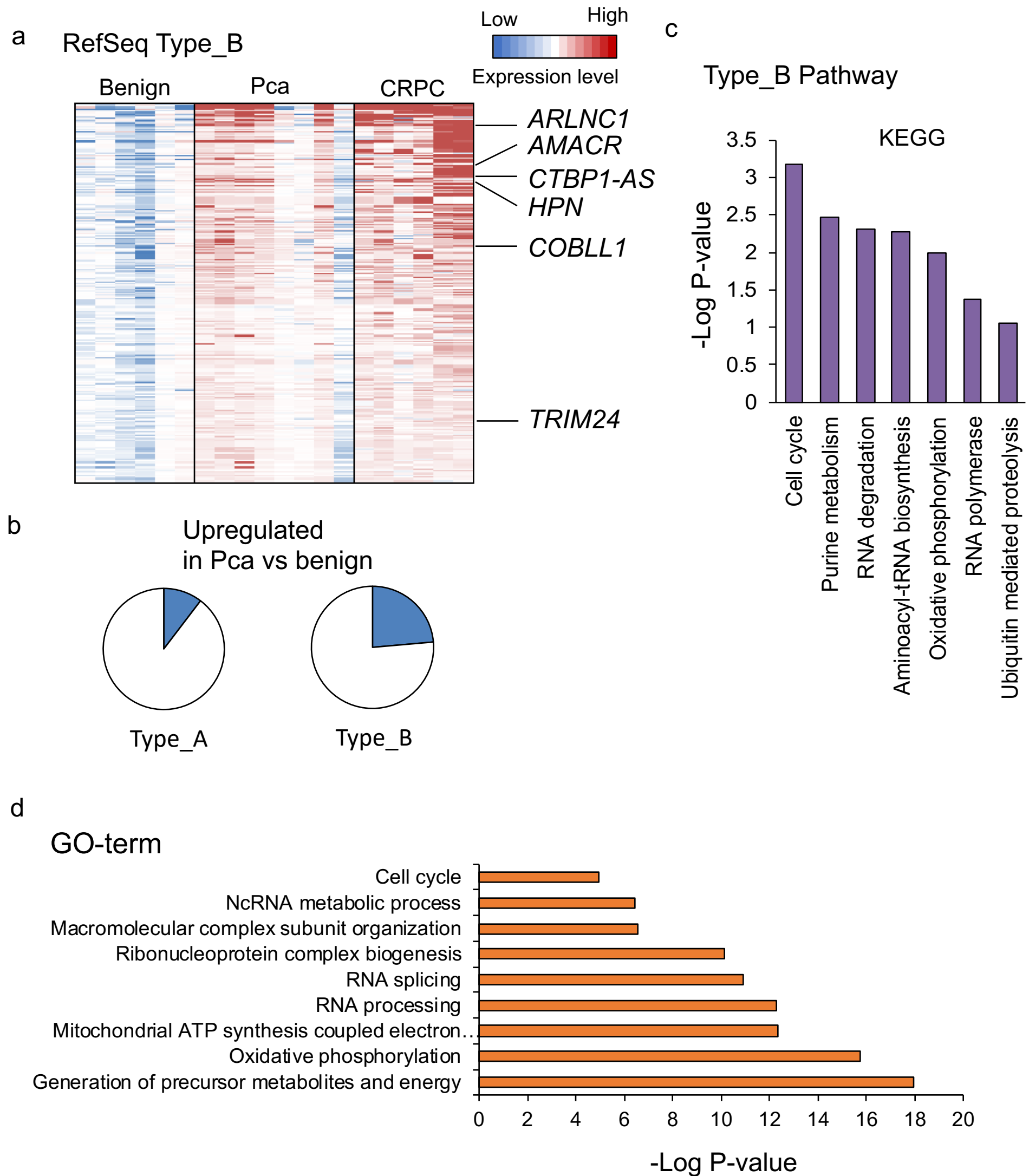
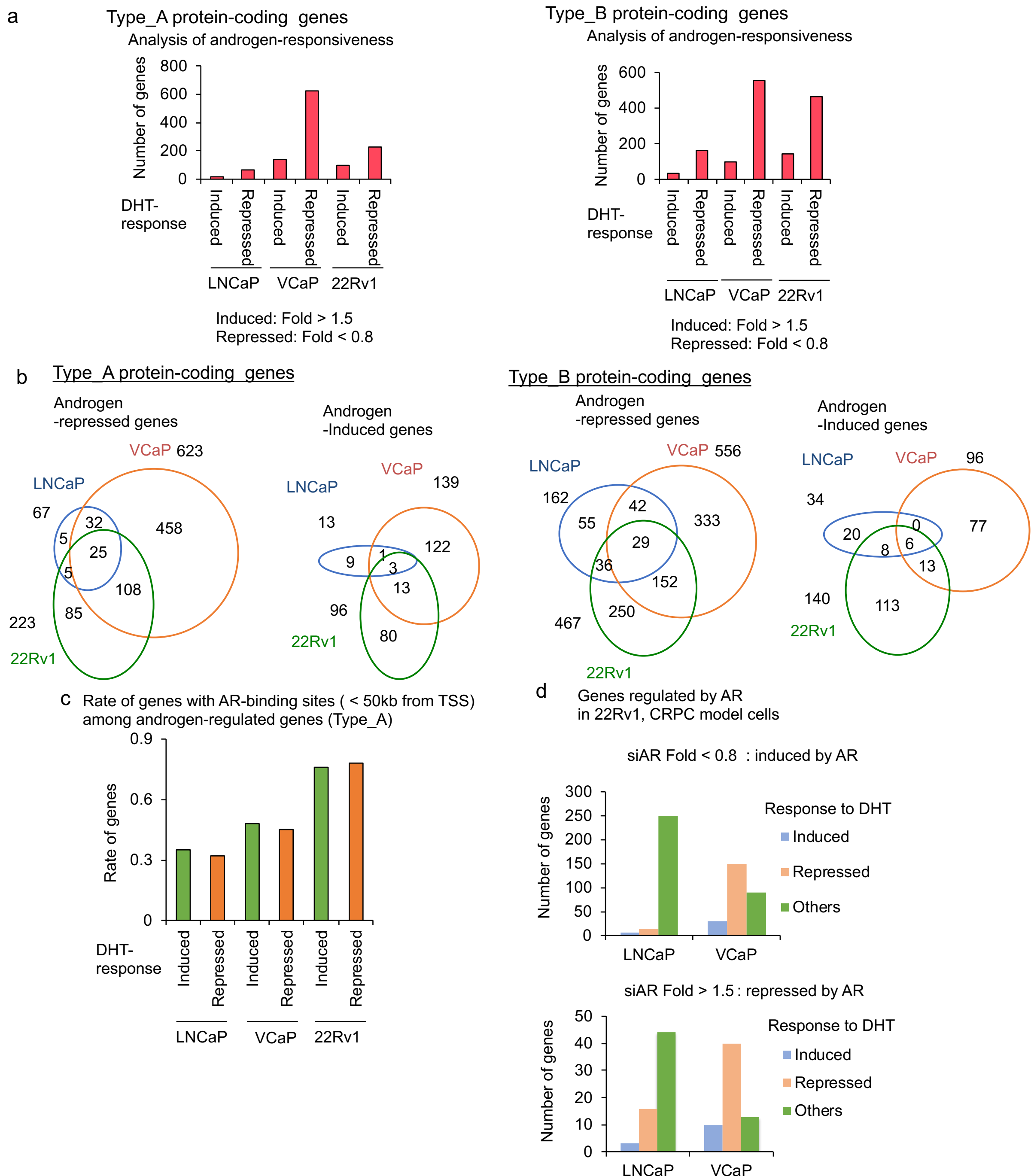




Supplementary Figure 1. Another type of genes highly expressed in CRPC tissues.

- (a) Identification of additional genes involved in CRPC development. Expression level relative to benign prostate tissue is visualized as heatmap. RefSeq genes of Type_B, which are upregulated between CRPC and benign prostate significantly ($P < 0.05$, Mann-Whitney test). Some genes, which were previously reported to be associated with prostate cancer progression, are indicated.
- (b) The rate of genes among Type_A and B whose expression levels are elevated in prostate cancer (Pca) compared with benign prostate tissues significantly ($P < 0.05$, Mann-Whitney test). We found that genes upregulated in Pca compared with benign prostate tissues are more enriched in Type_B rather than Type_A ($P < 0.0001$, Chi-square test).
- (c) KEGG pathway analysis of Type_B protein coding genes.
- (d) Gene Ontology (GO)-term analysis of Type_B protein coding genes.



Supplementary Figure 2. Regulation of upregulated genes in CRPC tissues by androgen in prostate cancer cells.

(a) The numbers of genes upregulated in CRPC tissues and regulated by androgen in prostate cancer cells. We used RNA-seq data to examine each gene of Type_A and Type_B is induced (Fold > 1.5) or repressed (Fold < 0.8) by DHT (10 nM) treatment in LNCaP, VCaP, and 22Rv1 cells.

