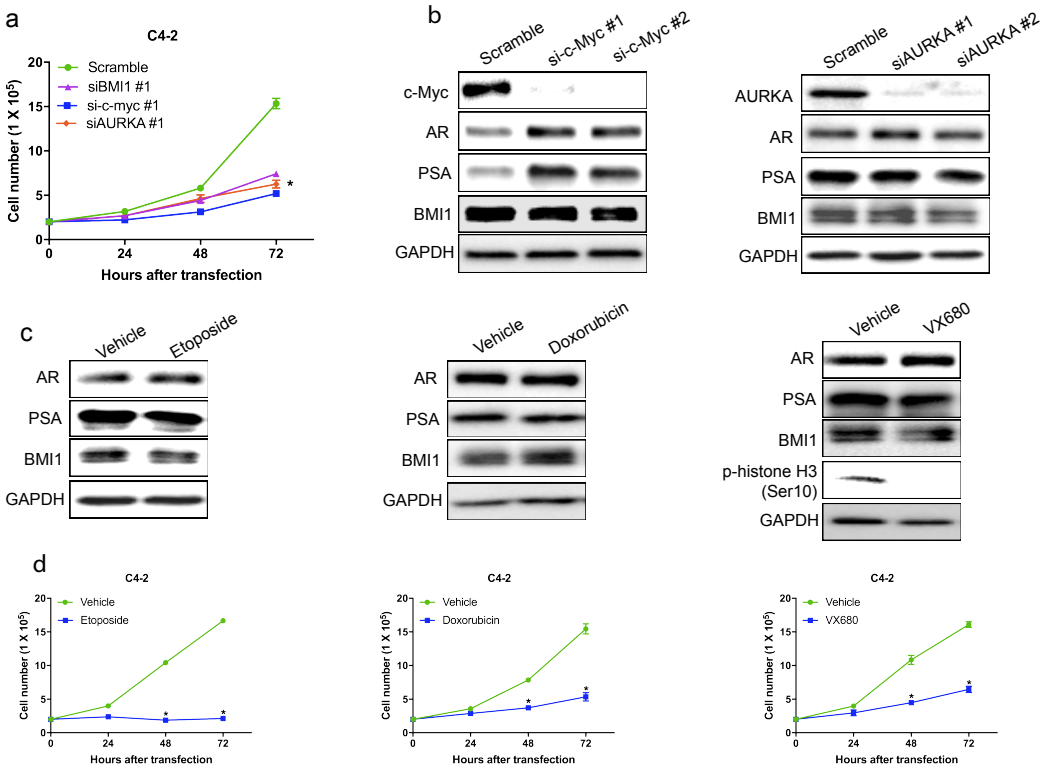
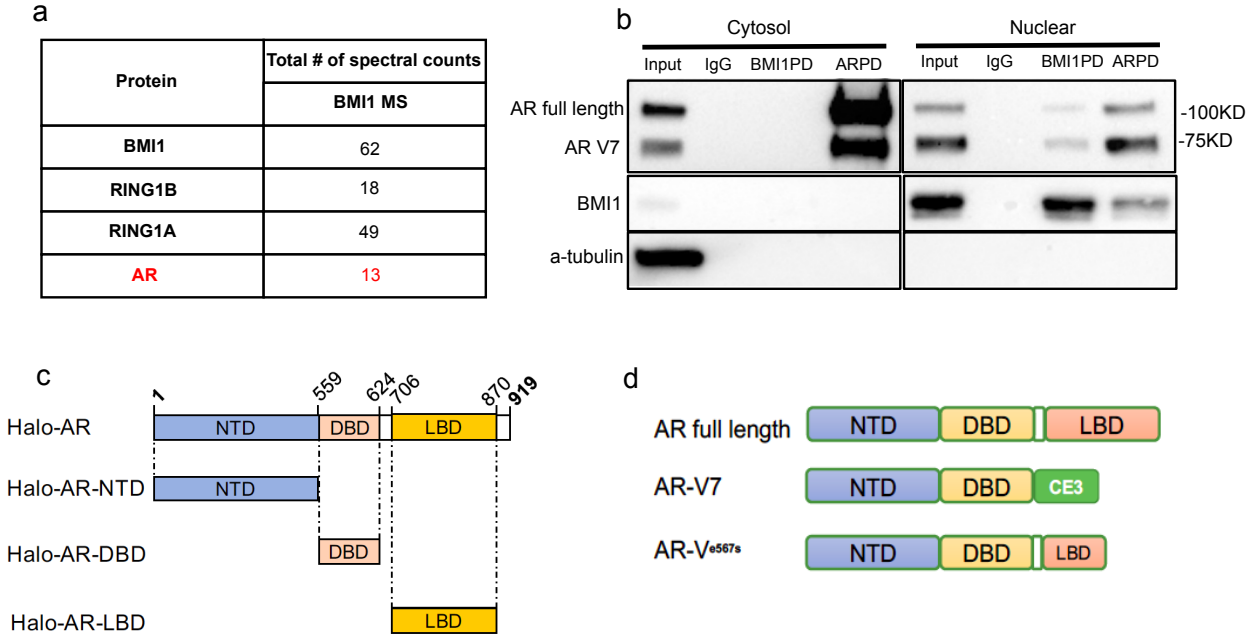


Supplementary Figure 1 | BMI1 knockdown inhibits cell growth, and cell growth arrest has no effect on AR and PSA levels

