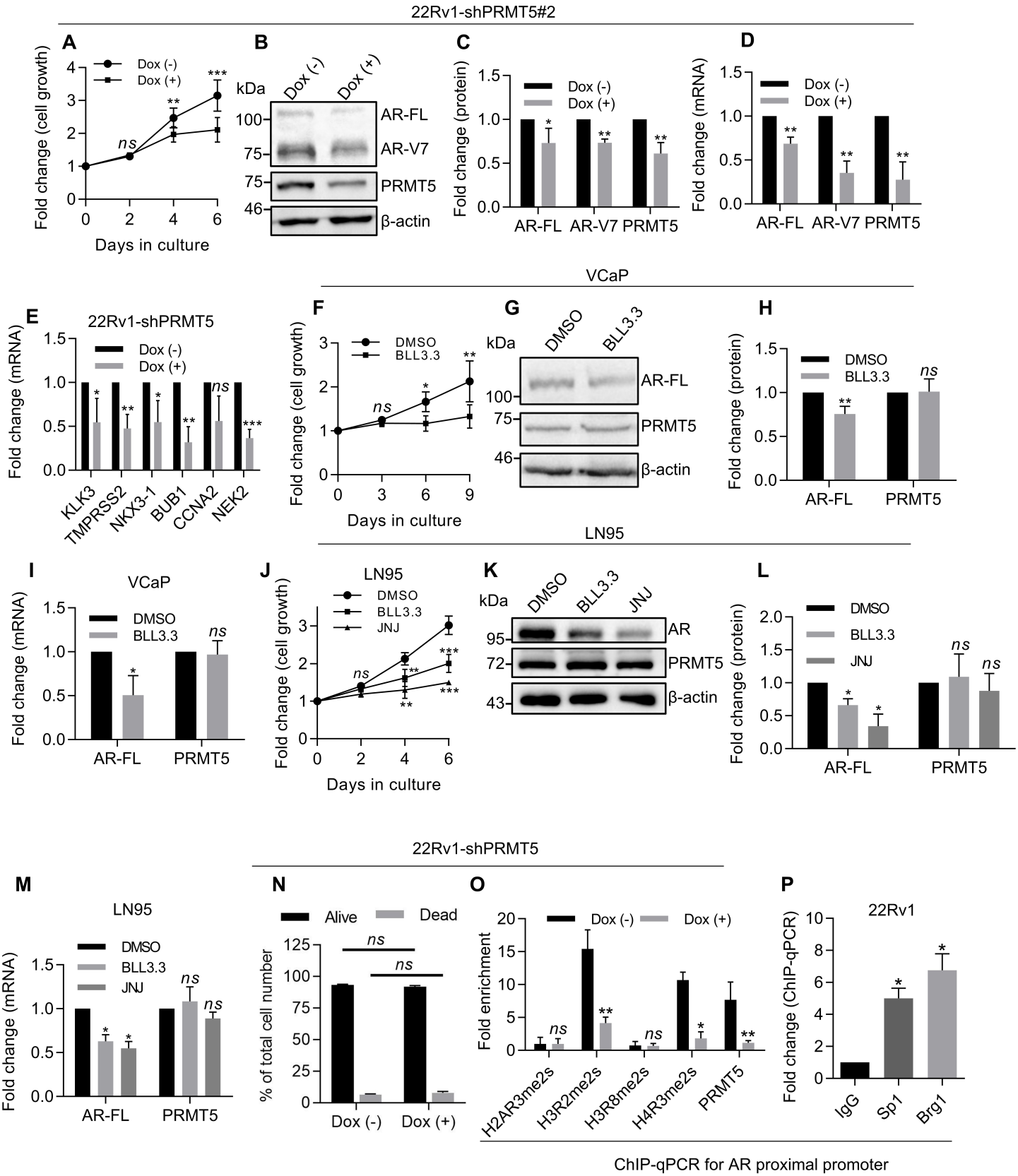
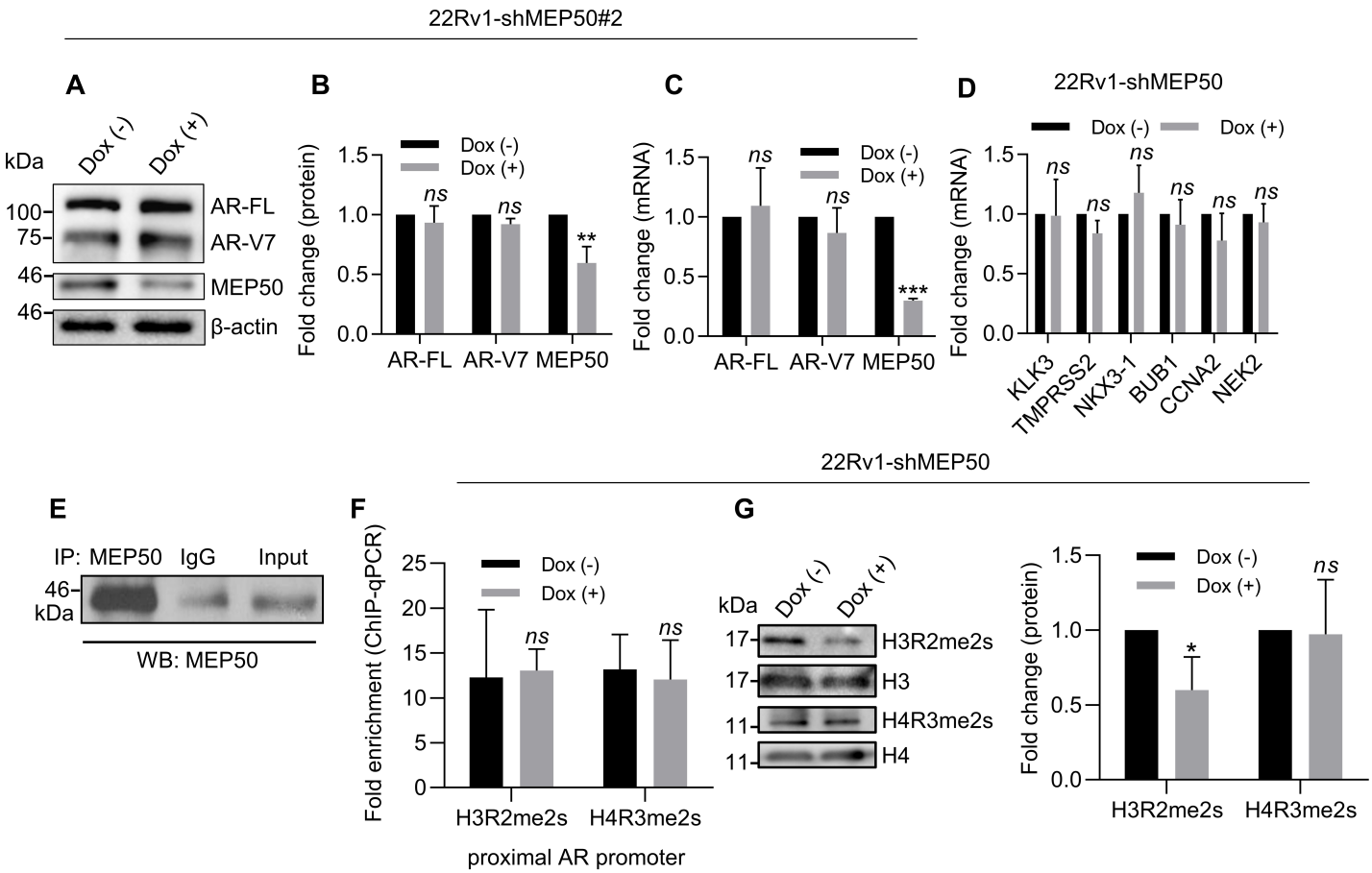
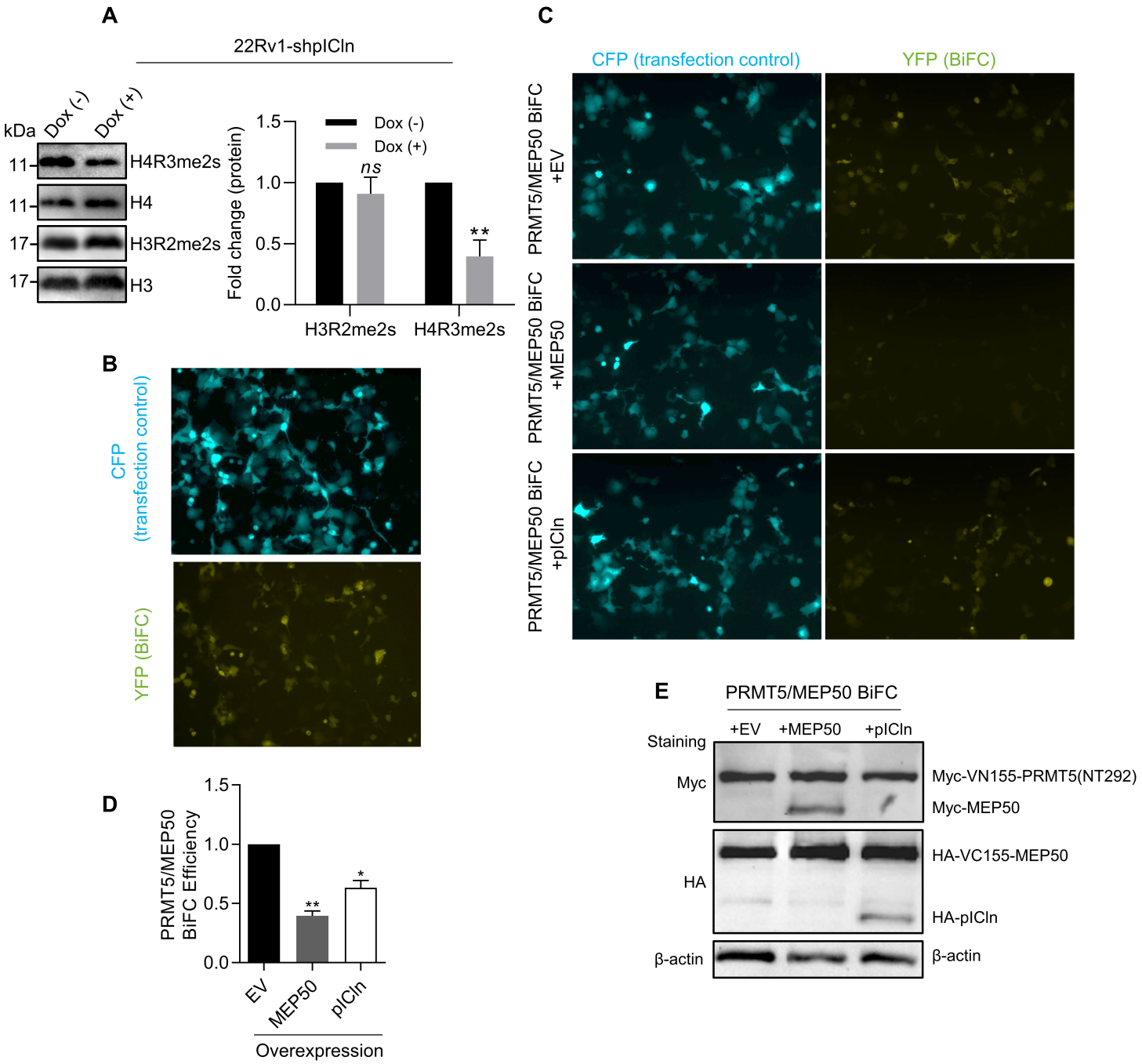

22Rv1-shPRMT5#2
A
Fold change (cell growth)
Dox (-)
Dox (+)
ns
Days in culture
B
kDa
Dox (-)
Dox (+)
100
75
75
46
AR-FL
AR-V7
PRMT5
β-actin
C
Fold change (protein)
Dox (-)
Dox (+)
AR-FL AR-V7 PRMT5
D
Fold change (mRNA)
Dox (-)
Dox (+)
AR-FL AR-V7 PRMT5
VCaP
E
22Rv1-shPRMT5
Fold change (mRNA)
Dox (-)
Dox (+)
KLK3
TMPRSS2
NKX3-1
BUB1
CCNA2
NEK2
F
Fold change (cell growth)
DMSO
BLL3.3
Days in culture
G
kDa
DMSO
BLL3.3
100
75
46
AR-FL
PRMT5
β-actin
H
Fold change (protein)
DMSO
BLL3.3
AR-FL
PRMT5
LN95
I
VCaP
Fold change (mRNA)
DMSO
BLL3.3
AR-FL
PRMT5
J
LN95
Fold change (cell growth)
DMSO
BLL3.3
JNJ
Days in culture
K
kDa
DMSO
BLL3.3
JNJ
95
72
43
AR
PRMT5
β-actin
L
Fold change (protein)
DMSO
BLL3.3
JNJ
AR-FL
PRMT5
22Rv1-shPRMT5
M
LN95
Fold change (mRNA)
DMSO
BLL3.3
JNJ
AR-FL
PRMT5
N
Alive
Dead
% of total cell number
Dox (-)
Dox (+)
O
Fold enrichment
Dox (-)
Dox (+)
H2AR3me2s
H3R2me2s
H3R8me2s
H4R3me2s
PRMT5
P
22Rv1
