Supplemental Data

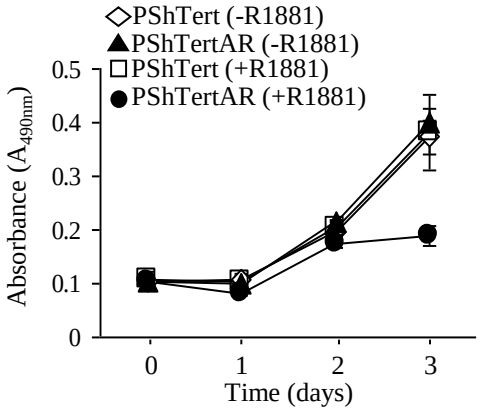
Supplemental Table 1. Antibodies used for PPAA

Antibodies for signal transduction proteins
14-3-3 Beta，Akt，alpha-tubulin，ATF-1，Bad，Bak，Bax，Bcl-2，Bcl-6，Bcl-xL，beta-catenin，BID，Calretinin，CaMKKa，cdc2 p34，Cdc25B，Cdc25C，cdc42，cdk2，Cdk4，Cdk6，c-Flip，CHK1，c-IAP2，cPKCα，CREB，cyclin B1，cyclin D1，cyclin E，DRG1，E2F-1，E-cadherin，Eg5 (H-300)，EGFR，eIF4B，Endoglin，Ep-CAM，Epo，ERa，Erb (H-150)，ERCC1，ERK，E-Selectin，Estrogen Receptor α，Factor XIII B，FAS，FGF-8，FOXM1，GAPDH，HCAM，HCAM，HER2，HIF-1α，HIF-2α，HIF-3α，H-Ras，Hsp90，ICAM-1，IGFBP5，IL-1β，KAI1，KLF6，K-Ras，L-Selectin，Lyn，Maspin，MDM2，Mesothelin，MetRS，N-cadherin，NEP，NFkB p65，NFkB50，NF-κB p52，Nkx-3.1，NM23，Notch4，Osteopontin，p27，p27，p38，p53，p63，patched，PCNA，PDEF，PSCA-1，PSCA-2，PSM，PTEN，Rab 7，Rap 1，RIP，SK3 (H-45)，SLUG，SRC-1 (M-341)，Stat 3，Stat 1，Survivin，Syk，TDP1，TFIIH p89，TGF-β，TNF-α，TTF-1，Twist，uPA，uPAR，VCAM-1，VEGF, Vimentin，WT1，XIAP，β-actin
Antibodies specific for phosphoproteins
p-AKT，p-beta-catenin，p-cdc2，p-c-Jun，p-CREB，p-eIF4B，p-ERK，p-ERK5，p-FAK，p-GSK-3α/β，p-HGF R/c-MET，p-P38，p-p53，p-p70 S6 Kinase，p-p90RSK，p-PDK1，p-PKC α/βII，p-PKCa，p-PKCδ，p-PTEN，p-Rb (Ser780)，p-Rb (Ser807/811)，p-Smad1/5 (Ser463/465)，p-stat3，p-stat5

Supplemental Table 2. Primers in the study

Name	Sequence
P1	5’- TTGGTACCCTCTTGTTTTTCTTTGGA-3’
P2	5’-CGGGTACCAAATAGA AAGCTAAAACA-3’
P3	5’-TCGGTACCTCCAACCCAGAGAGTT-3’
P4	5’-TTGGTACCTTGCCCGGCTAACCTTTCC-3’
P5	5’-AAGGTACCGCCAATGGGAAGGGAGTG-3’
P6	5’-TTGGTACCTATGCTGGGTGTAGGTCC-3’
P7	5’-CCAGATCTACCAACAGCCGTTCCGCCG-3’
P8	5’-TAGATCTGCTCCCTCCTTATTGGCCTGT-3’
P9	5’-TTAGATCTCTTCACCAGGCAGCAGCT-3’
P10	5’-AAAATAAGGCGAAGATCA-3’
P11	5’-TTCCAGTGACTTCCCGAC-3’
P12	5’-GACAGTCAGCCGCATCTTCTT-3’
P13	5’-CAATACGACCAAATCCGTTGAC-3’
P14	5’- CGATCGCCCTGGAAACGCATT-3’
P15	5’-CCAGCAGAAACCAACAGCCGT-3’
P16	5’-TCCTCCTCTTCCTCAATCTCG-3’
P17	5’-AAGGCAACTTTCGGAACGG-3’

