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Integrated Analysis of Genetic Abnormalities of the Histone Lysine Methyltransferases in Prostate Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

CD 1,2 Yangjun Zhang
BE 1,2 Libin Yan
FG 1,2 Weimin Yao
F 1,2 Ke Chen
AG 1,2 Hua Xu
AG 1,2 Zhangqun Ye

1 Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China
2 Institute of Urology of Hubei Province, Wuhan, Hubei, P.R. China

Corresponding Author: Hua Xu, e-mail: xuhuawhu@163.com
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Background: The histone methyltransferase (HMT) family includes histone lysine methyltransferases (HKMTs) and histone/protein arginine methyltransferases (PRMTs). The role of HMT gene variants in prostate cancer remains unknown. Therefore, this study aimed to evaluate HMT gene variants in the pathogenesis and prognosis of human prostate cancer, using *in vitro* cell studies and bioinformatics analysis.

Material/Methods: Integrative bioinformatics analysis of the expression of 51 HMT genes in human prostate cancer was based on datasets from the Cancer Genome Atlas (TCGA). Correlation and regression analysis were used to identify critical HMTs in prostate cancer. Kaplan-Meier and the area under the receiver operating characteristics curve (AUROC) were performed to evaluate the function of the HMTs on prognosis. Gene expression and function of 22Rv1 human prostate carcinoma cells were studied.

Results: The HMT genes identified to have a role in the pathogenesis of prostate cancer included the EZH2, SETD5, PRDM12, NSD1, SETD6, SMYD1, and the WHSC1L1 gene. The EZH2, SETD5, and SMYD1 genes were selected as a prognostic panel, with the SUV420H2 HMT gene. SETD2, NSD1, and ASH1L were identified as critical genes in the development of castration-resistant prostate cancer (CRPC), similar to mixed-lineage leukemia (MLL) complex family members. Knockdown of the SETD5 gene in 22Rv1 prostate carcinoma cells *in vitro* inhibited cancer cell growth and migration.

Conclusions: HMT gene variants may have a role in the pathogenesis of prostate cancer. Future studies may determine the role of HMT genes as prognostic biomarkers in patients with prostate cancer.

MeSH Keywords: Epigenesis, Genetic • Histone-Lysine N-Methyltransferase • Prostatic Neoplasms

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Background

Worldwide, prostate cancer (adenocarcinoma) is the fourth most common cancer and is the second most common cancer in men [1–3]. Prostate cancer is also clinically heterogeneous and can be indolent or may have a highly aggressive course, requiring different treatment approaches [4,5]. Increasing advances have been made in understanding the molecular basis for prostate cancer and distinguishing the different subtypes and the variable clinical outcomes. For example, androgen receptor-driven ETS gene fusion is an important molecular mechanism that allows the subclassification of prostate cancer, as are the activated gene fusions or mutations of RAS and RAF gene family members, a subtype of ETS-negative tumors [6]. An improved understanding of the genetic alterations that drive of prostate cancer at the molecular level may lead to new therapies that target for specific subtypes of prostate cancer.

The histone methyltransferase (HMT) family includes histone lysine methyltransferases (HKMTs) and histone/protein arginine methyltransferases (PRMTs). HMTs play crucial roles in epigenetic regulation, by controlling histone lysine methylation and demethylases. Currently, approximately 51 HMTs have been acknowledged identified in humans, with the primary functions being to catalyze the translocation of methyl moieties from S-adenosylmethionine (SAM) to histone lysine sites (Supplementary Table 1) [7,8]. Histone methylation can result in different biological effects according to the degree of histone methylated, including damage to chromatin structure, gene transcription, and alterations in cell mitosis, which may accelerate the development and progression of cancers [9]. There is now increasing evidence to validate the genetic roles of HMTs, including abnormal histone methylation, which may contribute to the genesis and progression of prostate cancer. For example, abnormal expression of the enhancer of zeste homolog 2 (EZH2) gene, resulting in an imbalance of H3K27me3 (trimethylation at lysine 27 of histone 3), has been shown to be associated with progression of prostate cancer and with castration-resistant prostate cancer (CRPC) [10,11].

Although increasing advances have been made in the understanding of epigenetic regulation of cancers, the roles of HMTs in prostate cancer remain unknown. Therefore, the aims of this preliminary study were to evaluate HMT gene variants in the pathogenesis and prognosis of human prostate cancer, using *in vitro* cell studies and bioinformatics analysis, including the roles the 51 known HMT genes. The study included the analysis of data available from the Cancer Genome Atlas (TCGA) database, which allowed differential mRNA expression, copy number alteration (CNA), somatic gene mutation, and DNA methylation state to be identified with clinical outcome, including progression-free survival (PFS), in patients with prostate cancer.

Material and Methods

Data sources

The dataset of gene expression, copy number alteration (CNA), DNA methylation, gene mutation and clinic data for 51 human histone methyltransferases (HMTs) of 550 prostate adenocarcinoma samples available from the Cancer Genome Atlas (TCGA) (including 52 matched tumor and adjacent normal tissues) used in this study were downloaded from The University of California, Santa Cruz (UCSC) Cancer Genomics Browser (*https://genome-cancer.ucsc.edu/*). A total of 399 tumor samples, with clinical data, were chosen to perform an integrated analysis of 51 HMTs. Gene expression data were $\log^2$ (x+1) transformed from the RNA-Seq by Expectation-Maximization (RSEM) count estimates and DNA methylation data, shown as the β-value, and were profiled using the Infinium HumanMethylation27 platform (Illumina, San Diego, CA, USA).

The Gleason scoring system of histological grade was used as an indicator of the degree of tumor aggression and prognosis of prostate cancer, with increasing Gleason scores reflecting worse prognosis. Two patients with a Gleason score of 10 were incorporated into the patient group with a Gleason score of 9. For CNA data, homozygous deletion (Homedel), heterozygous deletion (Hetloss), diploid, low-level amplification (Gain) and high-level amplification (Amp) were indicated as –2, –1, 0, 1, and 2 respectively. Also, a gene copy number dataset of 150 previously published metastatic prostate cancer samples was downloaded from cBioPortal for Cancer Genomics (*http://www.cbioportal.org*) to compare CNAs in prostate cancer of different stage further, as tumor stage is also an indicator of prognosis (Supplementary Table 2).

Statistical analysis

Statistical analysis was performed using the R software (version 3.3.4), GraphPad Prism (version 7.01) and SPSS (version 18.0). A two-tailed paired t-test was performed to compare the different expression levels of 51 HMT genes in 52 matched prostate tumor tissues and adjacent normal tissues. The correlation analysis for CNA, mRNA expression and DNA methylation, mRNA expression of 51 HMTs in 399 sequenced prostate cancer patients were performed by Spearman correlation, Kendall rank correlation, and Pearson correlation analysis. Comparison between HMT expression profiles in prostate cancer samples with Gleason score was conducted by R statistical software using one-way analysis of variance (ANOVA). Correlation analysis and clustering methods analyzed the association between androgen receptor (AR) expression and HMT gene expression. Kaplan-Meier survival curve was conducted to investigate the impact of genetic alterations of different HMTs on patient survival. Univariate and multivariate Cox regression analysis was

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performed to identify factors associated with patient prognosis in 399 samples of prostate cancer by using the survival package in the R statistical software package.

Generation of a prognostic HMT gene signature

The set of 399 patients with transcription, DNA methylation, CNA, mutation and clinical survival information was divided into groups for each gene that included high and low gene expression or methylation, deletion or amplification, diploid state, mutational and wild-type HMT genes, respectively. Each of the 51 gene expression features demonstrated a fit using a univariate Cox proportional-hazards analysis model without any adjustment, and 13 candidate features were generated at a p-value <0.05 using the Wald test (Wald chi-squared test). These candidate features were further analyzed using multivariate Cox proportional hazards analysis model, with adjustment for age, T-stage, Gleason score, and androgen receptor (AR) expression. Eight features with significant correlation coefficients were identified, and a panel of the top four most significant HMT genes was finally identified.

The risk score of the panel of the top four most significant HMT genes was calculated according to the gene expression values and risk factors, to divide the patients with prostate cancer into two groups according to their scores, a high-risk and a low-risk group. Prediction across this panel was then pooled and performance assessed using the area under the receiver operating characteristics curve (AUROC). Kaplan-Meier plots were generated to evaluate their impact on clinical outcome. For CNA, features were selected to fit a univariate Cox proportional hazards analysis if frequencies of each type CNA (deletion or amplification) were more than 3%. Significant genes were identified to fit a multivariate Cox proportional hazards analysis model to assess their value as prognostic cancer biomarkers. Univariate and multivariate Cox proportional hazards analysis models were performed to analyze the prognostic value of DNA methylation and mutation in prostate cancer.

Data visualization

Data visualization tools including the ggplot2 data visualization package, pheatmap package in R, progression-free survival (PFS), receiver operator characteristic (ROC) curves, and meta packages of R (vision 3.3.4). Box plots of gene expression, histograms of changes in copy number, and Kaplan-Meier plots were visualized by GraphPad Prism (version 7.01). The mutational spectrum of the KMT2C and KMT2D genes was generated by the Oncoprint and MutationMapper tools (*http://www.cbioportal.org/tools.jsp*) on the cBioportal website [12].

Cell culture and transfection and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The human prostate cancer cell line 22Rv1 was obtained from American Type Cell Culture (ATCC) (Catalog No. CRL-2505). Cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA), as recommended by the manufacturer. The target sequence of the SETD5 gene was chosen as follows: GCUUGGAGGCUGAGGAGUU. The oligonucleotides corresponding to this target sequence were annealed and cloned into the EcoR1 and AgeI sites of the pLkO.1 plasmid (Addgene, Cambridge, MA, USA). The efficiency of SETD5 interference was determined by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) using SYBR Premix Ex Taq II and SYBR Green I (Takara Biotechnology, Dalian, China). Quantitative primers of the SETD5 gene were as follows:
Forward: GGACTGCCTTATGCTACGA;
Reverse: GCCATCCTGAGAAAGACCACA.

Cell proliferation and migration assays

Cell proliferation of the human prostate cancer cell line 22Rv1 was evaluated by the MTS colorimetric cell proliferation assay (Sigma, St Louis, MO, USA) and a colony formation assay, according to the manufacturer's instructions, with each experiment performed in triplicate. A cell migration assay was used to assess the cell invasion capacity (Corning, NY, USA) with the assay performed in duplicate.

Results

Identification of the expression profiles of histone methyltransferase (HMT) gene mRNA in prostate cancer

The mRNA levels of the 51 histone methyltransferase (HMT) genes in 52 matched prostate tumor tissues and adjacent normal tissues were analyzed and presented as box plots. The Student's t-test was used to determine the significance of the gene expression levels. Compared with adjacent normal prostate tissues, mRNA levels of prostate cancer tissue of 14 HMT genes were identified that were significantly increased, including DOT1L, EZH2, EHMT1, KMT2B, PRDM10, PRDM12, PRDM15, SETD6, SETD7, SMYD2, SMYD3, SMYD5, SUV39H2, and WHSC1) ($p<0.001$). Compared with adjacent normal tissues, mRNA levels of prostate cancer tissue of nine HMT genes were identified that were significantly decreased, including EZH1, MECOM, PRDM11, PRDM16, PRDM5, PRDM6, PRDM8, SETD4, and SMYD4 ($p<0.001$) (Figure 1). Also, mRNA expression levels of the PRDM13, PRDM14, PRDM7, PRDM9,

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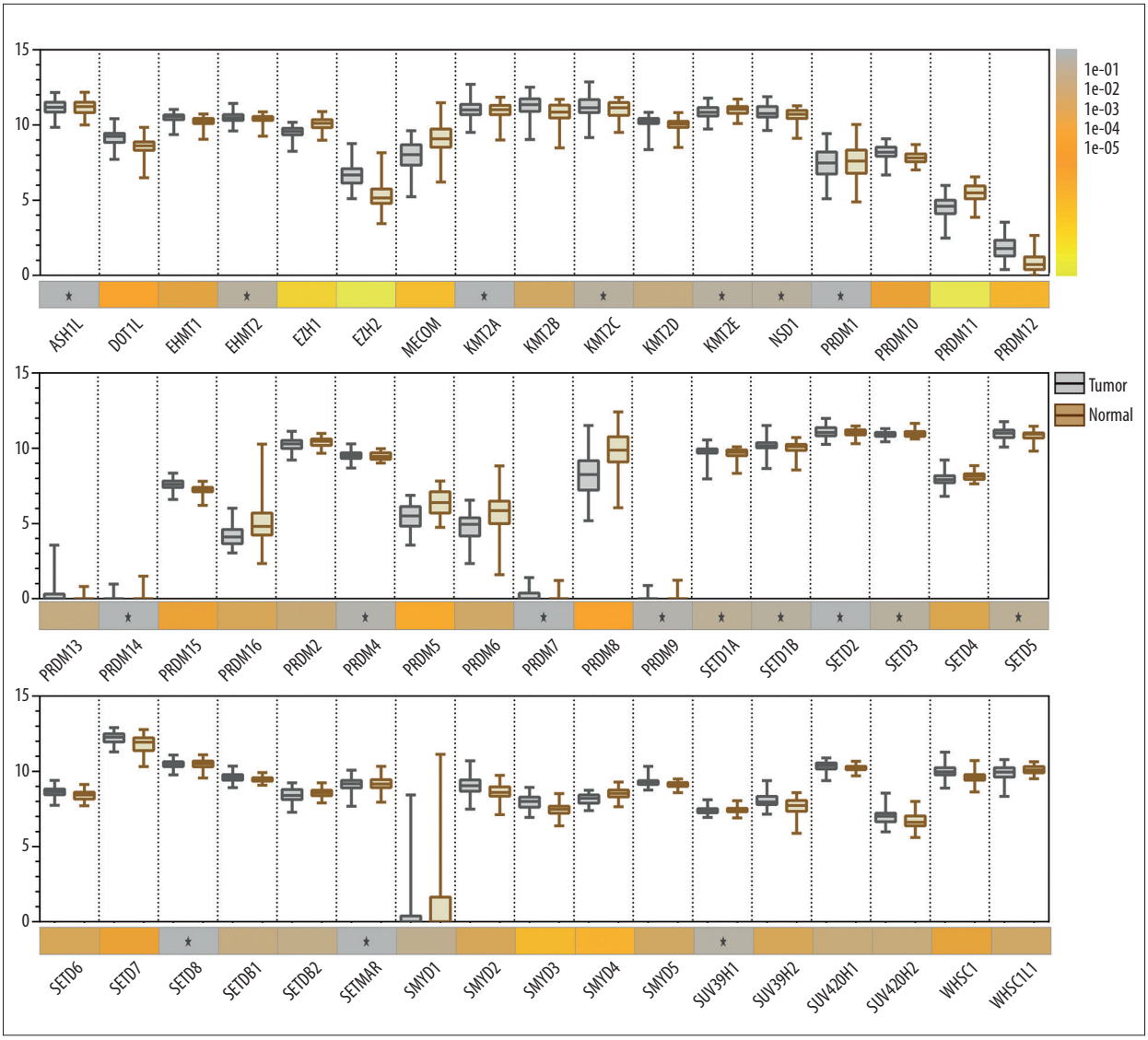

Figure 1. Box plots showing histone lysine methyltransferase (HKMT) mRNA levels in 52 matched prostate cancer tissues (grey) and adjacent normal tissues (brown). Paired t-test was performed to compare the different expression profiles between tumor and adjacent normal tissues. Statistical significance is shown as the p-values by different grayscales of the lower color bars (p>0.05 are specifically shown by * inside the sub-boxes). There was reduced mRNA expression of several genes, including PRDM7, PRDM9, PRDM13, PRDM14, and SMYD1.

and SMYD1 genes were at very low levels in both tumor tissues and normal prostate tissues. Twenty-one significantly fold-change (FC) genes with false discovery rates (FDR) with a p-value of <0.05 were found using the Limma R software package for analyzing data from gene expression, including six 2.0-fold-change, three 1.5-fold-change, and eleven 1.2-fold-change genes, which were in accordance with 23 HMT genes previously described in the literature (Supplementary Table 3).

To compare the differences between HMT gene expression profiles between Gleason score 6, 7, 8, and 9 prostate cancers, one-way analysis of variance (ANOVA) was performed, which identified 11 HMT genes that were significantly expressed: WHSC1, EZH2, PRDM12, KMT2D, SETD5, PRDM8, EHMT1, SUV420H2, PRDM10, PRDM6, and DOT1L (p <0.001) (Supplementary Figure 1, Supplementary Table 4). Among those genes, PRDM6 and PRDM8 both showed a significantly negative correlation with the Gleason score. The EZH2, PRDM12, SUV420H2, WHSC1, PRDM10 and SETD5 genes showed a significantly positive correlation with the Gleason score (Supplementary Figure 1, Supplementary Figure 2). These findings indicated that alteration in mRNA expression levels of these genes in prostate cancer tissue samples were prognostic indicators in these cases of prostate cancer.

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Table 1. Frequency of HMTs copy number alterations and mutations (%).

Gene	Location	Amp	Gain	Diploid	Hetloss	Homdel	Mutation
SETDB2	13q14.2	0.00	0.81	54.88	27.64	16.67	0.24
PRDM1	6q21	0.00	0.41	67.07	18.70	13.82	0.00
PRDM13	6q16.2	0.00	1.22	66.06	19.11	13.62	0.00
PRDM7	16q24.3	0.20	1.02	60.57	29.88	8.33	0.47
WHSC1L1	8p11.23	2.64	11.38	52.44	27.44	6.10	0.71
PRDM5	4q27	0.00	2.85	87.60	6.30	3.25	0.24
EZH1	17q21.2	0.00	1.02	86.18	10.16	2.64	0.24
SETD5	3p25.3	0.20	6.91	85.77	4.47	2.64	0.24
SETD6	16q21	0.20	1.22	73.98	21.95	2.64	0.00
PRDM11	11p11.2	0.20	4.47	88.82	4.27	2.24	0.00
PRDM6	5q23.2	0.20	2.64	87.20	8.13	1.83	0.00
KMT2A	11q23.3	0.41	5.89	85.77	6.30	1.63	1.18
PRDM15	21q22.3	0.00	4.67	87.40	6.50	1.42	0.24
SETD1B	12q24.31	0.81	4.88	87.20	5.69	1.42	0.00
SETD2	3p21.31	0.00	7.11	87.80	3.66	1.42	0.94
SETMAR	3p26.1	0.20	7.32	86.79	4.27	1.42	0.00
SMYD2	1q32.3	0.00	6.30	88.41	3.86	1.42	0.24
KMT2C	7q36.1	0.41	18.70	75.41	4.27	1.22	5.41
SETD3	14q32.2	0.41	2.64	89.02	6.71	1.22	0.00
PRDM10	11q24.3	0.81	6.10	87.60	4.47	1.02	0.24
SMYD4	17p13.3	0.00	1.42	80.08	17.48	1.02	0.00
PRDM2	1p36.21	0.20	0.61	89.23	9.15	0.81	0.47
SETD8	12q24.31	0.61	4.27	87.40	6.91	0.81	0.00
SMYD5	2p13.2	0.41	2.64	90.24	5.89	0.81	0.00
DOT1L	19p13.3	0.20	1.22	90.24	7.72	0.61	0.71
PRDM9	5p14.2	0.61	6.30	89.43	3.05	0.61	0.71
SETD1A	16p11.2	0.41	6.10	86.79	6.10	0.61	0.47
SMYD3	1q44	0.41	5.89	90.04	3.05	0.61	0.00
SUV39H2	10p13	0.41	3.46	86.59	8.94	0.61	0.00
SUV420H1	11q13.2	2.44	7.32	89.02	0.61	0.61	0.24
WHSC1	4p16.3	1.02	2.85	89.43	6.10	0.61	0.24
ASH1L	1q22	1.42	6.10	90.04	2.03	0.41	1.88
EHMT2	6p21.31	0.20	3.66	91.46	4.27	0.41	0.24
EZH2	7q36.1	0.41	18.70	79.07	1.42	0.41	0.24
KMT2B	19q13.12	0.41	2.03	92.89	4.27	0.41	0.47

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Table 1 continued. Frequency of HMTs copy number alterations and mutations (%).