(**a**) 2×10^5 C4-2 cells were plated in six-well plates, grown without antibiotics for 12h, and then transfected with siBMI1, si-c-Myc, siAURKA or Scramble control, the number of cells was counted at indicated time-points. *p<0.05 vs. Scramble (Mean ± SEM, n=3). (**b**) C4-2 cells were transfected with si-c-Myc, siAURKA or Scramble control and lysed after 48hrs, followed by immunoblot analysis with anti-AR, PSA, BMI1, c-Myc, AURKA and GAPDH antibodies. (**c**) C4-2 cells were treated with Etoposide (2μM), Doxorubicin (50nM) or VX680 (5 μM) for 48h. AR, PSA, BMI1, p-histone H3(Ser10) and GAPDH were tested by western blot. (**d**) 2×10^5 C4-2 cells were plated in six-well plates, grown for 12h, then treated with Etoposide (2μM), Doxorubicin (50nM) or VX680 (5 μM), the number of cells was counted at indicated time-points. *p<0.05 vs. Scramble (Mean ± SEM, n=3)

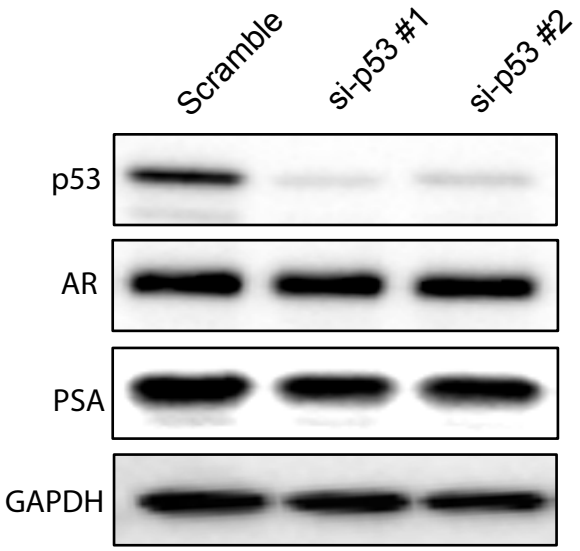
a

Protein	Total # of spectral counts
	BMI1 MS
BMI1	62
RING1B	18
RING1A	49
AR	13



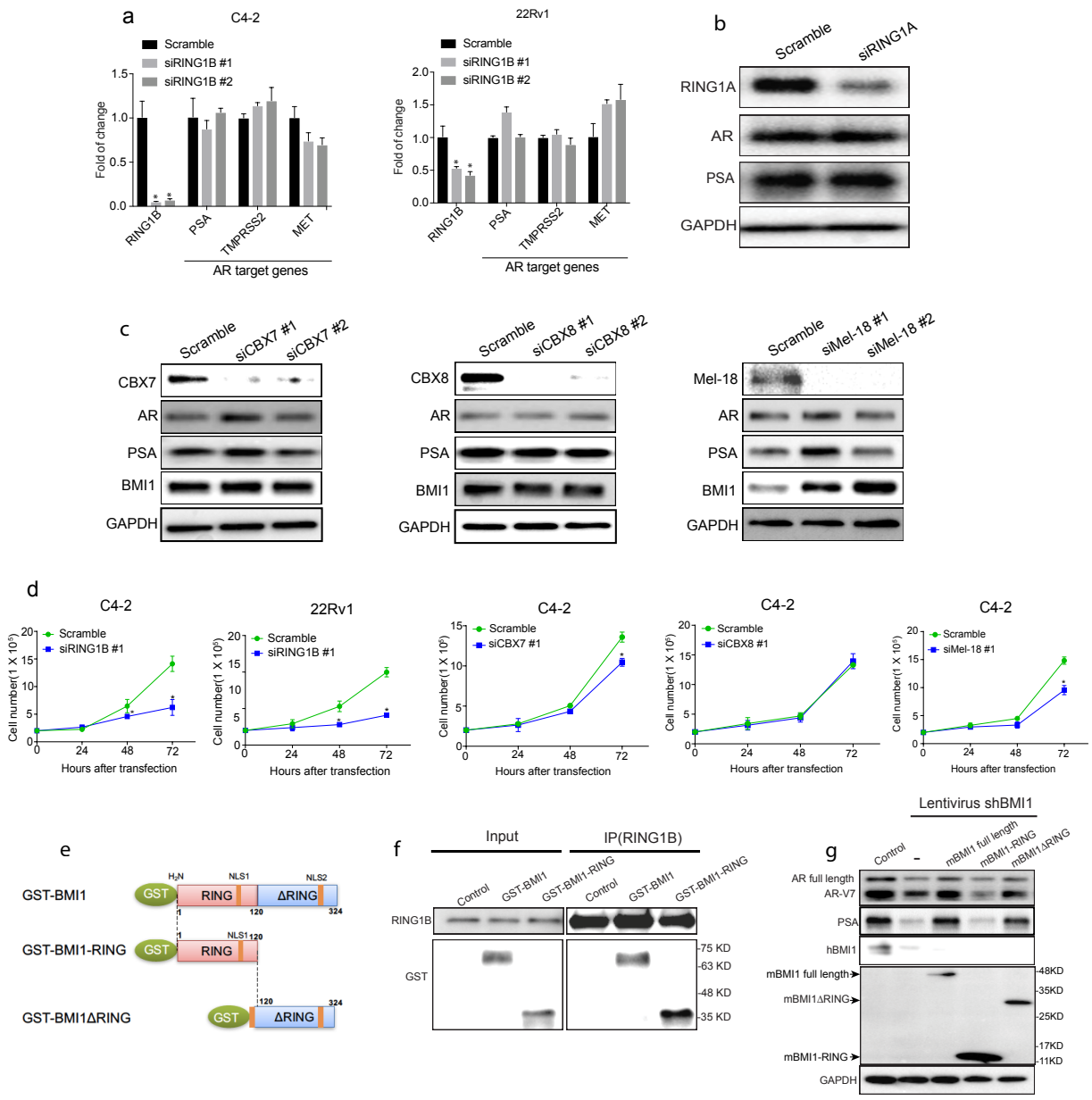
Supplementary Figure 2 | BMI1 interacts with AR

(a) Protein-protein interactions were identified by BMI1 pull-down experiment and Mass Spectrometry in VCaP cells. The number of peptides identified for each protein is indicated. **(b)** 22Rv1 cells were grown in charcoal-stripped serum for 48 hours; cytosol/nuclear fraction was subjected to pulldown assay using anti-BMI1 or anti-AR antibody; rabbit IgG used as control. **(c)** N-terminal domain (NTD), DNA binding domain (DBD), Ligand binding domain (LBD). **(d)** Domain structure of AR, cancer-associated missense mutations and splice variants: V7 and V^{e567s}.



