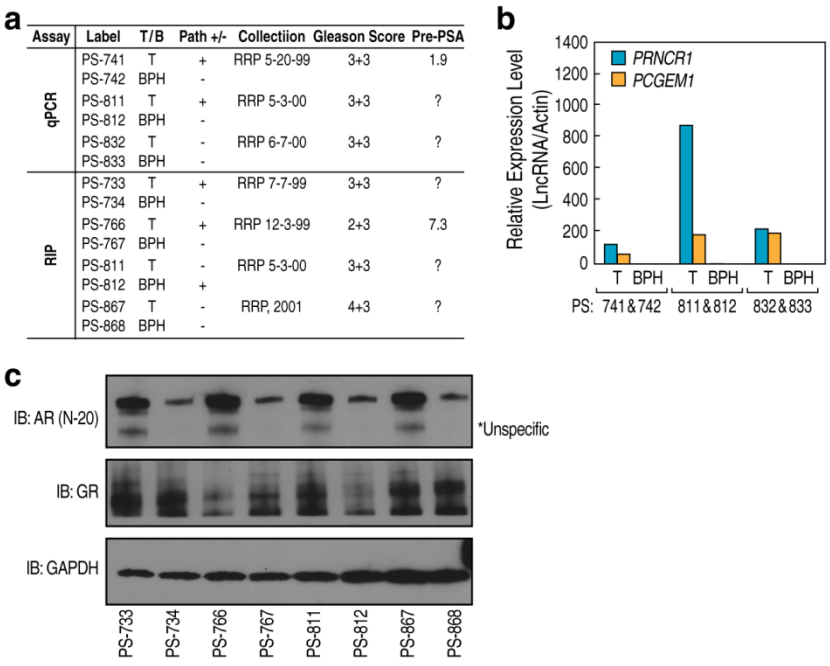
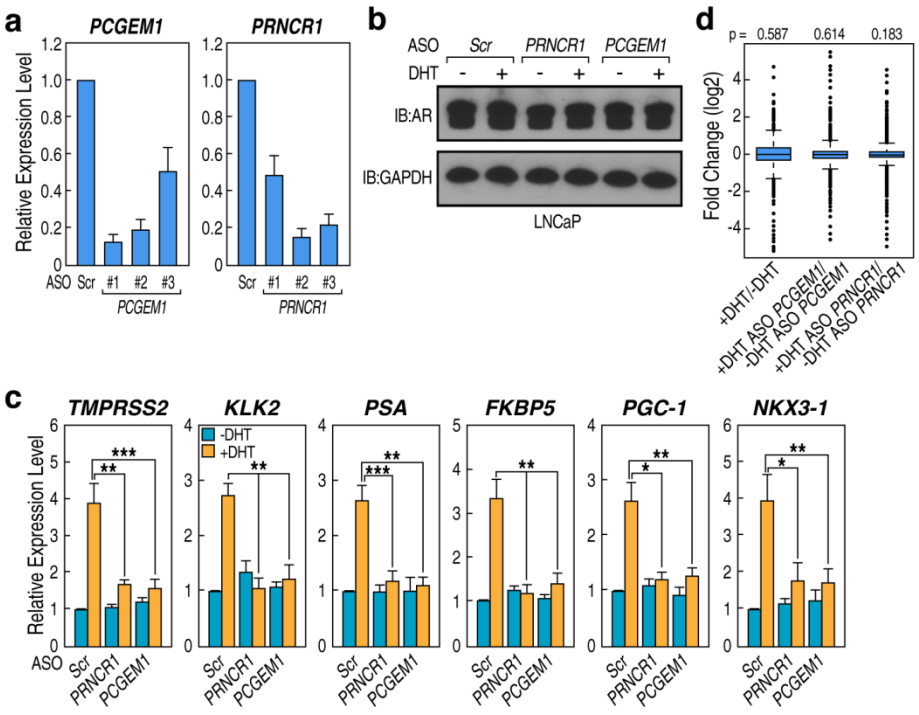
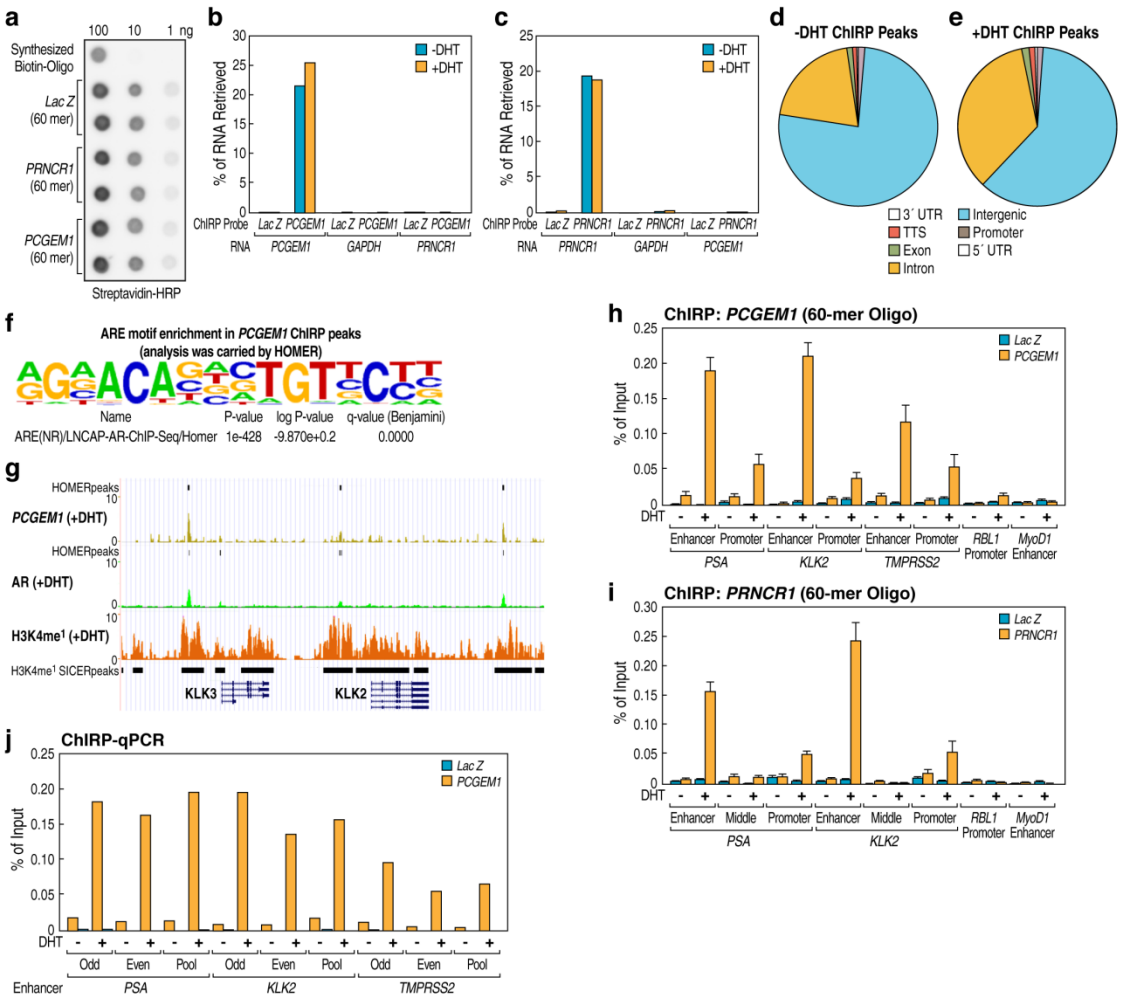


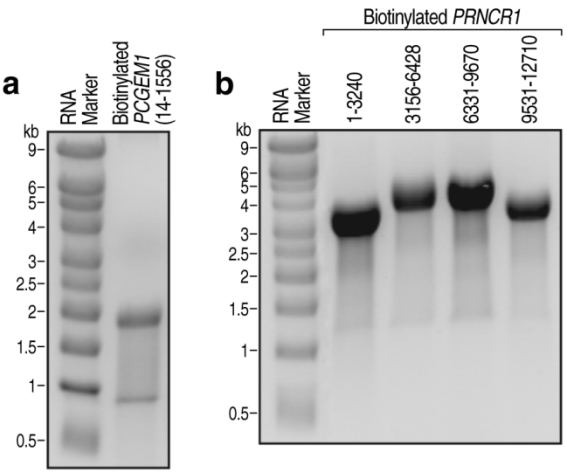
Supplementary Figure 1: **Human prostate tissues and characterization of AR, ER expression level. a**, Clinical information of human prostate cancer tissue (T) and benign prostatic hyperplasia (BPH) used in this study. **b**, Quantitative polymerase chain reaction (qPCR) analyses of *PCGEM1* and *PRNCR1* expression levels in paired benign prostatic hyperplasia (BPT) and tumor (T) derived from individual prostate cancer patients. **c**, Immunoblotting of lysates extracted from indicated tissue samples by AR N-20, GR or GAPDH antibodies.