Gene	Location	Amp	Gain	Diploid	Hetloss	Homdel	Mutation
KMT2E	7q22.3	0.81	18.70	78.25	1.83	0.41	0.94
PRDM16	1p36.32	0.41	1.63	89.43	8.13	0.41	0.24
PRDM8	4q21.21	0.41	2.64	92.89	3.66	0.41	0.71
SETD4	21q22.12	0.00	4.67	89.23	5.69	0.41	0.00
SETD7	4q31.1	0.00	3.25	91.26	5.08	0.41	0.00
SMYD1	2p11.2	0.41	2.24	91.46	5.49	0.41	0.00
PRDM12	9q34.12	1.22	10.57	85.37	2.64	0.20	0.47
PRDM4	12q23.3	1.02	5.28	88.82	4.67	0.20	0.00
EHMT1	9q34.3	1.42	10.57	85.37	2.64	0.00	0.00
KMT2D	12q13.12	0.20	3.25	91.87	4.67	0.00	4.94
MECOM	3q26.2	3.66	13.62	80.69	2.03	0.00	1.18
NSD1	5q35.2	0.81	4.27	91.67	3.25	0.00	0.47
PRDM14	8q13.3	6.10	24.80	68.29	0.81	0.00	0.47
SETDB1	1q21.3	1.42	6.71	91.26	0.61	0.00	0.47
SUV39H1	Xp11.23	1.02	2.64	90.85	5.49	0.00	0.00
SUV420H2	19q13.42	0.41	4.07	91.46	4.07	0.00	0.24

Amp – high-level amplification; Gain – low-level gain; Hetless – heterozygous deletion; Homdel – homozygous deletion. Genes were ranked based on the frequency of Homdel.

Changes in copy number alteration (CNA) of HMT genes in prostate carcinoma

Copy number alterations (CNAs) have previously been reported to be an important mechanism for activating oncogenes or inactivating tumor suppressor genes in the genesis and progression of human malignancy [13]. Therefore, CNAs of 51 HMT genes in 492 prostate cancer tissue samples were systematically analyzed, which identified six HMT genes: SETDB2, PRDM1, PRDM13, PRDM7, WHSC1L1, and PRDM5. These six genes showed homozygous deletion (Homedel) in more than 3% of prostate cancer tissue samples. Two HMT genes, PRDM14 and MECOM, showed high-level amplification (Amp) in more than 3% of prostate cancer samples (Table 1, Supplementary Figure 3).

Further analysis of 51 HMT CNAs in prostate cancer tissue samples, with different Gleason scores, showed that increasing CNA events in prostate cancer were significantly correlated with a Gleason score of between 6 to 9 (Figure 2A). The CNA frequencies of most HMT genes were positively correlated with the Gleason score, for both amplification and deletion alterations (Supplementary Figure 4). Also, with increasing Gleason score, the average CNA events of HMT genes in each tumor tissue sample were all incrementally increased, except for the homozygous deletion (Homedel), which had a more stable level in prostate cancer tissue samples with different Gleason scores (Figure 2C). The frequencies of CNA events in metastatic prostate cancer tissue samples were significantly increased when compared with primary prostate cancer tissue (Supplementary Figure 5). These data indicated that, in the cases in this study, prostate cancers that had many more CNA events of the HMT genes had an increased tumor grade and worse clinical outcome.

The correlations between copy number alterations and expression of 51 HMT genes were analyzed in the 399 prostate cancer tissue samples that underwent sequence analysis. To obtain a weighted ranking for the correlation coefficients, the Spearman correlation, Kendall rank correlation, and Pearson correlation analysis were performed (Table 2). As shown in Table 2, gene expression levels of almost all HMT genes were positively correlated with CNAs, including two HMT genes, WHSC1L1 and SETDB2 with correlation coefficients >0.5 (Spearman method, $p<0.0001$) and four HMT genes, SETD6, SMYD4, EZH2 and SETDB1 with correlation coefficients >0.3 (Spearman method, $p<0.0001$). To further analyze the impact caused by CNAs on gene expression levels of HMTs, the frequency of amplification

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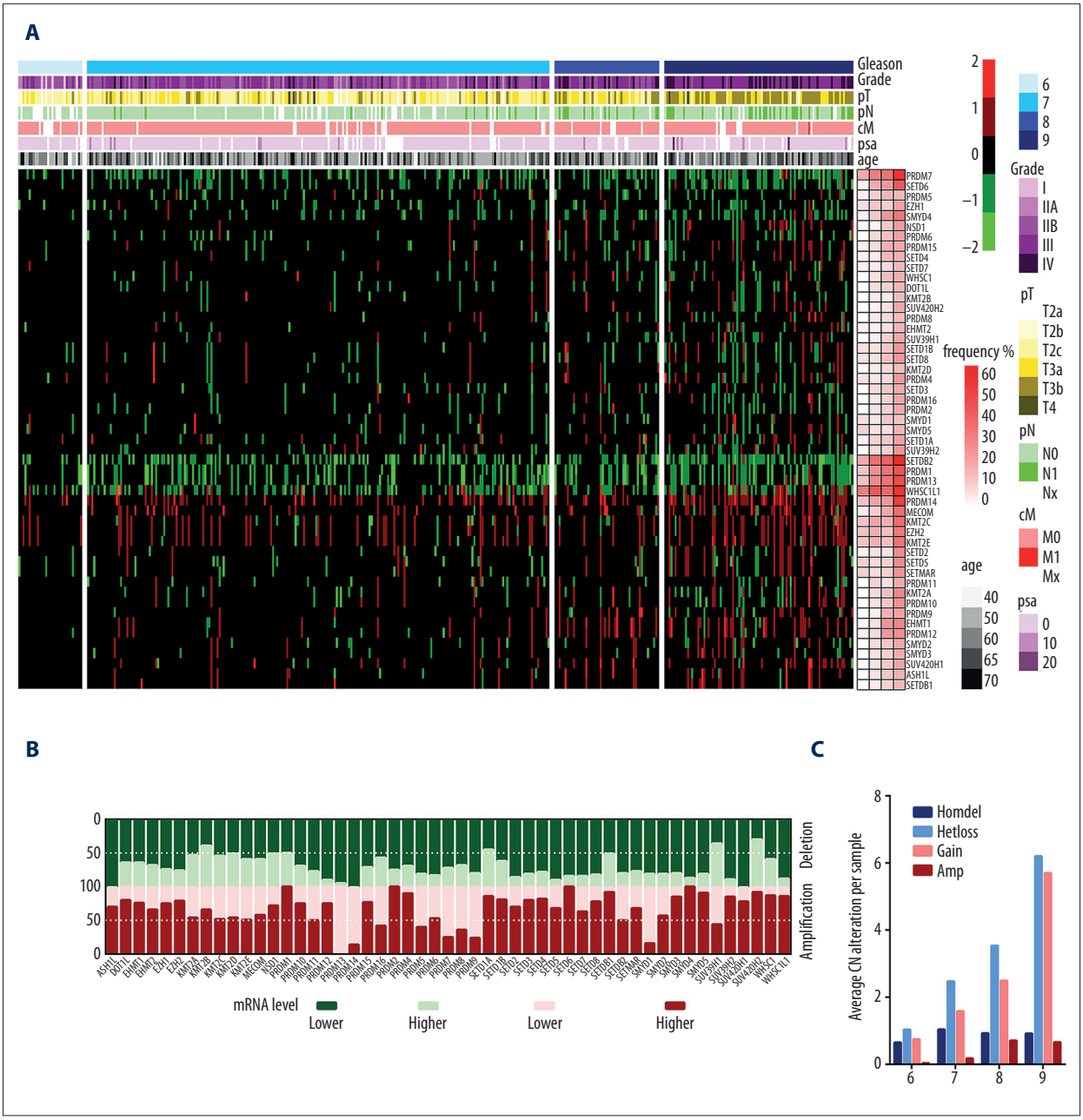

Figure 2. Expression profiles of altered copy numbers of histone lysine methyltransferase (HKMT) in samples of prostate cancer according to Gleason scores. (**A**) The pheatmap package in R shows that increasing copy number alteration (CNA) events of 51 histone methyltransferases (HMTs) in prostate cancer samples, with a Gleason score of 6 to 9, were significantly associated with the clinical stage. (**B**) A histogram shows the effect on HMT expression levels due to changes in gene copy numbers. The y-axis represents the proportion of genes expressed (higher or lower) associated with different types of copy number alterations. (**C**) A histogram shows that the average CNA events of HMTs in each sample are incrementally increased with an increase in Gleason score.

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Table 2. Associations between HMTs expression and CNAs or methylation state.

Gene	CNA/mRNA Correlation			Methylation/mRNA Correlation		
	Spearman	Pearson	Kendall	Spearman	Pearson	Kendall
WHSC1L1	0.577	0.577	0.460	–0.005	0.026	–0.004
SETDB2	0.555	0.535	0.435	0.122	0.183	0.083
SETD6	0.499	0.530	0.407	–0.232	–0.230	–0.157
SMYD4	0.400	0.410	0.325	NA	NA	NA
EZH2	0.347	0.363	0.283	–0.232	–0.197	–0.160
SETDB1	0.325	0.411	0.265	–0.179	–0.213	–0.119
SETD8	0.310	0.338	0.251	0.015	–0.013	0.011
SUV39H2	0.297	0.309	0.240	–0.043	–0.071	–0.029
SETD3	0.294	0.436	0.240	–0.068	–0.091	–0.045
EZH1	0.293	0.359	0.236	–0.089	–0.094	–0.059
EHMT1	0.280	0.355	0.228	NA	NA	NA
SUV420H1	0.277	0.462	0.226	–0.164	–0.160	–0.111
PRDM4	0.275	0.339	0.223	–0.024	0.033	–0.017
SETD4	0.265	0.302	0.216	0.305	0.259	0.205
SETD5	0.243	0.211	0.196	–0.118	–0.078	–0.078
KMT2C	0.243	0.283	0.195	NA	NA	NA
SMYD5	0.241	0.246	0.196	–0.116	–0.101	–0.081
PRDM13	0.237	0.174	0.216	0.346	0.327	0.266
PRDM12	0.217	0.205	0.176	0.356	0.356	0.244
SETD2	0.212	0.176	0.172	–0.161	–0.127	–0.112
PRDM2	0.209	0.216	0.171	–0.323	–0.360	–0.219
PRDM15	0.207	0.238	0.168	–0.087	–0.069	–0.058
SETMAR	0.196	0.229	0.158	–0.041	–0.053	–0.028
KMT2A	0.193	0.209	0.156	0.210	0.247	0.139
SMYD3	0.189	0.208	0.153	0.234	0.189	0.160
WHSC1	0.174	0.270	0.140	–0.133	–0.168	–0.088
PRDM10	0.170	0.191	0.138	–0.093	–0.130	–0.064
SETD1A	0.165	0.200	0.132	–0.071	–0.082	–0.046
SETD7	0.164	0.200	0.132	0.223	0.224	0.150
SETD1B	0.164	0.139	0.133	NA	NA	NA
KMT2E	0.160	0.169	0.129	0.129	0.048	0.087
SMYD2	0.158	0.252	0.129	–0.140	–0.215	–0.093
PRDM5	0.155	0.162	0.125	–0.523	–0.551	–0.365
PRDM6	0.154	0.157	0.124	–0.262	–0.265	–0.175

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Table 2 continued. Associations between HMTs expression and CNAs or methylation state.

Gene	CNA/mRNA Correlation			Methylation/mRNA Correlation		
	Spearman	Pearson	Kendall	Spearman	Pearson	Kendall
PRDM14	0.150	0.132	0.143	0.046	0.056	0.036
ASH1L	0.149	0.145	0.121	–0.298	–0.130	–0.205
PRDM11	0.142	0.164	0.114	0.055	0.054	0.035
NSD1	0.127	0.141	0.103	0.029	0.040	0.019
DOT1L	0.126	0.156	0.103	–0.053	–0.052	–0.034
PRDM9	0.122	0.055	0.118	–0.121	–0.013	–0.098
EHMT2	0.116	0.160	0.094	–0.128	–0.110	–0.085
SUV420H2	0.074	0.100	0.060	–0.050	–0.019	–0.032
KMT2B	0.072	0.055	0.058	0.052	0.028	0.034
KMT2D	0.059	0.050	0.048	0.030	0.025	0.021
PRDM16	0.058	0.121	0.047	NA	NA	NA
PRDM7	0.056	0.035	0.050	0.050	0.037	0.036
MECOM	0.048	0.059	0.038	–0.163	–0.139	–0.113
PRDM1	0.034	0.061	0.028	0.002	–0.080	0.003
SMYD1	0.006	0.030	0.006	0.007	–0.041	0.005
PRDM8	–0.011	–0.044	–0.009	–0.529	–0.551	–0.372
SUV39H1	–0.050	–0.014	–0.040	0.007	0.049	0.005

Genes were ranked based on the Spearman correlation coefficient. Significantly associated HMTs between gene expression with CNAs or methylation were highlighted.

and deletion with higher or lower HMT mRNA levels in tissue samples were calculated separately. The frequency of amplification with higher HMT mRNA levels and deletion with lower HMT mRNA levels, were significantly increased in prostate cancer tissue compared with the normal prostate tissue ($p<0.0001$, paired t-test). The difference between diploid tumor samples was non-significant (Figure 2B, Supplementary Table 5).

Methylation states of the HMT gene promoter CpG islands in prostate cancer

DNA methylation is a crucial regulator in tumor development, by altering mRNA expression. For 46 HMT genes, correlation analysis was performed to compare gene expression and promoter methylation in 399 prostate cancer tissue samples by three different statistical methods, the Spearman correlation analysis, Kendall rank correlation, and the Pearson correlation analysis. The results showed that the promoter methylation level of most HMT genes was negatively correlated with gene expression, including two HMT genes, PRDM8 and PRDM5, with an absolute value with the Spearman correlation coefficient >0.5 ($P<0.0001$) and one HMT gene, PRDM2 with the Spearman correlation coefficient >0.3 ($P<0.0001$). However, the methylation levels of the SETD4, PRDM12, and PRDM13 genes were all significantly correlated with gene expression levels, with a correlation coefficient >0.3 ($P<0.0001$) (Table 2).

The pheatmap package in R was plotted to show the promoter methylation profiles of 46 HMT genes in 50 paired prostate tumor and adjacent normal tissue samples. Most HMT genes had a lower promoter methylation level, but some HMT genes had a higher promoter methylation level, including the three genes, PRDM7, PRDM9, and SMYD1, which had low mRNA levels (Figures 1, 3A). Further quantitative analysis of the methylation changes between matched tumor and adjacent normal prostate tissues, identified PRDM14, NSD1, and PRDM8 as the top three differentially methylated HMT genes in prostate cancer, by paired t-test and correlation analysis (Supplementary Table 6). By combining the correlation analysis of mRNA and promoter methylation and the results of differential expression and methylation, the PRDM5 and PRDM8 genes were identified and underwent further analysis (Figure 3B, 3C).

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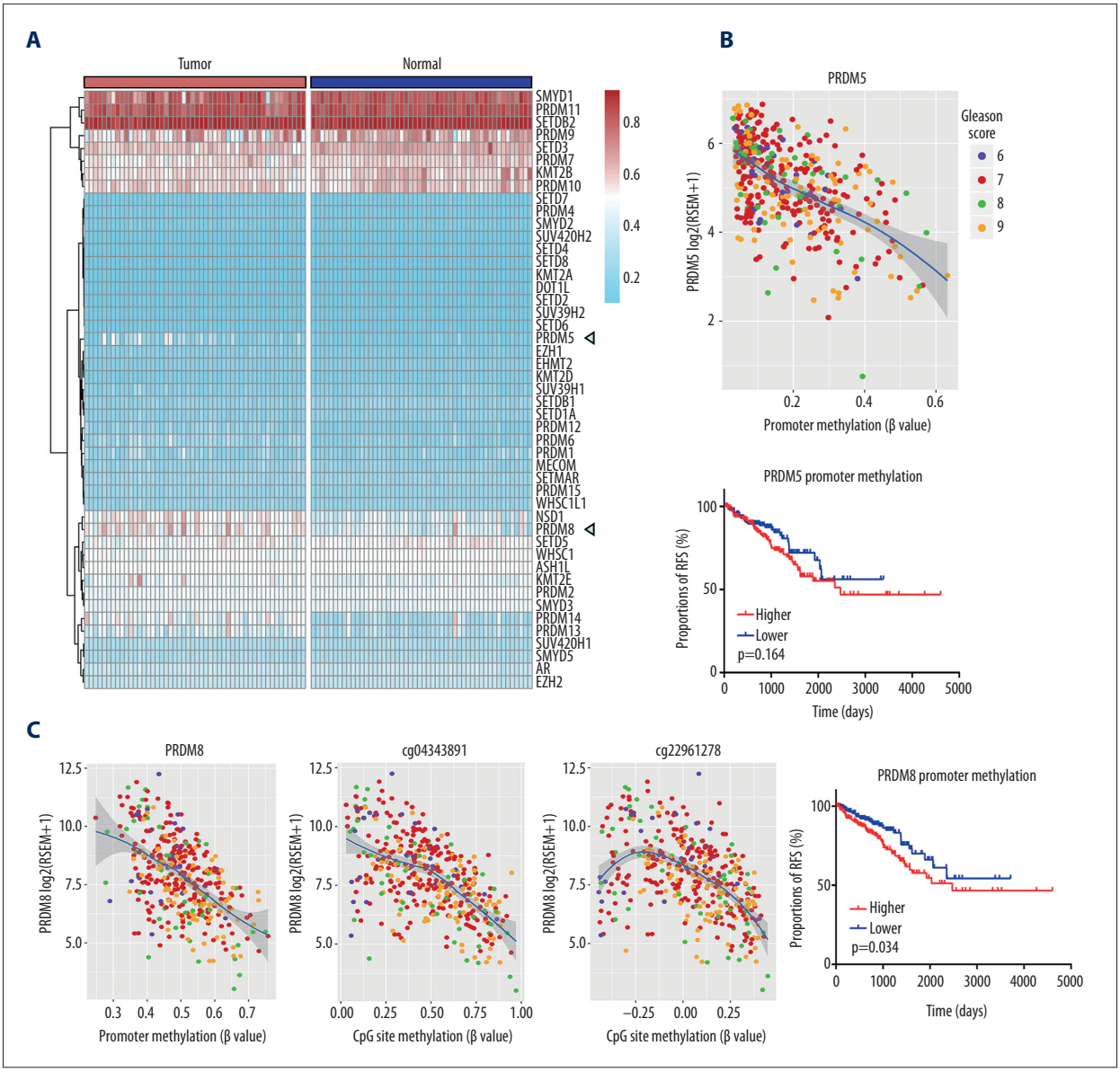

Figure 3. DNA methylation profiles of histone methyltransferases (HMTs) in prostate cancer (adenocarcinoma). (**A**) The pheatmap package in R showed different promoter methylation levels in 50 paired tumor tissues and corresponding adjacent tissues. Promoter CpG sites of most histone methyltransferases (HMTs) are expected at low methylation level and the difference between tumor and normal tissues are unexpected plain. PRDM5 and PRDM8 were indicated by triangle because their mRNA levels are both 1.2-fold-change from normal to tumor tissues and have a high correlation coefficient to the corresponding gene expression level. (**B, C**) Scatter diagrams showed the relationship between gene expression and its promoter methylation state (β value) in 399 prostate cancers. Survival analysis show samples with lower PRDM5 promoter methylation as well as PRDM8 have a better prognosis in some extent than higher ones. (**C**) Two CpG sites in the PRDM8 promoter which are both 1.2-fold-change between normal and tumor tissues have an increased correlation with PRDM8 mRNA levels compared with other CpG sites.

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Table 3. Summary of PRDM5 CpG sites.

CpG ID	Genetic location	Epigenetic location	β >0.3 in tumor(%)	β <0.1 in normal(%)	PRDM5 mRNA level in 399 PCa samples		
					β >0.3	β <0.1	p-Value
cg07070341	TSS200	Island	16	94	4.418	5.461	1.34E-14
cg18181323	TSS200	Island	8	98	3.996	5.418	2.34E-08
cg19294653	TSS200	Island	8	98	4.043	5.444	3.55E-11
cg22135105	TSS200	Island	22	98	4.438	5.506	8.41E-19
cg11171429	TSS1500	S_Shore	20	94	4.358	5.542	2.31E-18
cg13155001	1st Exon; 5'UTR	Island	10	100	4.661	5.201	6.39E-03
cg06329345	TSS1500	S_Shore	30	88		NA	
cg10416963	TSS1500	S_Shore	78	4		NA	

Methylation level of CpG sites of PRDM5 in 50 paired tumor and adjacent tissues were listed. Average PRDM5 mRNA level in the two groups (β >0.3 and β <0.1) were calculated and T.test was used to value the significance of difference.