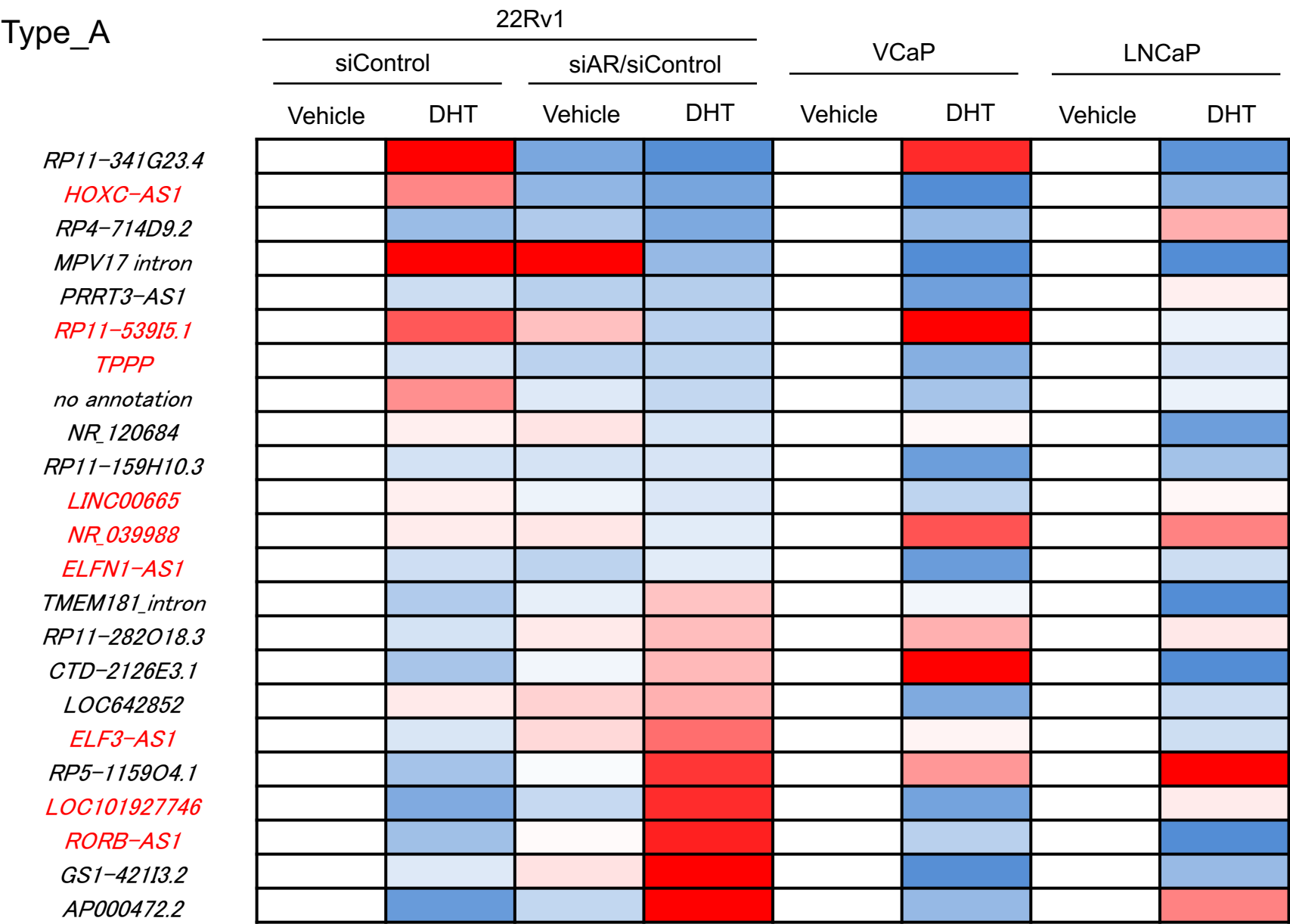
(b) Venn diagrams showed the overlap of repressed or induced genes in three prostate cancer cell lines.

(c) The rate of AR bindings among androgen-regulated genes (Type_A) identified in (a). TSS: transcription start site

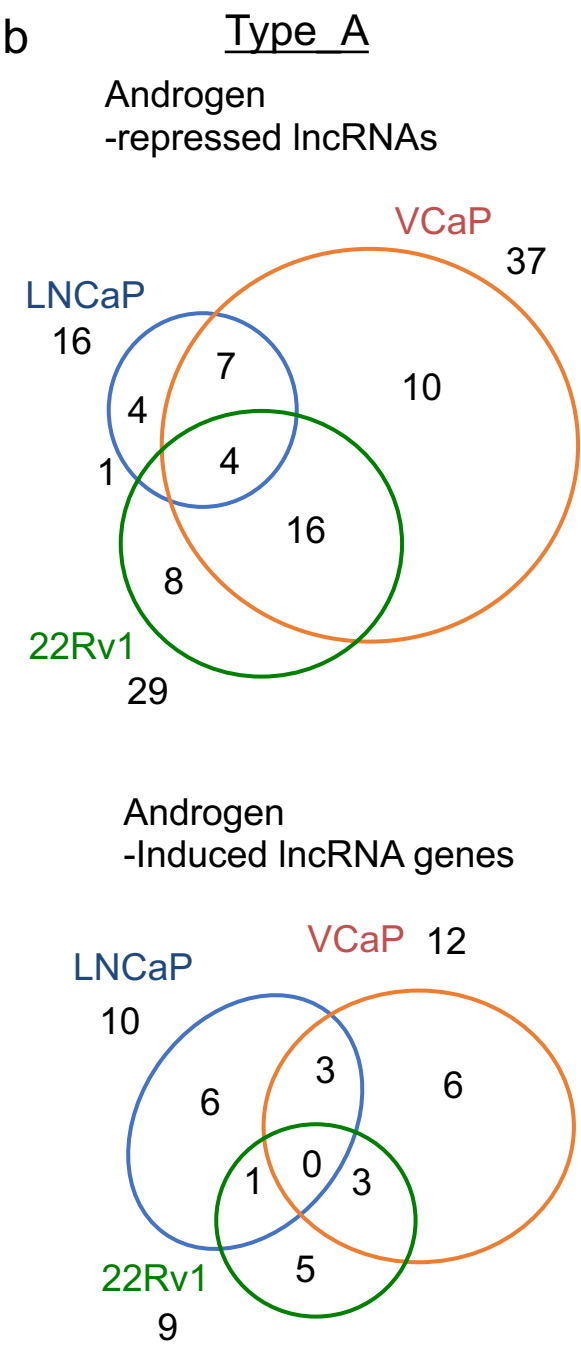
(d) AR-regulation of CRPC-associated genes in 22Rv1 cells is cell line specific. AR-regulated Type_A or Type_B genes in 22Rv1 cells, which are regulated by AR negatively (Fold > 1.5 by siAR) or positively (Fold < 0.8 by siAR), were used. Then we analyzed how many genes are regulated by DHT in LNCaP and VCaP cells. Androgen-induced (Fold > 1.5) or androgen-repressed (Fold < 0.8) genes were counted.

