Supplemental Figures.

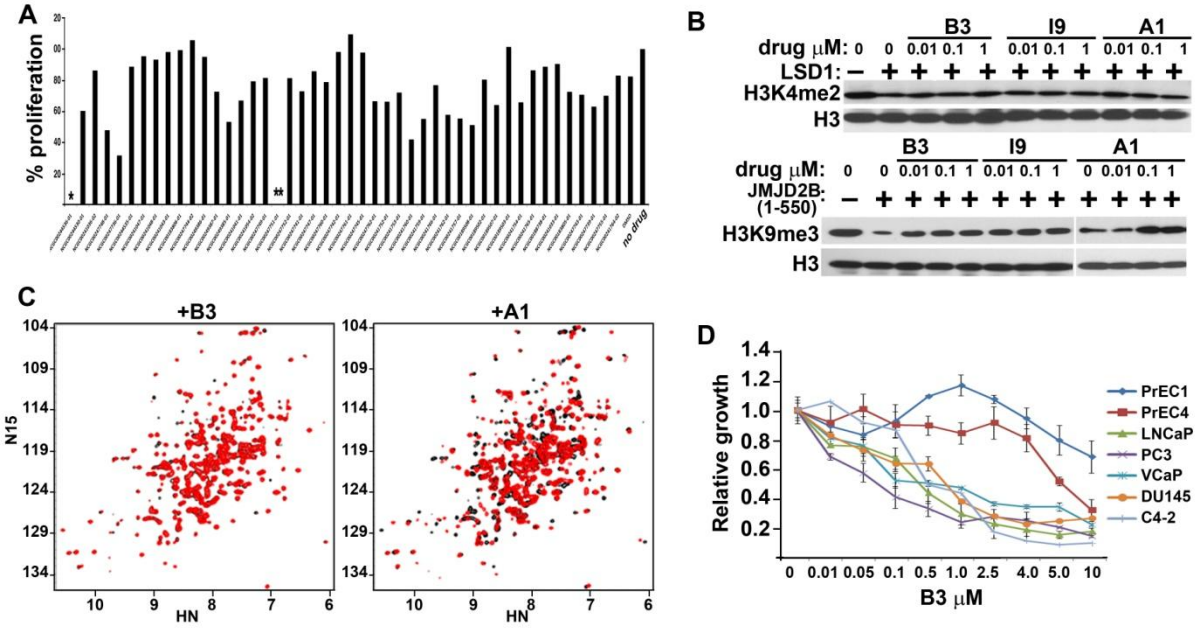

Figure S1, related to figure 1. Identification and characterization of novel inhibitors of KDM4.

(A) Relative growth of LNCaP cells in the presence of vehicle or small molecule inhibitors of KDM4 (5 μM). Equal amount of cells were seeded in 96-well plates and harvested 4 days later after compound treatment. The viable cells were measured by MTT assays. (B) Demethylase assay of LSD1 (upper panel) and KDM4B catalytic domain (lower panel) in the absence or presence of various concentrations of KDM4 inhibitors. Total histone (Sigma) was incubated with purified LSD1 or KDM4B that was expressed in bacterial *E coli* cultures. The remaining H3K4me3 or H3K9me3 were measured by Western blot. (C) 15N-H HSQC spectrum of KDM4A in the presence (red contours) and absence (black contours) of compound B3 (left panel) and A1 (right panel). Addition of B3 and A1 caused shifts in specific cross-peaks of the spectrum of the catalytic domain of KDM4A, and the shift patterns were markedly different for the two compounds, showing that both compounds bind specifically to the domain and suggesting that the binding modes are distinct. (D) Growth response curves of prostate cell lines to various concentrations of KDM4 inhibitor B3. N=6, mean ± SD.

