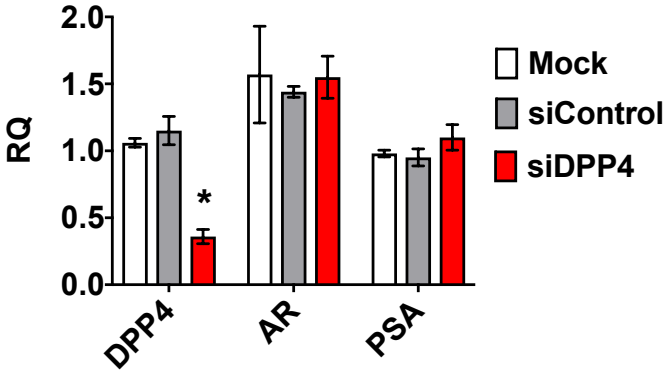
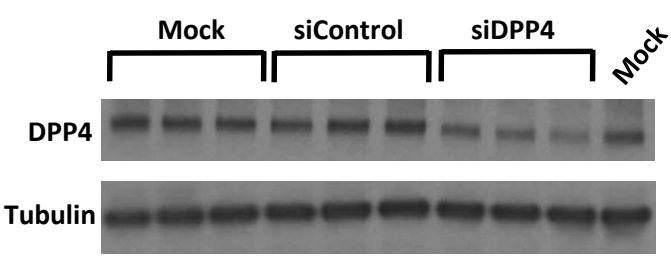
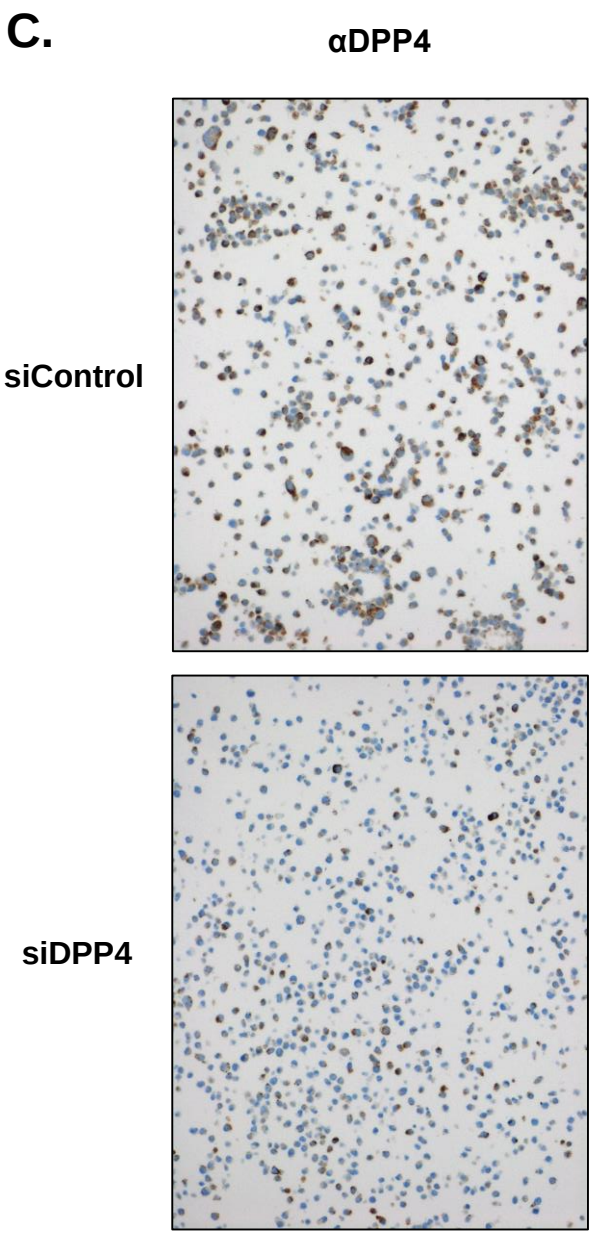
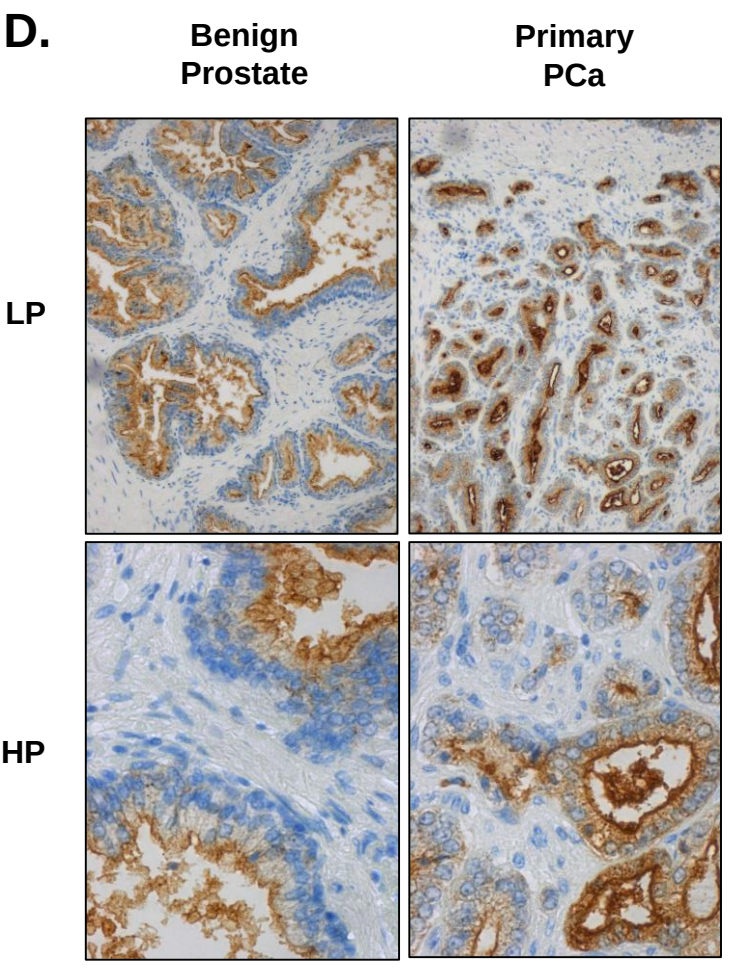
# Supplementary Figure S1