Supplementary Figure 2: Impaired activation of selected AR target genes upon *PCGEM1* or *PRNCR1* knock-down. a, qRT-PCR analyses of *PCGEM1* (left panel) or *PRNCR1* (right panel) in LNCaP cells transfected with the indicated ASOs. **b**, Immunoblotting analysis showing the endogenous protein level of AR in LNCaP cells transfected with *PRNCR1*, *PCGEM1* or scrambled ASOs, and treated with or without DHT (100 nM) for 1 hr. **c**, Examination of AR target genes in LNCaP cells transfected with indicated ASO followed by DHT (100 nM) treatment for 4 hrs. **d**, Knock-down of either *PRNCR1* or *PCGEM1* had no effect on DHT-unresponsive genes (n=5,675, edgeR FDR > 0.01), that are located at a distance higher than 200kb from AR-bound H3K4me[1] enhancers, corresponding to the samples in **Fig. 1e**. Mean ± SEM (n=3, *p<0.05, **p<0.01 and ***p<0.01).



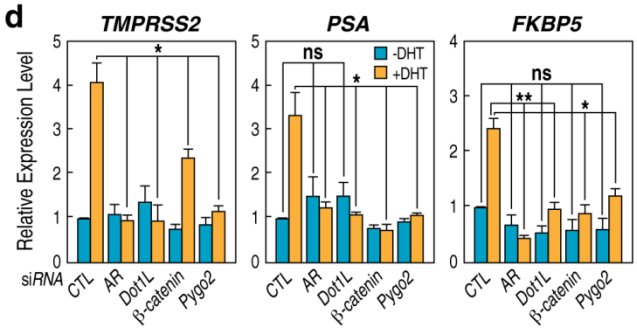
Supplementary Figure 3: Characterization and validation of ChIRP-Seq. a, Dot blot analysis was used to confirm the *in vitro* biotin labeling efficiency of ChIRP probes tiling *LacZ*, *PRNCR1* or *PCGEM1*. Single-stranded 60 nt *in vitro* biotin-labeled ChIRP probes (amount of oligonucleotides in nanogram listed on the top of the blot) were blotted on positively-charged Nylon membrane followed by detection using HRP-Conjugated Streptavidin. A synthesized biotinylated oligonucleotide was included as a positive control. **b** and **c**, Elutes from ChIRP assay using indicated probes from cell lysates of LNCaP cells with or without DHT (100 nM) treatment for 1 hr were subjected to RT-qPCR using primers targeting indicated RNA molecules. **d** and **e**, Genomic distribution of *PCGEM1* ChIRP-Seq peaks in LNCaP cells without (d) or with (e) DHT (100 nM) treatment for 1 hr (*n*= 323 and 2,142, respectively). **f**, *PCGEM1* binding sites (*n*=2,142) are strongly enriched for ARE motif, determined by ChIRP-Seq. **g**, *PCGEM1* occupancy at canonical *KLK2* and *KLK3*/*PSA* enhancers, overlaying regions of AR and H3K4me^{1} occupancy (display in UCSC browser). **h** and **i**, ChIRP–qPCR showing *PCGEM1* (h) or *PRNCR1* (i) occupancy on selected genomic regions in LNCaP cells treated with or without DHT (100 nM) for 1 hr. *RBL1* promoter and *MyoD1* enhancer served as negative controls. **j**, ChIRP–qPCR using Odd, Even or both (Pool) probe mixtures targeting *PCGEM1* to detect the occupancy of *PCGEM1* on selected genomic regions in LNCaP cells treated with or without DHT (100 nM) for 1 hr. Mean ± SEM for panel h and i (*n*=3).



c

PRNCR1 and *PCGEM1* Associated Transcriptional Factors/Coactivators

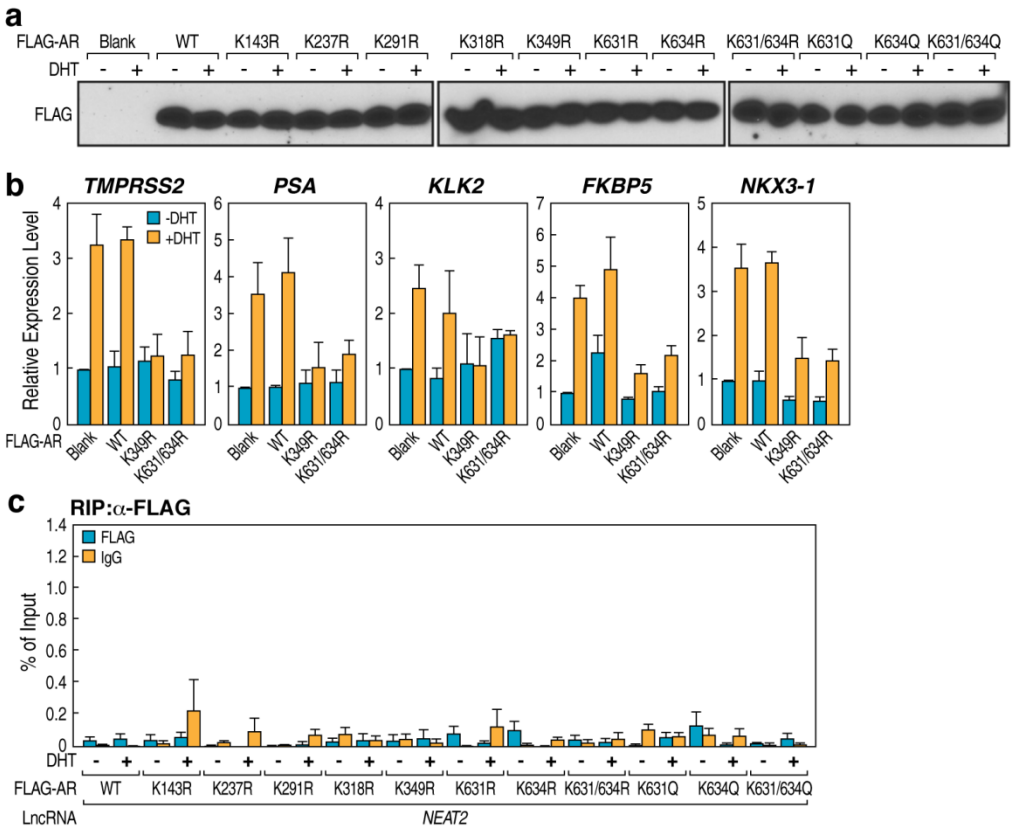
Protein	*Peptides*
Monoavidin Beads only	
protease, serine, 1 preproprotein	1
PREDICTED: hypothetical protein	1
Biotinylated *PRNCR1* LncRNA Pulldown	
DOT1-like, histone H3 methyltransferase	7
coactivator-associated arginine methyltransferase	4
growth arrest and DNA-damage-inducible, alpha	5
androgen receptor isoform 1	3
Biotinylated *PCGEM1* ncRNA Pulldown	
catenin (cadherin-associated protein), beta 1, 88kDa	7
androgen receptor isoform 1	6
pygopus homolog 2	7