Supplementary Figure 3 | P53 knockdown has no effect on AR signaling

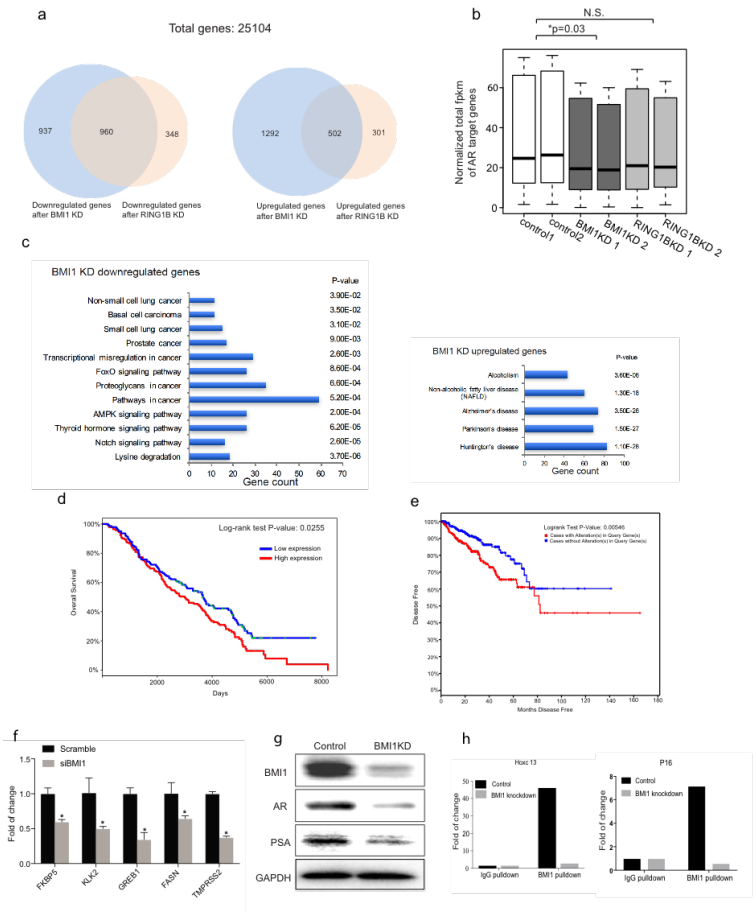
C4-2 cells were transfected with siP53 or Scramble and lysed after 48hrs, p53, AR, PSA, and GAPDH protein level was presented.



Supplementary Figure 4 | BMI1 regulates AR signaling independently of PRC1

(a) RING1B were depleted by 2 independent strands of siRNA; after 48h mRNA was extracted, RT- and quantitative PCR were performed to test AR target genes. *p<0.05 vs. Scramble (normalized to β-actin mRNA, Mean ± SEM, n=3). **(b)** C4-2 cells were transfected with siRING1A or Scramble and lysed after 48hrs; RING1A, AR and PSA protein level were presented. **(c)** C4-2 cells were transfected with siCBX7, siCBX8, siMel-18 or Scramble control and lysed

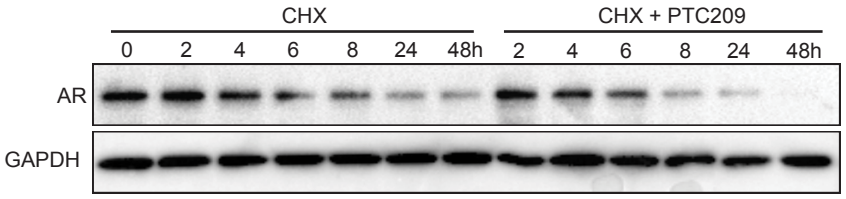
after 48hrs, followed by immunoblot analysis with anti-CBX7, CBX8, Mel-18, AR, PSA, BMI1 and GAPDH antibodies. **(d)** 2×10^5 C4-2 or 22Rv1 cells were plated in six-well plates, grown without antibiotics for 12h, and then transfected with indicated siRNA duplexes. The number of cells was counted at indicated time-points. *$p<0.05$ vs. Scramble (Mean ± SEM, n=3). **(e)** Schematic of BMI1 structures [1, 2, 3]. **(f)** GST-BMI1 or GST-BMI1-RING was incubated with RING1B for 12h followed by IPs with an anti-RING1B antibody. **(g)** 22Rv1 cells were infected with shBMI1 lentivirus, at 24hrs post infection cells were infected with flag-tagged mouse full length BMI1 lentivirus, mouse BMI1-RING lentivirus, or mouse BMI1ΔRING lentivirus as indicated; cells were lysed after another 24 hrs and probed by indicated antibodies. All experiments were biologically repeated at least three times. Representative images are shown.