Fold change (ChIP-qPCR)
IgG
Sp1
Brg1
ChIP-qPCR for AR proximal promoter

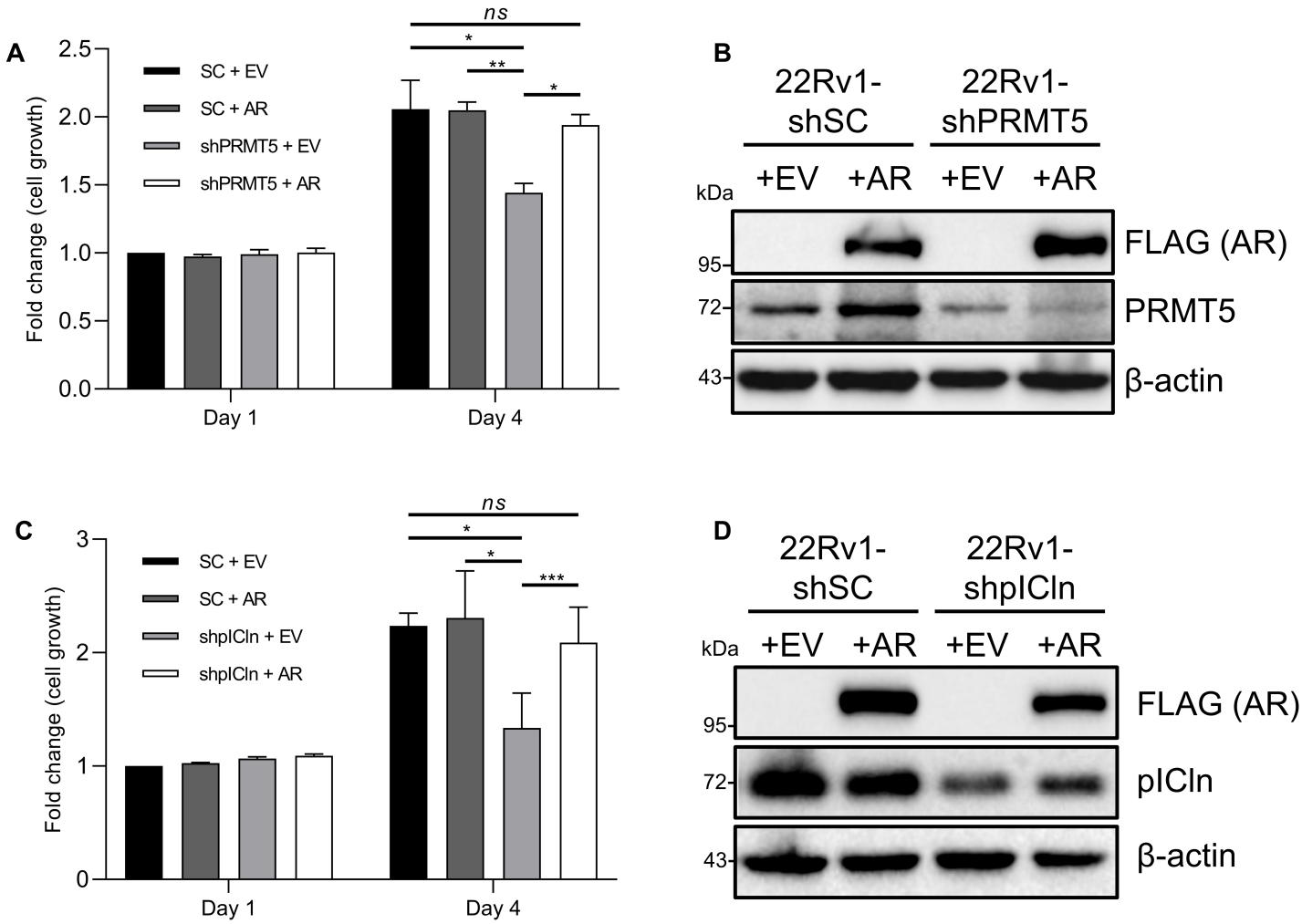
Supplementary Fig. S1. **PRMT5 promotes growth of CRPC cells via epigenetic activation of AR expression**. **A,** Growth curve (MTT assay) of 22Rv1 cells with doxycycline-inducible PRMT5 knockdown (22Rv1-shPRMT5#2) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. **B-C,** Representative western blot analysis (**B**) and quantification (**C**) of protein expression in cell lysates from Day 6 of **A**. **D,** qPCR analysis of gene expression in cells from Day 6 of **A**. **E,** qPCR of AR target genes in 22Rv1-shPRMT5 cell line after 6 days of PRMT5 knockdown. **F,** Growth curve (MTT assay) of VCaP cells incubated with 10 μM PRMT5 inhibitor (BLL3.3) or equal volume of vehicle (DMSO) for 9 days. **G-H,** Representative western blot analysis (**G**) and quantification (**H**) of protein expression in cell lysates from Day 9 of **F**. **I,** qPCR analysis of gene expression in cells from Day 9 of **F**. **J,** Growth curve (MTT