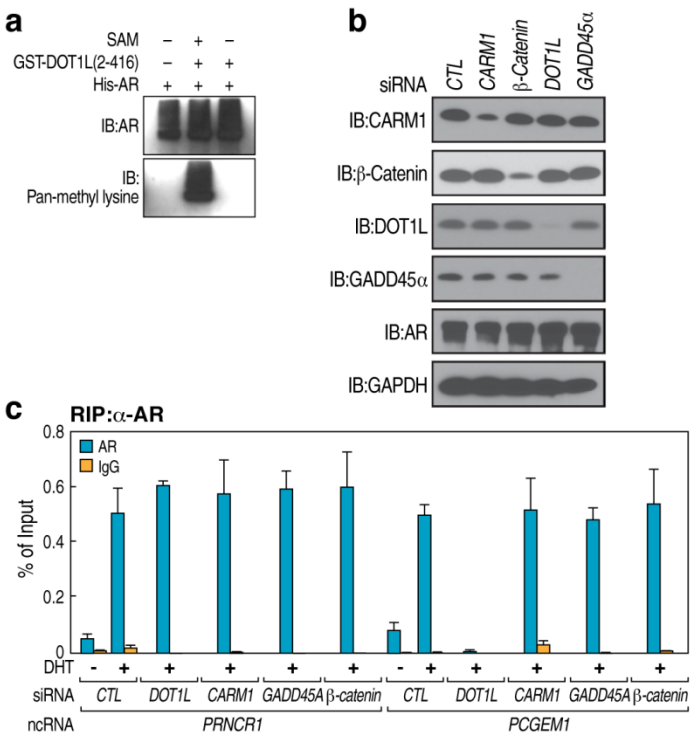
e

Residue	Conf	Sequence	Prec M/Z	Expe M/Z	Modifications
K143	0.065	AASKGLPQQL	1012.17	1105.62	Phospho(S)@3; Methyl(K)@4
K237	46.54	DNYLGGTSTISDNAKE	1684.74	1708.83	Dehydrated(D)@12; Deamidated(N)@13; Acetyl(K)@15
K291	2.446	KGSLLDDS	833.89	927.43	Methyl(K)@1; Phospho(S)@3
K318	0.05	TKGLEGESL	933.03	1026.56	Methyl(K)@2; Phospho(S)@8
K349	0.57	LSLYKSGALDE	1195.34	1288.59	Methyl(K)@5; Phospho(S)@6
K631	19.29	KLKKLGNLKLQEEGEA	1798.1	1854.86	Acetyl(K)@1; Oxidation(K)@9
K634	0.05	LGARKLKKL	1026.32	1083.64	Oxidation(K)@5; Acetyl(K)@8

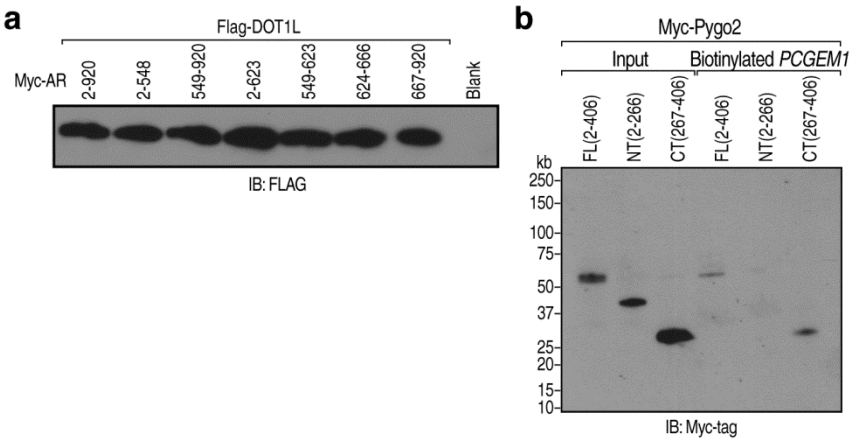
Supplementary Figure 4: Identification of proteins associated with *PRNCR1* and *PCGEM1*. **a** and **b**, Denaturing agarose gel electrophoresis of *in vitro* transcribed biotinylated-*PCGEM1* (a) or *PRNCR1* fragments (b) as indicated. **c**, A list of *PRNCR1* and *PCGEM1* lncRNA-associated proteins identified from MS analysis **d**, qRT-PCR analyses of selected AR targets in LNCaP cells transfected with the indicated siRNAs, and incubated without or with DHT (100 nM) for 4 hrs. **e**, Selective lysine post-translational modifications of *PRNCR1*- and *PCGEM1*-associated AR. The site numbers are for human AR. See the **Supplementary Tables 1-3** for further information. Mean ± SEM (n=3, *p<0.05 and **p<0.01).



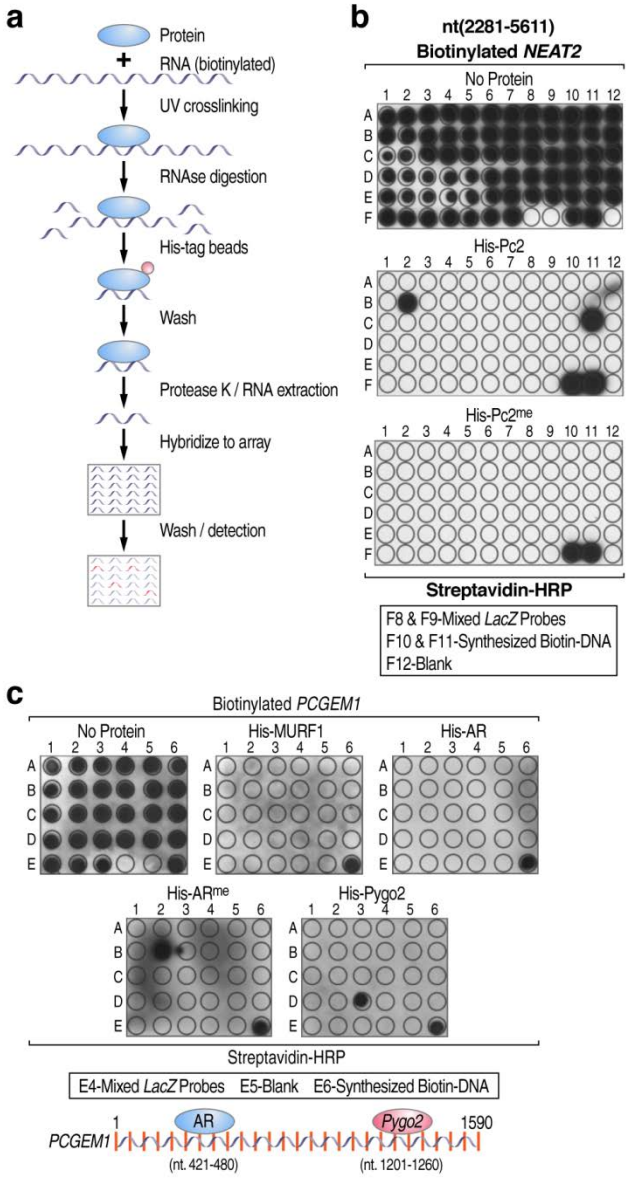
Supplementary Figure 5: The effect of lysine methylation and acetylation of AR on its transcriptional activity. a, Immunoblotting analysis showing the exogenous expression of wild-type AR or mutant in LNCaP cells transfected with Flag-tagged AR or blank vector, and treated with or without DHT (100 nM) for 1 hr. **b**. qRT-PCR analyses of selected AR target genes in LNCaP cells transfected with blank vector or FLAG-tagged AR expression vector (wild-type *vs*. K→R mutant), and treated with or without DHT (100 nM) for 4 hr. **c**, LNCaP cells expressing Flag-tagged AR or indicated mutants were treated with or without DHT (100 nM) for 1 hr and subjected to RIP assay to detect *NEAT2* binding. Mean ± SEM for panel b (*n*=3).



