Supplemental

**Tissue prostate specific antigen (PSA) facilitates refractory prostate tumor progression via enhancing ARA70-regulated androgen receptor transactivation**

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Figure Legend for Supplemental Figures:

Suppl. Fig. 1. The interaction of PSA and ARA70. (a) Interaction of PSA and ARA70 using GST pull-down assays. GST-PSA recombinant protein was incubated with $[^{35}S]$-methionine labeled ARA70, and the protein complex was precipitated by anti-GST antibody. Our results indicate the GST-PSA could interact with ARA70 with GST used as negative control. (b) Co-precipitation of endogenous ARA70, PSA, and AR in high passage LNCaP cells. Using anti-ARA70 monoclonal antibody, we precipitated the ARA70 binding protein complex from 1000 μg LNCaP protein lysate. The results indicated that PSA-ARA70-AR complex exists in prostate cancer cells. (c) The confocal microscope showed that ARA70 and PSA could be colocalized in the cytosol of high passage LNCaP cells treated with 1 nM DHT. PSA, ARA70 and AR are first recognized by goat anti-PSA, mouse anti-ARA70 and rabbit anti-Giantin antibody, and secondly developed into green (Alexa Fluors 488), red (TEXAS-RED) and blue (Alexa Fluors 647) fluorescence. PSA and ARA70 mainly existed in the cytosol, while AR could be located in both cytosol and nuclear in the high passage LNCaP cell treated with 1 nM DHT. When overlapping PSA green color to the ARA70 red color in Merge 1, PSA shows diffused in cytosol and colocalized with ARA70. Merge 2 shows that PSA colocalized with ARA70 and AR in the cytosol. (d) Using the confocal microscope, we found that the PSA (green color) is not restricted inside of the Golgi’s apparatus (blue).

Suppl. Fig. 2 (a). The endogenous expression levels of AR, PSA and ARA70 in LNCaP, CWR22rvl, PC-3 and COS-1 were examined using Western blotting assay. b. LNCaP, CWR22rvl, PC-3 and Du145 cells were transfected with pCDNA3-vector or pCDNA3-PSA. The expression of
PSA in these stable cell clones was determined by Western Blot assay. c. PSA alone, without ARA70, could not enhance AR transactivation using MMTV-luciferase assay in COS-1 cells (lane 7 vs lane 2). d. The knockdown efficiency of ARA70siRNA, compared with its scramble siRNA, was demonstrated by assaying ARA70 protein levels.