A



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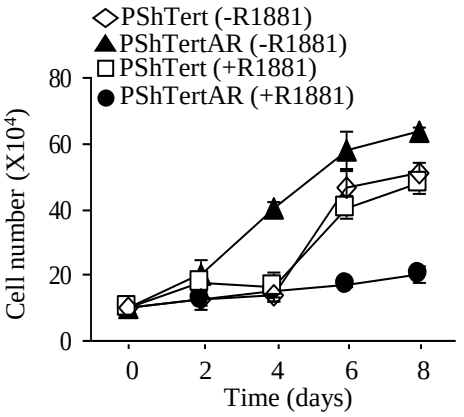

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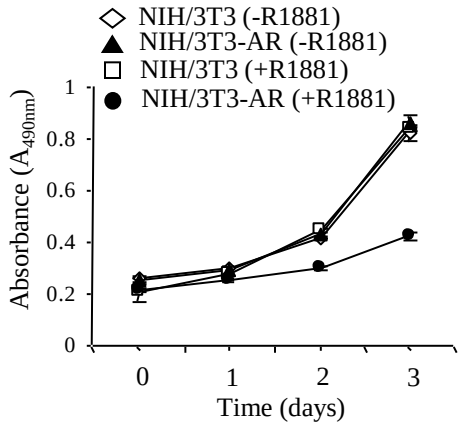

FIG. S1. Androgen inhibits prostate stromal cell proliferation.

(A) Androgen inhibits PShTertAR cell proliferation at low concentration of R1881 (0.1 nM). (B) Androgen inhibits PShTertAR cell proliferation by cell counting method with 10 nM R1881. (C) Cell proliferation was inhibited by androgen in NIH/3T3-AR cells but not NIH/3T3 cells.

