for PRMT5-mediated activation of *AR* transcription in CRPC cells

MEP50 is considered a canonical cofactor of PRMT5 (8–11). We next established Dox-inducible MEP50 knockdown stable cell lines in 22Rv1 (22Rv1-shMEP50 and 22Rv1-shMEP50#2) to examine the effect of MEP50 knockdown on AR expression. Unexpectedly, MEP50 knockdown affected neither AR-FL/AR-V7 expression (**Fig. 2A–C**; Supplementary Figs. S2A–S2C) nor the expression of AR target genes (Supplementary Fig. S2D). However, MEP50 knockdown de-repressed expression of involucrin (*IVL*) mRNA (**Fig. 2C**), confirming that PRMT5/MEP50 represses *IVL* transcription (33). We also confirmed that MEP50 knockdown had no effect on AR expression in LNCaP cells (**Fig. 2D–F**). ChIP-qPCR analysis revealed that MEP50 did not bind to the *AR* proximal promoter in these cell lines (**Fig. 2G** and **H**), although MEP50 antibody efficiently immunoprecipitated MEP50 (Supplementary Fig. S2E) and MEP50 bound to the *IVL* promoter (**Fig. 2G** and **H**). In addition, MEP50 knockdown in 22Rv1 did not significantly change H4R3me2s and H3R2me2s levels at *AR* proximal promoter (Supplementary Fig. S2F). Notably, MEP50 knockdown decreased the total cellular level of H3R2me2s but did not significantly affect the total level of H4R3me2s (Supplementary Fig. S2G). Contrary to the lack of cell death following PRMT5 knockdown (Supplementary Fig. S1I), MEP50 knockdown induced both cell death (**Fig. 2I**), and $G_1$ cell-cycle arrest (**Fig. 2J**) in 22Rv1 cells, indicating that PRMT5 and MEP50 might have distinct roles in cell proliferation. Taken together, MEP50 does not appear to participate in the regulation of *AR* transcription by PRMT5.

### pICln participates in epigenetic activation of *AR* transcription

The surprising finding that MEP50 was not involved in *AR* transcription regulation in HNPC and CRPC cells prompted us to search for PRMT5-interacting proteins that might cooperate with PRMT5 to regulate *AR* transcription (17, 34, 35). First, we found that only pICln, but not RIOK1 and COPR5, bound to the same *AR* proximal promoter region as PRMT5 did (**Fig. 3A**). Next, we established Dox-inducible pICln knockdown cell lines in 22Rv1 and LNCaP cells (22Rv1-shpICln and LNCaP-shpICln) to further interrogate a role of pICln in AR regulation. Indeed, pICln knockdown significantly suppressed cell proliferation (**Fig. 3B** and **C**), inhibited AR expression at both protein and mRNA levels in 22Rv1 (**Fig. 3D–F**) and LNCaP (**Fig. 3G–I**), and decreased its binding and the H4R3me2s level at the *AR* promoter (**Fig. 3J**). However, pICln knockdown did not affect PRMT5 binding nor the H3R2me2s level (**Fig. 3J**). Consistently, at the total cellular level, pICln knockdown decreased H4R3me2s but did not affect H3R2me2s (Supplementary Fig. S3A). In contrast, PRMT5 knockdown decreased pICln binding to the *AR* promoter (**Fig. 3K**). These results suggest that PRMT5 recruits pICln to the *AR* promoter, and PRMT5/pICln interaction is required for H4R3 methylation at the *AR* promoter. Contrary to PRMT5 knockdown, pICln knockdown induced cell death (**Fig. 3L**) and $G_2$ cell-cycle arrest in 22Rv1 cells (**Fig. 3M**). Thus, pICln has additional roles in cell proliferation and survival independently of PRMT5. Nonetheless, our results demonstrate that pICln is required for PRMT5-mediated H4R3 methylation to activate *AR* transcription.

To investigate whether pICln binds to the N-terminal region of PRMT5 as MEP50 does (14, 16), we utilized the BiFC assay (19). Coexpression of PRMT5(NT292)-VN155 and VC155-pICln in COS-1 cells resulted in YFP fluorescence, indicating that pICln interacted with N-terminal fragment of PRMT5 (Supplementary Fig. S3B). To determine whether pICln might bind to a similar site in PRMT5 as MEP50 does, we performed the BiFC competition assay, in which VN155-PRMT5 and VC155-MEP50 were coexpressed with MEP50 or pICln. Indeed, overexpression of MEP50 or pICln similarly decreased BiFC efficiency of the PRMT5-MEP50 BiFC interaction (Supplementary Figs. S3C–S3E), suggesting that pICln may indeed function as a cofactor by binding to the N-terminus of PRMT5 like MEP50 (16).

We next aimed to check whether the antiproliferative effect of PRMT5 or pICln knockdown is mediated through the regulation of AR expression. For this purpose, we performed AR rescue assays. We transfected 22Rv1-shPRMT5, 22Rv1-shpICln, and 22Rv1-shSC cells with the plasmid encoding FLAG-AR expression or the empty vector control. Remarkably, exogenously expressed AR completely restored cell proliferation in 22Rv1-shpICln and 22Rv1-shPRMT5 cells but did not affect cell proliferation in 22Rv1-shSC (Supplementary Figs. S4A–S4D) as observed previously in LNCaP-shPRMT5 cells (7). This observation suggests that the inhibition of 22Rv1 cell proliferation upon PRMT5 or pICln knockdown is primarily mediated through downregulation of AR expression.

### PRMT5 and pICln regulate the AR signaling independently of MEP50

The above results suggest distinct regulatory roles of PRMT5, MEP50, and pICln in cell proliferation, cell cycle progression, and cell death. To further understand their roles in genome-wide gene regulation, we performed RNA-seq of 22Rv1 cells with and without knockdown of PRMT5, MEP50, or pICln. We identified 6,730 out of



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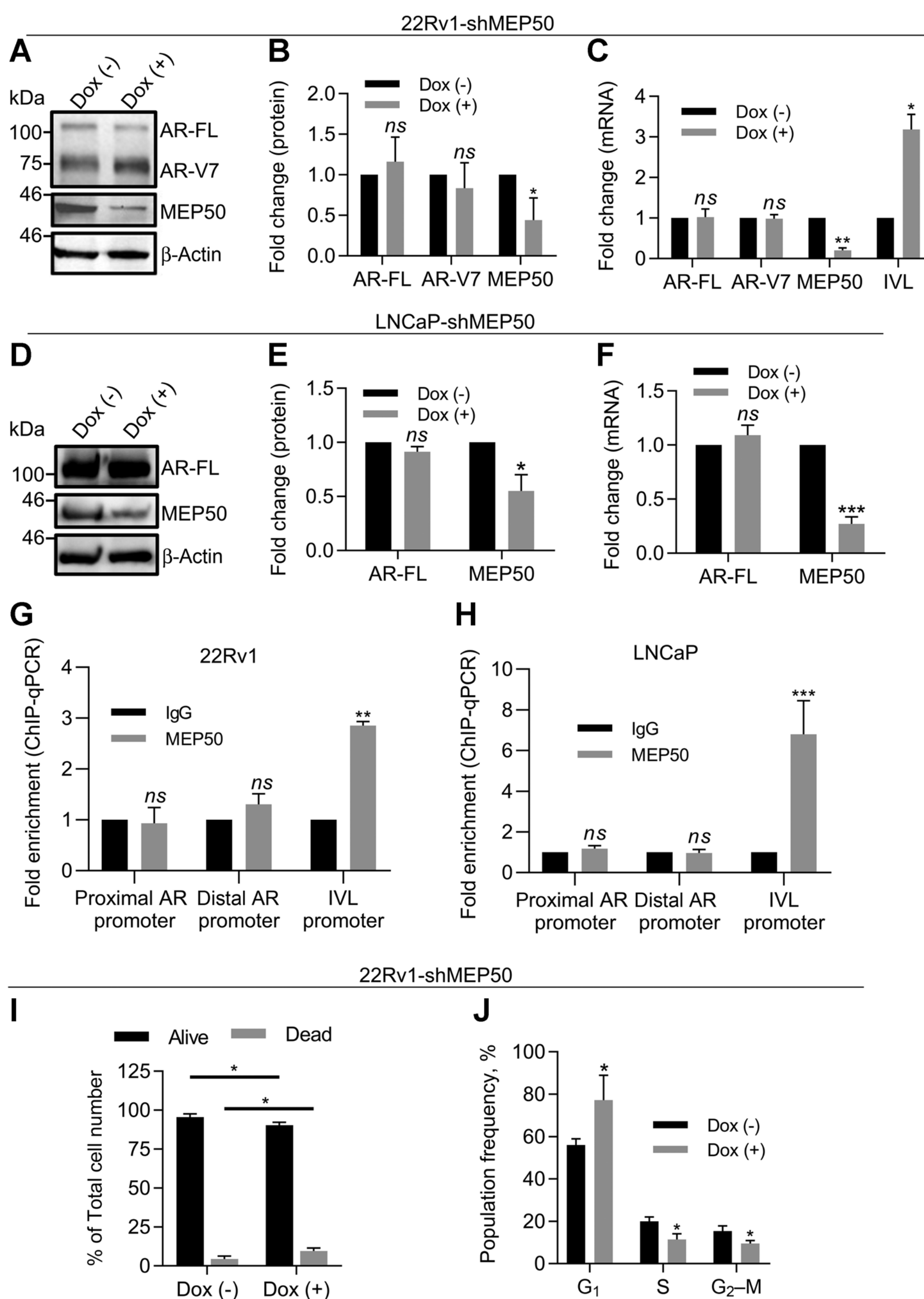


**Figure 2.**

MEP50 is not required for PRMT5-mediated activation of *AR* transcription in CRPC cells. **A** and **B,** Representative Western blot images (**A**) and quantification (**B**) of protein expression in cell lysates of 22Rv1 cells with doxycycline-inducible MEP50 knockdown (22Rv1-shMEP50) incubated in the presence [Dox (+)] or absence [Dox (−)] of doxycycline for 6 days. **C,** qPCR analysis of gene expression in cells from **A**. **D** and **E,** Representative Western blot images (**D**) and quantification (**E**) of protein expression in cell lysates of LNCaP cells with doxycycline-inducible MEP50 knockdown (LNCaP-shMEP50) incubated in the presence [Dox (+)] or absence [Dox (−)] of doxycycline for 6 days. **F,** qPCR analysis of gene expression in cells from **D**. **G** and **H,** ChIP-qPCR assay of MEP50 binding to the proximal *AR* promoter or control gene *IVL* promoter was performed with nonspecific IgG binding as a control in 22Rv1 (**G**) and LNCaP (**H**) cells. **I,** Trypan blue cell viability analysis in 22Rv1-shMEP50 cells after 6 days of MEP50 knockdown. **J,** Flow cytometry analysis of cells following PI staining at Day 6 of MEP50 knockdown in 22Rv1-shMEP50 (sub-$G_1$ cells were gated out). For Western blotting, cell cycle, cell viability, and qPCR analysis, statistical significance of group difference was determined for "Dox (−) vs. Dox (+)." For ChIP-qPCR, values were normalized to the corresponding IgG control, and indicated statistical significance of group difference was determined for "specific IP vs. IgG IP." Results are mean ± SD from three independent experiments. For Western blotting of AR, the AR N-20 antibody (sc-816; Santa Cruz Biotechnology) was used. Student *t* test with Welch's correction was performed to determine statistical significance. ns, nonsignificant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

23,334 genes, which had at least one differentially expressed transcript (DET) upon PRMT5 knockdown, including 3,426 genes with upregulated transcripts and 3,304 genes with downregulated ones (**Fig. 4A**). Following MEP50 knockdown, 447 upregulated and 626 downregulated differentially expressed genes (DEG) overlapped with the PRMT5-knockdown DEGs (**Fig. 4A**). Notably, pICln knockdown led to more overlapped genes with the PRMT5-knockdown DEGs, including 1,033 upregulated and 1,361 downregulated genes (**Fig. 4A**). To confirm the regulation of the AR signaling by PRMT5 and pICln, we analyzed the enrichment of different sets of DEGs involved in AR signaling pathway, Gene Ontology GO:0030521. Consistently, genes of this pathway were significantly overrepresented among PRMT5- and pICln-knockdown DEGs but not among MEP50-knockdown DEGs (**Fig. 4B**). Compared with fold changes (in log scale with base 2) of selected AR signaling pathway DEGs identified by mRNA-seq (**Fig. 4C**, left), qPCR analysis confirmed that PRMT5 and pICln, but not MEP50, similarly regulate the expression of these genes (**Fig. 4C**, right). These results suggest that PRMT5 and pICln coregulate AR signaling in a MEP50-independent way.

The GO enrichment analysis explored many GO terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways significantly enriched in DEGs downregulated after the knockdown of PRMT5, pICln, or MEP50 (**Fig. 4D**). For example, GO:0051301 "cell division," GO:0007049 "cell cycle," and GO:0000086 "$G_2$–M transition of mitotic cell cycle" were shared by repressed genes after PRMT5/pICln/MEP50 knockdown. GO functions associated with $G_1$–S phase regulation were notably overrepresented in DEGs downregulated by PRMT5- or MEP50-knockdown, but not in the



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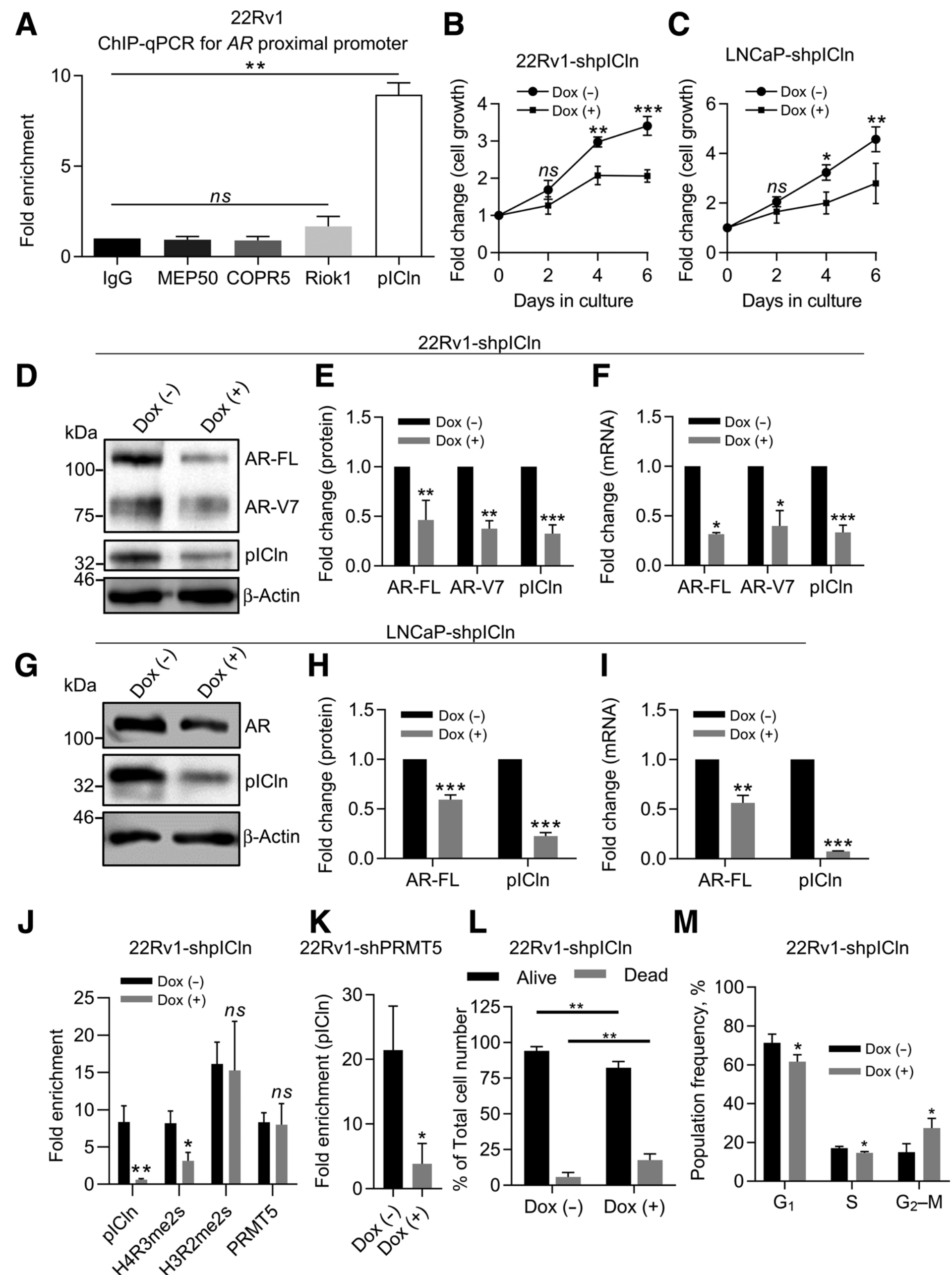


**Figure 3.**

pICln participates in epigenetic activation of AR transcription. **A,** ChIP-qPCR assay for binding of PRMT5-interacting proteins to the proximal *AR* promoter in 22Rv1 cells. Values were normalized to IgG control. **B** and **C,** Growth curve (MTT assay) of 22Rv1 (**B**) or LNCaP (**C**) cells with Dox-inducible pICln knockdown (shpICln). **D** and **E,** Representative Western blot images (**D**) and quantification (**E**) of protein expression in 22Rv1 after 6 days of pICln knockdown. **F,** qPCR analysis of gene expression in 22Rv1 after 6 days of pICln knockdown. **G** and **H,** Representative Western blot images (**G**) and quantification (**H**) of protein expression in LNCaP after 5 days of pICln knockdown. **I,** qPCR analysis of gene expression in LNCaP after 5 days of pICln knockdown. **J,** ChIP-qPCR assay for pICln and H4R3me2s presence at the proximal *AR* promoter in 22Rv1 upon pICln knockdown. **K,** ChIP-qPCR assay for pICln presence at the proximal *AR* promoter upon PRMT5 knockdown. **L,** Trypan blue cell viability analysis in 22Rv1-shpICln cells after 6 days of pICln knockdown. **M,** Flow cytometry analysis of fixed and stained with propidium iodide 22Rv1-shpICln cells after 6 days of pICln knockdown. For MTT, Western blotting, cell cycle, and qPCR analysis statistical significance of group difference was determined for "Dox (−) vs. Dox (+)." For ChIP-qPCR, values were normalized to the corresponding IgG control, and indicated statistical significance of group difference was determined for "specific IP vs. IgG IP" (**A**) or "Dox (−) vs. Dox (+)" (**J** and **K**). For all experiments, results are mean ± SD from three independent experiments. For Western blotting of AR, the AR N-20 antibody (sc-816; Santa Cruz Biotechnology) was used. Student *t* test with Welch's correction was performed to determine statistical significance. ns, nonsignificant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

pICln-knockdown group, for instance, GO:0000082 "$G_1$–S transition of mitotic cell cycle," GO:0000083 "regulation of transcription involved in $G_1$–S transition of mitotic cell cycle," and GO:0006270 "DNA replication" among others. This was consistent with our cell-cycle analysis results (**Figs. 1Q**, **2J**, and **3M**). However, we noticed that PRMT5- or MEP50-knockdown DEGs can be different, even though they were associated with the same GOs or KEGG pathways. But PRMT5 and pICln tended to mediate the same DEGs involved in some GOs and KEGG pathways, for example, GO:0006351 "transcription, DNA-templated" and GO:0006355 "regulation of transcription, DNA-templated" (**Fig. 4D**). Interestingly, pICln appears to have additional roles in regulating phosphatidylinositol signaling and NF-κB signaling in prostate cancer independently of PRMT5 (**Fig. 4D**). Taken together, our genome-wide gene expression analysis confirms the role of PRMT5/pICln in AR signaling in prostate cancer and reveals distinct regulatory roles of PRMT5, MEP50, and pICln in various cellular processes such as cell-cycle progression.

## PRMT5 and pICln expression positively correlates with AR in patients with CPRC

To investigate the clinical relevance of our findings, we examined the expression of AR, PRMT5, pICln, and MEP50 in HNPC and CRPC tissues. Nuclear PRMT5 and pICln expression was the highest in CRPC tissues with elevated AR expression (**Fig. 5A**), and nuclear PRMT5, pICln, and MEP50 expression correlates positively with AR expression (**Fig. 5B**). In general, correlation with AR expression was higher for PRMT5 and pICln compared with MEP50 (**Fig. 5B**;



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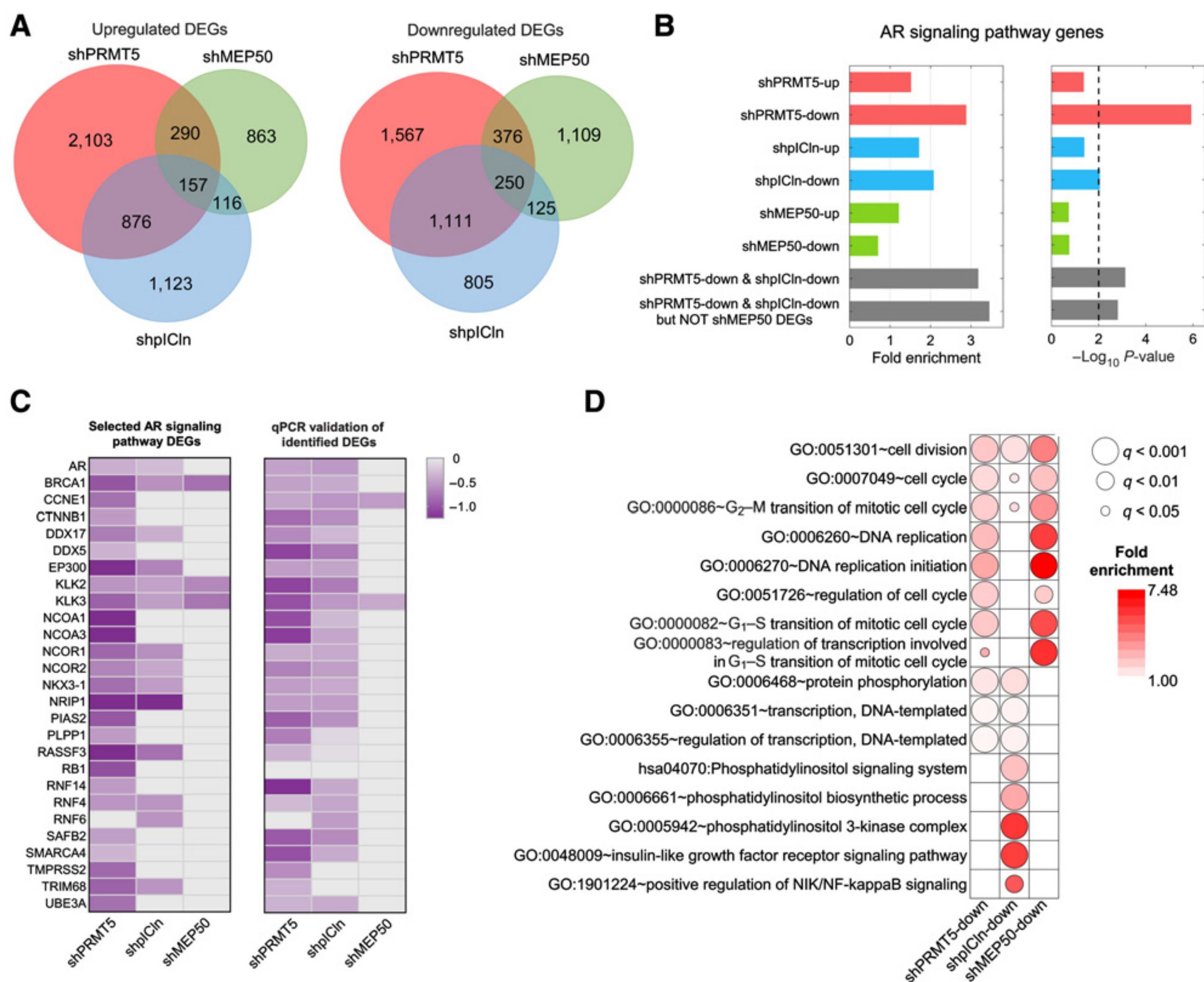

**Figure 4.**

PRMT5 and pICln regulate the AR signaling independently of MEP50. **A,** RNA-seq analysis of 22Rv1 cells upon 6 days of shRNA-mediated knockdown of PRMT5 (shPRMT5), MEP50 (shMEP50), or pICln (shpICln). Venn diagrams indicate overlap of upregulated and downregulated DEGs among three experiments. **B,** Presence of AR signaling pathway (GO:0030521) among identified upregulated and downregulated DEGs. **C,** Heatmap indicating expression fold change (FC, $\log_2$) of individual AR signaling pathway genes downregulated upon knockdown of PRMT5 (shPRMT5), MEP50 (shMEP50), and pICln (shpICln) from RNA-seq analysis (left) and qPCR validations (right). **D,** GO analysis of DEGs that were downregulated upon knockdown of PRMT5 (shPRMT5-down), pICln (shpICln-down), and MEP50 (shMEP50-down). Presented are selected GO terms significantly enriched in the DEG sets related to cell-cycle regulation, DNA replication, transcription, and phosphorylation. The color of each dot indicates the fold enrichment for the GO term, whereas the size of the dot indicates *q* value (FDR-corrected *P* value) of statistical significance of the enrichment.

Supplementary Figs. S5A–S5D). Notably, when samples were stratified by top and bottom 50% of AR staining ($AR^{high}$ and $AR^{low}$), the nuclear PRMT5/pICln expression was lower in $AR^{low}$ tissues compared with $AR^{high}$ (Supplementary Figs. S5E and S5F). However, PRMT5/MEP50 correlation was similar between $AR^{low}$ and $AR^{high}$ groups (Supplementary Figs. S5G–S5I). Consistent with the nuclear PRMT5/AR expression correlation in HNPC tissues (7), nuclear pICln expression also positively correlated with both nuclear PRMT5 and AR expression in these tissues (Supplementary Figs. S5J and S5K). These results further suggest that PRMT5 and pICln are strongly associated with higher AR expression.

Next, we retrieved 34 datasets from GEO and cBioportal including a total of 3,425 HNPC and 1,199 CRPC cases with mRNA expression profiles. PRMT5/AR and pICln/AR correlations were significantly higher than MEP50/AR correlation, confirming the role of PRMT5/pICln in AR signaling (**Fig. 5C**). Interestingly, comparable PRMT5/pICln and PRMT5/MEP50 correlations were observed, consistent with their distinct cellular roles. These results further support our finding that PRMT5 cooperates with pICln to activate transcription of AR in HNPC and CRPC tissues.

