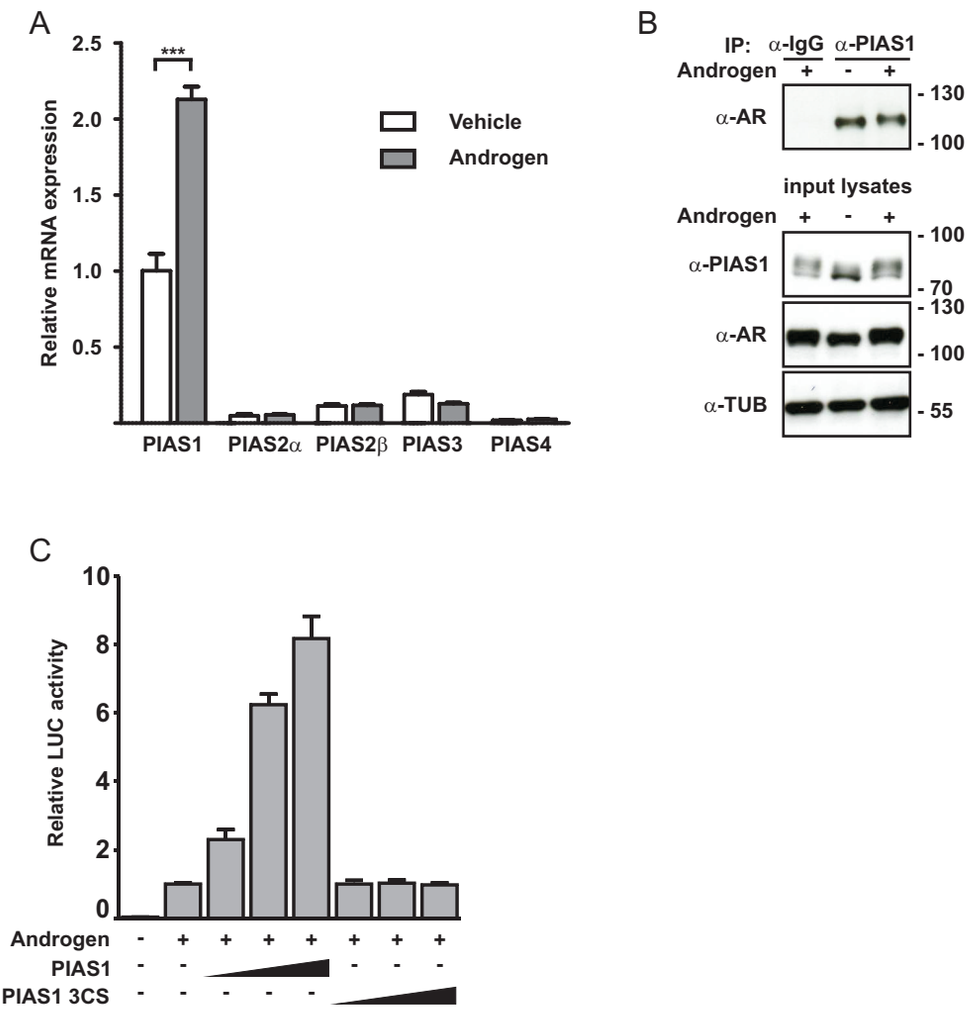
Supplementary Material

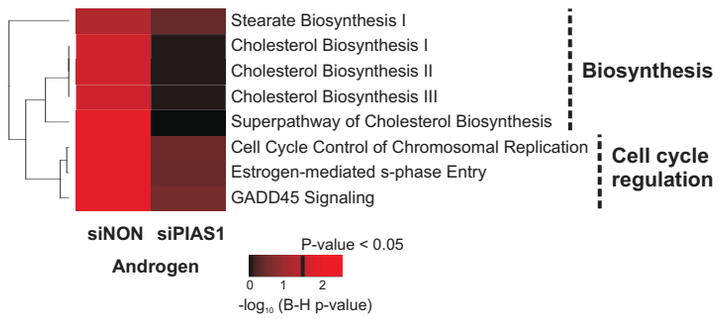
SUMO ligase PIAS1 functions as a target gene selective androgen receptor coregulator on prostate cancer cell chromatin

Sari Toropainen, Marjo Malinen,Sanna Kaikkonen, Miia Rytinki, Tiina Jääskeläinen, Biswajyoti Sahu, Olli A. Jänne and Jorma J. Palvimo