Supplementary Figure 5 | RNA-Seq analysis shows BMI1 regulates AR independently of PRC1

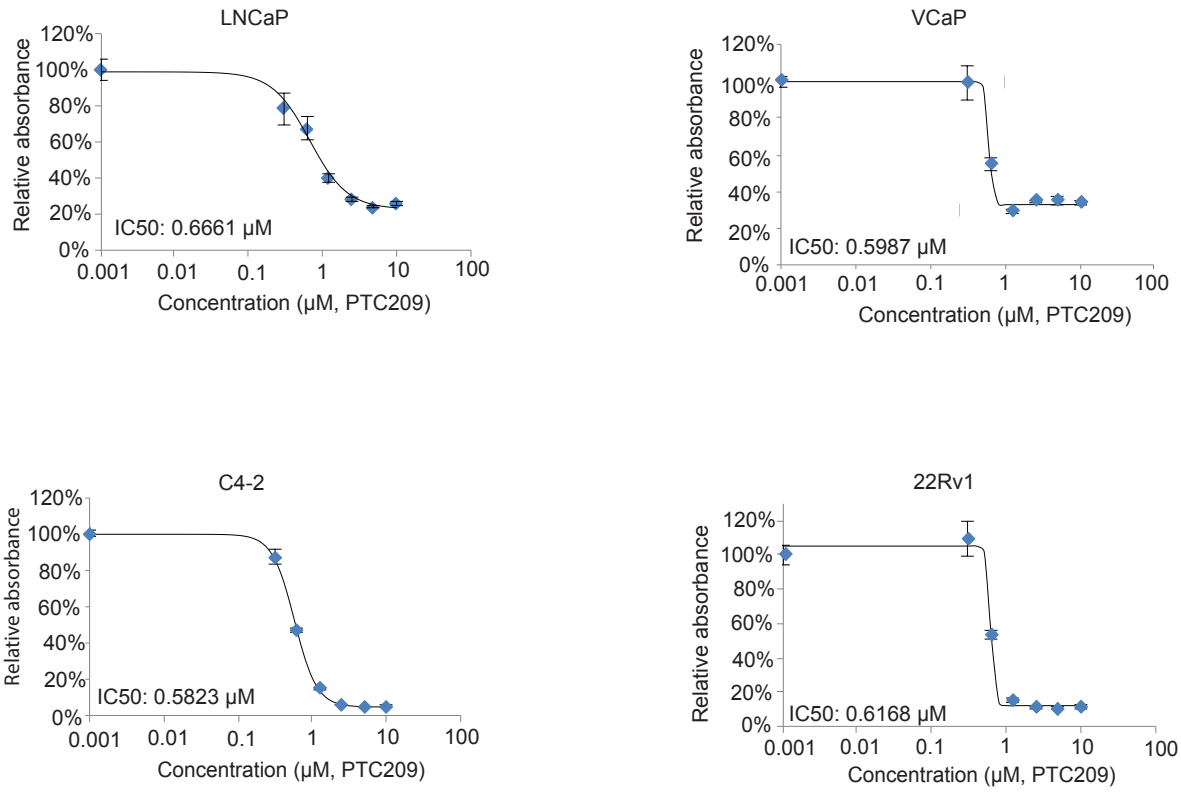
(a) Venn diagram shows that genes down or up regulated by BMI1 knockdown overlap with genes down or up regulated by RING1B knockdown, respectively. (**b)** Boxplot for expression level of 113 AR target genes collected from literatures (**Supplementary Table** 4). (**c)** KEGG pathways analysis shows that down regulated genes are associated with cancer pathways after BMI1 knock down. (**d)** Kaplan-Meier (KM) analysis of prostate cancer patient survival based on expression level of 53 genes that are most significantly activated by BMI1. Gene expression data were collected from the PROGgene database [4]. **(e)** Kaplan-Meier (KM) analysis of prostate cancer relapse based on mutation data of 53 genes that are most significantly activated by BMI1. TCGA

mutation data were collected from the Cbioportal database [5, 6]. **(f)** C4-2 cells were trasfected with BMI1 or control siRNA for 48 hours and then the total RNA was extracted, followed by RT- and quantitative PCR in order to measure the expression levels of AR activated genes. *$p<0.05$ vs. Scramble (normalized to β-actin, Mean ± SEM, n=3). **(g)** C4-2 were transfected with shBMI1 lentivirus or vector lentivirus, after 5 days total cell lysates were blotted for BMI1, AR and PSA, GAPDH as loading control. **(h)** BMI1 and IgG ChIP was conducted in C4-2 cells transfected with shBMI1 lentivirus or vector virus for 5 days. ChIP-qPCR was conducted using gene-specific primers.



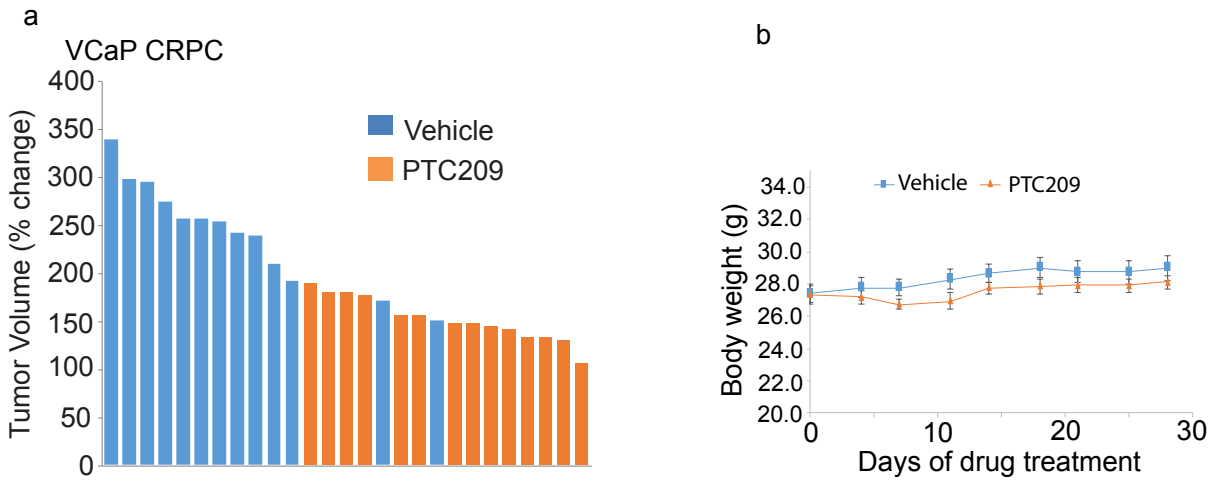
Supplementary Figure 6 | PTC209 treatment shortens AR half-life.