Analysis of the PRDM8 and PRDM5 differentially methylated HMT genes in prostate cancer

Promoter methylation of the PRDM8 gene was increased, even in the normal prostate tissues. However, the 1.5-fold-change was significantly increased in the tumor tissues resulting in significant alterations with gene expression ($p<0.0001$, using a t-test). Different methylation levels of the PRDM8 gene were also correlated with clinical outcome in prostate tumor samples from 399 patients. Also, two CpG sites (cg04343891 and cg22961278) of the PRDM8 promoter were both differently methylated and significantly associated with PRDM8 gene expression (Figure 3C).

Promoter methylation of the PRDM5 gene was decreased in the normal prostate tissues but was significantly increased in matched prostate cancer tissues. For PRDM5 gene expression, there were six CpG sites out of eight with a lower methylation level ($\beta \leq 0.1$). In more than 90% of adjacent normal prostate tissues, and in more than 5% of tumor tissues, a higher methylation level was found ($\beta \geq 0.3$). The average PRDM5 mRNA levels of the two groups ($\beta \geq 0.3$ and $\beta \leq 0.1$) in the 399 prostate cancer samples were compared for each CpG site. The results showed that PRDM5 was differently expressed between two groups for six CpG sites ($p<0.001$ using a t-test) (Table 3). These data support that PRDM5 was a methylation silenced gene in prostate cancer in the cases in this study, which highlights the need for further studies to determine the potential treatment approaches for patients with different PRDM5 promoter methylation states [14].

Mutations of HMT genes in prostate cancer

The KMT2C, KMT2D, ASH1L, KMT2A, and MECOM genes were the top five most frequently mutated HMT genes in prostate cancer in this study (Table 1, Figure 4A). Integrated analysis of mutation profiles of KMT2C and KMT2D were performed in 425 primary prostate cancer samples. The results showed that a total of 23 KMT2C gene mutations and 21 KMT2D gene mutations were identified (Figure 4B). A mutation map was performed to display the distribution and frequency of KMT2C and KMT2D mutations in 425 prostate cancers (Figure 4C). A mutualy exclusivity relationship for copy number alterations and mutations of the top five mutated HMTs in prostate cancers are shown in Figure 4D.

Co-expression of HMT genes with androgen receptors

Cluster analysis showed that high expression levels of several HMT genes, including ASH1L, SETD2, KMT2B, NSD1, KMT2C, SETD7, KMT2E, KMT2A, PRDM10, SETD5, PRDM2, PRDM4, and WHSC1L1 were significantly clustered in tumor tissue samples with high expression of androgen receptors when compared with tumor tissue samples with low expression of androgen receptors, which was determined by correlation analysis between androgen receptor and HMT mRNA levels (Figure 5, Supplementary Table 5). Notably, KMT2B, KMT2C, and KMT2E have been validated previously as having a role in the progression of castration-resistant prostate cancer (CRPC) [15]. These results showed that these genes, identified in the cases in this study, may be involved in the androgen receptor response pathway in prostate cancer, and their genetic alterations may function in the development of CRPC.

Alterations in HMT gene expression were associated with clinical outcome in patients with prostate cancer

To study the clinical effects HMT gene variations on clinical outcome in patients with prostate cancer, the association between progression-free survival (PFS), CNA, mRNA levels, mutation and

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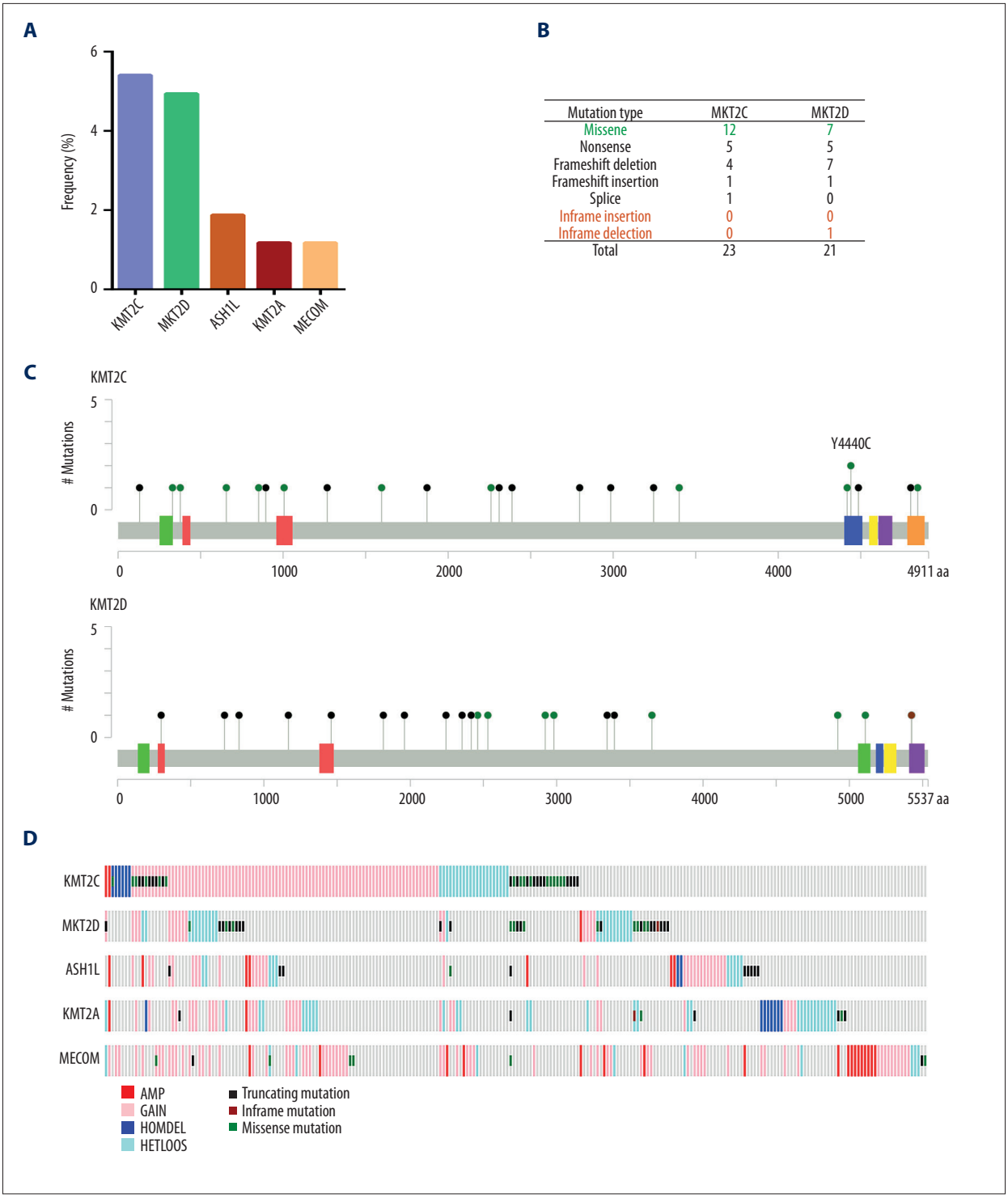

Figure 4. The KMT2C and KMT2D gene mutation spectrum in prostate carcinoma. (**A**) Bar graphs show the top five mutated histone methyltransferases (HMTs). (**B**) The frequency of each mutation type for KMT2C and KMT2D from 429 primary prostate adenocarcinomas. The data were obtained from The Cancer Genome Atlas (TCGA) database. (**C**) The images show protein domains and the positions of specific mutations of the KMT2C and KMT2D genes. A green dot indicates a missense mutation. The black dot indicates a truncating mutation include nonsense mutation, frameshift deletion, insertion, or splice. A brown dot indicates an in-frame insertion or deletion mutation. (**D**) Mutual exclusivity relationship for copy number alterations and mutation of the top five mutated HMTs in prostate cancers.

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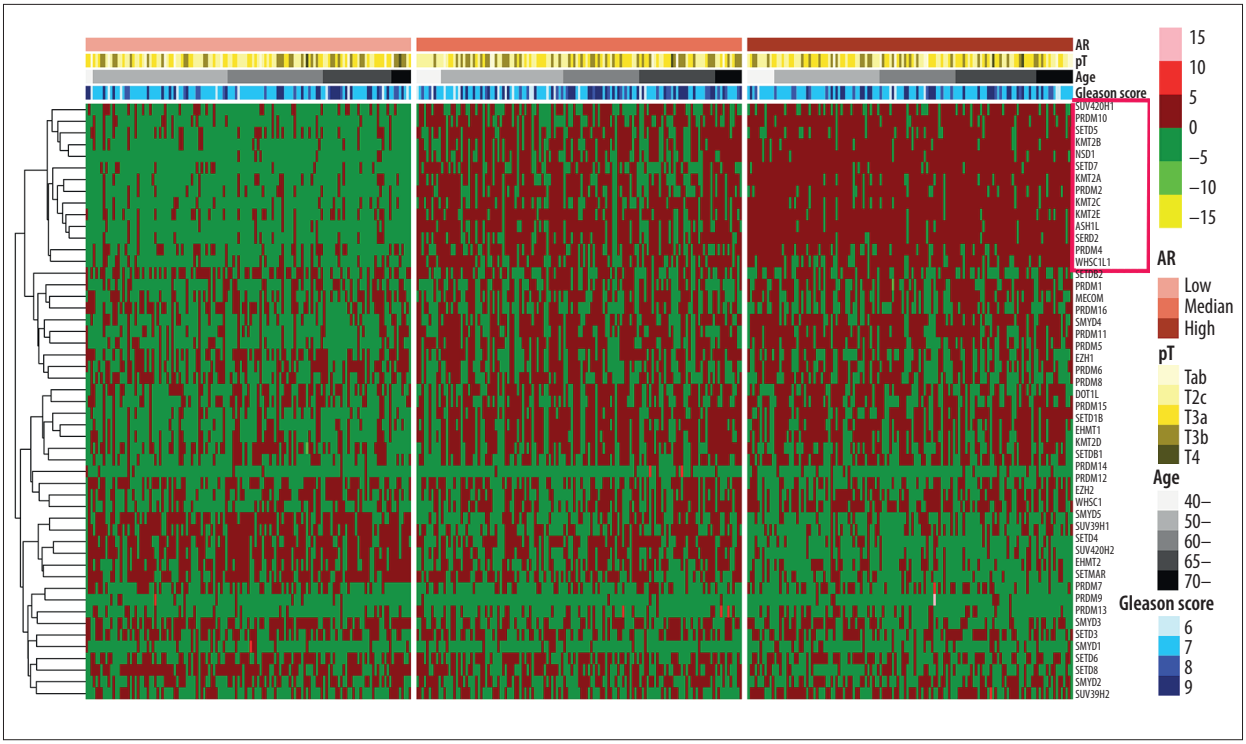

Figure 5. Co-expression of histone methyltransferases (HMTs) and androgen receptors. The pheatmap package in R shows that several histone methyltransferases (HMTs) (ASH1L, SETD2, KMT2B, NSD1, KMT2C, SETD7, KMT2E, KMT2A, PRDM10, SETD5, PRDM2, PRDM4, and WHSC1L1) were significantly clustering in the higher androgen receptor (AR) expression cluster.

promoter methylation levels were examined in the 399 prostate cancer samples. Univariate Cox proportional hazards analysis was performed primarily to find potential HMT genes that were most relevant for further multivariate Cox proportional-hazards analysis, with $p<0.05$. The results showed that amplification and deletion of most the most significant HMT genes were associated with poor clinical outcome (HR >1). Nine HMT genes with increased levels of promoter methylation, and sixteen HMT genes with increased mRNA levels were identified as having a significant association with clinical outcome in the cases of prostate cancer included in this study (Supplementary Table 7).

Patient age, the T-stage, Gleason score, and the expression level of the androgen receptor (AR) for 28 genetic alterations were identified by multivariate analysis ($p<0.1$) (Supplementary Table 8). The results showed that amplification of EHMT1, KMT2B, PRDM1, PRDM12, PRDM16, PRDM4, SETD1B, SETD8, SETDB1, SMYD1, SMYD2, SMYD5, and WHSC1L1 genes, and the deletion of EHMT2, SETD4, SETD6, SMYD1, SUV39H2, and WHSC1L1 genes, were significantly associated with reduced clinical outcome and PFS. Increased mRNA levels of EZH2, SETD5, and SUV420H2 genes, and lower mRNA levels of PRDM6, SMYD1, and PRDM16 genes were significantly associated with reduced clinical outcome and PFS in patients with prostate cancer. Increased methylation levels of NSD1 was significantly associated with a reduced patient overall survival (OS).

Of the 28 genetic alterations of HMT genes, mutations of KMT2C and KMT2D were investigated to show their clinical impact on prostate cancer samples using a forest plot (Figure 6A). The most relevant HMT genes were selected by integrating the score of mRNA, CNA and promoter methylation associated survival, CNA frequencies, differential expression and methylation, correlation of HMT gene expression and CNAs, androgen receptor expression and Gleason score (Figure 6B). Each of seven HMT genes, including EZH2, NSD1, RPDM12, SETD5, SETD6, SMYD1 and WHSC1L1, had a score of >3, indicating that these seven HMT genes might play important roles in oncogenesis in prostate cancer. For these seven HMT genes, Kaplan-Meier curves were plotted and the results showed that amplification of EZH2 and SETD5 were associated with clinical outcome (Figure 6C, Supplementary Figure 6).

The four-gene HMT prognostic signature included EZH2, SETD5, SMYD1, and SUV420H2

Expression of the four most significant HMT genes in terms of prognosis in prostate cancer, EZH2, SETD5, SMYD1, and SUV420H2, allowed a panel to be compiled to estimating their prognostic value for relapse of prostate cancer (Figure 7A). Kaplan-Meier curves were plotted to show the prognostic value of this gene panel in the Cancer Genome Atlas (TCGA) cohort (n=399), by dividing the samples a high-risk and low-risk

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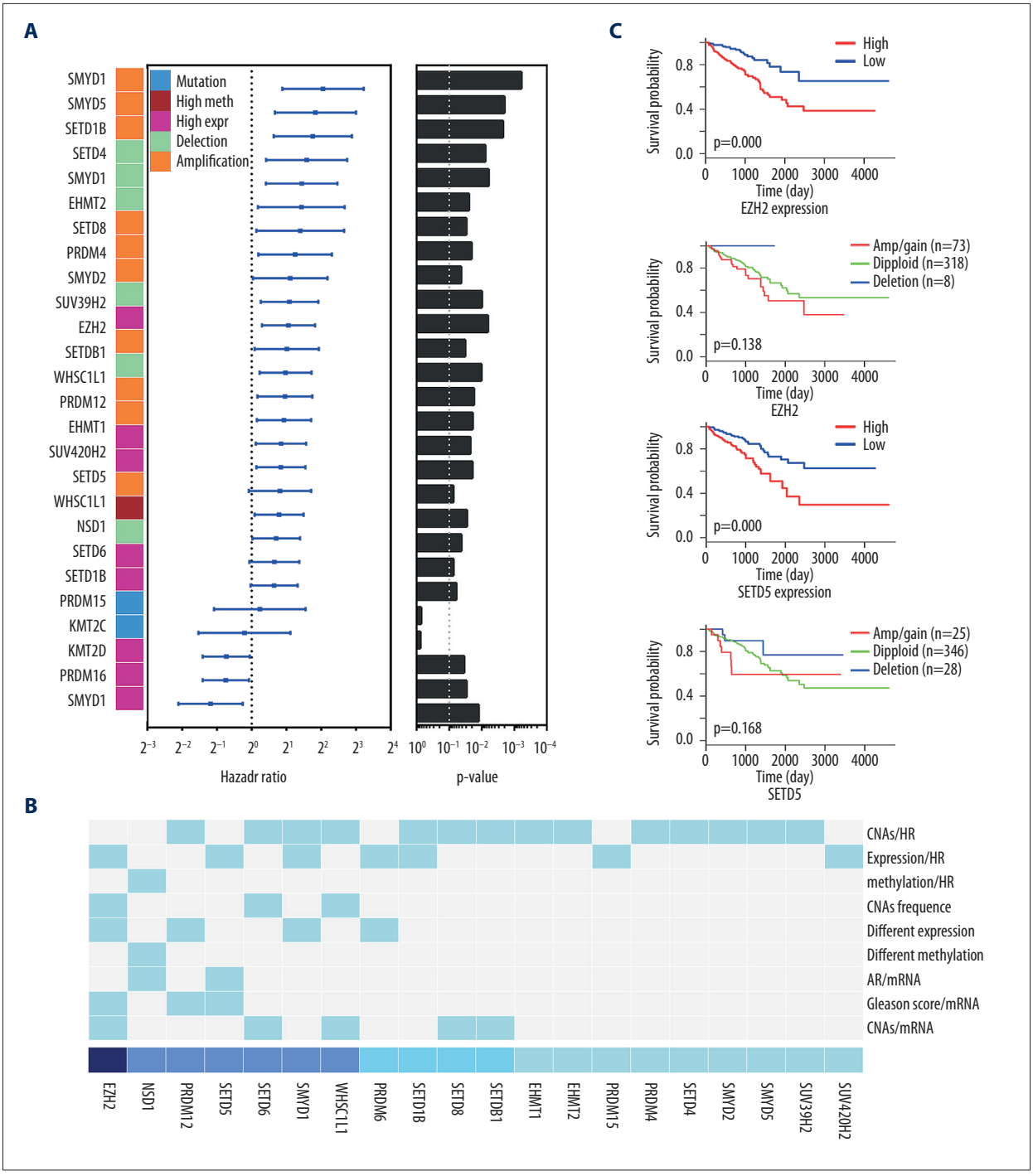

Figure 6. Genetic alterations of histone methyltransferases (HMTs) are associated with recurrence-free survival (RFS) of patients with prostate carcinoma. (**A**) The 28 genetic alterations (including mutation, amplification, deletion, increased gene expression, and methylation) of histone methyltransferases (HMTs) are shown by forest plot to demonstrate their impact on clinical survival, showing the hazard ratio (HR), 95% confidence interval (CI) and p-value (Wald test). (**B**) Critical HMT genes (EZH2, NSD1, RPDM12, SETD5, SETD6, SMYD1, and WHSC1L1) were analyzed using the mRNA score, copy number alterations (CNAs) and promoter methylation associated survival, CNA frequencies, differential expression and methylation, correlation of CNA, androgen receptor (AR) expression, and Gleason score with HMT expression. (**C**) Kaplan-Meier plot of the recurrence-free survival (RFS) show that two typical HMT genes (EZH2 and SETD5) have the same clinic outcome of amplification and increased expression or deletion and reduced expression.