a

Type_A

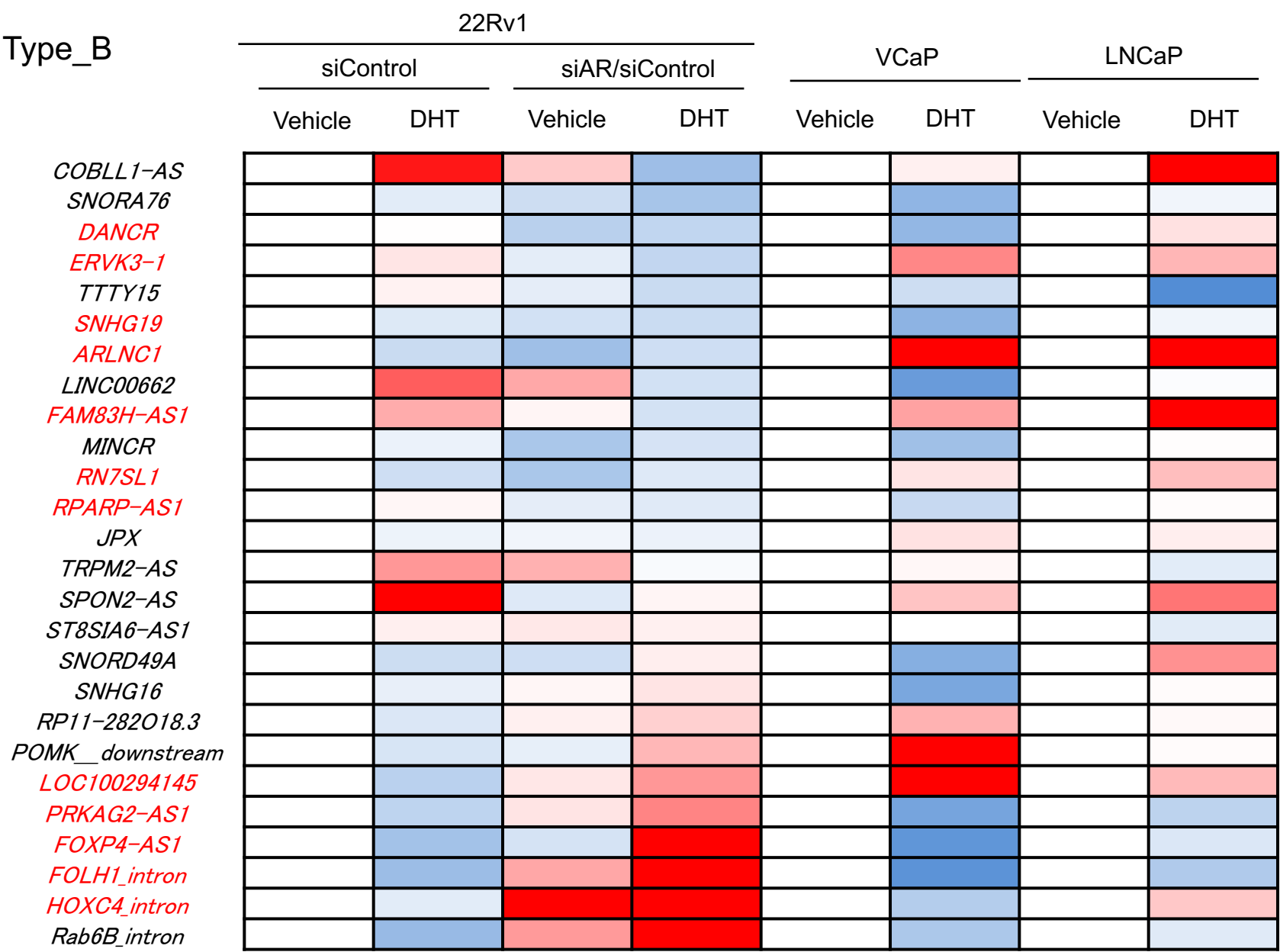


b

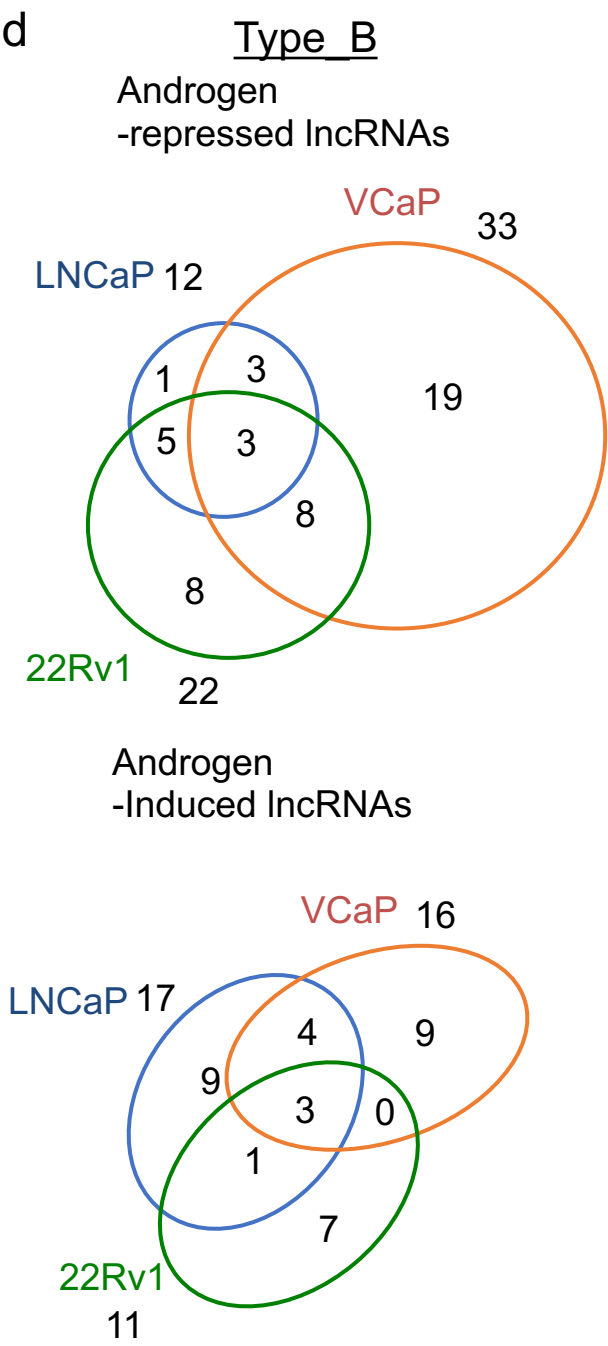


c

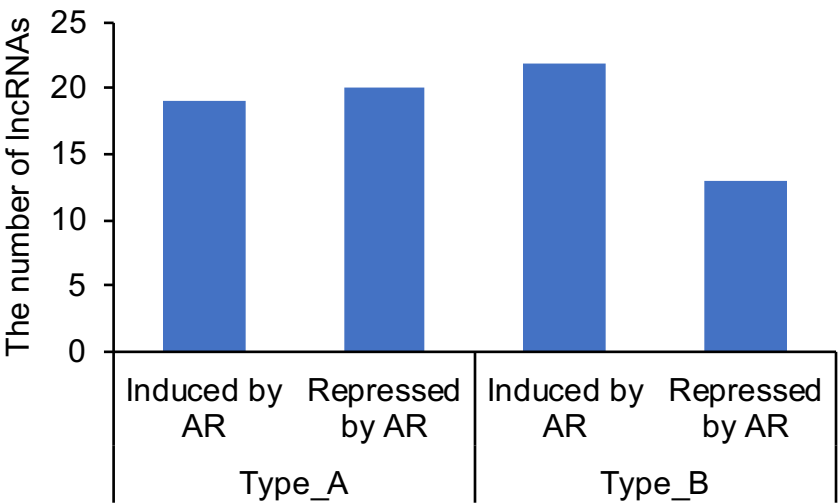
Type_B



d



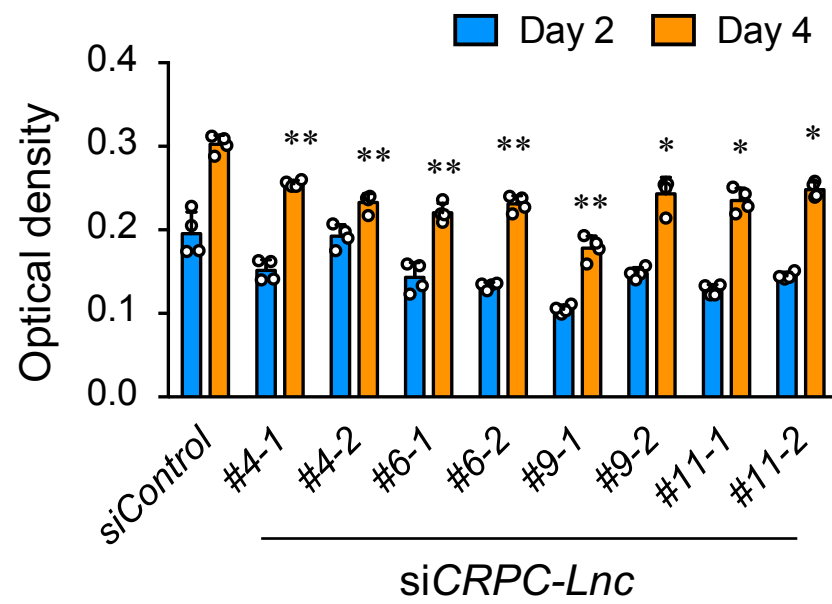
e



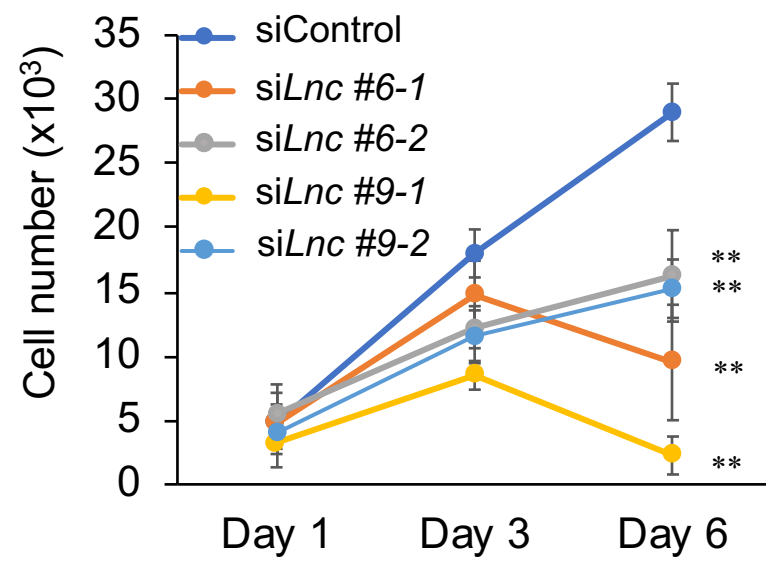
Supplementary Figure 3. Regulation of AR-regulated lncRNAs overexpressed in CRPC tissues.

(a, c) Androgen or AR-mediated regulation of lncRNAs overexpressed in CRPC tissues (Type_A and Type_B) is shown by heatmap. Fold changes relative to siControl or vehicle control are shown. RNA-seq data in prostate cancer cells were used for this analysis. 21 lncRNAs examined in qPCR analysis (Figure 4a) are highlighted by red letters. (b, d) Venn diagrams showed the overlap of androgen-regulated lncRNAs. We identified androgen-repressed (Fold < 0.8) or androgen-induced (Fold > 1.5) lncRNAs among lncRNAs overexpressed in CRPC tissues (Type_A and Type_B) by using RNA-seq data in prostate cancer cells. (e) Identification of AR-regulated lncRNAs in 22Rv1 cells. We used RNA-seq data to obtain AR-repressed (Fold > 1.5 by siAR) or AR-induced (Fold < 0.8 by siAR) lncRNAs.