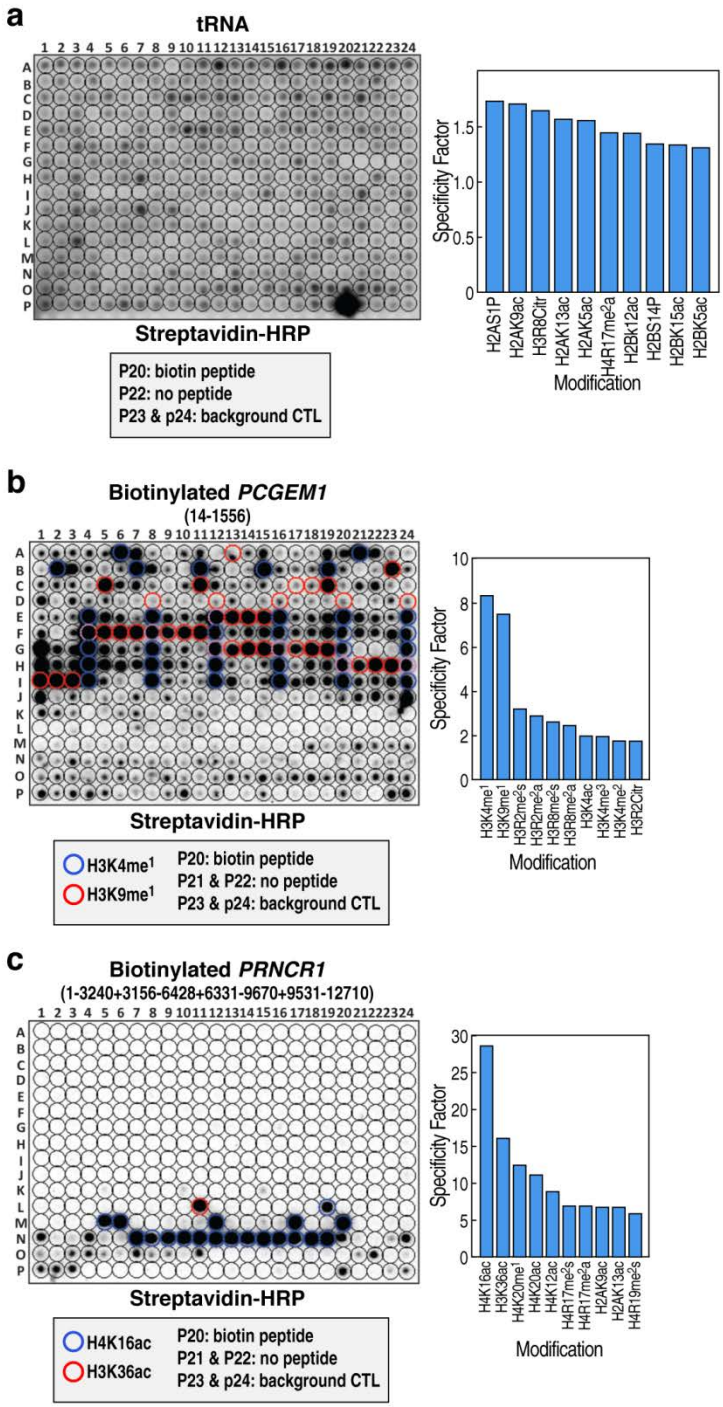
Supplementary Figure 6: DOT1L-mediated AR methylation is required for *PCGEM1* binding. a, *In vitro* methylation of His-tagged AR by recombinant DOT1L (2-416) in the presence of SAM. The signal was detected by Pan-methyl lysine antibody. **b**, LNCaP cells were transfected with indicated siRNAs, followed by immunoblotting using indicated antibodies. **c**, RIP assay in LNCaP cells transfected with the indicated siRNAs, and incubated without or with DHT (100 nM) for 1 hr. Mean ± SEM for panel c (*n*=3).



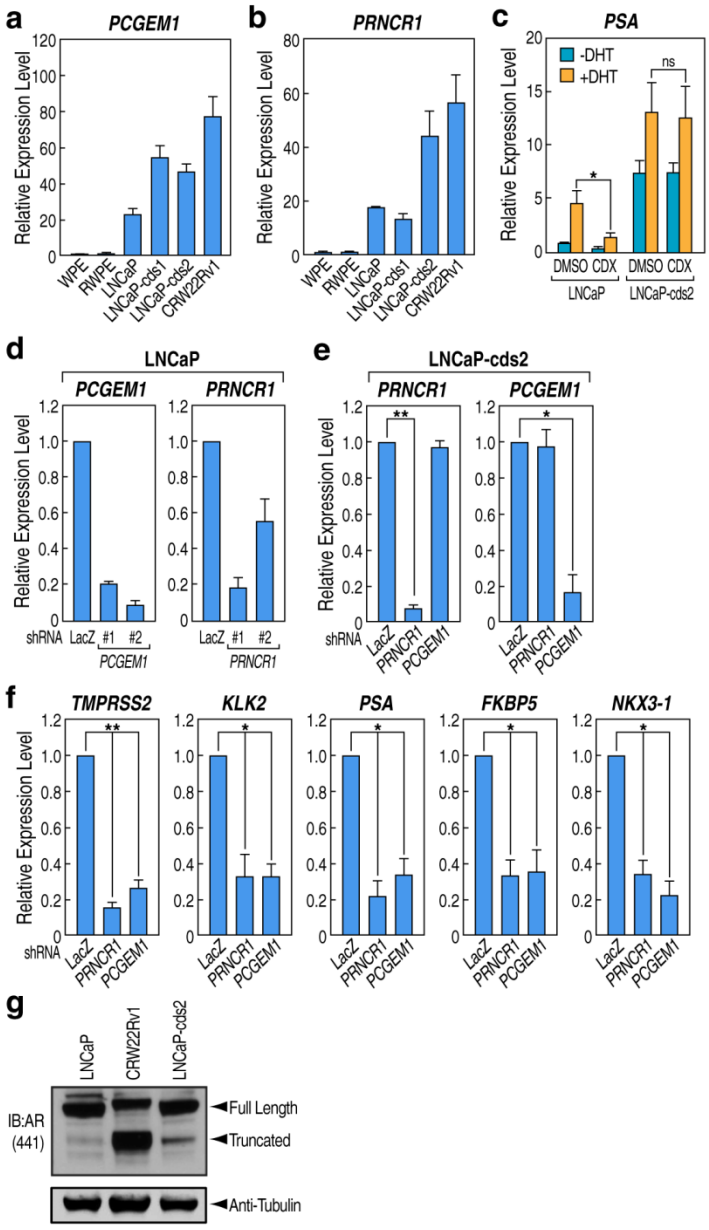
Supplementary Figure 7: Identification of *PCGEM1* binding domain of Pygo2. a, LNCaP cells were co-transfected with Myc-tagged AR fragment or Flag-tagged DOT1L (2-416) or blank vector, followed by immunoblotting using anti-Flag antibody. **b**, LNCaP cells were transfected with expression vectors expressing Myc-tagged Pygo2 full-length, N-terminal or C-terminal fragment as indicated. The cell lysates were incubated with *in vitro* transcribed biotinylated *PCGEM1* transcripts for RNA pulldown assay. The reactions were subjected to immunoblotting using anti-Myc antibody.



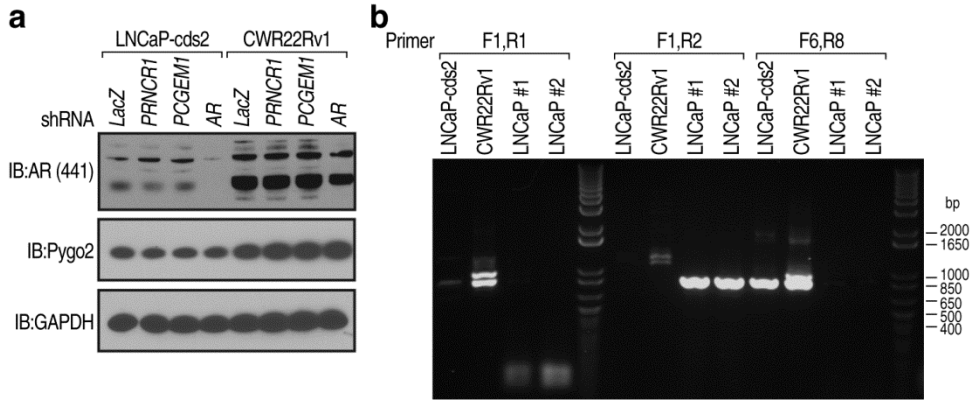
Supplementary Figure 8: Mapping RNA sequence responsible for AR and Pygo2 binding. a, Flow chart of *in vitro* RNA pulldown coupled with dot-blot assay. *In vitro* transcribed biotinylated RNA was formed secondary structure as described and incubated with His-tagged recombinant proteins, followed by UV crosslinking. After partial digestion of RNA, the RNA-protein complexes of interest were purified by His-tag magnetic beads. The recovered RNAs were hybridized to Nylon Membrane spotted with 60-mer antisense DNA oligonucleotides tiling along the indicated lncRNA sequence for detection of Streptavidin-HRP signals, aligned 1-27 from left to right, rows A-E. **b**, No protein control (top panel), His-Pc2 (middle panel) or methylated His-Pc2 (bottom panel) and *in vitro* transcribed *NEAT2* fragment were subjected to *in vitro* RNA pulldown coupled with dot-blot assay. **c**, Top panel: recombinant proteins as indicated and biotinylated *PCGEM1* transcripts were subjected to *in vitro* RNA pulldown assay, followed by dot-blot assay. Bottom panel: schematic graph illustrating the binding sites of *PCGEM1* recognized by AR and Pygo2, respectively.