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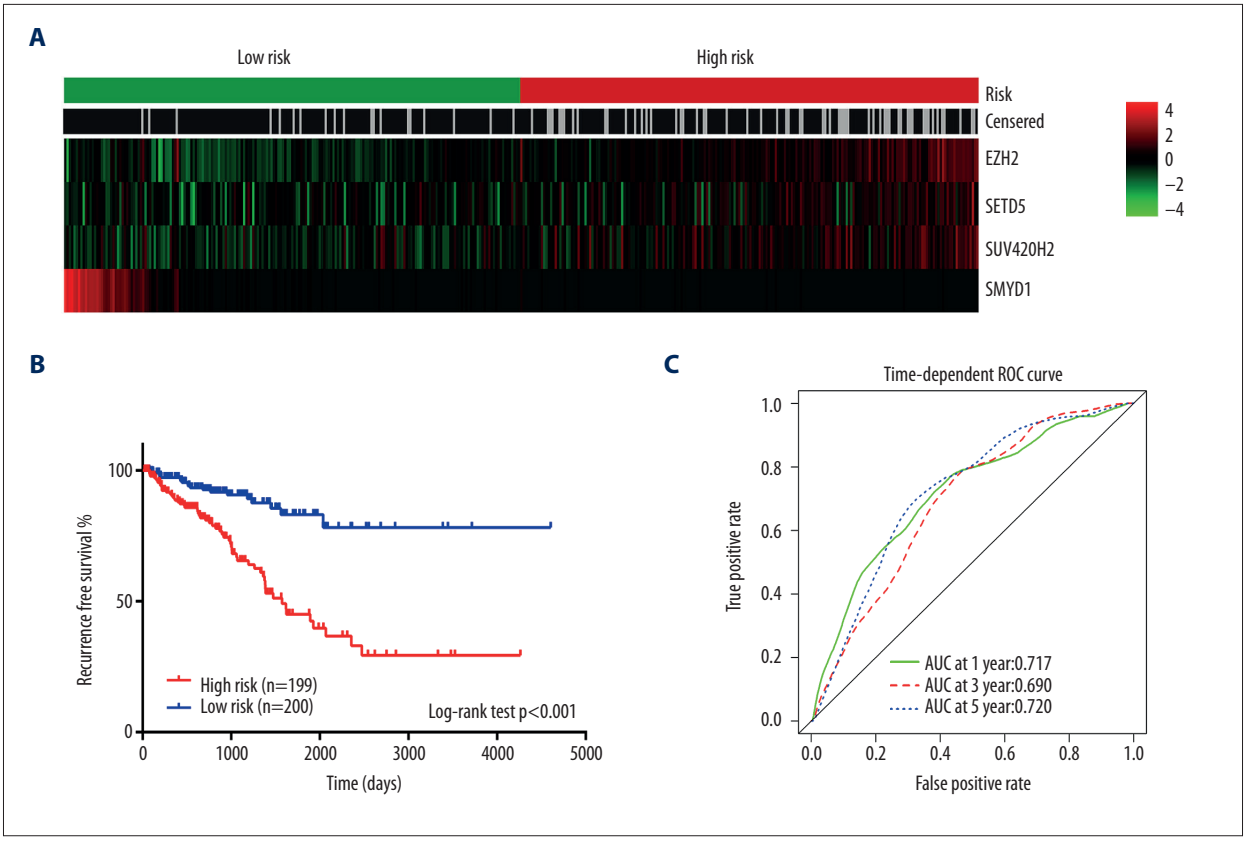

Figure 7. The histone methyltransferase (HMT) predictor-score analysis of 399 prostate cancer patients in the Cancer Genome Atlas (TCGA) cohort. (**A**) Expression profile of four histone methyltransferases (HMTs) in high-risk and low-risk patients. (**B**) Kaplan-Meier plot shows the recurrence-free survival (RFS) in high-risk and low-risk patients. (**C**) A time-dependent area under the receiver operating characteristics (AUROC) curve was used to assess the prognostic value of the four HMT gene prognostic signature (EZH2, SETD5, SMYD1, and SUV420H2). The AUC for the four HMT gene prognostic signature model at one, three, and five years were 0.717, 0.690, and 0.720, respectively via cross-validation (purple).

group (Figure 7B). A time-dependent area under the receiver operating characteristics (AUROC) curve was used to assess the prognostic value of the four-gene HMT prognostic signature (EZH2, SETD5, SMYD1, and SUV420H2). The area under the curve (AUC) for the four-gene HMT prognostic signature model at one, three, and five years were 0.717, 0.690, and 0.720, respectively (Figure 7C).

The SETD5 gene in the development and progression of prostate cancer

The SETD5 gene expression was studied in the 399 samples of prostate cancer from TCGA data. The study samples were divided into low-expression and high-expression groups for SETD5 in tissue samples of prostate cancer, with the median value as the cutoff point. Five characteristics were analyzed in the low-expression and high-expression groups. The results showed that high SETD5 expression was correlated with high Gleason scores, increase serum levels of prostate-specific antigen (PSA), advanced T-stage, and the presence of lymph node metastases (Table 4).

The validate these results in prostate cancer, SETD5 was selected. There was no difference in the expression of SETD5 between tumor and adjacent normal tissues, but there was a difference in the expression of SETD5 between tissue samples of primary prostate cancer and metastatic prostate cancer in the GSE3325 dataset [16]. Also, serial experiments were performed to validate the role of the SETD5 gene in the 22Rv1 prostate cancer cell line. Following the establishment of the SETD5 knockdown cell line, colony formation assay, the MTS proliferation assay, and the transwell assay, SETD5 gene knockdown reduced cancer cell growth and migration in the 22Rv1 cells (Figure 8).

Discussion

Integrated genomic analyses of 51 histone methyltransferase (HMT) genes were performed in prostate adenocarcinoma samples, which were collected from the Cancer Genome Atlas (TCGA) database. In this study, there were eight main findings. First, nine significant fold-change (FC) genes with false

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Table 4. Association between SETD5 expression and clinical characteristics in prostate cancer from TCGA Data portal.

Characteristics	Total (n=399)	SETD5 expression		p Value
		Higher	Lower	
Age (years)				
≤60	190	89	101	0.230
>60	209	111	98	
Gleason score				
G6–G7	256	109	147	0.000*
G8–G10	143	91	52	
T-stage				
T1–T2	167	70	97	0.006*
T3–T4	232	130	102	
Lymph node metastasis				
N0	288	142	146	0.039*
N1	54	35	19	
Nx	57	23	34	
Grade				
I–II	164	67	97	0.002*
III–IV	235	133	102	
PSA value				
0–4 ng/ml	375	182	193	0.019*
>4 ng/ml	24	18	6	

Two-tailed fisher's exact test was done with the SPSS software program to determine the statistical significance of the level of expression of SETD5 in different clinical characteristics, * p.value <0.05 was considered significant.

discovery rates (FDR) <0.05 were identified, including six 2.0-fold-change genes and three 1.5-fold-change genes. The expression of two HMT genes, PRDM6 and PRDM8, were significantly and negatively correlated with the Gleason score, while expression of the EZH2, PRDM12, SUV420H2, WHSC1, PRDM10 and SETD5 genes showed a significant positive correlation with the Gleason score. The mRNA levels of almost all HMT genes studied were significantly correlated with copy number, while expression of most HMT genes was negatively correlated with DNA methylation in prostate cancer in the cases in this study. An increased copy number alteration (CNA) in HMT genes in the prostate carcinoma tissue samples were positively correlated with a higher Gleason score and with a worse clinical outcome. Methylation of the PRDM8 gene was found to be significantly correlated with gene expression and clinical outcome. PRDM5, a methylation silencing gene, was identified as a potential gene for further investigation as a molecular target for prostate cancer. The SETD2 and ASH1L genes were found to have a potential role in the development of castration-resistant prostate cancer (CRPC). A panel of four HMT genes was identified as a gene prognostic signature, EZH2, SETD5, SMYD1, and SUV420H2, which could a potential prognostic biomarker panel. Also, knockdown of expression of the SETD5 gene reduced cell growth and migration of 22Rv1 human prostate carcinoma cells *in vitro*, indicating that the SETD5 gene may act as an oncogene in prostate cancer.

The HMT family includes histone lysine methyltransferases (HKMTs) and histone/protein arginine methyltransferases (PRMTs), which are enzymes that catalyze the transfer of methyl groups to specific substrates involving histones and specific proteins [7,17]. The expression of HMT gene alterations has been identified in several human cancers and are thought to have an oncogenic role, including in prostate cancer [8]. One of the most studied HMT genes is the enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), which is a protein-encoding gene. Genome and transcriptome alterations of EZH2 (amplification, overexpression, alternative splicing or mutation) have been validated to correlate with tumorigenesis and tumor progression in several types of cancers,

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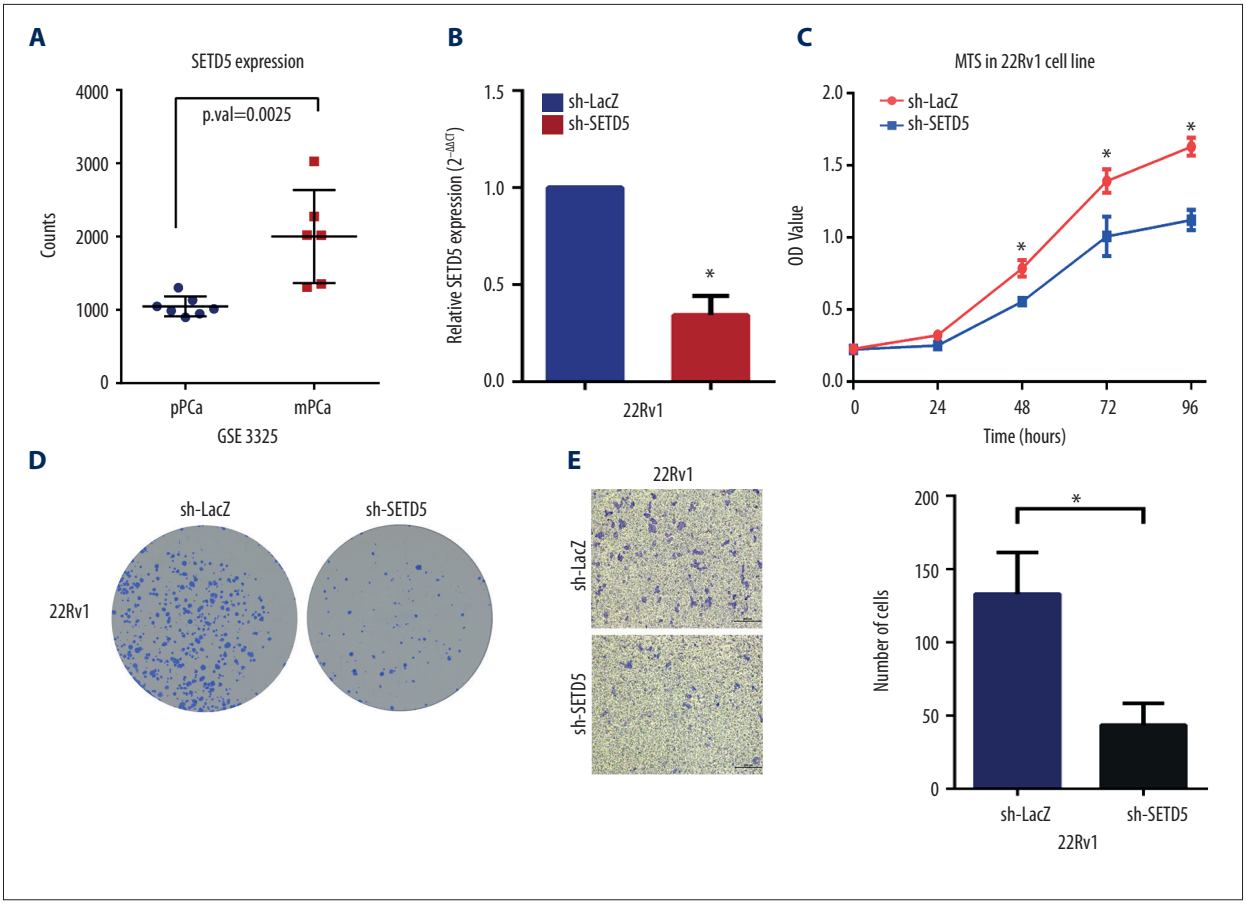

Figure 8. SETD5 gene knockdown reduced cell growth and migration in the 22Rv1 human prostate carcinoma cell line *in vitro*. (**A**) Differential expression of the SETD5 gene in primary prostate cancer (pPCa) and metastatic prostate cancer (mPCa) tissue samples. (**B**) The 22Rv1 human prostate carcinoma cells were stably transfected with lentivirus to knockdown the SETD5 gene. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) detected the SETD5 gene expression level. (**C**) The stable transfection of the 22Rv1 cells underwent a colony formation assay. (**D**) The MTS cell proliferation assay was performed in stably transfected 22Rv1 cells. (**E**) A transwell assay was used to evaluate the migration capacity of stably transfected 22Rv1 cells.

including prostate cancer [18–21]. Also, increased EZH2 mRNA and protein levels have previously been shown to be associated with more rapid progression of prostate cancer, which may result from silencing of a specific cohort of genes by hypermethylation of specific histones [20,22]. Another example of a prognostic gene is WHSC1 (also known as NSD2), which is an H3K36 histone methyltransferase [23]. Current studies indicate that the WHSC1 gene is frequently overexpressed in several cancers, including prostate cancer, and has as an oncogenic role [24,25]. In the present study, overexpression of WHSC1 was more common in high Gleason score prostate cancers and negatively correlated with patient prognosis (Supplementary Figure 1, Supplementary Table 7). The findings of this study validated earlier studies that for individual HMT genes, several HMTs were identified as potential oncogene or tumor suppressor genes in prostate cancer.

Genetic alterations, including DNA methylation, to contribute to cellular transformation and carcinogenesis and are characteristic of most human malignancies [26]. Gene-specific DNA hypermethylation of promoter regions is associated with the inactivation of genes and downregulation of transcription [27,28]. The PRDM8 and PRDM2 genes have been previously shown to be downregulated in invasive pituitary adenomas by whole-exome sequencing (WES), and they are both associated with cell proliferation and tumor recurrence [29]. Aberrant methylation of PRDM8 has been shown to be correlated with congenital dyskeratosis [30], but has not been previously studied in malignant tumors.

PRDM5 is a recognized tumor suppressor gene. Abnormal methylation of PRDM5 and levels of expression has been associated with carcinogenesis was validated in several cancers including cervical, colorectal, lung, and gastric cancers [31–33]. In some

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studies, PRDM5 has been identified as a target of epigenetic silencing, which supports the findings of the present study [34]. Differentially methylated CpG sites of PRDM13, which may contribute to more aggressive behavior in prostate cancer, were identified in tumor tissues of African American men [35]. In the present study, promoter methylation levels of PRDM13 and PRDM14 were significantly associated with clinical survival (Supplementary Figure 7). Therefore, it is possible that when epigenetic modification caused by DNA methylation is reversible, carcinogenesis and cancer promotion caused by altered DNA methylation might be targeted by anti-methylation in cancer treatment.

The SETDB2 gene is one of HMT genes for histone H3 lysine 9 (H3K9) methylation and contains a commonly deleted gene at 13q in chronic lymphocytic leukemia (CLL) [36,37]. In the present study, SETDB2 was the most frequently deleted HMT gene in prostate cancer, and this deletion was significantly related to clinical outcome (Supplementary Figure 3). Studies in patients with CLL with deletion of SETDB2 have shown an associated with disease progression [37]. A recently reported study showed that overexpression of SETDB2 may contribute to disease progression in gastric cancer [38]. These previous studies support the findings of the present study (Supplementary Table 5), and raise the possibility that CNAs function in tumorigenesis by several methods, regardless of their effects on gene expression.

The PRDM1 gene, localized at 6q21, has been previously shown to be a tumor suppressor gene in several human malignancies, especially in lymphoma. In diffuse large B-cell lymphoma, the PRDM1 gene can be inactivated by multiple mechanisms, including homozygous deletions, or missense or truncating mutations, which contribute to lymphomagenesis by blocking plasma cell differentiation [39,40]. Additionally, deficiency of PRDM1 contributes to the maintenance of the phenotype and pathogenesis of gliomas [41]. PRDM14, a transcriptional regulator localized at 8q13, is overexpressed in several cancers and was also the most amplified HMT gene in the present study. However, as well as being associated with clinical survival, amplification of PRDM14 minimally contributed to its mRNA level (Figure 2B, Supplementary Figure 3). Overexpression of PRDM14 mRNA and protein as well as gene amplification have been reported in breast cancer, which correlated with disease progression and poor clinical outcome, indicating that alterations of PRDM14 could be a potential target for the treatment of breast cancer [42,43]. In pancreatic cancer, overexpression of PRDM14 accompanied by deregulation of miR-125a-3p has been shown to be involved in cancer stem-like phenotypes and liver metastasis [44]. Similar results were also found in cases of non-small cell lung cancer (NSCLC) and leukemia [45,46].

The KMT2C and KMT2D genes, the most commonly mutated HMT genes in human cancers, were also validated in the present study [47]. KMT2C and KMT2D are essential for regulation of enhancer activity by H3K4 mono-methylation, with mutation promoting tumorigenesis by deregulation of enhancer activity [48,49]. Reduced expression of KMT2C and KMT2D has previously been shown to significantly correlate with improved outcome in pancreatic ductal adenocarcinoma, and *in vitro* studies have previously shown that the depleted level of these two HMTs attenuated cell proliferation by blocking cell-cycle and diminishing progression from G0-G1 [50]. In endometrioid endometrial carcinoma, the mutational status of KMT2C and KMT2D has been shown to correlate with the degree of myometrial invasion, which could be used to predict prognostic clinical outcome [51]. Also, a high level of expression of the KMT2D gene was associated with poor prognosis in patients with breast cancer, and KMT2D knockdown impaired proliferation and invasion in human breast cancer xenografts in mice [52]. In the present study, the expression of KMT2D was significantly associated with clinical outcome (Supplementary Table 7), suggesting that KMT2D might function as an oncogene in prostate cancer.

Increasing published evidence has now shown that abnormal epigenetic regulation caused by genetic alterations of HMTs, including CNAs, mutation, ectopic expression, and methylation, contribute to tumorigenesis and progression in malignancy. Abnormal epigenetic dysregulation can be treated with targeted drugs or other treatments, and targeting these abnormalities provide potential personalized treatment approaches for cancer patients. Several inhibitors of selective HMTs, including DOT1L, EZH2, WHSC1, have been previously validated with targeted antitumor effects [53]. Also, because mixed-lineage leukemia (MLL) complex family members act as a crucial co-activator of the androgen receptor (AR) in advanced prostate cancer, a small-molecule inhibitor may improve the clinical outcome of patients with castration-resistant prostate cancer (CRPC) by targeting the MLL complex [15]. The results of the present study from the analysis 51 HMT genes systematically identified several HMT genes that have frequent genetic alterations or relate to clinical outcome in prostate cancer, and a four-gene HMT prognostic signature (EZH2, SETD5, SMYD1, and SUV420H2) was identified.

Conclusions

The findings of this study have shown that epigenetic regulation of histone methyltransferases (HMTs) play important roles in the genesis and progression of prostate cancer. Further studies are required to determine the roles of HMTs in the diagnosis, treatment, and prognosis in prostate cancer.

Conflict of interest

None.

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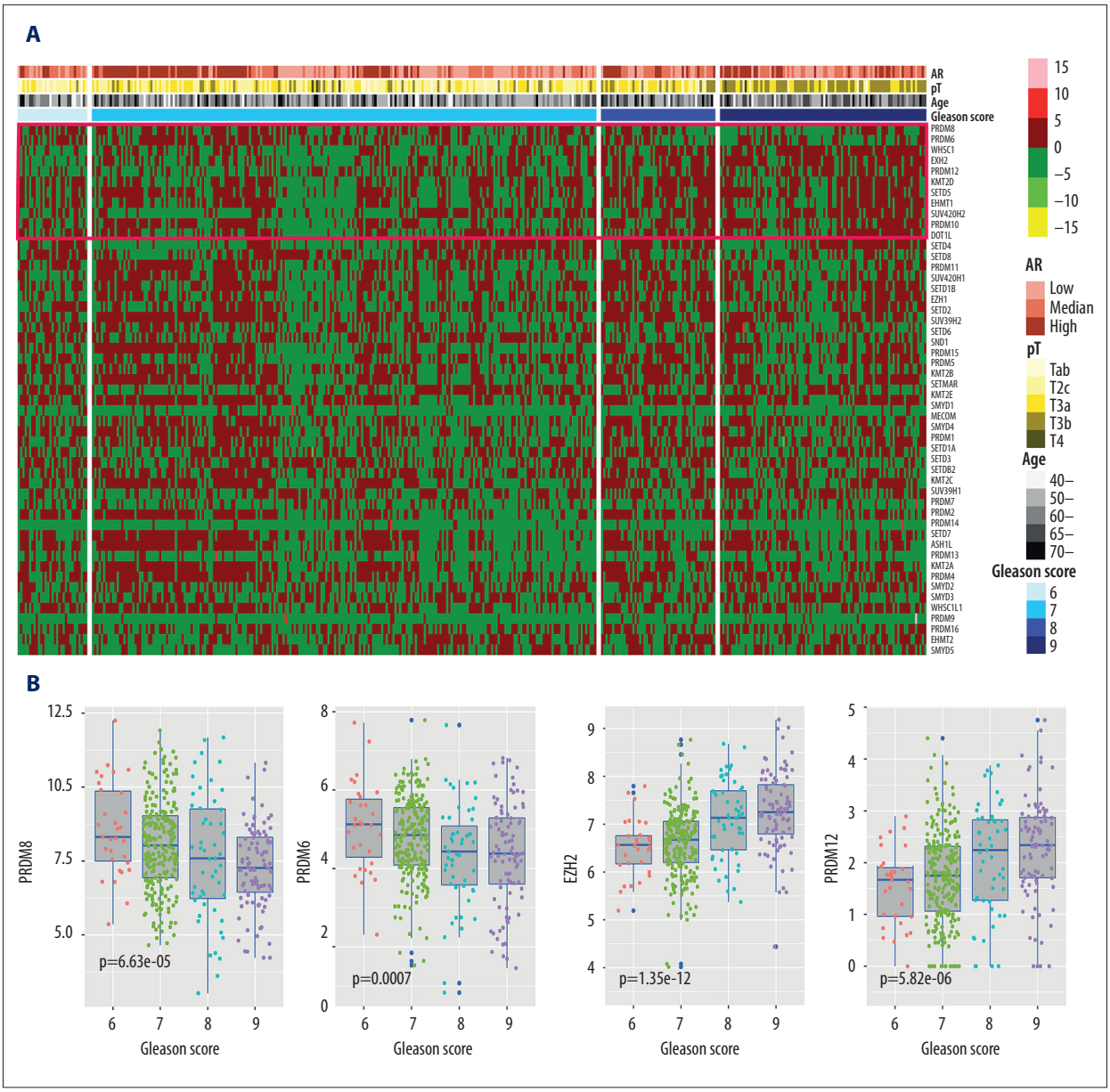
Supplementary Materials



Supplementary Figure 1. mRNA levels of 51 HMTs in different Gleason score prostate cancer samples. (**A**) Pheatmap of 51 HMTs expression profile in totally 399 prostate cancer samples. Normalized gene expression values by rows were median-centered prior to rows clustering based on Euclidean distance. One way ANOVA analysis were performed to compare the differences between Gleason score 6, 7, 8, and 9 prostate tumors. Significantly different expressed genes in prostate tumors are shown at the top, potential cancer related genes(p<0.0001) are indicated by a red box. (**B**) Box plot show significantly four genes (PRDM6, PRDM8, PRDM12, EZH2) expression level(y-axis) positive or negative related to different Gleason score(x-axis).