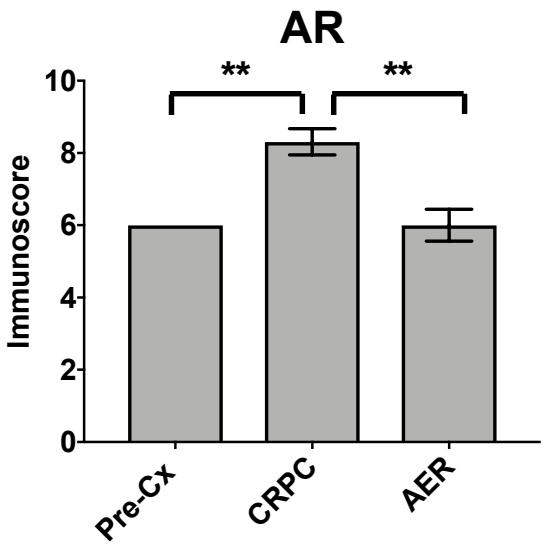



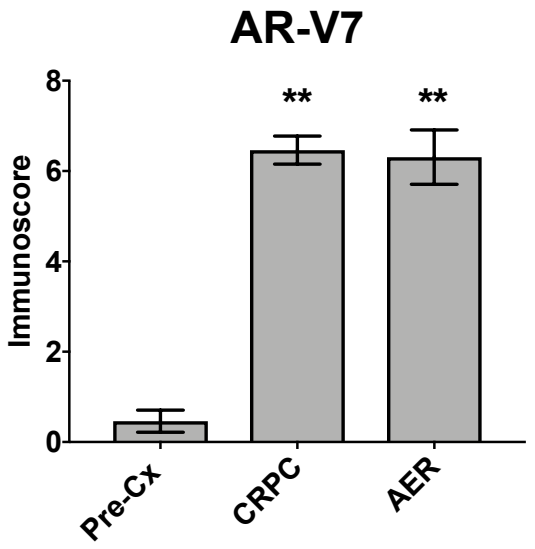



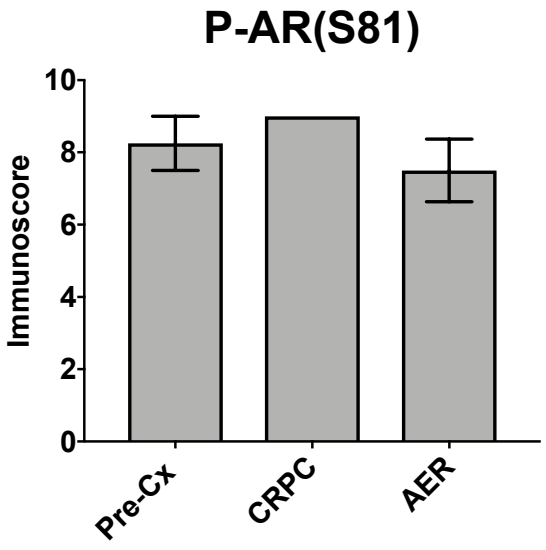



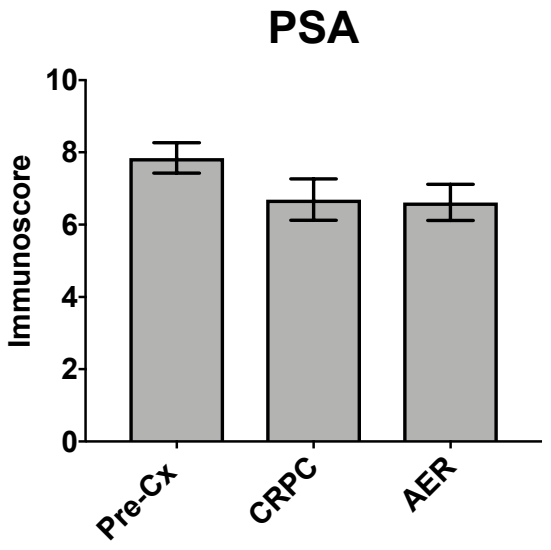
**Supplementary Figure S1 – Targeted siRNA knockdown of DPP4 decreases DPP4 antibody positivity in western blots and IHC. A)** RT-PCR showing RNA expression of *DPP4*, *AR*, and *PSA* 72hrs after mock transfection or transfection with a pooled set of nonsense siRNAs (siControl) or a pooled set of siRNAs to *DPP4* (siDPP4). Each column represents the expression levels from three biological replicates, with RT-PCR performed on each in technical triplicate. Bars = SEM.* = P<0.01, Mann Whitney U. RQ = Relative Quantification **B)** Western blot of cell lysate from VCaP cells transfected with above siRNAs probed with anti-DPP4 antibody D6D8K . **C)** Immunohistochemistry of VCaP cells transfected with above siRNAs and probed with anti-DPP4 antibody D6D8K. **D)** Immunohistochemistry of clinical samples probed with anti-DPP4 antibody D6D8K. LP-Low Power Magnification, HP-High Power Magnification.

**Supplementary Figure S2**



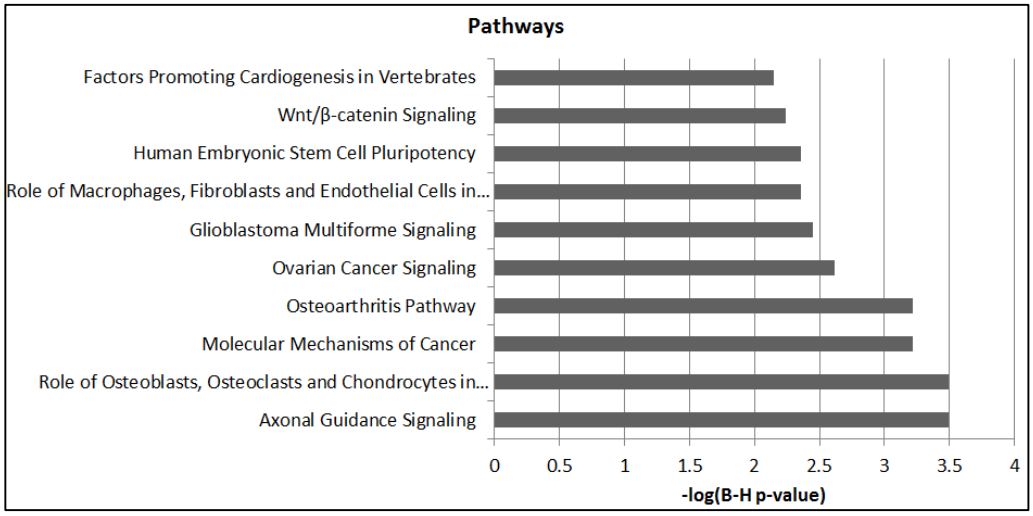



**Supplementary Figure S2 – Immunoscoring of AR, AR-V7, P-AR(S81), and PSA protein expression in serially biopsied VCaP xenografts.** Tumors were serial biopsied prior to cx (Pre-Cx), at tumor relapse (CRPC), and when tumors exceeded 2000mm$^3$ (Abi/Enza resistant = AER). For AR, AR-V7 and PSA, 13 tumors were immunostained and immunoscored as described in supplementary methods. For serine 81-phosphorylated AR, 4 tumors were scored. ** = P<0.0001, student's T test.