Supplementary Figure S1. PIAS1 is up-regulated by androgen, interacts with the AR and functions as an AR coactivator in VCaP prostate cancer cells. (A) Relative mRNA levels were measured by RT-qPCR from VCaP cells exposed to androgen (10 nM R1881) or vehicle (ethanol) for 6 h. The data were normalized to *GAPDH* levels and fold changes were calculated in reference to *PIAS1* vehicle sample. Data points represent the means of at least three biological replicates ±SDs. Student's t test was performed to determine the significance of differences between vehicle- and androgen-treated cells (***P<0.001). (B) VCaP cells exposed to vehicle (-) or androgen (+, R1881) for 2 h were immunoprecipitated with anti-PIAS1 antibody and normal rabbit IgG was used as a control antibody. The immunoprecipitates were analyzed by immunoblotting with anti-AR antibody, and the input cell lysates were immunoblotted with anti-PIAS1, anti-AR and anti-tubulin (α-TUB) antibodies. (C) VCaP cells were transfected with a pARE2-TATA-LUC together with increasing amounts of expression vectors (100-300 ng) encoding wild-type PIAS1 or SUMO E3 ligase-inactivated PIAS1 (PIAS1 3CS) as indicated. Empty vector was used to balance the total DNA amount in each well. One day after transfection, cells were grown in the presence or absence of androgen for 17 h. Luciferase (LUC) and β-galactosidase activities were measured. Relative LUC activity of AR in the presence of R1881 and in the absence of PIAS1 was set to 1. The columns represent the means ±SDs of a representative experiment with triplicate samples.

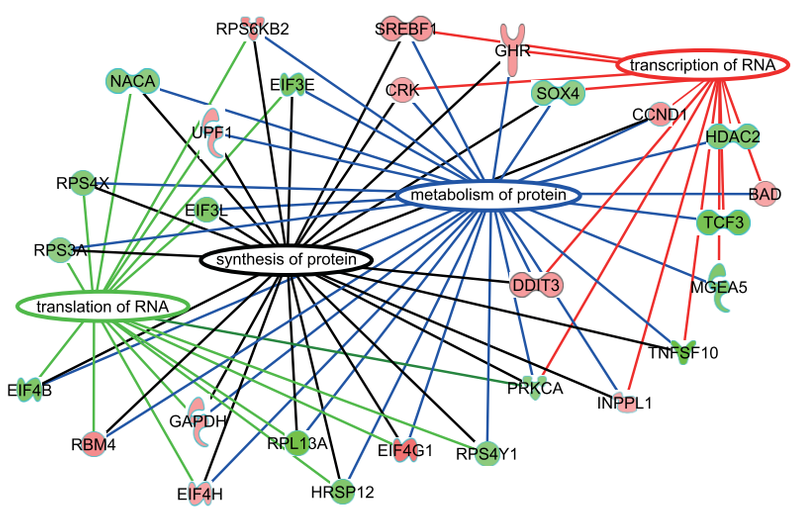
A



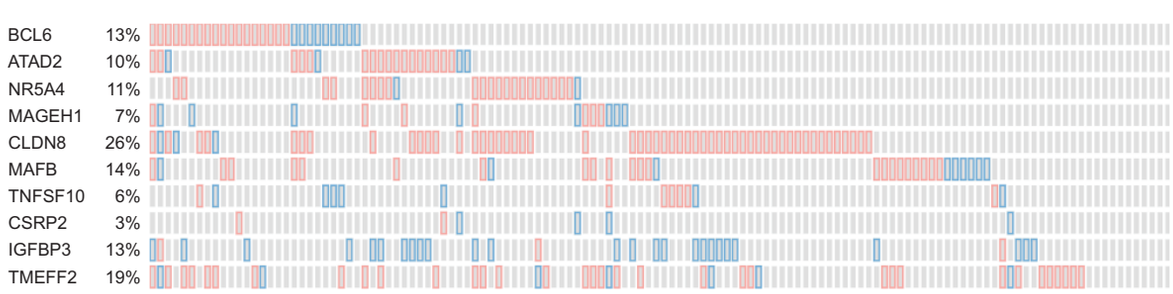
B

Ingenuity Canonical Pathways	-log10 (B-H P-value)
Cluster 4 pathaways	
Remodeling of Epithelial Adherens Junctions	3,37
Neuregulin Signaling	3,37
Xenobiotic Metabolism Signaling	2,34
PTEN Signaling	2,34
G Beta Gamma Signaling	2,07
PI3K/AKT Signaling	1,75
Endometrical Cancer Signaling	1,35
Integrin Signaling	1,33
Epithelial Adherens Junction Signaling	1,33
ErbB2-ErbB3 Signaling	1,33
Cluster 5 pathaways	
Superpathway of Cholesterol Biosynthesis	1,78
Purine Nucleotides De Novo Biosynthesis II	1,78

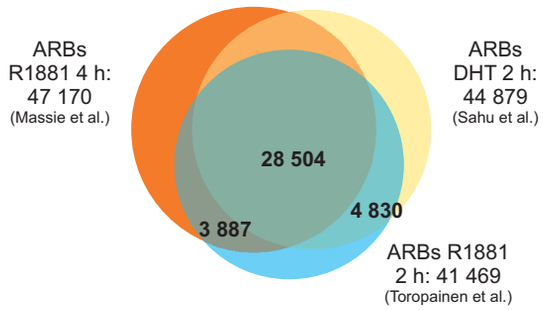
Supplementary Figure S2. PIAS1 influences AR-regulated signaling pathways. Pathways were analyzed by IPA. (A) Heat map of enriched canonical pathways from all androgen-regulated genes in siNON and siPIAS1 VCaP cells. (B) Pathway analysis of genes identified by unsupervised hierarchical clustering to clusters 4 and 5 (see Figure 1C). Threshold for pathways significantly enriched was set to $P < 0.05$ or $-\log_{10}$(B-H adjusted P-value) > 1.3).