C4-2 cells were treated with PTC209 (5μM) or PTC209 (5μM) + CHX (10μg ml^{-1}) for the time indicated. Total cell lysates were blotted for AR, while GAPDH as loading controls.



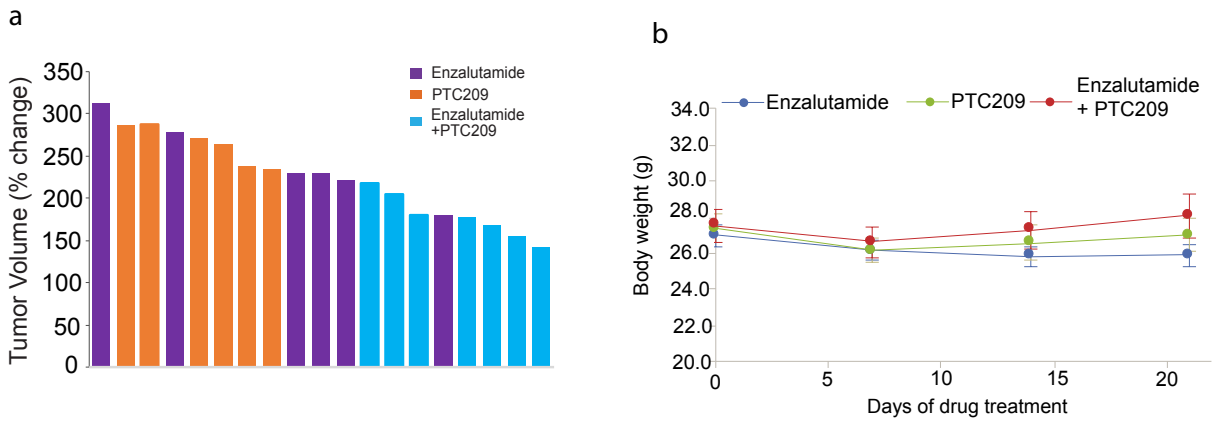
Supplementary Figure 7 | PTC209 inhibits prostate cancer cell proliferation.

SDA log-dose response analysis (IC50) of PTC209 in prostate cancer cell lines as indicated, Mean ± SEM.



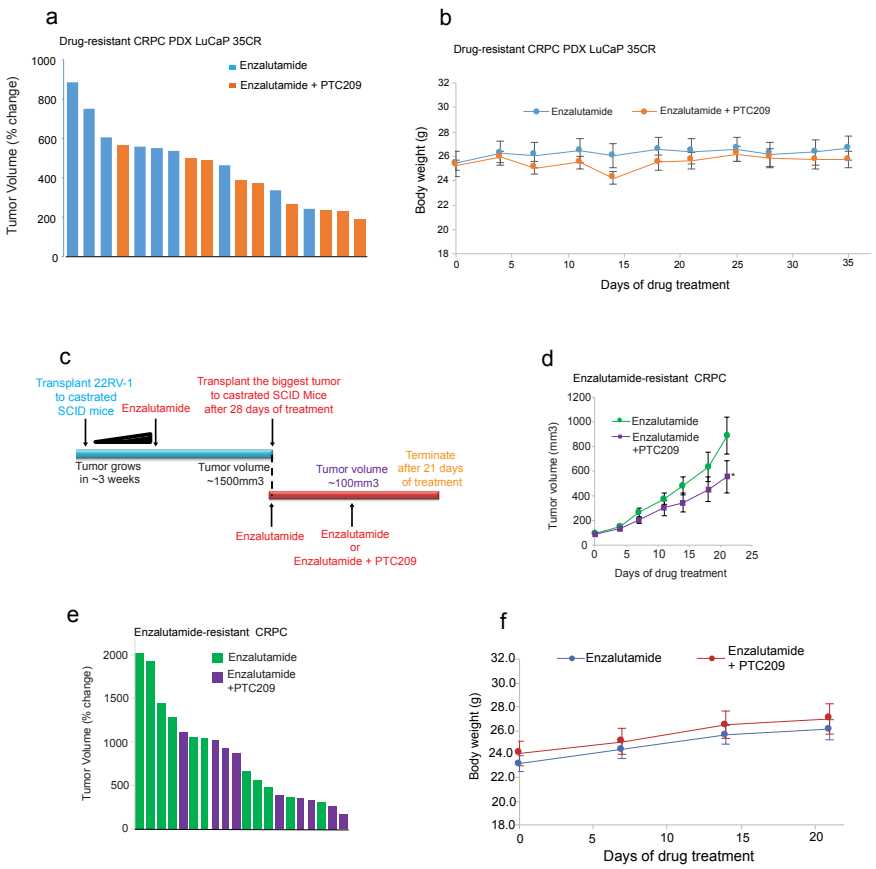
Supplementary Figure 8 | PTC209 treatment inhibits the growth of CRPC tumors

(a) Castrated mice carrying Castration-resistant VCaP xenograft received vehicle or PTC209 (60 mg kg^{-1} per day) 5 days per week. Waterfall plot of tumor volume response is shown. (**b)** Body weight of mice of different groups as indicated are shown, Mean ± SEM.