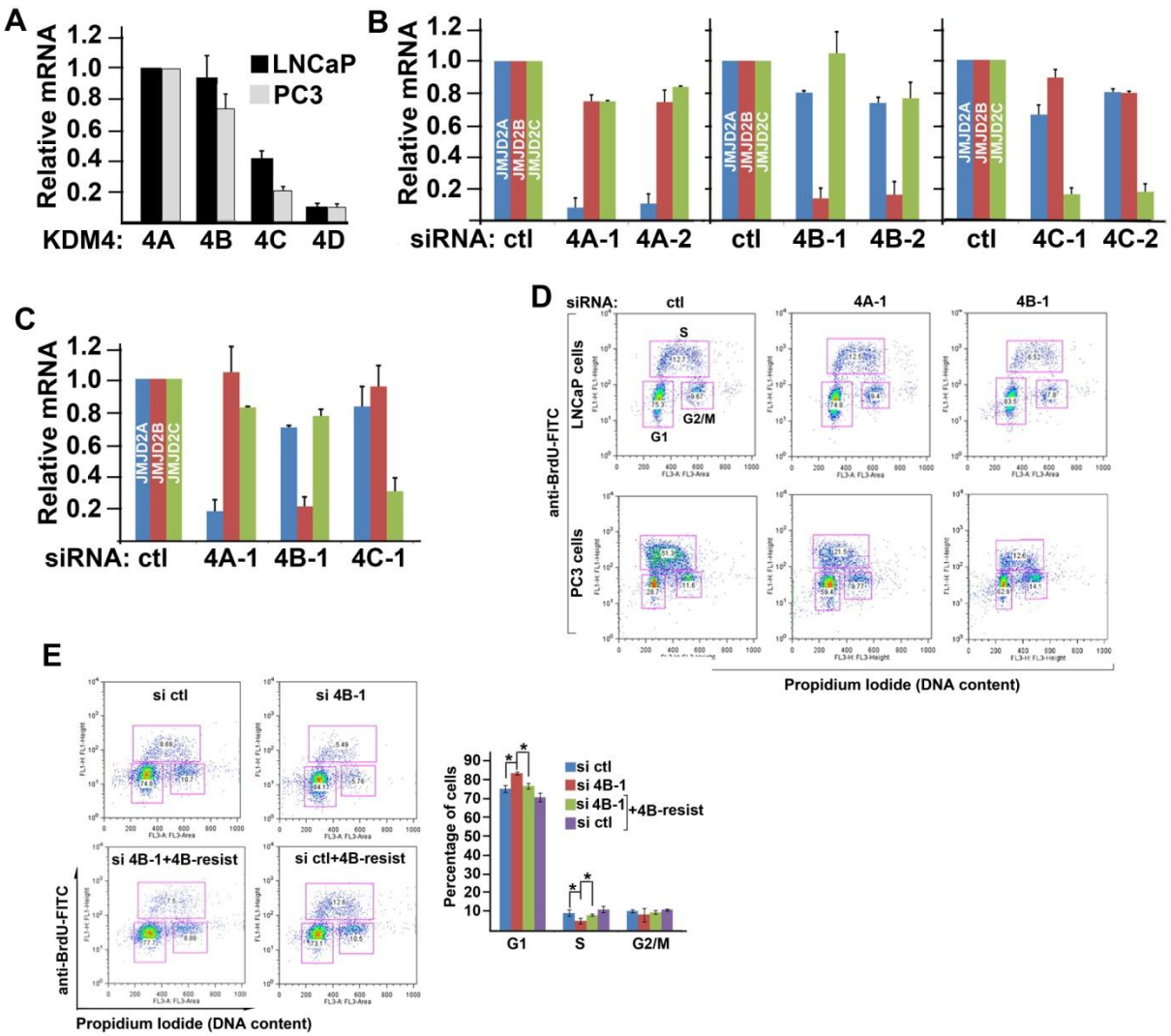

Figure S2, related to figure 2. Depletion of KDM4 proteins inhibits cell cycle progression. (**A**) Relative expression of KDM4A-4D in LNCaP and PC3 cells measured by qRT-PCR. (**B**) Relative mRNA of KDM4A-4C in LNCaP cells transfected with control (ctl), KDM4A-4C specific siRNA duplexes. Two independent siRNA duplexes were used for each KDm4 isoform. (**C**) Relative mRNA of KDM4A-4C in PC3 cells transfected with control, KDM4A-2C siRNAs. (D) FACS profiles of LNCaP and PC3 cells transfected with control (ctl), KDM4A siRNA, or KDM4B siRNA. (E) FACS profiles of LNCaP cells transfected with control, or KDM4B siRNA in the presence or absence of exogenously expressed siRNA-resistant KDM4B.