assay) of LN95 cells incubated with 10 μM PRMT5 inhibitor (BLL3.3 or JNJ) or equal volume of vehicle (DMSO) for 6 days. **K-L,** Representative western blot analysis (**K**) and quantification (**L**) of protein expression in cell lysates from Day 6 of **J**. **M,** qPCR analysis of gene expression in cells from Day 6 of **J**. **N,** Trypan blue cell viability analysis in 22Rv1-shPRMT5 cells after 6 days of PRMT5 knockdown. **O,** ChIP-qPCR analysis of histone methylation and PRMT5 binding at the proximal AR promoter at Day 6 of PRMT5 knockdown was performed with indicated antibodies. **P,** ChIP-qPCR analysis with antibodies of indicated specificity was performed using 22Rv1 cells lysates. Specific primers for the proximal region of AR promoter was used. For MTT, western blotting, cell cycle, and qPCR analysis, statistical significance of group difference was determined for 'DMSO vs BLL3.3' or 'Dox (-) vs Dox (+)'. For ChIP-qPCR, values were normalized to the corresponding IgG control. For **O**, statistical significance of group difference was determined for 'Dox (-) vs Dox (+)'. For **P**, indicated statistical significance of group difference was determined for 'specific IP vs IgG IP'. For all experiments, results are mean ± SD from 3 independent experiments. For western blotting of AR, the AR N-20 antibody (sc-816, Santa Cruz) was used. Student *t*-test with Welch's correction was performed to determine statistical significance of group difference. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



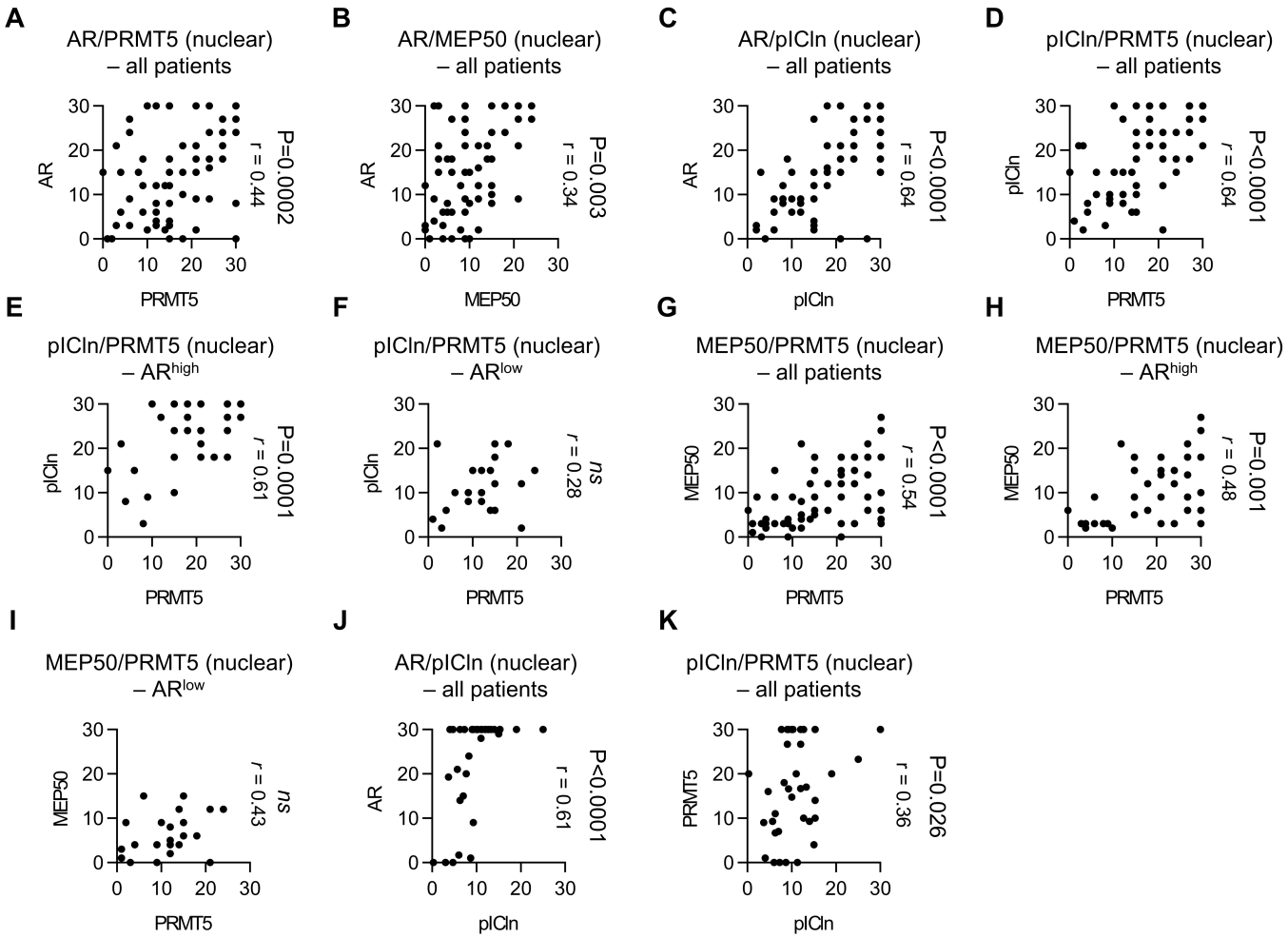
Supplementary Fig. S2. MEP50 is not required for PRMT5-mediated activation of AR transcription in CRPC cells. A-B, Representative western blot analysis (**A**) and quantification (**B**) of protein expression in cell lysates of 22Rv1 cells with doxycycline-inducible MEP50 knockdown (22Rv1-shMEP50#2) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. **C,** qPCR analysis of gene expression in cells from **A**. **D,** qPCR of AR target genes in 22Rv1-shMEP50 cells after 5 days of MEP50 knockdown. **E,** western blot analysis of