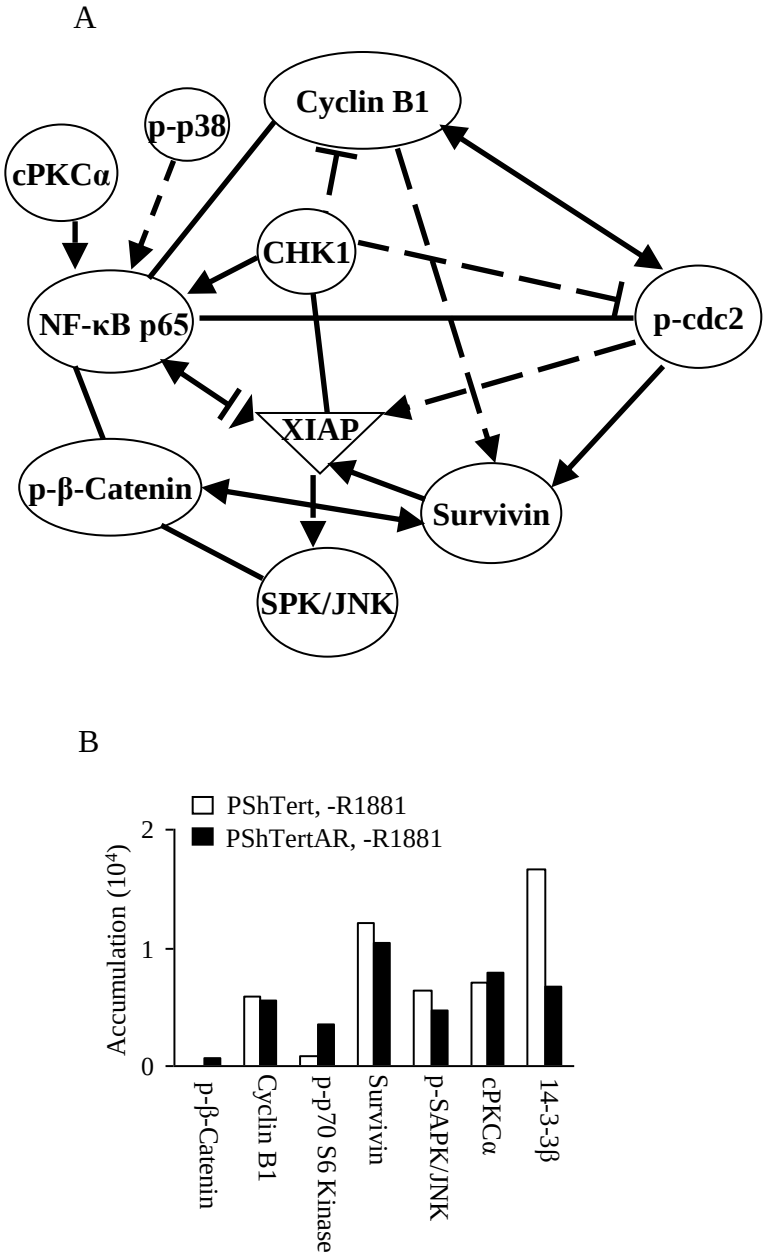

FIG. S2. Identification of signaling network proteins regulated by AR in prostate stromal cells.

(A) Differentially expressed proteins form an inter-related network using Ingenuity software IPA. (B) The difference in the levels of signal pathway proteins and phospho-proteins of PShTert and PShTertAR cells in the absence of androgen.