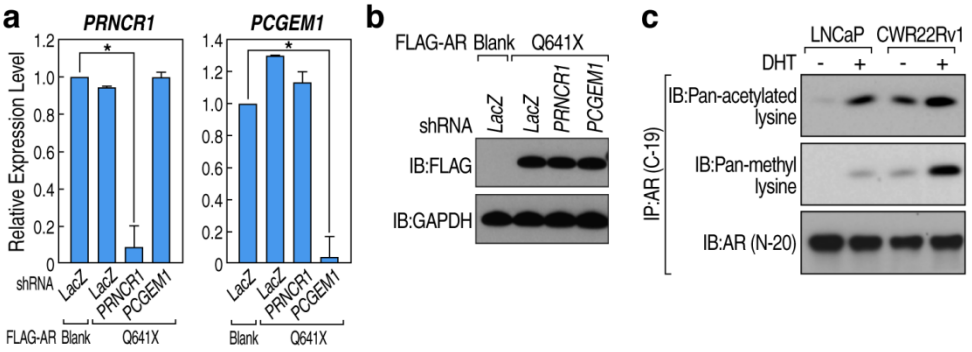
Supplementary Figure 9: Identification of histone marks recognized by *PRNCR1* and *PCGEM1*. a-c, Analysis of the binding specificity of yeast *tRNA* (a) or biotinylated *PCGEM1* (b) or biotinylated *PRNCR1* (c) on Modified histone peptide array. Left panel: a representative image of the array. Right panel: the binding specificity calculated by Array Analyses Software based on two duplicated arrays.



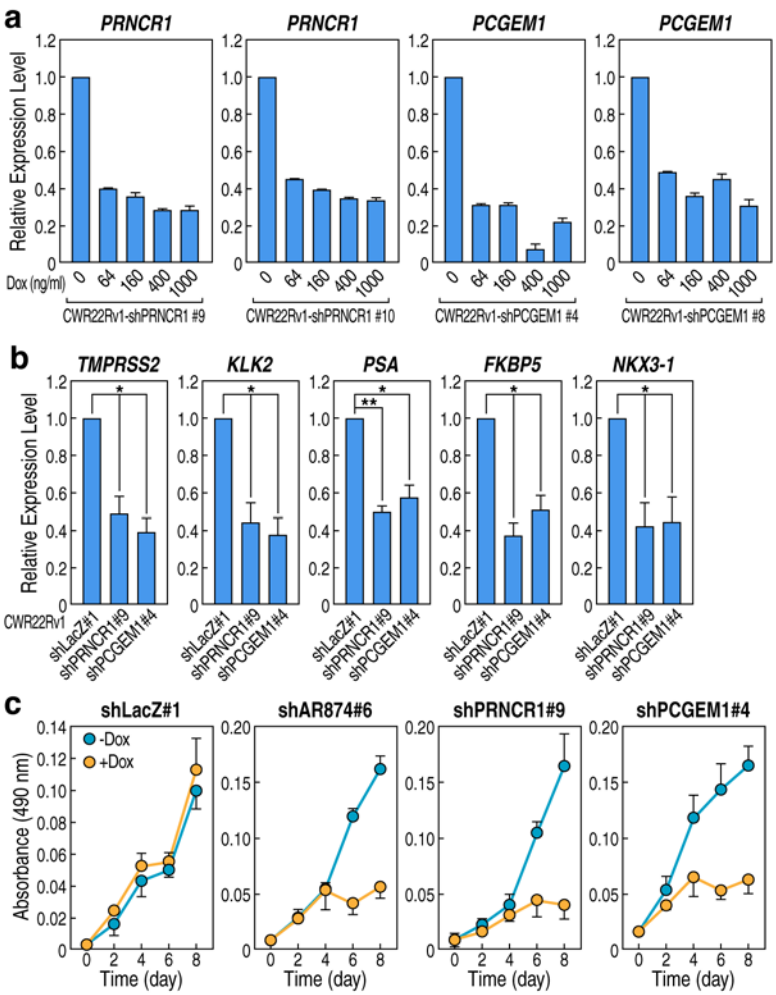
Supplementary Figure 10: Characterization of *PRNCR1* and *PCGEM1* in CRPC cells. a and **b,** qRT-PCR analyses of *PCGEM1* (a) and *PRNCR1* (b) in indicated cell lines. **c**, qRT-PCR analyses of *PSA* in LNCaP or LNCaP-cds2 cells lines pretreated with DMSO or Casodex (1 μM) followed by DHT (100 nM) or vehicle treatment for 4 hrs. **d**, qRT-PCR analyses showing knock-down of *PCGEM1* (left panel) or *PRNCR1* (right panel) in LNCaP cells with two independent shRNAs. **e**, qRT-PCR analyses in LNCaP-cds2 cells transduced with shRNAs targeting *LacZ*, *PCGEM1* or *PRNCR1* showing no reciprocal regulation between *PRNCR1* (left panel) and *PCGEM1* (right panel). **f**, qRT-PCR analyses of selected AR targets in LNCaP-cds2 cells transduced with lentiviruses harboring indicated TetO-inducible shRNAs, and incubated with 160 ng/ml Doxycycline for 2 days. **g**, Immunoblotting analysis of AR in LNCaP and CRPC cell lines using a specific antibody against N-terminus of AR (441). The amount of Tubulin was used as a loading control. Mean ± SEM (n=3, *p<0.05 and **p<0.01).



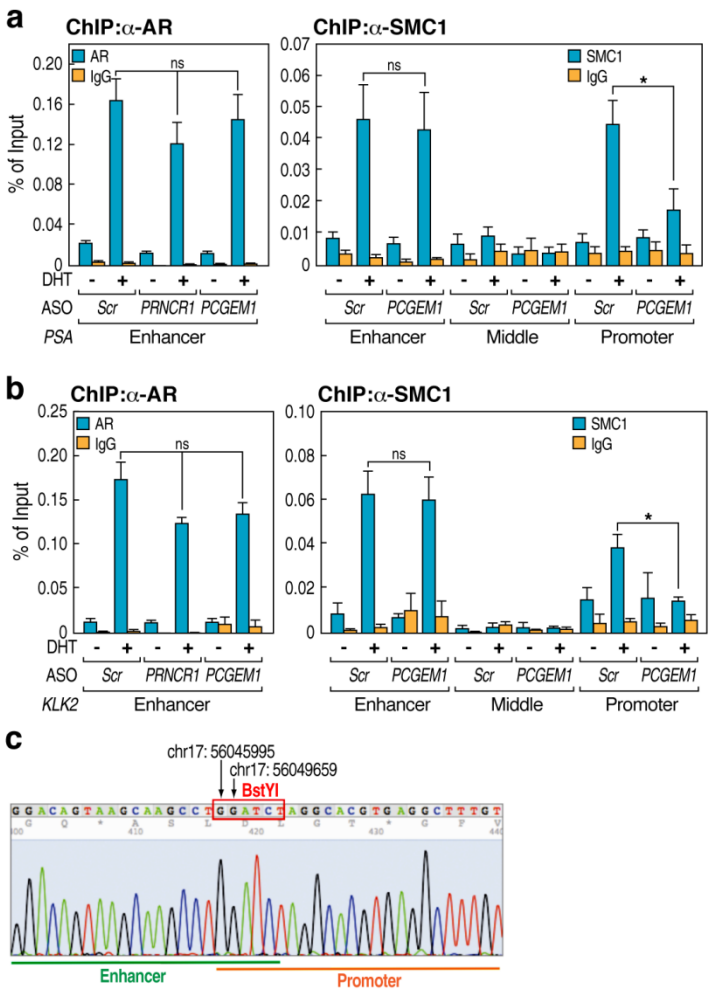
Supplementary Figure 11: Detection of truncated AR in CRPC cells. a, Immunoblotting analysis showing the level of indicated proteins in LNCaP-cds2 or CWR22Rv1 cells transduced with shRNA targeting *LacZ*, *PRNCR1*, or *PCGEM1*. A shRNA targeting N-terminus of AR served as positive control. **b**, Detection of the *AR* transcript variants by RT-PCR in various prostate cancer cell lines. 1 Kb Plus DNA Ladder from Invitrogen™ was used.



