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Supplemental Information

lncRNA HOXD-AS1 Regulates Proliferation and Chemo-Resistance of Castration-Resistant Prostate Cancer via Recruiting WDR5

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Supplemental Information

Supplemental Figures

Figure S1

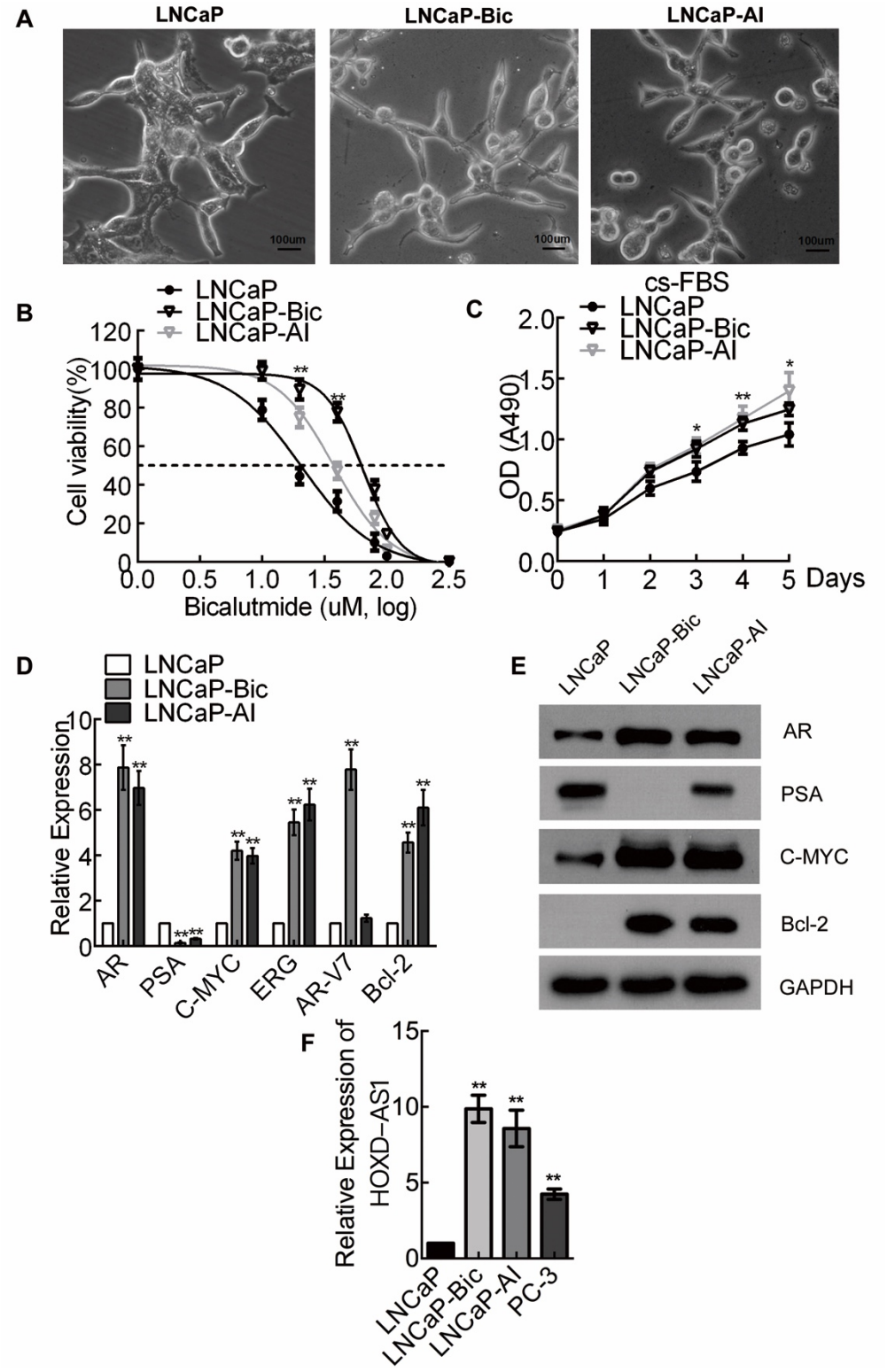


Figure S2

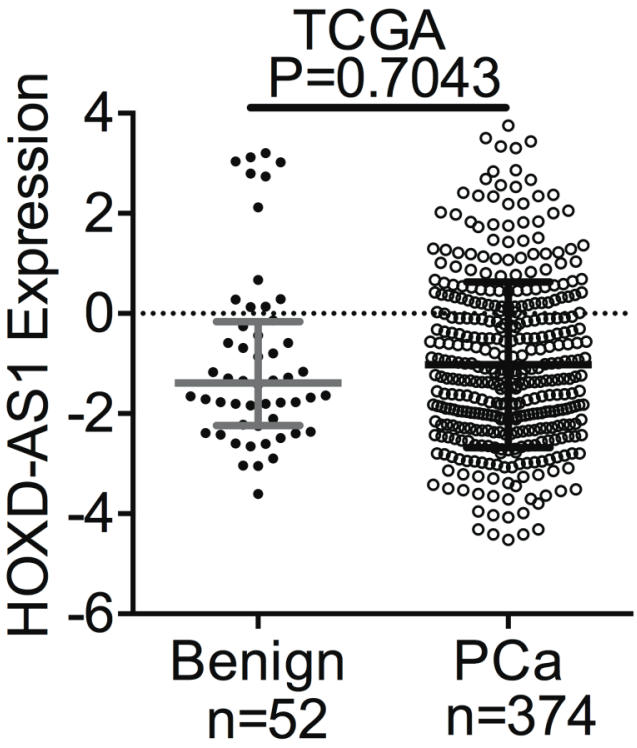


Figure S3

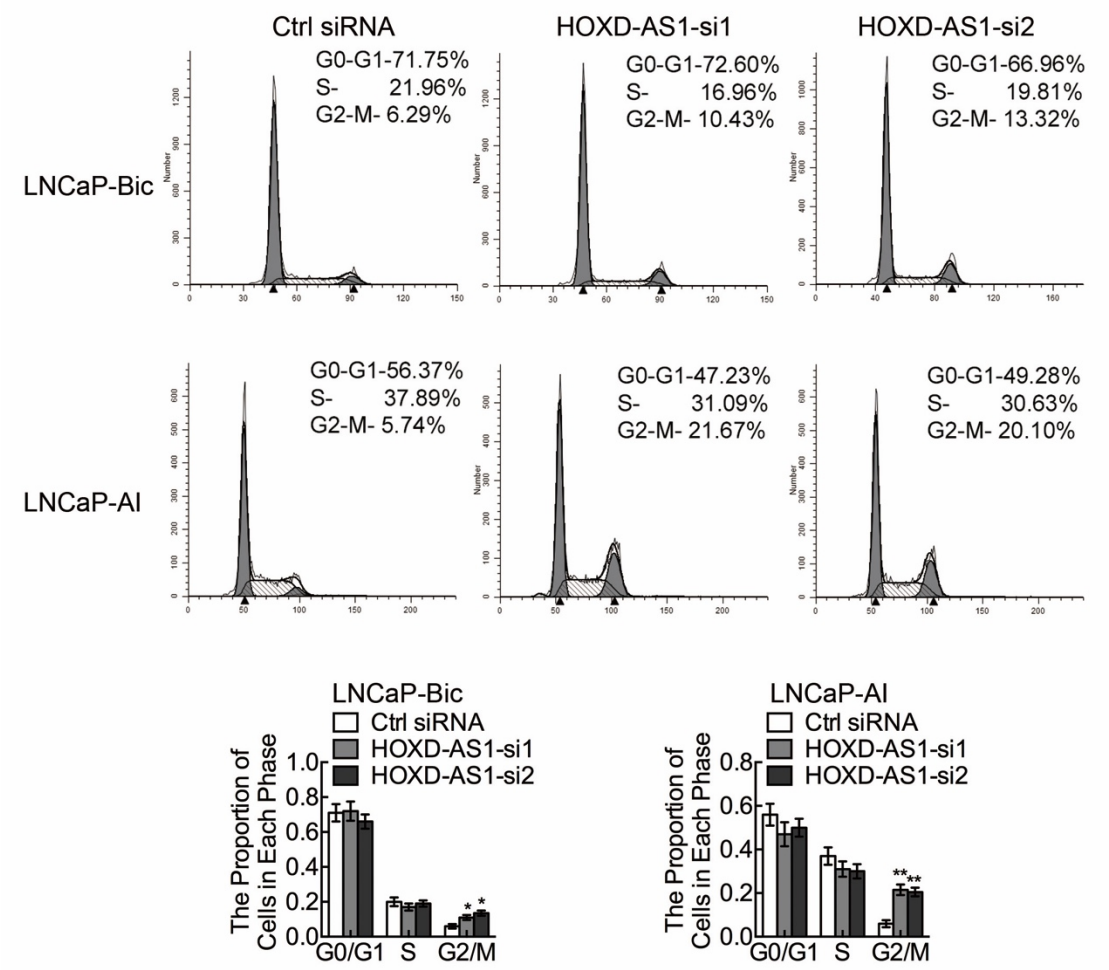

Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
LNCaP-Bic
G0-G1-71.75%
S- 21.96%
G2-M- 6.29%
G0-G1-72.60%
S- 16.96%
G2-M- 10.43%
G0-G1-66.96%
S- 19.81%
G2-M- 13.32%
LNCaP-AI
G0-G1-56.37%
S- 37.89%
G2-M- 5.74%
G0-G1-47.23%
S- 31.09%
G2-M- 21.67%
G0-G1-49.28%
S- 30.63%
G2-M- 20.10%
Number
LNCaP-Bic
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
The Proportion of Cells in Each Phase
G0/G1
S
G2/M
LNCaP-AI
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
The Proportion of Cells in Each Phase
G0/G1
S
G2/M