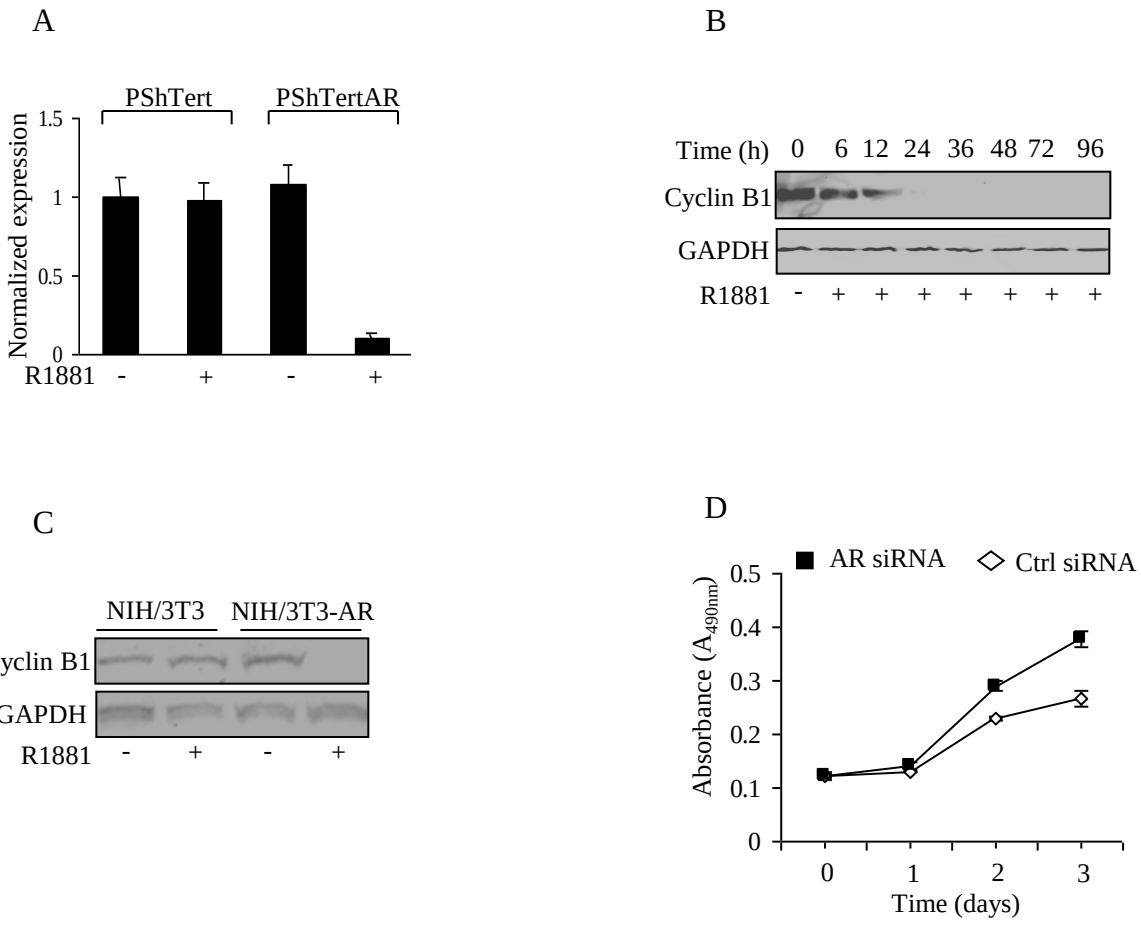

FIG. S3. Androgen repressed cyclin B1 expression in PShTertAR cells.
(A) qPCR shows that androgen inhibits cyclin B1 expression in PShTertAR cells . (B) The time course of cyclin B1 repression by androgen in PShTertAR cells. (C) cyclin B1 as well as cell proliferation were repressed by androgen in NIH/3T3-AR cells but not NIH/3T3 cells. (D) AR knockdown of the AR-positive primary cells promoted its growth even in androgen-containing medium.

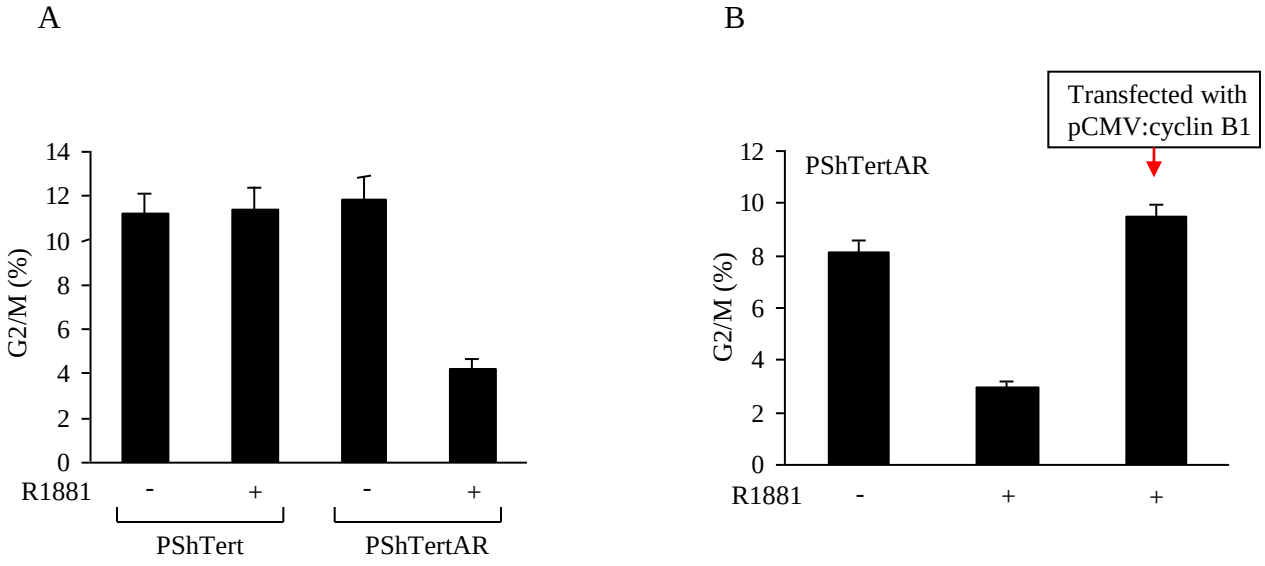

FIG. S4. FACS analysis for PShTert and PShTertAR cells upon androgen stimulation.
(A) FACS analysis for PShTert and PShTertAR cells in the presence and absence of androgen. (B) FACS analysis for PShTertAR cells in the presence of androgen and transfection with pCMV:cyclin B1.