We further investigated the relationship of PRMT5 and pICln mRNA expression with patients' survival in CRPC (24). Notably, patients with high expression of either PRMT5 or pICln had lower survival (**Fig. 5D**). These findings support that expression levels of PRMT5 and pICln may affect patient outcomes or potential responses to therapy, indicating their role in cancer progression.

Next, we examined whether nuclear-localized PRMT5 promotes cell proliferation. Antonysamy and colleagues reported that PRMT5



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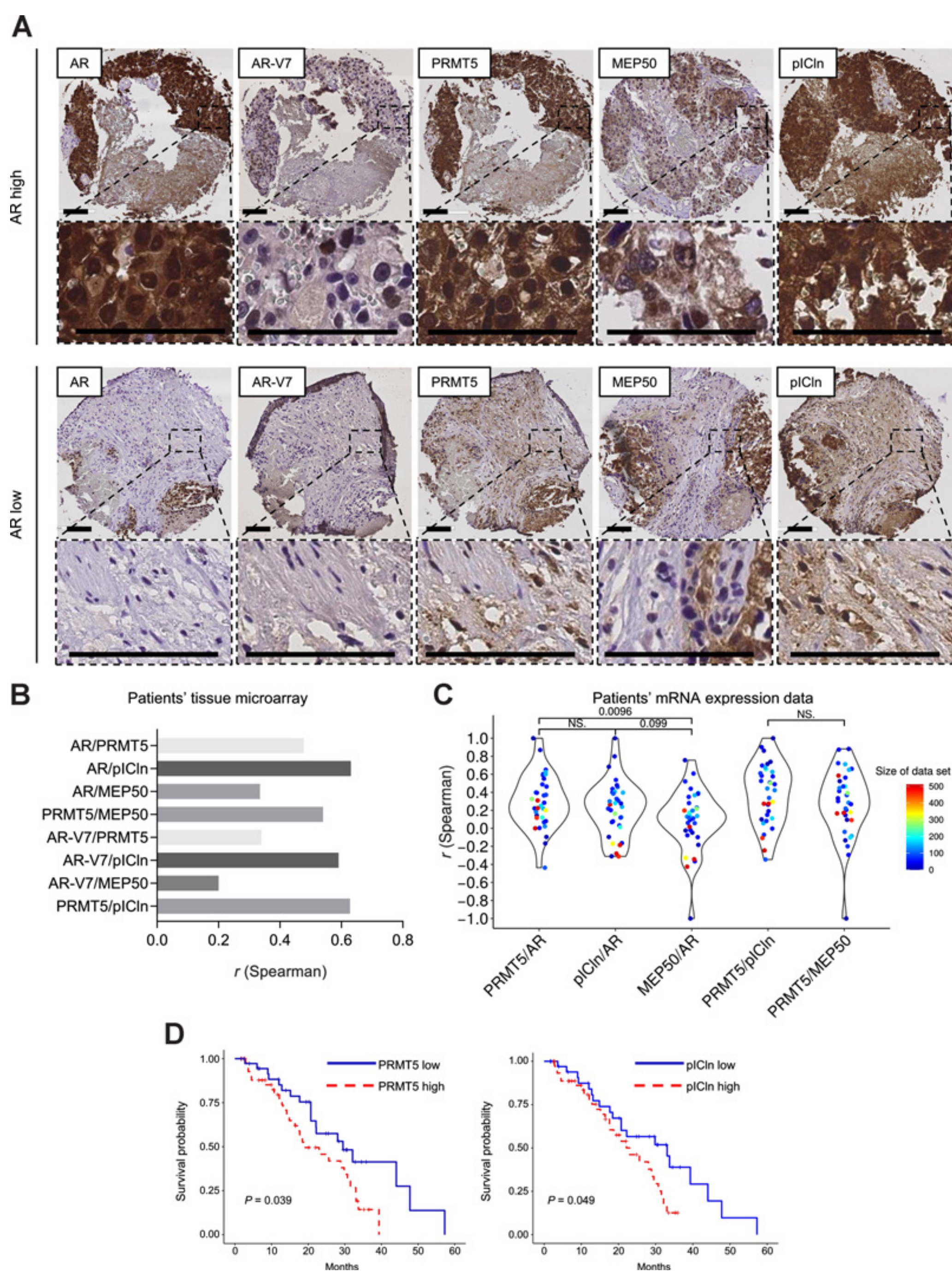


**Figure 5.**

PRMT5 and pICln expression positively correlates with AR in patients with CPRC. **A** and **B,** AR, AR-V7, PRMT5, pICln, and MEP50 protein expressions were analyzed by IHC in metastatic CRPC samples. **A,** Representative IHC images of AR, AR-V7, PRMT5, pICln, and MEP50 expression. Scale bar, 100 μm. **B,** Spearman correlations of protein-level expression of AR, AR-V7, PRMT5, pICln, and MEP50 in CRPC tissues. **C,** Spearman correlations of mRNA expression levels between AR, PRMT5, pICln, and MEP50. The mRNA expression data for 4,624 patient samples were obtained from 34 published datasets. Each dot denotes one dataset, representing the gene expression correlation between the pair of selected mRNAs. The dot color indicates the sample size of corresponding dataset. **D**, Kaplan–Meier curves comparing influences of mRNA expression levels of PRMT5 and pICln, respectively, on patients' survival. Red curves represent patients with high (top 50%) expression of PRMT5 and pICln, whereas blue ones are groups with low (bottom 50%) expression. The mRNA expression and patient survival data were downloaded from the cBioportal SU2C-PCF dataset.



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protein tended to aggregate in the absence of its cofactors (16). Thus, we reasoned that overexpression of PRMT5 alone may promote aggregate formation and decrease the cellular amount of active PRMT5, leading to reduced cell proliferation, which was previously observed in LNCaP cells by Gu and colleagues (36). In line with this, we performed overexpression of mutant shRNA-resistant PRMT5 fused with nuclear localization signal (NLS) or nuclear export signal (NES) or without localization signal in LNCaP or 22Rv1 cells on the background of PRMT5 knockdown. Consistent with a previous report by Gu and colleagues, NLS-PRMT5 decreased whereas NES-PRMT5 promoted cell proliferation in WT cells (Supplementary Figs. S6A and S6D). Conversely, NLS-PRMT5 promoted whereas NES-PRMT5 decreased cell proliferation in LNCaP-shPRMT5 or 22Rv1-shPRMT5 (Supplementary Figs. S6A and S6D). In cells with PRMT5 knockdown, NLS-PRMT5 promoted AR expression at both protein (Supplementary Figs. S6B and S6E) and mRNA level (Supplementary Figs. S6C and S6F). These observations further confirm that nuclear-localized PRMT5 promotes cell proliferation and AR expression in prostate cancer cells.

### Knockdown of PRMT5 or pICln suppresses CRPC tumor growth in mice

To determine whether targeting PRMT5 or its cofactor pICln can suppress the growth of CRPC tumors *in vivo*, we implanted 22Rv1-shPRMT5, 22Rv1-shpICln, and 22Rv1-shSC cells subcutaneously into male, precastrated NRG mice. When the average tumor volumes reached 100 $mm^3$, shRNA expression was induced by Dox treatment, and tumor growth was monitored. PRMT5 or pICln knockdown significantly suppressed tumor growth (**Fig. 6A** and **B**), consistent with the suppression of AR expression in xenograft tumors (**Fig. 6C**). Analysis of cleaved caspase-3 staining suggested slight induction of apoptosis by pICln knockdown but not by PRMT5 knockdown (**Fig. 6C**–**E**), confirming our *in vitro* findings. Ki-67 analysis showed that tumors with either PRMT5 or pICln knockdown had significantly lower proliferative index compared with scramble control (**Fig. 6F** and **G**). Taken together, these results demonstrate that PRMT5 and pICln also regulate AR expression and the growth of CRPC tumors *in vivo*.

### Targeting PRMT5 overcomes resistance to ASI treatment in CRPC cells and tumors

As resistance to androgen signaling inhibitor (ASI) treatment remains a clinical challenge for CRPC, we examined whether targeting PRMT5 can overcome the resistance to ASI. Because intracellular androgen synthesis by prostate cancer cells is one of the AR reactivation mechanisms in CRPC, and 22Rv1 produces CYP17A1 (37), we also treated 22Rv1 cells with the CYP17A1 inhibitor abiraterone. First, we performed MTT assay using either PRMT5 enzymatic inhibitors (BLL3.3 or JNJ-64619178) or ASI (abiraterone or enzalutamide) alone, in combination, or vehicle (DMSO). Notably, the combinational treatment decreased cell growth more effectively than either of drugs alone (**Fig. 7A**). However, using the Chou–Talalay method and software CompuSyn (http://www.combosyn.com/) to analyze the drug interaction, the combinational indexes for BLL3.3/abiraterone and BLL3.3/enzalutamide pair were 0.91 and 0.92, and for JNJ-64619178/abiraterone and JNJ-64619178/enzalutamide were 0.94 and 0.91 (Supplementary Fig. S7), respectively, indicating that PRMT5 inhibition in combination with ASI can achieve additive effect.

Next, we implanted 22Rv1-shPRMT5 cells in the castrated male mouse to evaluate the observed *in vitro* effect. After average tumor volumes reached 100 $mm^3$, PRMT5 knockdown was initiated (Dox), or treatment with ASI (abiraterone acetate or enzalutamide) started. Consistent with previous findings that 22Rv1 xenografts tumors in mice are resistant to ASI (38, 39), treatment of mice with either drug alone did not affect tumor growth (**Fig. 7B** and **C**). However, PRMT5 knockdown significantly suppressed tumor growth and showed better survival. Although combinational treatment was not more effective than PRMT5 knockdown alone in terms of tumor growth suppression (**Fig. 7B**), Ki-67 analysis suggested that combination of PRMT5 knockdown with ASI showed a better inhibition of tumor cell proliferation (**Fig. 7D** and **E**). Thus, PRMT5 targeting alone is effective to overcome the resistance of CRPC tumors to ASI in mice. The lack of additive effect of PRMT5 knockdown and ASI on tumor growth in the xenograft model is likely due to the fact that PRMT5 knockdown and ASI both act on the same AR signaling pathway. Alternatively, incomplete knockdown of PRMT5 in xenograft tumors may be an attributing factor (Supplementary Fig. S8). Analysis of cleaved caspase-3 staining suggested no significant induction of apoptosis by ASI treatment or PRMT5 knockdown (**Fig. 7F**), confirming our *in vitro* findings. Taken together, these results suggest that PRMT5 targeting is an effective treatment approach for ASI-resistant CRPC.

## Discussion

### PRMT5 regulates AR signaling through multiple mechanisms

PRMT5 has emerged as a putative oncogene in multiple human cancers (8). Although earlier studies suggested that PRMT5 promotes proliferation of cancer cells via epigenetic repression of tumor suppressors (8–11), we recently reported that PRMT5 epigenetically activates AR transcription in HNPC (7). Because AR drives prostate cancer development and progression, we investigated whether PRMT5 also regulates AR expression in CRPC. We present evidence demonstrating that PRMT5 activates transcription of AR and AR-V7 in multiple CRPC cell lines. First, knockdown or pharmacologic inhibition of PRMT5 in several CRPC cell lines (22Rv1, VCaP, C4–2, and LN95) reduced the expression of AR and AR-V7 at both mRNA and protein levels. Second, PRMT5 bound to the proximal promoter of *AR* and methylated H4R3 and H3R2. Third, transcriptomic analysis confirmed that PRMT5 regulated AR signaling in CRPC cells. Finally, PRMT5 expression positively correlates with the expression of AR and AR-V7 in CRPC tissues. Collectively, PRMT5 is overexpressed in prostate cancer tissues, and PRMT5-driven regulation of *AR* transcription is conserved in HNPC and CRPC cells.

PRMT5 may also regulate AR signaling through a nonepigenetic mechanism (40, 41). *TMPRSS2–ERG* fusion is present in nearly 50% of prostate cancer cases, and AR-driven expression of this fusion promotes prostate cancer growth (42). In *TMPRSS2–ERG*–positive VCaP cells, mass spectrometry identified PRMT5 as an interacting protein of ERG (41). Mechanistically, ERG mediated both the methylation of arginine 761 on AR by PRMT5 and the recruitment of PRMT5 to the AR target gene promoters. PRMT5-catalyzed methylation of AR attenuated AR binding to a subset of AR target genes, resulting in transcriptional repression of genes associated with prostatic epithelium differentiation. Thus, PRMT5 promoted cell proliferation in *TMPRSS2–ERG*–positive cells. However, PRMT5 knockdown did not inhibit growth of *TMPRSS2–ERG*–negative 22Rv1 cells. This contrasts with our observations that both pharmacologic inhibition and knockdown of PRMT5 significantly decreased proliferation of several CRPC cell lines, including *TMPRSS2–ERG*–negative 22Rv1 and C4–2. This discrepancy could be due to the use of heterogeneous pool of shRNA-expressing cells in their study whereas we used single-cell–derived



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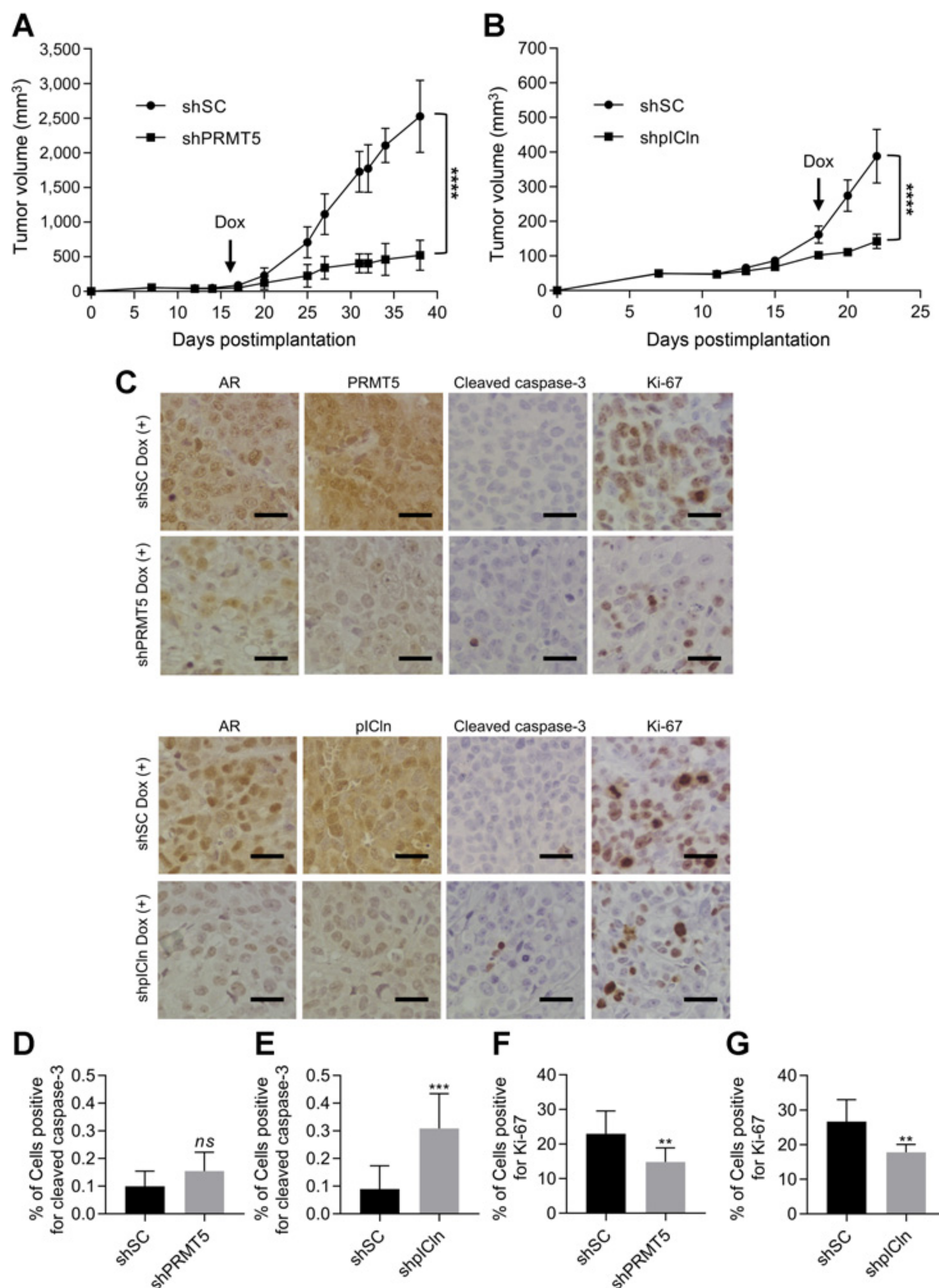


**Figure 6.**

Knockdown of PRMT5 or pICln suppresses CRPC tumor growth in mice. 22Rv1 cells with Dox-inducible knockdown of PRMT5 (shPRMT5), pICln (shpICln), or scramble control (shSC) were injected subcutaneously into right flanks of surgically castrated male NRG mice. Tumor-bearing mice were treated with doxycycline in drinking water once tumors reached nearly 100 $mm^3$. **A** and **B,** Tumor growth curves were determined and compared between treatment groups (ANOVA; ****, $P < 0.0001$). **C–G,** At the end of treatment, tumors were resected and probed for cleaved caspase-3 and Ki-67 using IHC. Presented are representative images (**C**) and the quantification of the percentage of positively stained cells out of the total number of cells (**D–G**). Scale bar, 40 μm. Results are mean ± SD ($n = 10$ per group). **D–G**, Student $t$ test was performed to determine statistical significance. ns, nonsignificant, $P > 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

stable clones that express shRNAs targeting different regions of PRMT5. In another study, ectopic overexpression of either PRMT5 or catalytically inactive PRMT5(R368A) mutant in *TMPRSS2—ERG*–negative PC3 cells enhanced luciferase activity of an androgen-responsive element-containing luciferase reporter (40), suggesting that PRMT5 might also function as a coactivator of AR independent of its methyltransferase activity. As PRMT5 may regulate prostate cancer cell growth via direct regulation of AR signaling and indirect modulation of other AR regulators, future investigation of these additional mechanisms will provide a full picture of PRMT5-driven regulation of prostate cancer cell growth. Further, genetic analysis for the role of PRMT5 in prostate cancer development and progression in mouse models will further validate PRMT5 as a therapeutic target for CRPC.

### PRMT5 interacts with pICln to epigenetically activate *AR* transcription independently of MEP50

The finding that MEP50, a canonical cofactor of PRMT5 (8), did not participate in PRMT5 regulation of *AR* transcription in LNCaP and 22Rv1 cells was surprising. This led to the discovery of pICln as a potential cofactor of PRMT5 to activate *AR* transcription. Transcriptomic analysis of PRMT5, pICln, and MEP50 target genes further confirmed that pICln, but not MEP50, cooperates with PRMT5 to regulate AR signaling in CRPC tissues (**Fig. 4**). Interestingly, pICln, but not MEP50, also cooperates with PRMT5 to activate transcription of multiple DNA damage response genes upon ionizing radiation (IR; ref. 12). Thus, pICln rather than MEP50 might be required for the activation of PRMT5 target genes. In contrast, MEP50 might form a complex with PRMT5 and pICln to repress gene transcription. For



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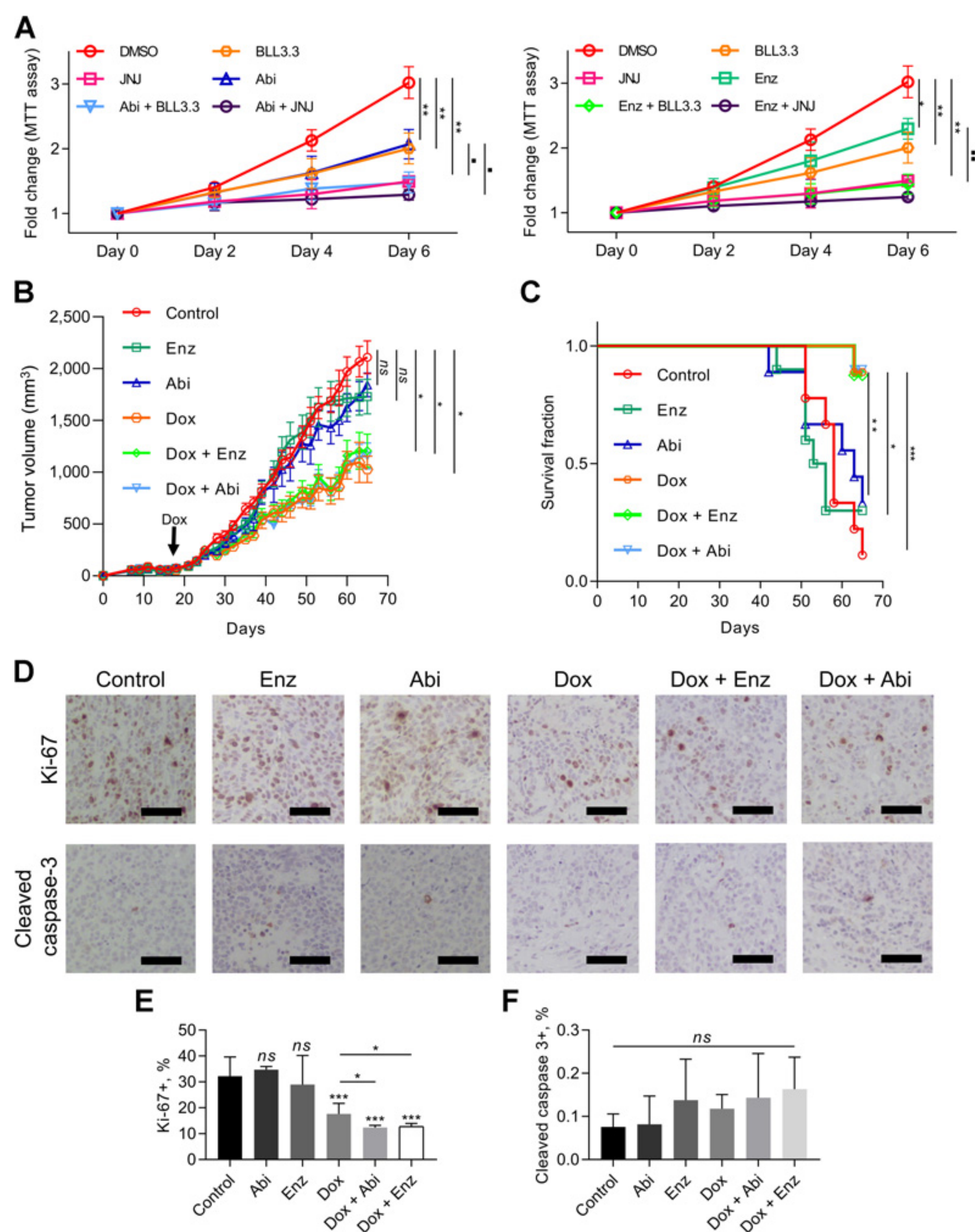


**Figure 7.**

Targeting PRMT5 overcomes resistance to ASI treatment in CRPC cells and tumors. **A,** Growth curve (MTT assay) of 22Rv1 cells incubated with 10 μmol/L PRMT5 inhibitor (BLL3.3 or JNJ-64619178, referred to as JNJ) or 10 μmol/L of either abiraterone acetate (Abi) or enzalutamide (Enz), or equal volume of vehicle (DMSO) for 6 days. Cell proliferation assays were performed at the indicated time points, and OD550 values were normalized to values from Day 0 for each cell line. ANOVA test with Welch's correction was performed to determine statistical significance. Stars represent significant difference with DMSO group, squares represent significant difference of "Abi" vs. "Abi + BLL3.3," "Abi vs. Abi + JNJ," "Enz vs. Enz + BLL3.3," or "Enz vs. Enz + JNJ" groups. Results are mean ± SD from three independent experiments. **B,** 22Rv1 cells with Dox-inducible knockdown of PRMT5 were injected subcutaneously into right flanks of surgically castrated male NRG mice. Once tumors reached nearly 100 $mm^3$, tumor-bearing mice were treated with doxycycline in drinking water, or abiraterone acetate per oral 200 mg/kg/day, or enzalutamide 25 mg/kg/day, or combination. Tumor growth curves were determined and compared between groups (ANOVA; *, $P < 0.05$). **C,** Survival of tumor–bearing mice is represented as Kaplan–Meier plot. **D–F,** At the end of treatment, tumors were resected and probed for cleaved caspase-3 and Ki-67 using IHC. Shown are representative images of IHC staining (**D**) and the quantified percentage of positively stained cells out of the total number of cells counted (**E** and **F**). Results are mean ± SD ($n = 10$ per group). Student *t* test was performed to determine statistical significance of difference vs. "Control" group. ns, nonsignificant, $P > 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$.



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example, *IVL* promoter was co-occupied by PRMT5, MEP50, and pICln, and knockdown of either MEP50 or pICln increased IVL expression (**Fig. 2C**; ref. 12). Future studies may examine whether the co-occupancy of target gene promoters by PRMT5 and PRMT5-interacting proteins, for example, MEP50, pICln, RIOK1, and COPR5, determines the transcriptional activation versus repression.

Several studies also demonstrated that PRMT5 may activate transcription of individual genes in a variety of tissues and conditions (12, 39–47). Consistent with recent transcriptomic analysis in LNCaP cells showing that majority of identified DEGs (1,136 of 2,035) was downregulated upon PRMT5 knockdown (12), similar number of upregulated and downregulated DEGs was identified in this study upon PRMT5 knockdown in 22Rv1 cells. Thus, PRMT5 likely functions as an epigenetic activator or repressor for different target genes. This notion is also supported by two recent transcriptomic studies in lung cancer cells A549 (48) and leukemia cells MOLM-13 (13). Because PRMT5 interacts with many chromatin remodelers (9), future studies focusing on the interplay between PRMT5 and PRMT5-interacting proteins including cofactors and other chromatin remodelers will likely shed new light on the epigenetic mechanism of PRMT5-mediated transcriptional regulation of target gene expression.

### Targeting PRMT5 as a novel approach for prostate cancer treatment

Although targeting AR signaling remains a mainstay of CRPC treatment (4), the inevitable emergence of resistance via AR reactivation limits the therapeutic efficacy of ASI (5, 6). Targeting AR protein expression instead may provide an alternative approach for the CRPC treatment and potentially overcome multiple AR reactivation mechanisms. In fact, targeting AR expression by promoting AR degradation effectively suppressed prostate cancer cell growth in several preclinical studies (49, 50). One of the AR degraders utilizing PROTAC technology is in a phase I clinical trial (51).