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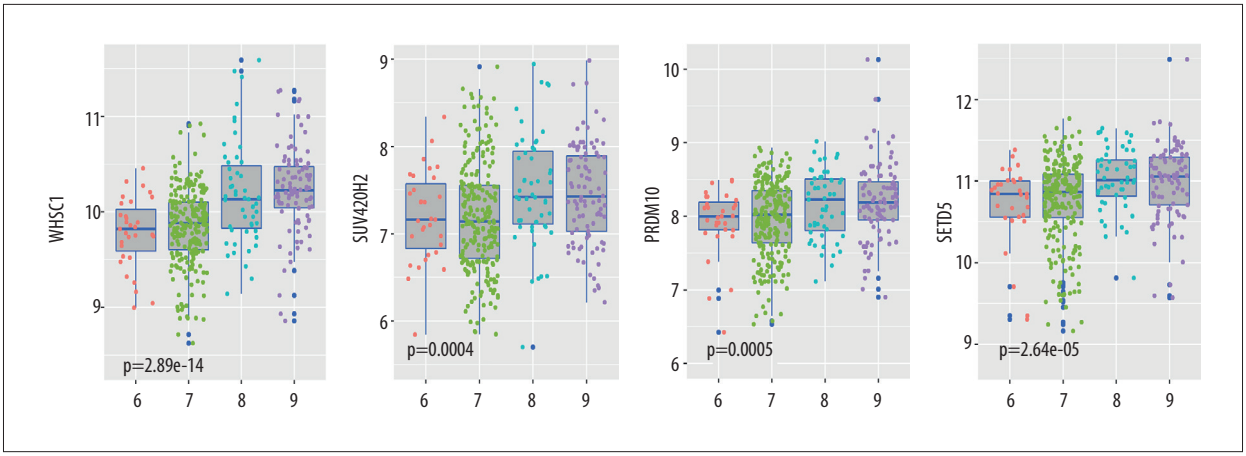
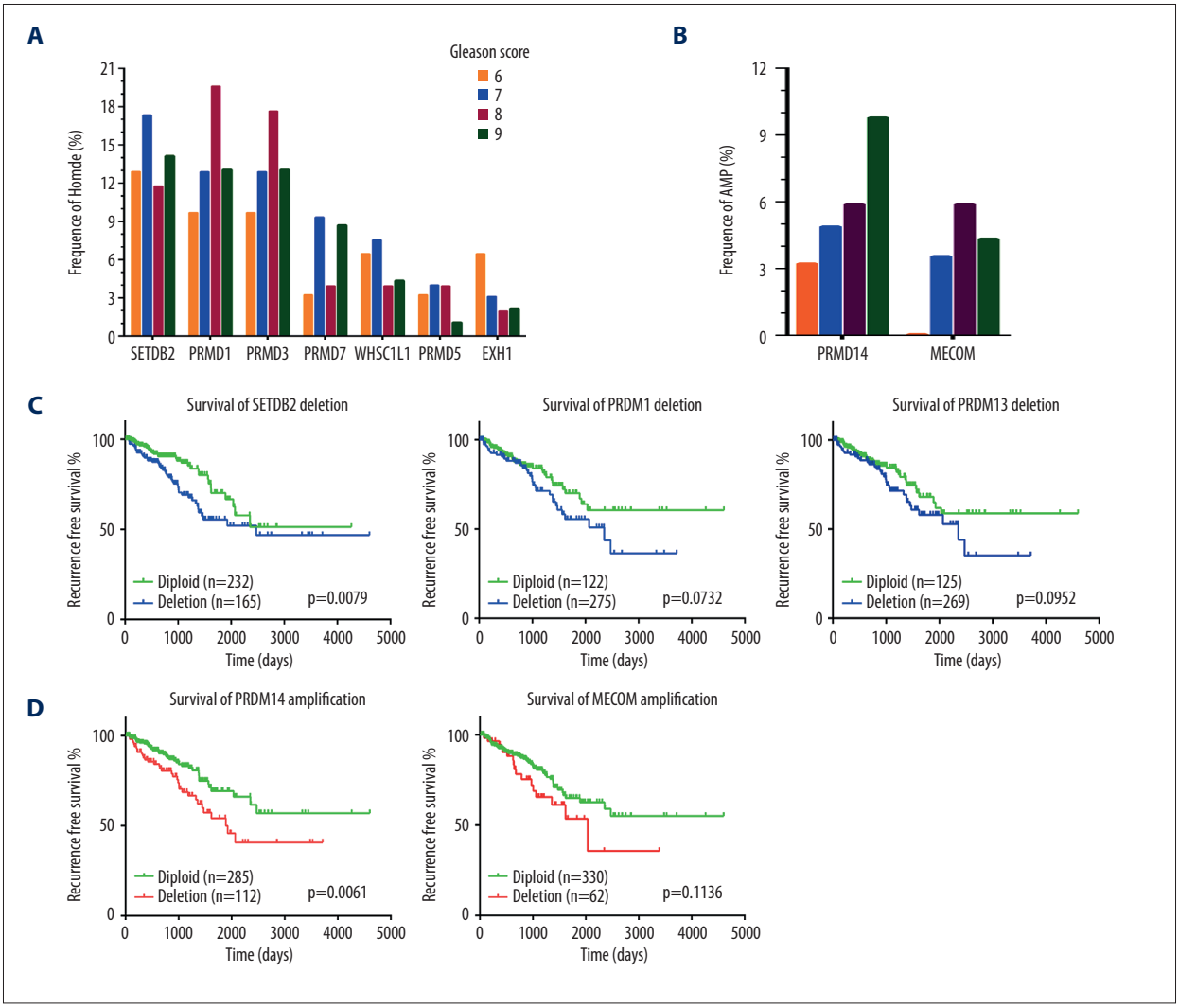


Supplementary Figure 2. mRNA levels of four HMTs in different Gleason score prostate cancers. Box plots show four genes (WHSC1, SUV420H2, PRDM10, SETD5) expression level (y-axis) significantly related to different Gleason score (x-axis).



Supplementary Figure 3. CNAs frequencies of HMTs and their impact on clinical survival. (**A, B**) Bar graphs showed the most frequently altered HMTs. (**C, D**) Kaplan-Meier plotS of RFS proportion shows that deletion of SETDB2 and amplification of PRDM14 were significantly relevant to clinical outcome.

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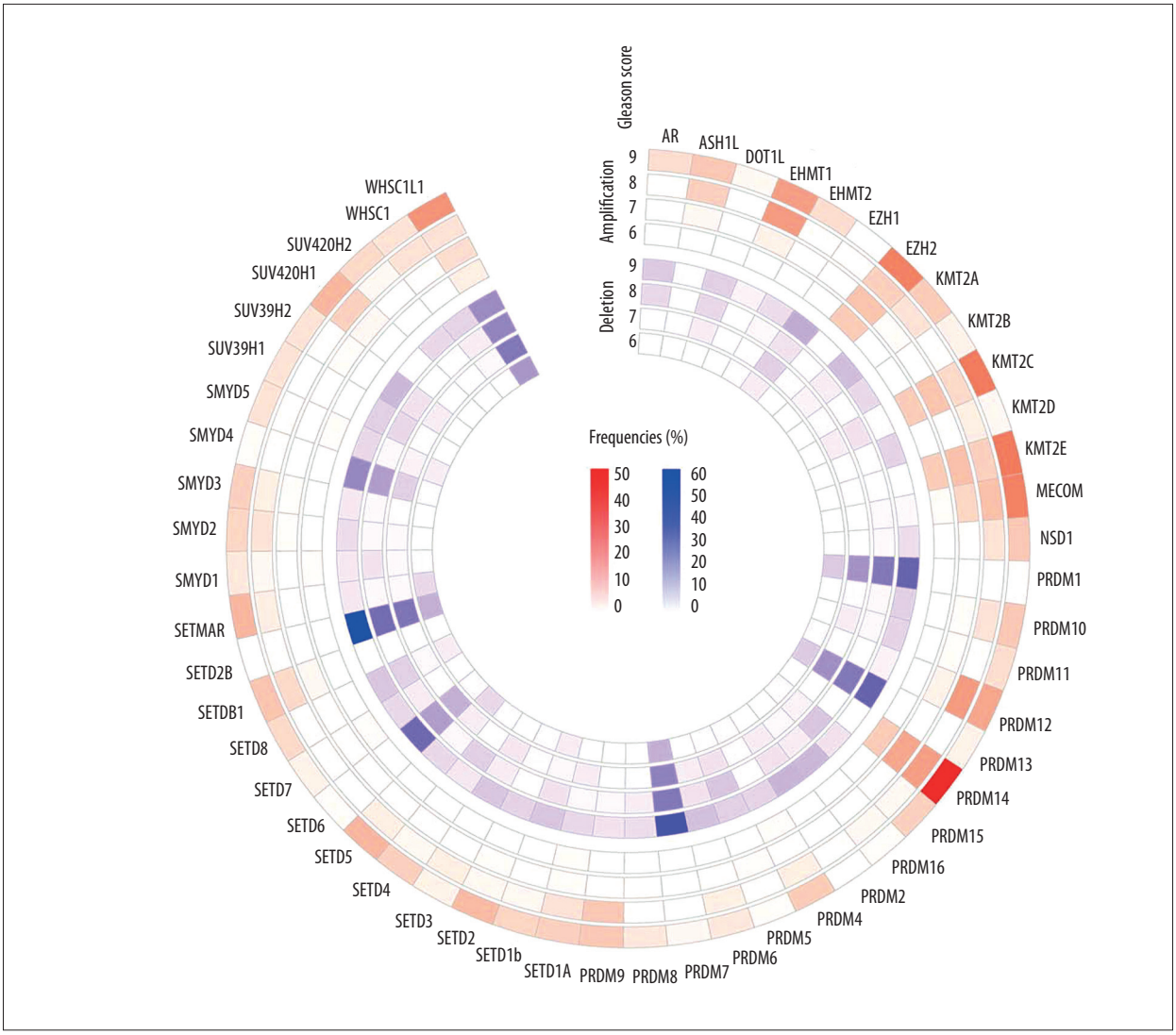

Supplementary Figure 4. HMTs copy number alteration profiles in different Gleason score prostate samples. Pheatmap shows that CNAs frequencies of HMTs were tendentiously positive related to Gleason score, interestingly, both for amplification and deletion alterations.

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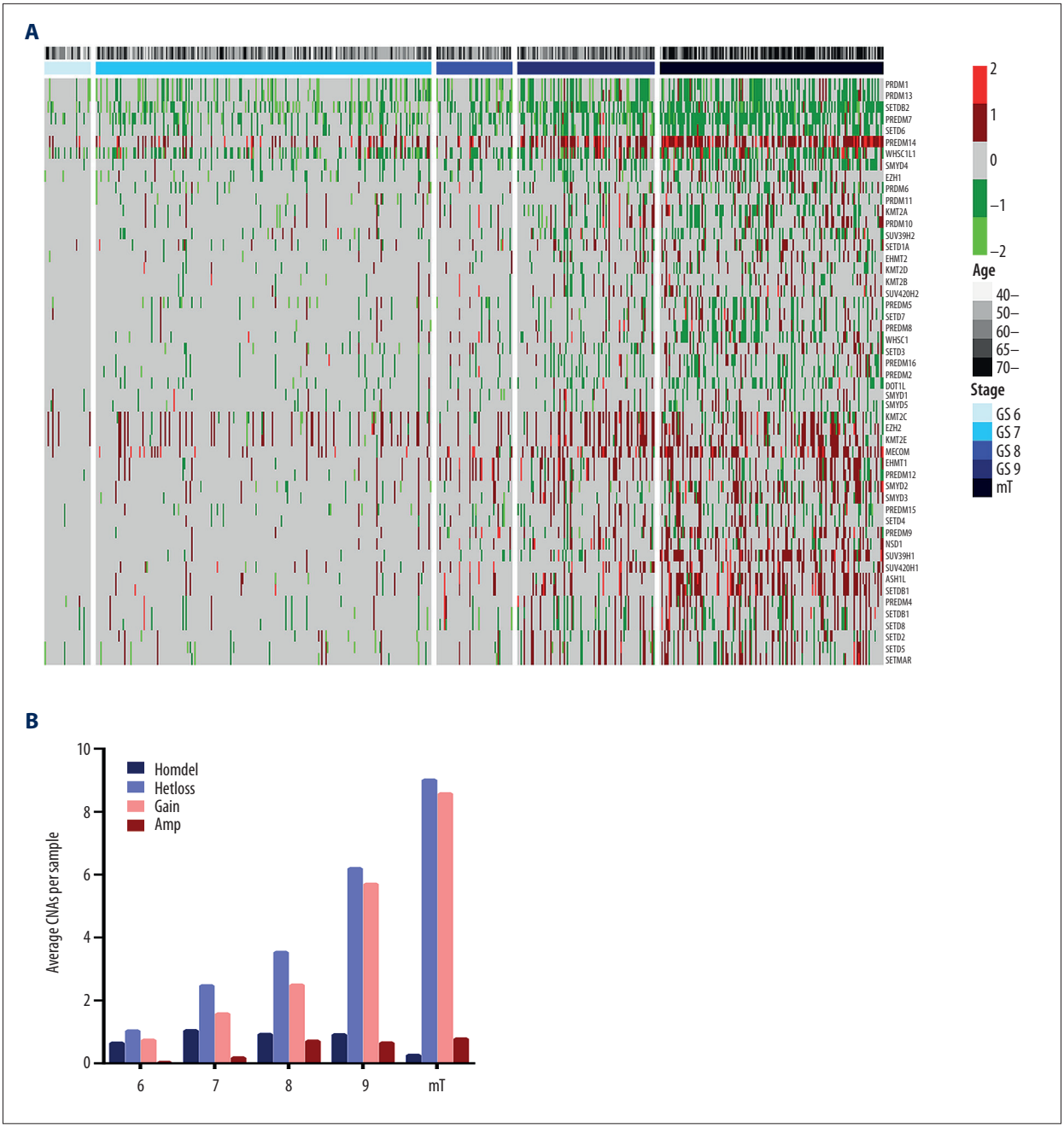

Supplementary Figure 5. HMTs copy number alteration profiles in different stage prostate cancers. (**A**) Pheatmap shows that increasing CNA events of 51 HMTs correlated with different stages of prostate cancers. (**B**) Histogram shown average CNAs per sample in different stage prostate cancers.

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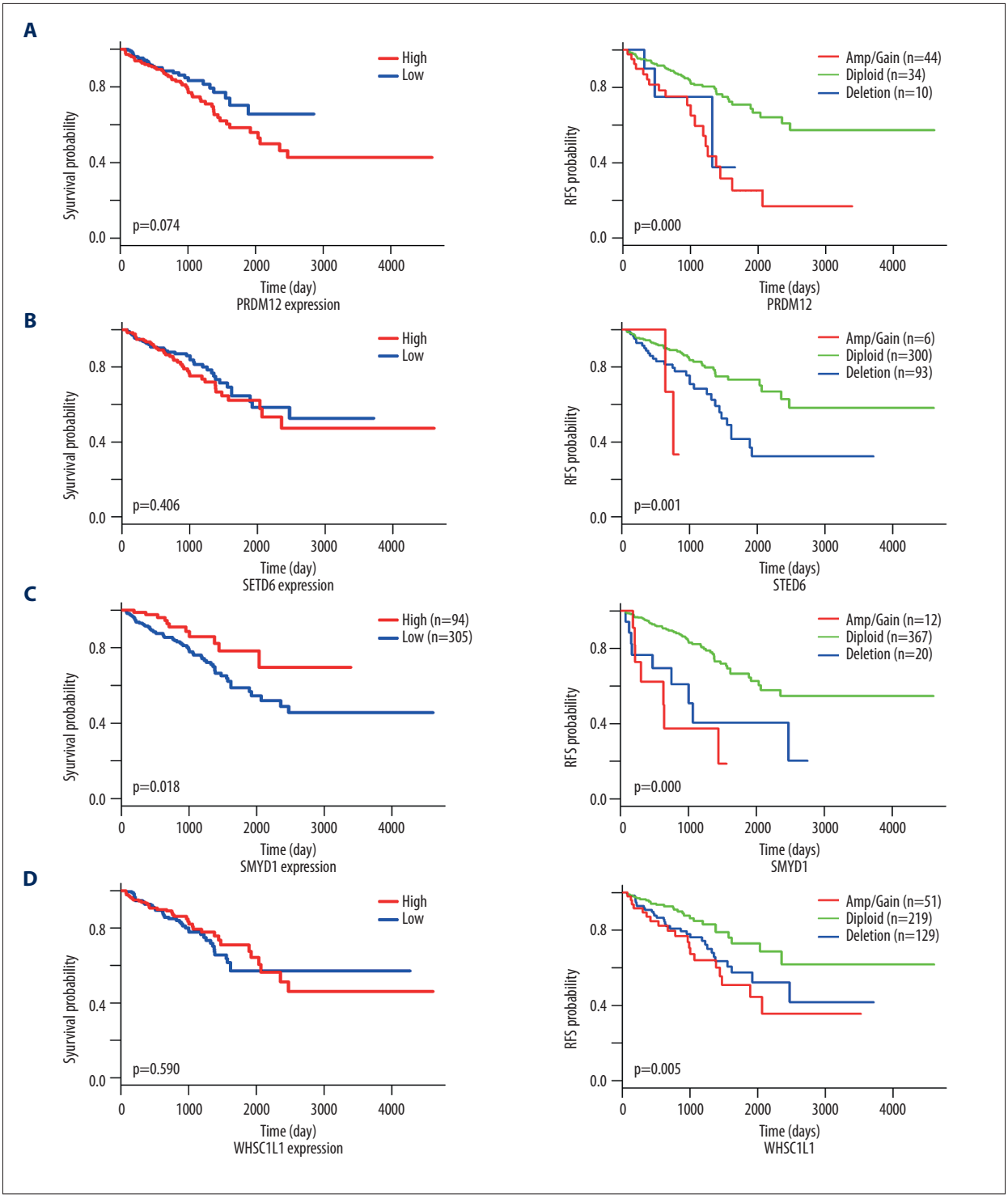

Supplementary Figure 6. Kaplan-Meier plots of RFS proportion show that different impacts caused by CNAs and ectopic gene expression of significant HMTs (PRDM12, SETD6, SMYD1, WHSC1L1) on clinical survival.