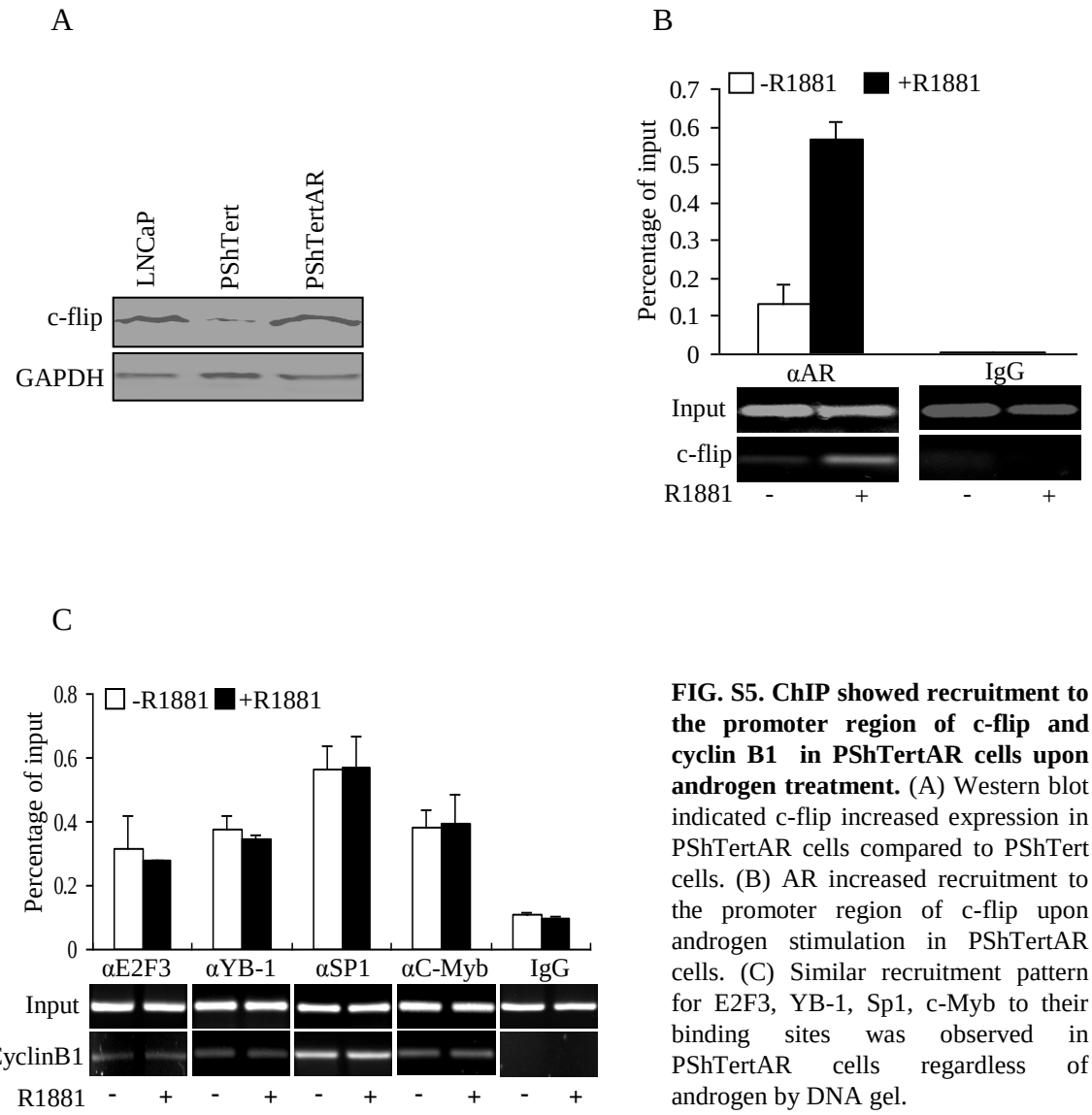

FIG. S5. ChIP showed recruitment to the promoter region of c-flip and cyclin B1 in PShTertAR cells upon androgen treatment. (A) Western blot indicated c-flip increased expression in PShTertAR cells compared to PShTert cells. (B) AR increased recruitment to the promoter region of c-flip upon androgen stimulation in PShTertAR cells. (C) Similar recruitment pattern for E2F3, YB-1, Sp1, c-Myb to their binding sites was observed in PShTertAR cells regardless of androgen by DNA gel.