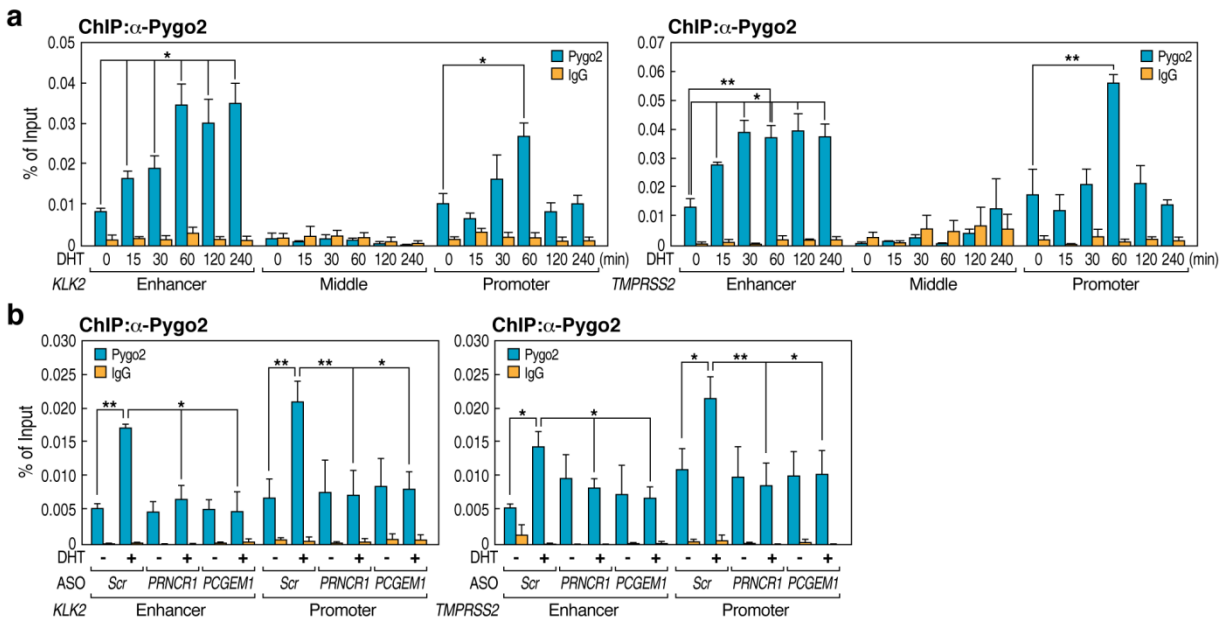
Supplementary Figure 12: Expression of truncated AR and shRNAs targeting *LacZ*, *PRNCR1* and *PCGEM1* in LNCaP cells. a and **b**, LNCaP cells co-transfected with lentiviral based TetO-inducible shRNA targeting *LacZ*, *PRNCR1* or *PCGEM1* and blank vector or AR-Q641X mutant, were incubated with Doxycycline (160 ng/ml) for 2 days and subjected to qRT-PCR analyses of cDNAs (a) and immunoblotting analysis of cell extracts using indicated antibodies (b). **c**, Full length AR in LNCaP or CRPC cells with or without DHT (100 nM, 1hr) were immunoprecipitated by AR C-19 antibody. The immunoprecipitates were detected by immunoblotting using indicated antibodies. Mean ± SEM (n=3 for a, b and n=6 for c, *p<0.05).



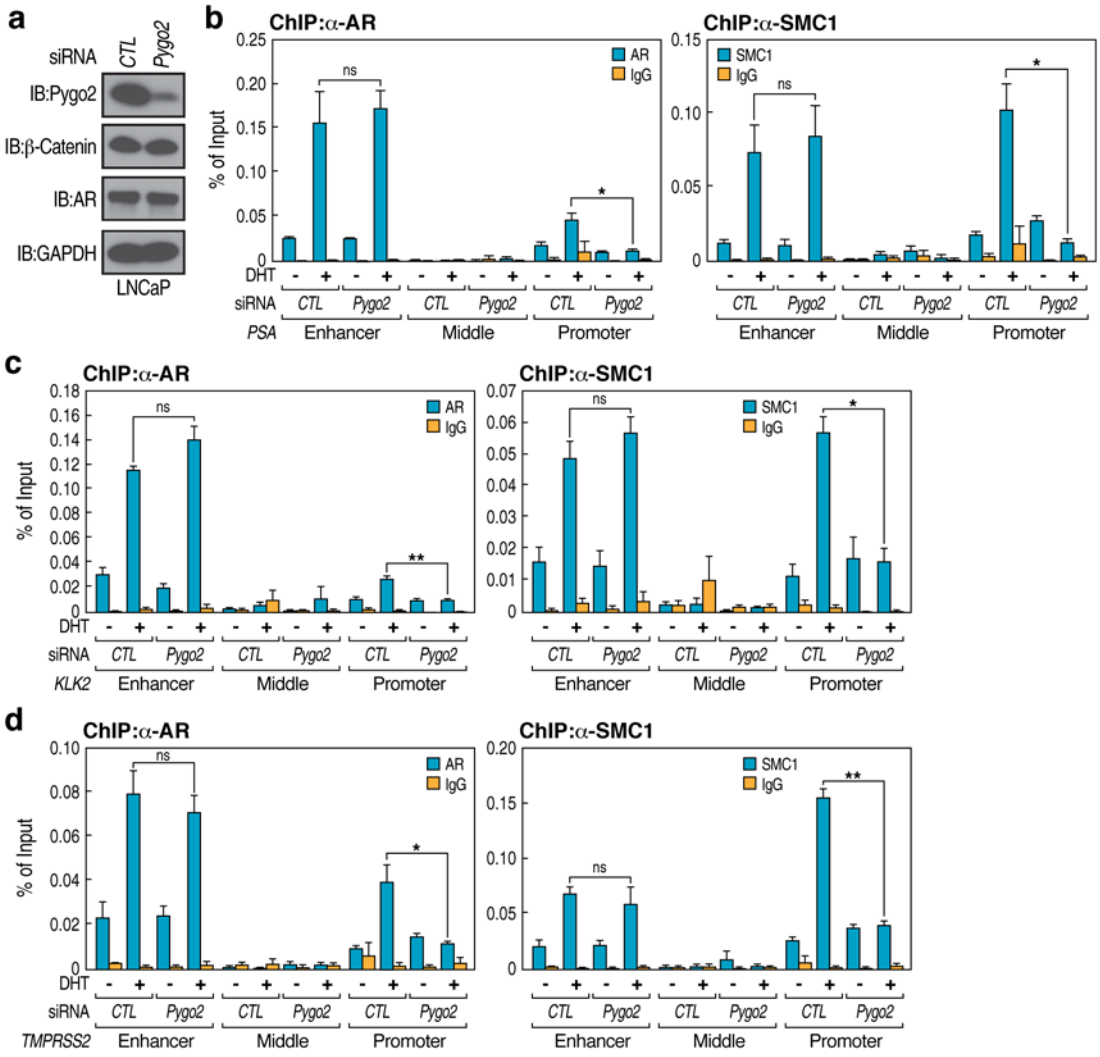
Supplementary Figure 13: The effect of PCGEM1 and PRNCR1 knock-down on CWR22Rv1 cell proliferation. **a,** qRT-PCR analyses showing Doxycycline-induced knock-down of *PRNCR1* (two left panels) and *PCGEM1* (two right panels) in two independent clones of CWR22Rv1 stable cell line harboring TetO-inducible shRNA against *PRNCR1* or *PCGEM1* at indicated concentration of Doxycycline. **b**, qRT-PCR analyses of selected AR targets in CWR22Rv1 cells stably expressing TetO-inducible shRNA targeting *LacZ*, *PRNCR1* or *PCGEM1* in the presence of Doxycycline (160 ng/ml) for 3 days. The single colony number of each cell lines was indicated. **c**, Cell proliferation assay in independent clones of CWR22Rv1 stable cell lines harboring TetO-inducible shRNA against *LacZ*, *AR*, *PRNCR1* or *PCGEM1*. The clone numbers were indicated. Mean ± SEM (n=3, *p<0.05 and **p<0.01).