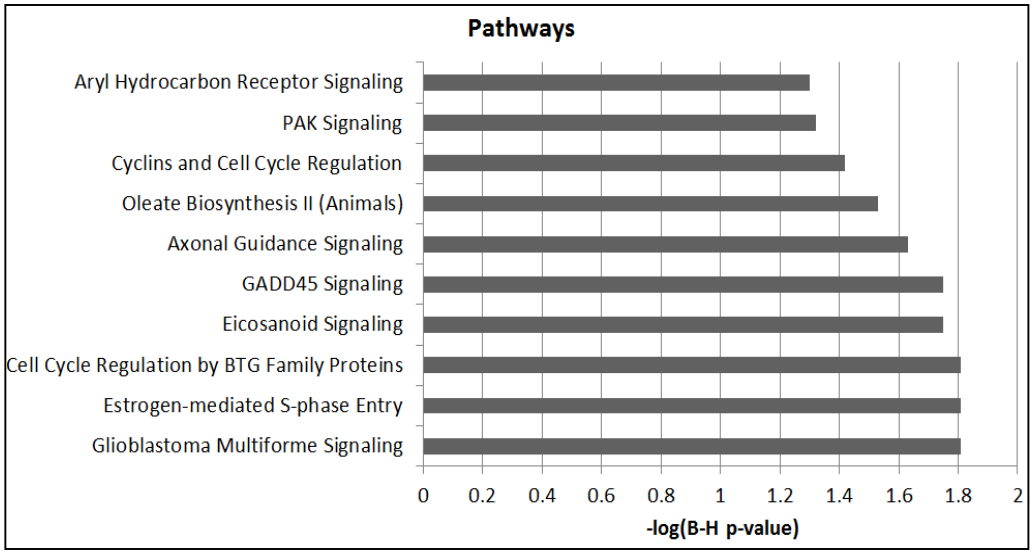
# Supplementary Figure S3

**A.**

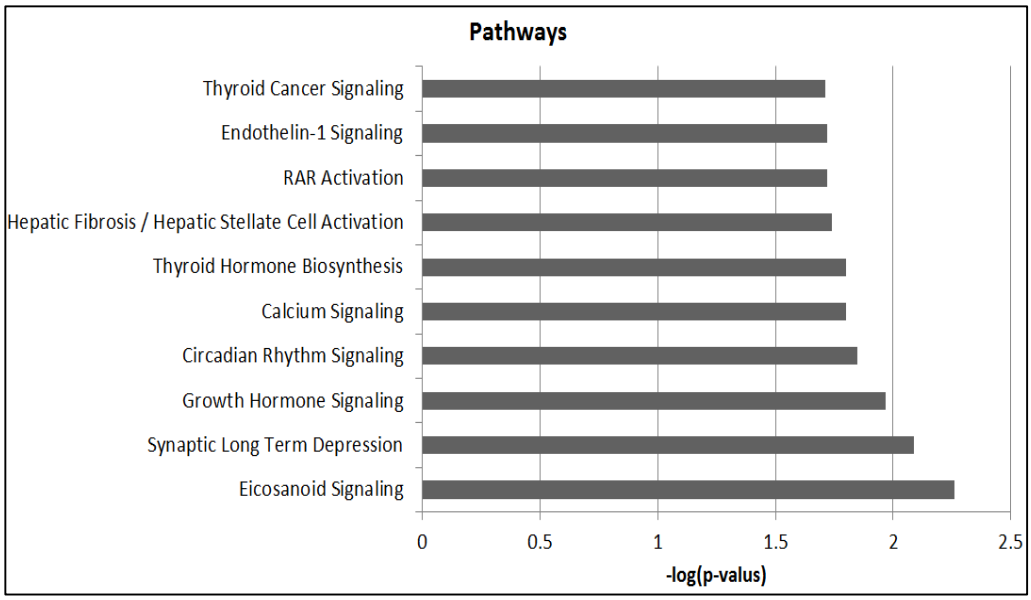
## AER vs. Pre-Cx



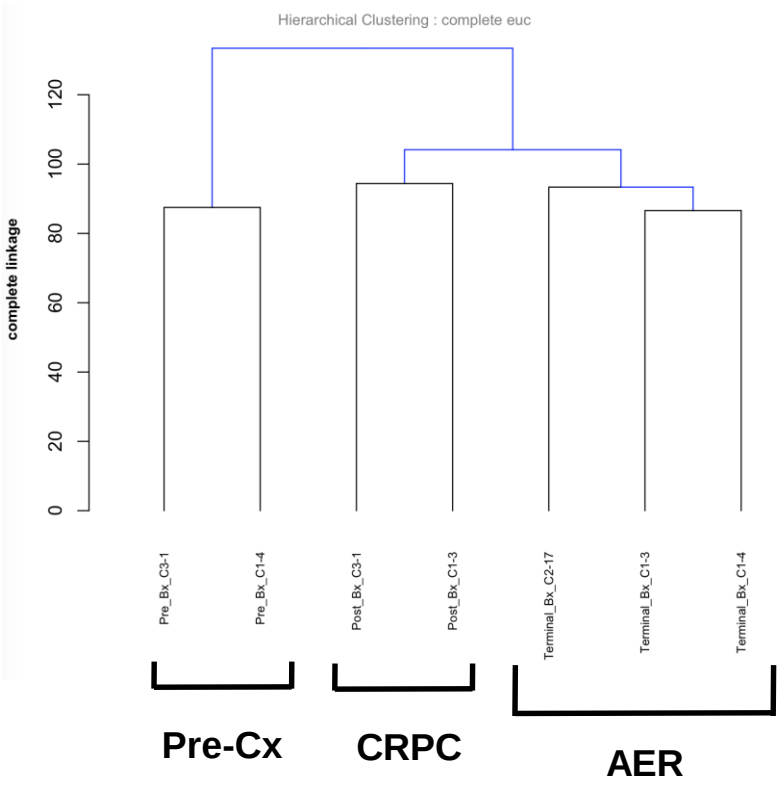

## CRPC vs. Pre-Cx



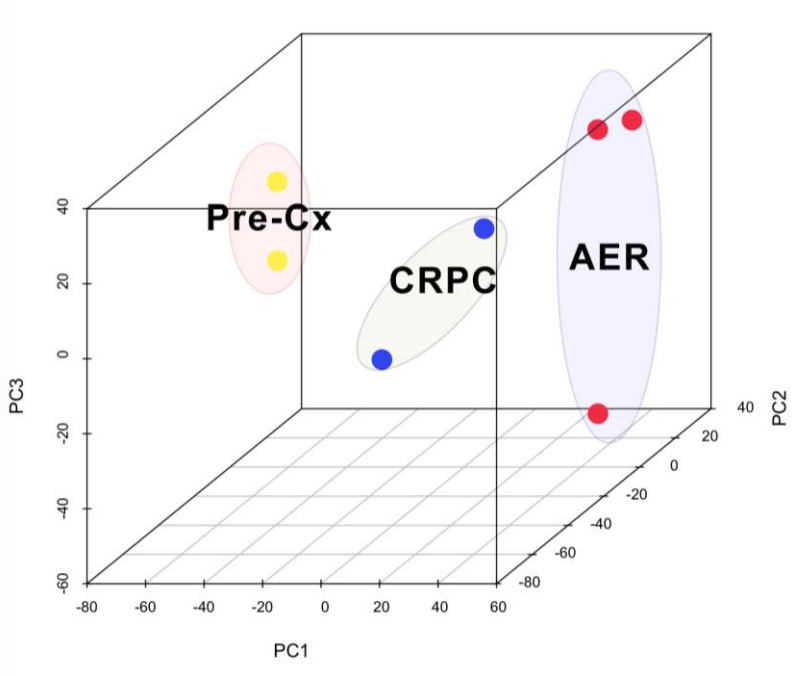

## AER vs. CRPC



**B.**

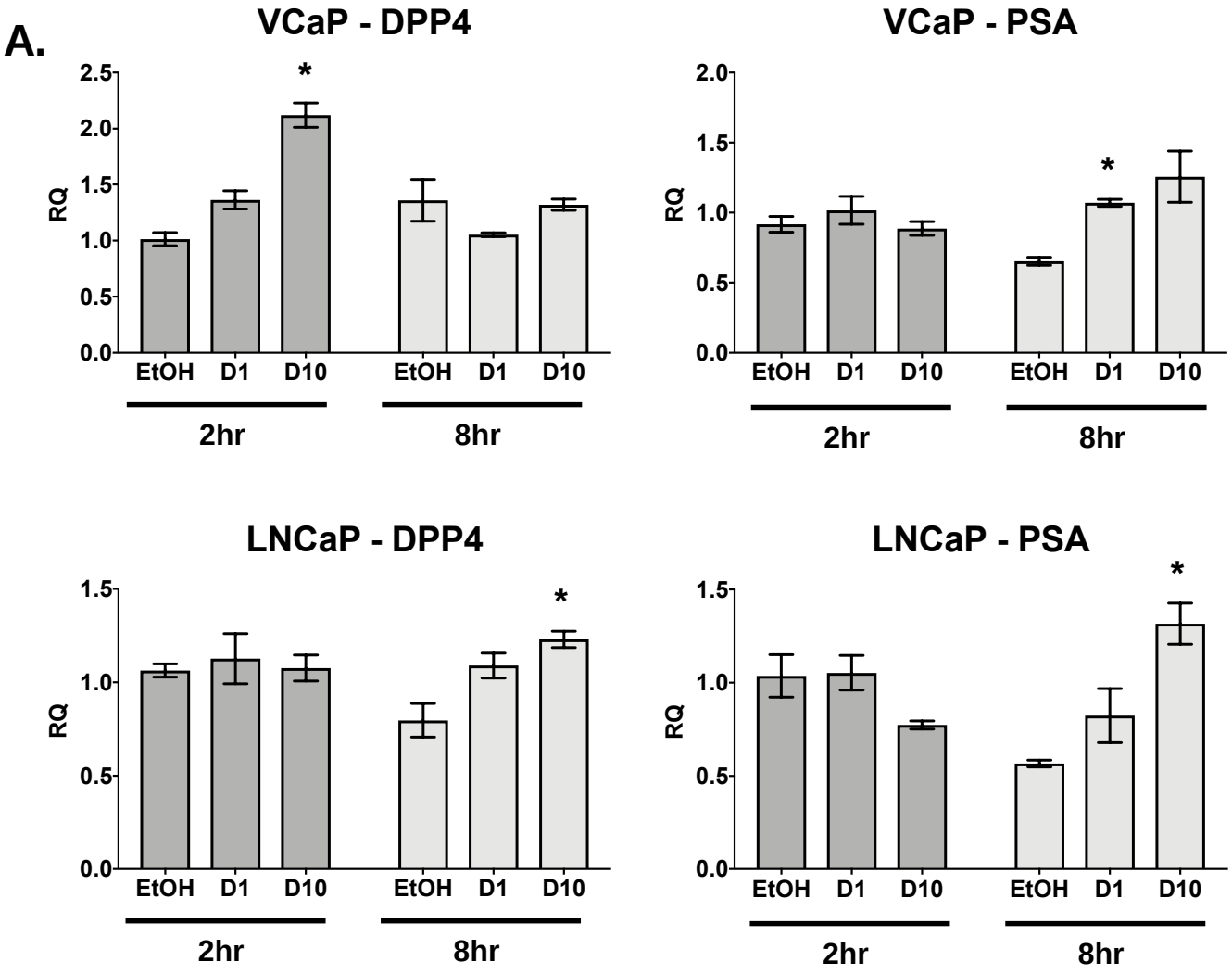

Hierarchical Clustering : complete euc
complete linkage
0
20
40
60
80
100
120
Pre_Bx_C3-1
Pre_Bx_C1-4
Post_Bx_C3-1
Post_Bx_C1-3
Terminal_Bx_C2-17
Terminal_Bx_C1-3
Terminal_Bx_C1-4
Pre-Cx
CRPC
AER

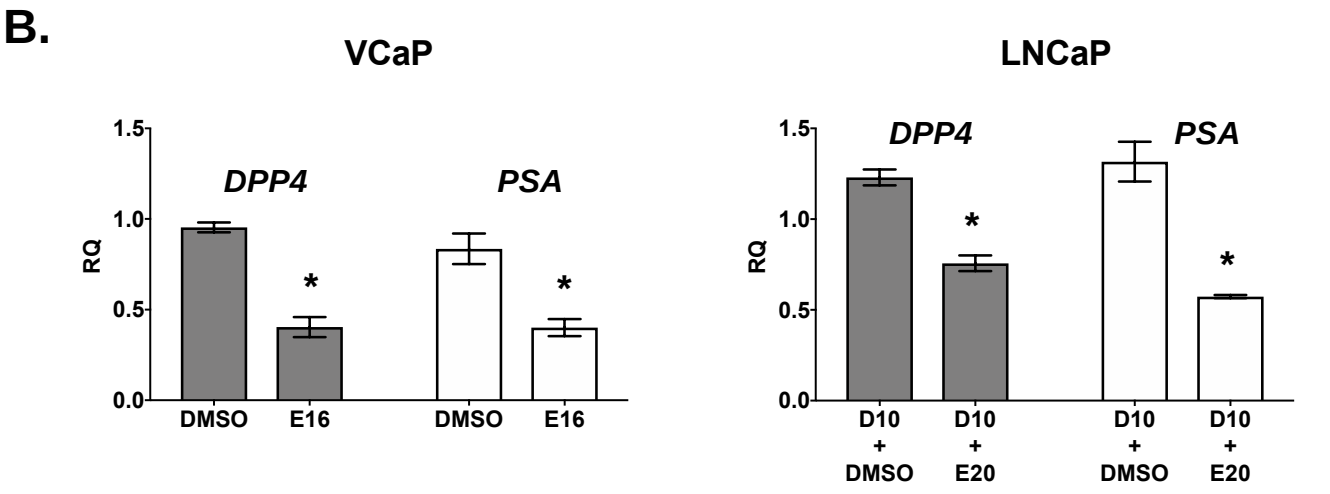
**C.**


Pre-Cx
CRPC
AER
PC3
40
20
0
-20
-40
-60
PC1
-80
-60
-40
-20
0
20
40
60
PC2
40
20
0
-20
-40
-60
-80

**Supplementary Figure S3 – A)** Ingenuity Pathway Analysis of AER vs. Pre-Cx, CRPC vs. Pre-Cx, and AER vs. CRPC serial biopsies based on genes with significant differential expression. **B)** Hierarchical clustering and **C)** Principal component analysis of Pre-Cx, CRPC, and AER, serial biopsies based on overall gene expression.

**Supplementary Figure S4**



**Supplementary Figure S4 – *DPP4* is an AR-stimulated gene whose expression can be blocked by enzalutamide.** RT-PCR showing RNA expression of *DPP4* and *PSA* following DHT stimulation and enzalutamide treatment. **A)** VCaP cells were cultured in 10% FBS and LNCaP cells were cultured in 5% CSS for two days, followed by stimulation with 1nM (D1) or 10nM (D10) DHT for 2 and 8 hrs prior to mRNA isolation. **B)** VCaP cells were cultured in 10% FBS with or without 16uM enzalutamide (E16) for 8hrs prior to RNA isolation. LNCaP cells were cultured in 5% CSS, followed by addition of 10nM DHT (D10) with or without 20uM enzalutamide (E20) for 8hrs prior to RNA isolation. Each column represents the expression levels from 3 biological replicates, with RT-PCR performed on each in technical triplicate. Bars = SEM. * = $p<0.05$, Student's t test with Welch correction with comparison made to EtOH control for a given time period in part A and and to DMSO control within a given gene for Part B. RQ = Relative Quantification