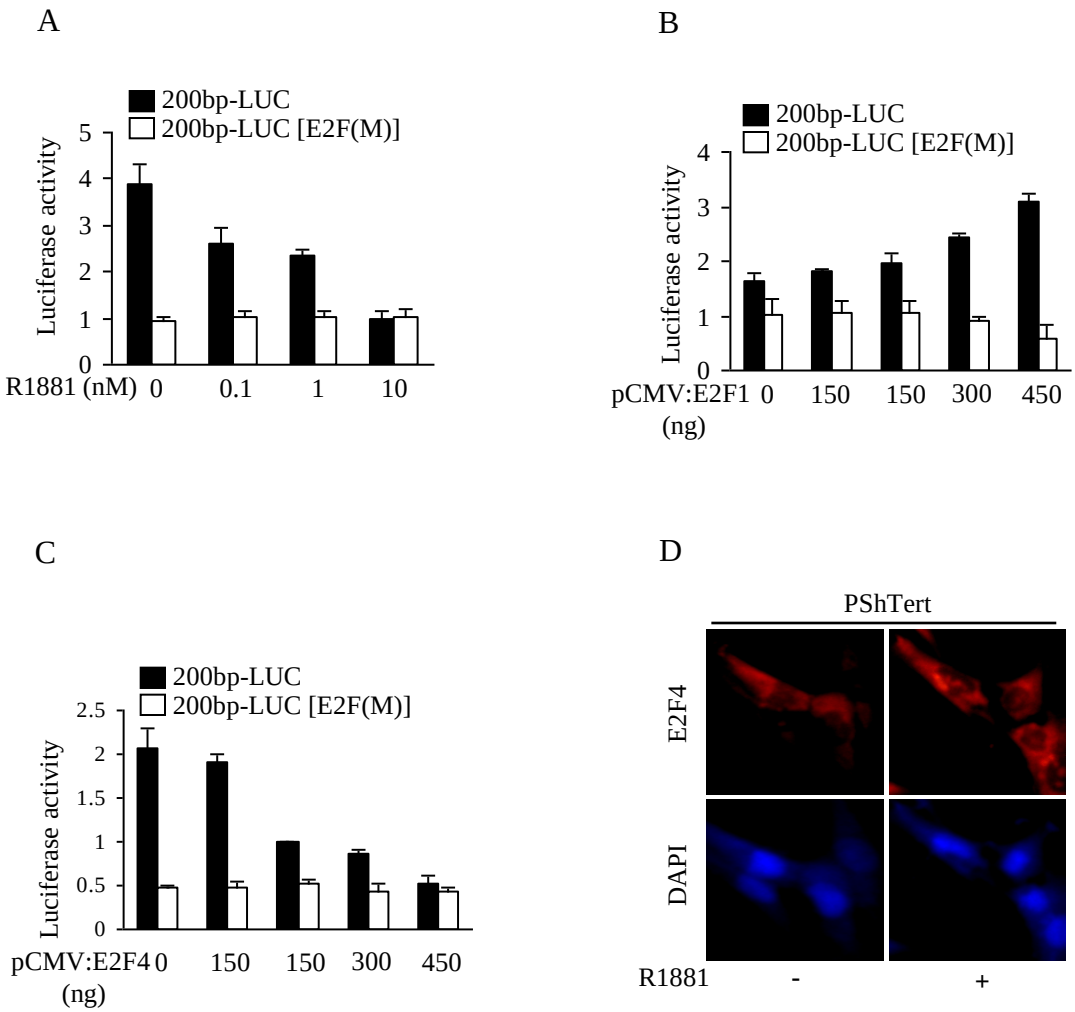

FIG. S6. Luciferase assay for 200 bp-LUC with E2F site mutation.
(A) Luciferase assay showed the 200 bp-LUC with E2F mutation lost transactivation by androgen. (B) The E2F mutated 200 bp-LUC lost activation by increasing E2F1. (C) The E2F mutated 200 bp-LUC also lost repression by increasing E2F4. (D) E2F4 is in cytoplasm regardless of androgen.