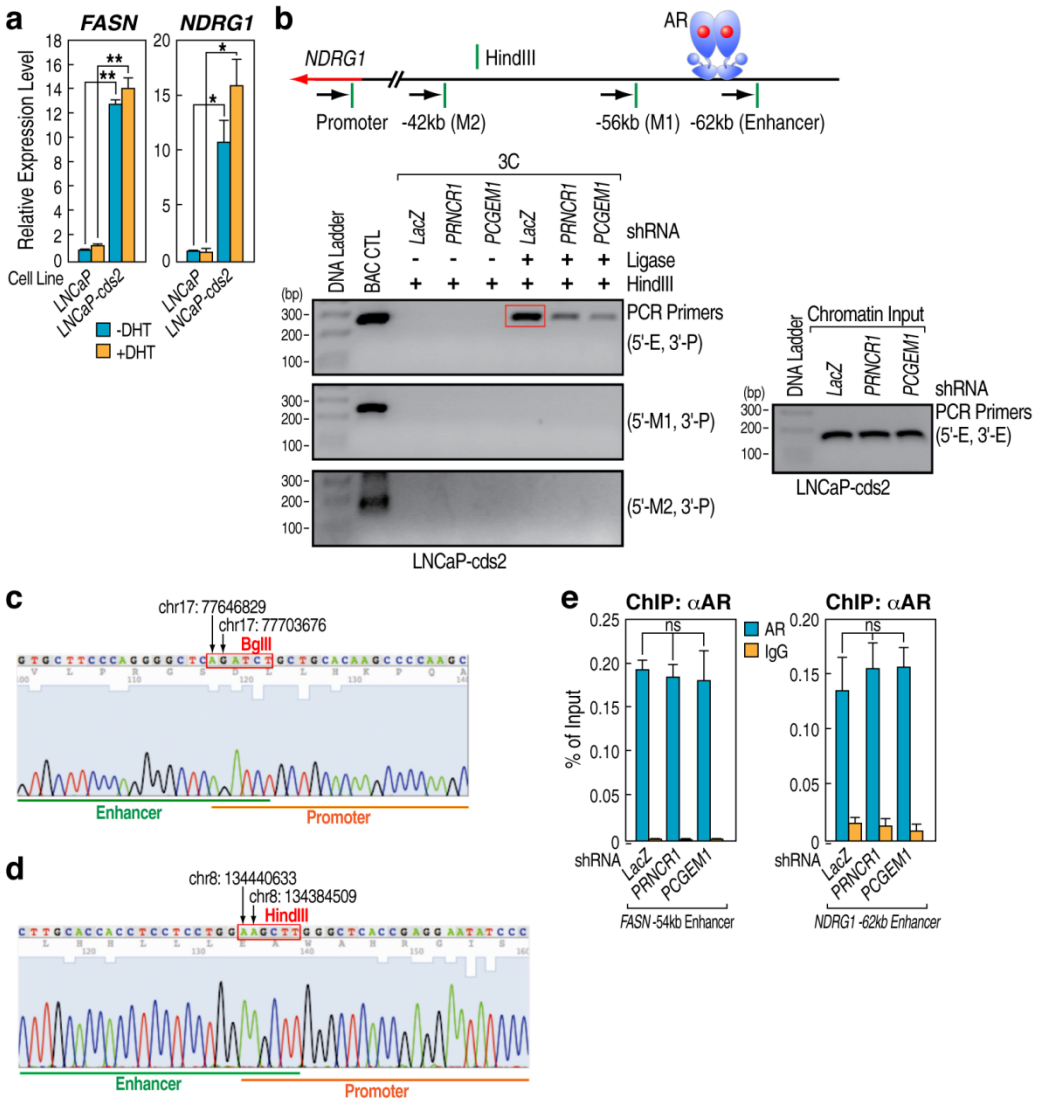
Supplementary Figure 14: Characterization of the involvement of *PRNCR1* and *PCGEM1* in enhancer: promoter looping event of AR target gene loci. a and **b**, ChIP–qPCR showing AR (left panel) or SMC1 (right panel) occupancy on *PSA* (a) and *KLK2* (b) loci in control *vs*. *PRNCR1* or *PCGEM1* ASO transfected LNCaP cells treated with or without DHT (100 nM) for 1 hr. **c**, Sanger sequencing of a positive ChIP-3C PCR product corresponding to sample in **Fig. 4a**. DNA sequence was aligned to enhancer (green) and promoter (red) of *PSA* loci with genomic position of ligating nucleotides highlighted by red. Mean ± SEM (n=3, *p<0.05).



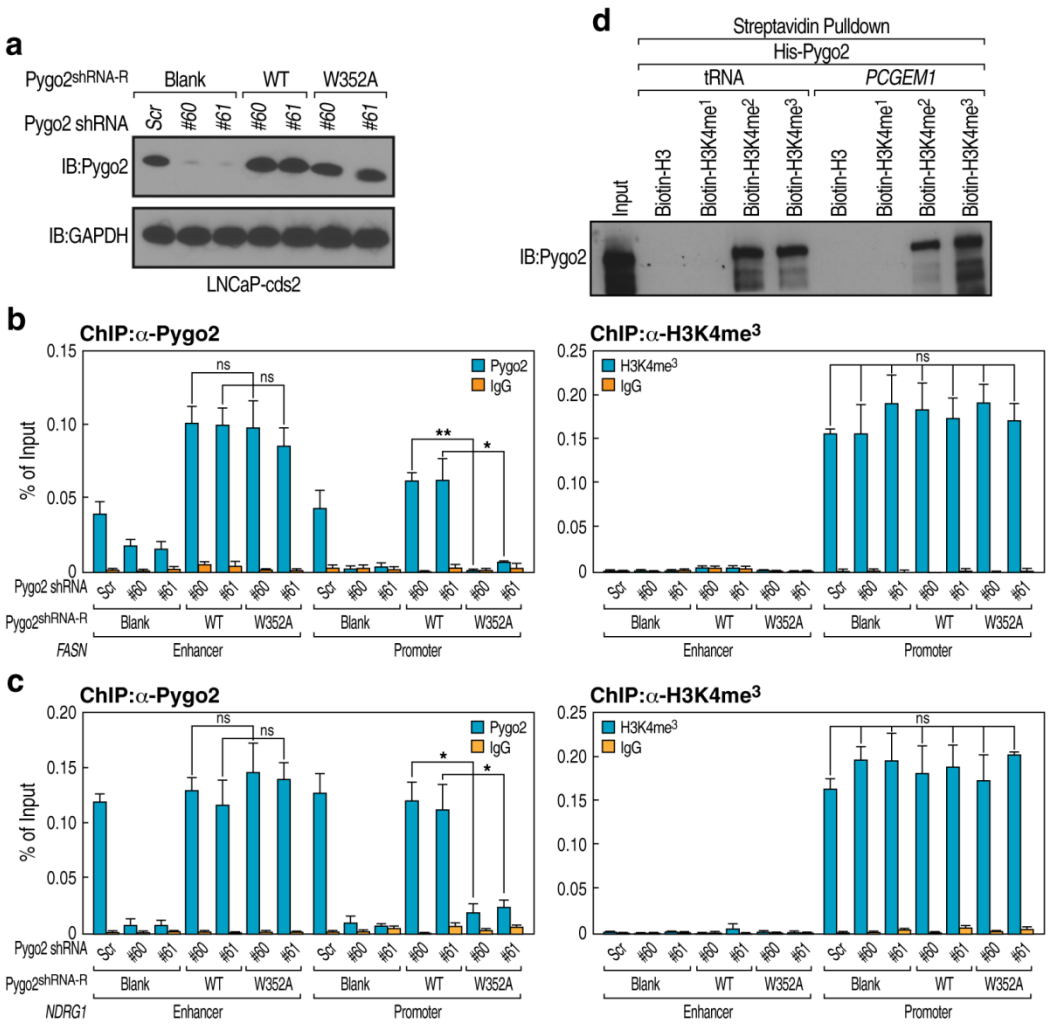
Supplementary Figure 15: The involvement of *PRNCR1* and *PCGEM1* in recruitment of Pygo2 to AR target gene loci. a, ChIP–qPCR showing Pygo2 occupancy on *KLK2* (left panel) and *TMPRSS2* (right panel) loci in LNCaP cells treated with DHT (100 nM) for indicated time points. **b**, ChIP–qPCR showing Pygo2 occupancy on *KLK2* (left panel) and *TMPRSS2* (right panel) loci in control *vs. PRNCR1* or *PCGEM1* ASO transfected LNCaP cells treated with or without DHT (100 nM) for 1 hr. Mean ± SEM (n=3, *p<0.05 and **p<0.01).