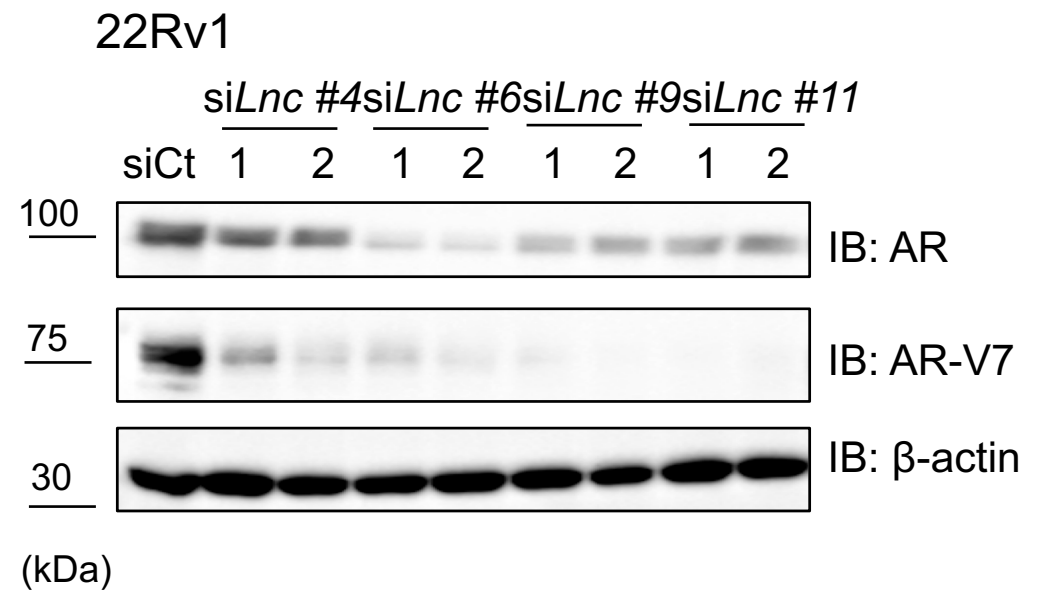
a 22Rv1
MTS assay



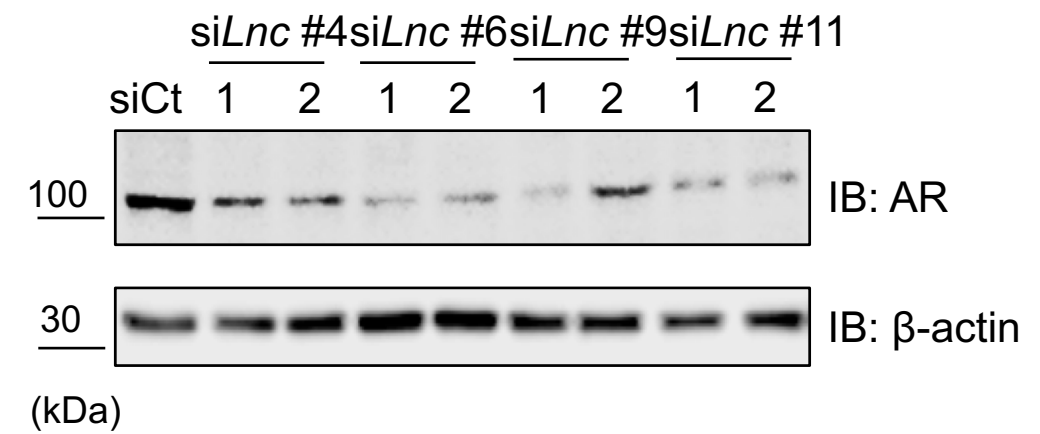
Cell growth assay



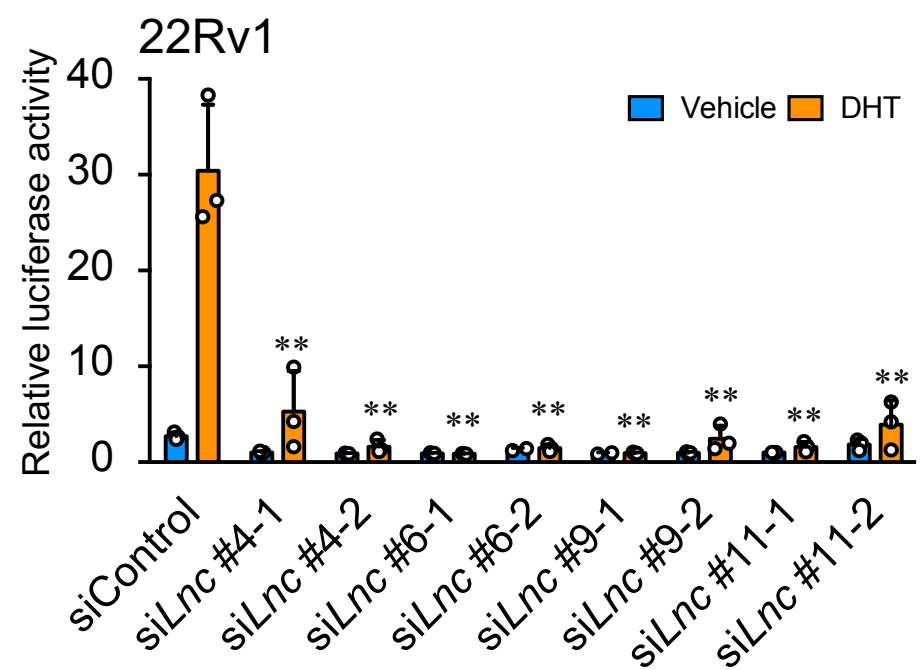
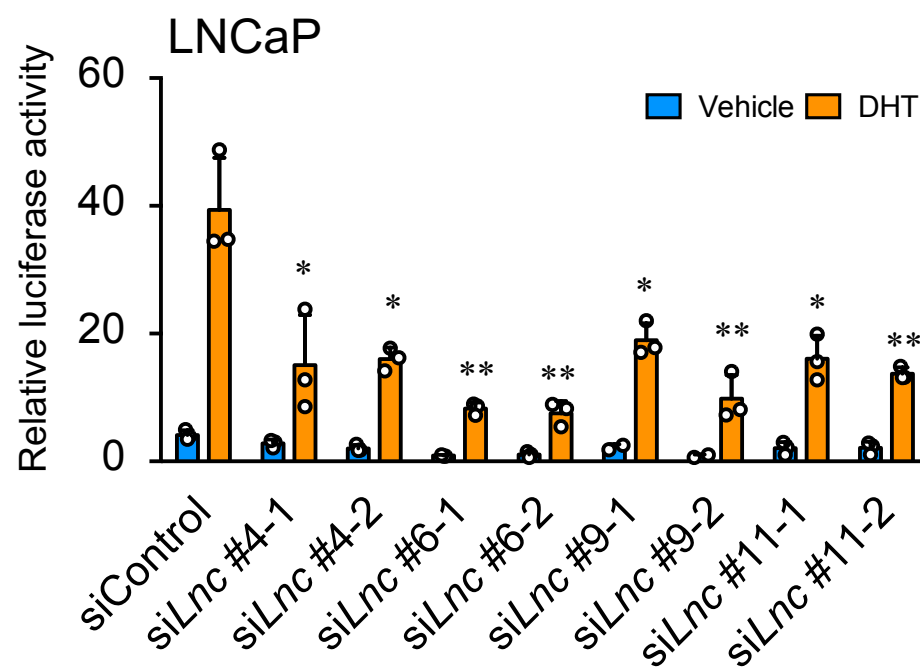
b



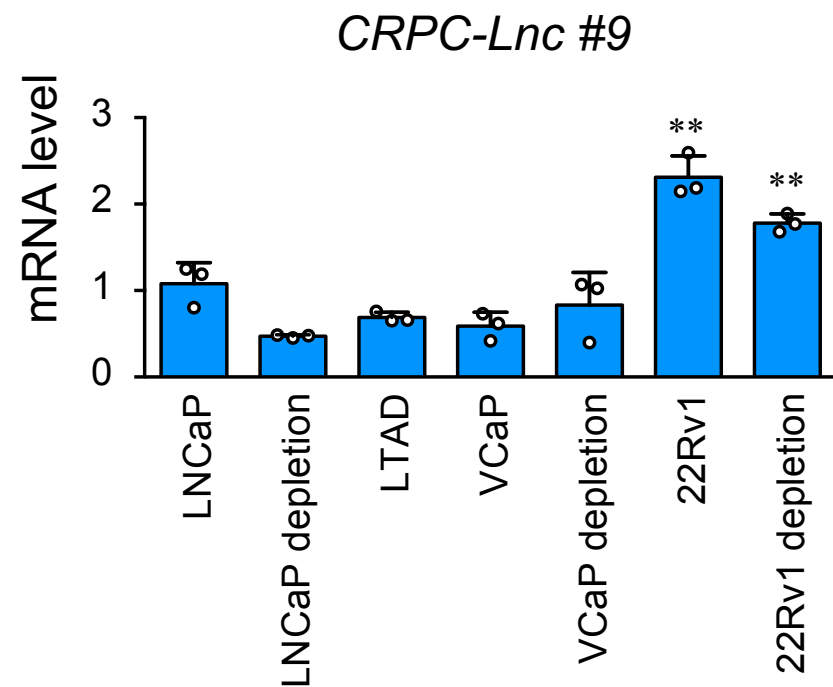
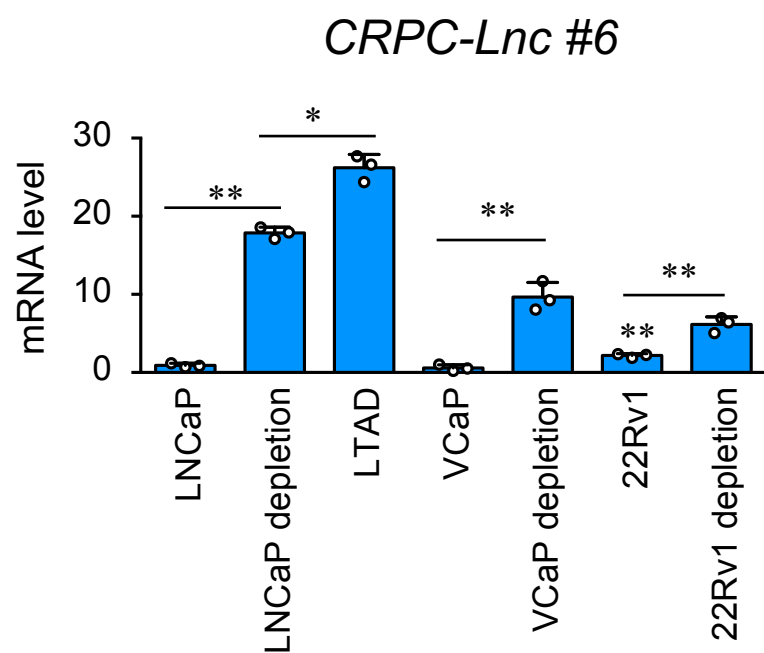
LNCaP



c MMTV-LUC



d



Supplementary Figure 4. Additional data about the role of *CRPC-Lncs* in cell growth and AR activity.