Figure S4

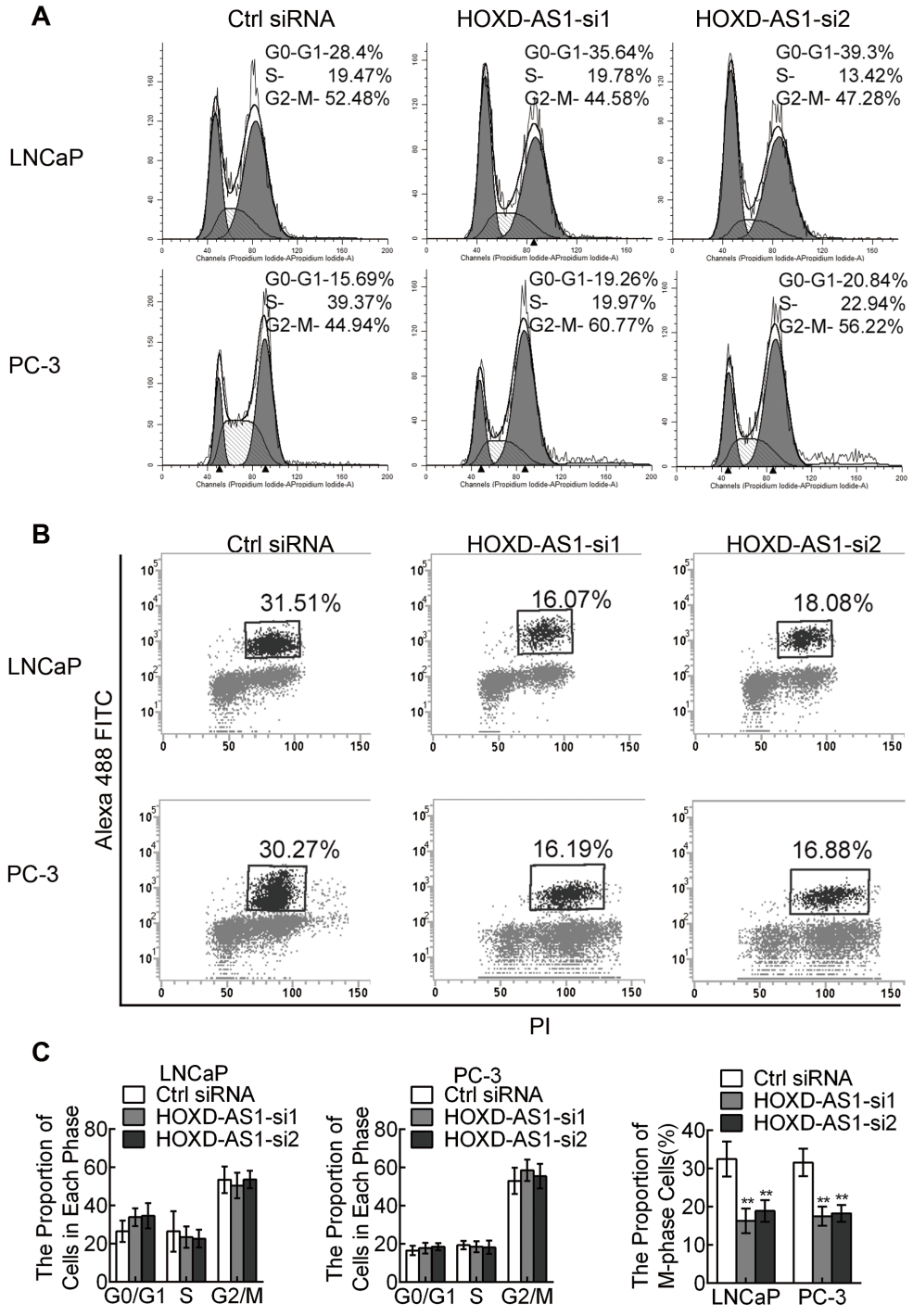

A
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
LNCaP
PC-3
G0-G1-28.4%
S- 19.47%
G2-M- 52.48%
G0-G1-35.64%
S- 19.78%
G2-M- 44.58%
G0-G1-39.3%
S- 13.42%
G2-M- 47.28%
G0-G1-15.69%
S- 39.37%
G2-M- 44.94%
G0-G1-19.26%
S- 19.97%
G2-M- 60.77%
G0-G1-20.84%
S- 22.94%
G2-M- 56.22%
Channels (Propidium Iodide-APropidium Iodide-A)
B
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
LNCaP
PC-3
31.51%
16.07%
18.08%
30.27%
16.19%
16.88%
Alexa 488 FITC
PI
C
LNCaP
PC-3
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
The Proportion of Cells in Each Phase
G0/G1 S G2/M
The Proportion of M-phase Cells(%)
LNCaP PC-3
**

Figure S5

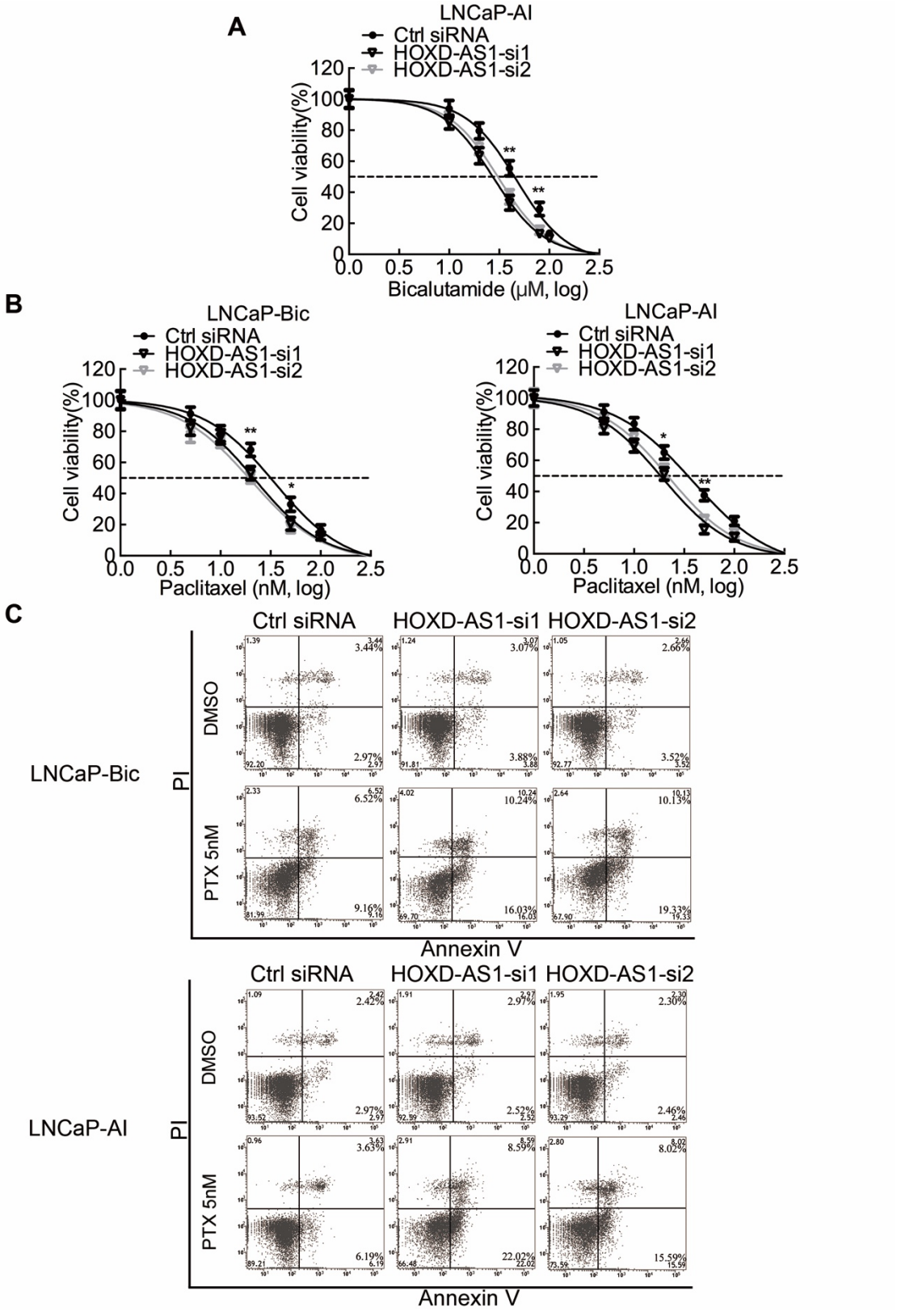

A
LNCaP-AI
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
Cell viability(%)
Bicalutamide (μM, log)
B
LNCaP-Bic
LNCaP-AI
Paclitaxel (nM, log)
C
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
LNCaP-Bic
LNCaP-AI
DMSO
PTX 5nM
PI
Annexin V