Given that epigenetic landscapes of CRPC and HNPC are largely distinct (52), the conserved role of PRMT5 in epigenetic activation of AR transcription in HNPC and CRPC is interesting and significant (7). As the vast majority of HNPC and CRPC demonstrate dependency on the AR signaling, targeting PRMT5 may offer an alternative or even more effective treatment for both HNPC and CRPC. In fact, PRMT5 targeting alone effectively suppressed CRPC growth (**Fig. 7**). In addition, because AR reactivation promotes resistance to the next-generation ASI, we explored whether targeting PRMT5 can overcome this resistance. Indeed, PRMT5 inhibition in combination with ASI showed additive suppression of CRPC cell growth *in vitro*. Interestingly, PRMT5 knockdown alone showed suppression of 22Rv1 xenograft tumor growth in mice as well as combination treatments (**Fig. 7B** and **C**), indicating that targeting PRMT5 might be an effective approach to overcoming resistance to ASI.

Three PRMT5 inhibitors are currently in clinical trials for leukemia and solid tumors (clinicaltrials.gov). As PRMT5 is an essential gene in normal organism processes, such as hematopoiesis and keratinocyte differentiation (53, 54), targeting PRMT5 may cause adverse effects. If so, targeted prostate-specific membrane antigen-based delivery of PRMT5 inhibitors will likely provide an alternative to suppress AR expression in prostate cancer specifically (55). Alternatively, targeting PRMT5/pICln interaction may provide another promising approach for both HNPC and CRPC by suppressing or even eliminating AR expression.

## Disclosure of Potential Conflicts of Interest

J. Huang reports grants from Duke University during the conduct of the study and personal fees from Kingmed, MoreHealth, OptraScan, Genetron, and Sisu Pharma outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

**E. Beketova:** Conceptualization, resources, data curation, software, formal analysis, funding acquisition, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. **S. Fang:** Data curation, software, formal analysis, investigation, visualization, methodology, writing-review and editing. **J.L. Owens:** Funding acquisition, validation, investigation, methodology, writing-review and editing. **S. Liu:** Data curation, software, formal analysis, investigation, visualization, methodology, writing-review and editing. **X. Chen:** Investigation, methodology. **Q. Zhang:** Investigation, methodology. **A.M. Asberry:** Funding acquisition, validation, investigation, writing-review and editing. **X. Deng:** Formal analysis, investigation, methodology. **J. Malola:** Investigation, methodology. **J. Huang:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, project administration, writing-review and editing. **C. Li:** Resources, project administration. **R. Pili:** Resources, project administration. **B.D. Elzey:** Resources, investigation. **T.L. Ratliff:** Supervision, project administration. **J. Wan:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, project administration, writing-review and editing. **C.-D. Hu:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, visualization, methodology, project administration, writing-review and editing.

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# Protein Arginine Methyltransferase 5 Promotes pICln-Dependent Androgen Receptor Transcription in Castration-Resistant Prostate Cancer

Elena Beketova, Shuyi Fang, Jake L. Owens, et al.

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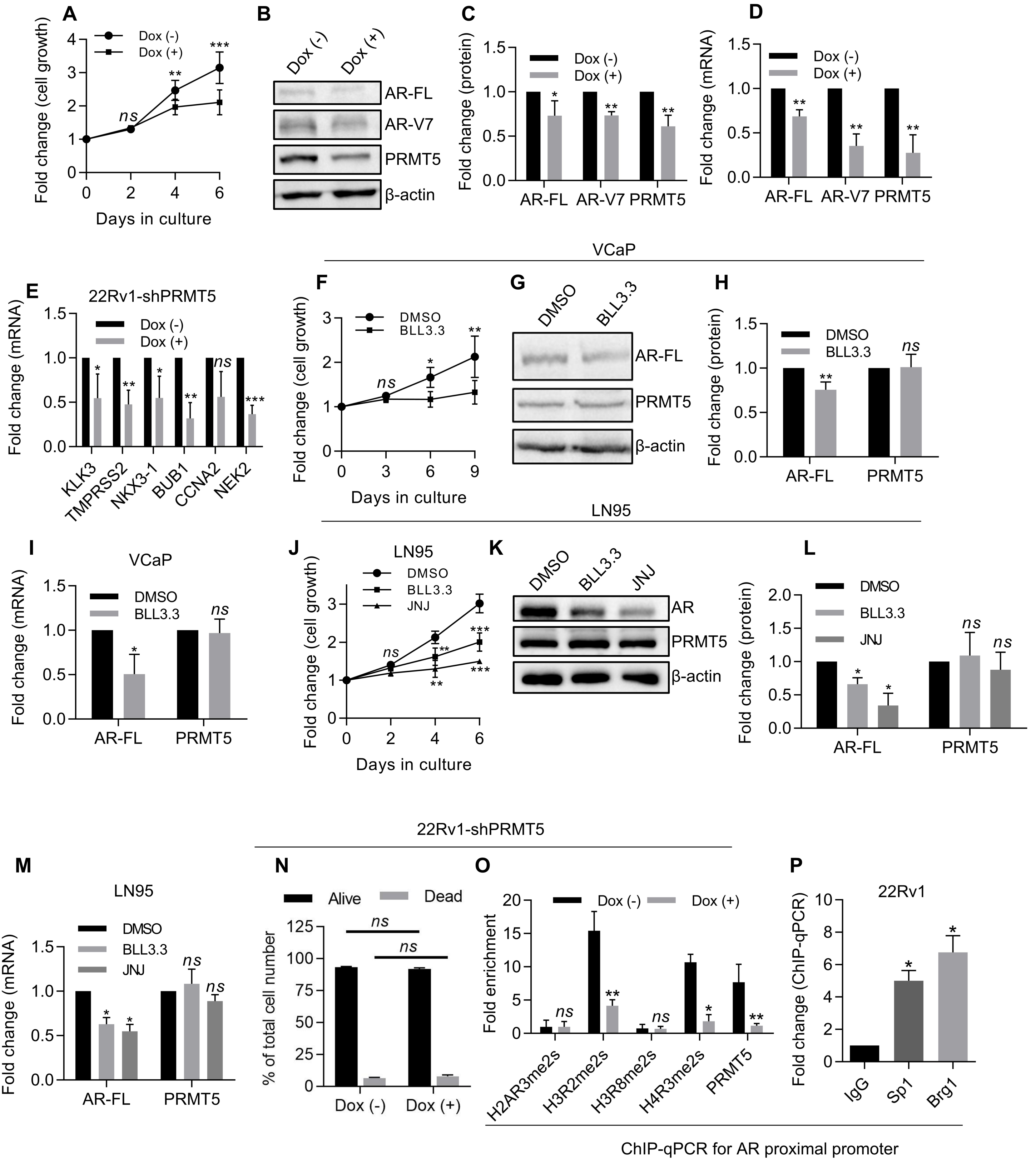
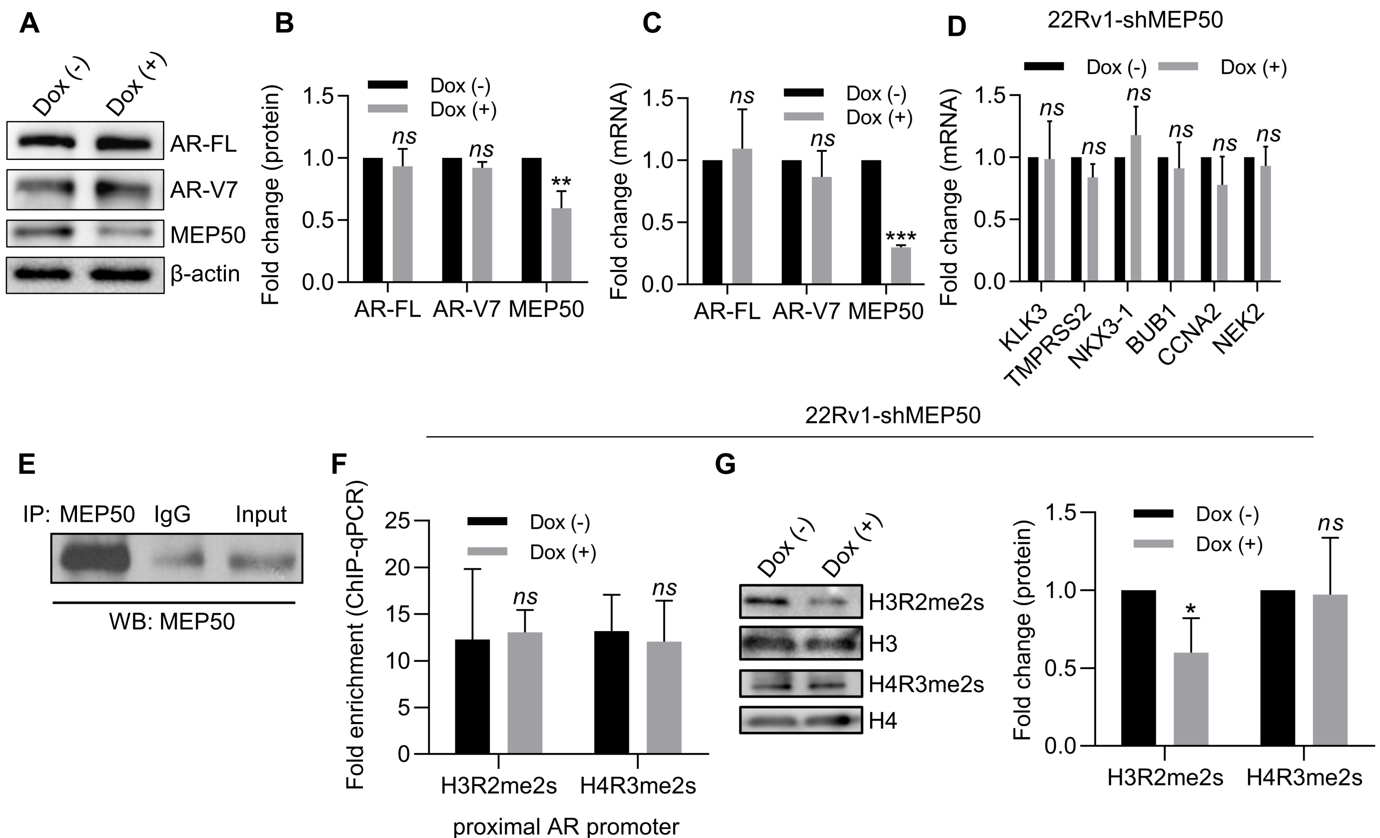
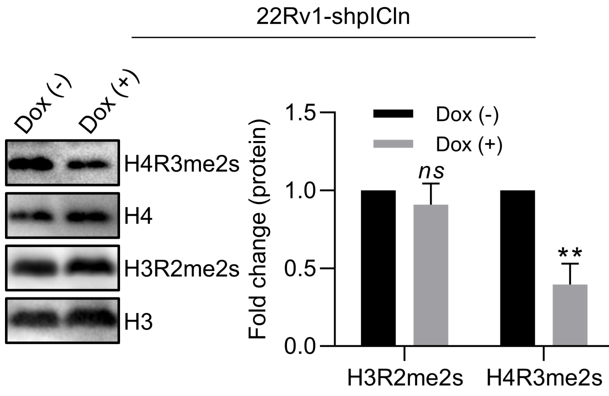


22Rv1-shPRMT5#2
A
Fold change (cell growth)
Dox (-)
Dox (+)
Days in culture
B
Dox (-)
Dox (+)
AR-FL
AR-V7
PRMT5
β-actin
C
Fold change (protein)
D
Fold change (mRNA)
AR-FL
AR-V7
PRMT5
E
22Rv1-shPRMT5
KLK3
TMPRSS2
NKX3-1
BUB1
CCNA2
NEK2
VCaP
F
DMSO
BLL3.3
G
H
LN95
I
VCaP
J
LN95
JNJ
K
AR
L
M
LN95
N
22Rv1-shPRMT5
Alive
Dead
% of total cell number
O
Fold enrichment
H2AR3me2s
H3R2me2s
H3R8me2s
H4R3me2s
P
22Rv1
Fold change (ChIP-qPCR)
IgG
Sp1
Brg1
ChIP-qPCR for AR proximal promoter

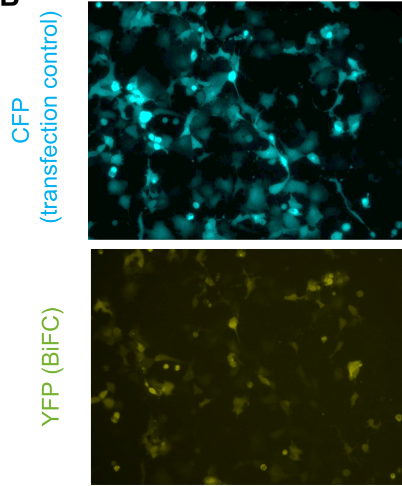
**Supplementary Fig. S1**. **PRMT5 promotes growth of CRPC cells via epigenetic activation of AR expression**. **A,** Growth curve (MTT assay) of 22Rv1 cells with doxycycline-inducible PRMT5 knockdown (22Rv1-shPRMT5#2) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. **B-C,** Representative western blot analysis (**B**) and quantification (**C**) of protein expression in cell lysates from Day 6 of **A**. **D,** qPCR analysis of gene expression in cells from Day 6 of **A**. **E,** qPCR of AR target genes in 22Rv1-shPRMT5 cell line after 6 days of PRMT5 knockdown. **F,** Growth curve (MTT assay) of VCaP cells incubated with 10 μM PRMT5 inhibitor (BLL3.3) or equal volume of vehicle (DMSO) for 9 days. **G-H,** Representative western blot analysis (**G**) and quantification (**H**) of protein expression in cell lysates from Day 9 of **F**. **I,** qPCR analysis of gene expression in cells from Day 9 of **F**. **J,** Growth curve (MTT assay) of LN95 cells incubated with 10 μM PRMT5 inhibitor (BLL3.3 or JNJ) or equal volume of vehicle (DMSO) for 6 days. **K-L,** Representative western blot analysis (**K**) and quantification (**L**) of protein expression in cell lysates from Day 6 of **J**. **M,** qPCR analysis of gene expression in cells from Day 6 of **J**. **N,** Trypan blue cell viability analysis in 22Rv1-shPRMT5 cells after 6 days of PRMT5 knockdown. **O,** ChIP-qPCR analysis of histone methylation and PRMT5 binding at the proximal AR promoter at Day 6 of PRMT5 knockdown was performed with indicated antibodies. **P,** ChIP-qPCR analysis with antibodies of indicated specificity was performed using 22Rv1 cells lysates. Specific primers for the proximal region of AR promoter was used. For MTT, western blotting, cell cycle, and qPCR analysis, statistical significance of group difference was determined for 'DMSO vs BLL3.3' or 'Dox (-) vs Dox (+)'. For ChIP-qPCR, values were normalized to the corresponding IgG control. For **O**, statistical significance of group difference was determined for 'Dox (-) vs Dox (+)'. For **P**, indicated statistical significance of group difference was determined for 'specific IP vs IgG IP'. For all experiments, results are mean ± SD from 3 independent experiments. Student *t*-test with Welch's correction was performed to determine statistical significance of group difference. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



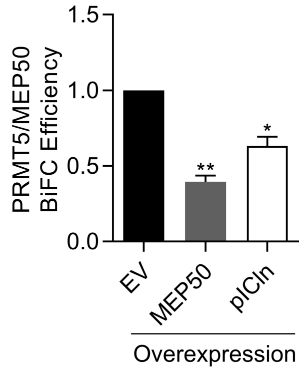

**Supplementary Fig. S2. MEP50 is not required for PRMT5-mediated activation of AR transcription in CRPC cells. A-B,** Representative western blot analysis (**A**) and quantification (**B**) of protein expression in cell lysates of 22Rv1 cells with doxycycline-inducible MEP50 knockdown (22Rv1-shMEP50#2) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. **C,** qPCR analysis of gene expression in cells from **A**. **D,** qPCR of AR target genes in 22Rv1-shMEP50 cells after 5 days of MEP50 knockdown. **E,** western blot analysis of immunoprecipitates of MEP50 from LNCaP cell lysate. **F,** ChIP-qPCR analysis of histone methylation at the proximal AR promoter at Day 6 of MEP50 knockdown was performed with indicated antibodies. **G**, Representative western blot analysis and quantification of H4R3me2s and H3R2me2s in cell lysates of 22Rv1-shMEP50 incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. For ChIP-qPCR, values were normalized to the corresponding IgG control. For western blotting, ChIP-qPCR, and qPCR analysis, statistical significance of group difference was determined for 'Dox (-) vs Dox (+)'. Results are mean ± SD from 3 independent experiments. Student *t*-test with Welch's correction was performed to determine statistical significance. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



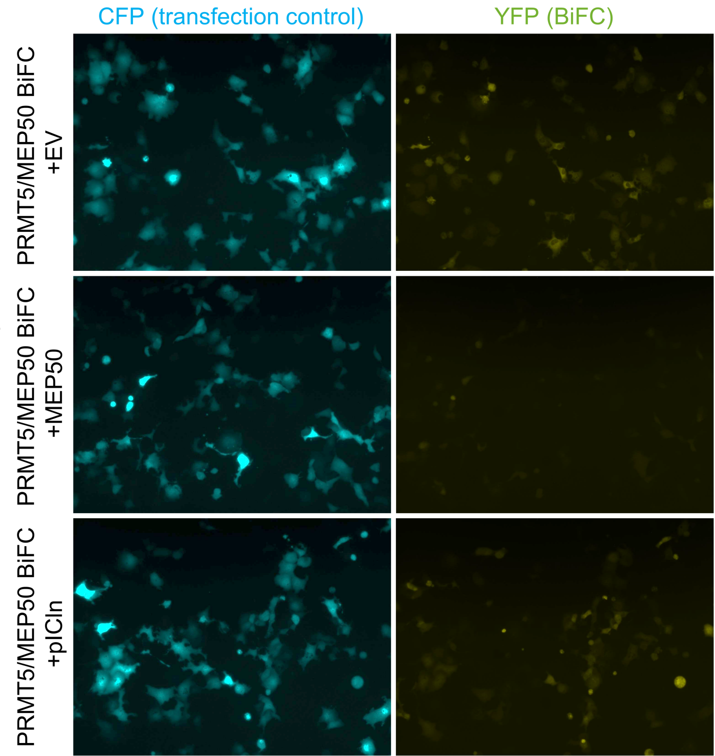
A
22Rv1-shpICln
Dox (-)
Dox (+)
H4R3me2s
H4
H3R2me2s
H3
Fold change (protein)
1.5
1.0
0.5
0.0
Dox (-)
Dox (+)
ns
**
H3R2me2s
H4R3me2s

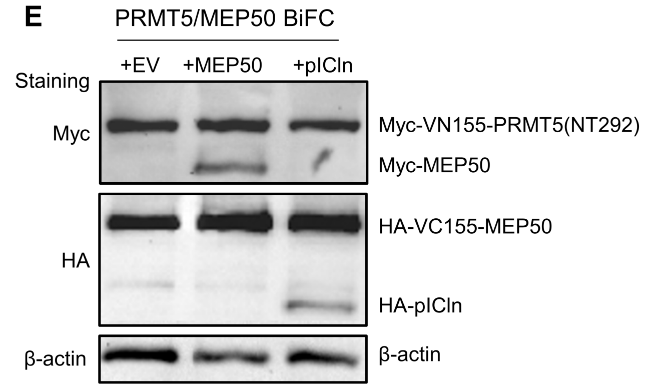
B
CFP (transfection control)
YFP (BiFC)

D
PRMT5/MEP50 BiFC Efficiency
1.5
1.0
0.5
0.0
**
*
EV
MEP50
pICln
Overexpression

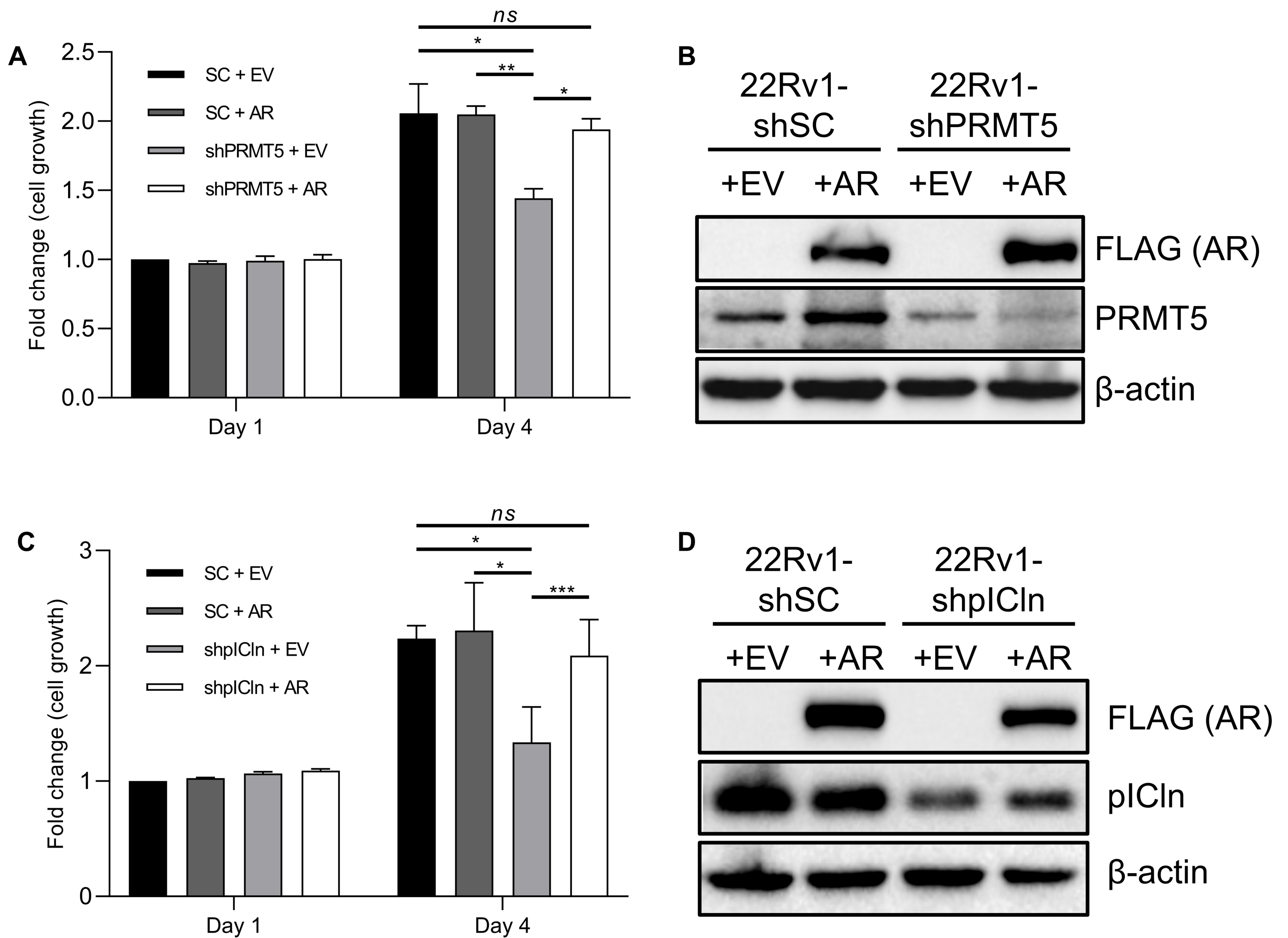



C
CFP (transfection control)
YFP (BiFC)
PRMT5/MEP50 BiFC +EV
PRMT5/MEP50 BiFC +MEP50
PRMT5/MEP50 BiFC +pICln

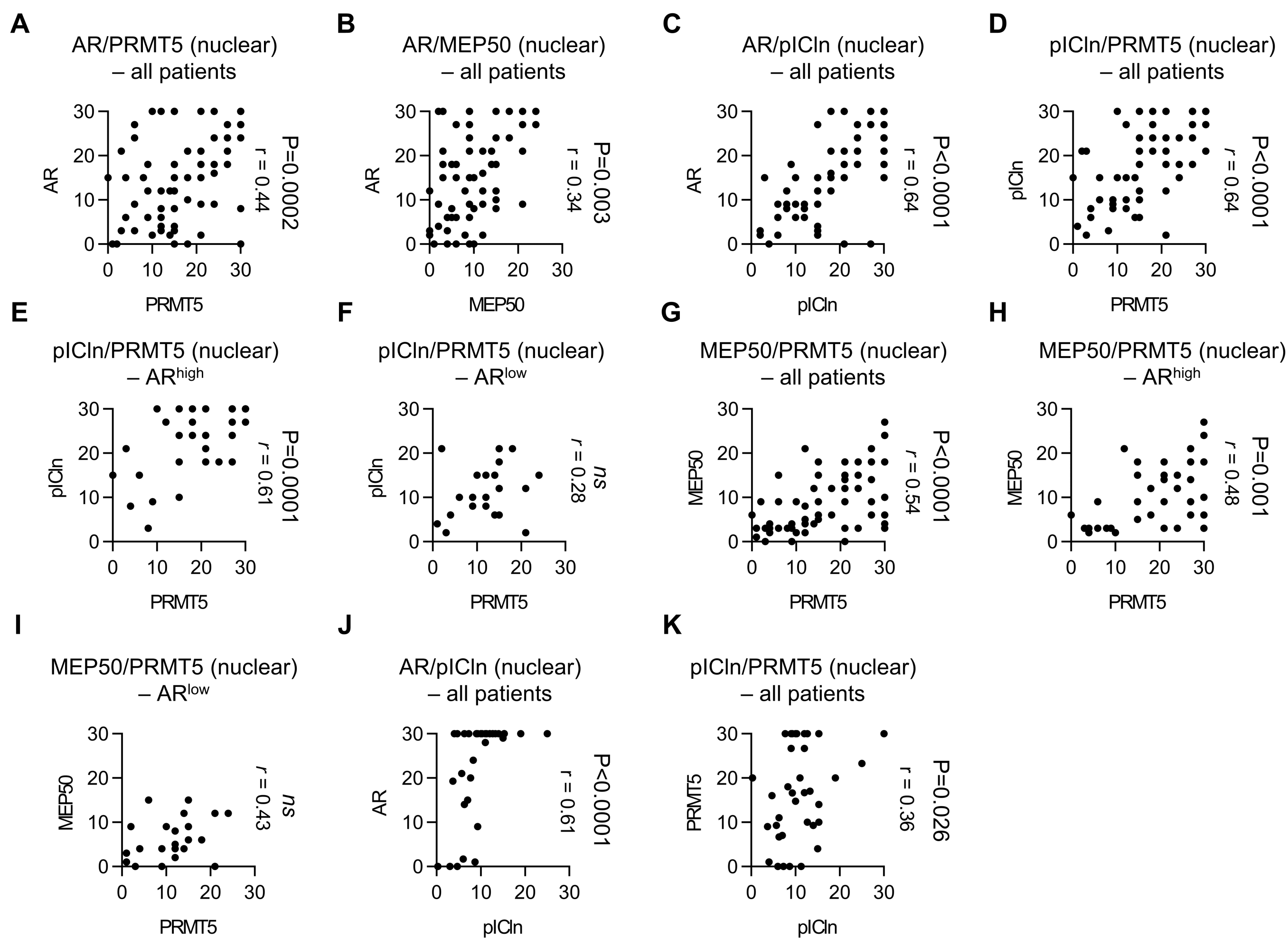



E
PRMT5/MEP50 BiFC
Staining
+EV
+MEP50
+pICln
Myc
HA
β-actin
Myc-VN155-PRMT5(NT292)
Myc-MEP50
HA-VC155-MEP50
HA-pICln
β-actin