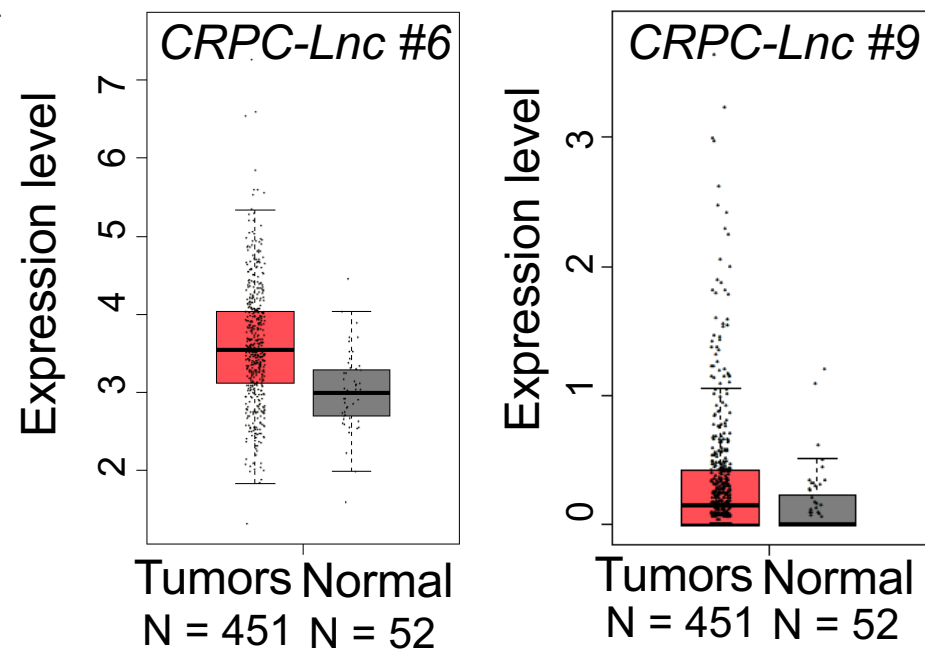
(a) Cell proliferation was attenuated by knockdown of *CRPC-Lncs* (#4, #6, #9, and #11) using two siRNAs. MTS assay (upper) and cell counting (lower) were performed to assess cell growth ability in 22Rv1 cells (N = 4). *Lnc*: *CRPC-Lnc*.

(b) AR expression is inhibited by silencing of *CRPC-Lncs* (#4, #6, #9, and #11) using two siRNAs. Western blot analysis was performed in 22Rv1 and LNCaP cells. IB: immunoblot. Values represent the mean $\pm$ S.D. *P < 0.05, **P < 0.01.

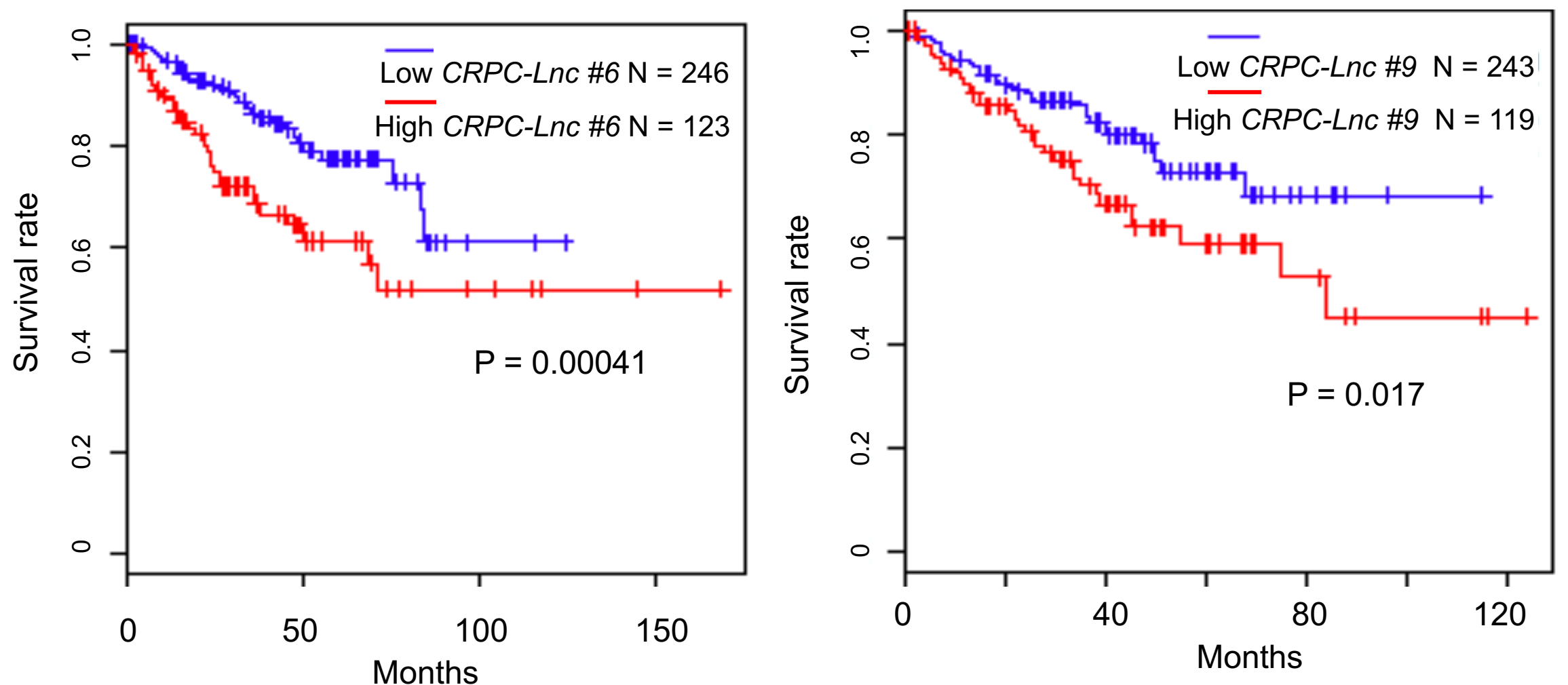
(c) AR activity was repressed by knockdown of *CRPC-Lncs* (#4, #6, #9, and #11) using two siRNAs. *MMTV-LUC* was transfected after 48 h treatment of siRNAs. After 24 h incubation, cells were treated with 10 nM DHT or vehicle. Luciferase assay was performed for evaluation of AR activity (N = 3). *Lnc*: *CRPC-Lnc*. Values represent the mean $\pm$ S.D. *P < 0.05, **P < 0.01.

(d) *CRPC-Lncs* were distinctly affected by AR overexpression and androgen-deprivation. For hormone deprivation treatment, LNCaP, VCaP, and 22Rv1 cells were cultured in phenol-red free medium with 10 % charcoal dextran stripped FBS for one week. Expression levels of *CRPC-Lnc* #6 and #9 were determined by qRT-PCR analysis (N = 3). Values represent the mean $\pm$ S.D. *P < 0.05, **P < 0.01. LTAD: long-term androgen deprivation cells.

a TCGA



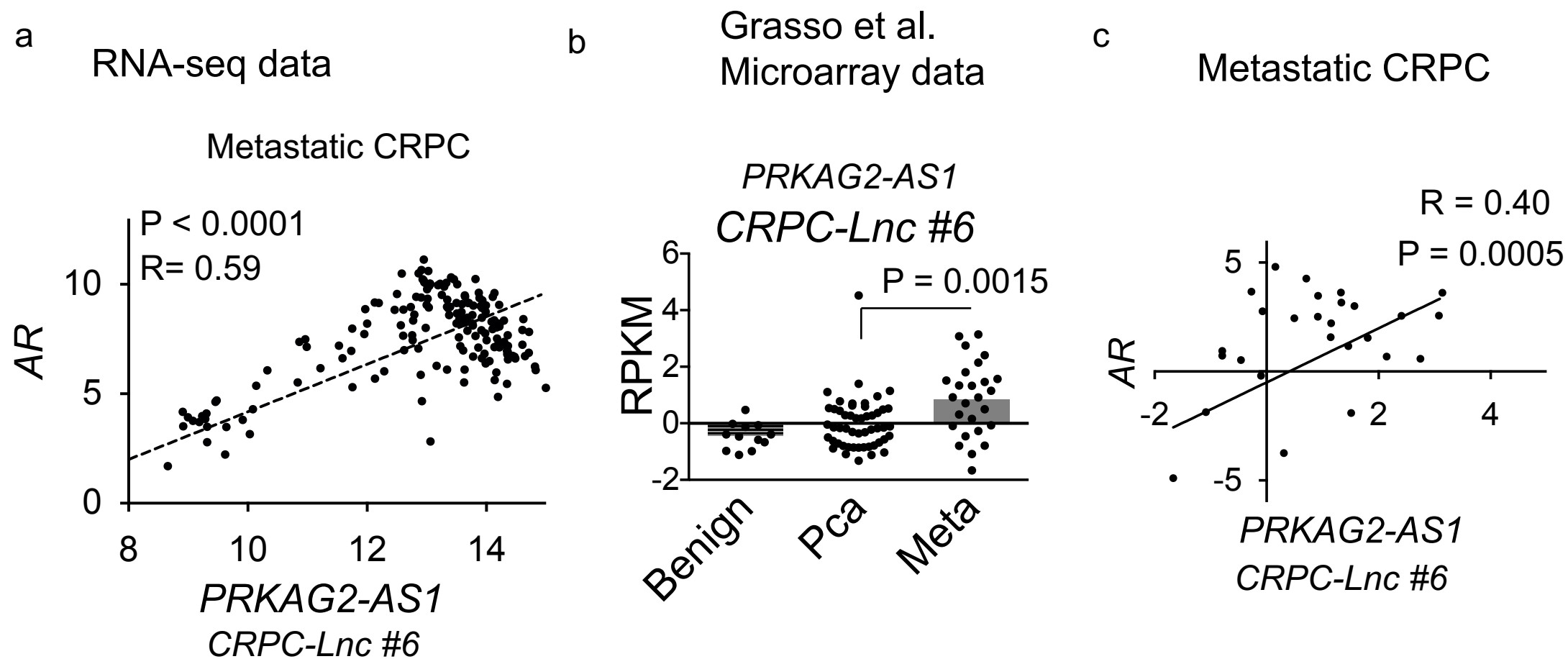
b



Supplementary Figure 5. High expression levels of *CRPC-Lncs* are correlated with poor prognosis of prostate cancer patients.