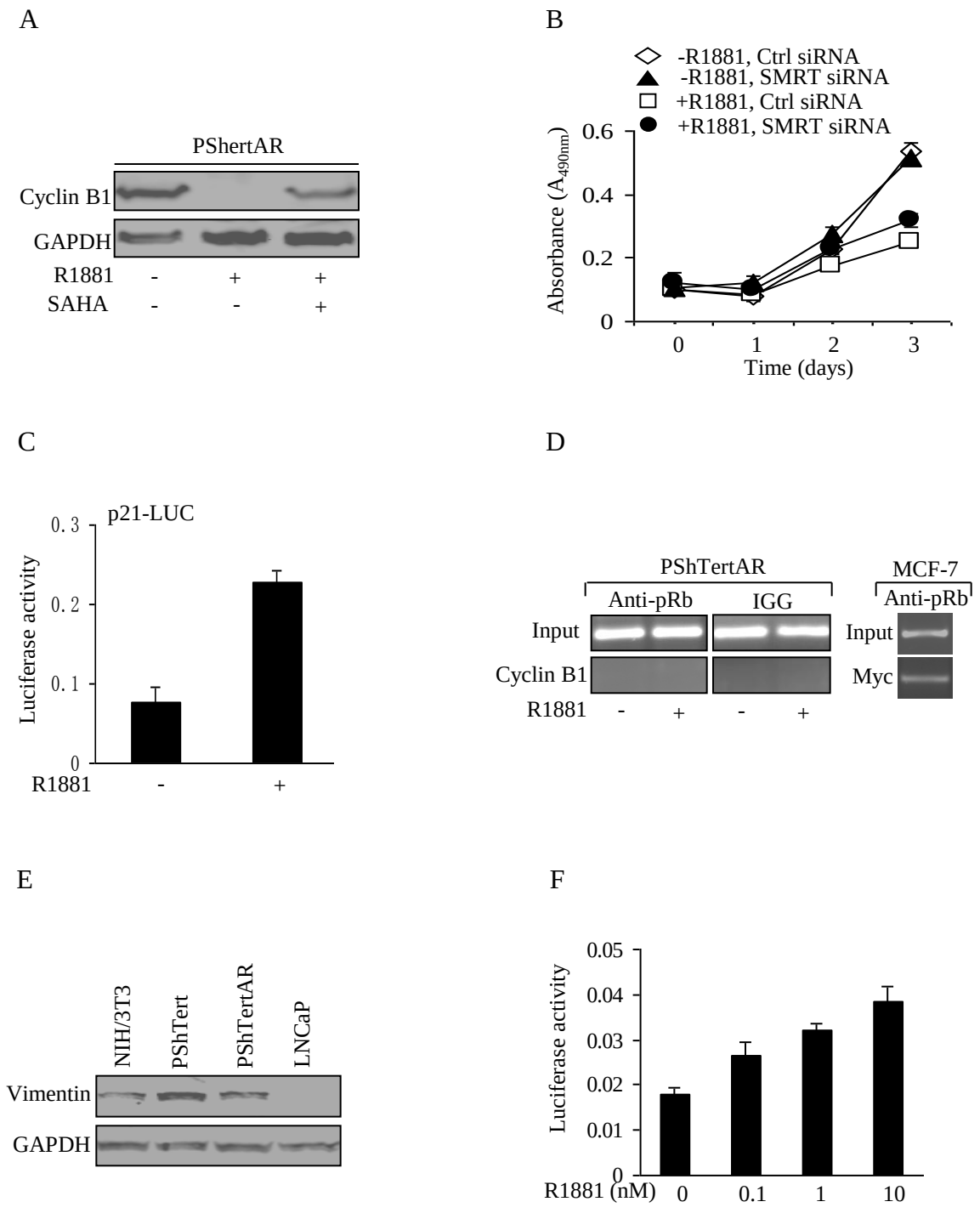

FIG. S7. Histone modification patterns in the cyclin B1 promoter region and Pocket proteins and the cell cycle inhibitors p21 on cyclin B1 regulation

(A) The cyclin B1 expression was recovered by HDAC inhibitor (SAHA) even in the presence of androgen. (B) SMRT knockdown partially reversed the growth repression of PShTertAR cells by androgen. (C) Luciferase assay with luciferase reporter containing p21 promoter showed increased transactivation by androgen. (D) Rb was not recruited to the cyclin B1 promoter in PShTertAR by ChIP. (E) Vimentin expression in PShTert and PShTertAR cells. (F) Luciferase assay showed androgen-mediated activation with 200 bp-LUC in LNCaP cells.