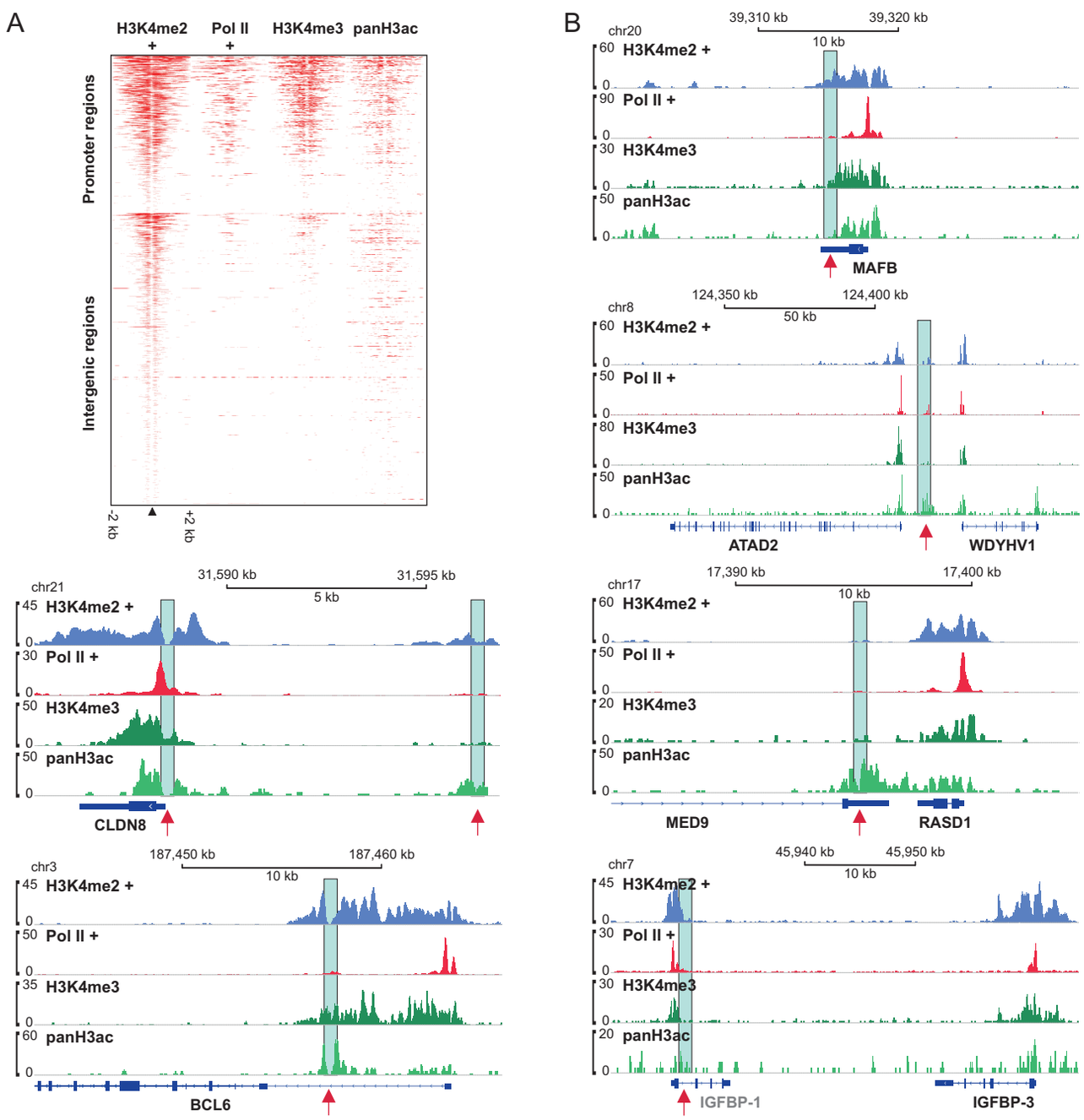
Supplementary Figure S3. Interaction map of genes in gene expression and protein synthesis molecular and cellular functions in Figure 1E as generated by IPA.