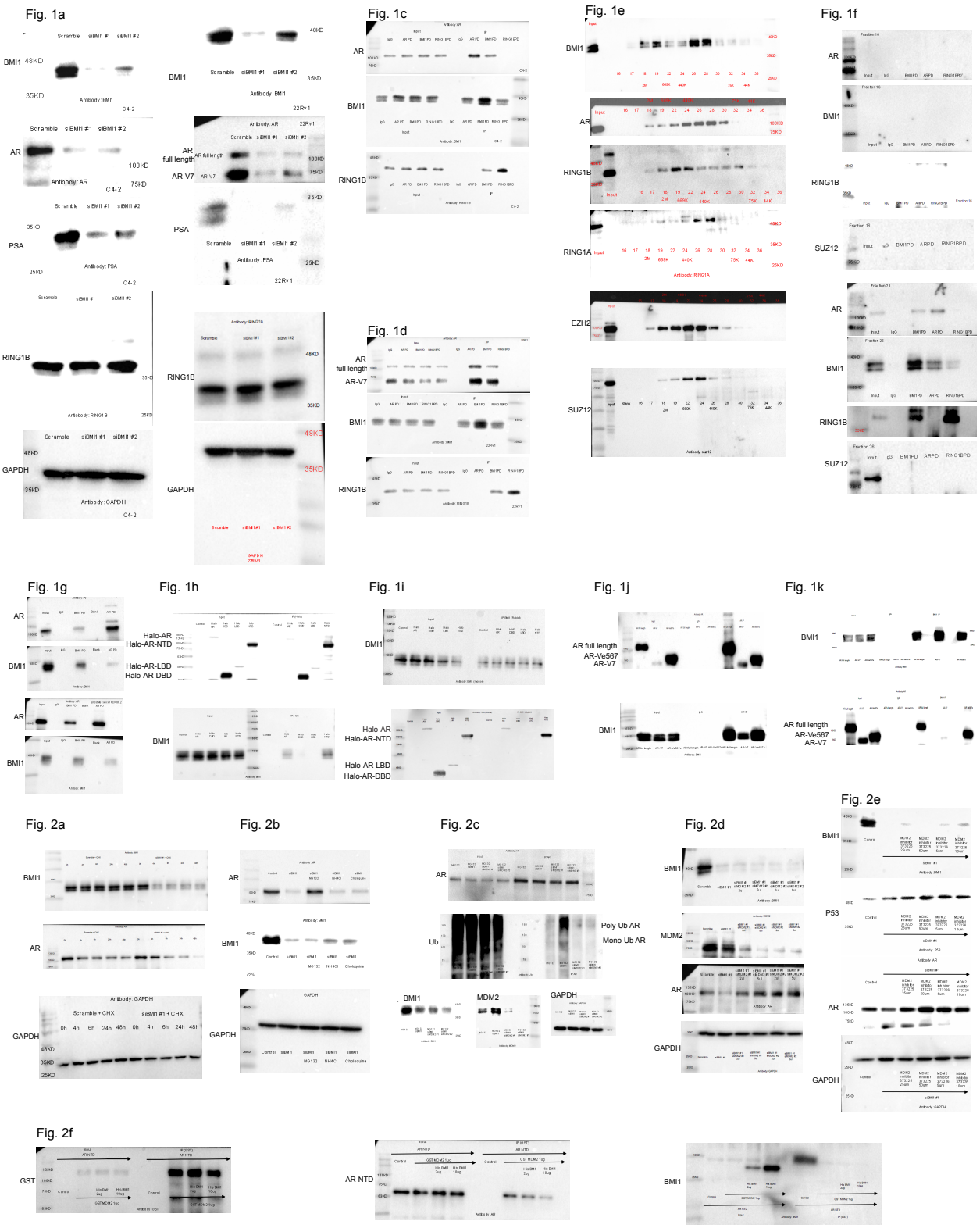
Supplementary Figure 9 | Combinatorial treatment of PTC209 and enzalutamide shows significantly better outcomes compared to each treatment alone.

(a) Castrated mice carrying CRPC xenograft received enzalutamide (10 mg kg^{-1} per day), PTC209 (60 mg kg^{-1} per day), or PTC209 (60 mg kg^{-1} per day) + enzalutamide (10 mg kg^{-1} per day) 5 days per week. Waterfall plot of tumor volume response is shown. **(b)** Body weight of mice of different groups as indicated are shown, Mean ± SEM.



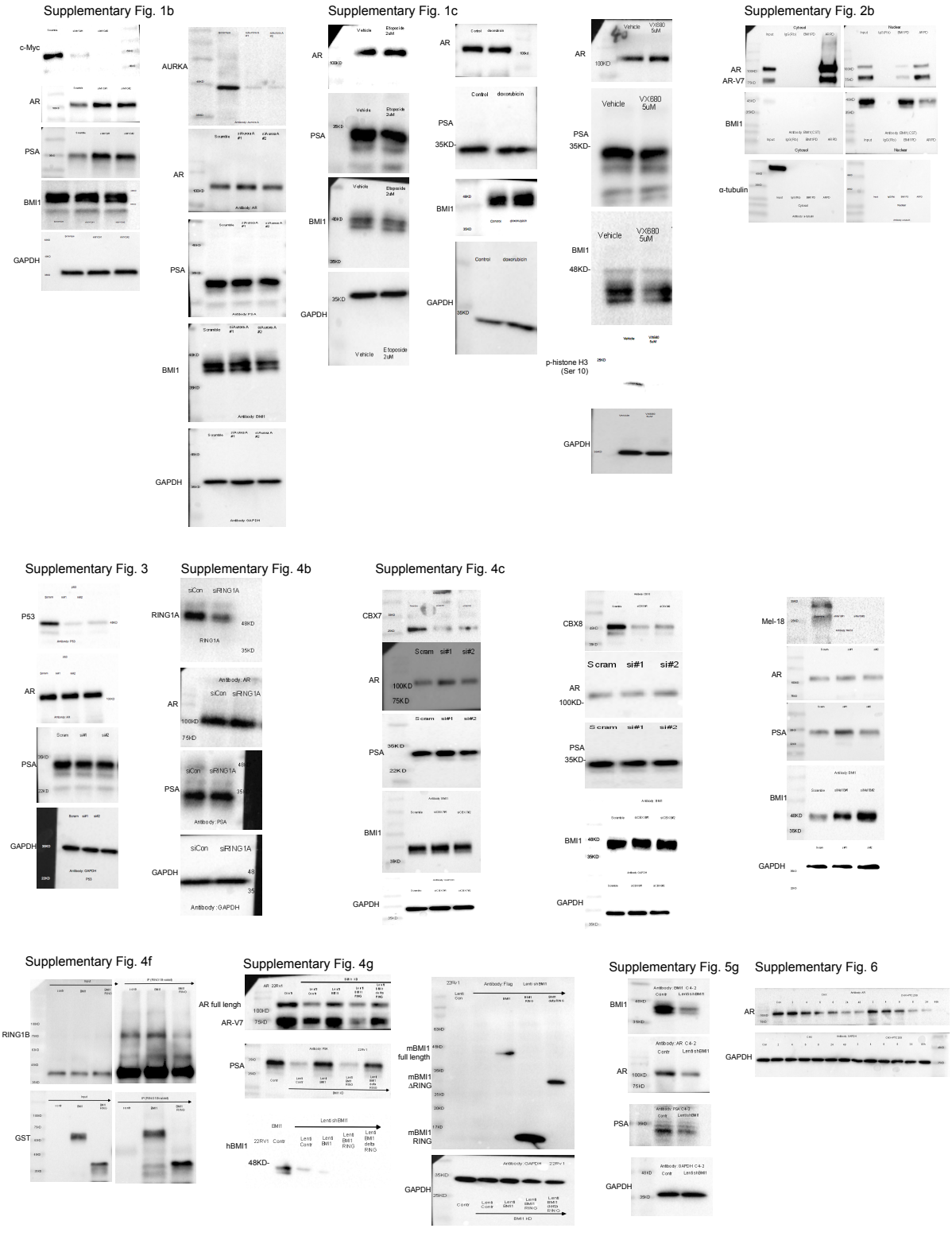
Supplementary Figure 10 | PTC209 inhibits drug-resistant CRPC progression *In Vivo*