(a) Expression level of *CRPC-Lnc #6* and *#9* in prostate tumors and normal prostate tissues in the Cancer Genome Atlas (TCGA) cohort. We analyzed the expression levels of *CRPC-Lncs* by using Gene Expression interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>).

(b) High expression levels of *CRPC-Lnc #6* and *#9* in prostate tumors are correlated with poor prognosis. We analyzed the disease-free survival of patients by using GEPIA. We set the cut off at 75% for high expression and 50 % for low of 451 tumors. P-value was calculated by log rank test.

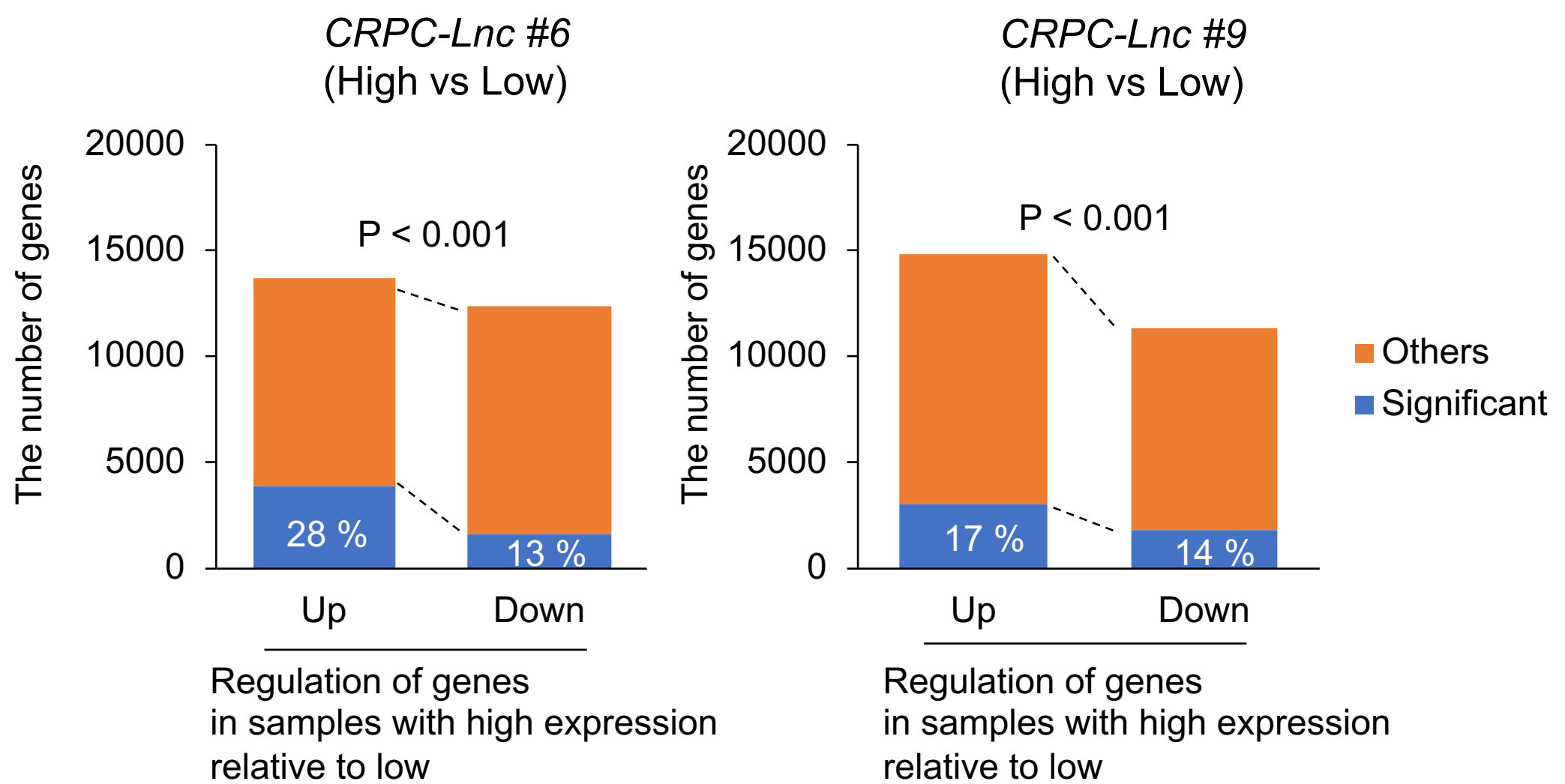


Supplementary Figure 6. The expression level of CRPC-Lnc #6 is associated with AR mRNA.

(a) We analyzed the expression levels of CRPC-Lnc #6 (*PRKAG2-AS1*) and AR by using cbiportal RNA-seq data (Kumar et al.¹). Spearman correlation analysis was performed to determine the correlation.

(b) CRPC-Lnc #6 is upregulated in metastatic CRPC tissues compared with Pca and benign. We used publicly available microarray data (Grasso et al.²) to analyze the expression levels of CRPC-Lnc #6 (*PRKAG2-AS1*). Meta: metastatic CRPC. Mann-Whitney U test was used to determine the P-value.

(c) We analyzed the correlation of AR with CRPC-Lnc #6 in metastatic CRPC tissues (N = 27) in microarray data (Grasso et al.²). Spearman correlation analysis was performed to determine the correlation.



Supplementary Figure 7. The general effect of *CRPC-Lncs* on global gene expression.

The expression levels of *CRPC-Lnc* #6 and #9 in 154 prostate cancer tissues were analyzed using exon array data publicly available (Taylor et al.³). We then divided samples to two groups, “high expression” and “low expression” of *CRPC-Lncs* and compared the global expression profile between them. The rates of significantly upregulated genes ($P < 0.01$, tow-sided t-test) among all upregulated genes in high expression of *CRPC-Lncs* were larger than downregulated genes ($P < 0.0001$, chi-square test).