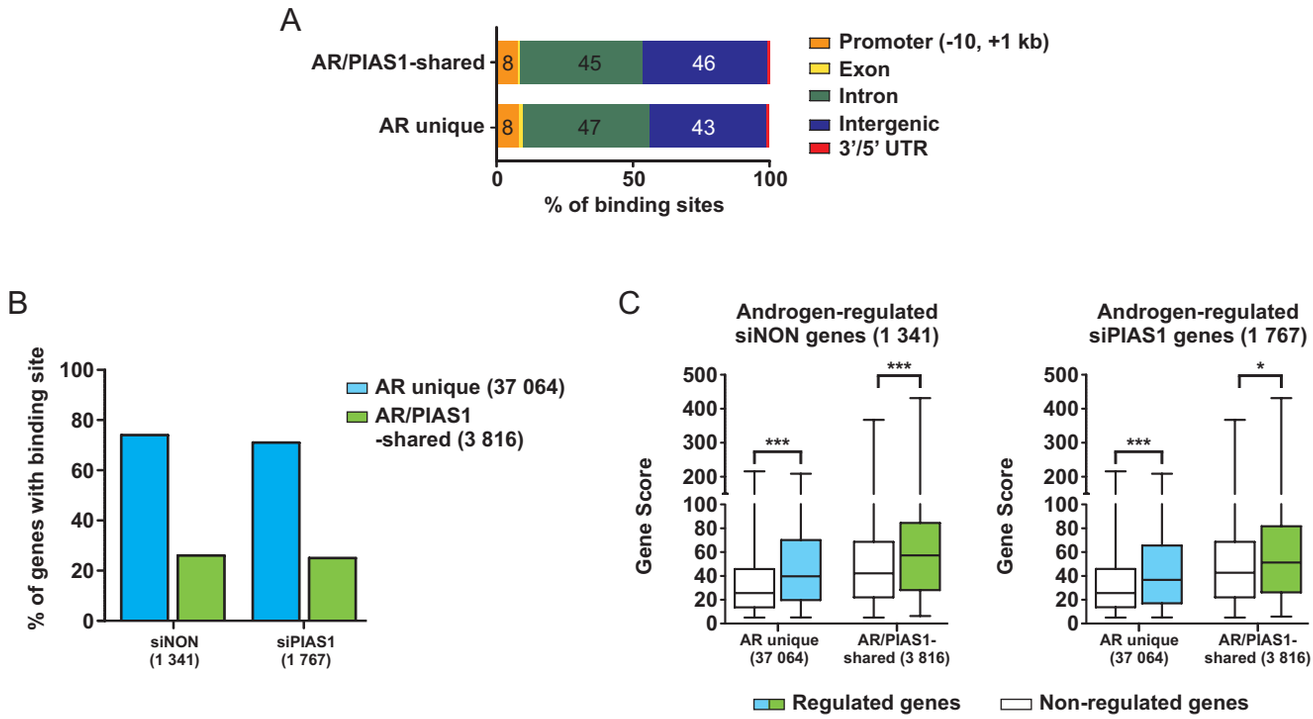
Supplementary Figure S4. The cBioPortal (http://www.cbioportal.org/public-portal/) MSKCC dataset (Taylor *et al.* Cancer. Cell. 2010) demonstrates the importance of select androgen-regulated genes significantly affected by PIAS1 depletion in human prostate cancer. The mRNA expression z-scores compared to normal prostate were selected for mRNA expression data profile (a z-score threshold ±2).



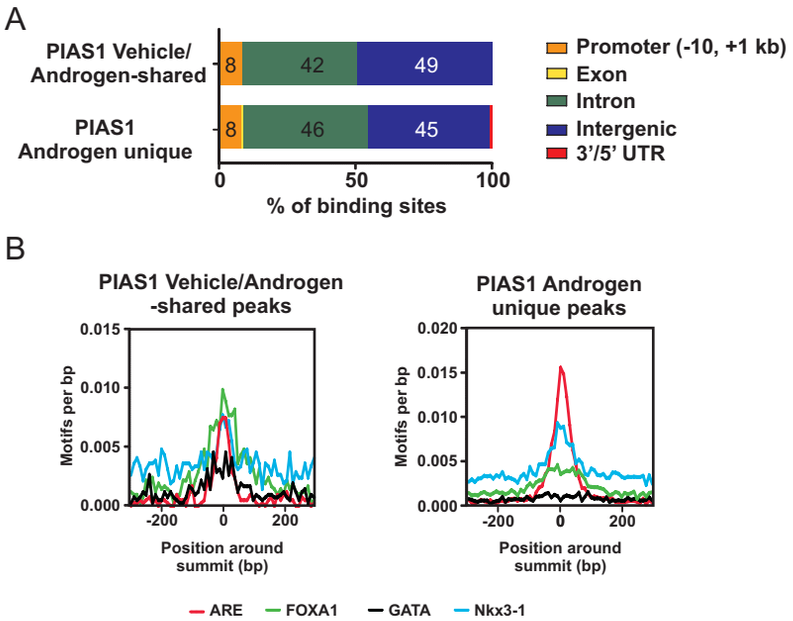
Supplementary Figure S5. Meta-analyses of AR cistromes in VCaP cells. Overlap analysis of AR-binding sites (ARBs) identified in this study (R1881 for 2 h) *vs.* cells exposed to R1881 for 4 h (Massie *et al*. EMBO J. 2011) and cells exposed to DHT for 2 h (Sahu *et al*. EMBO J. 2011).