Figure S6

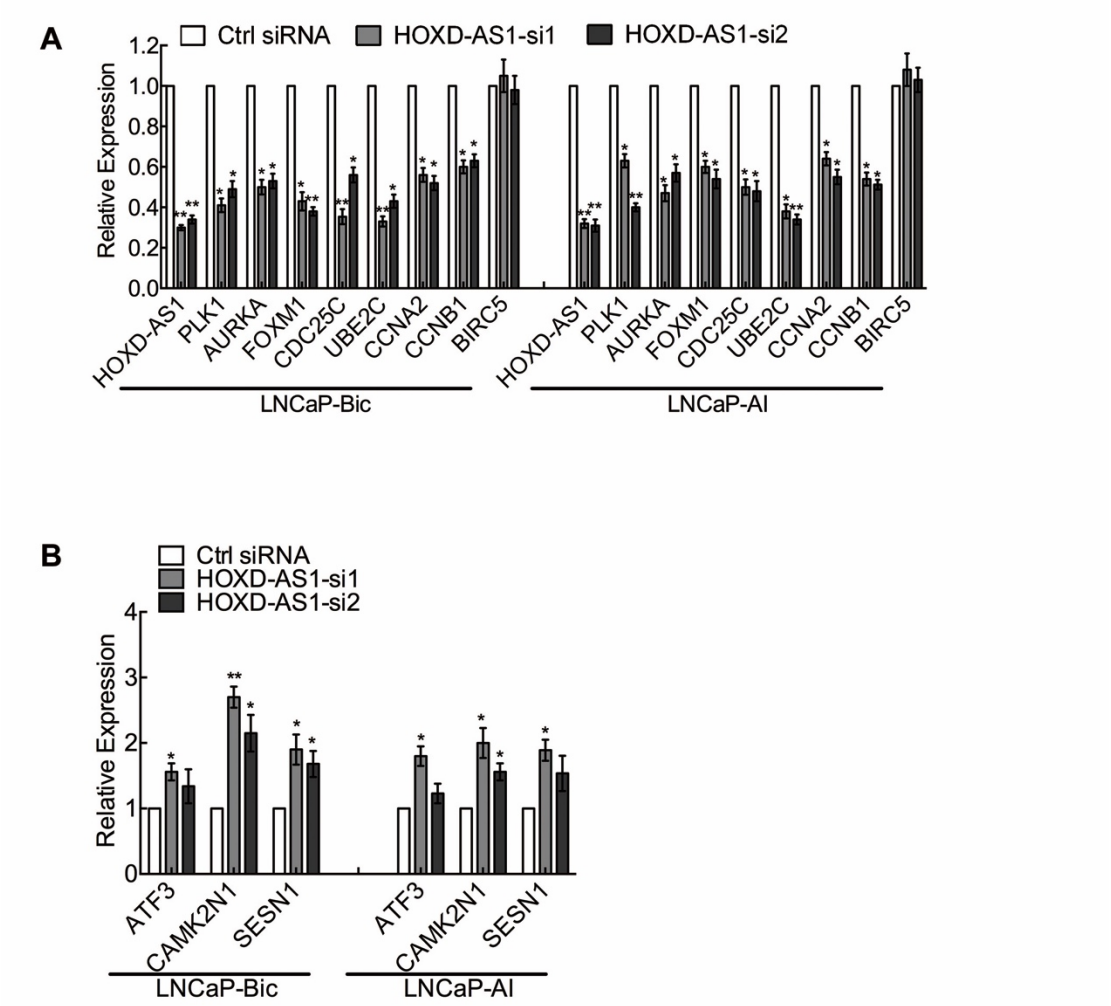

A
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
Relative Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
HOXD-AS1
PLK1
AURKA
FOXM1
CDC25C
UBE2C
CCNA2
CCNB1
BIRC5
LNCaP-Bic
LNCaP-AI
B
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
Relative Expression
4
3
2
1
0
ATF3
CAMK2N1
SESN1
LNCaP-Bic
LNCaP-AI

Figure S7

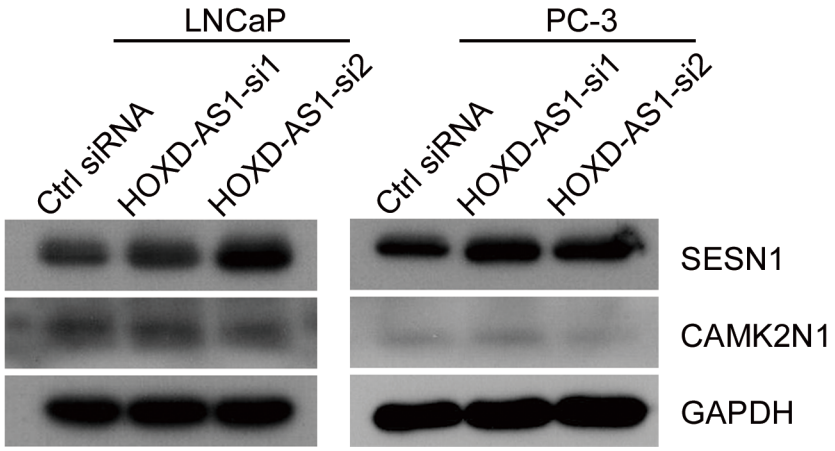
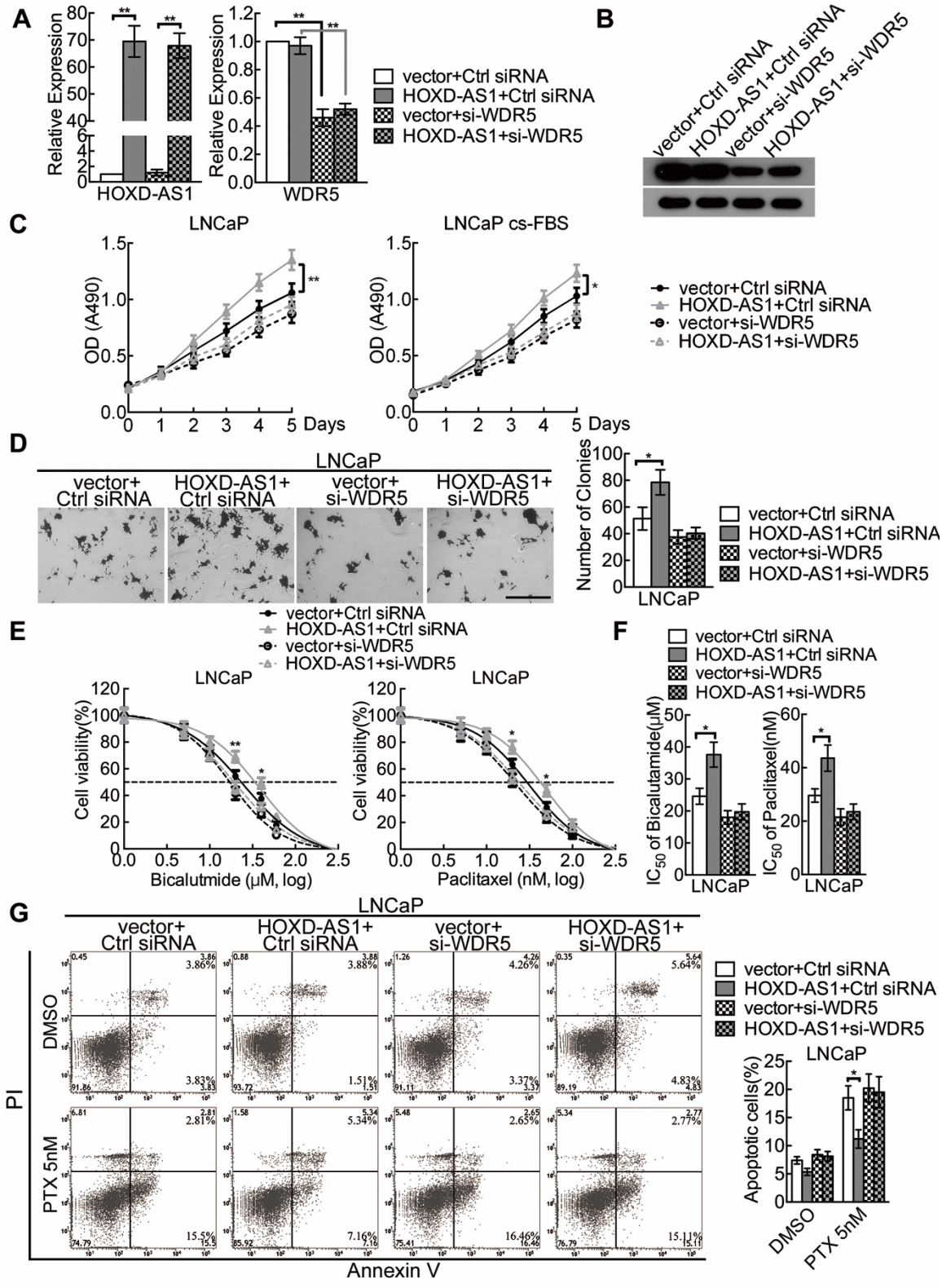