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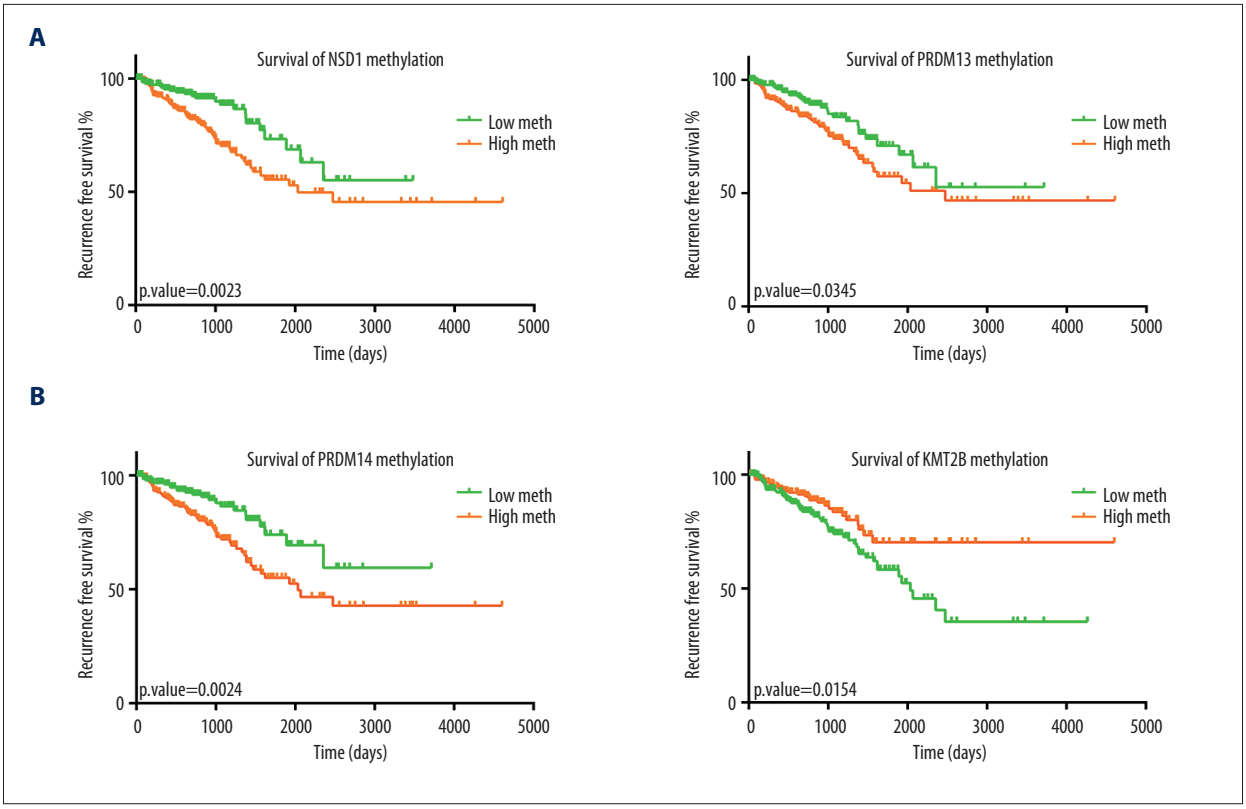

Supplementary Figure 7. Kaplan-Meier plots of RFS proportion show that different impactS caused by methylation level of significant HMTs (NSD1, PRDM13,PRDM14, KMT2B) on clinical survival.

Supplementary Table 1. Summary of identified human HMTs and their histone substrates.

Official symbol	Other aliases	Gene ID	Gene location	Histone substrates
ASH1L	KMT2H; ASH1L1	55870	1q22	H3K4me1/3, H3K36me2
DOT1L	DOT1; KMT4	84444	19p13.3	H3K79me1/2/3
EHMT1	GLP1; KMT1D	79813	9q34.3	H3K9me1/2
EHMT2	G9A; KMT1C	10919	6p21.31	H3K9me1/2
EZH1	KMT6B	2145	17q21.2	H3K27me2/3
EZH2	KMT6A	2146	7q36.1	H3K27me2/3
KMT2A	MLL	4297	11q23.3	H3K4me1/2/3
KMT2B	MLL2	9757	19q13.12	H3K4me1/2/3
KMT2C	MLL3	58508	7q36.1	H3K4me1/2/3
KMT2D	MLL4	8085	12q13.12	H3K4me1/2/3
KMT2E	MLL5	55904	7q22.3	H3K4
MECOM	PRDM3; MDS1-EVI1	2122	**3q26.2**	H3K9me1
NSD1	KMT3B	64324	5q35.2	H3K36me1/2
PRDM1	BLIMP16	639	6q21	
PRDM10	PFM7	56980	11q24.3	
PRDM11	PFM8	56981	11p11.2	

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Official symbol	Other aliases	Gene ID	Gene location	Histone substrates
PRDM12	PFM9	59335	9q34.12	
PRDM13	PFM10	59336	6q16.2	
PRDM14	PFM11	63978	**8q13.3**	
PRDM15	PFM15	63977	21q22.3	
PRDM16	MEL1; PFM13	63976	1p36.32	H3K9me1
PRDM2	RIZ; KMT8	7799	1p36.21	H3K9me1/2/3
PRDM4	PFM1	11108	12q23.3	
PRDM5	PFM2	11107	4q27	H3K9me2
PRDM6		93166	5q23.2	
PRDM7	PFM4; ZNF910	11105	16q24.3	
PRDM8	PFM5	56978	4q21.21	H3k9me3
PRDM9	PFM6; MEISETZ	56979	5p14.2	H3K4me3
SETD1A	KMT2F; SET1A	9739	16p11.2	H3K4me1/2/3
SETD1B	KMT2G; SET1B	23067	12q24.31	H3K4me1/2/3
SETD2	HYPB; SET2	29072	3p21.31	H3K36me1/2/3
SETD3	C14orf154	84193	14q32.2	
SETD4	C21orf18	54093	21q22.12	
SETD5		55209	3p25.3	
SETD6		79918	16q21	
SETD7	KMT7; SET7/9	80854	4q31.1	H3K4me1
SETD8	KMT5A; PR-SETD7	387893	12q24.31	H4K20me1
SETDB1	ESET; KMT1E	9869	1q21.3	H3K9me1/2/3
SETDB2	CLL8; KMT1F	83852	13q14.2	H3K9
SETMAR	METNASE	6419	3p26.1	H3K36
SMYD1	KMT3D; ZMYND18	150572	2p11.2	H3K4
SMYD2	KMT3C; ZMYND14	56950	1q32.3	H3K4; H3K36me2
SMYD3	KMT3E; ZMYND1;	64754	1q44	H3K4me2/3
SMYD4	ZMYND21	114826	17p13.3	
SMYD5	RRG1; ZMYND23	10322	2p13.2	
SUV39H1	KMT1A	6839	Xp11.23	H3K9me2/3
SUV39H2	KMT1B	79723	10p13	H3K9me2/3
SUV420H1	KMT5B; CGI-85	51111	**11q13.2**	H4K20me2/3
SUV420H2	KMT5C	84787	19q13.42	H4K20me2/3
WHSC1	NSD2; MMSET	7468	4p16.3	H3K36me1/2
WHSC1L1	NSD3	54904	**8p11.23**	H3K36me1/2; H3K4

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Supplementary Table 2. Number of samples from TCGA used in our analyses. For the RNA-Seq data and methylation data the number of tumor and normal samples are indicated. We also indicate the number of tumor samples with mutation data, and with copy number variation (CNV) data.

Acronym	Tumor type	Data type	Paired samples		Total tumor samples	Metastatic PRAD
			Normal	Tumor		
PRAD	Prostate adenocarcinoma	RNA seq data	52	52	497	0
		CpG data(46HMTs)	50	50	499	0
		CNV data	0	0	492	150
		Mutation data	0	0	425	0
		Quadruple overlap	0	0	399	0

Supplementary Table 3. Summary of expression fold-changed genes with false discovery rates (FDR) <0.05.

	Gene	logFC	AveExpr	t	P.value	FDR	B
2.0FC	PRDM8	–1.526	9.088	–5.666	1.30E-07	8.08E-07	6.880
	MECOM	–1.216	8.501	–6.737	9.03E-10	9.65E-09	11.736
	PRDM16	–1.129	4.769	–4.080	8.80E-05	2.54E-04	0.592
	SMYD1	–1.033	1.099	–2.254	2.63E-02	4.55E-02	–4.668
	PRDM12	1.016	1.348	7.227	8.37E-11	2.18E-09	14.071
	EZH2	1.433	5.944	8.778	3.43E-14	1.78E-12	21.754
1.5FC	PRDM5	–0.932	5.892	–5.874	5.10E-08	3.79E-07	7.790
	PRDM11	–0.904	4.968	–6.843	5.42E-10	9.40E-09	12.235
	PRDM6	–0.800	5.200	–3.475	7.44E-04	1.93E-03	–1.424
1.2FC	EZH1	–0.542	9.792	–6.731	9.28E-10	9.65E-09	11.709
	SMYD4	–0.371	8.315	–4.951	2.84E-06	1.23E-05	3.885
	PRDM15	0.320	7.400	5.011	2.21E-06	1.05E-05	4.127
	PRDM10	0.337	7.990	4.292	3.96E-05	1.47E-04	1.353
	WHSC1	0.367	9.820	5.064	1.77E-06	9.20E-06	4.345
	SETD7	0.429	11.984	4.351	3.16E-05	1.26E-04	1.571
	KMT2B	0.432	11.025	3.438	8.42E-04	1.99E-03	–1.540
	SMYD2	0.433	8.849	4.118	7.65E-05	2.34E-04	0.725
	SUV39H2	0.455	7.832	4.273	4.26E-05	1.48E-04	1.284
	SMYD3	0.509	7.713	5.885	4.84E-08	3.79E-07	7.841
	DOT1L	0.567	8.862	5.649	1.40E-07	8.08E-07	6.807

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Supplementary Table 4. Summary for association of Gleason score and AR expression with HMTs expression profile.

Gleason score/HMTs expression (one-way ANOVA, top 11 HMTs were filled with yellow color)		AR/HMTs expression (top 13 significant HMTs with the green padding were also in the same clustering)		
Gene	p.Value	Gene	p.Value	Coefficients
ASH1L	4.49E-01	ASH1L	4.94E-155	0.871
DOT1L	7.57E-04	DOT1L	1.43E-06	0.214
EHMT1	2.78E-04	EHMT1	9.08E-46	0.579
EHMT2	8.27E-01	EHMT2	2.19E-12	0.308
EZH1	2.11E-02	EZH1	9.07E-01	0.005
EZH2	1.35E-12	EZH2	1.23E-03	0.145
KMT2A	4.68E-01	KMT2A	5.77E-97	0.766
KMT2B	1.40E-01	KMT2B	1.24E-128	0.832
KMT2C	3.70E-01	KMT2C	5.65E-122	0.820
KMT2D	2.60E-05	KMT2D	5.63E-32	0.494
KMT2E	1.92E-01	KMT2E	4.56E-105	0.785
MECOM	2.21E-01	MECOM	1.31E-06	0.215
NSD1	5.49E-02	NSD1	8.13E-127	0.829
PRDM1	3.20E-01	PRDM1	7.06E-13	0.315
PRDM10	4.92E-04	PRDM10	1.21E-87	0.741
PRDM11	2.71E-03	PRDM11	6.96E-12	0.301
PRDM12	5.82E-06	PRDM12	1.80E-01	0.060
PRDM13	4.62E-01	PRDM13	2.76E-01	0.049
PRDM14	4.33E-01	PRDM14	7.13E-01	0.017
PRDM15	5.90E-02	PRDM15	6.48E-04	0.152
PRDM16	6.83E-01	PRDM16	7.48E-01	0.014
PRDM2	3.85E-01	PRDM2	2.77E-70	0.686
PRDM4	4.69E-01	PRDM4	3.72E-54	0.620
PRDM5	9.29E-02	PRDM5	3.45E-19	0.387
PRDM6	6.89E-04	PRDM6	4.26E-03	0.128
PRDM7	3.84E-01	PRDM7	5.38E-03	0.125
PRDM8	6.63E-05	PRDM8	8.01E-01	0.011
PRDM9	5.35E-01	PRDM9	7.02E-01	0.017
SETD1A	3.29E-01	SETD1A	3.63E-02	0.094
SETD1B	9.31E-03	SETD1B	1.11E-44	0.573
SETD2	2.64E-02	SETD2	3.47E-129	0.833
SETD3	3.48E-01	SETD3	8.77E-01	0.007
SETD4	1.29E-03	SETD4	2.56E-22	0.417

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Gleason score/HMTs expression (one-way ANOVA, top 11 HMTs were filled with yellow color)		AR/HMTs expression (top 13 significant HMTs with the green padding were also in the same clustering)		
Gene	p.Value	Gene	p.Value	Coefficients
SETD5	2.64E-05	SETD5	6.88E-78	0.712
SETD6	5.42E-02	SETD6	7.32E-01	0.015
SETD7	4.45E-01	SETD7	9.24E-106	0.787
SETD8	2.48E-03	SETD8	5.01E-09	0.258
SETDB1	1.24E-02	SETDB1	1.60E-13	0.323
SETDB2	3.50E-01	SETDB2	1.61E-02	0.108
SETMAR	1.63E-01	SETMAR	2.40E-34	0.511
SMYD1	1.97E-01	SMYD1	9.40E-01	0.003
SMYD2	4.78E-01	SMYD2	2.22E-01	0.055
SMYD3	5.14E-01	SMYD3	1.14E-09	0.269
SMYD4	3.06E-01	SMYD4	9.99E-20	0.392
SMYD5	9.71E-01	SMYD5	8.87E-21	0.402
SUV39H1	3.77E-01	SUV39H1	4.30E-19	0.386
SUV39H2	4.21E-02	SUV39H2	1.03E-05	0.196
SUV420H1	6.57E-03	SUV420H1	3.81E-42	0.559
SUV420H2	3.88E-04	SUV420H2	7.67E-26	0.447
WHSC1	2.89E-14	WHSC1	4.06E-31	0.488
WHSC1L1	5.19E-01	WHSC1L1	6.19E-47	0.585

Supplementary Table 5. Summary of correlation between CNAs frequencied and gene expression levels.

Gene	Amplification		Deletion		Diploid		Total amplification	Total deletion	Amplification and expressed higher (%)	Amplification and expressed lower (%)	Deletion and expressed higher (%)	Deletion and expressed lower (%)
	Higher	Lower	Higher	Lower	Higher	Lower						
	Expression											
ASH1L	19	8	0	8	183	181	27	8	70.37	29.63	0.00	100.00
DOT1L 4	1	11	19	179	185	5	30	80.00	20.00	36.67	63.33	
EHMT1	35	11	3	5	183	162	46	8	76.09	23.91	37.50	62.50
EHMT2	8	4	5	10	185	187	12	15	66.67	33.33	33.33	66.67
EZH1	3	1	13	37	161	184	4	50	75.00	25.00	26.00	74.00
EZH2	58	15	2	6	178	140	73	8	79.45	20.55	25.00	75.00
KMT2A	12	10	14	15	174	174	22	29	54.55	45.45	48.28	51.72
KMT2B	6	3	10	6	190	184	9	16	66.67	33.33	62.50	37.50
KMT2C	39	35	10	11	153	151	74	21	52.70	47.30	47.62	58.38
KMT2D	6	5	8	8	186	186	11	16	54.55	45.45	50.00	50.00
KMT2E	39	37	3	4	158	158	76	7	51.32	48.68	42.86	57.14
MECOM	36	26	3	4	169	161	62	7	58.06	41.94	42.86	57.14
NSD1	16	6	5	5	188	179	22	10	72.73	27.27	50.00	50.00

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Gene	Amplification		Deletion		Diploid		Total amplification	Total deletion	Amplification and expressed higher (%)	Amplification and expressed lower (%)	Deletion and expressed higher (%)	Deletion and expressed lower (%)
	Higher	Lower	Higher	Lower	Higher	Lower						
	Expression											
PRDM1	2	0	62	60	139	136	2	122	100.00	0.00	50.82	49.18
PRDM10	18	6	6	13	180	176	24	19	75.00	25.00	31.58	68.42
PRDM11	8	8	6	20	171	186	16	26	50.00	50.00	23.08	76.92
PRDM12	33	11	1	9	179	166	44	10	75.00	25.00	10.00	90.00
PRDM13	0	5	7	118	189	80	5	125	0.00	100.00	5.60	94.40
PRDM14	16	96	0	2	270	15	112	2	14.29	85.71	0.00	100.00
PRDM15	14	4	8	18	177	178	18	26	77.78	22.22	30.77	69.23
PRDM16	3	4	13	16	179	184	7	29	42.86	57.14	44.83	55.17
PRDM2	2	0	9	26	173	189	2	35	100.00	0.00	25.71	74.29
PRDM4	18	2	6	13	184	176	20	19	90.00	10.00	31.58	68.42
PRDM5	4	6	7	29	164	189	10	36	40.00	60.00	19.44	80.56
PRDM6	7	6	6	26	167	187	13	32	53.85	46.15	18.75	81.25
PRDM7	1	3	42	103	160	90	40	145	25.00	75.00	28.97	71.03
PRDM8	4	7	5	10	182	191	11	15	36.36	63.64	33.33	66.67
PRDM9	6	19	2	8	347	17	25	10	24.00	76.00	20.00	80.00
SETD1A	20	3	14	11	185	166	23	25	86.96	13.04	56.00	44.00
SETD1B	13	3	11	17	179	176	16	28	81.25	18.75	39.29	60.71
SETD2	17	7	3	16	176	180	24	19	70.83	29.17	15.79	84.21
SETD3	8	2	6	25	172	186	10	31	80.00	20.00	19.35	80.65
SETD4	14	3	4	13	183	182	17	17	82.35	17.65	23.53	76.47
SETD5	17	8	3	25	166	180	25	28	68.00	32.00	10.71	89.29
SETD6	6	0	15	78	121	179	6	93	100.00	0.00	16.13	83.87
SETD7	7	4	3	14	181	190	11	17	63.64	36.36	17.65	82.35
SETD8	11	3	6	25	171	183	14	31	78.57	21.43	19.35	80.65
SETDB1	26	2	1	1	196	173	28	2	92.86	7.14	50.00	50.00
SETDB2	1	1	34	131	67	165	2	165	50.00	50.00	20.61	79.39
SETMAR	17	8	5	16	175	178	25	21	68.00	32.00	23.81	76.19
SMYD1	2	10	4	16	279	88	12	20	16.67	83.33	20.00	80.00
SMYD2	12	9	4	16	174	184	21	20	57.14	42.86	20.00	80.00
SMYD3	18	3	3	11	185	179	21	14	85.71	14.29	21.43	78.57
SMYD4	5	0	9	58	141	186	5	67	100.00	0.00	13.43	86.57
SMYD5	11	1	5	19	179	184	12	24	91.67	8.33	20.83	79.17
SUV39H1	4	5	13	7	187	183	9	20	44.44	55.56	65.00	35.00
SUV39H2	12	2	4	29	168	184	14	33	85.71	14.29	12.12	87.88
SUV420H1	26	7	0	6	186	174	33	6	174.79	21.21	0.00	100.0
SUV420H2	13	1	10	4	194	177	14	14	92.86	7.114	71.43	28.57
WHSC1	14	2	9	12	185	177	16	21	87.50	12.50	42.86	57.14
WHSC1L1	44	7	18	111	81	138	51	129	86.27	13.73	13.95	86.05

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Supplementary Table 6. Summary for differential promoter methylation HMTs in paired normal and tumor tissues.