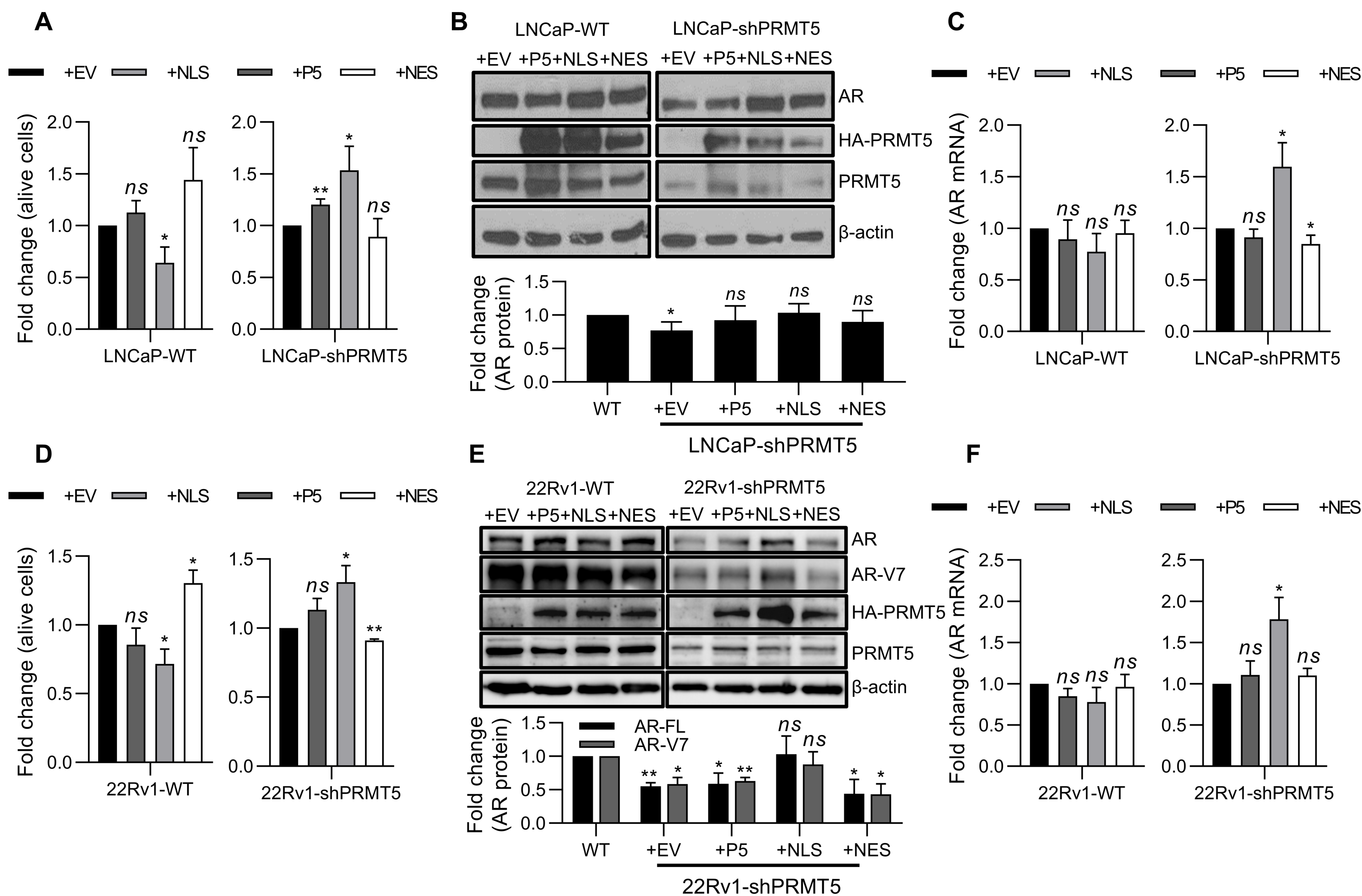
**Supplementary Figure S3. pICln is a novel PRMT5 binding partner participating in epigenetic activation of AR transcription. A**, Representative western blot analysis and quantification of H4R3me2s and H3R2me2s in cell lysates of 22Rv1 cells with doxycycline-inducible pICln knockdown (22Rv1-shpICln) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. **B,** COS-1 cells were co-transfected with BiFC plasmids to co-express VC155-pICln and VN155-PRMT5(NT292) along with the plasmid expressing Cerulean fluorescent protein (CFP) as a transfection control. Images shown were taken 48 h post-transfection. YFP fluorescence indicates reconstituted Venus as a result of PRMT5/pICln interaction. 20x magnification. **C-D,** COS-1 cells were co-transfected with BiFC plasmids to co-express VC155-MEP50 and VN155-PRMT5(NT292) and the plasmid expressing MEP50 (+MEP50) or pICln (+pICln) as well as the plasmid expressing Cerulean (CFP) as a transfection control. +EV, empty vector, or Myc-MEP50 overexpression plasmid (+MEP50), or HA-pICln overexpression plasmid (+pICln). **C,** Representative fluorescence images acquired 48 h after transfection. 20x magnification. **D,** Quantification of PRMT5/MEP50 BiFC efficiency (ratio of Venus/CFP fluorescence intensities) from **C**. **E,** Western blot analysis of protein expression in lysates of cells from **C**. Membrane probing is indicated on the left, and detected proteins are indicated on the right. Results are mean ± SD from 3 independent experiments. Student *t*-test with Welch's correction was performed to determine statistical significance between groups. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.



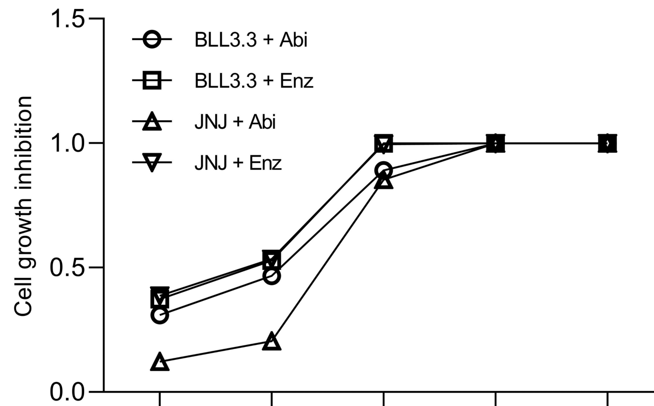

**Supplementary Fig. S4. AR re-expression restores cell proliferation after PRMT5 or pICln knockdown in 22Rv1. A,** 22Rv1 cells with Dox-inducible expression of scramble control (22Rv1-shSC) or PRMT5 shRNA (22Rv1-shPRMT5) were treated with Dox and transfected with either empty vector (+EV) or plasmid for FLAG-AR expression (+AR). MTT assay was performed at Day 1 and Day 4 of treatment. **B**, Western blot analysis of protein expression in cell lysates at Day 4 of **A**. **C,** 22Rv1 cells with Dox-inducible expression of scramble control (22Rv1-shSC) or pICln shRNA (22Rv1-shpICln) were treated with Dox and transfected with either empty vector (+EV) or plasmid for Flag-AR expression (+AR). MTT assay was performed at Day 1 and Day 4 of treatment. **D**, Western blot analysis of protein expression in cell lysates at Day 4 of **C**.




**Supplementary Fig. S5. PRMT5 and pICln expression positively correlates with AR in CRPC and HNPC tissues. A-K,** Correlation analysis (Spearman) of corresponding protein pairs in CRPC and HNPC tissue microarrays (**A-I**, CRPC tissue microarray; **J and K**, HNPC tissue microarray). For **E-F, H-I** same analysis was performed for data stratified based on AR expression. *ns* $P > 0.05$

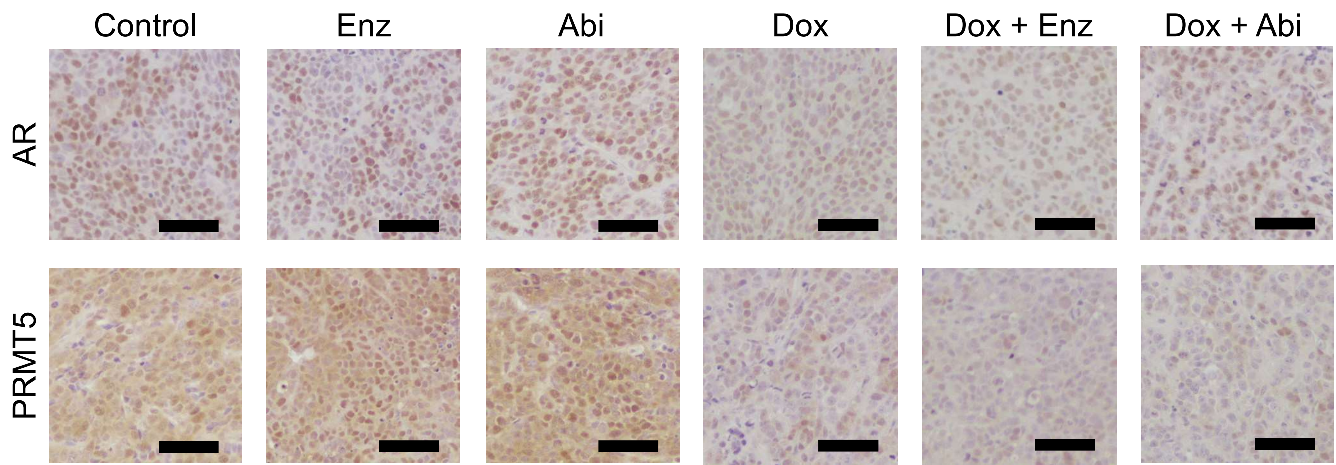



**Supplementary Fig. S6. Nuclear-localized PRMT5 promotes cell proliferation and AR expression in LNCaP and 22Rv1. A-C**, Wild-type LNCaP (LNCaP-WT) or LNCaP with Dox-inducible knockdown of PRMT5 (LNCaP-shPRMT5) were transfected with empty vector (+EV), or constructs for overexpression of PRMT5 (+P5), nuclear-localized PRMT5 (+NLS), or cytoplasmic PRMT5 (+NES). **A**, Alive cell number was analyzed using Trypan Blue staining after 4 days of transfection. **B**, Representative western blot and quantification of protein expression in cell lysates from **A**. **C**, qPCR analysis of *AR* expression in cells from **A**. **D**, Wild-type 22Rv1 (22Rv1-WT) or 22Rv1 with Dox-inducible knockdown of PRMT5 (22Rv1-shPRMT5) cells were similarly transfected with the plasmids indicated in **A**. Alive cell number was analyzed using Trypan Blue staining after 4 days of transfection. **E**, Representative western blot and quantification of protein expression in cell lysates from **D**. **F**, qPCR analysis of *AR* expression in cells from **D**. For western blot, statistical significance of group difference was determined for comparison with 'WT' group. For Trypan Blue staining and qPCR, statistical significance of group difference was determined for comparison with '+EV' group. For all experiments, results are mean ± SD from at least 3 independent experiments. Brown-Forsythe and Welch ANOVA was performed to determine statistical significance of group difference. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.




| | | | | | |
|---|---|---|---|---|---|
| BLL3.3 (μM) | 4.25 | 8.5 | 17 | 34 | 68 |
| JNJ-64619178 (μM) | 5.5 | 11 | 22 | 44 | 88 |
| Abiraterone (μM) | 4 | 8 | 16 | 32 | 64 |
| Enzalutamide (μM) | 10 | 20 | 40 | 80 | 160 |

**Supplementary Fig. S7**. **PRMT5 targeting and ASI have some additive effect in 22Rv1.** MTT assay of 22Rv1 cells incubated with indicated concentrations of PRMT5 inhibitor (BLL3.3 or JNJ-64619178, referred to as JNJ) or either abiraterone acetate (Abi) or enzalutamide (Enz) for 72 hours.




**Supplementary Fig. S8**. **Expression of AR and PRMT5 in 22Rv1 xenografts.** 22Rv1 cells with Dox-inducible knockdown of PRMT5 were injected subcutaneously in right flanks of surgically castrated male nude mice. Once tumors reached ~100 $mm^3$, tumor-bearing mice were treated with doxycycline in drinking water, or abiraterone acetate per oral 200 mg/kg/day, or enzalutamide 25 mg/kg/day, or combination. At the end of treatment tumors were resected and probed for AR and PRMT5. Representative IHC images are shown. Scale bar indicates 100 μm.

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REVIEW ARTICLE

# PRMT5: a putative oncogene and therapeutic target in prostate cancer

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**Abstract**

Protein arginine methyltransferase 5 (PRMT5) was discovered two decades ago. The first decade focused on the biochemical characterization of PRMT5 as a regulator of many cellular processes in a healthy organism. However, over the past decade, evidence has accumulated to suggest that PRMT5 may function as an oncogene in multiple cancers via both epigenetic and non-epigenetic mechanisms. In this review, we focus on recent progress made in prostate cancer, including the role of PRMT5 in the androgen receptor (AR) expression and signaling and DNA damage response, particularly DNA double-strand break repair. We also discuss how PRMT5-interacting proteins that are considered PRMT5 cofactors may cooperate with PRMT5 to regulate PRMT5 activity and target gene expression, and how PRMT5 can interact with other epigenetic regulators implicated in prostate cancer development and progression. Finally, we suggest that targeting PRMT5 may be employed to develop multiple therapeutic approaches to enhance the treatment of prostate cancer.

## Introduction

Protein arginine methyltransferase 5 (PRMT5) is a type II enzyme of the emerging family of protein arginine methyltransferases (PRMTs) that can methylate arginine residues of histones and non-histone substrates [1]. Arginine methylation is a ubiquitous post-translational modification across species [2]. Nine arginine methyltransferases have been described to date, and their function has been reviewed elsewhere [1, 3]. However, PRMT5 is the most widely studied type II enzyme, and PRMT9 is the only other type II enzyme. Biochemical and structural studies suggest that PRMT5 forms an octameric complex with the methylosome protein 50 (MEP50) for its catalytic activity [4–6]. In general, PRMT5 is considered an epigenetic repressor of target gene transcription via symmetric dimethylation of histones H4R3 (H4R3me2s), H3R8 (H3R8me2s), and H2AR3 (H2AR3me2s) [4–6]. However, several studies suggest that symmetric dimethylation of histones may activate gene transcription via dimethylation of H3R2 (H3R2me2s), H3R8 (H3R8me2s), and H4R3 (H4R3me2s) [7–9]. Other than epigenetic regulation of gene transcription, PRMT5 post-translationally methylates many signaling molecules such as NF-κB, EGFR, p53, and others [6]. As a result, PRMT5 is a critical regulator of cellular proliferation, differentiation, cell cycle progression, DNA damage response (DDR), and cell death [4–6]. As a critical regulatory protein, PRMT5 is overexpressed in multiple cancers [4]. Mechanistic studies have suggested that PRMT5 may function as an oncogene through (1) epigenetic repression of tumor suppressor genes or cell cycle regulators or (2) post-translational regulation of signaling molecules. In this review, we will focus on recent progress in prostate cancer. We present evidence that PRMT5 functions as an epigenetic activator to promote transcription of DDR genes and androgen receptor (AR) in prostate cancer cells. Further, we discuss the identification of pICln as a novel cofactor of PRMT5 to activate transcription of these target genes independent of MEP50. These novel findings suggest potential PRMT5-based targeting approaches for prostate cancer treatment.

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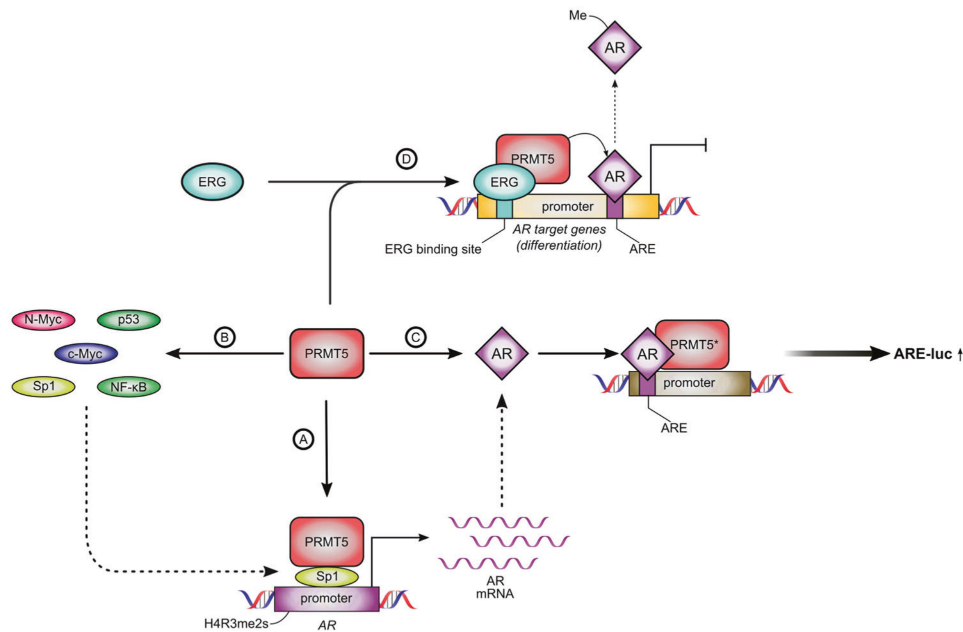


**Fig. 1 Mechanisms of PRMT5-driven regulation of AR signaling in prostate cancer.** PRMT5 is implicated in the regulation of AR signaling at multiple steps: (A) PRMT5 is recruited to the *AR* promoter by Sp1 to symmetrically dimethylate H4R3 thus promoting *AR* transcription. (B) PRMT5 can modulate activity of transcriptions factors that regulate AR expression. (C) PRMT5 can function as an AR co-activator independently of its methyltransferase activity to enhance activation of AR target gene expression. (D) PRMT5 can methylate AR in an ERG-dependent manner leading to decreased recruitment of AR to AR target gene promoters of differentiation-promoting genes and increased cell proliferation. *mechanism (C) is independent of PRMT5 enzymatic activity.

## PRMT5-driven regulation of AR signaling in prostate cancer

Androgen/AR signaling is the major driver of the normal prostate function and prostate cancer growth and progression [10]. Due to the critical role of AR signaling in prostate cancer, AR remains the primary therapeutic focus for this disease. Indeed, androgen deprivation therapy (ADT) suppresses the production of androgens or inhibits AR signaling and is the standard of care for metastatic prostate cancer [11]. However, AR signaling plasticity leads to the emergence of the therapeutic resistance and the development of castration-resistant prostate cancer (CRPC) via multiple mechanisms of AR reactivation, including emergence of gain-of-function mutations, AR gene amplification, and expression of ligand-independent splice variants [12]. Thus, understanding the regulatory mechanisms of AR expression and activity is necessary to develop novel approaches for prostate cancer treatment.

### Epigenetic regulation of AR transcription

Epigenetic mechanisms are implicated in both positive and negative regulation of *AR* transcription [13]. As early as 2000, it was demonstrated that the level of *AR* promoter DNA methylation negatively correlates with AR expression [14]. Since then, multiple mechanisms such as histone methylation and expression of non-coding RNAs were identified to contribute to *AR* transcription [13]. One of the first reports indicating the potential implication of histone methylation on *AR* transcription was published in 2012 [15]. In this study, treatment of LNCaP cells with adenosine dialdehyde, the inhibitor of multiple methyltransferases, caused downregulation of AR expression, decrease of H3K9 methylation, and inhibition of cell growth. However, that study did not address the effect of general methyltransferase inhibition on other methylation marks.

In 2017, it was shown that PRMT5 binds to the *AR* promoter and symmetrically dimethylates H4R3 at the *AR* promoter in hormone-naïve prostate cancer (HNPC) cell line LNCaP [16] (Fig. 1A). Targeting PRMT5 via either pharmacological inhibition or short hairpin RNA (shRNA)-mediated knockdown caused decrease of *AR* mRNA and protein expression accompanied by the decrease of cell proliferation in both cell culture and in LNCaP xenograft model. It was demonstrated that transcription factor Sp1 recruits PRMT5 to the *AR* promoter as PRMT5 does not have a DNA binding domain. Furthermore, it was shown that PRMT5 similarly promotes *AR* transcription in CRPC cells in a pICln-dependent manner [17]. Targeting PRMT5 in CRPC cells with different mechanisms of AR reactivation decreased cell proliferation and downregulated the

expression of AR, AR splice variants, and AR target genes. As PRMT5-driven methylation of histones can also promote deposition of transcription activation marks such as H3K4me3 [7, 9, 18], the potential interplay between arginine methylation and other chromatin modification marks in the context of *AR* transcription regulation requires further investigation.

### Regulation of transcription factor-mediated AR transcription

In addition to direct regulation of *AR* transcription by methylation of histones at the proximal *AR* promoter, PRMT5 may also control *AR* transcription indirectly via modulation of AR-regulating transcription factors (Fig. 1B). For example, **Sp1** is a major transcription factor to activate expression of *AR* [19]. In acute myeloid leukemia, PRMT5 downregulation causes downregulation of Sp1 expression likely via de-repression of miRNA miR-29b [8]. Interestingly, Sp1 recruits PRMT5 to the *AR* proximal promoter region to activate *AR* transcription in prostate cancer cells [16]. Given that expression of miR-29b is significantly lower in prostate cancer compared to normal tissues [20], future studies are needed to investigate if this positive feedback loop plays an essential role in the regulation of AR expression in prostate cancer cells.

Like Sp1, **c-Myc** is another positive regulator of *AR* transcription and a prominent oncogene in prostate cancer [21]. c-Myc can also recruit PRMT5 to its target genes in glioblastoma [22]. Interestingly, c-Myc has been shown to upregulate PRMT5 transcription [23], and, vice versa, PRMT5 has been shown to upregulate c-Myc expression in lymphoma [24], suggesting another potential positive feedback loop mechanism. **N-Myc**, another transcription factor of Myc family, was implicated in progression of prostate adenocarcinoma to neuroendocrine prostate cancer (NEPC) [25]. Importantly, N-Myc simultaneously interacts with AR and EZH2 to promote transcriptional repression of AR target genes during prostate cancer neuroendocrine differentiation [26]. In neuroblastoma, PRMT5 functions as a key regulator of N-Myc protein stability [27]. With the prominent role of N-Myc in NEPC, further studies are needed to investigate if PRMT5 also regulates N-Myc stability in prostate cancer and if targeting PRMT5 can suppress the growth of NEPC via downregulation of N-Myc expression.

Another transcription factor implicated in regulation of *AR* transcription in prostate cancer is **NF-κB**. It was shown to be capable of both transcriptional activation and repression of *AR*, indicating the context-dependent role of this protein [28, 29]. In several models, NF-κB is activated by PRMT5 methylation enhancing NF-κB binding to target genes [30, 31]. Thus, it is likely that in prostate cancer with PRMT5 overexpression [16], PRMT5 promotes AR expression via NF-κB activation. Interestingly, **p53** is implicated in regulation of AR expression as transcriptional repressor [32], while PRMT5-mediated methylation inactivates p53 in lymphoma model [33]. However, several studies show that PRMT5 activates p53 in the context of DNA damage which we discuss below. In summary, PRMT5 is involved in regulation of AR expression at several levels, including direct transcription activation via association with *AR* promoter and modulation of AR-regulating transcription factors.

### Regulation of AR transcriptional activity and target gene expression

Apart from regulating AR expression, PRMT5 can regulate AR activity directly by interacting with AR protein and modulating AR function as a transcription factor (Fig. 1C, D). The study by Hosohata et al. [34] suggested that PRMT5 may function as an AR co-activator independently of PRMT5 methyltransferase activity. Overexpression of either wild-type PRMT5 or the catalytically inactive PRMT5(R368A) mutant in PC3 cells enhanced luciferase activity of an androgen-responsive element (ARE)-containing luciferase reporter (Fig. 1C). However, a later report by Mounir et al. [35] indicated that in VCaP cells, PRMT5-mediated methylation of AR attenuated AR binding to a subset of AR target genes (Fig. 1D). This methylation led to the repression of genes associated with prostatic epithelium differentiation and promoted VCaP cell proliferation. The interaction of PRMT5 and AR was mediated by ERG and only occurred in TMPRSS2-ERG-positive cell lines such as VCaP but not TMRSS2-ERG-negative cell lines such as 22Rv1. However, PRMT5 may also interact with AR in TMRSS2-ERG-negative PC3 via the PRMT5-interacting protein MEP50, which was also reported to act as AR co-activator [36, 37]. Taken together, these studies demonstrate that PRMT5 can regulate AR activity via non-epigenetic mechanisms in a context-dependent manner.

## PRMT5-mediated regulation of the DDR in prostate cancer

The genome is constantly exposed to both endogenous and environmental stresses that cause damage to DNA. DDR is an evolutionarily conserved cellular response which maintains genome integrity through coordinated regulation of cell cycle arrest, DNA damage repair, and apoptosis [38]. DNA double-stranded breaks (DSBs) are the most cytotoxic DNA lesions. Incorrect or incomplete repair of DSBs promotes accumulation of mutations and cancer development.