LNCaP
PC-3
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
SESN1
CAMK2N1
GAPDH

Figure S8


A
Relative Expression
HOXD-AS1
WDR5
vector+Ctrl siRNA
HOXD-AS1+Ctrl siRNA
vector+si-WDR5
HOXD-AS1+si-WDR5
B
vector+Ctrl siRNA
HOXD-AS1+Ctrl siRNA
vector+si-WDR5
HOXD-AS1+si-WDR5
C
LNCaP
LNCaP cs-FBS
OD (A490)
Days
D
LNCaP
vector+ Ctrl siRNA
HOXD-AS1+ Ctrl siRNA
vector+ si-WDR5
HOXD-AS1+ si-WDR5
Number of Clonies
E
Cell viability(%)
Bicalutmide (μM, log)
Paclitaxel (nM, log)
F
IC50 of Bicalutamide(μM)
IC50 of Paclitaxel(nM)
G
DMSO
PTX 5nM
PI
Annexin V
Apoptotic cells(%)

Supplemental Figure Legends

Figure S1. Construction of castration resistant LNCaP sublines. (A) The morphology of LNCaP, LNCaP-bic and LNCaP-AI cells. The scale bar is equal to 100μm. (B) The viability of LNCaP, LNCaP-bic and LNCaP-AI cells treated with different concentration of bicalutamide measured by MTT. The results are presented as the means ± SD of values obtained in three independent experiments. (C) The proliferation of LNCaP, LNCaP-bic and LNCaP-AI in androgen deprived cultural medium measured by MTT. The results are presented as the means ± SD of values obtained in three independent experiments. (D and E) The castration resistance related genes were detected by real time qPCR and Western blotting. The results are presented as the means ± SD of values obtained in three independent experiments. (F) The expression of HOXD-AS1 in LNCaP, LNCaP-Bic, LNCaP-AI and PC-3 cells was detected by real time qPCR. The results are presented as the means ± SD of values obtained in three independent experiments. *$p < 0.05$, **$p < 0.01$.

Figure S2. The expression of HOXD-AS1 between benign prostate and prostate cancer from TCGA. The whiskers indicate mean ± SD in the plot.

Figure S3. Knockdown of HOXD-AS1 inhibits castration resistant prostate cancer cell proliferation by inducing G2/M cell cycle arrest. LNCaP-Bic and LNCaP-AI cells were transfected with HOXD-AS1 siRNAs or control siRNA for 48h and the cell cycles were analyzed by flow cytometry. All histograms show the percentage (%) of cell populations in each phase from three independent experiments. *$p < 0.05$, **$p < 0.01$.

Figure S4. The influence of knockdown HOXD-AS1 on prostate cancer cells synchronized at M-phase. (A) LNCaP and PC-3 cells were transfected with HOXD-AS1 siRNAs or control siRNA for 48h, then treated with 50ng/ml nocodazole for 16h and the cell cycles were measured by flow cytometry. (B) LNCaP and PC-3 cells were transfected with HOXD-AS1 siRNAs or control siRNA for 48h, then treated with 50ng/ml nocodazole for 16h, the proportion of M-phase cells were measured by flow cytometry. (C) The histogram showed the percentage (%) of cells in different phase from three independent experiments. *$p < 0.05$, **$p < 0.01$.

Figure S5. Downregulation of HOXD-AS1 represses castration-resistance and chemo-resistance of prostate cancer cells. (A) The viability of LNCaP-AI cells transfected with HOXD-AS1 siRNAs or control siRNA, treated with bicalutamide for 120h and analyzed by MTT assay. The results are presented as the means ± SD of values obtained in three independent experiments. (B) The viability of LNCaP-Bic and LNCaP-AI cells transfected with HOXD-AS1 siRNAs or control siRNA, treated with paclitaxel for 48h and analyzed by MTT assay. The results are presented as the means ± SD of values obtained in three independent experiments. (C) The LNCaP-Bic and LNCaP-AI cells transfected with control or HOXD-AS1 siRNAs were treated with 5 nM paclitaxel for 48h. The percentage of apoptotic cells was analyzed by flow cytometer. *$p < 0.05$, **$p < 0.01$.

Figure S6. (A and B) The differentially expressed genes in the microarray were verified in LNCaP-Bic and LNCaP-AI cells by real time qPCR. The results are presented as the means ± SD of values obtained in three independent experiments.

Figure S7. The expression of SENS1 and CAMK2N1 after HOXD-AS1 knockdown in LNCaP and PC-3 cells was detected by western blotting. GAPDH was used as internal control.

Figure S8. The in vitro effect of combined overexpression of HOXD-AS1 and knockdown of WDR5 in LNCaP cells. (A) The efficiency of combined overexpression of HOXD-AS1 and knockdown of WDR5 was detected by real time qPCR. The results are presented as the means ± SD of values obtained in three independent experiments. (B) The efficiency of combined overexpression of HOXD-AS1 and knockdown of WDR5 was detected by western blotting. (C) The influence of enforced expression of HOXD-AS1 combined with knockdown of WDR5 on viability of LNCaP cells in normal medium and androgen ablated medium, as measured by MTT. The results are presented as the means ± SD of values obtained in three independent experiments. (D) The influence of enforced expression of HOXD-AS1 combined with knockdown of WDR5 on colony formation ability of LNCaP cells. The scale bar is equal to 600μm. The histogram showed the the mean± SD of clonies from three independent experiments. (E) The effect of overexpression of HOXD-AS1 combined with knockdown of WDR5 on sensitivity of bicalutamide and paclitaxel in LNCaP cells. The results are presented as the means ± SD of values obtained in three independent experiments. (F) Calculation of IC_{50} of bicalutamide and paclitaxel of LNCaP cells co-transfected with HOXD-AS1 vector and WDR5 siRNA. (G) The LNCaP cells co-transfected with HOXD-AS1 expression vector and WDR5 siRNA were treated with 5 nM paclitaxel for 48h. The percentage of apoptotic cells was analyzed by flow cytometer. The histogram showed the percentage (%) of late and early apoptotic cells from three independent experiments.

Tables

Table S1. Characteristics of patients from the TCGA Prostate adenocarcinoma (PRAD) cohort.

	PCa profiles (%)	Tumor tissues for survival analysis (%)
Patients(N)	374	309
Age(Year)		
Median(range)	61.23(42-78)	61.27(42-78)
Mean±SD	60.98±6.72	60.92±6.78
Gleason Score	374	309
6	26(7)	22(7)
7	230(61.5)	187(60.4)
8	43(11.5)	37(12)
9	72(19.2)	61(20)
10	3(0.8)	2(0.6)
T stage	368	309
T2	161(43.7)	136(44)
T3	202(54.9)	169(54.7)
T4	5(1.4)	4(1.3)
Lymph nodes status N(%)	316	309
Negative	270(85.4)	270(87.4)
Positive	46(14.6)	39(12.6)

Patients with not available clinical data were excluded for further analysis.

Table S2. The primers used in real time qPCR are listed as follows.