Gene	Differential methylation analysis of 46 HMTs						Paired T-test
	logFC	AveExpr	t	P.value	adj.P.Val	B	
PRDM14	−0.201	0.320	−11.345	1.30E-19	6.09E-18	33.276	1.75E-17
NSD1	−0.142	0.421	−9.479	1.49E-15	3.50E-14	23.928	2.44E-13
PRDM8	−0.146	0.432	−8.138	1.20E-12	1.89E-11	17.253	6.68E-12
EZH1	−0.024	0.103	−7.529	2.41E-11	2.83E-10	14.277	1.61E-10
PRDM13	−0.121	0.306	−7.276	8.19E-11	7.70E-10	13.062	6.16E-12
PRDM6	−0.065	0.158	−6.626	1.81E-09	1.42E-08	9.998	6.49E-10
PRDM12	−0.047	0.119	−6.586	2.18E-09	1.47E-08	9.813	3.76E-10
SETD3	0.061	0.604	6.222	1.18E-08	6.93E-08	8.149	3.26E-08
PRDM5	−0.093	0.117	−5.224	9.70E-07	5.07E-06	3.821	2.09E-06
KMT2B	0.057	0.551	4.548	1.54E-05	7.22E-05	1.136	5.32E-06
PRDM15	−0.012	0.157	−4.393	2.81E-05	1.20E-04	0.554	2.38E-05
SETD5	0.037	0.474	3.924	1.61E-04	6.31E-04	−1.122	6.03E-05
PRDM7	0.041	0.537	3.845	2.13E-04	7.71E-04	−1.391	1.73E-04
WHSC1	0.023	0.463	3.619	4.67E-04	1.57E-03	−2.136	2.21E-04
SETDB1	0.014	0.140	3.088	2.61E-03	8.19E-03	−3.749	1.03E-04
EHMT2	0.004	0.097	2.887	4.78E-03	1.40E-02	−4.307	3.23E-04
MECOM	−0.016	0.151	−2.837	5.52E-03	1.53E-02	−4.439	3.26E-03
KMT2E	−0.031	0.417	−2.721	7.69E-03	1.72E-02	−4.741	5.31E-03
SETD1A	0.009	0.127	2.725	7.60E-03	1.72E-02	−4.731	6.35E-03
SMYD1	0.050	0.805	2.747	7.14E-03	1.72E-02	−4.674	5.86E-03
PRDM9	0.071	0.586	2.735	7.39E-03	1.72E-02	−4.705	9.94E-04
SUV420H1	−0.008	0.225	−2.700	8.16E-03	1.74E-02	−4.795	2.43E-03
PRDM2	0.007	0.431	2.583	1.13E-02	2.30E-02	−5.087	3.74E-03
PRDM11	0.019	0.845	2.497	1.42E-02	2.77E-02	−5.293	9.22E-03
PRDM4	−0.004	0.062	−2.420	1.73E-02	3.26E-02	−5.473	1.23E-02
PRDM10	0.025	0.564	2.390	1.87E-02	3.38E-02	−5.542	2.59E-02
DOT1L	0.002	0.036	2.290	2.42E-02	4.20E-02	−5.766	9.03E-03
SMYD5	0.008	0.230	1.982	5.02E-02	8.43E-02	−6.401	3.76E-02
SETD7	0.003	0.057	1.902	6.00E-02	9.73E-02	−6.551	2.41E-02
SETMAR	−0.005	0.173	−1.523	1.31E-01	2.05E-01	−7.188	8.77E-02
SETD4	0.001	0.032	1.425	1.57E-01	2.39E-01	−7.331	1.30E-01
KMT2D	0.003	0.079	1.318	1.91E-01	2.71E-01	−7.476	5.25E-02
SETDB2	0.005	0.905	1.335	1.85E-01	2.71E-01	−7.454	1.62E-01
WHSC1L1	−0.004	0.151	−1.244	2.17E-01	2.99E-01	−7.571	2.39E-01
SUV39H2	0.001	0.041	1.191	2.36E-01	3.09E-01	−7.634	2.32E-01
AR	0.005	0.316	1.191	2.36E-01	3.09E-01	−7.634	1.74E-01
PRDM1	−0.014	0.148	−1.147	2.54E-01	3.23E-01	−7.686	2.81E-01

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Gene	Differential methylation analysis of 46 HMTs						Paired T-test
	logFC	AveExpr	t	P.value	adj.P.Val	B	
SETD2	0.001	0.036	0.996	3.22E-01	3.98E-01	–7.847	2.55E-01
ASH1L	0.003	0.473	0.978	3.31E-01	3.98E-01	–7.865	2.85E-01
SUV420H2	–0.001	0.025	–0.908	3.66E-01	4.30E-01	–7.931	3.20E-01
SMYD2	–0.002	0.062	–0.828	4.10E-01	4.70E-01	–8.001	3.54E-01
SMYD3	–0.002	0.413	–0.685	4.95E-01	5.29E-01	–8.109	4.27E-01
EZH2	–0.001	0.295	–0.703	4.84E-01	5.29E-01	–8.097	3.90E-01
SETD6	–0.001	0.039	–0.717	4.75E-01	5.29E-01	–8.086	3.54E-01
SETD8	0.000	0.030	–0.373	7.10E-01	7.42E-01	–8.275	6.77E-01
KMT2A	0.000	0.030	0.107	9.15E-01	9.35E-01	–8.340	8.71E-01
SUV39H1	0.000	0.115	0.057	9.55E-01	9.55E-01	–8.344	9.52E-01

Supplementary Table 7A. Summary of uniivariate analysis of RFS for **amplification of 51 HMTs** in prostate cancer.

Gene	p-Value	HR	95% CI		Count amplification	Count diploid	Count percentage
			Lower	Higher			
PRDM14	0.0081	1.860	1.185	2.919	112	285	28.2
WHSC1L1	0.0051	2.401	1.330	4.332	51	219	18.9
EHMT1	0.0001	3.133	1.876	5.232	46	345	11.8
PRDM12	0.0002	3.022	1.795	5.090	44	345	11.3
SUV420H1	0.0075	2.390	1.336	4.273	33	360	8.4
SETDB1	0.0031	2.860	1.543	5.303	28	369	7.1
SETD1A	0.0157	2.520	1.286	4.938	23	351	6.1
SMYD2	0.0188	2.723	1.304	5.686	21	358	5.5
PRDM4	0.0015	3.808	1.882	7.703	20	360	5.3
PRDM15	0.0445	2.214	1.095	4.478	18	355	4.8
SETD1B	0.0006	4.815	2.279	10.173	16	355	4.3
WHSC1	0.0467	2.299	1.098	4.813	16	362	4.2
SETD8	0.0100	3.722	1.596	8.682	14	354	3.8
SUV420H2	0.0499	2.409	1.099	5.281	14	371	3.6
SMYD5	0.0009	5.127	2.330	11.284	12	363	3.2
SMYD1	0.0005	5.729	2.599	12.630	12	367	3.2
EHMT2	0.0278	2.969	1.281	6.882	12	372	3.1
KMT2B	0.0311	3.242	1.300	8.084	9	374	2.3
PRDM16	0.0029	7.508	2.674	21.079	7	363	1.9
DOT1L	0.0358	3.657	1.323	10.106	5	364	1.4
PRDM1	0.0219	9.340	2.227	39.178	2	275	0.7
SETD3	0.2315	0.362	0.050	2.622	10	358	2.7

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Gene	p-Value	HR	95% CI		Count amplification	Count diploid	Count percentage
			Lower	Higher			
PRDM7	0.3568	0.000	0.000	Inf	4	250	1.6
SETD4	0.0502	2.263	1.083	4.731	17	365	4.5
PRDM8	0.0507	2.393	1.096	5.227	11	373	2.9
PRDM13	0.0664	3.720	1.147	12.065	5	269	1.8
ASH1L	0.0749	1.998	0.993	4.017	27	364	6.9
PRDM9	0.0840	1.953	0.971	3.926	25	364	6.4
SETD6	0.0854	4.711	1.119	19.832	6	300	2.0
PRDM2	0.1026	9.419	1.273	69.698	2	362	0.5
NSD1	0.1187	1.887	0.905	3.937	22	367	5.7
MECOM	0.1318	1.543	0.898	2.653	62	330	15.8
SMYD4	0.1402	3.589	0.868	14.839	5	327	1.5
EZH2	0.1436	1.470	0.891	2.426	73	318	18.7
KMT2E	0.1859	1.418	0.857	2.347	76	316	19.4
PRDM10	0.1868	1.649	0.821	3.311	24	356	6.3
KMT2C	0.1870	1.418	0.856	2.349	74	304	19.6
SETMAR	0.1931	1.746	0.801	3.809	25	353	6.6
KMT2A	0.1991	1.674	0.802	3.494	22	348	5.9
SETD5	0.2196	1.688	0.774	3.679	25	346	6.7
SMYD3	0.2284	1.742	0.754	4.024	21	364	5.5
PRDM5	0.2589	1.894	0.688	5.217	10	353	2.8
SUV39H1	0.2972	1.975	0.620	6.296	9	370	2.4
SETD2	0.3418	1.538	0.666	3.551	24	356	6.3
PRDM11	0.3802	1.543	0.620	3.839	16	357	4.3
SETD7	0.6018	1.383	0.433	4.411	11	371	2.9
SETDB2	0.6704	1.601	0.211	12.166	2	232	0.9
PRDM6	0.6765	1.292	0.406	4.117	13	354	3.5
EZH1	0.6774	1.570	0.216	11.403	4	345	1.1
KMT2D	0.7397	1.224	0.385	3.895	11	372	2.9
SUV39H2	0.9204	0.943	0.295	3.016	14	352	3.8

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Supplementary Table 7B. Summary of uniivariate analysis of RFS for **deletion of 51 HMTs** in prostate cancer.

Gene	p-Value	HR	95% CI		Count amplification	Count diploid	Count percentage
			Lower	Higher			
DOT1L	0.0363	2.053	1.103	3.821	30	364	7.6
EHMT2	0.0356	2.801	1.211	6.478	15	372	3.9
PRDM7	0.0055	1.881	1.205	2.934	145	250	36.7
SETD4	0.0042	3.905	1.778	8.576	17	365	4.5
SETD6	0.0016	2.161	1.364	3.423	93	300	23.7
SETDB2	0.0082	1.833	1.164	2.886	165	232	41.6
SMYD1	0.0102	2.847	1.409	5.756	20	367	5.2
SUV39H2	0.0014	2.710	1.558	4.711	33	352	8.6
SUV420H2	0.0121	2.947	1.411	6.159	14	371	3.6
WHSC1L1	0.0154	1.886	1.129	3.151	129	219	37.1
SETD5	0.1644	0.481	0.151	1.531	28	346	7.5
PRDM8	0.3963	0.573	0.140	2.341	15	373	3.9
EZH2	0.0977	0.000	0.000	Inf	8	318	2.5
SUV420H1	0.1404	0.000	0.000	Inf	6	360	1.6
SETDB1	0.1916	0.000	0.000	Inf	2	369	0.5
EZH1	0.0528	1.783	1.026	3.096	50	345	12.7
PRDM15	0.0550	2.129	1.053	4.307	26	355	6.8
PRDM6	0.0598	2.008	1.031	3.914	32	354	8.3
SETD1A	0.0611	2.085	1.032	4.214	25	351	6.6
PRDM1	0.0788	1.512	0.959	2.383	122	275	30.7
SETD7	0.0805	2.162	0.989	4.727	17	371	4.4
KMT2B	0.0874	2.118	0.971	4.620	16	374	4.1
PRDM4	0.0918	2.234	0.965	5.171	19	360	5.0
PRDM9	0.0966	2.675	0.972	7.362	10	364	2.7
PRDM13	0.1008	1.473	0.932	2.327	125	269	31.7
SUV39H1	0.1021	2.042	0.937	4.449	20	370	5.1
PRDM11	0.1118	1.846	0.916	3.719	26	357	6.8
SMYD5	0.1304	1.855	0.884	3.892	24	363	6.2
SETD1B	0.1357	1.774	0.879	3.580	28	355	7.3
SETD8	0.1572	1.672	0.856	3.269	31	354	8.1
SMYD3	0.1598	2.058	0.828	5.119	14	364	3.7
SETD3	0.1602	1.595	0.860	2.956	31	358	8.0
EHMT1	0.2166	2.283	0.712	7.313	8	345	2.3
PRDM12	0.2324	2.215	0.690	7.111	10	345	2.8
SMYD4	0.2552	1.373	0.808	2.334	67	327	17.0
NSD1	0.2914	1.994	0.625	6.357	10	367	2.7
PRDM2	0.3186	1.405	0.740	2.668	35	362	8.8

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Gene	p-Value	HR	95% CI		Count amplification	Count diploid	Count percentage
			Lower	Higher			
PRDM14	0.3336	3.197	0.436	23.424	2	285	0.7
KMT2D	0.3915	1.524	0.615	3.777	16	372	4.1
ASH1L	0.4391	1.635	0.513	5.211	8	364	2.2
PRDM16	0.4450	1.327	0.658	2.676	29	363	7.4
KMT2C	0.5010	0.685	0.214	2.197	21	304	6.5
SETD2	0.6543	1.218	0.526	2.817	19	356	5.1
WHSC1	0.7368	0.844	0.307	2.321	21	362	5.5
SETMAR	0.7739	0.865	0.314	2.382	21	353	5.6
PRDM5	0.7968	0.904	0.415	1.971	36	353	9.3
SMYD2	0.8241	1.111	0.447	2.762	20	358	5.3
KMT2A	0.8987	1.056	0.457	2.441	29	348	7.7
PRDM10	0.9515	0.965	0.303	3.077	19	356	5.1
MECOM	0.9671	1.025	0.318	3.305	7	330	2.1
KMT2E	0.9844	0.986	0.239	4.064	7	316	2.2

Supplementary Table 7C. Summary of univariate analysis of RFS for **methylation level of 51 HMTs** in prostate cancer.

Gene	p-Value	HR	95% CI	
			Lower	Higher
PRDM14	0.0023	2.00E+01	2.93E+00	1.37E+02
PRDM13	0.0181	1.17E+01	1.57E+00	8.66E+01
PRDM6	0.0184	5.91E+01	2.28E+00	1.53E+03
PRDM8	0.0211	1.58E+01	1.56E+00	1.60E+02
KMT2B	0.0383	1.56E-02	3.31E-04	7.31E-01
NSD1	0.0441	3.10E+01	1.07E+00	8.99E+02
SETDB2	0.0572	6.91E-02	4.83E-03	9.87E-01
SETDB1	0.0580	3.52E-05	1.11E-09	1.11E+00
SETD4	0.0873	1.31E+13	5.38E-02	3.18E+27
EZH2	0.1045	7.80E-10	3.21E-21	1.90E+02
KMT2E	0.1152	1.45E+01	6.10E-01	3.45E+02
PRDM4	0.1323	4.90E+03	1.71E-01	1.40E+08
SUV39H1	0.1694	3.77E+01	2.58E-01	5.50E+03
PRDM12	0.1911	1.19E+01	3.34E-01	4.23E+02
SMYD3	0.2266	2.15E+04	1.62E-03	2.87E+11
MECOM	0.2358	4.09E+01	1.15E-01	1.45E+04
DOT1L	0.2610	3.25E+05	2.83E-04	3.73E+14
SETD6	0.2710	4.95E-05	3.38E-13	7.26E+03

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Gene	p-Value	HR	95% CI	
			Lower	Higher
SETD3	0.3030	1.56E-01	4.77E-03	5.09E+00
PRDM9	0.3091	5.09E-01	1.39E-01	1.86E+00
SETD5	0.3255	1.49E-01	3.32E-03	6.66E+00
WHSC1L1	0.3297	1.86E+02	7.11E-03	4.86E+06
PRDM1	0.3832	4.35E+00	1.70E-01	1.12E+02
SUV420H2	0.3911	8.48E+07	1.16E-09	6.20E+24
KMT2D	0.4038	4.02E-04	3.62E-12	4.46E+04
SUV39H2	0.4289	6.77E+06	1.22E-10	3.77E+23
SETD1A	0.4393	9.61E-03	6.83E-08	1.35E+03
PRDM5	0.5427	1.69E+00	3.16E-01	9.06E+00
SMYD2	0.5584	2.99E+01	1.02E-03	8.79E+05
SETD7	0.5756	2.92E-04	1.06E-16	8.05E+08
EZH1	0.6304	1.22E+01	4.84E-04	3.09E+05
PRDM15	0.6484	6.84E-02	6.01E-07	7.78E+03
PRDM11	0.6665	3.32E-01	2.30E-03	4.80E+01
PRDM10	0.7125	4.99E-01	1.26E-02	1.98E+01
SETD2	0.7127	5.25E-04	1.16E-21	2.37E+14
SMYD5	0.7344	2.17E-01	3.15E-05	1.49E+03
SETD8	0.7427	2.28E-03	7.04E-20	7.36E+13
PRDM7	0.7496	5.91E-01	2.37E-02	1.48E+01
SMYD1	0.7786	7.48E-01	1.00E-01	5.57E+00
ASH1L	0.8598	6.00E-01	1.78E-03	2.02E+02
SETMAR	0.8750	2.48E+00	3.02E-05	2.03E+05
EHMT2	0.8931	1.75E-01	1.51E-12	2.02E+10
SUV420H1	0.8952	2.14E+00	2.59E-05	1.77E+05
WHSC1	0.9032	7.11E-01	2.96E-03	1.71E+02
KMT2A	0.9108	2.45E+01	1.39E-23	4.30E+25
PRDM2	0.9111	2.29E+00	1.16E-06	4.51E+06
EHMT1	NA	NA	NA	NA
KMT2C	NA	NA	NA	NA
PRDM16	NA	NA	NA	NA
SETD1B	NA	NA	NA	NA
SMYD4	NA	NA	NA	NA

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Supplementary Table 7D. Summary of uniivariate analysis of RFS for **expression level of 51 HMTs** in prostate cancer.

Gene	p-Value	HR	95%CI		Coef.	Wald test
			Lower	Higher		
EZH2	0	2.897	1.755	4.78	1.064	17.31
SETD5	3.00E-04	2.343	1.478	3.715	0.852	13.13
WHSC1	4.00E-04	2.392	1.478	3.87	0.872	12.61
SETD1B	0.0054	1.931	1.215	3.07	0.658	7.74
SUV420H2	0.0054	1.93	1.214	3.067	0.657	7.74
PRDM6	0.0066	0.526	0.331	0.836	–0.642	7.38
PRDM15	0.0101	1.829	1.155	2.898	0.604	6.62
KMT2D	0.0119	1.793	1.137	2.827	0.584	6.32
SMYD1	0.0207	0.471	0.249	0.891	–0.753	5.35
PRDM10	0.0235	1.682	1.073	2.639	0.52	5.13
KMT2B	0.0269	1.666	1.06	2.618	0.51	4.9
PRDM16	0.0395	0.617	0.39	0.977	–0.483	4.24
DOT1L	0.0397	1.617	1.023	2.556	0.481	4.23
PRDM8	0.076	0.658	0.415	1.045	–0.418	3.15
PRDM12	0.0764	1.522	0.956	2.422	0.42	3.14
SUV39H1	0.099	1.475	0.93	2.34	0.389	2.72
KMT2C	0.1048	1.448	0.926	2.264	0.37	2.63
SETD8	0.1267	0.705	0.45	1.104	–0.35	2.33
KMT2E	0.1411	1.402	0.894	2.2	0.338	2.17
NSD1	0.1462	1.392	0.891	2.175	0.331	2.11
EHMT1	0.1524	1.386	0.886	2.166	0.326	2.05
SETD3	0.1529	0.722	0.461	1.129	–0.326	2.04
PRDM1	0.1609	1.378	0.88	2.156	0.32	1.97
SETD4	0.1734	1.376	0.869	2.178	0.319	1.85
PRDM11	0.2041	0.745	0.472	1.174	–0.295	1.61
SETDB2	0.2374	1.313	0.836	2.063	0.272	1.4
MECOM	0.2825	0.78	0.497	1.227	–0.248	1.15
SETDB1	0.2992	1.267	0.811	1.98	0.236	1.08
SUV39H2	0.3051	0.788	0.5	1.242	–0.238	1.05
SETD2	0.3203	1.256	0.801	1.968	0.228	0.99
PRDM14	0.3581	1.44	0.661	3.137	0.365	0.84
SETD6	0.4063	1.207	0.774	1.883	0.188	0.69
SMYD5	0.4071	1.209	0.772	1.892	0.19	0.69
SETMAR	0.4329	0.836	0.535	1.307	–0.179	0.61

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Gene	p-Value	HR	95%CI		Coef.	Wald test
			Lower	Higher		
SUV420H1	0.4691	1.179	0.755	1.841	0.165	0.52
ASH1L	0.5269	1.156	0.738	1.811	0.145	0.4
KMT2A	0.5758	1.136	0.727	1.775	0.127	0.31
WHSC1L1	0.59	0.885	0.567	1.381	–0.123	0.29
PRDM5	0.6124	0.889	0.565	1.4	–0.117	0.26
EHMT2	0.631	1.115	0.715	1.741	0.109	0.23
PRDM7	0.6638	0.899	0.555	1.455	–0.107	0.19
SMYD3	0.6794	1.098	0.704	1.714	0.094	0.17
EZH1	0.7453	0.929	0.595	1.45	–0.074	0.11
PRDM9	0.8192	0.907	0.392	2.097	–0.098	0.05
SMYD4	0.8539	1.043	0.665	1.637	0.042	0.03
SMYD2	0.8758	1.036	0.664	1.617	0.035	0.02
SETD7	0.8837	1.034	0.657	1.629	0.034	0.02
PRDM13	0.8883	1.038	0.619	1.741	0.037	0.02
PRDM2	0.9321	0.981	0.626	1.536	–0.019	0.01
PRDM4	0.982	1.005	0.645	1.568	0.005	0
SETD1A	0.9988	1	0.641	1.562	0	0

Supplementary Table 8A. Summary of multivariate analysis of RFS for amplification of 21 HMTs in prostate cancer.