**Supplementary Figure S5**

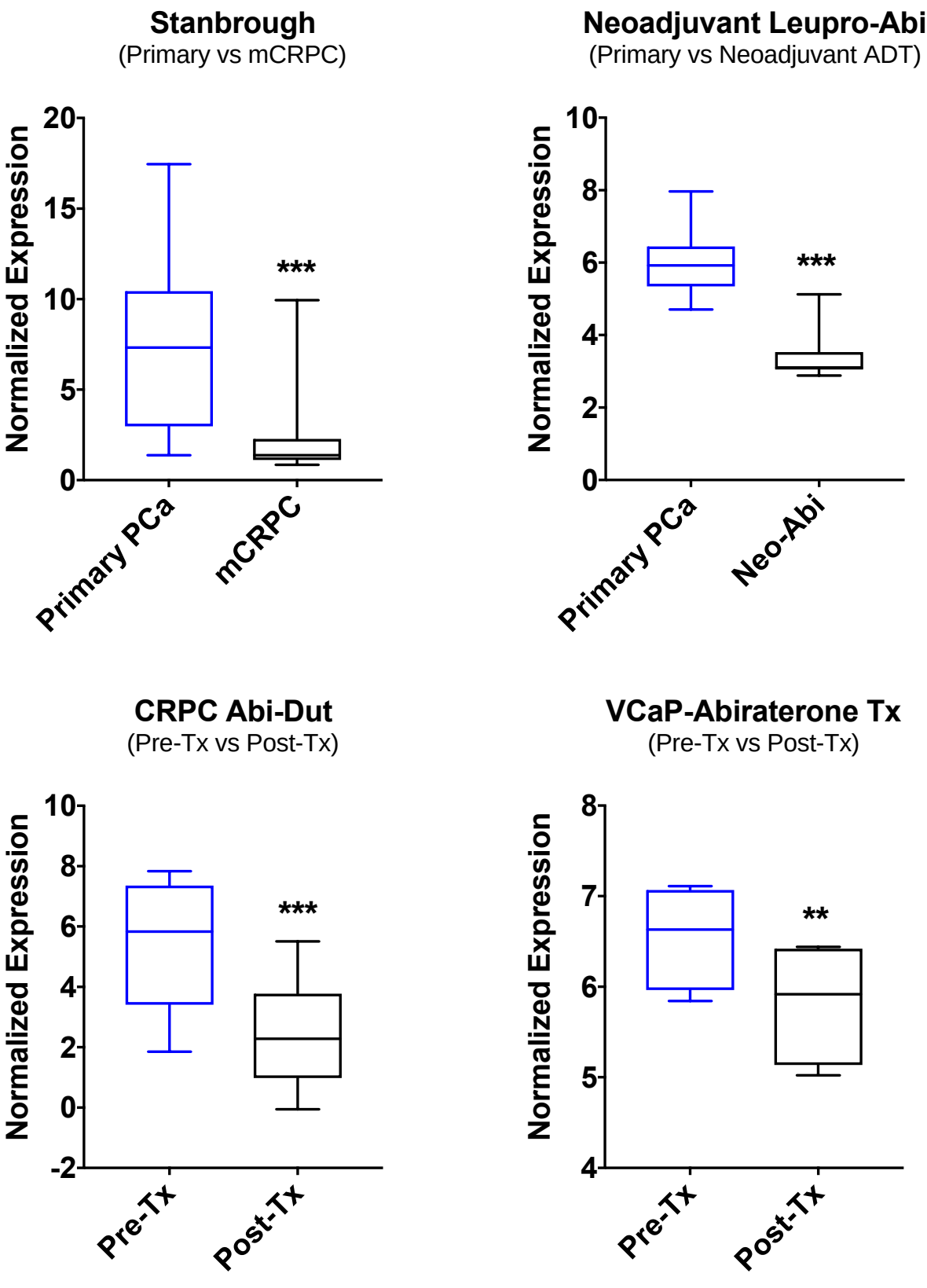
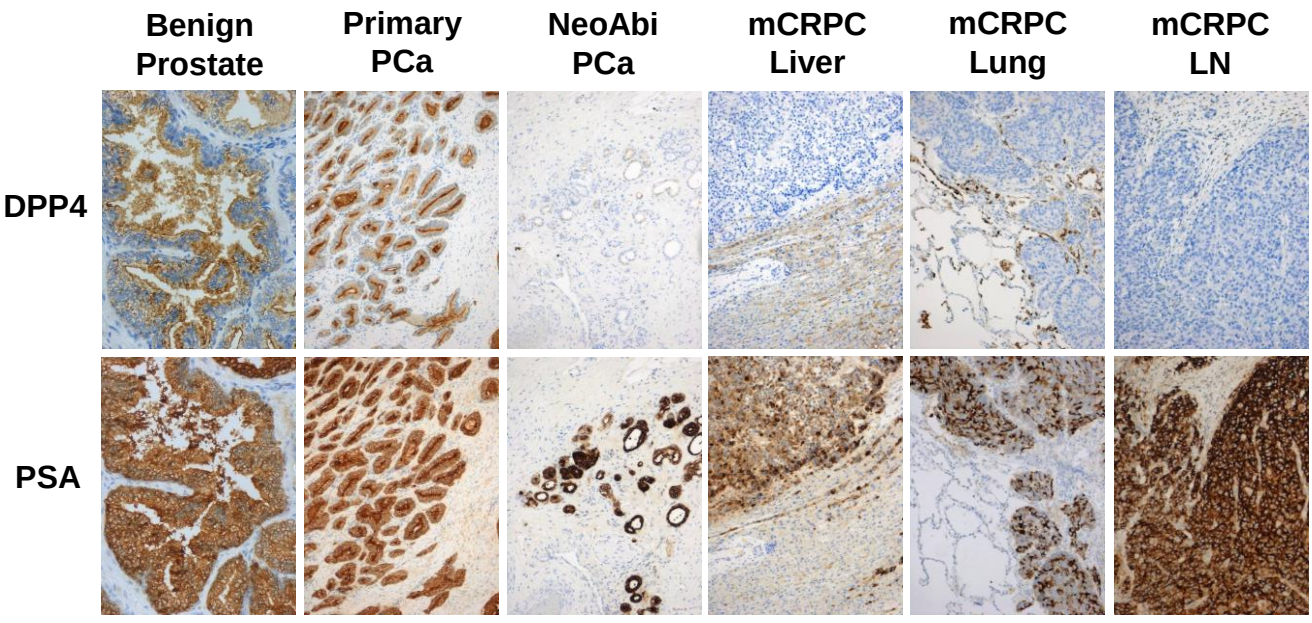


**Figure S5 – DPP4 expression is decreased in Post-ADT therapy samples in several clinical and one preclinical data set compared to Pre-Cx. A)** RNA expression levels of *DPP4* obtained through Affymetrix microarray (Stanbrough) or RNA sequencing (CRPC Abi-Dut, Neoadjuvant Leupro-Abi, and VCaP Abiraterone-Tx) of material from several clinical and preclinical studies comparing Pre-ADT samples to resistant Post-ADT samples.*** = P<0.0001, ** = <0.001, limma for RNA sequencing studies and two-tailed student's t-test for microarray study. Abi – Abiraterone, Dut – Dutasteride, Leupro – Leuprolide.