Primer Name	Sequence 5'-3'
HOXD-AS1 Forward	ACCTGCCTCTACTACTGCAAA
HOXD-AS1 Reverse	GCAAAGACAATATAAGGGCCC
NEAT1 Forward	TTCTAAATTGAGCCTCCGGTC
NEAT1 Reverse	CTGCAAGCTCCATCTACAAGG
GAS5 Forward	GCTTAAGTGCCTGCATTCCG
GAS5 Reverse	TTGCCATTAACCGATGTCGAG
MEG3 Forward	TTCACCTACCTCACAGGGCTG
MEG3 Reverse	TTATTGAGAGCACAGTGGGGT
CDKN1A Forward	AGCGATGGAACTTCGACTTTG
CDKN1A Reverse	GGGAAGGTAGAGCTTGGGCAG
ELF3 Forward	ATCCCACTGATGGCAAGCTCT
ELF3 Reverse	CGAGACAGTCCCAGTACTCTT
SESN1 Forward	CTCTTGCCTCATTCACATTCG

SESN1 Reverse	GTAATGTCACAGATGCAGTAG
CAMK2N1 Forward	TGCAGGACACCAACAACTTCT
CAMK2N1 Reverse	TCAATAACAACCCGCTTGCTC
ATF3 Forward	CATCACAAAAGCCGAGGTAGC
ATF3 Reverse	AGGCACTCCGTCTTCTCCTTC
PLK1 Forward	CCGCCCAACCATTAACGAGCT
PLK1 Reverse	ACCTTGGTGGAATGGTCAGGC
PTTG1 Forward	GGAGTGCCTCTCATGATCCTT
PTTG1 Reverse	AGGAGACTGCAACAGATTGGA
AURKA Forward	CAAATGCCCTGTCTTACTGTC
AURKA Reverse	ATGGAGCATGTACTGACCACC
CDC25C Forward	TTTCTGAAGAAGCCCATCGTC
CDC25C Reverse	ACCTGTCCTCTTCACGCAGAC
NEK2 Forward	AGGATTACCATCGACCTTCTG
NEK2 Reverse	GCTCTCCTAATTGTCGCCCTC
CCNA2 Forward	GAAGAAACAGCCAGACATCAC
CCNA2 Reverse	GTAGTTCACAGCCAAATGCAG
UBE2C Forward	ACTCAAGATTCTAGCAAGCCC
UBE2C Reverse	GCATGTGTGTTCAAGGGACTA
FOXM1 Forward	GAGGACCTTTTAAGACACCCA
FOXM1 Reverse	GGCTGAAATCCAGTCCCCCTA
BIRC5 Forward	CTTTCTCAAGGACCACCGCAT
BIRC5 Reverse	CAAGTCTGGCTCGTTCTCAGT
CCNB1 Forward	TTGGAGAGGTTGATGTCGAGC
CCNB1 Reverse	AGAAGGAGGAAAGTGCACCAT
U6 Forward	CTCGCTTCGGCAGCACATATAC
U6 Reverse	AACGCTTCACGAATTTGCGTGTC
MALAT1 Forward	GACGGAGGTTGAGATGAAGC
MALAT1 Reverse	ATTCGGGGCTCTGTAGTCCT
HOTTIP Forward	ATCAAGGTTGGCCGCTGACTC
HOTTIP Reverse	TGGTCTGTTGGTTAGCACCTG
AR Forward	TGAGCAGAGTGCCCTATCCCA
AR Reverse	CTGGGGTGGAAAGTAATAGTC
AR-V7 Forward	CCATCTTGTCGTCTTCGGAAATGTTA
AR-V7 Reverse	TTTGAATGAGGCAAGTCAGCCTTTCT
PSA Forward	GTATCACGTCATGGGGCAGTG
PSA Reverse	GTTGGCCACGATGGTGTCCTT
C-MYC Forward	AATAGAGCTGCTTCGCCTAGA
C-MYC Reverse	GAGGTGGTTCATACTGAGCAAG
GPADH Forward	CAAGGCTGAGAACGGGAAG

GPADH Reverse	TGAAGACGCCAGTGGACTC

Table S3. The primers used in ChIP-real time qPCR are listed as follows.

Primer Name	Sequence 5'-3'
PLK1-P Forward	TGGGTCCGGGTTTAAAGGCTG
PLK1-P Reverse	GCTCCTCCCCGAATTCAAACG
AURKA-P Forward	CAAGGCGTCGGGTTTGTTGTC
AURKA-P Reverse	AAGTCTTCCAAGAGCTCAGCC
CDC25C-P Forward	AAAGAGGGAAGGAGGGAGGGA
CDC25C-P Reverse	CTACTCTCCTCAGGGACTCGT
FOXM1-P Forward	AGCCCGGAATGCCGAGACAA
FOXM1-P Reverse	GGCACCGGAGCTTTCAGTTTG
UBE2C-P Forward	GGCAGCATCATCTACCAATCG
UBE2C-P Reverse	TGATCCAGCCAATGAGACGCT
CCNA2-P Forward	CCCCAGCCAGTTTGTTTCTCC
CCNA2-P Reverse	CCGCGACTATTGAAATGGACC
CCNB1-P Forward	CGCCCTGGAAACGCATTCTCT
CCNB1-P Reverse	AGAAGAGCCAGCCTAGCCTCA
BIRC5-P Forward	TGTTGGGATTACAGGCGTGAG
BIRC5-P Reverse	TGTGCCGGGAGTTGTAGTCCT
Negative control F	GTAATCAGGAAACTGCATAC
Negative control R	CTCAAGACTCAATAGTGATC

Table S4. The probes used for ChIRP are listed as follows.

Probe Name	Sequence 5'-3'
HOXD-AS1-probe1	CTGCCCTACAAATACCATAT
HOXD-AS1- probe2	CCTTCTTTCAGAAACTTGGC
HOXD-AS1- probe3	GCTGCTAACATTGCTGAACA
HOXD-AS1- probe4	AGAATGGCCAGCTGCAAAAC
HOXD-AS1- probe5	CCGAGTCTCAGAAGCAGAAA
HOXD-AS1- probe6	CCGAGACTTCTAATAGCTCG
HOXD-AS1- probe7	TGCATCCATAGGCAGAATTT
HOXD-AS1- probe8	CGCATCTCTATTTGGTTTGA
HOXD-AS1- probe9	GCCTTCTTTCTAGACACAAT
HOXD-AS1- probe10	ATTGGTTCTCGGGATACTTG
TERC-probe-1	AGGGTTAGACAAAAAATGGCCA
TERC-probe-2	AATGAACGGTGGAAGGCGGCAG
TERC-probe-3	GTTCGGGGGCTGGGCAGGCGAC
TERC-probe-4	GAGAGACCCGCGGCTGACAGAG

TERC-probe-5	ACGTCCCACAGCTCAGGGAATC

Table S5. The primers used for ChIRP-real time qPCR are listed as follows.

Primer Name	Sequence 5'-3'
PLK1-chirp-F	TGGGTCCGGGTTTAAAGGCTG
PLK1-chirp-R	GCTCCTCCCCGAATTCAAACG
AURKA-chirp-F	ACGGCTGAGCTCTTGGAAGAC
AURKA-chirp-R	CTCGTCCGCCACTGAGATATC
CDC25C-chirp-F	AAAGAGGGAAGGAGGGAGGGA
CDC25C-chirp-R	CTACTCTCCTCAGGGACTCGT
FOXM1-chirp-F	TTCGGAGCTACGGCCTAACG
FOXM1-chirp-R	CTGGGACTCCATTGCTGCAT
UBE2C-chirp-F	GGCAGCATCATCTACCAATCG
UBE2C-chirp-R	TGATCCAGCCAATGAGACGCT
CCNA2-chirp-F	CCCCAGCCAGTTTGTTTCTCC
CCNA2-chirp-R	CCGCGACTATTGAAATGGACC
CCNB1-chirp-F	CGCCCTGGAAACGCATTCTCT
CCNB1-chirp-R	AGAAGAGCCAGCCTAGCCTCA
BIRC5-chirp-F	TGTTGGGATTACAGGCGTGAG
BIRC5-chirp-R	TGTGCCGGGAGTTGTAGTCCT
GAPDH-RNA-F	CAAGGCTGAGAACGGGAAG
GAPDH-RNA-R	TGAAGACGCCAGTGGACTC
GAPDH-DNA-F	GTTTCCAGGAGTGCCTTTGTG
GAPDH-DNA-R	ATTAGGGCAGACAATCCCGGC
TERC-RNA-F	CGCTGTTTTTCTCGCTGACT
TERC-RNA-R	GCTCTAGAATGAACGGTGGAA
WNT-1-F	AGGGCTGGAATTTCAAAGGT
WNT-1-R	TTCTCCTCAGGATGTACCCG

Supplemental Material and Method

HOXD-AS1 cloning and vector transfection

The HOXD-AS1 was amplified by PCR using primer F- GTTTGTGCCGCGCGCCCGCCAGACC and R-TGACACTTTGAAAAAAATATTTTAT. And then cloned into pCDNA3.1(+) vector. Co-transfection of vector and WDR5 siRNA was performed by using Lipofectamine 3000 (Life Technologies, Waltham, MA, USA) according to the manufacture's protocol.

The full-length blots of manuscript are presented.