Multivariate		Age	Pathology T	Gleason score	AR expression	EHMT1 amplification
Coefficient		–0.017	0.788	0.988	0.198	0.646
Hazard ratio		0.983	2.198	2.687	1.219	1.908
95%CI	Lower	0.948	1.139	1.59	0.767	1.112
	Higher	1.019	4.241	4.538	1.938	3.274
p-Value		0.35	0.0189	0.0002	0.4019	**0.0191**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**EHMT2 amplification**
Coefficient		–0.004	0.717	0.906	0.239	0.666
Hazard ratio		0.996	2.049	2.476	1.27	1.947
95%CI	Lower	0.961	1.089	1.473	0.795	0.83
	Higher	1.033	3.854	4.16	2.029	4.57
p-Value		0.8407	0.0261	0.0006	0.3177	0.1257

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Multivariate		Age	Pathology T	Gleason score	AR expression	PRDM12 amplification
Coefficient		–0.007	0.676	0.921	0.325	0.665
Hazard ratio		0.993	1.967	2.512	1.384	1.945
95%CI	Lower	0.959	1.037	1.492	0.871	1.125
	Higher	1.029	3.73	4.23	2.2	3.362
p-Value		0.7062	0.0383	0.0005	0.1691	**0.0172**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM14 amplification**
Coefficient		–0.007	0.757	0.984	0.226	0.329
Hazard ratio		0.993	2.131	2.675	1.254	1.39
95%CI	Lower	0.959	1.136	1.61	0.797	0.875
	Higher	1.029	3.999	4.446	1.972	2.209
p-Value		0.7034	0.0184	0.0001	0.3271	0.1637
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM15 amplification**
Coefficient		–0.005	0.654	0.952	0.132	0.436
Hazard ratio		0.995	1.923	2.59	1.141	1.546
95%CI	Lower	0.958	1.019	1.534	0.704	0.743
	Higher	1.033	3.629	4.372	1.849	3.22
p-Value		0.7942	0.0436	0.0004	0.5931	0.2442
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM4 amplification**
Coefficient		–0.006	0.779	0.923	0.263	0.867
Hazard ratio		0.994	2.179	2.517	1.301	2.379
95%CI	Lower	0.959	1.164	1.502	0.801	1.143
	Higher	1.031	4.082	4.219	2.112	4.953
p-Value		0.7471	0.0149	0.0005	0.2881	**0.0205**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SETD1A amplification**
Coefficient		–0.015	0.872	0.893	0.073	0.391
Hazard ratio		0.985	2.392	2.442	1.076	1.479
95%CI	Lower	0.947	1.217	1.43	0.662	0.722
	Higher	1.024	4.702	4.171	1.749	3.029
p-Value		0.4491	0.0114	0.0011	0.7672	0.2851
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SETD1B amplification**
Coefficient		–0.003	0.796	0.907	0.152	1.218
Hazard ratio		0.997	2.216	2.477	1.164	3.381
95%CI	Lower	0.961	1.179	1.467	0.711	1.55
	Higher	1.034	4.166	4.18	1.906	7.372
p-Value		0.8521	0.0135	0.0007	0.5451	**0.0022**

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Multivariate		Age	Pathology T	Gleason score	AR expression	SETD8 Amplification
Coefficient		0	0.8	0.898	0.2	0.97
Hazard ratio		1	2.226	2.456	1.221	2.638
95%CI	Lower	0.964	1.182	1.453	0.747	1.101
	Higher	1.038	4.191	4.152	1.996	6.323
p-Value		0.9897	0.0132	0.0008	0.4256	**0.0296**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SETDB1 amplification**
Coefficient		–0.009	0.812	0.941	0.158	0.701
Hazard ratio		0.991	2.253	2.563	1.171	2.016
95%CI	Lower	0.957	1.209	1.559	0.744	1.062
	Higher	1.027	4.2	4.214	1.842	3.826
p-Value		0.6255	0.0106	0.0002	0.4956	**0.0319**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SMYD1 amplification**
Coefficient		–0.004	0.788	0.906	0.15	1.423
Hazard ratio		0.996	2.198	2.475	1.162	4.149
95%CI	Lower	0.959	1.139	1.443	0.716	1.844
	Higher	1.034	4.243	4.244	1.885	9.336
p-Value		0.8369	0.0189	0.001	0.5434	**0.0006**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SMYD2 amplification**
Coefficient		0	0.798	0.955	0.181	0.77
Hazard ratio		1	2.221	2.6	1.198	2.159
95%CI	Lower	0.965	1.16	1.556	0.751	1.026
	Higher	1.037	4.253	4.343	1.91	4.546
p-Value		0.9835	0.0161	0.0003	0.4483	**0.0426**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SMYD5 Amplification**
Coefficient		0.004	0.745	0.929	0.163	1.274
Hazard ratio		1.004	2.106	2.532	1.177	3.574
95%CI	Lower	0.967	1.119	1.484	0.729	1.595
	Higher	1.042	3.966	4.32	1.901	8.01
p-Value		0.8462	0.0211	0.0007	0.5045	**0.002**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SUV420H1 amplification**
Coefficient		–0.006	0.835	0.897	0.155	0.394
Hazard ratio		0.994	2.304	2.452	1.168	1.483
95%CI	Lower	0.96	1.237	1.48	0.742	0.809
	Higher	1.03	4.289	4.064	1.838	2.718
p-Value		0.7365	0.0085	0.0005	0.5016	0.2028

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Multivariate		Age	Pathology T	Gleason score	AR expression	SUV420H2 amplification
Coefficient		–0.014	0.885	0.927	0.072	0.422
Hazard ratio		0.986	2.422	2.528	1.075	1.524
95%CI	Lower	0.949	1.263	1.51	0.664	0.673
	Higher	1.025	4.642	4.231	1.741	3.455
p-Value		0.4852	0.0077	0.0004	0.7691	0.3126
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**WHSC1 amplification**
Coefficient		–0.014	0.786	1.051	0.152	0.439
Hazard ratio		0.986	2.194	2.86	1.165	1.551
95%CI	Lower	0.951	1.142	1.702	0.733	0.732
	Higher	1.022	4.218	4.806	1.851	3.286
p-Value		0.4366	0.0184	0.0001	0.5191	0.2522
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**WHSC1L1 amplification**
Coefficient		0.001	0.486	1.027	0.21	0.565
Hazard ratio		1.001	1.627	2.792	1.233	1.76
95%CI	Lower	0.956	0.709	1.378	0.686	0.946
	Higher	1.049	3.732	5.657	2.216	3.272
p-Value		0.95	0.251	0.0044	0.4836	0.0742

Supplementary Table 8B. Summary of multivariate analysis of RFS for deletion of 10 HMTs in prostate cancer.

Multivariate		Age	Pathology T	Gleason score	AR expression	DOT1L deletion
Coefficient		–0.003	0.742	0.971	0.304	0.441
Hazard ratio		0.997	2.101	2.64	1.356	1.554
95%CI	Lower	0.962	1.118	1.585	0.847	0.815
	Higher	1.033	3.945	4.397	2.17	2.963
p-Value		0.8719	0.021	0.0002	0.2053	0.1802
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**EHMT2 deletion**
Coefficient		0.004	0.775	0.951	0.176	0.992
Hazard ratio		1.004	2.17	2.588	1.193	2.695
95%CI	Lower	0.968	1.156	1.548	0.743	1.135
	Higher	1.042	4.074	4.327	1.916	6.398
p-Value		0.81	0.016	0.0003	0.4662	**0.0246**

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Multivariate		Age	Pathology T	Gleason score	AR expression	PRDM7 deletion
Coefficient		0.003	0.796	0.947	0.196	0.333
Hazard ratio		1.003	2.217	2.577	1.216	1.395
95%CI	Lower	0.969	1.19	1.563	0.773	0.881
	Higher	1.039	4.133	4.25	1.914	2.208
p-Value		0.8706	0.0122	0.0002	0.397	0.1559
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SETD4 deletion**
Coefficient		–0.006	0.733	0.991	0.177	1.097
Hazard ratio		0.994	2.081	2.694	1.194	2.996
95%CI	Lower	0.959	1.107	1.598	0.738	1.336
	Higher	1.031	3.91	4.545	1.932	6.716
p-Value		0.7515	0.0229	0.0002	0.4706	**0.0077**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SETD6 deletion**
Coefficient		0	0.762	0.911	0.24	0.491
Hazard ratio		1	2.143	2.486	1.271	1.634
95%CI	Lower	0.966	1.146	1.486	0.805	1.018
	Higher	1.035	4.006	4.159	2.008	2.622
p-Value		0.9999	0.017	0.0005	0.3036	**0.042**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SETDB2 deletion**
Coefficient		–0.007	0.835	0.928	0.125	0.322
Hazard ratio		0.993	2.305	2.531	1.133	1.38
95%CI	Lower	0.96	1.237	1.529	0.721	0.868
	Higher	1.028	4.298	4.189	1.782	2.195
p-Value		0.6953	0.0086	0.0003	0.5873	0.1739
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SMYD1 deletion**
Coefficient		0	0.755	0.96	0.309	0.998
Hazard ratio		1	2.128	2.612	1.362	2.714
95%CI	Lower	0.964	1.135	1.558	0.848	1.329
	Higher	1.038	3.99	4.378	2.189	5.543
p-Value		0.9902	0.0185	0.0003	0.2012	**0.0061**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SUV39H2 deletion**
Coefficient		–0.013	0.774	0.976	0.187	0.755
Hazard ratio		0.987	2.169	2.655	1.205	2.129
95%CI	Lower	0.953	1.15	1.588	0.761	1.199
	Higher	1.022	4.092	4.437	1.909	3.778
p-Value		0.4618	0.0168	0.0002	0.4265	**0.0099**

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Multivariate		Age	Pathology T	Gleason score	AR expression	SUV420H2 deletion
Coefficient		0	0.77	0.89	0.289	0.553
Hazard ratio		1	2.16	2.435	1.335	1.738
95%CI	Lower	0.964	1.147	1.443	0.831	0.808
	Higher	1.037	4.071	4.108	2.144	3.736
p-Value		0.9946	0.0172	0.0009	0.232	0.1572
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**WHSC1L1 deletion**
Coefficient		–0.001	0.681	1.184	0.121	0.677
Hazard ratio		0.999	1.976	3.267	1.129	1.968
95%CI	Lower	0.959	1.028	1.867	0.671	1.173
	Higher	1.04	3.8	5.716	1.899	3.303
p-Value		0.9534	0.0411	0	0.6489	**0.0103**

Supplementary Table 8C. Summary of multivariate analysis of RFS for higher promotor methylation of 6 HMTs in prostate cancer.

Multivariate		Age	Pathology T	Gleason score	AR expression	PRDM14 high_meth
Coefficient		–0.009	0.785	0.916	0.188	0.32
Hazard ratio		0.991	2.193	2.498	1.207	1.377
95%CI	Lower	0.957	1.174	1.508	0.769	0.823
	Higher	1.027	4.095	4.139	1.894	2.303
p-Value		0.6314	0.0137	0.0004	0.4131	0.223
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM13 high_meth**
Coefficient		–0.006	0.791	0.971	0.171	0.317
Hazard ratio		0.994	2.206	2.641	1.187	1.373
95%CI	Lower	0.96	1.182	1.608	0.755	0.86
	Higher	1.029	4.119	4.338	1.864	2.19
p-Value		0.7279	0.013	0.0001	0.4581	0.1839
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM6 high_meth**
Coefficient		–0.004	0.816	0.967	0.191	0.079
Hazard ratio		0.996	2.262	2.631	1.211	1.082
95%CI	Lower	0.962	1.208	1.594	0.771	0.675
	Higher	1.031	4.235	4.341	1.901	1.734
p-Value		0.8298	0.0108	0.0002	0.4059	0.7441
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM8 high_meth**
Coefficient		–0.004	0.826	0.933	0.184	0.324
Hazard ratio		0.996	2.285	2.542	1.201	1.383
95%CI	Lower	0.963	1.227	1.542	0.766	0.869
	Higher	1.031	4.257	4.189	1.883	2.199
p-Value		0.8314	0.0092	0.0003	0.4236	0.1712

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Multivariate		Age	Pathology T	Gleason score	AR expression	KMT2B high_meth
Coefficient		–0.003	0.86	0.897	0.229	–0.417
Hazard ratio		0.997	2.363	2.452	1.257	0.659
95%CI	Lower	0.963	1.274	1.486	0.8	0.409
	Higher	1.033	4.382	4.045	1.975	1.061
p-Value		0.8776	0.0064	0.0004	0.3215	0.086
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**NSD1 High_meth**
Coefficient		–0.006	0.852	0.889	0.161	0.547
Hazard ratio		0.994	2.345	2.432	1.175	1.728
95%CI	Lower	0.961	1.26	1.476	0.749	1.06
	Higher	1.028	4.364	4.009	1.843	2.818
p-Value		0.7268	0.0071	0.0005	0.4839	**0.0284**

Supplementary Table 8D. Summary of multivariate analysis of RFS for higher mRNA level of 16 HMTs in prostate cancer.

Multivariate		Age	Pathology T	Gleason score	AR expression	DOT1L high_exp
Coefficient		–0.004	0.854	0.916	0.163	0.254
Hazard ratio		0.996	2.35	2.5	1.177	1.289
95%CI	Lower	0.962	1.26	1.504	0.747	0.802
	Higher	1.032	4.382	4.157	1.855	2.071
p-Value		0.8373	0.0072	0.0004	0.4813	0.2941
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**EZH2 high_exp**
Coefficient		–0.006	0.858	0.731	0.185	0.736
Hazard ratio		0.994	2.357	2.076	1.203	2.088
95%CI	Lower	0.961	1.263	1.23	0.768	1.23
	Higher	1.028	4.4	3.506	1.887	3.544
p-Value		0.7334	0.0071	0.0063	0.4195	**0.0064**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**KMT2B high_exp**
Coefficient		–0.004	0.877	0.923	-0.026	0.387
Hazard ratio		0.996	2.403	2.516	0.975	1.472
95%CI	Lower	0.962	1.288	1.526	0.563	0.839
	Higher	1.031	4.484	4.149	1.688	2.584
p-Value		0.8266	0.0059	0.0003	0.9268	0.1781
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**KMT2D high_exp**
Coefficient		–0.004	0.857	0.906	0.087	0.333
Hazard ratio		0.996	2.357	2.474	1.091	1.396
95%CI	Lower	0.962	1.264	1.492	0.677	0.851
	Higher	1.031	4.393	4.102	1.758	2.289
p-Value		0.8135	0.007	0.0004	0.721	0.1865

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Multivariate		Age	Pathology T	Gleason score	AR expression	PRDM10 high_exp
Coefficient		–0.003	0.829	0.94	0.054	0.266
Hazard ratio		0.997	2.291	2.56	1.055	1.304
95%CI	Lower	0.963	1.23	1.551	0.617	0.755
	Higher	1.033	4.267	4.227	1.804	2.255
p-Value		0.878	0.009	0.0002	0.8443	0.3412
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM12 high_exp**
Coefficient		–0.005	0.841	0.923	0.231	0.224
Hazard ratio		0.995	2.319	2.517	1.26	1.251
95%CI	Lower	0.962	1.245	1.512	0.799	0.773
	Higher	1.03	4.322	4.188	1.988	2.024
p-Value		0.7926	0.0081	0.0004	0.3195	0.362
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM15 high_exp**
Coefficient		–0.003	0.856	0.909	0.153	0.448
Hazard ratio		0.997	2.354	2.483	1.165	1.566
95%CI	Lower	0.964	1.264	1.507	0.742	0.981
	Higher	1.033	4.383	4.092	1.83	2.5
p-Value		0.8865	0.007	0.0004	0.5069	0.0604
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM16 high_exp**
Coefficient		–0.006	0.898	0.948	0.175	-0.514
Hazard ratio		0.994	2.454	2.581	1.191	0.598
95%CI	Lower	0.96	1.315	1.571	0.759	0.377
	Higher	1.029	4.579	4.24	1.87	0.949
p-Value		0.747	0.0048	0.0002	0.4462	**0.0293**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM6 high_exp**
Coefficient		–0.003	0.851	0.897	0.208	-0.506
Hazard ratio		0.997	2.343	2.452	1.231	0.603
95%CI	Lower	0.963	1.259	1.486	0.785	0.377
	Higher	1.032	4.36	4.046	1.931	0.964
p-Value		0.8603	0.0072	0.0004	0.366	**0.0345**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**PRDM8 high_exp**
Coefficient		–0.005	0.857	0.916	0.208	-0.284
Hazard ratio		0.995	2.356	2.499	1.231	0.753
95%CI	Lower	0.962	1.261	1.504	0.785	0.47
	Higher	1.03	4.401	4.155	1.93	1.206
p-Value		0.7952	0.0072	0.0004	0.3658	0.2375

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Multivariate		Age	Pathology T	Gleason score	AR expression	SETD1B high_exp
Coefficient		–0.008	0.861	0.903	0.059	0.453
Hazard ratio		0.992	2.365	2.468	1.06	1.573
95%CI	Lower	0.958	1.271	1.496	0.661	0.957
	Higher	1.028	4.401	4.071	1.701	2.584
p-Value		0.6715	0.0066	0.0004	0.8076	0.0738
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SETD5 high_exp**
Coefficient		–0.005	0.784	0.903	0.033	0.584
Hazard ratio		0.995	2.191	2.466	1.033	1.794
95%CI	Lower	0.961	1.176	1.496	0.646	1.1
	Higher	1.031	4.082	4.067	1.651	2.926
p-Value		0.7935	0.0135	0.0004	0.8915	**0.0193**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SMYD1 high_exp**
Coefficient		–0.009	0.862	1.002	0.191	–0.819
Hazard ratio		0.991	2.369	2.723	1.21	0.441
95%CI	Lower	0.957	1.269	1.662	0.772	0.232
	Higher	1.027	4.423	4.461	1.896	0.838
p-Value		0.623	0.0068	0.0001	0.405	**0.0124**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SUV39H1 high_exp**
Coefficient		–0.007	0.86	0.925	0.22	0.329
Hazard ratio		0.993	2.363	2.521	1.246	1.39
95%CI	Lower	0.958	1.267	1.525	0.792	0.866
	Higher	1.028	4.407	4.167	1.958	2.23
p-Value		0.6758	0.0068	0.0003	0.3412	0.1728
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**SUV420H2 high_exp**
Coefficient		–0.009	0.852	0.847	0.382	0.586
Hazard ratio		0.991	2.344	2.334	1.465	1.796
95%CI	Lower	0.957	1.257	1.402	0.909	1.087
	Higher	1.027	4.37	3.884	2.361	2.966
p-Value		0.6247	0.0073	0.0011	0.1165	**0.0222**
Multivariate		**Age**	**Pathology T**	**Gleason score**	**AR expression**	**WHSC1 high_exp**
Coefficient		–0.005	0.815	0.831	0.146	0.429
Hazard ratio		0.995	2.259	2.297	1.157	1.535
95%CI	Lower	0.961	1.21	1.357	0.735	0.913
	Higher	1.03	4.217	3.888	1.821	2.581
p-Value		0.7789	0.0105	0.002	0.5276	0.1057

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