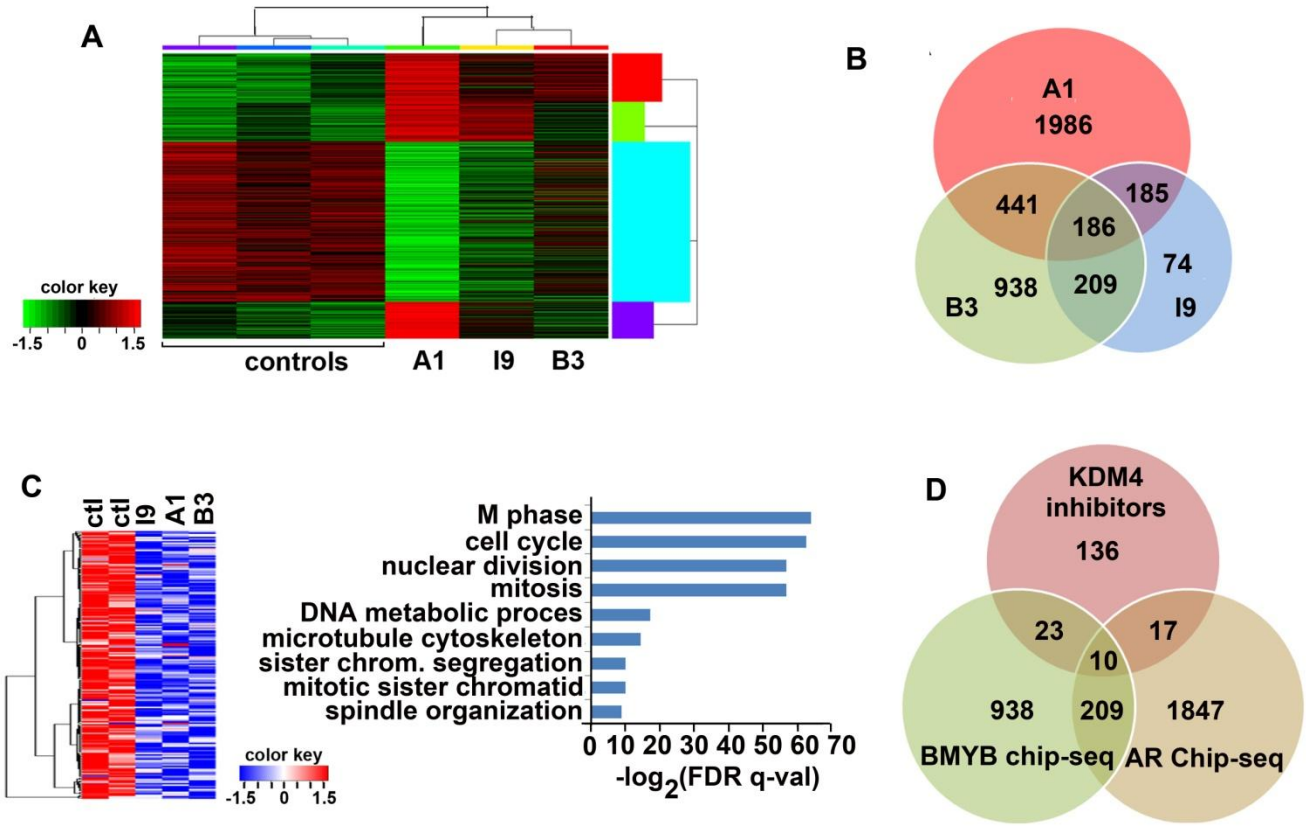

Figure S3, related to figure 3. KDM4 inhibitors downregulate expressions of critical cell cycle genes. (A) Cluster analysis of genes differentially expressed in vehicle and KDM4 inhibitors (A1, I9, and B3) treated LNCaP cells. (**B**) Venn diagram showing common downregulated genes in A1, B3, and I9-treated LNCaP cells. (**C**) Selected heat map of differentially expressed transcripts of common-downregulated genes from (B). Gene ontology analysis (DAVID, NIH) indicated that cell-cycle related genes are among the top ones affected by the compounds. (**D**) Venn diagram showing the number of genes from (A) that are regulated by AR, BMYB, or both.