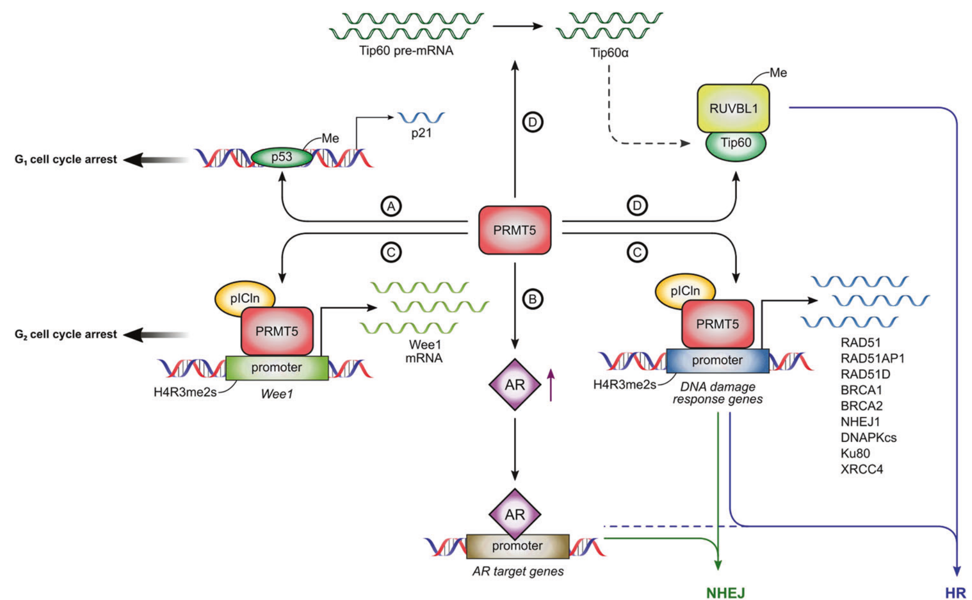


**Fig. 2 Role of PRMT5 in DNA damage response in prostate cancer.** (A) PRMT5 can regulate cell cycle progression via methylation of p53 or (C) epigenetic activation of *Wee1* transcription. (B) PRMT5 activates AR expression which can promote both HR and NHEJ. (C) PRMT5 functions as a master epigenetic regulator for a number of DSBs repair genes promoting both HR and NHEJ. (D) PRMT5 regulates DSB repair pathway choice and promotes HR through symmetrically dimethylating RUVBL1 or through modulating splicing of Tip60.

Conversely, cancer therapies such as radiation therapy (RT) and chemotherapy kill cancer cells by inducing extensive DSBs and apoptosis. Thus, mutations in DDR proteins contribute to increased incidence of cancer whereas therapeutic agents targeting DDR regulators are used as anti-cancer agents alone or in combination with chemotherapy and RT.

## Regulation of the cell cycle

p53 is a critical transcription factor of cell cycle arrest and apoptosis in response to DNA damage. However, how p53 can selectively activate these cellular responses remained a central question in the field. In 2008, Jansson et al. demonstrated that PRMT5 can methylate p53 at arginine residues 333, 335 and 337 to activate transcription of *p21* and promote $G_1$ cell cycle arrest in response to DNA damaging agent etoposide (Fig. 2A). This finding represented the first report to demonstrate a direct role for PRMT5 in DDR [39]. Since then, multiple studies have demonstrated that PRMT5 plays a pivotal role in DDR (Fig. 2A–D). In most cancer cells, including prostate cancer, PRMT5 knockdown induces $G_1$ arrest, suggesting a positive regulatory role of $G_1$ progression by PRMT5.

In the case of DNA damage, PRMT5 plays a different role in the regulation of the cell cycle. In prostate cancer cells, PRMT5 is required for DNA damage-induced $G_2$ arrest due to epigenetic activation of *WEE1* [40]. In other cancer cells, PRMT5 regulates $G_2$ arrest via methylation of RAD9 [41] and accumulation of KLF4 [42]. These mechanisms may contribute to $G_2$ arrest in prostate cancer cells as well. Future studies will further define the role of PRMT5 in cell cycle arrest in prostate cancer cells.

## Epigenetic regulation of DSB repair gene expression

One major discovery regarding the role of PRMT5 in DDR is that PRMT5 functions as a master epigenetic activator of DSB repair genes in prostate cancer cells (Fig. 2C) [40]. PRMT5 is required for the repair of ionizing radiation (IR)- and etoposide-induced DSBs and activation of both homologous recombination (HR) and non-homologous enjoining (NHEJ). Interestingly, knockdown of PRMT5 increased spontaneous DSBs independent of external DNA damage inducers, indicating that PRMT5 is required to prevent or repair endogenous DSBs. Subsequent analysis identified several PRMT5 target genes involved in the DDR, including genes involved in HR (*RAD51, RAD51D, RAD51AP1, BRCA1, and BRCA2*) and NHEJ (*NHEJ1/XLF* and *DNAPKcs/PRKDC*). Upon DNA damage, PRMT5 was upregulated and recruited to the promoters of DDR genes to promote transcription. Surprisingly, PRMT5-mediated activation of these genes was independent of the canonical cofactor MEP50 but dependent on pICln. Upon recognition of DSBs, repair proteins (such as RAD51, BRCA1, and BRCA2) are transiently upregulated to facilitate repair through HR or NHEJ. Although this transient upregulation is required for cell survival following genotoxic stresses, little is known how proteins are quickly upregulated to promote repair of DNA damage [43]. Upregulated PRMT5 expression by DNA damage and subsequent activation of DSB repair genes likely contribute significantly to DSB repair at the protein level as well.

PRMT5-regulated DSB repair genes appear to be conserved in multiple cancer and normal human cells [40]. However, PRMT5 may be particularly critical to DSB repair in prostate cancer cells given its regulatory role in

transcription of *AR* [16], due to the fact that AR transcriptionally regulates expression of several target genes involved in NHEJ (Fig. 2B) [44–46]. Targeting PRMT5 in prostate cancer cells decreased expression of AR and AR target genes involved in NHEJ (Ku80/XRCC5, XRCC4, and DNAPKcs/PRKDC). Furthermore, PRMT5-mediated regulation of Ku80/XRCC5, XRCC4, and DNAPKcs/PRKDC was stronger in AR-expressing prostate cancer cells compared to cells of other origins (such as breast cancer or glioblastoma). Therefore, in prostate cancer cells, PRMT5 can regulate the expression of DDR genes directly as well as indirectly through regulating AR expression. Indeed, the correlation between PRMT5 and AR target genes involved in NHEJ was stronger in prostate cancer compared to most other cancers [40]. Interestingly, DNAPKcs/PRKDC is both an AR and PRMT5 target gene in prostate cancer cells [40]. As PRMT5 does not contain any DNA binding domain, it remains to be determined whether AR also recruits PRMT5 to the promoter of DNAPKcs/PRKDC.

### Splicing regulation of DDR genes

PRMT5 methylates several spliceosome proteins and is a critical regulator of alternative splicing [47]. In 2017, Braun et al. [48] demonstrated that, at least in malignant glioma cells, PRMT5 could modulate gene expression by regulating the splicing out of detained-introns (DIs). Additionally, in 2019 Tan et al. [49] reported that in hematopoietic stem cells, PRMT5 maintains appropriate splicing activity to prevent aberrant intron retention and exon skipping. Further, genes annotated as “DNA repair” were enriched when functional enrichment analysis was performed on alternative splicing events upon PRMT5 knockdown in hematopoietic stem cells [49]. Although there was little overlap between these differentially spliced genes in hematopoietic stem cells [49] and the validated PRMT5 target genes involved in DDR in prostate cancer cells (RAD51, RAD51AP1, RAD51D, BRCA1, BRCA2, NHEJ1, DNAPKcs, and WEE1) [40], RNA-seq analysis also identified many other genes involved in DDR in prostate cancer cells. Future research is needed to determine if PRMT5 may also regulate the expression of DDR genes in prostate cancer cells via splicing.

### Regulation of DSB repair pathways

DSBs can be efficiently repaired regardless of pathway choice. However, pathway choice affects the speed and accuracy of the repair [50]. HR and NHEJ are the two major pathways for DSB repair. Acetylation of H4K16 (H4K16Ac) catalyzed by Tip60 can relax local chromatin and facilitate the displacement of 53BP1 from DSBs, enabling BRCA1 binding, RPA filament formation, and subsequent DSBs repair via HR.

Recently, it was discovered that in osteosarcoma cells, PRMT5 might promote the DSBs repair via promoting HR over NHEJ by methylating R205 on RUVBL1, a member of the Tip60 complex (Fig. 2D). Mechanistically, the methylation of RUVBL1 increases the Tip60’s acetylase activity resulting in increased H4K16ac. Additionally, PRMT5 is required for appropriate splicing of Tip60, which promotes Tip60 acetyltransferase activity, demotes the binding of 53BP1 to DSBs, and promotes HR [51]. PRMT5-deficient hematopoietic progenitor cells have reduced levels of full-length Tip60, reduced Tip60 acetyltransferase activity, and defective HR.

Although it remains to be determined if PRMT5 also regulates the appropriate splicing of Tip60 or methylates RUVBL1in prostate cancer cells, PRMT5 may likely have distinct roles in DSB repair choice in a cell type-dependent manner. For example, knockdown of RUVBL1 had no effect on IR-induced 53BP1 foci in hematopoietic cells [51], suggesting that the role of PRMT5 in DSB repair choice may be different in various cell types. In prostate cancer cells, PRMT5 activates transcription of *RAD51*, and targeting PRMT5 causes downregulation of RAD51 protein [40]. However, in osteosarcoma cells, depletion of PRMT5 impaired HR without affecting the expression of RAD51 [52]. Future studies may elucidate if PRMT5 regulates DSB repair choice via methylation of RUVBL1 or Tip60 splicing in prostate cancer cells.

In summary, it is clear that PRMT5 is a critical regulator of DDR in prostate cancer. PRMT5 regulates the function of other proteins associated with DDR (p53 [39, 53], E2F1 [54, 55], FEN1 [56], RAD9 [41], KLF4 [42], and TDP1 [57]). Future characterization of these PRMT5 substrates and identification of additional substrates and target genes will shed new light on both the epigenetic and non-epigenetic roles of PRMT5 in the DDR in prostate cancer cells.

## Regulation of PRMT5 activity and target gene expression by interacting proteins

PRMT5 can interact with several proteins in addition to MEP50, and these interacting proteins may regulate the enzymatic activity and substrate specificity [58–62]. Studies utilizing purified recombinant proteins suggest that MEP50 is an obligate cofactor of PRMT5 required for methyltransferase activity while proteins such as RioK1 and pICln alter substrate specificity [59, 62]. However, in the context of prostate cancer, pICln [17, 40], but not MEP50 [16], mediates PRMT5 activity towards histones at the promoters of AR and DDR genes.

Recently, we demonstrated that PRMT5 and pICln bind to the promoters of multiple DNA damage response genes to symmetrically dimethylate histone H4R3, and this recruitment of PRMT5/pICln and histone methylation is enhanced upon DNA damage [40]. Mechanistically, while overall expression of pICln and MEP50 did not change upon DNA damage, subcellular distribution of pICln and MEP50 changed oppositely: pICln accumulated in the nucleus while MEP50 localized in the cytoplasm. As pICln and MEP50 can both enhance PRMT5 activity and H4R3 methylation is involved in both activation and repression of target gene expression [4–6, 40], the selection of its interacting proteins may likely determine the activation or repression of PRMT5 target gene expression. Indeed, MEP50, pICln, and PRMT5 all bound to the promoter of PRMT5-repressed gene involucrin [63] and mediated repression of involucrin transcription. The transcriptional activation of target gene expression by PRMT5/pICln was also demonstrated in activation of *AR* transcription in CRPC cells [17]. It is also interesting to note that whereas only H4R3me2s was observed at the proximal promoters of *AR* and DDR genes, H4R3me2s, H3R2me2s, H3R8me2s, and H2AR3me2s were all detected at the proximal promoter of *IVL* [17, 40]. Thus, the composition of PRMT5-centered complexes and the types of histone arginine methylations will likely determine transcriptional activation versus repression of target genes. Genome-wide analysis of PRMT5 target genes and their relationship with its cofactors as well as histone modifications are needed in future studies.

## Interplay of PRMT5 with epigenetic regulators in prostate cancer cells

### PRMT5 and other arginine methyltransferases

Out of nine protein arginine methyltransferases, four were shown to monomethylate and asymmetrically dimethylate the same histone residues as PRMT5: H2AR3 by **PRMT7**, H3R2 by **PRMT6**, H3R8 by **PRMT2**, H4R3 by **PRMT1**, **PRMT6**, and **PRMT7**.

While **PRMT1** and PRMT5 deposit different types of methylation marks (asymmetrical vs symmetrical dimethylation) and possibly act in the opposite ways [64, 65], inhibition of both PRMT5 and PRMT1 had synergistic effect in lung and pancreatic cells [66]. However, their potential competition was not explored in the context of prostate cancer. It remains to be established if inhibition of both enzymes is a better therapeutic approach, especially in methylthioadenosine phosphorylase (MTAP)-deficient prostate cancer [67]. There is no direct evidence suggesting interplay between **PRMT2** and PRMT5, and their relationship remains to be investigated. **PRMT6** was suggested to be an oncogene in prostate cancer via activation of PI3K/Akt pathway, possibly by increasing asymmetrical dimethylation of H3R2 on the target gene promoters [68]. Interestingly, PRMT6 knockdown increased AR expression in prostate cancer cells, though whether PRMT6 directly regulates *AR* transcription via asymmetrical dimethylation of H3R2 or H4R3 remains to be determined [16]. However, PRMT5-driven symmetrical dimethylation of H3R2 enhanced the binding of H3R2 by the epigenetic reader WDR5 [18, 69], while asymmetrical dimethylation of H3R2 (mark that is catalyzed by PRMT6) prevented the binding of WDR5 in biochemical assay [70]. These observations suggest that PRMT5 and PRMT6 may play opposite roles via regulation of target gene expression by depositing different types of arginine methylation, at least on H3R2 in prostate cancer cells.

The role of **PRMT7** was not explored in the context of prostate cancer. However, PRMT7 expression is detected in prostate cancer (Protein Atlas), and PRMT7-mediated H4R17 monomethylation can allosterically promote PRMT5-mediated H4R3 dimethylation [71]. Additionally, multiple evidences suggest at least partial overlap of PRMT7 with PRMT5 function [18] while retaining unique substrates [72]. Future research elucidating genome-wide differential binding of PRMT5 and PRMT7 and their substrates in prostate cancer cells may establish whether co-targeting PRMT5 and PRMT7 is a better approach than targeting single enzyme.

Apart from functional overlap in the epigenetic regulation, other arginine methyltransferases might interact with PRMT5 via post-translational modification of non-histone substrates. **PRMT4** (also known as co-activator-associated arginine methyltransferase 1, CARM1) is an established co-activator of androgen receptor [73]. A 2006 study demonstrated that in hormone-naïve LNCaP cells, PRMT4 binding increased AR transcriptional activity and promoted cell proliferation and survival. Since PRMT5 may also function as AR co-activator, as discussed above [34], it will be beneficial to explore the possible combinational effect of PRMT4 and PRMT5 targeting on AR signaling.

### PRMT5 and lysine methylation

Histone lysine methylation is a post-translational modification that has been linked to a variety of cellular processes such as transcription regulation, DNA replication, and DNA repair [74]. In prostate cancer, **EZH2**, the enzymatic component of polycomb repressive complex 2 (**PRC2**), is an established oncogene [75]. Importantly, EZH2 functions as an oncogene in prostate cancer both as a part of PRC2 and in PRC2-independent manner. Notably, independently of PRC2, EZH2 may function as a co-activator

of AR-mediated transcription in LNCaP-abl model [76] and as an epigenetic activator of *AR* transcription in LNCaP cells [77] while in NEPC models EZH2 suppressed AR expression and signaling [78]. Thus, targeting EZH2 could be an effective approach to either repress AR signaling in AR-dependent prostate cancer or restore AR signaling in AR-independent prostate cancer. In fact, dual targeting of AR and EZH2 was explored in CRPC cell models [79]. While direct PRMT5/PRC2 or PRMT5/EZH2 interaction was not investigated in prostate cancer, in leukemia model, PRMT5 colocalized with PRC2 at the promoters of tumor suppressors *RBL2* and *ST7*, and PRMT5/PRC2 interaction was mediated by BRD7. This interaction was associated with transcriptional repression of *RBL2* and *ST7* [80]. Additionally, in lymphoma cells PRMT5 promotes expression of PRC2 through epigenetic repression of *RBL2* [81], which possibly can lead to even further suppression of genes co-regulated by PRMT5 and PRC2. Based on in vitro evidence, it was suggested that SUZ12 directly interacts with MEP50 to recruit PRMT5 to histone H2A substrate [82]. However, contrary to the evidence that PRMT5 cooperates with PRC2 to repress gene transcription, observation in AML cells demonstrated that PRMT5-driven histone H3R8 symmetric dimethylation prevented methylation of H3K27 by PRC2 and activated multiple gene expression [83]. Taken together, these observations suggest that interaction of PRMT5 and PRC2, and transcriptional outcome of this interaction is highly context-dependent. Given the importance of PRC2 and EZH2 in prostate cancer, it is imperative to investigate this interaction in detail. EZH2 also functions as an AR co-activator outside of PRC2 [84], and thus exploring the effect of co-targeting PRMT5 and EZH2 on AR signaling is warranted.

In addition to research on PRC2/PRMT5 axis, significant research effort was devoted to investigating the interplay of **WDR5** and PRMT5. WDR5 recruits H3K4 methyltransferase complexes MLL1–4 to chromatin and functions as an oncogene in prostate cancer to promote AR recruitment to its target genes [85]. Despite well-characterized functional interaction of PRMT5 and WDR5, this interaction has not been investigated in prostate cancer. It was demonstrated that PRMT5-driven methylation of H3R2 in vitro [70], in lymphoma [18], lung cancer [9], and ovarian cancer [86] cells enhances binding of WDR5 to promote H3K4 trimethylation and activation of target gene transcription. On the contrary, in erythroleukemia cells and bone marrow, PRMT5-driven recruitment of WDR5 lead to transcriptional repression of γ-globin gene expression [69] again suggesting context-dependent outcome of PRMT5-driven histone methylation. Elucidation of composition of PRMT5-containing protein complexes at the regulatory elements of target genes may shed light on this discrepancy.

## PRMT5 and lysine acetylation

The connection between histone acetylation and gene expression modulation was discovered as early as 1964, and since then, a variety of histone acetyltransferases and histone deacetylases was described [87]. **p300/CBP** is a well-known co-activator of AR transcriptional activity and was shown to be a potential driver of prostate cancer progression [88]. In prostate cancer cells, PRMT5 was present in the same complex with p300/CBP and nucleolin, and this interaction was facilitated by p300/CBP-interacting transactivator with E/D-rich carboxy-terminal domain-2 (CITED2) [89]. The formation of the complex promoted methylation and acetylation of nucleolin by PRMT5 and p300/CBP, respectively, and subsequent nucleolin nuclear export to promote AKT-mediated EMT and prostate cancer metastasis via increased translation of AKT mRNA. Interestingly, PRMT5- and p300/CBP-containing complex also included both RioK1 and MEP50, though their functional involvement remains to be determined.

Another lysine acetyltransferase demonstrated to interact with and be regulated by PRMT5 is **TIP60**, as discussed above. Notably, both TIP60 and p300/CBP acetylate H4K5. In vitro, acetylation of H4K5 enhances methylation of H4R3 by PRMT5/MEP50 complex [90]. However, another study suggested that non-acetylated histone H4 was methylated by purified PRMT5 in complex with Brg1 or hBrm more efficiently than H4K5-acetylated H4 [91]. The same study also found that PRMT5 was present in a complex containing c-Myc, histone deacetylase (HDAC) 2, and Brg1 on the two Myc target gene promoters to repress gene transcription, demonstrating the interplay between PRMT5-mediated histone methylation, histone acetylation/deacetylation, and chromatin remodeling. As Brg1 and PRMT5 bind to the *AR* promoter and catalyze H4R3 dimethylation to activate *AR* transcription [16], the status of H4K5 acetylation on the *AR* promoter requires further investigation.

Support for the interplay between histone methylation and acetylation also comes from a recent study demonstrating that in lymphoma, PRMT5-mediated H3R8 symmetric dimethylation is coupled with HDAC2- or HDAC3-mediated deacetylation at H2BK12, H3K9, H3K14, and H4K8 on the promoter regions of three target miRNAs [24] that regulate expression of cyclin D1 and c-Myc. Interestingly, methylation of H4R3 by PRMT5/MEP50 may also impact the acetylation of H4K5 by TIP60, at least in vitro [92]. In short, interplay between PRMT5-mediated methylation and other marks has been explored extensively; future research will likely provide more insight into complex epigenetic regulation. However, biochemical evidence from in vitro studies must be cautiously taken as epigenetic regulation is heavily dependent on chromatic status and protein complex composition in cells.

### PRMT5 and DNA methylation

DNA methylation, mediated by DNA methyltransferases (DNMTs), is essential for the maintenance of various cellular processes such as DNA repair, recombination, replication, and gene expression [93]. In cancer, including prostate cancer, alterations of DNA methylation often lead to promoter hypo- and hypermethylation at oncogenes and tumor suppressor genes, respectively [93]. Although changes of DNA methylation status were well described in prostate cancer, and accumulating evidence suggests connection between PRMT5 activity and such alterations, this relationship has not been explored in prostate cancer specifically.

The direct relationship between PRMT5-driven histone methylation and DNA methylation was first observed in erythroid cells [94]. In this study, H4R3 methylation by PRMT5 at the γ-globin promoter caused recruitment of **DNMT3a** to the same region via interaction of H4R3me2s with DNMT3a. Methylation of CpG islands in this region was PRMT5-dependent, a phenomenon also observed in gastric cancer [95]. However, it was not elucidated whether DNMT3a interacted with methylated histones or directly with PRMT5. In addition to potentially recruiting DNA methylases to its target regions, PRMT5 can also be recruited to already methylated DNA regions. In breast cancer cells, PRMT5/MEP50 was recruited to methylated DNA via interaction with methyl CpG binding domain 2 (**MBD2**) protein and coincided with increased methylation of histone H4R3 [96]. If both mechanisms are active in prostate cancer, it suggests a possible DNA methylation – histone H4R3 methylation positive feedback loop. Future research needs to investigate the relationship between other histone methylation marks mediated by PRMT5 and DNA methylation status.

## Therapeutic potential of PRMT5 targeting for prostate cancer treatment

Although prostate cancer is well managed with a 10-year survival of over 90% [97], localized high-risk prostate cancer and metastatic prostate cancer remain the major clinical challenge. The gold standard treatment for localized high-risk prostate cancer is RT in combination with ADT whereas metastatic HNPC patients are treated with the first line ADT, and CRPC patients are treated with the second generation of AR signaling inhibitors (ASI) abiraterone and enzalutamide [97]. Unfortunately, 30–50% of high-risk prostate cancers recur and can eventually progress to metastatic disease [97]. Despite the initial positive response of most HNPC to ADT, almost all HNPC cases progress to CRPC within 2 years. Further, evidence has emerged that nearly 20% of CRPC progress to NEPC after ASI [98]. NEPC is an aggressive cancer with a median survival of 1 year. Currently, NEPC is considered the end-stage of the disease with no effective treatment option. Additionally, significant adverse effects are associated with RT and ADT/ASI, diminishing the quality of life. Thus, there is an urgent need to develop novel treatment strategies or improve the existing treatment efficacy. Given the importance of PRMT5 in regulation of radiation-induced DSB repair and AR signaling in prostate cancer cells, targeting PRMT5 may be explored as a novel AR targeting approach for treatment of metastatic prostate cancer or as a radiosensitization approach for treatment of localized prostate cancer.

### Targeting PRMT5 as a monotherapy for prostate cancer treatment

AR is the major driver of prostate cancer development and progression, and targeting AR remains the mainstay of treatment for metastatic prostate cancer. Recent findings that PRMT5 regulates AR signaling at multiple levels discussed above suggest that targeting PRMT5 may be used as a novel approach to treat prostate cancer. Several inhibitors for PRMT5 have been developed by multiple labs [99–101]. Currently, five inhibitors are in clinical phase I trials in the US: GSK3326595, JNJ-64619178, PF-06939999, PRT543, and PRT811 (clinicaltrials.gov). GSK3326595 and JNJ-6461918 are catalytic inhibitors of PRMT5, with GSK3326595 competing with peptide substrates in the substrate binding pocket and JNJ-64619178 binding competitively with both the SAM and substrate binding pockets. These trials enroll patients with hematological malignancies and solid tumors and are expected to be complete in 2021–3.

Given that knockdown of PRMT5 significantly inhibited prostate cancer cell growth and suppressed xenograft tumor growth in mice [16, 17], it is possible that catalytic PRMT5 inhibitors may phenocopy the effect of PRMT5 knockdown in preclinical models. As PRMT5 not only regulates AR transcription but also regulates AR activity and target gene expression [34, 35], targeting PRMT5 with these inhibitors may likely offer additivity or synergy to current ADT and ASI therapies. As HNPC/CRPC growth and survival are dependent on AR signaling, targeting PRMT5 may similarly suppress the growth of CRPC cells via downregulation of the AR signaling. Interestingly, MTAP deletion, which renders cells heavily dependent on PRMT5, may represent a particularly useful patient stratum for PRMT5-specific therapy [102]. As 10–70% of prostate cancer tissues have MTAP deletion [67], this subset of patients may be effectively treated with PRMT5 inhibitors alone. Future preclinical evaluation of these PRMT5 inhibitors should include prostate cancer cells with and without MTAP

deletion. If these preclinical studies validate and confirm the therapeutic potential of PRMT5 inhibition, future clinical trials will enable evaluation of the therapeutic effect of these PRMT5 inhibitors to treat metastatic prostate cancer.

### PRMT5 targeting as a radiosensitization approach

RT is used to treat more than 50% of human cancers, and the clinical efficacy is limited by adverse toxicity to surrounding tissues. Co-administration of an effective radiosensitizer would decrease the IR dose required for the same therapeutic effect. For prostate cancer, ADT has been used as such a radiosensitizer, and several large clinical studies confirmed the clinical benefit of RT in combination with adjuvant or neoadjuvant ADT for localized high-risk disease when compared with RT or ADT alone [97]. Although the mechanism was initially unclear, recent studies suggest that AR transcriptionally activates several genes involved in NHEJ and HR [44–46].