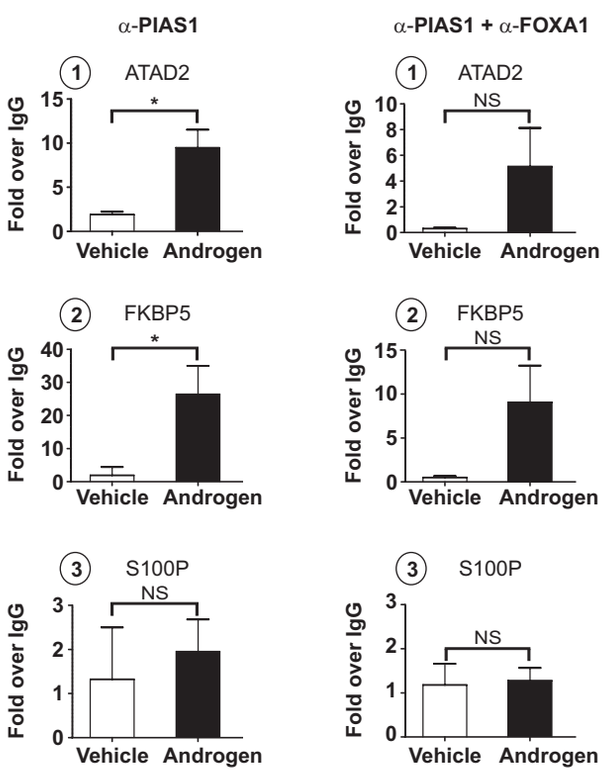
Supplementary Figure S6. Comparison of active enhancer/promoter marks and Pol II occurrence in VCaP cells. VCaP cells exposed to androgen (+, R1881) for 2 h were immunoprecipitated with anti-H3K4me2 or anti-RNA polymerase II (Pol II) antibody. (Nucleosome free regions within H3K4me2 mark were identified using findPeaks tool with FC>4.) (A) Heat map showing H3K4me2, Pol II, H3K4me3 (Yu *et al*. Cancer Cell 2010, GSM353620) and panH3ac (Yu *et al*. Cancer Cell 2010, GSM353629) tag densities for promoter (-10, +1kb) and intergenic regions in a window ±2 kb. (B) ChIP-seq track examples of H3K4me2 (violet), Pol II (red), H3K4me3 (dark green) and panH3ac-binding (light green) events in the same growth-associated AR target loci as shown in Figure 6. H3K4me3 and panH3ac were ChIP-seq analyzed from VCaP cells grown in regular medium. Red arrows depict the sites co-occupied by AR, PIAS1, FOXA1 and SUMO2/3.