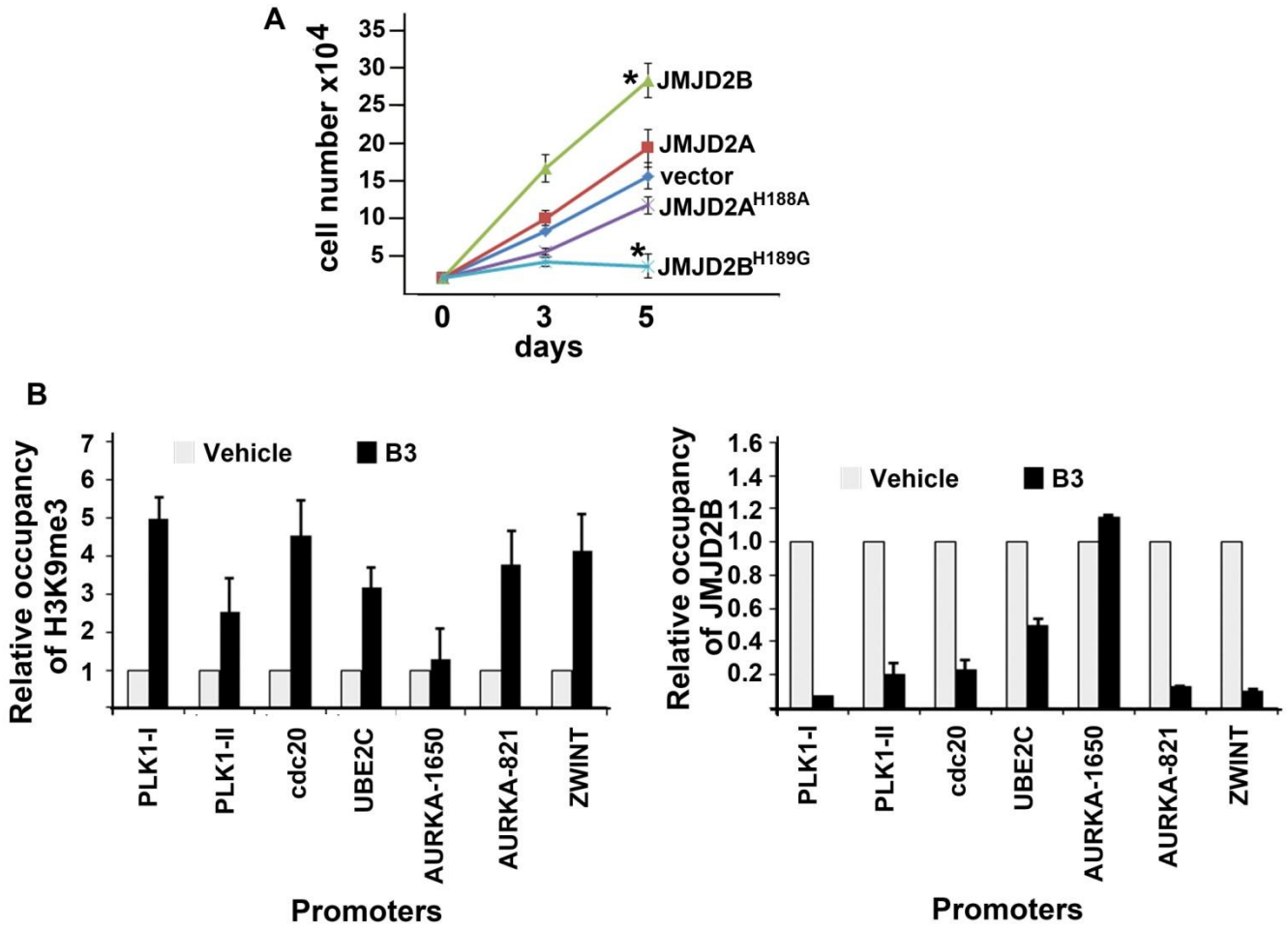

Figure S4, related to figure 5. (A) KDM4B^{H189G} is a dominant-negative form. KDM4A, KDM4B, and their demethylase-inactive form was ectopically expressed LNCaP cell using lenti-viral transduction, respectively. KDM4A and KDM4B promoted LNCaP cell growth whereas their demethylase-inactive forms inhibited cell growth. (B) **B3 inhibits binding of KDM4B to the promoters of cell cycle genes.** Relative occupancy of H3K9me3 (left panel) and KDM4B (right panel) on the indicated promoters in LNCaP cells treated with vehicle or B3 as measured by ChIP-qPCR assays. N=3, mean ± SD.