Because PRMT5 epigenetically activates *AR* transcription in prostate cancer cells [16], Owens et al. [40] recently evaluated whether targeting PRMT5 can radiosensitize prostate cancer cells and discovered that PRMT5 functions as a master epigenetic activator of multiple DDR genes in an AR-independent manner as discussed above. Significantly, targeting PRMT5 by either knockdown or inhibition with their PRMT5 inhibitor BLL3.3 increased radiation-induced DSBs and increased cell death. Further, PRMT5 expression correlates positively with most of its DDR target genes in prostate and other cancers. Because PRMT5 regulates expression of DSB repair genes (HR, NHEJ, $G_2$ arrest) and the expression of AR, PRMT5 may be a more effective radiosensitization target as opposed to ADT for treatment of localized high-risk prostate cancer patients. Preclinical studies utilizing mouse models to mimic localized, high-risk prostate cancer could assess PRMT5 inhibition as a radiosensitization approach. If successful, PRMT5 targeting would represent a novel mode of action for control of this disease stage.

## Concluding remarks and future perspectives

The cloning of the human PRMT5 gene was reported in 1999 [103]. However, the role of PRMT5 in human cancers has only been reported in recent years. Results from our lab and several others have clearly suggested that PRMT5 plays a critical role in DDR and the AR signaling in prostate cancer. Notably, PRMT5 expression is significantly higher in prostate cancer tissues compared to benign hyperplasia, and expression of PRMT5 highly correlated with AR at both protein and mRNA level [16]. While PRMT5 was generally considered an epigenetic repressor of tumor suppressors and cell cycle regulators in human cancers, we have provided compelling evidence that PRMT5 also functions as an epigenetic activator of DDR genes and *AR* [16, 17, 40]. Consistent with our findings, several recent RNA-seq studies have also identified comparable number of genes that are activated or repressed by PRMT5 knockdown [83, 104], although whether these genes are direct targets of PRMT5 remains to be determined. Another surprising finding is identification of pICln, but not MEP50, as a potential cofactor of PRMT5 to transcriptionally activate DDR and AR gene expression.

As activation of *AR* transcription by PRMT5 requires the participation of Sp1 and Brg1 [16, 17], PRMT5 may likely form higher-order complexes with other transcriptional regulators such as transcription factors and chromatin remodelers to determine the transcriptional output, and in a locus-specific manner. Additionally, the interplay between PRMT5 and other epigenetic regulators (e.g., other PRMTs, HDACs, KMTs, DNMTs, Fig. 3) should have a similarly important role in controlling the transcription of PRMT5 target genes. Future research focusing on the analysis of PRMT5-centered and locus-specific complexes and the functional relationship with other transcriptional and epigenetic regulators will shed new light on the epigenetic role and mechanisms of PRMT5 in prostate cancer. Additionally, it remains to be investigated whether and how PRMT5 promotes prostate cancer growth and progression by regulating key prostate cancer modulators (Rb, p53, N-Myc, and EZH2 [4–6]) in the nucleus or through regulating other unidentified signaling molecules in the cytoplasm in prostate cancer [105]. Importantly, these mechanistic studies will likely offer new avenues for development of novel therapeutics. This may be particularly important given that targeting PRMT5 with the catalytic inhibitors may cause some unwanted side effects due to its many normal cellular roles [4–6]. Although targeted delivery of PRMT5 inhibitors could potentially circumvent this problem, identification of pICln as a novel cofactor of PRMT5 to activate transcription of AR and DDR genes suggests that targeting the interaction of PRMT5 with pICln may offer a specific and unique approach to treat prostate cancer as a monotherapy or as a radiosensitizer.

As our understanding of prostate cancer biology and pathology continues to evolve and new therapies are developed, exploration of PRMT5's role in the context of prostate cancer development and progression is warranted. One emerging clinical challenge is the development of ASI-induced NEPC. As most NEPC cells lose the expression or activity of AR [98], it would be essential to determine if PRMT5 targeting may promote NEPC development via AR downregulation. Conversely, targeting PRMT5 in combination with the current platinum chemotherapy may offer a better treatment approach for

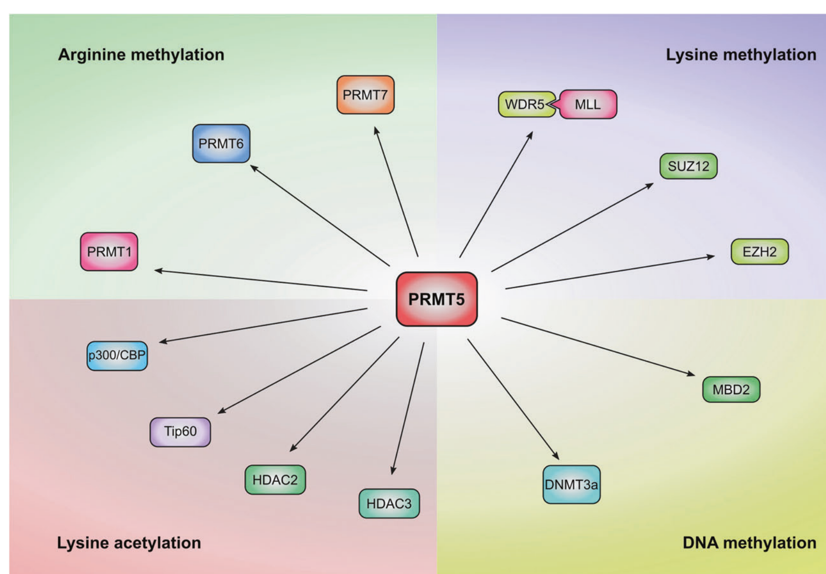


**Fig. 3 Potential interplay of PRMT5 with other epigenetic regulators.** PRMT5 can interact with and regulate multiple epigenetic regulatory molecules leading to various cellular effects in various types of cancer cells. Investigation of these interplays in the context of prostate cancer will shed new light on the roles and mechanisms of PRMT5 in prostate cancer.

NEPC. Likewise, co-targeting AR expression (PRMT5 inhibitors) and AR activity (e.g., abiraterone, enzalutamide, and darolutamide) may offer additive or even synergistic effect on the suppression of CRPC and prevention of progression to NEPC.

In conclusion, the role of PRMT5 in prostate cancer is beginning to emerge. The evidence discussed above clearly suggests that targeting PRMT5 may be explored as a novel AR targeting approach and as a radiosensitization approach. Future effort in elucidating the role and the underlying mechanism of PRMT5 in the context of prostate cancer development, progression, and therapeutic intervention will likely lead to development of novel and specific therapeutic approaches for prostate cancer management.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

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**2018 SBUR Abstract (Jake Owens Poster Presentation)**

**Title:** PRMT5 is a master epigenetic regulator of the DNA damage response and is a novel therapeutic target for prostate cancer radiosensitization

**Authors:** Jake L. Owens, Elena Beketova, Samantha Tinsley, Andrew Asberry, Sheng Liu, Xuehong Deng, Chenglong Li, Jun Wan, and Chang-Deng Hu

**Background:** Radiation therapy (RT) is one of two curative treatments for prostate cancer and kills cells by inducing double-strand breaks (DSBs) in DNA. Following RT, tumors regrow for about 60% of patients with high-risk disease within 5 years. The only clinical approach to enhance RT is androgen deprivation therapy (ADT), which targets androgen receptor (AR) signaling. The use of ADT is limited due to side effects; thus, identification of novel therapeutic targets to enhance RT will save lives of prostate cancer patients. We recently reported that PRMT5 epigenetically activates AR expression and our finding led us to investigate if targeting PRMT5 may enhance RT.
**Methods:** To evaluate if targeting PRMT5 may sensitize prostate cancer cells to radiation, we performed a clonogenic assay of irradiated cells. To determine if PRMT5 is required for repair of radiation-induced DSBs, we performed foci analysis in cancer and non-cancer cell lines via immunocytochemistry. We then used RNA-seq, qPCR, western blot, and ChIP to evaluate a potential epigenetic role of PRMT5 in activating the expression of genes critical to DSB repair. To extend our findings, we analyzed clinical data from tens-of-thousands of cancer patients encompassing 43 cancer types to assess if PRMT5 expression correlates with the expression of its putative target genes.
**Results:** We found that targeting PRMT5 sensitizes prostate cancer cells to radiation independent of the AR status. RNA-seq analysis identified genes that enriched in DSB repair and G2 arrest. Mechanistically, we determined that PRMT5 functions as an epigenetic activator of several genes involved in the DNA damage response (DDR). Upon radiation, PRMT5 is quickly upregulated and recruited to the promoters of DDR genes to activate gene expression, including BRCA1, BRCA2, and RAD51. Additionally, targeting PRMT5 decreases expression of these proteins and hinders repair of radiation-induced DSBs in multiple cell types, suggesting that PRMT5 plays a conserved role in DDR. Clinically, PRMT5 expression positively correlates with the expression of putative target genes involved in DSB repair across all 43 cancer types analyzed.
**Conclusions:** PRMT5 acts as a master epigenetic activator of genes involved in DDR and is critical for cells to survive radiation treatment. Given that PRMT5 is often overexpressed in cancer and its expression correlates with its target genes involved in DSB repair. Our findings suggest that PRMT5 is a therapeutic target in cancer to improve radiation therapy.

**2018 SBUR Abstract (Elena Beketova Poster Presentation)**

**Title:** PRMT5 promotes androgen receptor transcription in castration resistant prostate cancer cells.

**Authors:** Elena Beketova, Jake L. Owens, Andrew Asberry, Sheng Liu, Xuehong Deng, Chenglong Li, Jun Wan, and Chang-Deng Hu

**Background:** Metastatic prostate cancer is commonly treated with the first-line treatment option androgen deprivation therapy (ADT). Following ADT, the disease inevitably progresses within 18-24 months to a lethal stage called castration resistant prostate cancer (CRPC). In general, androgen receptor (AR) reactivation is the major cause of CRPC, and it occurs through multiple mechanisms such as AR gene amplification, expression of androgen-independent mutants or ligand-independent splice variants, and intratumoral androgen synthesis. Current CRPC therapies that target androgen synthesis or full-length AR are not curative and only prolong survival by 4-5 months. Thus, development of novel therapeutic approaches for CRPC treatment is in urgent need. Recently it was demonstrated that protein arginine methyltransferase 5 (PRMT5), an emerging oncogene in various cancers that symmetrically dimethylates arginine residues of numerous substrates, regulates hormone-naïve prostate cancer (HNPC) growth in AR-dependent manner. Mechanistically, it was demonstrated that PRMT5 epigenetically activates AR transcription. Considering the role of AR signaling in CRPC and that PRMT5 regulates AR in HNPC, we aimed to determine whether PRMT5 also regulates expression of AR in CRPC.

**Methods:** Short hairpin RNA (shRNA) against PRMT5 and small molecule inhibitor BLL3.3 were used to target PRMT5 in CRPC cell lines C4-2 (AR overexpression), 22Rv1 (AR-V7 expression) and VCaP (AR gene amplification). AR and AR target genes expression were analyzed using Western Blot and/or RT-qPCR. Cell proliferation was measured using MTT assay. Chromatin immunoprecipitation was used to analyze presence of PRMT5 and PRMT5-mediated methylation marks at the proximal AR promoter. 22Rv1 lines with doxycycline-inducible expression of PRMT5-targeting shRNA and control shRNA were established for use in xenograft studies.

**Results**: PRMT5 targeting reduced cell proliferation and downregulated the expression of both AR full length and V7 at the protein and mRNA levels in all CRPC cell lines tested. Consistently, expression of AR target genes regulated by either full length AR or AR-V7 was decreased. PRMT5 and H4R3me2s were present at the AR proximal promoter and decreased upon PRMT5 knockdown. PRMT5 knock down significantly reduced growth of 22Rv1 xenografts in castrated NRG male mice.

**Conclusions**

Taken together, these results suggest that PRMT5 acts as epigenetic activator for both full length and spliced variants of AR in CRPC cells. Basing on these findings, we propose that targeting PRMT5 may present a novel treatment approach for CRPC via eliminating the expression of AR and its splice variants.

**2019 SUBR (Elena Beketova Oral Presentation)**

**Title:** Targeting PRMT5 as a novel approach for the treatment of castration-resistant prostate cancer

**Authors:** Elena Beketova, Jake L. Owens, Andrew Asberry, Sheng Liu, Xuehong Deng, Chenglong Li, Jun Wan, and Chang-Deng Hu

**Background:** Emergence of castration-resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT) is one of the biggest challenges in prostate cancer therapy. Androgen receptor (AR) reactivation via various mechanisms is the driver of the ADT resistance. Current CRPC therapies that target AR signaling are not curative and only prolong survival by 4-5 months. Thus, the development of novel approaches for CRPC treatment is in urgent need.

Recently it was shown that protein arginine methyltransferase 5 (PRMT5), an emerging epigenetic enzyme and putative splicing regulator, is required for the hormone-naïve prostate cancer (HNPC) growth. Mechanistically, it was demonstrated that in HNPC PRMT5 epigenetically activates AR transcription. Considering the role of AR in CRPC and that PRMT5 regulates AR in HNPC, we aimed to determine whether PRMT5 regulates AR expression in CRPC.

**Methods:** shRNA against PRMT5 and inhibitor BLL3.3 were used to target PRMT5 in CRPC cells C4-2 (AR overexpression), 22Rv1 (AR-V7 expression) and VCaP (AR gene amplification). Transcriptome-wide gene expression was measured via RNA-seq. AR and AR target genes expression were analyzed using Western Blot and RT-qPCR. Cell proliferation was measured using MTT assay. Chromatin immunoprecipitation was used to analyze presence of PRMT5 and associated histone methylation marks at the AR promoter. 22Rv1 lines with shRNA inducible expression were established for use in xenograft studies.

**Results:** PRMT5 targeting reduced cell proliferation and decreased the protein and mRNA levels of both AR full length and V7 in all CRPC cell lines tested. Consistently, expression of full length AR or AR-V7 target genes was decreased. PRMT5 and H4R3me2s were present at the AR promoter. To further explore the role of PRMT5 in CRPC, we performed RNA-seq analysis in 22Rv1 upon PRMT5 knockdown. Interestingly, 293 genes were down- and 329 genes were upregulated upon PRMT5 knockdown contrary to the common perception of PRMT5 as an epigenetic suppressor. Additionally, exon mapping revealed differential up- and down-regulation of AR isoforms in PRMT5 knockdown samples suggesting that PRMT5 regulates AR splicing. PRMT5 knockdown significantly reduced the growth of 22Rv1 xenografts in castrated NRG male mice.

**Conclusions:** Our results suggest that PRMT5 acts as a regulator of AR expression in CRPC cells via both epigenetic regulation of transcription and mRNA splicing. Based on these findings, we propose that targeting PRMT5 may present a novel treatment approach for CRPC via eliminating AR and its splice variants expression.

**2019 SBUR (Jake Owens Poster Presentation)**

**Title:** PRMT5 cooperates with pICln to function as a master epigenetic activator of DNA double-strand break repair genes

**Authors:** Jake L. Owens, Elena Beketova, Samantha Tinsley, Andrew Asberry, Sheng Liu, Xuehong Deng, Chenglong Li, Jun Wan, and Chang-Deng Hu

**Background:** Efficient repair of DNA double-strand breaks (DSBs) is critical for cell survival. Upon recognition of DSBs, repair proteins are upregulated and recruited to the sites of damage to facilitate repair. Although the recruitment and action of repair proteins are well characterized, little is known about how their expression is induced upon DNA damage. Targeting repair proteins is a common approach for cancer treatment, therefore proteins responsible for the upregulation of repair proteins are potential therapeutic targets to improve DNA-damaging therapies.

Protein arginine methyltransferase 5 (PRMT5) is an enzyme involved in cancer development and progression. MEP50 is believed to be the obligate cofactor of PRMT5 and required for PRMT5 activity. Here, we determined that targeting PRMT5 sensitizes prostate cancer cells to DNA damage. However, further studies including RNA-seq and analysis of clinical patient data suggested PRMT5 also plays a conserved role in repair of DSBs independently of MEP50 and be a therapeutic target across cancer.

**Methods:** Clonogenic assays and foci analysis via immunocytochemistry were used to assess if targeting PRMT5 sensitizes prostate cancer cells to DNA damage. To determine if PRMT5, along with interacting proteins, acts as an epigenetic activator of DNA damage response (DDR) genes, we used RNA-seq, qPCR, western blot, and ChIP. To extend our findings, we analyzed clinical cancer patient data from The Cancer Genome Atlas Pan-Cancer analysis.

**Results:** Here, we characterize pICln as a novel epigenetic cofactor of PRMT5. Under normal conditions, a complex involving PRMT5:pICln maintains basal expression of DDR genes. DNA damage induces both PRMT5 protein upregulation and pICln nuclear translocation which leads to an increase in the PRMT5:pICln interaction in the nucleus. The PRMT5:pICln complex is then recruited to the promoters of genes involved in the DDR where it epigenetically activates expression of target genes. PRMT5:pICln target genes, including repair proteins, are then upregulated at the mRNA and protein level to facilitate the repair of DSBs. In clinical cancer data sets, both PRMT5 and pICln, but not MEP50, correlated positively with DDR genes in almost all cancers independently of any correlation with AR.

**Conclusions:** PRMT5 cooperates with pICln and independently of its obligate cofactor MEP50 to function as a master epigenetic activator of DDR genes. Targeting PRMT5/pICln may be explored in combination with DNA-damaging therapies for cancer treatment.

# Curriculum Vitae

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## Education / Degrees Awarded:

9/1979-7/1984: Bachelor in Medical Science (Equivalent to ***M.D.***)
Faculty of Medicine, Bengbu Medical College, Bengbu, China
9/1984-7/1987: ***M.S.*** (Cancer Immunology)
Department of Microbiology and Immunology, College of Medicine, Tongji Medical University, Wuhan, China
4/1994-3/1997: ***Ph. D***. (Molecular Biology)
Department of Physiology II, Kobe University School of Medicine, Japan

## Research/Working Experience:

9/1984-7/1987: ***Graduate Student (M.S.)*** in the Department of Microbiology & Immunology, Tongji Medical University, Wuhan, China.
Study of anti-tumor mechanisms of a new Chinese herb in cell culture and animal models.
7/1987-9/1991: ***Lecturer*** in the Department of Epidemiology, School of Public Health, Tongji Medical University, Wuhan, China.
(1). Mutagenicity of trichloromethane in drinking water
(2). Epidemiological investigation of drinking water and cancer incidence in Wuhan, China.
9/1991-3/1994: ***Visiting Research Associate*** in the Department of Molecular Oncology, Kyoto University School of Medicine, Kyoto, Japan.
(1). Spontaneous and induced acquisition of tumorigenicity in nude mice by lymphoblastoid cell line derived from patients with xeroderma pigmentosum group A.
(2). Subtractive isolation of genes contributing to the acquisition of tumorigenicity by lymphoblastoid cell line derived from xeroderma pigmentosum group A patient.
4/1994-3/1997: ***Graduate Student (Ph.D.)*** in the Department of Physiology II, Kobe University School of Medicine, Kobe, Japan
(1). Identification of cysteine-rich domain in Raf-1 as a novel Ras binding domain for activation by Ha-Ras and Rap1A.

(2). Activation mechanisms of Ras effectors (Raf-1, B-Raf, adenylyl cyclase).

4/1997-8/2000: ***Assistant Professor*** in the Department of Physiology II, Kobe University School of Medicine, Kobe, Japan.

(1). Differential regulation of Raf kinase activity by Ha-Ras and Rap1A.

(2). Identification and characterization of novel Ras effectors, (RalGDS, AF-6, PLC-ε) and regulators (RA-GEF-1, RA-GEF-2).

(3). Activation mechanisms of Ras effectors.

9/2000-6/2003: ***Research Investigator/Specialist*** in the Department of Biological Chemistry and Howard Hughes Medical Institute, University of Michigan School of Medicine.

(1). Development of bimolecular fluorescence complementation (BiFC) and multicolor BiFC assays for visualization of protein-protein interactions in living cells.

(2). Functional analysis of cross-family transcription factor interactions among bZIP, Rel, Smad and Myc/Max families.

7/2003-6/2009: ***Assistant Professor*** in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.

(1) Development and improvement of BiFC-based technologies

(2) BiFC analysis of AP-1 dimers in living cells and *C. elegans*

(3) AP-1 in prostate cancer development and therapeutic responses

7/2009- 7/2015: ***Associate Professor*** (tenured) in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.

(1) Development and improvement of BiFC-based technologies

(2) AP-1 in prostate cancer development and progression

(3) Mechanisms and targeting of radiation-induced neuroendocrine differentiation in prostate cancer

(4) Protein arginine methyltransferase 5 (PRMT5) in prostate cancer development, progression and therapeutic response

8/2015- present: ***Professor*** (tenured) in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.

(1) Mechanisms and targeting of radiation-induced neuroendocrine differentiation (NED) in prostate cancer

(2) Role and targeting of protein arginine methyltransferase 5 (PRMT5) in castration resistant prostate cancer (CRPC) and neuroendocrine prostate cancer (NEPC)

(3) Development of high throughput screens for small molecule inhibitors targeting protein-protein interactions

(4) Development of BiFC-based cDNA library screens for interacting proteins

08/2013-present: Program Co-Leader of the Cell Identity and Signaling (CIS) program of the Purdue University Center for Cancer Research (PCCR)

08/2013-present: Executive Committee Member of PCCR

08/2010-present: Co-Leader of the Prostate Cancer Discovery Group of PCCR
2011-2018: Director of Pharmacy Live Cell Imaging Facility (PLCIF)
2016-present: Director of Small Animal Radiation Facility (PCCR)
7/2016-present: Showalter Faculty Scholar of Purdue University
7/2020- Steve and Lee Ann Taglienti Chair in Pharmacy

## Current Professional Memberships

| | |
|---|---|
| 2001- Present | American Association for Cancer Research |
| 2009- Present | Society for Basic Urological Research |
| 2010- Present | American Urological Association |
| 2015-present | Radiation Research Society |

## Awards:

| | |
|---|---|
| 09/91-09/92: | Fellowship of JSPS<br>Source: **J**apan **S**ociety for the **P**romotion of **S**cience (JSPS) |
| 09/92-09/93: | Kyoto University Alumni Fellowship<br>Source: Kyoto University |
| 04/94-03/97 | Senshukai Scholarship (Ph.D. student)<br>Source: Kobe Senshukai Scholarship Foundation |
| 04/98-03/99 | President Young Investigator Award<br>Source: Kobe University |
| 04/98-03/99 | Young Investigator Award<br>Source: JSPS |
| 04/99-03/01 | Young Investigator Award<br>Source: Hyogo Prefecture Science and Technology Association |
| 07/03-08/06 | Walther Assistant Professor |
| 07/16-06/21 | University Showalter Faculty Scholar Award of Purdue University |
| 04/17 | Pharmaceutical Sciences Teacher of the Year in the College of Pharmacy |
| 10/17 | Seed for Success Award (EVPRP) |
| 5/18 | Lafayette Lions Club Award for Outstanding Achievements in Cancer Research (State Award) |
| 5/19 | 2019 Chaney Faculty Scholar Award (Research Award in the Purdue University College of Pharmacy) |
| 2020 | Award for Exceptional Teaching and Instructional Support during the COVID-19 Pandemic |

## Professional Services:

### *Reviewer for Grant Applications*

2004 Reviewer of MAES (The Maryland Agricultural Experiment Station at the University of Maryland)

| | |
|---|---|
| 2005 | Reviewer for NSF Advisory Panel for Molecular and Cell Biology |
| 2006-2008 | American Heart Association (MCB Panel) |
| 2007-2011 | Qatar National Research Fund (QNRF) |
| 2008 | UK Cancer Research |
| 2008 | UK Diabetes |
| 2009 | Welcome Trust |
| 2008-present | Pennsylvania Department of Health (PADOH) |
| 2010-2019 | Department of Defense, Prostate Cancer Research Program (Immunology, Endocrine, Experimental Therapeutics panels) |
| 2015-present | Florida Department of Health |
| 2015 | NIH, RTB study section (IAR) |
| 2016 | NCI (DP5) |
| 2019/2020 | NIH, RTB study section (March and July) |
| 2021 | NIH, DMP (March), OTB (June) |

***Reviewer for Professional Journals***

Combinatory Chemistry and HTS, Zebrafish, Journal of Biological Chemistry, Molecular and Cellular Biology, Nature Biotechnology Nature Methods, Molecular Cell, Molecular Biology of the Cell, PNAS, BMC Biotechnology, BMC Biology, Biotechniques, Biochemistry, ACS Chemical Biology, Chemistry & Biology, Journal of Innovative Optical Health Sciences, TIBS, TIBT, Current Cancer Drug Targets, Journal of Cell Science, PLoS One, Ontarget, Oncogene, Redox Biology, Cancer Letters, and etc

***Editorial Board Member:***

2007- Perspective in Medicinal Chemistry
2011- American Journal of Cancer Research
2013- Journal of Biological Methods (Founding Editorial Member)
2014- Frontier in Surgical Oncology (review editor)
2015- Journal of Drug Research and Development

***Organizer/Program Committee Member/Session Chair of Conferences, Symposiums, and Workshops***

- Organizer of Tristate Worm Meeting at Purdue (2006)
- Session Chair of Optical Molecular Imaging of the 2008 PIBM
- Session Chair of Imaging Technology Symposium of the 2008 4$^{th}$ Modern Drug Discovery and Development Summit
- Program Member of the 2009 PIBM Program Committee
- Organizer of 2010 Bimolecular Fluorescence Complementation Workshop (Purdue University)

- Member of the Scientific Program Committee and Moderator of Breakout Panel Discussion of the 2013 Drug Discovery Chemistry-Sixth Annual Protein-Protein Interactions, San Diego
- Organizer, Program Committee Member and Session Chair of the 2013 Hefei Prostate Cancer Translational Medicine and Personalized Medicine Symposium
- Session Co-chair of the 2016 Spring SBUR Symposium
- Session Co-chair of the 2019 Fall SBUR Symposium

***Member of Big Ten Cancer Research Consortium (BTRC) GU Clinical Trial Working Group*** (2013-present)

***Consultation on BiFC technology***

Since 2003, we have been providing BiFC plasmids, letters of support and consultations to many BiFC users worldwide. The lab provided BiFC plasmids to more than 200 labs prior to 2007. To facilitate the request process, we deposited 11 BiFC plasmids to Addgene in 2007, and 2282 samples have been distributed via Addgene as of August 1, 2019.