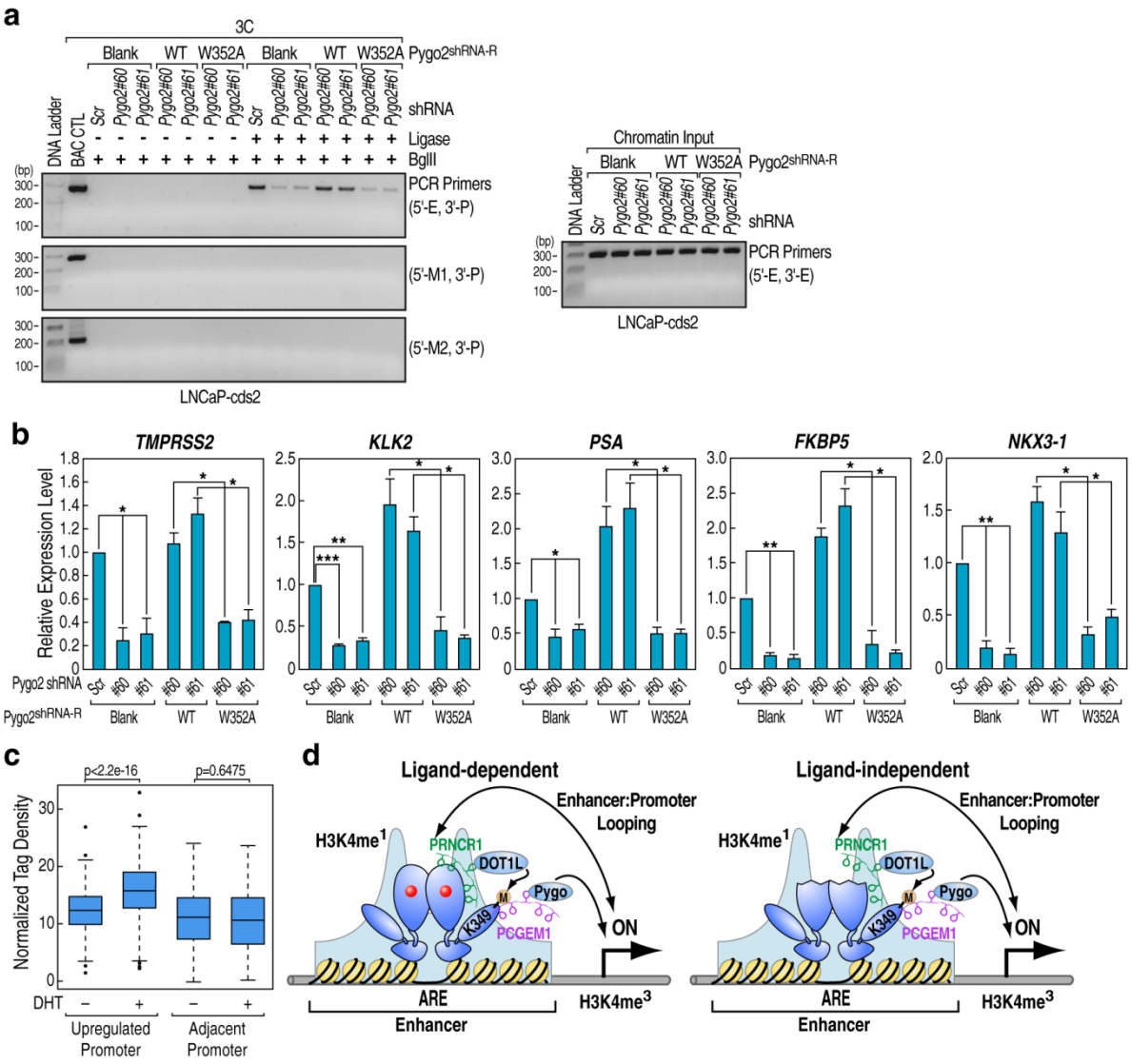
Supplementary Figure 16: Pygo2 is required for DHT-induced enhancer: promoter looping of AR target gene loci. a, Immunoblotting analysis showing the level of indicated proteins in control *vs*. *Pygo2* knock-downed LNCaP cells. **b**, ChIP–qPCR showing AR (left panel) or SMC1 (right panel) occupancy in LNCaP cells transfected with indicated siRNAs and treated with or without DHT (100 nM) for 1 hr. **c** and **d**, ChIP–qPCR showing AR (left panels) and SMC1 (right panels) occupancy on *KLK2* (c) and *TMPRSS2* (d) loci in control *vs.* Pygo2 knock-downed LNCaP cells treated with or without DHT (100 nM) for 1 hr. Mean ± SEM (n=3, *p<0.05 and **p<0.01).



Supplementary Figure 17: Additional data on the functional roles of *PRNCR1* and *PCGEM1* in enhancer: promoter looping. a, qRT-PCR analyses showing the expression of *FASN* (left panel) and *NDRG1* (right panel) in LNCaP *vs*. LNCaP-cds2 cells with or without DHT treatment (100 nM) for 4 hrs. **b,** Long-distance interaction between gene promoter and AR-bound site was determined by the 3C assay on *NDRG1* locus in LNCaP-cds2 cells transduced with TetO-inducible shRNA against *LacZ*, *PRNCR1* or *PCGEM1*. The ligated DNA was PCR amplified with primers as indicated and a representative PCR product was sequenced (red box). BAC containing corresponding regions of *NDRG1* locus was used as positive control. **c** and **d,** Sanger sequencing of positive 3C PCR products corresponding to samples in **Fig. 4f** and **b** above. DNA sequences were aligned to enhancer (green) and promoter (red) of *FASN* and *NDRG1* locus respectively, with genomic position of ligating nucleotides highlighted by red. **e**, ChIP–qPCR showing AR occupancy on *FASN* (left panel) and *NDRG1* (right panel) enhancers in LNCaP-cds2 cells transduced with lentiviruses that express the TetO-inducible shRNA against *LacZ*, *PRNCR1* or *PCGEM1*. Mean ± SEM (n=3, *p<0.05 and **p<0.01).



Supplementary Figure 18: Recognition of H3K4me^3 by PHD domain of Pygo2 is critical for enhancer: promoter looping. a, Immunoblotting analysis of Pygo2 in LNCaP-cds2 cells transduced with lentivirus that express the indicated shRNAs plus lentivirus that express RNAi-resistant form of Pygo2 (wild-type, W352A mutant) or blank vector. GAPDH served as loading control. **b** and **c**, ChIP–qPCR showing Pygo2 (left panels) and H3K4me^3 (right panels) occupancy on *FASN* (b) or *NDRG1* (c) loci in LNCaP-cds2 cells transduced with lentivirus that express the indicated shRNAs plus lentivirus that express RNAi-resistant form of Pygo2 (wild-type, W352A mutant) or blank vector. **d**, His-tagged recombinant Pygo2 were incubated with *tRNA* or *in vitro* transcribed *PCGEM1* in the presence of Biotinylated histone peptides as indicated. After Streptavidin pull down, elutes were subjected to immunoblotting using antibody targeting Pygo2. Mean ± SEM (n=3, *p<0.05, **p<0.01 and ***p<0.001).



Supplementary Figure 19: Regulation of ligand-induced enhancer: promoter looping and gene activation events by lncRNA/Pygo2. a, 3C assay was performed in LNCaP-cds2 cells transduced with lentiviruses harboring shRNA against Pygo2 (#60 or #61) and cDNAs encoding wild-type or W352A mutant Pygo2. **b**, qRT-PCR analyses of selected AR targets in LNCaP-cds2 cells transduced with lentiviruses harboring shRNA against Pygo2 (#60 or #61) and cDNAs encoding wild-type Pygo2 or the W352A mutant. **c**, Global occupancy of Pygo2 was analyzed by ChIP-Seq assay in LNCaP cells indicating the normalized intensity of Pygo2 binding to 220 AR-upregulated gene promoters or 204 adjacent non-regulated gene promoters with or without DHT treatment (100 nM, 1hr). **d**, The graphic illustration of the proposed roles of *PCGEM1* and *PRNCR1* in ligand-dependent (left panel) and ligand-independent (right panel) AR target gene activation.