Table S1, related to experimental procedure. Sequences of siRNA duplexes and qRT-PCR primers.

siRNA		
JMJD2A-1	5’-CGAAACTTCAGTAGATACATT	
JMJD2A-2	5’-GTGATGATGAGACATCTGATT	
JMJD2B-1	5’-CAAATACGTGGCCTACATA TT	
JMJD2B-2	5’-CTCTTCACGCAGTACAATATT	
JMJD2C-1	5’-CATTACATGCTTTCG ACATTT	
JMJD2C-2	5’-CTCATACCAGAGATGTGTTTT	
qRT-PCR	**forward**	**reverse**
JMJD2A	AGCTTGCTTAAAGGCTGACG	GAAGTTTCAGTGAGCGGGAG
JMJD2B	ATCTTGACCATGTCCTTCCG	TCAACTGCGCAGAATCTACC
JMJD2C	CTTTCCTGCAAGTGCACAAT	CCATGCTGGTTTTAATCATGG
JMJD2D	GTGGGAGTGAAGAGCACACA	CAGTTACCCAGGAGAGCAGC
P21	CCGAAGTCAGTTCCTTGTGG	CATGGGTTCTGACGGACAT
PLK1	CACAGTGTCAATGCCTCCA	TTGCTGACCCAGAAGATGG
TPX1	ACATCTGAACTACGAAAGCATCC	GGCTTAACAATGGTACATCCCTTA
BIRC5	GCCCAGTGTTTCTTCTGCTT	AACCGGACGAATGCTTTTTA
AURKA	CGCCCTGTAGGATACTGCTT	CAAATATCCCCGCACTCTG
UBE2C	CATGATGTCTGGCGATAAAGG	GGTTCTGATGCTCCCCACT
GADD45G	CAGCCAAAGTCTTGAACGTG	CCTGGATCAGCGTAAAATGG
ChIP-qPCR	**forward**	**reverse**
PLK1 (II)	CCCCGAGGTAGAGGAAGATT	AAGCTCCTGCGGTTCACTT
PLK1(I)	TGATTGCTGGAGGAATTGTG	GGTGTAAGCCTCCCACAGTC
UBE2C	TGCCCGAGGGAAATTGG	CTTACTCCGCGTGGGAACA
AURKA(1650)	CGGGCTGATTACCTTGAACT	AAGCACAGGGAAAGCCTCTT
AURKA(621)	CTAAGGACTGGGTGGGAATG	GCCTCTACTTTGCGTTTCCTAA
CDC20	GGAATGTACCCTAAGTAGCTCTGG	CCTAGAAGGGGAAGGAGAGC
ZWINT	GACTGTGACTTCAAAGTAATCTTAGGG	GCTGCCTCCATCTTTCCAG

Table S2, related to experimental procedure and figure 6. Clinicopathologic characteristics of human prostate cancers.

	Patient age	PSA (ng/ml)	Clinical stage	Pathologic stage	GS	LN
1	70	11	T1c	pT3q	4+3=7	Negative
2	50	9	T2a	pT2c	4+4=8	Negative
3	62	6.5	T2a	pT3b	4+5=9	Negative
4	65	7	T1c	pT2c	3+4=7	Negative

Supplemental experiment procedures.

Antibodies. Antibodies against H3K9me3 (ab8898), Histone 3(ab1012), and p21(ab7980-1) were purchased from Abcam. Antibodies against HA(sc-805), cyclinB1(sc-752), cyclinA(sc-75), Brdu (sc-32323), and GAPDH were from Santa Cruz. Anti-AR(PG-21) antibody was from Millipore. Antibodies against KDM4B, KDm4A, cdc20 were from Cell Signaling. Anti-Flag and anti-PLK1 antibodies were from Sigma-Aldridge. Anti-UBE2C antibody was from Protein Technique. Flag and anti-PLK1 antibodies were from Sigma-Aldridge.

Cell lines. LNCaP and C4-2 were cultured in 5% FBS T medium; Du145, PC-3 and PNT1A were cultured in 10% FBS RPMI medium. PrEC1 and PrEC4 were cultured in PrEGM™ media (Lonza). Hela, MDAMB2, MCF-7, VCaP, and 293T cells were cultured in 10% FBS DMEM medium. For androgen stimulated cell growth, LNCaP cells were cultured in 10% GIBCO® Charcoal Stripped FBS for at least 24 hours before addition of final 2 nM R1881.

Plasmids and lentiviral constructs. KDM4A and KDM4A^{H188A} expression plasmids were described previously (*32*). KDM4B and KDM4B^{H189G} were obtained from Addgene. PLK1-luc was constructed by subcloning PCR-amplified human PLK1 promoter fragments into pGL2-basic (Promega, USA) using KpnI and XhoI sites. Site-specific mutations were made using quick-change kit (Stratagene). Mutations were confirmed by DNA sequencing.