Fig.5a

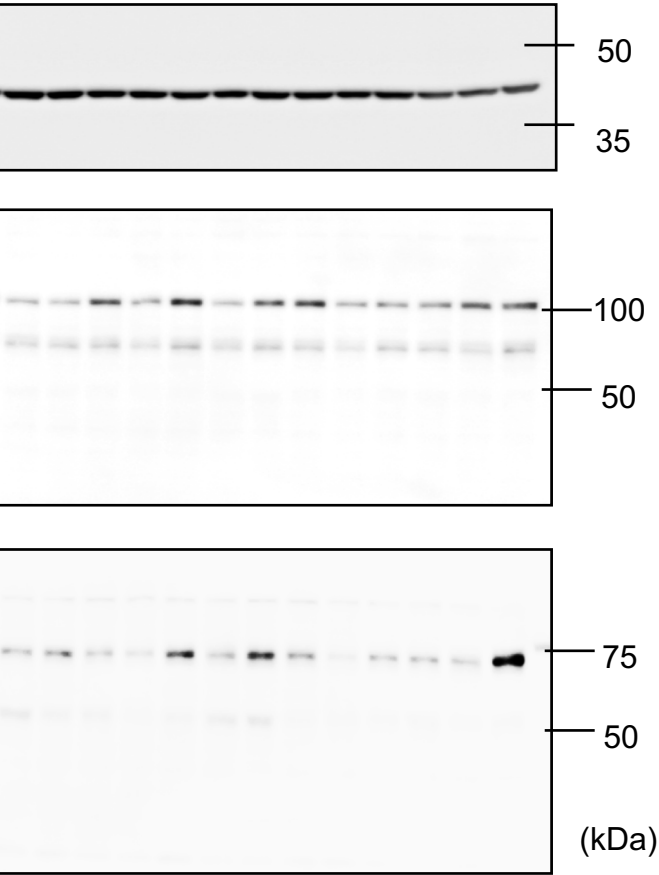


Fig.5b

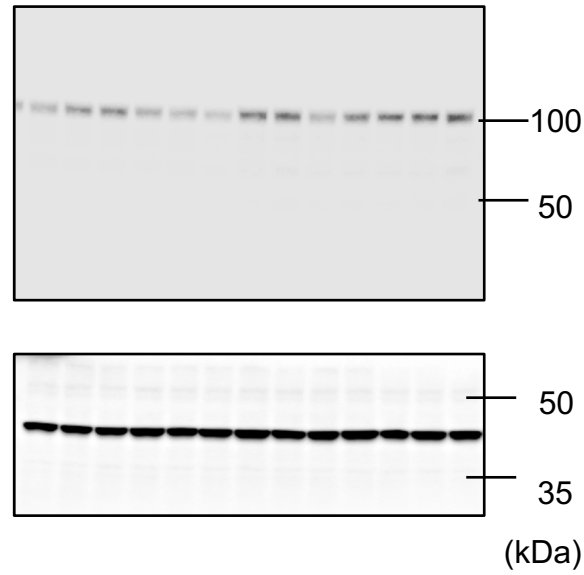


Fig.5g

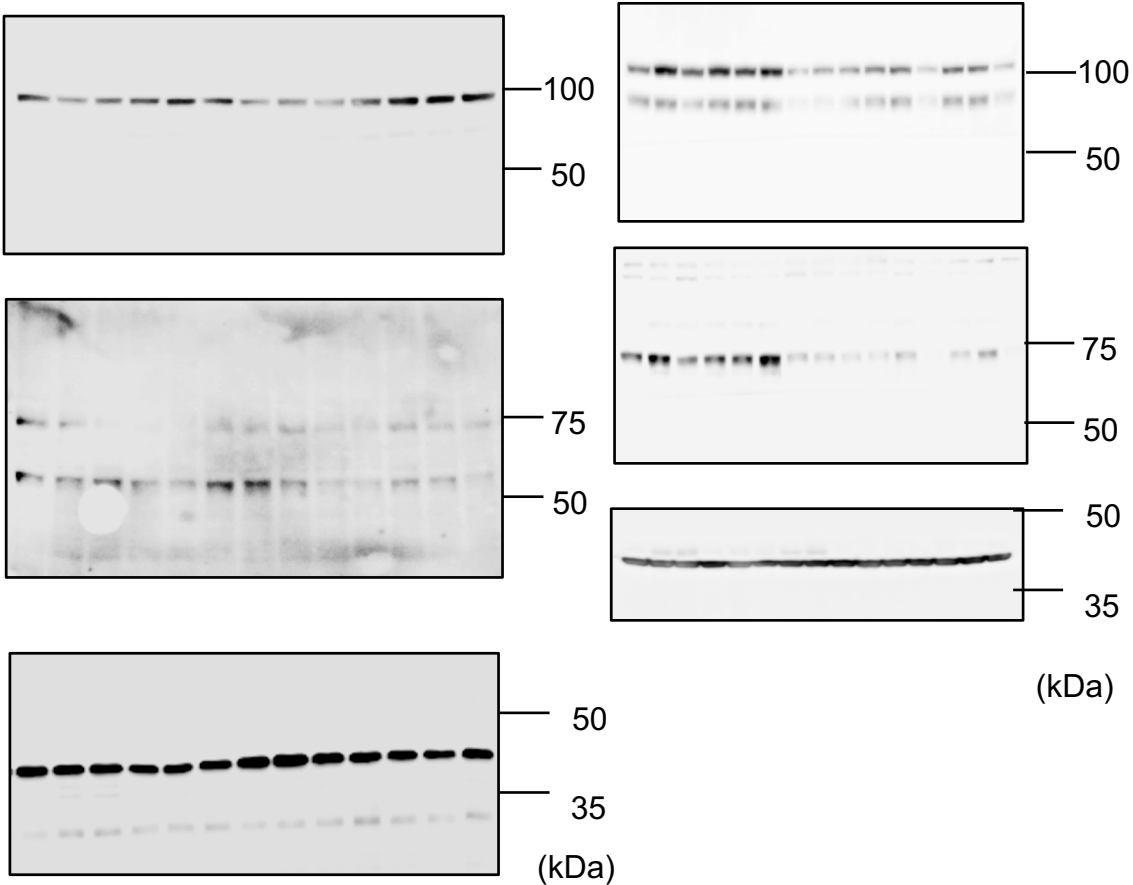


Fig.6b

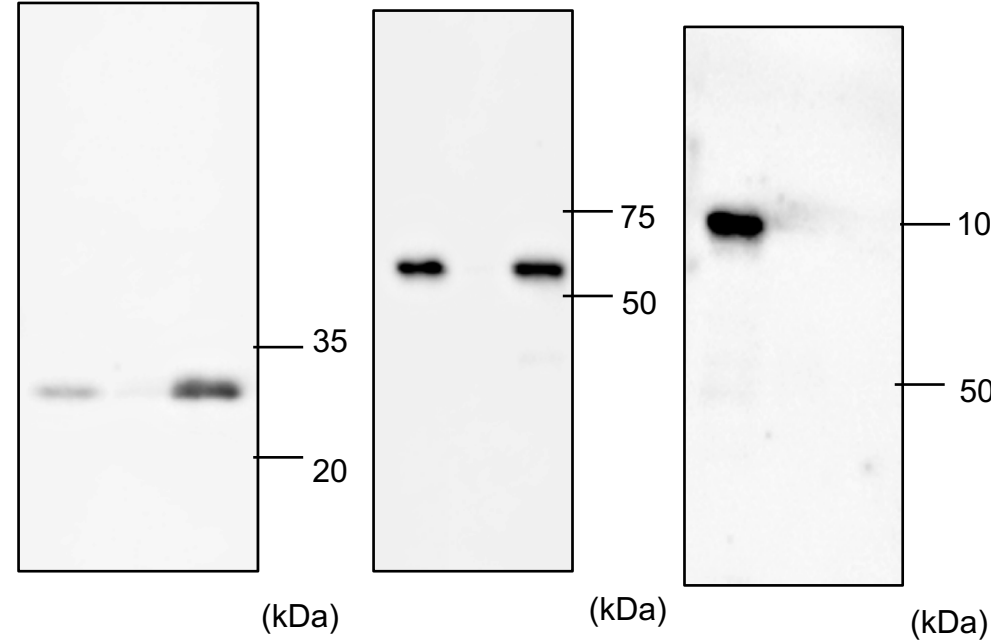


Fig.6g

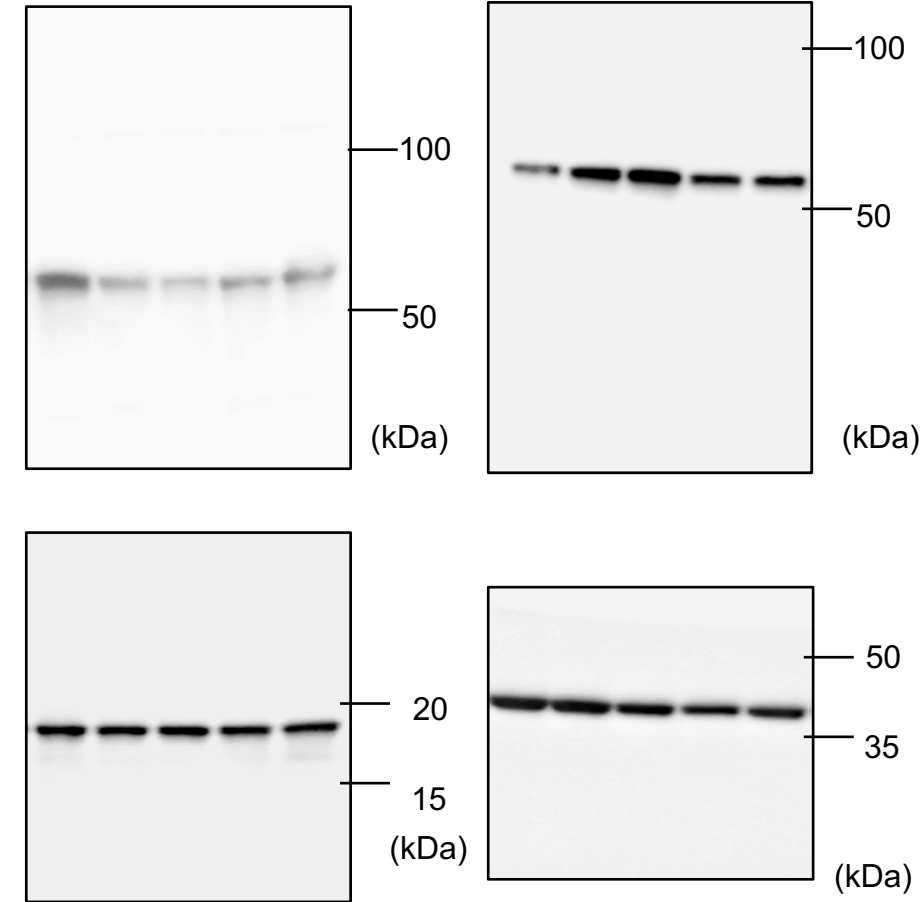


Fig.6f

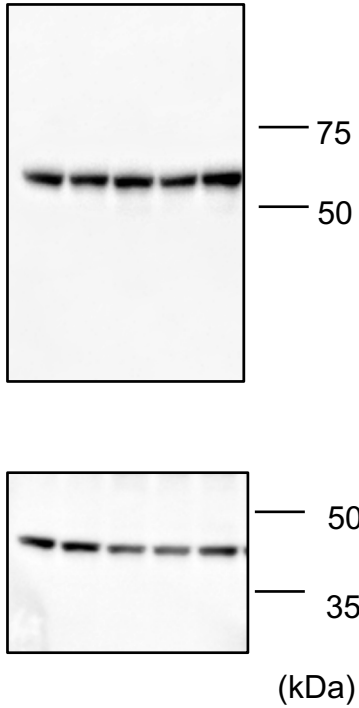


Fig.7c

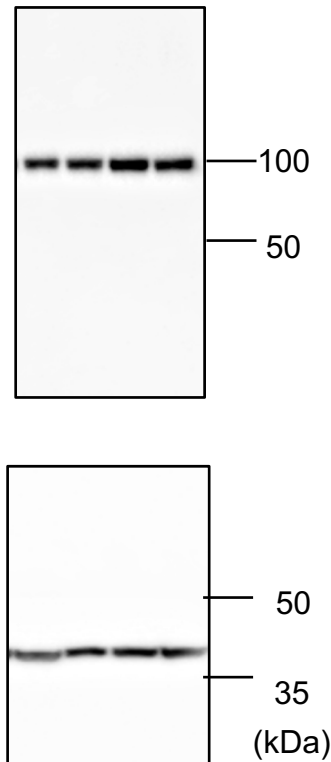
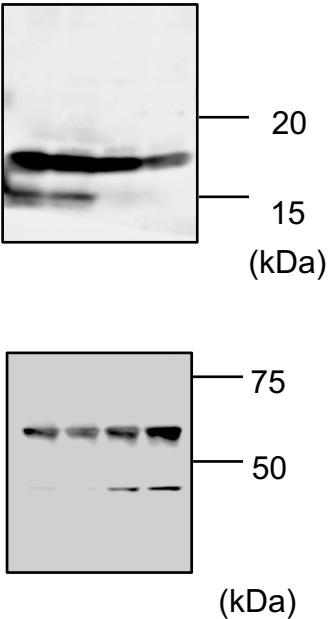
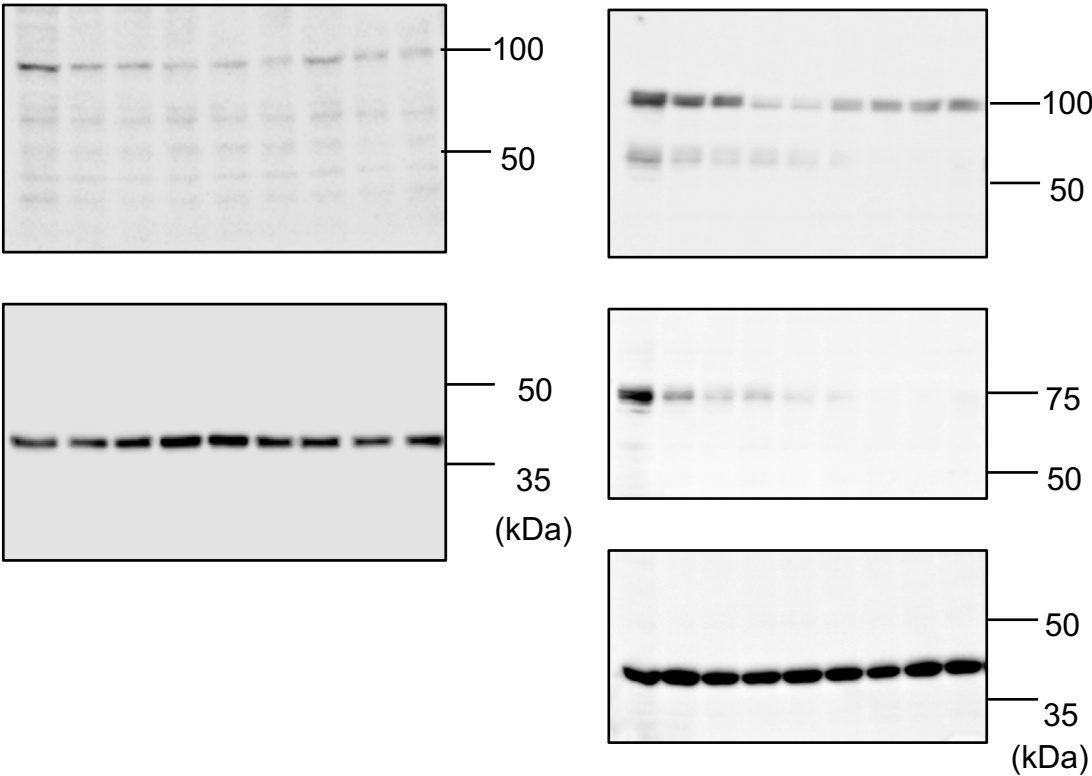


Fig.7f



Supplementary Fig. S4



Supplementary Figure 8. Full scan images of immunoblotting in Figures.

Supplementary Table 1. Clinical characteristics of CRPC tissue samples used for RNA-seq analysis

	Case 1	Case 2	Case 3	Case 4	Case 5
Age	84	86	65	80	68
Methods to obtain tissues	TURP	TURP	Biopsy	Autopsy	TURP
PSA nadir	0.01	0.11	0.54	15.97	< 0.01
Time to PSA failure (days)	1761	840	3317	161	162
Serum PSA	0.01	15.05	8.01	25.13	5.9
First-treatment	MAB	MAB	Brachytherapy	MAB	RARP
Surgical castration	1	0	1	1	0
LHRH agonist	0	1	1	0	1
Casodex	1	1	0	1	1
Enzaltamide	1	0	0	0	1
Abiraterone	0	0	0	0	1
Radiotherapy	1	0	0	1	1

TURP: transurethral resection of the prostate, MAB: maximal androgen blockade, RARP: robot-assisted laparoscopic radical prostatectomy, 0: no, 1: yes We obtained two samples (lymph node and liver metastases) from Case 4.

Supplementary Table 2. Sequences of RNA FISH probes

CRPC-Lnc #6

Probe

number	Sequence
1	ttccagattccccacttg
2	gcacgaacgggcttactg
2	ctccgagctggttattctg
3	actgcgcaaaccttgatgaa
5	ttccaggctggcttcatt
6	ctctgtctcgagactggg
7	taaaccagacagggtcc
8	aggatggctgcttggtg
6	caggagaagaccagaagtc
7	aaatcctctagcaagaagcc
8	caatacagtcttgctgggtc
9	tatcttgagcattcagtggg
10	tccccctttctacttctaac
11	agtggggacagaggctaaat
20	gacaattgggaataattt
21	gctaaacagatgacacaa
22	gacaaggattaaaactgt
12	ccctggcaaatagatcatttt
13	ccagttctcatcaaataggg
17	gccccattttcctttaa
14	agacgtctcttcacaccaac
17	caccagtggaatgaggg
18	atcgcggtttctccttc
29	acactgtccataaagaaaac
30	gtacacattataacaattgc
31	gtatacagatcaatcaacaa
34	aatgtaggaagcactaaagt
35	gcaggcaatacagtaaaatg

AR_intron 3

1	gctacttgagatgattctttg
2	tatctgagtctcatttgatgtg
3	ttgagtgtataagaacctcaa

4 ttgcatccaaatgttatgagg
 5 caggctgcttaataatggagaa
 6 actttccagctaaattactagt
 7 tatggcataccaagttagtag