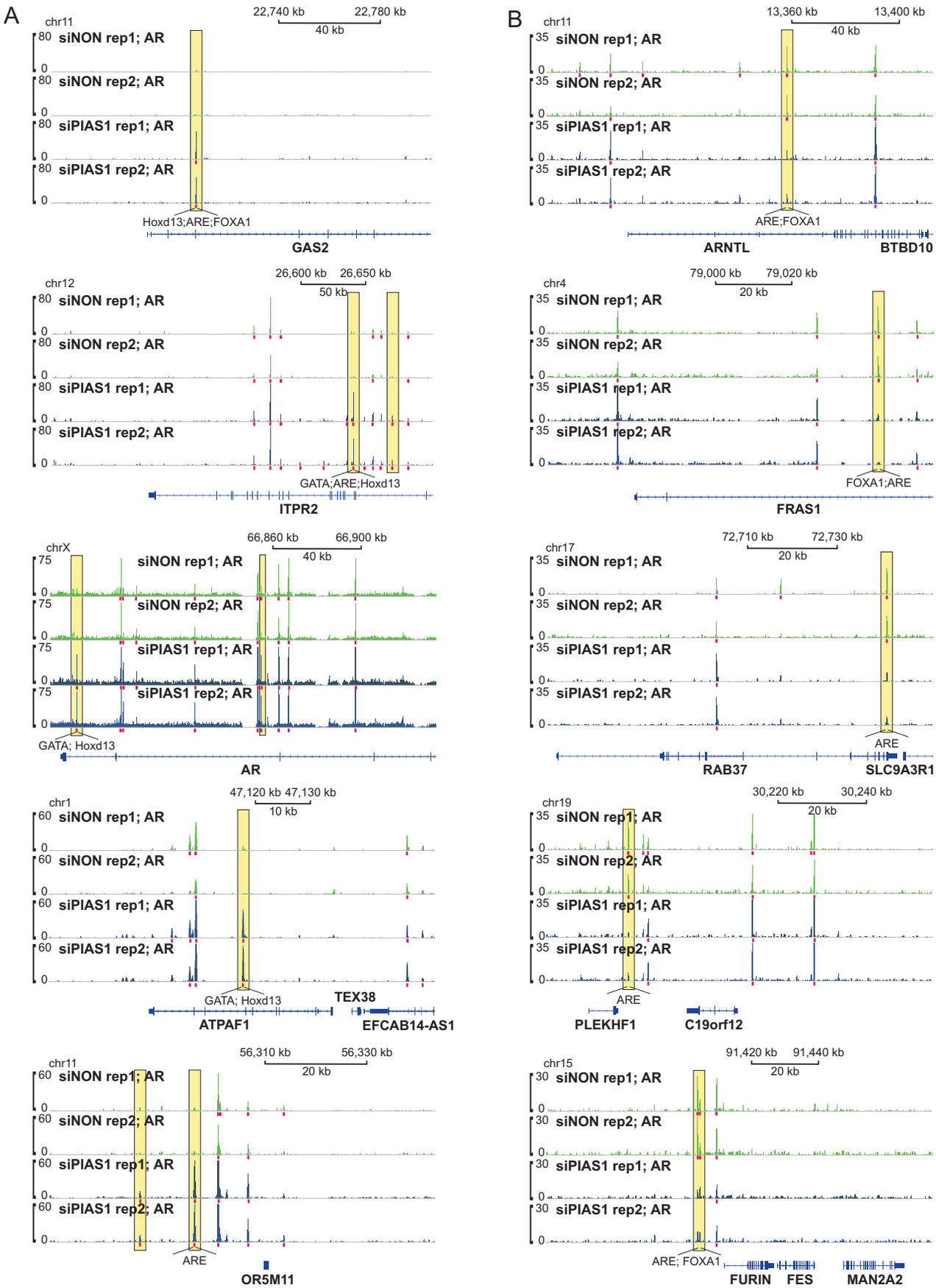
Supplementary Figure S7. Genomic location of AR and PIAS1 peaks and their relation to nearby genes in androgen-exposed VCaP cells. (A) Distribution of AR- and PIAS1-binding sites in different genomic locations. Non-overlapping sites were further analyzed using get-DifferentialPeak tool with FC>3 to achieve final categories. (B) AR/PIAS1-shared sites and AR unique sites were associated with androgen-regulated genes in siNON (1 341) and siPIAS1 (1 767) cells in a search window of ±50 kb of their TSS. (C) Gene scores (>5) for the genes linked to nearby peaks were calculated by taking into consideration the peak depth (normalized tags), the distance and the peak count. The significances were calculated between androgen-regulated and non-regulated gene scores by one-way ANOVA (Kruskal-Wallis test) (***P<0.001 and *P<0.05).



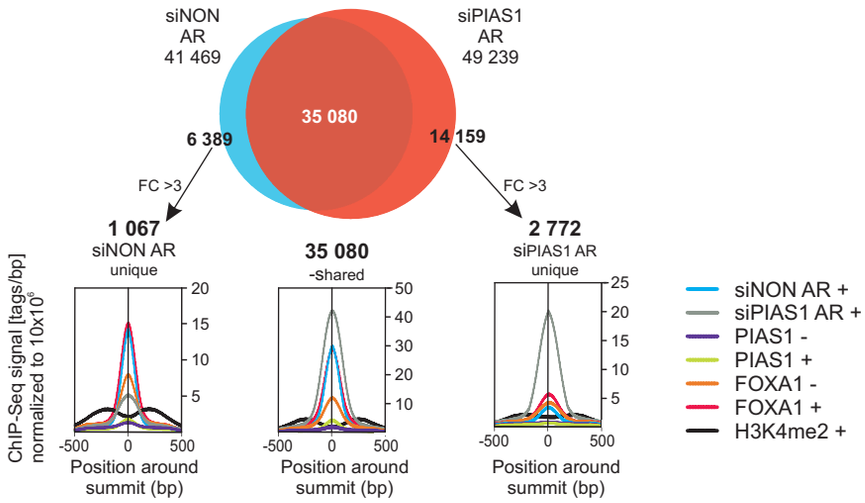
Supplementary Figure S8. Genomic locations and motif analyses of PIAS1-binding sites in VCaP cells. (A) Distribution of PIAS1-binding sites in vehicle- or androgen-exposed cell in different genomic locations. (B) Presence of ARE, FOXA, GATA and Nkx3-1 motifs within ±300 bp from the center of the indicated peak groups are shown. Initial *de novo* motif analysis was performed on all PIAS1 (both vehicle and R1881) binding sites on ±100 bp of the PIAS1 peak center.