Figure 3G

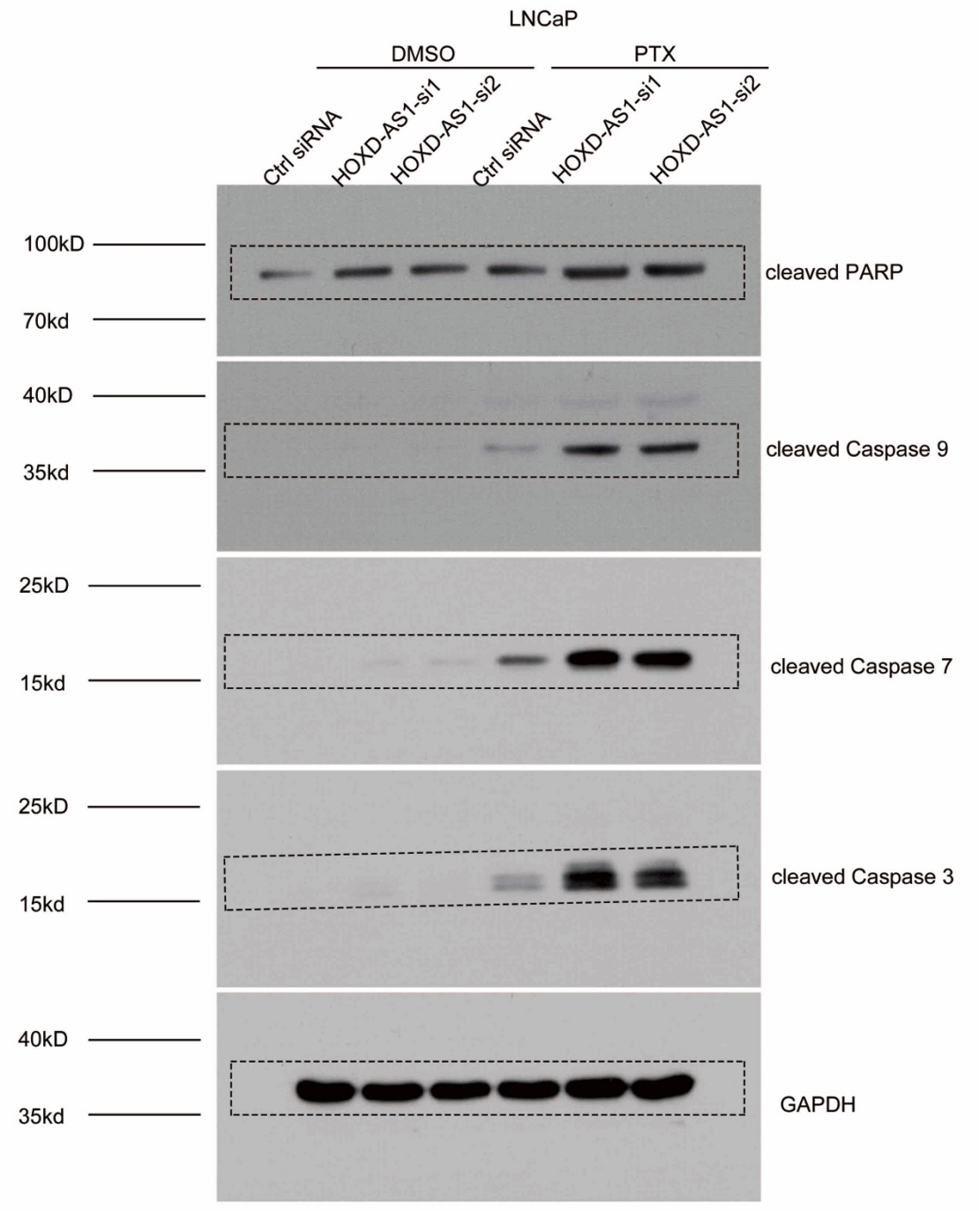


Figure 3G

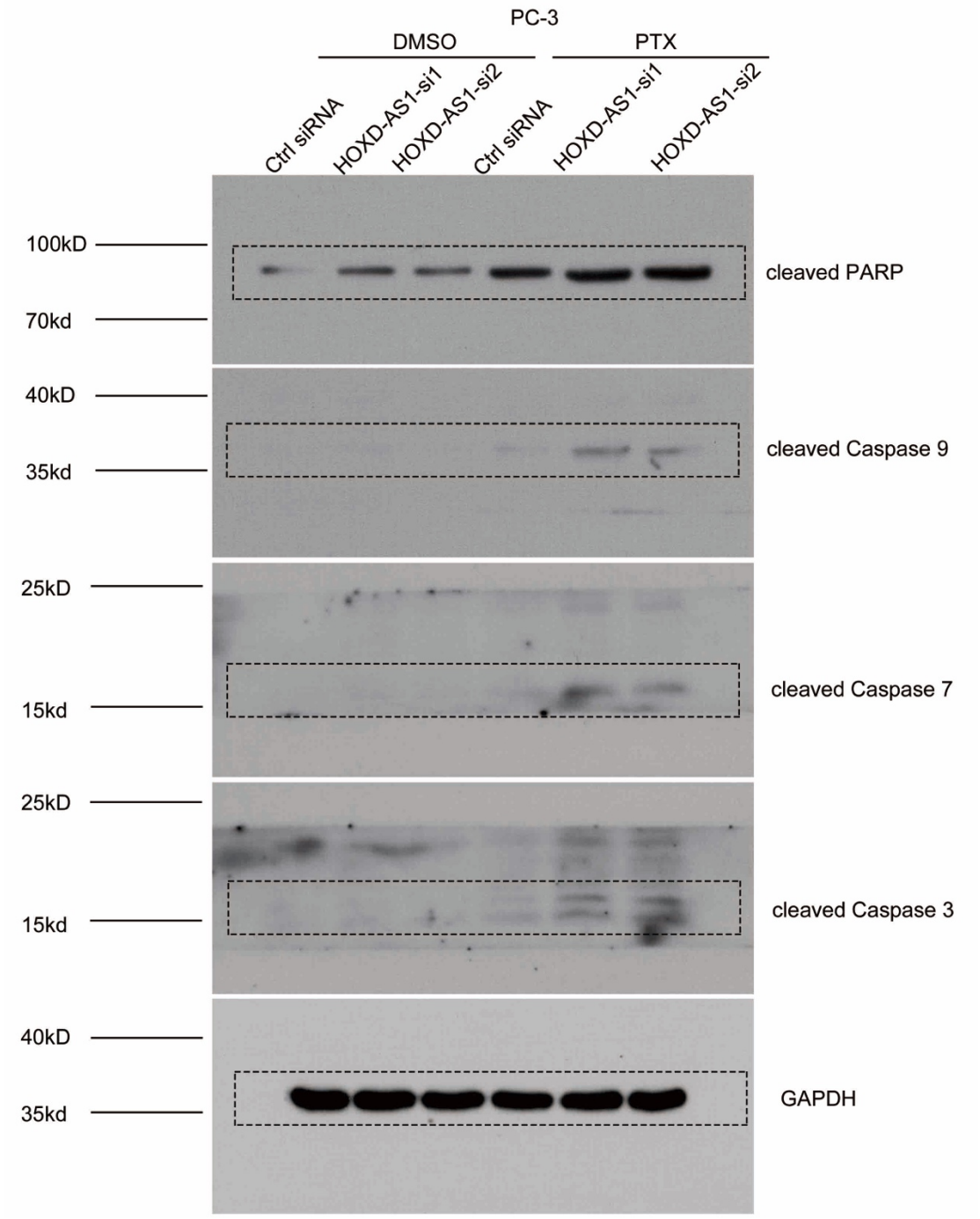

PC-3
DMSO
PTX
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
100kD
70kd
cleaved PARP
40kD
35kd
cleaved Caspase 9
25kD
15kd
cleaved Caspase 7
25kD
15kd
cleaved Caspase 3
40kD
35kd
GAPDH