 8 acttttcgttgaggtaatca
 9 ggaacgtggagataagttcatt
 10 ctgtgcattgaatgtgtaagt
 11 agtctactccttaaggaataga
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 15 tttatcctaactatggactgtg
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 31 tcaatcattggatggtttctac
 32 ccacgttgtaattctttatga
 33 aggagaatggaagaaatccag
 34 aaaagaagggtgaagggtacg
 35 agtggaaattcaagggtgtt
 36 cccttaagaactacagtttag
 37 attttgagcactcacagtaaa
 38 ttggaaaatattgcctatggc
 39 ttcttaacaacgtgatcccaa

40	gaagaatggaacctgattccaa
41	ccctcaacaatcaatttttact
42	cggtgcttacatgtttttaatt
43	aagcattattactcagactgga
44	cactcagagaccaagaaagact
45	ccacttgtaagacagaagtgag
46	cataggaagcagcatgtaaagt
47	aatcagatgcctaagacagatc
48	tgttgactgtagcagtctaaat

Supplementary Table 3. Primer sequences for qRT-PCR

Primer	Sequence
#1: <i>NR_039988</i>	F: CAGTGAGCAGAACCAGTCCA R: TAGAAGCAGGGCTTTCCTCA
#2: <i>LINC00665</i>	F: TCCTGACCTCTGACCCGTAT R: ATGGTAGTCGATCCGCTGTC
#3: <i>DANCR</i>	F: TGCAGCTGCCTCAGTTCTTA R: ACCTGCTACACTGCCCTGTT
#4: <i>FAM83H-AS1</i>	F: GCGACAGGAGAATGGACAGT R: GGAGGTAGCAGTGAGCCAAG
#5: <i>LOC101927746</i>	F: TTCACACTTGCAGCAGATCC R: CTTTTTCACGTTTCCCGTGT
#6: <i>PRKAG2-AS1</i>	F: GACCCTGTCTGGGTTTAGCA R: ACTACCCCCTCCCCTTTTCT
#7: <i>RPARP-AS1</i>	F: GCCCGGCCTATTACTTTTC R: TTTTGGCTTCTCCCATGAC
#8: <i>FOXP4-AS1</i>	F: AAAACCACGTGCACCAAAC R: AGCAGACCTGGAGCTGTCAT
#9: <i>HOXC-AS1</i>	F: CATGTCCACCCATAAGCAGA R: GTGTCGCAGAGATGGAGTTG
#10: <i>SNHG19</i>	F: GCACCACTTTGTCCCTTAGC R: ATAGACACAGCGCCACCT
#11: <i>ELFN1-AS1</i>	F: GCCTCAGCCACAATCGTAAT R: CAGGTTCTTCAGCCAGGAAG
#12: <i>ERVK3-1</i>	F: TGGCCATGCTAGCTGTAGTG R: GAGTTGGTCACGACCCTCAT
<i>RP11-539I5.1</i>	F: GCAGCAGCTACCACACAAAG R: TTGGCTAACAGGCCCATAAC
<i>TPPPP</i>	F: ACTGGACAGGGTCAAGGATG R: GCTCAGAAGGATGCCTGAAC
<i>ELF3-AS1</i>	F: AGAATCCACACGGAATCCAC R: CTGACTGAACCCAAGCCATT
<i>RORB-AS1</i>	F: CAGCACTGCTCTTCTGCTTG R: GACAGGAGCAGTTTGGAAGC
<i>FOLH1-intron</i>	F: AGGGAAGAGGTCAGAGCACA R: GCTTCAGCTTCGTAGCCACT

<i>LOC100294145</i>	F: GGTCTGTACCTGAGCCTGGA R: CATGCTGAGGTTCCCAATTT
<i>HOXC-intron</i>	F: ATCTGTTTCTCCCCCTGCTT R: AGGTCTTGTCTCCTGGCTCAGA
<i>ARLNC1</i>	F: ACCTTGTCCACTGGAACCTCG R: ATCCGCAAAGGCACTATTTTC
<i>RN7SL1</i>	F: GGAGTTCTGGGCTGTAGTGC R: ATCAGCACGGGAGTTTTGAC