(a&b) Castrated mice carrying LuCaP 35CR received Enzalutamide (10mg kg^{-1} per day) or PTC209 (60 mg kg^{-1} per day) + Enzalutamide (10mg kg^{-1} per day), 5 days per week (n=9 per group). Caliper measurements were taken every 4 days to obtain. **(a)** Waterfall plot of tumor volume response is shown. (**b)** Body weight of mice of different groups as indicated are shown, Mean ± SEM. **(c)** Enzalutamide-resistant CRPC mouse xenograft experimental design is illustrated. **(d-f)** Castrated mice carrying Enzalutamide-resistant CRPC xenograft received Enzalutamide (10 mg kg^{-1} per day), Enzalutamide (10 mg kg^{-1} per day) + PTC209 (60 mg kg^{-1} per day) 5 days per week (n=9 per group); **(d)** Mean tumor volume ± SEM; **(f)** Waterfall plot of tumor volume response is shown. *P<0.05, Enzalutamide+PTC209 vs. Enzalutamide. (f) Body weight of mice of different groups as indicated are shown, Mean ± SEM.



Supplementary Figure 11 | Unprocessed blot images for the western results in Fig. 1 and 2

Supplementary Figure 12 | Unprocessed blot images for the western results in Fig. 3, 5 and 6



Supplementary Figure 13 | Unprocessed blot images for the western results in Supplementary Figures

Supplementary Table 1 | Antibodies

	Host	Company	Cat#	Purpose(dilution)
AR	rabbit	Millipore	06-680	IP, Immunoblot(1:1000)
BMI-1(D20B7)	rabbit	Cell signaling	6964s	IP, Immunoblot(1:1000)
MDM2(SMP14)	mouse	Santa Cruz	sc-965	Immunoblot(1:1000)
RING1A	rabbit	Cell signaling	2820s	Immunoblot(1:1000)
RING1B	rabbit	proteintech	16031-1-AP	IP, Immunoblot(1:1000)
Ubiquityl-Histone H2A(K119)	rabbit	Cell signaling	8240s	Immunoblot(1:1000)
Histone H3	rabbit	Cell signaling	9715s	Immunoblot(1:1000)
Histone H2A (D603A)	rabbit	Cell signaling	12349s	Immunoblot(1:1000)
Prostate-Specific Antigen (PSA)	rabbit	DAKO	A0562	Immunoblot(1:1000)
Ubiquitin (P4D1)	mouse	Cell signaling	3936s	Immunoblot(1:1000)
GFP (B-2)	mouse	Santa Cruz	sc-9996	Immunoblot(1:1000)
His-probe (H-3)	mouse	Santa Cruz	sc-8036	Immunoblot(1:1000)
HA-probe (F-7)	mouse	Santa Cruz	sc-7392	Immunoblot(1:1000)
GST (I-5)	rabbit	Santa Cruz	sc-459	Immunoblot(1:1000)
GST (B-14)	mouse	Santa Cruz	sc-138	Immunoblot(1:1000)
DYKDDDK Tag	rabbit	Cell signaling	147935	Immunoblot(1:1000)
Flag (M2.Ab)	mouse	Cell signaling	F1804	Immunoblot(1:1000)
SUZ12	rabbit	Cell signaling	3737s	Immunoblot(1:1000)
EZH2	rabbit	Cell signaling	5246	Immunoblot(1:1000)
p53	mouse	Santa Cruz	sc-126	Immunoblot(1:1000)
CBX7	mouse	Santa Cruz	sc-376274	Immunoblot(1:1000)
CBX8	mouse	Santa Cruz	sc-374332	Immunoblot(1:1000)
Mel-18	mouse	Santa Cruz	sc-390868	Immunoblot(1:1000)
c-Myc	mouse	Santa Cruz	sc-40	Immunoblot(1:1000)

Supplementary Table 1 | Antibodies (Continued)