Figure 5G

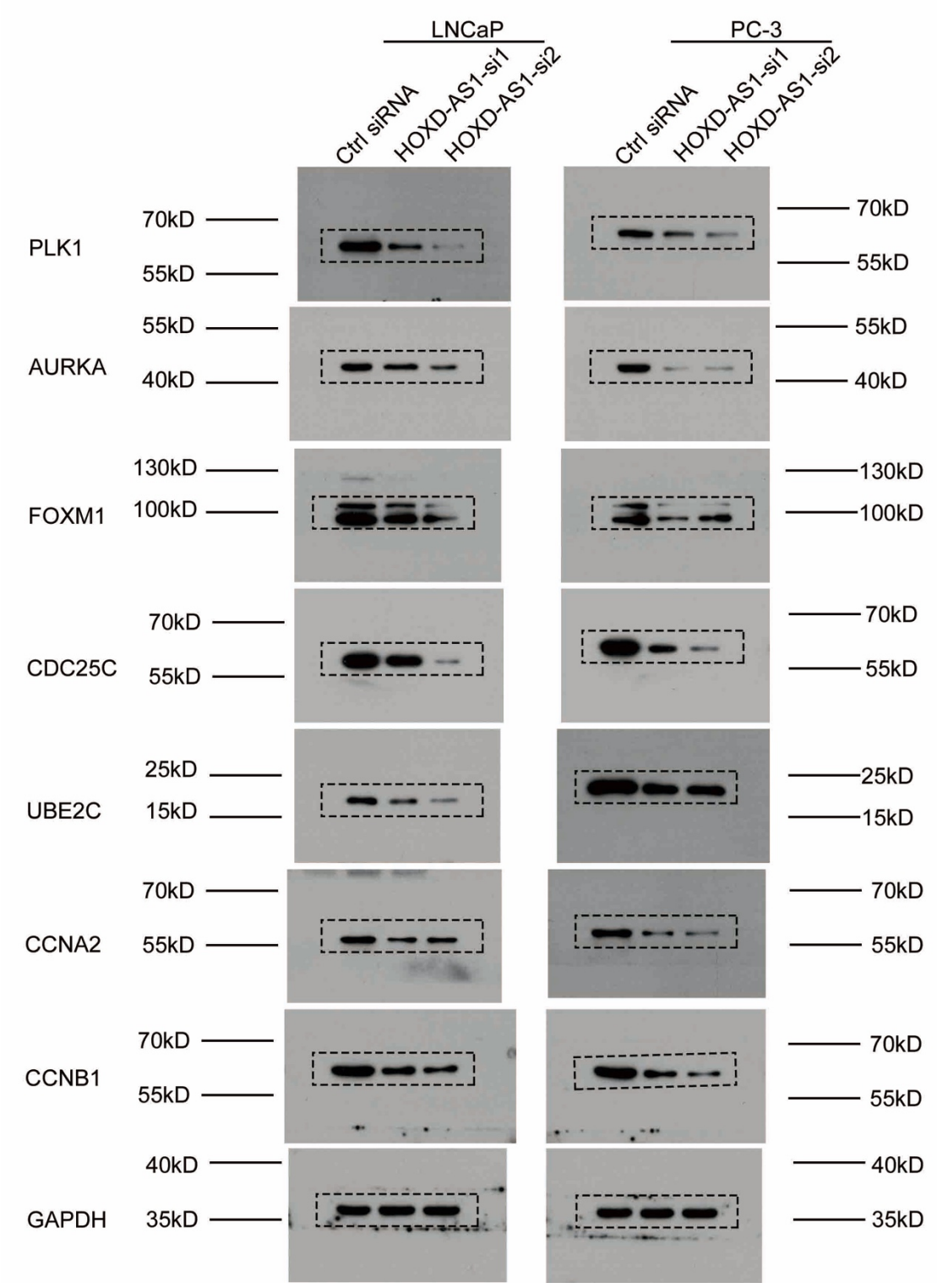

LNCaP
PC-3
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
Ctrl siRNA
HOXD-AS1-si1
HOXD-AS1-si2
PLK1
70kD
55kD
70kD
55kD
AURKA
55kD
40kD
55kD
40kD
FOXM1
130kD
100kD
130kD
100kD
CDC25C
70kD
55kD
70kD
55kD
UBE2C
25kD
15kD
25kD
15kD
CCNA2
70kD
55kD
70kD
55kD
CCNB1
70kD
55kD
70kD
55kD
GAPDH
40kD
35kD
40kD
35kD

Figure 6B

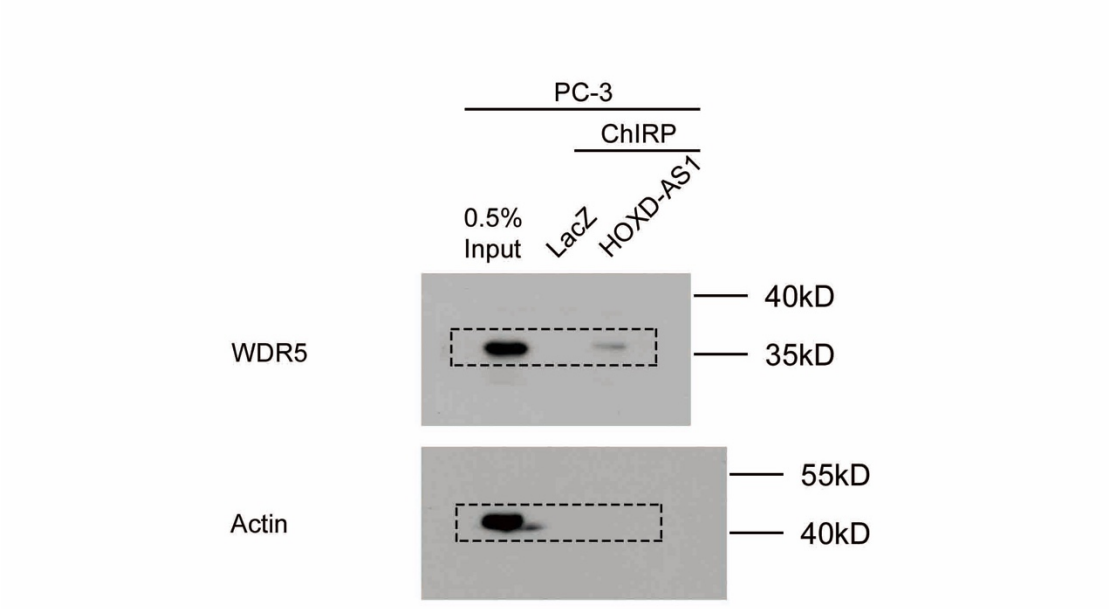

PC-3
ChIRP
0.5% Input
LacZ
HOXD-AS1
WDR5
40kD
35kD
Actin
55kD
40kD

Figure 6D

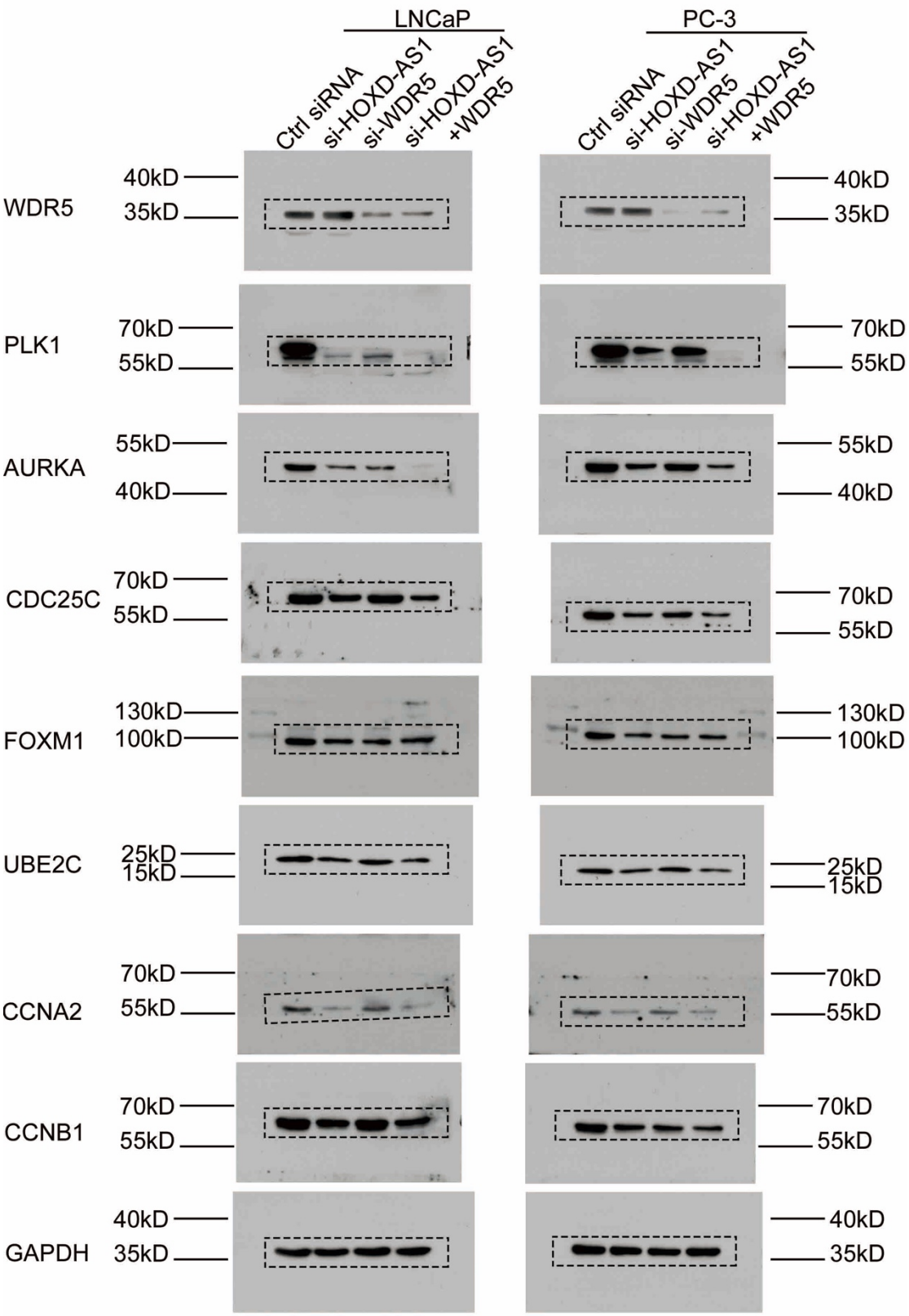

LNCaP
PC-3
Ctrl siRNA
si-HOXD-AS1
si-WDR5
si-HOXD-AS1 +WDR5
WDR5
40kD
35kD
PLK1
70kD
55kD
AURKA
55kD
40kD
CDC25C
70kD
55kD
FOXM1
130kD
100kD
UBE2C
25kD
15kD
CCNA2
70kD
55kD
CCNB1
70kD
55kD
GAPDH
40kD
35kD