immunoprecipitates of MEP50 from LNCaP cell lysate. **F,** ChIP-qPCR analysis of histone methylation at the proximal AR promoter at Day 6 of MEP50 knockdown was performed with indicated antibodies. **G**, Representative western blot analysis and quantification of H4R3me2s and H3R2me2s in cell lysates of 22Rv1-shMEP50 incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. For ChIP-qPCR, values were normalized to the corresponding IgG control. For western blotting, ChIP-qPCR, and qPCR analysis, statistical significance of group difference was determined for 'Dox (-) vs Dox (+)'. Results are mean ± SD from 3 independent experiments. For western blotting of AR, the AR N-20 antibody (sc-816, Santa Cruz) was used. Student *t*-test with Welch's correction was performed to determine statistical significance. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.


A
22Rv1-shpICln
kDa
Dox (-)
Dox (+)
11
H4R3me2s
11
H4
17
H3R2me2s
17
H3
Fold change (protein)
1.5
1.0
0.5
0.0
Dox (-)
Dox (+)
ns
**
H3R2me2s
H4R3me2s
B
CFP (transfection control)
YFP (BiFC)
C
CFP (transfection control)
YFP (BiFC)
PRMT5/MEP50 BiFC +EV
PRMT5/MEP50 BiFC +MEP50
PRMT5/MEP50 BiFC +pICln
D
PRMT5/MEP50 BiFC Efficiency
1.5
1.0
0.5
0.0
**
*
EV
MEP50
pICln
Overexpression
E
PRMT5/MEP50 BiFC
+EV
+MEP50
+pICln
Staining
Myc
Myc-VN155-PRMT5(NT292)
Myc-MEP50
HA
HA-VC155-MEP50
HA-pICln
β-actin
β-actin

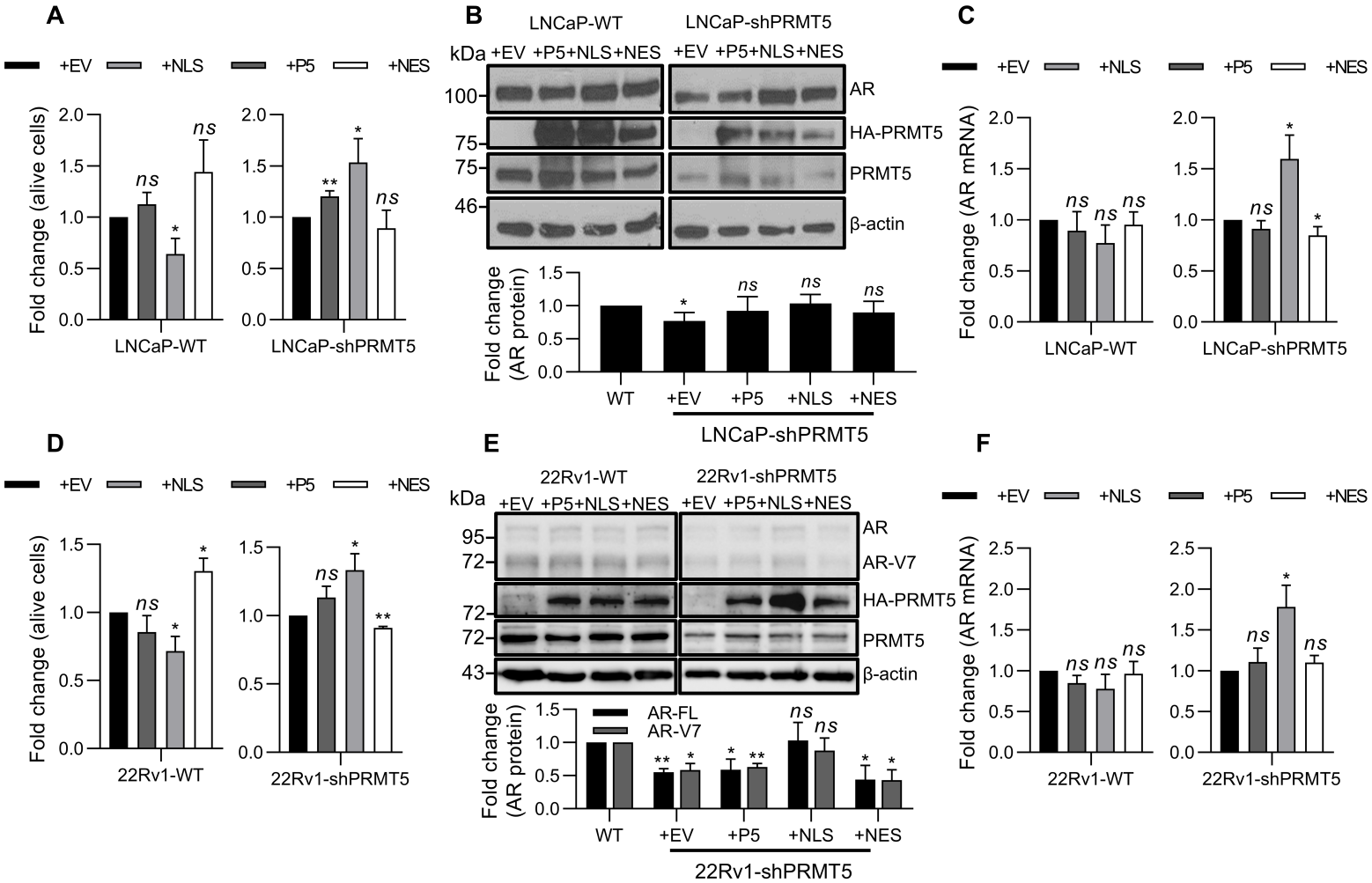
Supplementary Figure S3. pICln is a novel PRMT5 binding partner participating in epigenetic activation of AR transcription. A, Representative western blot analysis and quantification of H4R3me2s and H3R2me2s in cell lysates of 22Rv1 cells with doxycycline-inducible pICln knockdown (22Rv1-shpICln) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. **B,** COS-1 cells were co-transfected with BiFC plasmids to co-express VC155-pICln and VN155-PRMT5(NT292) along with the plasmid expressing Cerulean fluorescent protein (CFP) as a transfection control. Images shown were taken 48 h post-transfection. YFP fluorescence indicates reconstituted Venus as a result of PRMT5/pICln interaction. 20x magnification. **C-D,** COS-1 cells were co-transfected with BiFC plasmids to co-express VC155-MEP50 and VN155-PRMT5(NT292) and the plasmid expressing MEP50 (+MEP50) or pICln (+pICln) as well as the plasmid expressing Cerulean (CFP) as a transfection control. +EV, empty vector, or Myc-MEP50 overexpression plasmid (+MEP50), or HA-pICln overexpression plasmid (+pICln). **C,** Representative fluorescence images acquired 48 h after transfection. 20x magnification. **D,** Quantification of PRMT5/MEP50 BiFC efficiency (ratio of Venus/CFP fluorescence intensities) from **C**. **E,** Western blot analysis of protein expression in lysates of cells from **C**. Membrane probing is indicated on the left, and detected proteins are indicated on the right. Results are mean ± SD from 3 independent experiments. For western blotting of AR, the AR N-20 antibody (sc-816, Santa Cruz) was used. Student *t*-test with Welch's correction was performed to determine statistical significance between groups. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.