## Invited Seminars/Presentations

07/27/21 — Place: Brooke Army Medical Center, Department of Clinical Investigation (San Antonio)
Title: Neuroendocrine differentiation: An emerging mechanism of therapy resistance and tumor recurrence

03/18/21 — Place: 2021 6th International Conference on Pharmacy and Pharmaceutical Science (Tokyo)
Title: Protein arginine methyltransferase 5 (PRMT5): An emerging oncogene and therapeutic target in prostate cancer
**Invited Keynote Speaker** (http://www.icpps.org/keynote.html)

09/15/20 — Place: 2020 5th International Conference on Pharmacy and Pharmaceutical Science (Tokyo)
Title: Treatment-induced neuroendocrine differentiation in prostate cancer: Therapeutic challenges and opportunities
**Invited Keynote Speaker** (http://www.icpps.org/keynote.html)

07/08/19 — Place: Purdue-SEU Biotechnology and Data Science Symposium
Title: Bimolecular fluorescence complementation (BiFC): From single molecular visualization to genome-wide investigation

06/07/18 — Place: Department of Radiation Oncology, Chinese University of Sciences and Technology First Affiliated Hospital
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation

05/31/18 — Place: Jinan University School of Medicine
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation

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| 05/30/18 | Place: Sun Yat-sen University Cancer Center<br>Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance |
| 05/24/18 | Place:  Department of Urology, Wannan Medical College Yiji Shan Hospital<br>Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation |
| 03/28/18 | Place: Utsunomiya University Center for Biosciences Research and Education<br>Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation |
| 03/19/18 | Place: Xuhui Hostpital of Fudan University Zhongshan Hospital<br>Title: Neuroendocrine differentiation of prostate cancer: From basic research to drug discovery |
| 03/12/18 | Place: Bengbu College of Medicine<br>Title: Neuroendocrine differentiation of prostate cancer: Translational medicine research and training of physician scientists |
| 09/14/17 | Place: University of Colorado Denver Cancer Center<br>Title: Neuroendocrine differentiation: Am emerging mechanism of therapy resistance and tumor recurrence |
| 07/04/17 | Place: China Jiliang University School of Pharmacy<br>Title: Title: Title: Bimolecular fluorescence complementation (BiFC): From basic research to drug discovery |
| 06/16/17 | Place: Hong Kong University School of Chinese Medicine<br>Title: Bimolecular fluorescence complementation (BiFC): From basic research to drug discovery |
| 06/12/17 | Place: Jinan University School of Medicine<br>Title: Protein arginine methyltransferase 5 (PRMT5): An emerging oncogene and therapeutic target in prostate cancer |
| 05/15/17 | Place: Northwesten University School of Medicine, Department of Pathology<br>Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance |
| 10/11/2016 | Place: Chromatin and Epigenetics Symposium (Purdue)<br>Title: PRMT5 is a master epigenetic activator of DNA damage response and a therapeutic target for prostate cancer radiosensitization (presented by Jake Owens) |
| 05/10/16 | Place: 2016 American Urological Association (AUA) meeting<br>Title: Protein arginine methyltransferase 5 (PRMT5) is a novel epigenetic regulator of androgen receptor in prostate cancer |
| 01/07/16: | Place: Jinan University the first affiliated hospital<br>Title: How to conduct scientific research |
| 12/27/15: | Place: Northwest University of Agriculture and Forestry<br>Title: Bimolecular fluorescence complementation (BiFC): Current status and future perspectives |
| 01/05/15: | Place: Tongling First People's Hospital |

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| | Title: Advances in prostate cancer diagnosis and treatment- A comparative analysis between China and America |
| 12/29/14 | Place: Jinan University the first affiliated hospital<br>Title: Targeting PRMT5 for prostate cancer radiosensitization |
| 05/18/14 | Place: Mayo Clinic, Departments of Radiation Oncology<br>Title: Mechanism and targeting of radiotherapy-induced neuroendocrine differentiation for prostate cancer treatment |
| 03/25/14 | Place: Tongling 4th Hospital, Wannan Medical College<br>Title: Advances in prostate cancer diagnosis and treatment |
| 02/27/14 | Place: UCLA, Departments of Pathology and Laboratory Medicine<br>Title: Targeting neuroendocrine differentiation as a novel radiosensitization approach for prostate cancer treatment |
| 10/9//13 | Place: Cancer Hospital, Hefei Institutes of Physical Science Chinese Academy of Sciences<br>Title: Development of radiosensitizers: An urgent need for prostate cancer radiotherapy |
| 05/24/13 | Place: Hefei Chinese Academy of Sciences Cancer Hospital<br>Title: Impact of neuroendocrine differentiation in prostate cancer radiotherapy |
| 05/20/13 | Place: Huazhong University of Science and Technology Union Hospital Cancer Institute<br>Title: Radiation-induced neuroendocrine differentiation in prostate cancer: From bench to bedside |
| 05/17/13 | Place: Jinan University School of Medicine<br>Title: Neuroendocrine differentiation (NED) in prostate cancer cells: From basic science to clinical practice |
| 05/14/13 | Place: Northwestern Agriculture and Forestry University (NWAFU): 2013 Purdue-NWAFU Center Symposium<br>Title: Bimolecular fluorescence complementation (BiFC): Current Status and Future Perspectives |
| 04/17/13 | Place: 2013 Drug Discovery Chemistry in San Diego: Sixth Annual Protein-Protein Interactions (Targeting PPI for Therapeutic Interventions)<br>Title: Bimolecular fluorescence complementation (BiFC) as a novel imaging-based screening for inhibitors of protein-protein interactions. |
| 02/05/13 | Place: Tongji Hospital, Huazhong University of Science and Technology<br>Title: Neuroendocrine differentiation (NED): A therapeutic challenge in prostate cancer management |
| 10/25/12 | Place: Wright State University Department of Biochemistry and Molecular Biology<br>Title: Bimolecular fluorescence complementation (BiFC): An imaging tool for visualization of molecular events |
| 06/06/12 | Place: Jiangshu University School of Medical Technology and Laboratory Medicine |

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| | Title 1: Mechanisms and targeting of radiation-induced neuroendocrine differentiation |
| | Title 2: Bimolecular fluorescence complementation (BiFC): Past, Present and Future |
| 06/4/12 | Place: Chinese Academy of Sciences (Hefei) |
| | Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future |
| 05/31/12 | Place: Tongling Traditional Chinese Medicine Hospital |
| | Title: Recent advances in prostate cancer diagnosis and treatment |
| 05/18/12 | Place: Shanghai Center for Plant Stress Biology of Chinese Academy of Sciences |
| | Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future |
| 04/25/12 | Place: University of Western Ontario |
| | Title: Radiotherapy-induced neuroendocrine differentiation: Implications in prostate cancer progression and treatment |
| 03/13/12 | Place: Mayo Clinic Department of Urology |
| | Title: Mechanisms and targeting of therapy-induced neuroendocrine differentiation for prostate cancer treatment |
| 07/11/11 | Place: Jinan University Medical School |
| | Title: Bimolecular fluorescence complementation: An emerging technology for biological research |
| 07/10/11 | Place: Sun-Yat-sun University Medical School |
| | Title: Mechanisms and targeting of therapy-resistant prostate cancer |
| 02//09/11 | Place: Tulane University Medical School |
| | Title: Mechanisms and targeting of therapy-resistant prostate cancer |
| 01/17/11 | Place: Penn State University College of Medicine |
| | Title: Bimolecular fluorescence complementation (BiFC): Current Challenges and Future Developments |
| 12/07/10 | Place: Purdue University BiFC Workshop |
| | Title: Bimolecular fluorescence complementation: principle, experimental design and data analysis |
| 11/18/10 | Place: UT Austin College of Pharmacy |
| | Title: Bimolecular fluorescence complementation (BiFC) analysis of AP-1 dimierzation in living cells and *C. elegans* |
| 09/28/10 | Place: Nanjing University Medical School |
| | Title: Multicolor bimolecular fluorescence complementation (BiFC): A novel high throughput screening method for protein-protein interactions |
| 09/25/10 | Place: Wannan Medical College |
| | Title: Mechanisms and targeting of therapy-resistant prostate cancer |
| 09/16/10 | Place: Wuhan Institute of Virology |

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| | Title: | Bimolecular fluorescence complementation (BiFC): Current Status and Future Perspectives |
| 09/13/10 | Place: | Beijing University Cancer Hospital |
| | Title: | Mechanisms and targeting of therapy resistant prostate cancer |
| 09/08/10 | Place: | Purdue University BIG Symposium |
| | Title: | Fluorescence complementation: An emerging tool for visualization of molecular events in living cells and animals |
| 10/16/09 | Place: | Southern China Agriculture University |
| | Title: | Principle and applications of bimolecular fluorescence complementation (BiFC) |
| 10/19/09 | Place: | Sun Yat-sen University Zhongshan Medical School |
| | Title: | Principle and applications of bimolecular fluorescence complementation (BiFC) |
| 10/26/09 | Place: | Bengbu Medical College |
| | Title: | Principle and applications of bimolecular fluorescence complementation (BiFC) |
| 10/28/09 | Place: | Nanjing University Medical School |
| | Title: | Seeing is believing: visualization of protein-protein interactions using bimolecular fluorescence complementation (BiFC), |
| 05/07/09 | Place: | University of Chicago Graduate Program of Physiology |
| | Title: | Bimolecular fluorescence complementation (BiFC) analysis in living cells and living animals, |
| 02/02/09 | Place: | Indiana University Medical School, Department of Biochemistry |
| | Title: | Ionizing radiation-induced neuroendocrine differentiation: implication in prostate cancer therapy |
| 12/08/08 | Place: | University of Virginia Cancer Center |
| | Title: | Ionizing radiation-induced neuroendocrine differentiation: implication in prostate cancer therapy |
| 11/25/08 | Place: | $7^{th}$ International Conference on Photonics and Imaging in Biology and Medicine (Wuhan, China), Nov 24-27, 2008 |
| | Title: | Fluorescence complementation: an emerging technology in biomedical research (presentation and panel discussion) |
| 10/15/08 | Place: | $4^{th}$ Modern Drug Discovery & Development Summit (San Diego, 10/15/08-10/17/08) |
| | Title: | Multicolor bimolecular fluorescence complementation in drug discovery |
| 11/29/07 | Place: | UMDNJ-SOM Stratford |
| | Title: | Bimolecular fluorescence complementation (BiFC) analysis of AP-1 dimerization in living cells and living animals |
| 11/28/07 | Place: | The Children's Hospital of Philadelphia and the University of Pennsylvania |
| | Title: | Molecular regulation and targeting of ATF2 nucleocytoplasmic shuttling |

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| 11/13/07 | Place: Department of Biochemistry, Purdue University<br>Title: AP-1 biology, pathology, and technology |
| 10/30/07 | Place: Fluorescent proteins and Biosensors Symposium at HHMI Janelia Farm<br>Title: BiFC-FRET, a novel assay for visualization of ternary complexes in living cells |
| 08/07/07 | Place: International Microscopy & Microanalysis 2007 at Ft. Lauderdale<br>Title: Bimolecular fluorescence complementation (BiFC) and beyond |
| 02/09/07 | Place: Montana State University Department of Microbiology<br>Title: Functional analysis of AP-1 dimerization by bimolecular fluorescence complementation |
| 11/01/06 | Place: Vanderbilt University Institute of Chemical Biology<br>Title: Visualization of AP-1 protein interactions in living cells and in living animals using an improved BiFC system |
| 10/04/06 | Place: University of Illinois at Chicago School of Medicine<br>Title: Bimolecular fluorescence complementation: principle and applications |
| 07/17/06 | Place: Huazhong University of Science and Technology Tongji Medical College<br>Title: Bimolecular fluorescence complementation: principle and applications |
| 03/14/06 | Place: University of Toronto Western Research Institute<br>Title: Visualization of AP-1 protein interactions in living cells and in living animals using an improved BiFC system |
| 09/30/05 | Place: Eli Lilly, Indianapolis<br>Title: Identification of new fluorescent protein fragments for BiFC analysis under physiological conditions |
| 03/10/05 | Place: Purdue University, School of Health Science, Purdue University<br>Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions |
| 09/02/04 | Place: Illinois State University, Department of Biology<br>Title: Role of *C. elegans* Fos and Jun homologs in development. |
| 08/13/04 | Place: Cold Spring Harbor (Cold Spring Harbor Image Course)<br>Title: Seeing is believing: visualization of transcription factor interactions in living cells and in living animals using a novel using bimolecular fluorescence complementation (BiFC) approach |
| 05/07/04 | Place: Purdue University, Department of Chemistry<br>Title: Seeing is believing: visualization of transcription factor interactions in living cells and in living animals |
| 01/14/04 | Place: Purdue University, Department of Biological Science<br>Title: Seeing is believing: visualization of transcription factor interactions in living cells and in living animals |

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| 12/04/03 | Place: | Indiana University at Bloomington, Department of Biology |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions |
| 11/07/03 | Place: | Purdue Cancer Center (Purdue Cancer Center Director's Advisory council) |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions in cancer research |
| 09/04/03 | Place: | Purdue Cancer Center (Annual Scientific Retreat) |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions |
| 03/11/03 | Place: | Cincinnati Children's Hospital, Division of Experimental Hematology |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells |
| 03/04/03 | Place: | Harvard Medical School, MGH, Laboratories of Photomedicine |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells |
| 02/24/03 | Place: | Medical University of South Carolina, School of Pharmacy Department of Pharmaceutical Science |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells |
| 02/19/03 | Place: | University of Texas M.D. Anderson Cancer Center, Department of Molecular Therapeutics |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells |
| 02/06/03 | Place: | Ohio State University, School of Medicine Department of Physiology and Cell biology |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells |
| 12/28/02 | Place: | Purdue University Cancer Center |
| | Title: | Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells |
| 07/20/00 | Place: | Bengbu Medical College, Bengbu, China |
| | Title: | Recent progress in the activation mechanisms of Raf by Ras |
| 07/15/00 | Place: | Tongji Medical University, Wuhan, China |
| | Title: | Cloning and functional characterization of a novel type phospholipase C (PLC-ε) |

## Development of Intellectual Property

- A novel fluorescent protein for protein-protein interaction studies, 65557.P1.US Patent filed on July 16, 2010

- Methods for identifying protein-protein interactions, 66261-01-2013 US Patent filed on June 13, 2013
- Methods for identifying protein-protein interactions, 66261-02-2014 US Patent filed on June 14, 2014
- Bimolecular fluorescence complementation (BiFC)-based screen for discovery of PRMT5 inhibitors. Provisional Patent Application No 62/121,627 filed on February 27, 2015
- Methods and composition matters for the treatment of a ca cancer using an inhibitor of PRMT5. Provisional Patent Application No 69244-01, filed on Dec 14, 2020

## Publications

### *a. Peer-reviewed Research Articles*

Que Z, Olivero-Acosta MI, Zhang J, Eaton M, Tukker AM, Chen X, Wu J, Xie J, Xiao T, Wettschurack K, Yunis L, Shafer JM, Schaber JA, Rochet JC, Bowman AB, Yuan C, Huang Z, **Hu CD**, Trader DJ, Skarnes WC, Yang Y. Hyperexcitability and pharmacological responsiveness of cortical neurons derived from human iPSCs carrying epilepsy-associated sodium channel Nav1.2-L1342P genetic variant. *J. Neuroci* (2021), doi: 10.1523/JNEUROSCI.0564-21.2021. Online ahead of print.

Han, H., Jiang, G., Kumari, R., Silic, M., Owens, J.L., Hu, C.D., Mittal, S. K. and Zhang, G. Loss of *smarcad1a* accelerates tumorigenesis of malignant peripheral nerve shealth tumors in zeabrafish. *Genes, Chromosomes and Cancer* (2021), 60:743-761.

Fang, S., Liu, S., Shen, J., Lu, A.Z., wang, A.K.Y., Zhang, Y., Li, Ko., Liu, J., Yang, L., Hu, C.D., and Wan, J. Updated SARS-CoV-2 single nucleotide variants and mortality association. *J. Med Virol* (2021), 93:6525-6534.

Beketova, E., Owens, J.L. and Hu, C.D. Protein arginine methyltransferase 5 (PRMT5): A putative oncogene and therapeutic target in prostate cancer. *Cancer Gene Therapy* 2021, doi: 10.1038/s41417-021-00327-3. Online ahead of print.

Fang, S., Li, K., Shen, J., Liu, S., Liu, J., Yang, L., Hu, C.D., and Wan, J. GESS: a database of global evaluation of SARS-CoV-2/hCoV-19 sequence. *Nucleic Acids Res* (2021), 49(D1):D706-D714

Jin, B., Prins, G.S., Adam, R., Sfanos, K.S., Li, B., Hu, C.D., Dong, Y., and You, Z. Novel discoveries in urology: big data to microbiome – highlights of the society for basic urological research 2019 annual meeting. *Am J clin Exp Urol* (2020), 8:73-75

Beketova, E., Fang, S., Owens, J.L., Liu, S., Chen, X., Zhang, Q., Asberry, A.M., Deng, X., Maloa, J., Huang, J., Li, C., Pili, R., Elzey, B.D., Ratliff, T.L., Wan, J. and Hu, C.D. Protein arginine methyltransferase 5 promotes pICln-dependent androgen receptor transcription in castration-resistant prostate cancer. *Cancer Res* (2020), 80:4904-4917

Liu, S., Shen, J., Fang, S., Li, K., Liu, J., Yang, L., Hu, C.D., and Wan, J. Genetic spectrum and distinct evolution patterns of SARS-CoV-2. *Front Microbiol* (2020), 11:593548

Jia, Z., Yue, F., Chen, X., Narayanan, N., Qiu, J., Syed, S.A., Imbalzano, A.N., Deng, M., Yu, P., Hu, C.D. and Kuang, S. Protein arginine methyltransferase PRMT5 regulates fatty acid metabolism and lipid droplet biogenesis in white adipose tissues. *Advanced Science* (2020), 7:20202602

Owens, J.L., Beketova, E., Tinsley, S.L., Asberry A.M., Deng, X., Huang, J., Li, C., Wan, J. and Hu, C.D. PRMT5 cooperates with pICln to function as a master epigenetic activator of DNA double-strand break repair genes. *iScience* (2020), 23:100750

Li, Y.H., Tong, K.L., Lu, J.L., Lin, J.B., Li, Z.Y., Yang, J., Sang, Y., Ghodbane, A., Liu, N., Gao, X.J., Tam, M.S., Hu, C.D. Zhang, H.T., and Zha, Z.G. PRMT5-TRIM21 interaction regulates the senescence of osteosarcoma cells by targeting the TXNIP/p21 axis. *Aging* (2020), 12:2507

Doyle T.B., Muntean, B.S., Ejendal, K.F., Hayes, M.P., Soto-Velasquez, M. Martemyanov, K.A., Dessauer, C.W., Hu, C.D., and Watts, V.J. Identification of novel adenylyl cyclase 5 (AC50 signaling networks in D1 and D2 medium spiny neurons using bimolecular fluorescence complementation screening. *Cells* (2019), 8:1468.

Vickman, R.E., Yang, J., Atallah, N., Cresswell, G.M., Zheng, F., Zhang, C., Doerge, R.W., Crist, S.A., Mesecar, A.D., Hu, C.D., and Ratliff, T. L. Cholesterol sulfotransferase SULT2B1b modulates sensitivity to death receptor ligand TNF alpha in castration resistant prostate cancer. *Molecular Cancer Research* (2019), 17:1253-1263.

Zeng, L., Wang, W.H., Arrington, J., Shao, G., Geahlen, R.L., Hu, C.D. and Tao, W.A. Identification of upstream kinases by fluorescence complementation mass spectrometry. *ACS Central Sci*, 3:1078-1085 (2017).

Deng, X., Shao, G., Zhang, H.T., Li, C., Zhang, D., Cheng, L., Elzey, B.D., Pili, R., Ratliff, T.L., Huang, J., Hu, C.D. Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. *Oncogene*, 36:1223-1231 (2017)

Vickman, R.E., Christ, S.A., Kerian, K., Eberlin, L., Coos, R.G., Burcham, G.N., Buhman, K.K., Hu, C.D., Mesecar, A.D., Cheng, L., Ratliff, T.L. Cholesterol sulfonation enzyme, SULT2B1b, modulates AR and cell growth properties in prostate cancer. *Mol Cancer Res*, 14:776-786 (2016)

Zhang, H., Zeng, L., Tao, A.W., Zha, Z., and Hu, C.D. The E3 ubiquitin ligase CHIP mediates ubiquitination and protesomal degradation of PRMT5. *Biochem Biophys Acta,* 1863:336-346 (2016)

Xu, D., Zhan, Y., Qi, Y., Cao, B., Bai, S., Xu, W., Gambhir, S.S., Lee, P., Sartor, O., Flemington, E.K., Zhang, H., Hu, C.D., and Dong, Y. Androgen receptor splice variants dimerize to transactivate target genes. *Cancer Res*, 75:3663-3671 (2015)

Hu, C.D., Choo, R., Huang, J. Neuroendocrine differentiation in prostate cancer: a mechanism of radioresistance and treatment failure. *Front Oncol*, 5:90 (2015)

Suarez, C.D., Deng, X., and Hu, C.D. Targeting CREB inhibits radiation-induced neuroendocrine differentiation and increases radiation-induced cell death in prostate cancer cells. *Am J Cancer Res,* 4:850-861 (2014)

Zhang, H., Zha, Z. and Hu, C.D. Transcriptional activation of PRMT5 by NF-Y is required for cell growth and negatively regulated by the PKC/c-Fos signaling in prostate cancer cells. *Biochem Biophys Acta,* 1839:1330-1340 (2014)

Hsu, C. and Hu, C.D. Transcriptional activity of c-Jun is critical for the suppression of AR function. *Mol. Cell. Endocrinol*. 372:12-22 (2013)

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Hsu, C. and Hu, C.D. Critical role of an N-terminal end nuclear export signal in regulation of ATF2 subcellular localization and transcriptional activity. *J. Biol. Chem*. 287:8621-8632 (2012)

Deng, X., Elzey, B.D, Poulson, J.M., Morrison, W.B., Ko, S.C., Hahn, N.M., Ratliff, T.L., and Hu, C.D. Ionizing radiation induces neuroendocrine differentiation in vitro, in vivo and in human prostate cancer patients. *Am. J. Cancer. Res.* 1:834:844 (2011)

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Hiatt, S.M., Duren, H.M. Shyu, Y., Ellis, R.E., Hisamoto, N., Matsumoto, K., Kariya, K., Kerppola, T.K., and Hu, C.D.* *C. elegans* FOS-1 and JUN-1 regulate *plc-1* expression to control ovulation. *Mol. Biol. Cell* 20:3888-3895 (2009)

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Wang ,KZQ, Wara-Asparati, N., Boch, J.A., Yoshida, Y., Hu, C.D., Galson, D.L., and Auron, P.E. TRAF6 activation of PI3 kinase-dependent cytoskeletal changes is cooperative with Ras and mediated by an interaction with cytoplasmic c-Src. *J. Cell Sci*. 119:1579-1591 (2006).

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***b. Invited Peer-reviewed Review Articles***

Hu, C.D. , Choo, R., and Huang, J. Neuroendocrine differentiation in prostate cancer: a mechanism of radioresistance and treatment failure. *Front Oncol*, Apr 14;5:90. Doi: 10.3389/fonc.2015.00090 (2015)

Kodama, Y. and Hu, C.D. Bimolecular fluorescence complementation (BiFC): A 5-year update and future perspectives. *Biotechniques*, 53:285-298 (2012)

Shyu, Y. and Hu, C.D. Recent advances in fluorescence complementation-based technologies. *Trends Biotechnol.* 26:622-630 (2008)

Hu, C.D., Zhang, X.-H., and Bi, E.-H. Role of macrophages in the modulation of NK activity. *Foreign Medicine, Part of Immunology*, 10, 16-20 (1987) (*in Chinese*).

***c. Invited Review Article (Not peer-reviewed)***

Shyu, Y., Akasaka, K., and Hu, C.D*. Bimolecular fluorescence complementation (BiFC): A colorful future in drug discovery. Sterling-Hoffman Life Science Journal, July, 2007. (http://www.sterlinglifesciences.com/newsletter/articles/article006.html).

***d. Book Chapters***

Pratt, E.P.S., Owens, J.L., Hockerman, G.H., and Hu, C.D. Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interactions and assessment of subcellular localization in live cells. High resolution imaging of proeins in tissues and cells: light and electron microscopy methods and protocols (Ed, Schwartzbach, S.D., Skalli, O., and Schikorski, T.), Springer (2015).

Ejendal, K.F.K., Conley, J.M., Hu, C.D. and Watts, V.J. Bimolecular fluorescence complementation analysis of G protein-coupled receptor dimerization in living cells. *Methods Enzymol*., 521:259-279 (2013).

Kodama, Y. and Hu, C.D.* Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interaction: How to calculate signal-to-noise ratio. *Methods Cell Biol*., 113: 107-121 (2013).

Vidi, P.A., Przybyla, J., Hu, C.D., and Watts, V.J. Visualization of G protein-couple receptor (GPCR) interactions in living cells using bimolecular fluorescence complementation (BiFC). *Curr. Protoc. Neurosci.*, Unit 5.29.1-5.29.15 April 2010.

Hu, C.D., Grinberg, A.V. and Kerppola, T.K. Visualization of Protein Interactions in Living Cells Using Bimolecular Fluorescence Complementation (BiFC) Analysis. (ed. Coligan JE, Dunn BM, Speicher DW, Wingfield PT) *Curr. Protoc. Protein Sci.* 41:19.10.1-19.10.21. Hoboken, John Willey & Sons, 2005.

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Hu, C.D., Grinberg A., and Kerppola TK. Visualization of protein interaction in living cells using bimolecular fluorescence complementation (BiFC) analysis. In *Curr. Protoc. Cell Biol.* (ed. Bonifacino JS, Dasso M, Harford JB, Lippincott-Schwartz J, Yamada KM) pp. 21.3.1-21.3.21. Hoboken, John Willey & Sons, 2005

Kataoka, T., Kariya, K., Yamawaki-Kataoka, Y., Hu, C.D., Shirouzu, M., Yokoyama, S., Okada, T., and Shima, F. Isoprenylation-dependent and independent interaction of Ras with its effectors. In Kuzumaki, N. Cytoskeleton and G-Protein in the Regulation of Cancer. *Hokaido University Medical Library Series*, 37, 141-146 (1998).