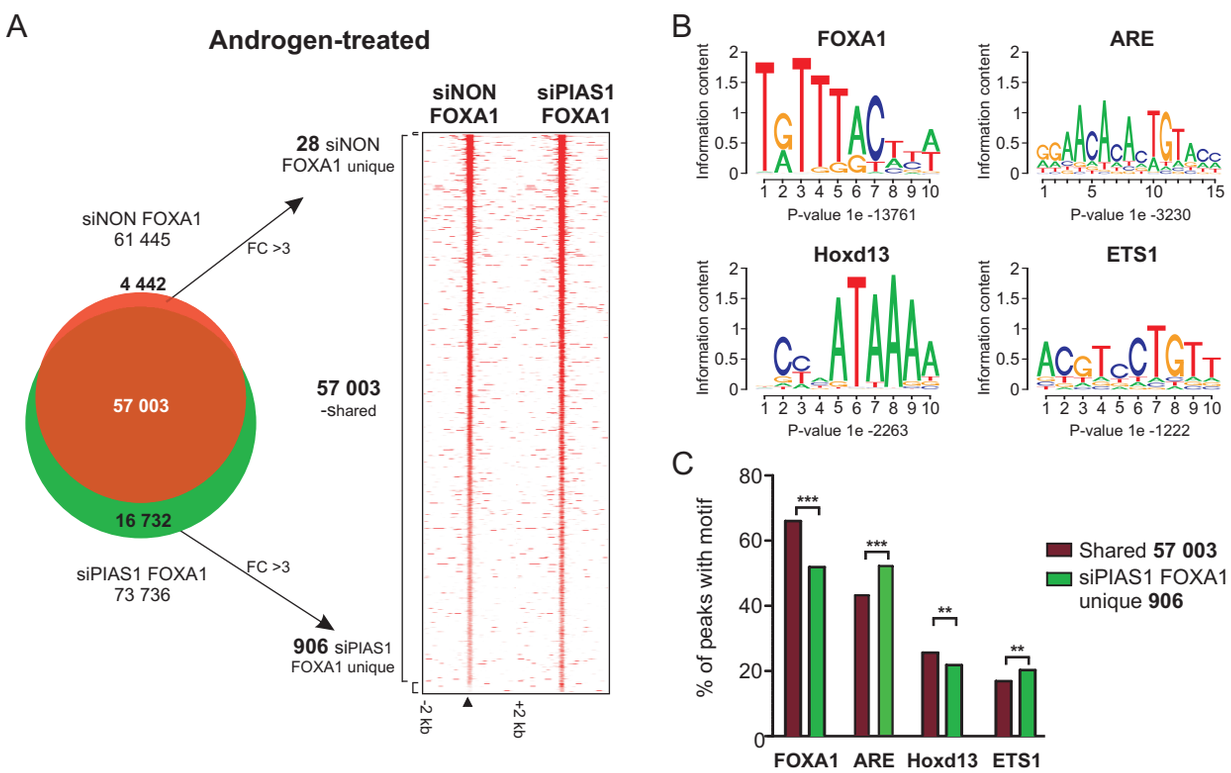
Supplementary Figure S9. Evidence for direct interaction of PIAS1 and FOXA1 on the chromatin. VCaP cells were treated with or without androgen for 2 h and chromatin was sequentially immunoprecipitated with anti-PIAS1 and anti-FOXA1 antibody and analyzed by qPCR with primers specific for genomic regions of *ATAD2* and *FKBP5* exhibiting (based on ChIP-seq data) PIAS1 and FOXA1 co-occupancy or genomic region of S100P showing only FOXA1 occupancy. Student's t test was used to analyze the significance of androgen treatment (*P<0.05).



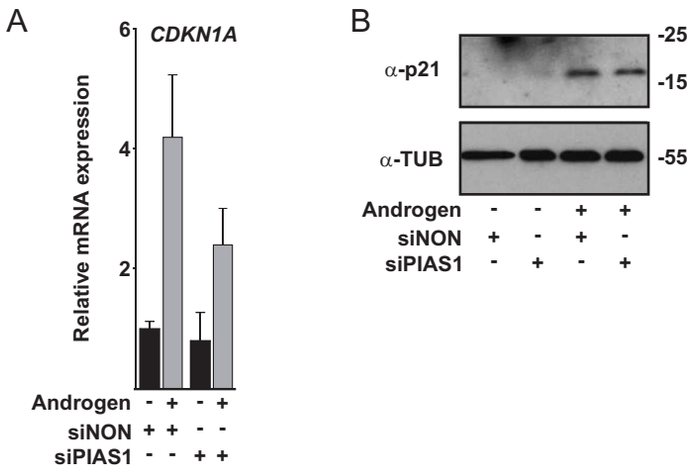
Supplementary Figure S10. ChIP-seq examples of PIAS1 depletion-induced new AR-binding sites (A) and PIAS1-dependent AR-binding sites (B) are yellow-boxed in replicates 1 and 2 (rep1 and rep2). Predicted binding motifs are indicated under the boxes.



Supplementary Figure S11. The new ARBs uncovered by PIAS1 depletion do not generally overlap with the PIAS1 or FOXA1 cistrome. Average tag density maps showing AR (siNON or siPIAS1 cells), PIAS1, FOXA1 and H3K4me2 tag densities for the siNON/siPIAS1-shared, the siNON and the siPIAS1 unique sites (-, vehicle: +, androgen exposure).