	Host	Company	Cat#	Purpose(dilution)
AURKA	rabbit	Millipore	04-1037	Immunoblot(1:1000)
p-Histone H3 (Ser 10)	rabbit	Santa Cruz	Sc-8656-R	Immunoblot(1:1000)
Halo tag	mouse	Promega	G921	Immunoblot(1:1000)
GAPDH(6cs)	mouse	Santa Cruz	sc-32233	Immunoblot(1:5000)
α-tubulin	Mouse	Cell Signaling	3873	Immunoblot(1:2000)
normal mouse IgG		Santa Cruz	sc-2025	IP,ChIP
normal rabbit IgG		Santa Cruz	sc-2027	IP,ChIP
Clean-Blot IP Detection Reagent (HRP)		Thermo Scientific	21230	IP
Goat anti-Mouse IgG (H+L)-HRP		GenDEPOT	SA001-500	IP, Immunoblot(1:3000)
Goat anti-Rabbit IgG (H+L)-HRP		GenDEPOT	SA002-500	IP, Immunoblot(1:3000)

Supplementary Table 2 | Oligonucleotide primers for RT-PCR

Gene Name	Forward primer	Reverse primer
BMI1	CCA TTGAATTCTTTGACCAGA A	CTGCTGGGCATCGTA AGTATC
AR	CAAATCACCCCCCAGGAAT	CACTGGAATAATGCTGAAGAGTAGCA
PSA	ACCTGCACCCGGAGAGCT	TCACGGACAGGGTGAGGAAG
TMPRSS2	AGAATCGGTGTGTTCGCCTC	CTCGTTCCAGTCGTCTTGGC
RING1B	ATGGCACACAGAACAATGGA	ATGTGGCAACCCAAAATGAT
MET	CGCTGACTTCTCCACTGGTT	TACACTCCCCATTGCTCCTC
FKBP5	TCTCATGTCTCCCCAGTTCC	TTCTGGCTTTCACGTCTGTG
KLK2	GCTGCCCATTGCCTAAAGAAG	TGGGAAGCTGTGGCTGACA
GREB1	AAGGAGGGCTGGAAACAAAT	CATTGTGGCCATTGTCATCT
FASN	CAGCCATGGAGGAGGTGGTGATT	CGAAGAAGGAGGCATCAAACCTA
β-actin	GACAGGATGCAGAAGGAGATCACT	GCGCTCAGGAGGAGCAATG

Supplementary Table 3 | Oligonucleotide primers for ChIP-qPCR

Gene Name	Forward primer	Reverse primer
PSA	GCCTGGATCTGAGAGAGATATCATC	ACACCTTTTTTTTTCTGGATTGTTG
FKBP5	GGTTCCTGGGCAGGAGTAAG	AACGTGGATCCCACACTCTC
TMPRSS2 enhancer	TGGAGCTAGTGCTGCATGTC	CTGCCTTGCTGTGTGAAAAA
Hoxc 13	GAGGGAACCCCAGGAGAC	CGCTCTCCACCTCTCAGC
P16	GCACTCAAACACGCCTTTGC	AGAGCCAGCGTTGGCAAGGA

Supplementary Table 4

Gene Symbol	Reference	Gene Symbol	Reference	Gene Symbol	Reference
ABCC4	[7]	GREB1	[8]	PATZ1	[8]
ABCG1	[8]	HERC3	[7]	PHF21A	[8]
ACAA1	[8]	HMGCR	[9]	PMEPA1	[7 8 9]
ACAT2	[8]	HMGCS1	[9]	PMS2	[8]
ACOT7	[8]	HOXC13	[8]	POLE2	[8]
ACSL3	[7]	HPGD	[9]	PPAP2A	[8]
ADAM7	[7]	IDI1	[8]	PPP2R2A	[8]
AR	[9]	INSIG1	[9]	PTGER4	[7]
ATAD2	[8]	IQGAP2	[8]	PTPN21	[9]
ATP1B1	[8]	JARID2	[8]	RAB3B	[9]
BIRC5	[8]	KCNN2	[8]	RFC3	[8]
C1orf116	[8]	KLK2	[7 8 9]	RHOU	[9]
CAMKK2	[8]	KLK3	[7 8 9]	SCAP	[9]
CCNE1	[8]	KLK4	[9]	SDC4	[8]
CCNG2	[8]	LDLR	[9]	SESN1	[8]
CDC25A	[8]	MAF	[7 9]	SGK1	[9]
CDC6	[8]	MAFB	[8]	SGK3	[8]

Supplementary Table 4 (Continued)

Gene Symbol	Reference	Gene Symbol	Reference	Gene Symbol	Reference
CDKN1A	[8]	MALT1	[8]	SI	[10]
CEBPD	[8]	MCCC2	[8]	SLC45A3	[9]
CENPN	[7 9]	MCM3	[8]	SMAD1	[8]
CXCR4	[9]	MCM4	[8]	SMPD4	[8]
DBI	[9]	MCM5	[8]	SNRK	[8]
DDC	[10]	MED28	[7]	SPDEF	[8]
DHRS7	[8]	MET	[10]	SQLE	[8]
DTL	[8]	MICAL1	[9]	STK39	[8]
EAF2	[7 9]	MPHOSPH9	[7]	STX3	[8]
EBP	[9]	NBR1	[8]	TAOK3	[8]
EIF4A3	[8]	NCAPD3	[8 9]	TMPRSS2	[7 9]
ELL2	[7 8]	NDRG1	[9]	TULP4	[8]
ELOVL5	[8]	NFAT5	[8]	UAP1	[8]
ENDOD1	[8]	NFKB1	[8]	VGLL4	[8]
EPHA1	[8]	NKX3-1	[7 8 9]	WIPI1	[9]
FASN	[9]	NNMT	[7]	WWC1	[9]
FEN1	[8]	ODC1	[8]	ZBTB10	[7]
FKBP5	[7 8 9]	OPRK1	[10]	ZBTB43	[8]
FZD5	[8 9]	ORC5	[8]	ZFAND5	[8]
GNMT	[7]	ORM1	[9]	ZNF350	[9]
GRB10	[8]	PAK2	[8]		

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