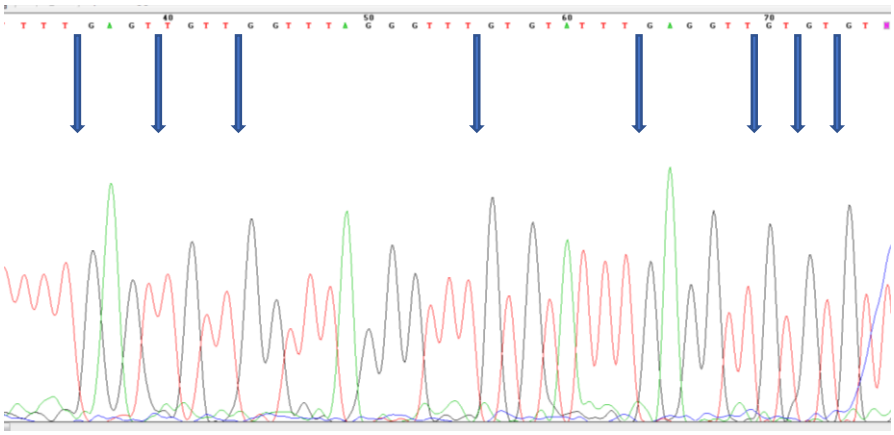
# Supplementary Figure S6



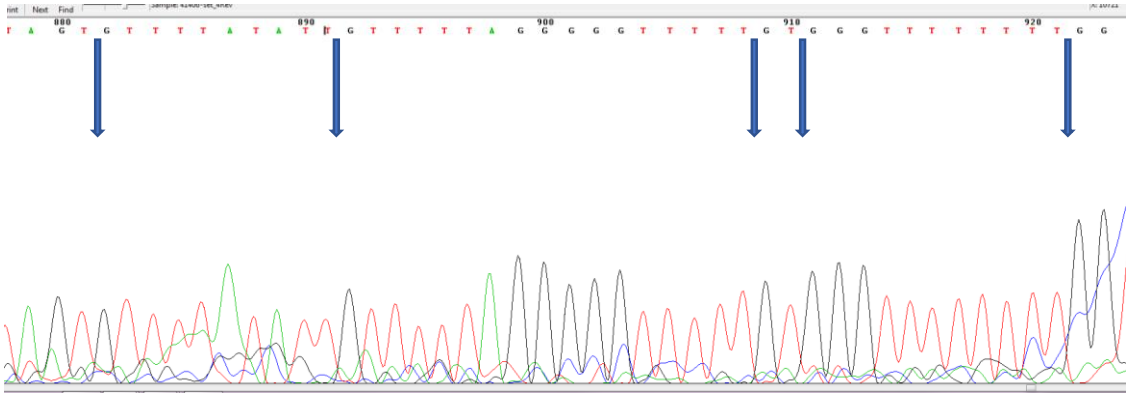
**Supplementary Figure S6 – DPP4 expression is decreased in clinical CRPC sections, despite normal levels of PSA.** Representative images of DPP4 and PSA immunohistochemistry from sections of benign prostate, hormone-naïve primary prostate cancer (Primary PCa), neoadjuvant leuprolide plus abiraterone-treated primary prostate cancer (Neo-Abi PCa), and metastatic castration-resistant prostate cancer (mCRPC). LN – lymph node.

# Supplementary Figure S7

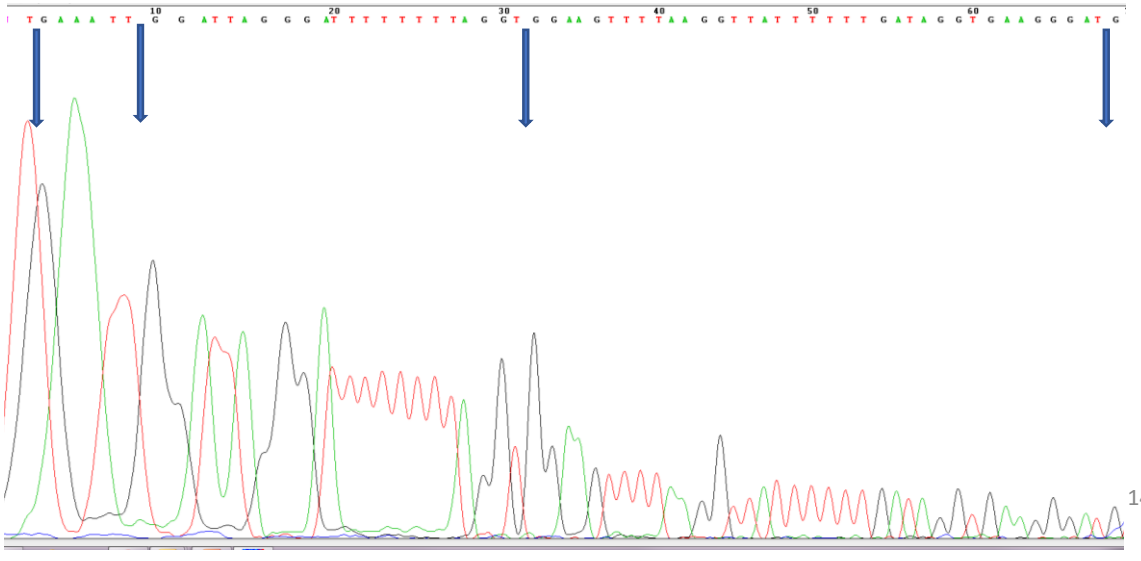
CpG islands 9-16 in promoter region (position -390/-348)



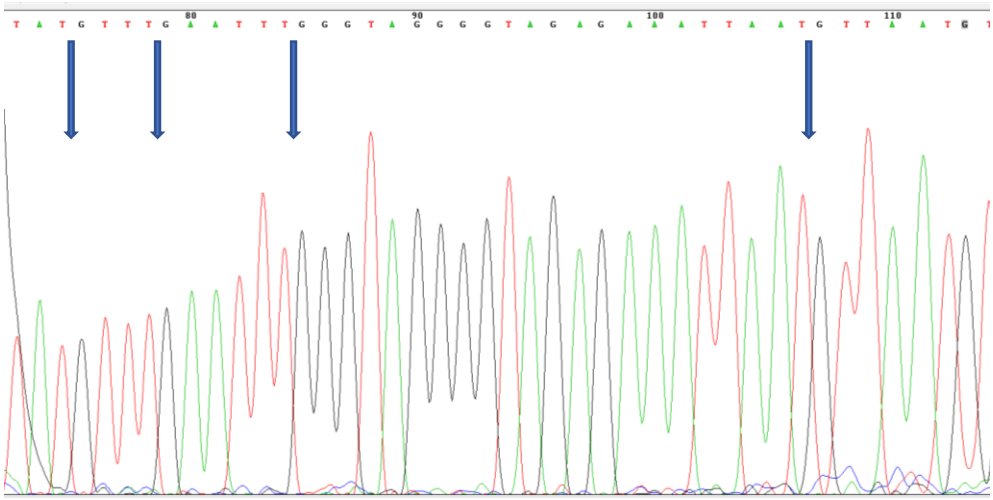
CpG islands 23-27 in promoter region (position -290/-245)



CpG islands 70-73 in exon 1 (position 87/153)