Supplementary Figure S12. Effect of PIAS1 depletion on the FOXA1 cistrome. VCaP cells were transfected with siNON or siPIAS1 and exposed to androgen (R1881) for 2 h before performing ChIP-seq as described in Materials and Methods. (A) Venn diagram showing overlap of FOXA1 cistrome in non-silenced and PIAS1 silenced cells (left panel). Non-overlapping sites were further analyzed using getDifferentialPeak tool with FC>3 to achieve final categories. Heat map showing FOXA1-binding site tag densities for siNON/siPIAS1-shared and unique regions in a window ±2 kb (right panel). (B) The four most enriched motifs of FOXA1-binding sites in VCaP cells. *De novo* motif analysis was performed to all FOXA1 (siNON)-binding sites with a window ±100 bp from the peak center. (C) Distribution of the most enriched *de novo* motifs in siNON/siPIAS1-shared and siPIAS1 unique FOXA1-binding sites. Statistical significances were calculated by χ^2-test (***P<0.001 and **P<0.01).



Supplementary Figure S13. Effect of PIAS1 depletion on p21 expression in VCaP cells. VCaP cells were transfected with control (siNON) or PIAS1 (siPIAS1) siRNA and then cells were exposed to vehicle (-) or androgen (+, R1881) for 16 h. (A) mRNA was analyzed by RT-qPCR with spesific primers for *CDKN1A*. Measurements were normalized to *GAPDH* levels. (B) Immunoblotting of cell lysates with anti-p21 and anti-tubulin antibodies.

Supplementary Table S1. RT-qPCR primers used in this study.

Gene	Primer sequences (5'-3')
PIAS1	AGCGACCCAGCCGACCAAT
	CCGCAGTCAACTTCTCTTTA
PIAS2α	CCAGCCAACCGTGTACAAAAATAG
	TTCTTTGTTCTCCTGGCAAATC
PIAS2β	CCAGCCAACCGTGTACAAAAATAG
	CTGGTGGTGGTGACAGACGTA
PIAS3	CTGTCCCTTGTCGTGCCCT
	CGACCTTCTTCTTATTCTCTG
PIAS4	GGCTACTACCCCTCCAATAA
	TTCTCCTTGACCAGTGCCTT
BCL6	GAAGCAAGGCATTGGTGAAG
	CTCTGCTTCACTGGCCTTAA
ATAD2	CGAGTACTCCTGTGGCTTGC
	TTGACTTTTTGCGAATTTTCC
NR5A2	TGGCAAAACTTCGTTCTCTC
	GCATTGACTTGTTCCTGGAC
MAGEH1	TGAAGAGGCTACAAGCATCC
	ATTGTTGCGGTTCTCTTCTG
CLDN8	CGTGAGGCAGGCTAACATCA
	AGCAGCACACATCAGTCCTC
MAFB	CCCGACCGAACAGAAGACAC
	GTGAGGGTGGTGGTGATGG
TNFSF10	CCTCAGAGAGTAGCAGCTCACA
	CAGAGCCTTTTCATTCTTGGA
CSRP2	GTGTACCACGCAGAAGAGGT
	GTAGCCTTTTGGCCCATACT
IGFBP-3	AAGTTGACTACGAGTCTCAG
	AATCAGTTCACCACAAACAGA
TMEFF2	AAGGAGACATCCACCTGTGA
	GAGGGGATTGAAGTTGGTTT
CDKN1A	GCAGACCAGCATGACAGATTT
	GGATTAGGGCTTCCTCTTGGA
GAPDH	TGGGGAAGGTGAAGGTCGG
	TCTCAGCCTTGACGGTGCC

Supplementary Table S2. qChIP primers used in this study.

Gene	From TSS	Primer sequences (5'-3')
ATAD2	-404	CTCTGTGGACACACCGTATTT
	-576	TTGACCTAGCCATTGCACTT
C6orf81 (FKBP5)	-14692	CTGTGAATGTGGGCTAATGG
	-14235	AAGTCTGAGTCCCTGGTTTAC
S100P	2668	GAGGCCCATGAAGTTAGCAG
	2808	TCTGCGAGAGGGAGTTTCAT

Supplementary Table S3. Duplicate similarities of AR, PIAS1 and FOXA1 ChIP-seq samples exposed to vehicle (-) or R1881 (+) for 2 h. Total numbers of defined peaks are indicated along with peak numbers of shared peaks between two replicates before and after tag cut-off of 10.

ChIP-seq	**Rep1 (FE4)**	**Rep2 (FE4)**	**Shared (FE4)**	**Shared (FE4, tag >10)**
siNON AR (+)	79 951	47 203	45 389	41 469
siPIAS1 AR (+)	81 065	66 664	55 720	49 239
siNON FOXA1 (+)	90 956	111 750	85 702	61 445
siPIAS1 FOXA1 (+)	108 777	116 512	95 753	73 736
PIAS1 (-)	8 286	4 490	3 322	933
PIAS1 (+)	12 313	12 085	9 012	3 904
FOXA1 (-)	101 257	99 242	81 600	58 364
FOXA1 (+)	110 543	105 381	90 470	69 780