## Current and Past Grant Support at Purdue University as PI or Co-PI

**Active Grant Support**

Title: Development and preclinical evaluation of a novel targeted radionuclide therapy for metastatic castration resistant prostate cancer
Source: DoD (2020 PCRP)
Role: PI
Grant Period: 07/01/21-06/30/24
Total Cost:
Goal: The goal of this project is to develop a novel targeted radiation therapy by co-delivering $^{177}$Lu and a radiosensitizer into prostate cancer cells.

Title: Role and targeting of PRMT5 in prostate cancer
Source: NCI RO1
Role: Contact PI (**Multi-PI** with Chenglong Li and Jiaoti Huang)
Total Cost Requested:
Grant Period: 06/09/2017-05/31/2022
Goal: The goal of this proposal is to elucidate the molecular mechanisms by which PRMT5 promotes prostate cancer cell growth, improve the potency of BLL3.3, and conduct a preclinical evaluation of PRMT5 inhibition for castration resistant prostate cancer treatment.

Title: Co-targeting of androgen synthesis and androgen receptor expression as a novel treatment for castration resistant prostate cancer
Source: DoD (2015 PCRP)
Role: PI
Grant Period: 08/01/16-07/30/21
Total Cost:
Goal: The goal of this project is to evaluate whether co-targeting of androgen synthesis by abiraterone and androgen receptor expression via PRMT5 inhibition is an effective treatment for CRPC.

## Past Grant Support at Purdue University (2003-2018):

### *External Funding*

Title: Temporal and spatial interaction patterns of bZIP proteins in living *C. elegans*
Source: National Science Foundation (MCB 0420634)
Role: PI
Grant Period: 06/04/07 – 07/30/08
Total Cost:
Goals: The goal of this REU was to support Summer High School Student Research on the funded NSF *C. elegans* project.

Title: Regulation of *c-jun* transcription by ATF2 in cardiomyocyte in response to stress
Source: American Heart Association (AHA 0655570Z)
Role: PI
Grant Period: 07/01/06 – 06/30/08
Total Cost:
Goals: The goal of this project was to study the role of ATF2 subcellular localization in regulating *c-jun* transcription in rat cardiomyocytes in response to hypoxia and oxidative stress.

Title: Interplay of CREB and ATF2 in radiation-induced prostate cancer transdifferentiation

Source: DoD Prostate Cancer Idea Development Award (PC073981)
Role: PI
Grant Period: 06/01/08-05/30/11
Total Cost:
Goals: The goal of this project was to determine how CREB and ATF2 oppose each other at the transcriptional level to regulate radiation-induced neuroendocrine differentiation in prostate cancer cells.

Title: Improvement of BiFC technology and its application in the TLR signal transduction pathway (International collaborative project)
Source: Natural Science Foundation of China
Role: PI
Grant Period: 01/01/11-12/31/13
Total Cost:
Goal: The goal of this project was to collaborate with Dr. Yayi Hou at Nanjing University to apply BiFC technologies to study the TLR signaling in immune system.

Title: D2 receptor-induced sensitization of adenylate cyclase
Source: NIH RO1 (National Institute of Mental Health)
Role: Co-Investigator (PI: Val Watts)
Grant Period: 08/15/11-04/31/14
Total Cost:
Goal: The goal of this RO1 grant was to investigate the molecular mechanisms underlying D2 receptor-induced sensitization of adenylate cyclase. As a Co-Investigator, Dr. Hu provided his expertise in BiFC technology to help the analysis of D2 receptor interacting proteins.

Title: New mechanism for modulating opioid receptor mediated analgesia
Source: Showalter Trust Award
Role: Co-PI (PI: Richard van Rijn)
Total Cost:
Grant Period: 07/01/14-06/30/16
Goal: The goal of the project is to study the mechanisms and regulation of opioid receptors and to develop agents targeting protein-protein interactions using BiFC-based technologies.

Title: Targeting PRMT5 as a novel radiosensitization approach for primary and recurrent prostate cancer radiotherapy
Source: DoD (2011 PCRP)
Role: PI
Grant Period: 08/01/12-07/30/16
Total Cost:
Goal: The goal of this grant is to determine that PRMT5 is a novel therapeutic target for prostate cancer radiotherapy.

Title: Identification of the Ac5 sensitization interactome using BiFC

Source: NIH R21 (National Institute of Mental Health)
Role: Multi-PI with Val Watts
Total Cost:
Role: Multi-PI
Grant Period: 07/19/13-06/15/17
Goal: The goal of this project is to develop BiFC-based cDNA library screening for identification of Ac5 interacting proteins.

Title: Targeting neuroendocrine differentiation for prostate cancer radiosensitization
Source: DoD (2012 PCRP)
Grant Period: 09/30/13-09/30/17
Total Cost:
Role: PI
Goal: The goal of this grant is to use CREB targeting as a model to determine whether targeting radiation-induced NED can be explored as a novel radiosensitization approach for prostate cancer radiotherapy.

Title: Development of novel small molecule inhibitors targeting protein arginine methyltransferase 5
Source: CTSI (Indiana Drug Discovery Alliance)
Period: 12/01/14-12/30/17 (No cost extension for current year)
Total amount awarded:
Role: PI
Goal: The goal of this project is to discover inhibitors for disruption of PRMT5/MEP50 interaction using BiFC-based screening.

Title: Developing novel therapeutic strategies for castration-resistant prostate cancer
Source: DOoD (2013 PCRP)
Total Cost:
Role: Co-PI (PI: Kavita Shah)
Grant Period: 08/01/14-07/30/18
Goal: The goal of this project is to determine whether targeting LIMK2 can be used to treat CRPC.

Title: Request of a Nikon A1RSI confocal microscope
Source: NIH (S10 OD027043-01)
Period: 09/20/19-09/19/20
Role: Co-Investigator (PI: Robert Stahelin)
Total Cost:
Goal: The goal of this support is to acquire a new Nikon confocal microscope for the Pharmacy Live Cell Imaging Facility (PLCIF), which was established and run by Dr. Hu (2011-2018)

***<u>Internal Funding</u>***

Title: Discovery of novel therapeutic targets for neuroendocrine prostate cancer
Source: Department of MCMP Research Enhancement Award, Purdue University
Period: 04/01/17-12/31/19
Total amount awarded:
Role: PI
Goal: The goal of this award is to discovery altered ion channels in neuroendocrine prostate cancer as therapeutic targets

Title: Discovery of inhibitors to disrupt the interaction of PRMT5 with its cofactor pICln for prostate cancer treatment
Source: Purdue University Center for Cancer Research
Period: 08/01/18-03/30/20
Total amount awarded:
Role: PI (Co-I: Dr. Wen Jiang)
Goal: This support is to develop a BiFC-based high throughput screen assays for identification of inhibitors to disrupt the PRMT5/pICln interaction.

Title: Deep neural network-assisted protein structure modeling for drug development from low resolution 3D cryo-electron microscopy maps
Source: Purdue Institute for Drug Discovery
Period: 12/01/18-11/30/20
Total amount awarded:
Role: Co-PI with Dr. Daisuke Kihara (computational biologist) and Dr. Wen Jiang (cryo-EM expert)
Goal: This support is to develop a deep learning method to predict cryo-EM structures using PRMT5/MEP50 and PRMT5/pICln interactions as a model and to identify novel interfaces for drug discovery.

Title: Targeted RO1: Molecular and genetic analysis of PRMT5 in neuroendocrine prostate cancer
Source: EVPRP Targeted RO1
Period: 12/01/15-10/31/19
Total amount awarded:
Role: PI
Goal: The goal of this project is to generate preliminary data for a RO1 proposal to determine the role of PRMT5 and its cofactor MEP50 in neuroendocrine differentiation of prostate cancer cells and validate whether targeting PRMT5/MEP50 is an effective therapeutic approach for neuroendocrine prostate cancer

Title: Biochemical and cryo-EM analysis of PRMT5 in complex with its cofactor pICln
Source: Purdue University Center for Cancer Research
Period: 05/01/18-04/30/19
Total amount awarded:

Role: PI
Goal: This support is to solve cryo-EM structure of PRMT5 in complex pICln, a novel cofactor for PRMT5.

Title: Generation of MEP50 transgenic mice for prostate cancer research
Source: Purdue University Center for Cancer Research
Period: 05/01/18-11/30/18
Total amount awarded:
Role: PI
Goals: This support is to generate MEP50 transgenic mice for prostate cancer research.

Title: PRMT5 in prostate cancer development, progression and therapy response
Source: EVPRP Targeted RO1
Period: 12/01/15-05/30/17
Total amount awarded:
Role: PI
Goals: The goal of this project is to generate genetically modified mouse models (PRMT5 transgenic mice and PRMT5 Floxed mice) for prostate cancer research.

Title: Discovery of PRMT5 target genes in neuroendocrine prostate cancer
Source: Purdue University Center for Cancer Research
Period: 12/01/16-06/30/17
Total amount awarded:
Role: PI
Goals: The goal of this grant is to perform RNA-seq and ChIP-seq to identify target genes of PRMT5 contributing to the development of neuroendocrine prostate cancer.

Title: Mass spectrometric identification of pCREB interacting proteins in prostate cancer cells LNCaP
Source: Purdue Cancer Center Small Grant (Indiana Elks, Inc)
Role: PI
Grant Period: 03/01/08-02/28/09
Total Cost:
Goals: The goal of this project was to identify cytoplasmic interacting proteins of pCREB using mass spectrometry.

Title: Identification of interacting proteins and phosphorylation of ATF2 implicated in prostate cancer transdifferentiation
Source: Purdue Research Foundation
Role: PI
Grant Period: 06/01/08-05/30/09
Total Cost:
Goals: The goal of this PRF support was to use mass spectrometry to identify interacting proteins and phosphorylation of ATF2 in the cytoplasm in radiation-induced neuroendocrine cells and to determine how ATF2 nuclear import is impaired by ionizing radiation.

Title: Targeting of prostate cancer transdifferentiation and proliferation via a novel DNA nanotube-based nucleic acid delivery
Source: Lilly Seed Grant
Role: PI
Grant Period: 01/01/09-12/31/10
Total cost:
Goal: The goal of this grant was to collaborate with Dr. Chengde Mao to develop DNA nanotube-based delivery of siRNAs.

Title: Targeting neuroendocrine differentiation as a novel therapeutics in prostate cancer treatment
Source: Purdue Research Foundation
Role: PI
Grant Period: 08/01/2010-07/30/2011
Total cost:
Goal: The goal of this project was to support graduate student Chris Suarez to study the role of radiation-induced neuroendocrine differentiation in radioresistance.

Title: Ionizing radiation induces neuroendocrine differentiation in nude mice prostate cancer xenograft models: Implication in disease progression
Source: Purdue University Center for Cancer Research
Role: PI
Grant Period: 01/01/09-12/31/11
Total Cost:
Goals: The goal of this project was to use xenograft nude mice prostate cancer cell models to investigate whether CREB and ATF2 contribute to radiation-induced neuroendocrine differentiation *in vivo* and to determine whether radiation induces changes of pCREB and ATF2 subcellular localization.

Title: Generation of cytoplasmic-localized ATF2 transgenic mice for prostate cancer research
Source: Purdue University Center for Cancer Research
Role: PI
Grant Period: 06/01/10-05/30/11
Total cost:
Goal: The goal of this support was to supplement the cost for making a transgenic mouse strain using the shared transgenic mouse facility

Title: Chromogranin A, a novel biomarker to monitor radiation-induced neuroendocrine differentiation in prostate cancer patients
Source: The Indiana Clinical and Translational Science Institute (CTSI)-Purdue Project Development Program
Role: PI
Grant Period: 06/01/10-05/30/12
Total cost:

Goal: The goal of this support was to conduct a pilot clinical study to determine the effect of radiotherapy on neuroendocrine differentiation in prostate cancer patients.

Title: Acquisition of an Nikon A1 Confocal Microscope
Source: Lilly Seed Grant, College of Pharmacy
Role: PI
Grant Period: 07/01/11-06/30/12
Total amount awarded:
Goal: The goal of this support was to acquire Nikon A1 confocal microscope to set up a Pharmacy Live Cell Imaging Facility

Title: Ultrahigh performance liquid chromatography (UHPLC) coupled to high resolution mass spectrometry
Source: Office of the Vice President for Research (OVPR) Laboratory Equipment Program
Role: Co-PI (PI: Andy Tao)
Period: Purchased by May 31, 2014
Total amount awarded:
Goal: The goal of this internal support was to acquire UHPLC.

Title: Generation of PRMT5 transgenic mice for prostate cancer research
Source: Purdue University Center for Cancer Research Shared Resource Grant
Period: 12/01/15-12/31/16
Total amount awarded:
Role: PI
Goal: The goal of this project is to use the transgenic mouse facility to generate PRMT5-overexpressing mice.

## Past Grant Support at Kobe University as PI (1998-2001):

Title: Regulation of Rap1A activity by phosphorylation
Source: Kobe University, President Young Investigator Award
Role: PI
Grant Period: 04/01/98-03/30/99
Total Cost: ~(for supplies)
Goals: The goal of this project was to investigate whether phosphorylation of Rap1A by PKA affects the ability of Rap1A to antagonize the function of Ras in activating Raf-1.

Title: Effect of phosporylation on the regulation of Rap1A activity
Source: Ministry of Education, Science, Sports, and Culture of Japan
Role: PI
Grant Period: 04/1/98 - 03/30/99
Total Cost: ~(for supplies)

Goals: The goal of this project was to investigate whether phosphorylation of Rap1A by PKA affects the ability of Rap1A to activate downstream effectors such as Raf-1 and B-Raf.

Title: Activation mechanism of phospholipase C (PLC-ε) by Ras
Source: Hyogo Science and Technology Association
Role: PI
Grant Period: 04/01/00 – 03/30/01
Total Cost: ~ (for supplies)
Goals: The goal of this project was to investigate whether Ras regulates catalytic activity of PLC $\varepsilon$ directly by their physical interaction. The approach was to use *in vitro* reconstitution system.

Title: Regulation of a novel phospholipase C (PLC-ε) by Ras
Source: Japan Society for the Promotion of Science
Role: PI
Grant Period: 04/01/00 – 03/30/01
Total Cost: ~(for supplies)
Goals: The goal of this project was to investigate how Ras regulates catalytic activity of PLC $\varepsilon$ and determine whether membrane anchoring of PLC-ε by Ras is sufficient for the activation of PLC-ε. This project was primarily focused on the studies in cells.

*Note: Research grants in Japan do not provide personnel support. All faculty members and staff are supported by the government. Postdoctoral fellows and graduate students can only be supported by fellowships.*

## Fellowships/Awards received by trainees

- Susan Fox, Ross Fellowship (08/2003-07/2005): ~
- Susan Fox, 2nd place of graduate student presentation
  2004 Walther Cancer Institute Annual Retreat (Aug. 5-7)
- John Y Shyu, graduate student, Travel Award from 15th International Worm Meeting (June 25-29, 2005, Los Angeles) ($866)
- Susan Fox, graduate student, Travel Award from 15th International Worm Meeting (June 25-29, 2005, Los Angeles) ($866)
- Zeina Shtaih, Pharmacy Student, Summer Research Fellowship (2005 Breast Cancer Research Program),
- Jonathan Smith, Pharmacy Student, Summer Research Fellowship (2005 Breast Cancer Research Program),
- Jonathan Smith, NSF, Summer Research Fellowship (REU),(IC)
- Apinya Supatkul, Prepharmacy Student, 2006 Summer Research Fellowship
- John Shyu, 1st Place of 2007 Purdue University Graduate Student Research Competition

- Holli Duren, Travel Award from 16th International Worm Meeting (June 27-July 1, 2007, UCLA)
- John Shyu, John Koo Travel Award for Fall 2007
- Holli Duren, Kienly Award for outstanding graduate student teaching assistant 2007, MCMP
- Holli Duren, 2007 PRF Summer Fellowship
- Holli Duren, 2008-2009 PRF Fellowship
- Chris Suarez, Purdue University Doctoral Fellowship (08/2007-07/2009): ~
- Susan Fox, Bilsland Dissertation Fellowship (07/2008-12/2008): ~
- John Shyu, Bilsland Dissertation Fellowship (07/2008-12/2008): ~
- Holli Duren, 2008-2009 Graduate Student Award for Outstanding Teaching at Purdue University
- Holli Duren, 2009 Charles J. Paget Travel Award:
- Yutaka Kodama, 04/01/09-03/31/10 TOYOBO Postdoctoral Fellowship
- Akhil Shenoy (Texas AM U) , 06/01/09-07/26/09, Purdue SROP:
- Yutaka Kodama, 04/01/10-03/31/12, JSPS Postdoctoral Fellowship

- Holli Duren, Bilsland Dissertation Fellowship (01/01/2010-06/30/2010):
- Chih-chao Hsu, Ronald W. Dollens Graduate Scholarship in Life Sciences (08/2010-05/2011):
- Yeo Jin Choi, Purdue University College of Pharmacy 2010 Summer Undergraduate Research Fellowship:
- Chris Suarez, 2010 PRF Fellowship:
- Chih-chao Hsu, Travel Award for conference attendance from PULSe, (2012)
- Chih-chso Hsu, 2011 PRF Fellowship:
- Chris Suarez, 2011 Paget Travel Award from MCMP department,
- Chris Suarez, 2012 AACR Minority Scholar in Cancer Research Award for participation in the Advances in Prostate Cancer Research conference (Feb 6-9, 2012),
- Chih-chao Hsu, Bilsland Dissertation Fellowship (09/01/12-12/31/12):
- Huantin Zhang (visiting student from Jinan University, China): Graduate Student Study Abroad Scholarship: (2012)
- Huantin Zhang (visiting student form Jinan University, China): China Scholarship Council (CSC): (awarded for two years 10/2013-9/2015, but stay for one year)
- Limin Zhang (PharmD student): 2014 Summer Undergraduate Research Fellowship (Lilly Endowment Fellowship):
- Jake Owens, Ross Graduate Fellowship (2014-2015),

- Athena He: 2016 LSAMP Summer Undergraduate Research Fellowship:
- Jonathan Malola: 2017 College of Pharmacy Summer Undergraduate Research Fellowship:
- Jake Owens, CTSI Predoctoral fellowship (07/01/17-06/30/19): /year plus tuition remission
- Jake Owens, 2nd place of Presentation Award at the 2017 Indiana Urological Research Symposium:
- Elena Beketova, 2018 Purdue Research Foundation (PRF) Graduate Fellowship: plus tuition remission
- Elena Beketova, 2018 Purdue University Center for Cancer Research Travel Award to 2018 AACR meeting,
- Jake Owens, 2018 MCMP Koo Travel Award to 2018 SBUR meeting,

- Samantha Tinsley, Purdue University Graduate School Andrew Fellowship (08/2017-07/2018): year plus tuition remission
- Ji Yang, China Council Scholarship (10/01/18-03/31/20):
- Yi Liu, China Council Scholarship (01/12/19-01/11/20):
- Jonathan Malola (3/23/2019): Outstanding Nuclear Pharmacy Student Scholarship from the 2019 NANP:
- Jake Owens (8/15/19-12/31/19): Bilsland Dissertation Fellowship

- Elena Beketova (7/1/19-6/30/20): Purdue University Cancer Center SIRG Research Assistantship
- Elena Beketova (8/15/20-12/31/20): Bilsland Dissertation Fellowship

- Andrew Asberry (8/1/19-7/31/21): Purdue Institute for Drug Discovery Training Program (NIH T32): Full stipend/supplement/tuition

## Teaching Experience

**Lectures and labs**

| | |
|---|---|
| 5/1985-6/1987: | Microbiology and Immunology labs (medical students) |
| 7/1987-8/1991: | Epidemiology lectures and labs in the Department of Epidemiology, School of Public Health, Tongji Medical University, Wuhan |
| 4/1997-8/2000: | Physiology and Molecular Biology lab (medical students) in the Department of Physiology II, Kobe University |
| 8/2003-present: | As a faculty member in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy, I have been involved in the teaching of the following courses. The class size for the courses ranges from 5~15 for graduate students, 30-40 for BSPS students, and 150 ~205 for professional pharmacy students. The total number of lecture hours |

taught is approximately 40h/year. Teaching evaluation scores have been 4.5~4.8/5.0. In April 2017, I received the first teaching award of the Pharmaceutical Sciences Teacher of the Year, which was completely nominated and voted by BSPS graduates in the College of Pharmacy.

***Courses Taught***

*Professional Pharmacy Students*:
MCMP 305 (Biochemistry I, 2004-2006)
MCMP 304 (Biochemistry II, 2005-2008)
MCMP 440 (Pathophysiology, 2006-2012)
PHRM 824 (Principles of Pathophysiology and Drug Action, 2012-present)
PHRM 302 (Integrated Lab, Neoplasia module, 2005-2012)
PHRM 820 (Professional Program Laboratory, Neoplasia module, 2012-2015)

*Graduate students:*
MCMP 618/690G (Molecular Targets of Cancer, 2007-present)
MCMP 617/690N (Molecular Targets of Neurological Disorders, 2007-present)
MCMP 514 (Biomolecular Interactions-Theory and Practice, 2009-present)
MCMP 696 (Seminars in Medicinal Chemistry and Molecular Pharmacology, 2006-2008)
MCMP 599 (Cumulative written examinations, 2015-present)

*Undergraduate students (BS in Pharmaceutic Sciences):*
PHRM 460 (Drug Discovery and Development I, 2013-present)

MCMP 544 (Drug Classes and Mechanisms, 2015-present)

*Medical students (Indiana School of Medicine):*
LCME 504 (Molecular Cell Biology, guest lecture of Molecular Biology of Cancer, 2013-2015)

***Courses Served as Coordinator***

*PHRM 824 (Principles of Pathophysiology and Drug Action, 2013-present)*
*MCMP 440 (Pathophysiology, 2011-2012)*
*MCMP 696 (Seminars in Medicinal Chemistry and Molecular Pharmacology, 2006-2008)*
MCMP 599 (Cumulative written examinations, 2015-2017)

**Supervision of graduate, professional and undergraduate student research**

| | |
|---|---|
| 07/1987-08/1991 | Supervised 6 undergraduate students at Tongji Medical University |
| 04/1997-08/2000 | Co-supervised 7 Ph.D. students for thesis research with Professor Tohru Kataoka and supervised 5 undergraduate summer research at Kobe University. |
| 09/2000-06/2003 | Supervised two undergraduate students at University of Michigan |
| 07/2003-present | (1) Served as thesis adviser of 14 Ph.D. students (12 graduated) and 2 master students (graduated) and co-adviser of 5 Ph.D. students (4 graduated)<br>(2) Served as a thesis committee member of 54 graduate students<br>(3) Served as a committee member of 43 oral preliminary examination<br>(4) Supervised 40 graduate students for lab rotations<br>(5) Supervised 35 professional and undergraduate student research<br>(6) Supervised 4 high school students for summer research |

**Supervision of postdoctoral fellows, visiting scholars and technicians**

| | |
|---|---|
| 07/2003-present | Supervised 14 postdoctoral fellows, visiting scholars and technicians |

**Current lab members: 7**

The lab has 1 technician, 1 postdoc, 2 PhD students, 2 undergraduate students and 1 visiting scholar

## Service Experience

**Major Administrative Services in the Purdue University Center for Cancer Research**

| | |
|---|---|
| 2010-2013 | **Seminar Director** of Purdue University Center for Cancer Research |
| 2012- 2016 | **Executive Committee Member** of Obesity and Cancer Discovery Group, Purdue University Center for Cancer Research |
| 2010-Present | **Co-leader** of Prostate Cancer Discovery Group of Purdue University Center for Cancer Research |
| 2012- Present | **Co-Director** of Indian Basic Urological Research (IBUR) monthly meetings |
| 2013- Present | **Executive Committee Member** of Purdue University Center for Cancer Research |
| 2013- Present | **Co-leader**, Cell Identity and Signaling (CIS) Program of Purdue University Center for Cancer Research |
| 2013-present | Member of Big Ten Clinical Trial GU Working Group |
| 2016- Present | **Director** of Small Animal Radiation Facility |

**Major Administrative Services at Purdue University**

| | |
|---|---|
| 2007-2009 | PULSe Graduate Program Admission Committee |
| 2007-2009 | PULSe Graduate Program Recruitment Committee |
| 2008-present | Bindley Imaging Committee (BIG) |
| 2010 | Faculty Search Committee for a Cancer biology and Pharmacology position in the College of Veterinary Medicine |
| 2012-present | PULSe Graduate Program Curriculum Committee |
| 2016-present | Review Panel Member of CTSI PDT (Project Development Team) |

**Major Administrative Services in the College of Pharmacy**

| | |
|---|---|
| 2009-2013 | Member of Assessment Committee |
| 2011-2018 | **Director** of Pharmacy Live Cell Imaging Facility (PLCIF) |
| 2011-2018 | **Chair** of PLCIF Committee |
| 2012-2014 | Member of Grade Appeal Committee |
| 2012-present | Faculty Liaison for Core-Pharmacy Courses Taught by Other Schools (BIOL110/111) |
| 2013-2014 | Member of Honor Degree Policy Committee |
| 2013-2016 | Member of Curriculum committee |
| 2014-present | Member of Pharm.D. Academic Standards and Readmissions Committee |
| 2017-2019 | Member of Area Promotion Committee |
| 2017-2019 | Member of Nomination and Awards Committee |
| 2017-present | Member of Strategic Plan Research and Innovation Task Force |
| 2019-present | Member of Assessment Committee |

**Major Administrative Services in the Department of Medicinal Chemistry and Molecular Pharmacology**

| | |
|---|---|
| 2005-2011 | Member of Facility and Instrumentation Committee |
| 2008-2009 | Member of Strategy Plan Task Force |
| 2009 | Member of Biochemistry Task Force |
| 2010 | Member of Business Manger Search Committee |
| 2011 | Member of Faculty Search Committee (Pharmacology) |
| 2012 | Member of Faculty Search Committee (Pharmacology) |
| 2012 | Member of Faculty Search Committee (Epigenetics) |
| 2010-2015 | Member of Graduate Admissions and Recruiting Committee |
| 2012-2017 | Member of Graduate Assessment Committee |
| 2015-2017 | **Chair** of Graduate Assessment Committee |
| 2016 | **Chair** of faculty search committee (Cancer Biology) |
| 2017 | **Chair** of faculty search committee (Cancer Biology) |
| 2018 | **Chair** of faculty search committee (Cancer Biology) |
| 2017-present | Member of Heads Advisory Committee |
| 2018 | Member of Curriculum Committee |