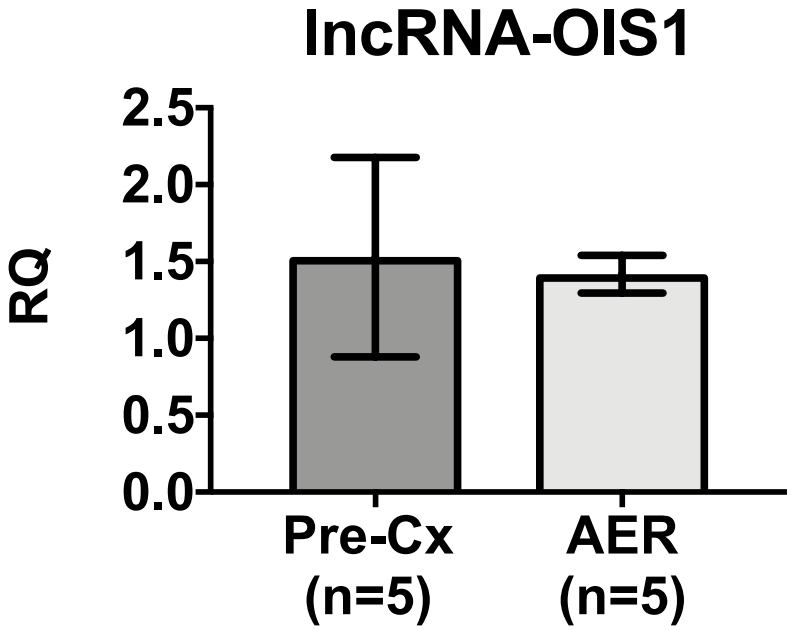


CpG islands 80-83 in promoter region (position 247/280)



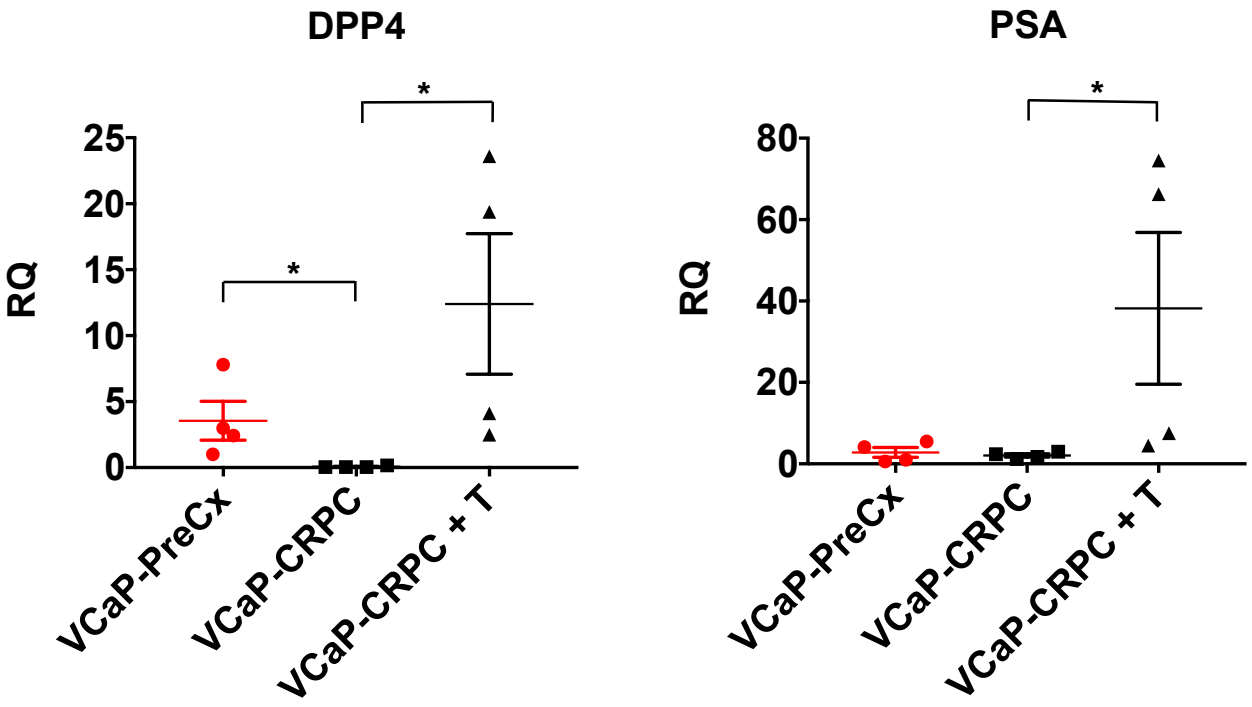
**Supplementary Figure S7 – CpG islands in the *DPP4* promoter and exon 1 are unmethylated.** Representative results of Sanger sequencing of bisulfite-converted DNA from the promoter/exon 1 region of the *DPP4* gene from four Pre-Cx and five AER VCaP tumors that underwent RNA sequencing. Blue arrows mark positions of individual CpG islands. All of the sequenced islands in all Pre-Cx and AER samples showed conversion of CpG cytosines to uracil (converted to thymine following amplification), indicating all sequenced CpG islands were unmethylated and the methylation status of the DPP4 promoter does not change with progression.

**Supplementary Figure S8**

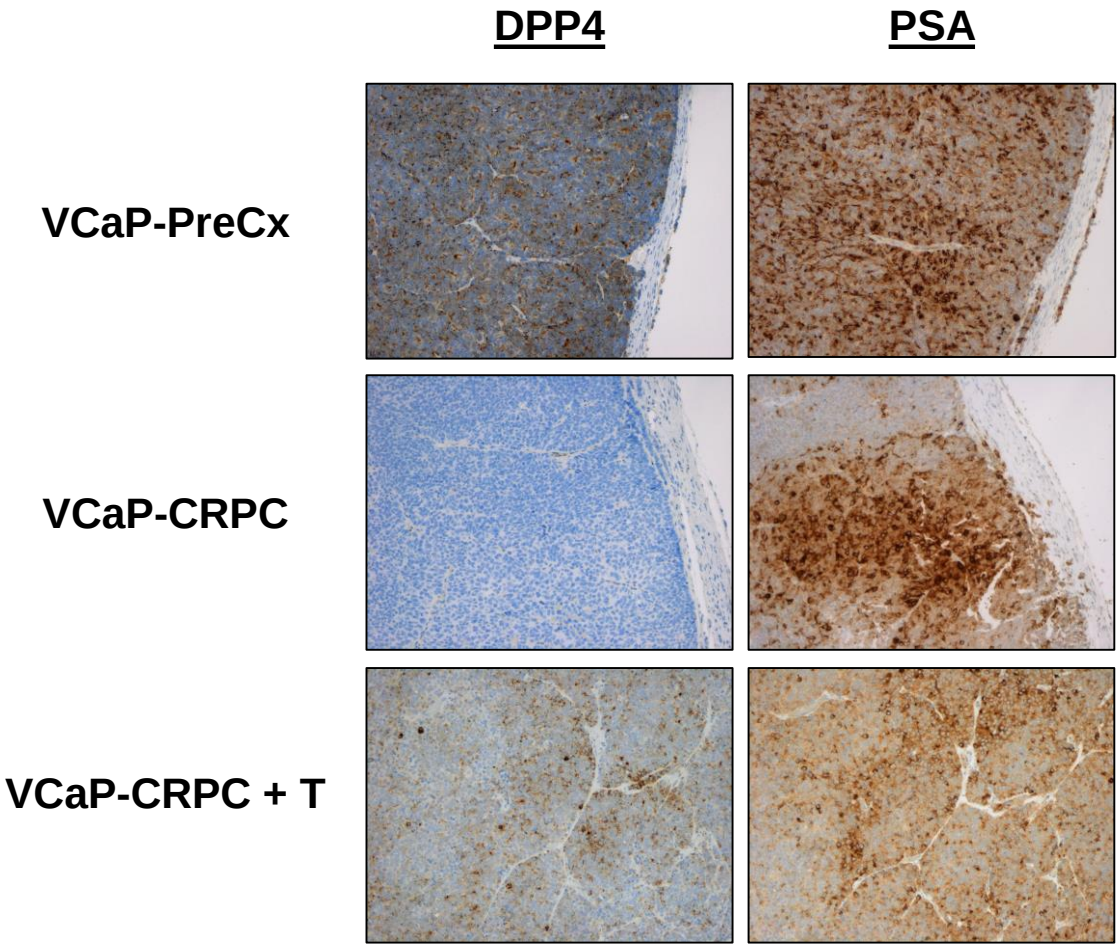


**Supplementary Figure S8 – lncRNA-OIS1 is expressed at similar levels in Pre-Cx and AER VCaP tumors.** RT-PCR showing RNA expression of lncRNA-OIS1 in Pre-Cx and AER biopsies of VCaP xenografts. Each column represents the expression levels from five biological replicates, with RT-PCR performed on each in technical triplicate. Bars = SEM. P= 0.1508, Mann Whitney U. RQ = Relative Quantification