Figure 6D

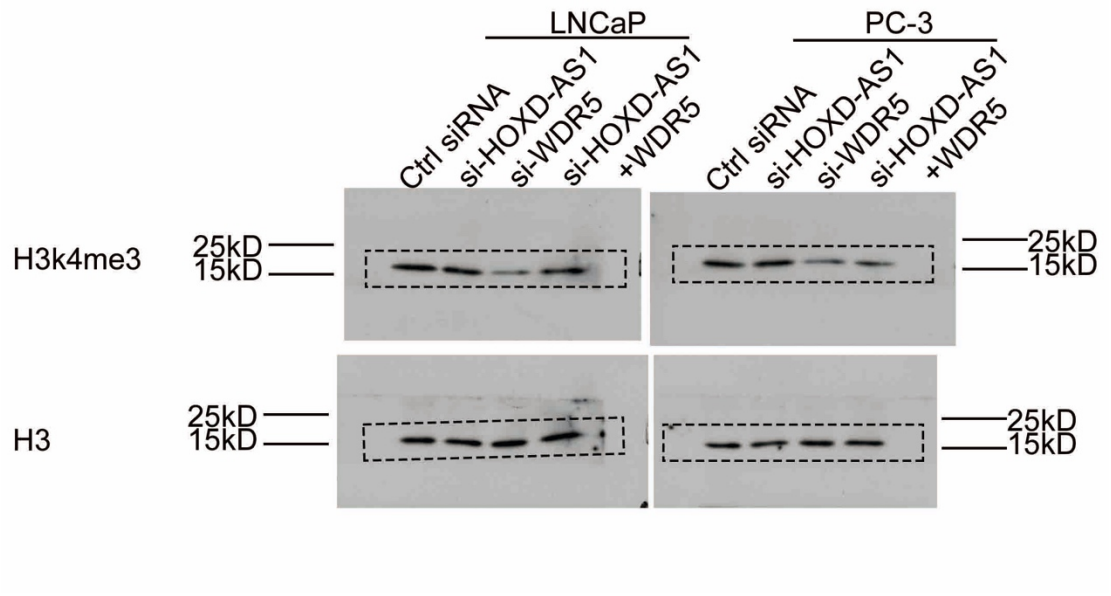

LNCaP
PC-3
Ctrl siRNA
si-HOXD-AS1
si-WDR5
si-HOXD-AS1 +WDR5
Ctrl siRNA
si-HOXD-AS1
si-WDR5
si-HOXD-AS1 +WDR5
H3k4me3
25kD
15kD
25kD
15kD
H3
25kD
15kD
25kD
15kD

Figure S1E

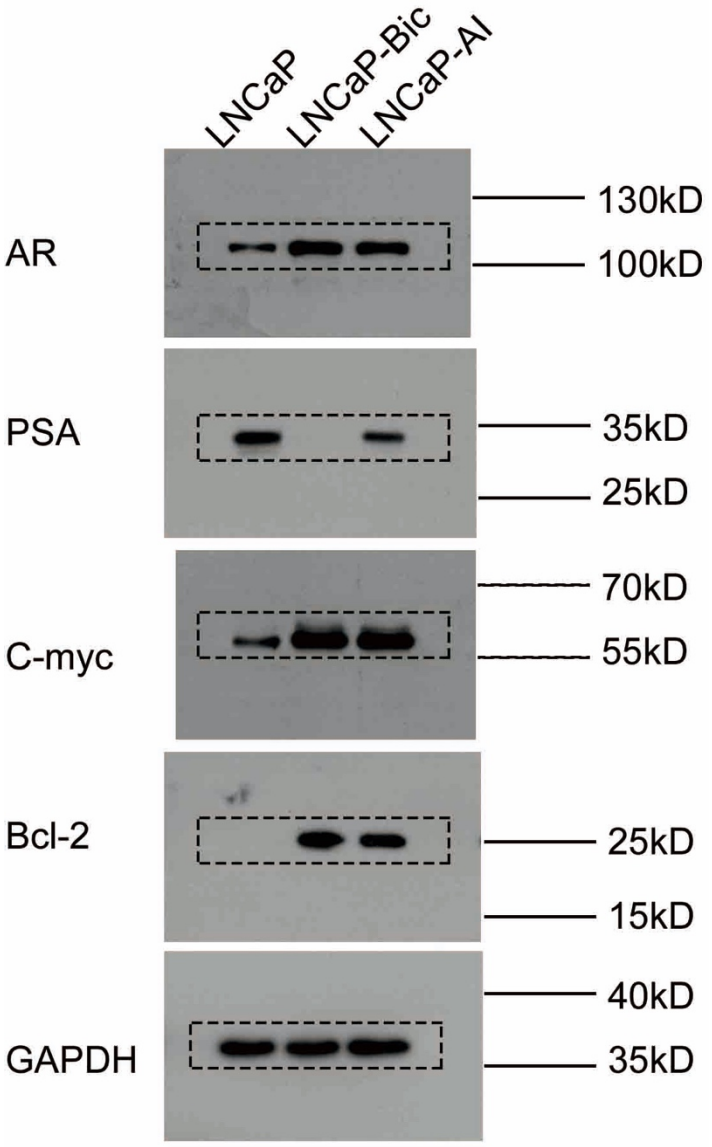

LNCaP
LNCaP-Bic
LNCaP-AI
AR
130kD
100kD
PSA
35kD
25kD
C-myc
70kD
55kD
Bcl-2
25kD
15kD
GAPDH
40kD
35kD

Figure S7

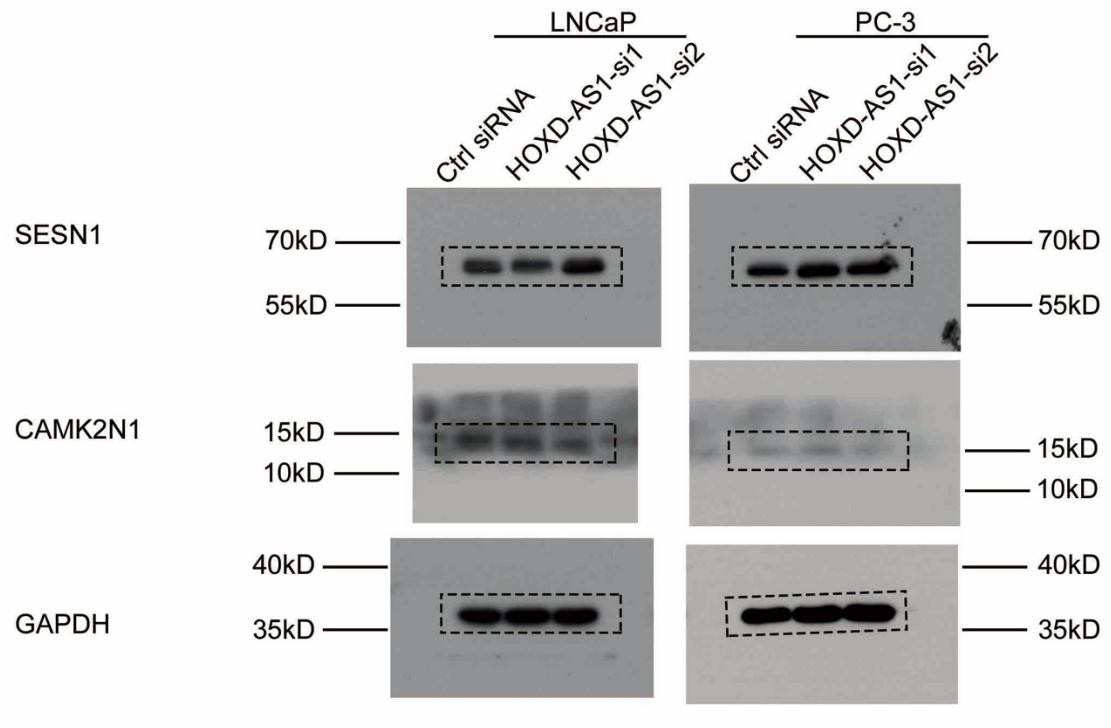


Figure S8B