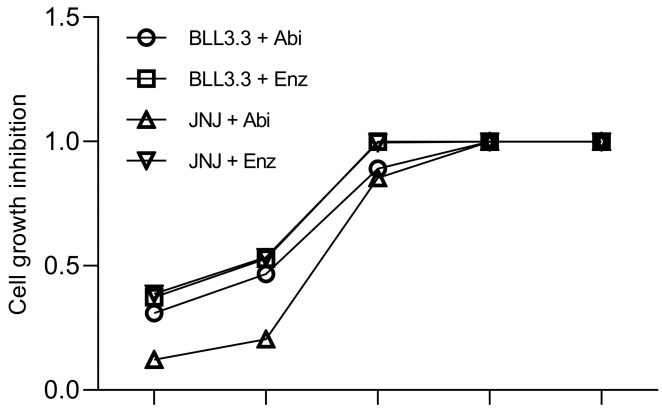
Supplementary Fig. S4. AR re-expression restores cell proliferation after PRMT5 or pICln knockdown in 22Rv1. A, 22Rv1 cells with Dox-inducible expression of scramble control (22Rv1-shSC) or PRMT5 shRNA (22Rv1-shPRMT5) were treated with Dox and transfected with either empty vector (+EV) or plasmid for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



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

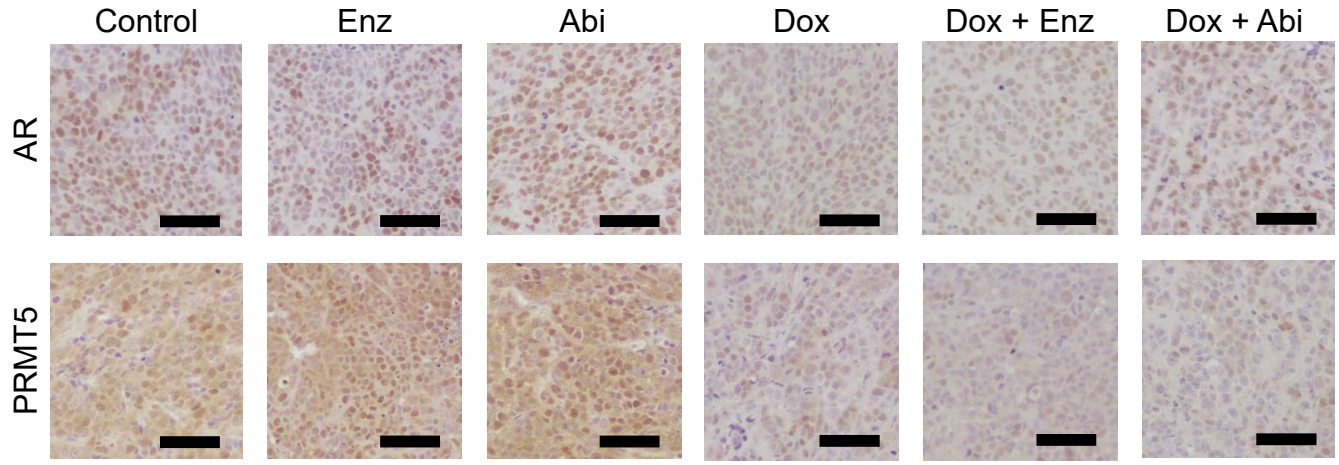


Supplementary Fig. S6. Nuclear-localized PRMT5 promotes cell proliferation and AR expression in LNCaP and 22Rv1. A-C, Wild-type LNCaP (LNCaP-WT) or LNCaP with Dox-inducible knockdown of PRMT5 (LNCaP-shPRMT5) were transfected with empty vector (+EV), or constructs for overexpression of PRMT5 (+P5), nuclear-localized PRMT5 (+NLS), or cytoplasmic PRMT5 (+NES). **A**, Alive cell number was analyzed using Trypan Blue staining after 4 days of transfection. **B**, Representative western blot and quantification of protein expression in cell lysates from **A**. **C**, qPCR analysis of *AR* expression in cells from **A**. **D**, Wild-type 22Rv1 (22Rv1-WT) or 22Rv1 with Dox-inducible knockdown of PRMT5 (22Rv1-shPRMT5) cells were similarly transfected with the plasmids indicated in **A**. Alive cell number was analyzed using Trypan Blue staining after 4 days of transfection. **E**, Representative western blot and quantification of protein expression in cell lysates from **D**. **F**, qPCR analysis of *AR* expression in cells from **D**. For western blot, statistical significance of group difference was determined for comparison with 'WT' group. For Trypan Blue staining and qPCR, statistical significance of group difference was determined for comparison with '+EV' group. For all experiments, results are mean ± SD from at least 3 independent experiments. For western blotting of AR, the AR N-20 antibody (sc-816, Santa Cruz) was used. Brown-Forsythe and Welch ANOVA was performed to determine statistical significance of group difference. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.



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

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



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