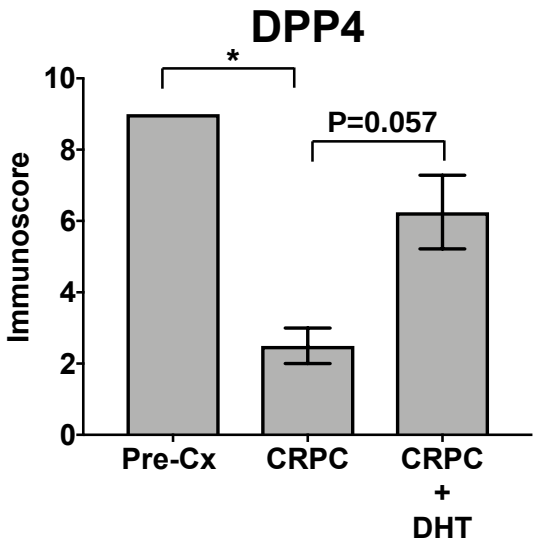
A. **Supplementary Figure S9**

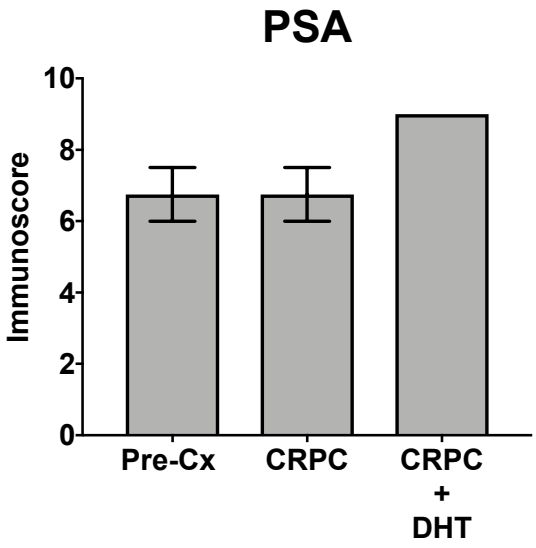



B.



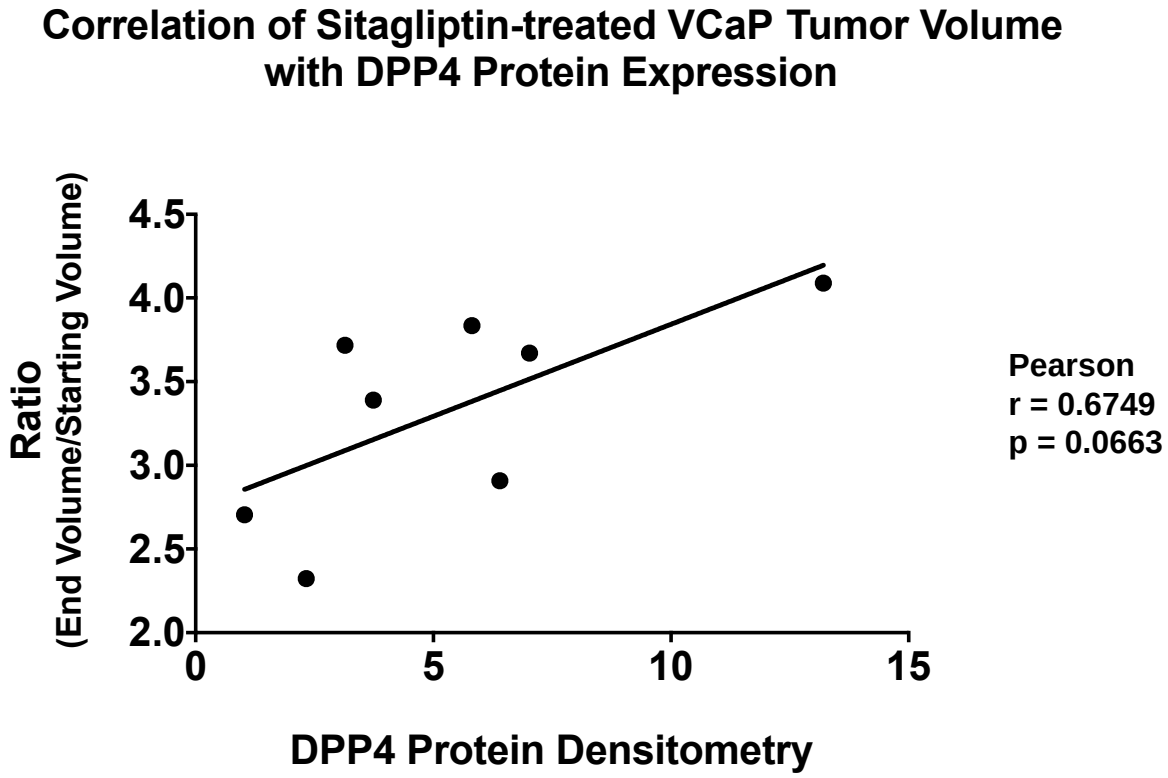
C.


DPP4
*
P=0.057
Immunoscore
10
8
6
4
2
0
Pre-Cx
CRPC
CRPC + DHT


PSA
Immunoscore
10
8
6
4
2
0
Pre-Cx
CRPC
CRPC + DHT

**Supplementary Figure S9 – Androgen treatment of VCaP CRPC xenografts restores DPP4 expression. A)** RT-PCR of DPP4 and PSA transcript in Pre-Cx and CRPC VCaP xenograft tumors and in CRPC VCaP xenograft tumors following 3 days of daily IP administration of testosterone (200mg/kg). Each column represents the expression levels of xenograft tumors from four mice, with RT-PCR performed on each in technical triplicate. Bars = standard error of the mean (SEM). * = P<0.03, Mann-Whitney U. RQ = Relative Quantification. T – Testosterone **B)** Representative images of DPP4 and PSA immunohistochemistry from Pre-Cx, CRPC, and CRPC tumors treated with testosterone. **C)** IHC of DPP4 and PSA from four Pre-Cx, four CRPC, and 4 CRPC+DHT tumors were immunoscored as described in methods. * = P<0.05, Mann-Whitney U.

**Supplementary Figure S10**



**Supplementary Figure S10 –The volume ratio (End Volume/Starting Volume) of VCaP tumors shows a trend toward positive correlation with tumor DPP4 protein levels.** The ratio of the end volume to starting volume of VCaP tumors treated with sitagliptin were compared to densitometric levels of DPP4 protein observed by western blot of tumor lysates. Pearson's coefficient of determination.