SUPPORTING INFORMATION

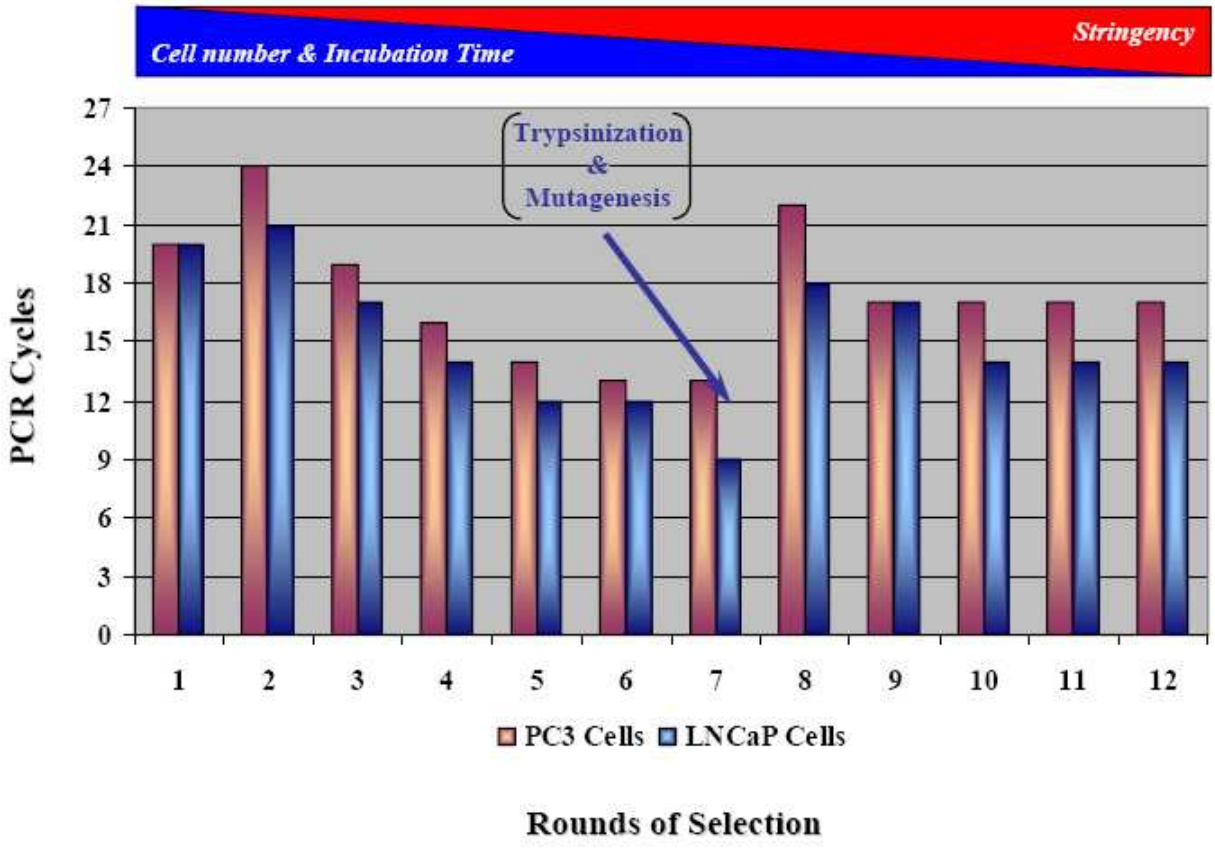

Figure S1. Progress of the selections identified by the number of PCR cycles. Stringency was increased by diminishing both the number of PC3 (pink) and LNCaP (blue) cells and the incubation time. After round 7, mutagenic PCR was used to explore the sequence-neighborhood of the selected libraries, and extensive trypsinization of the PC3 and LNCaP cells was applied to eliminate non-internalized RNAs.

Apt truncation. We truncated the selected sequences by analyzing the predicted secondary structure. Using a method reported to determine the essential sequences,[1] we got the minimized lengths of XEO2 and XEO6 required for specific binding. For the truncated form of XEO2, hereinafter referred to as XEO2mini, a total of 43 nucleotides were removed from its parent XEO2 Apt. The truncated Apt XEO6mini was created by the removal of 27 nucleotides from its parent Apt XEO6. The predicated secondary structures of XEO2 and XEO2mini (boxed) are shown in Figure S2.

Notably, compared to XEO2, XEO2mini showed enhanced intracellular uptake into PC3 cells, but diminished intracellular uptake into LNCaP (Figure S3), RWPE-1, and BPH cells (Table 2), indicating an increase in internalization specificity. A possible reason may be that the XEO2 fragment that binds to the non-specific antigen was eliminated during truncation, while the XEO2 fragment that binds to the specific antigen was retained within the truncated Apt XEO2mini, resulting in the increased internalization specificity. The intracellular distribution of cy3-labeled XEO2mini was show in Figure S4.

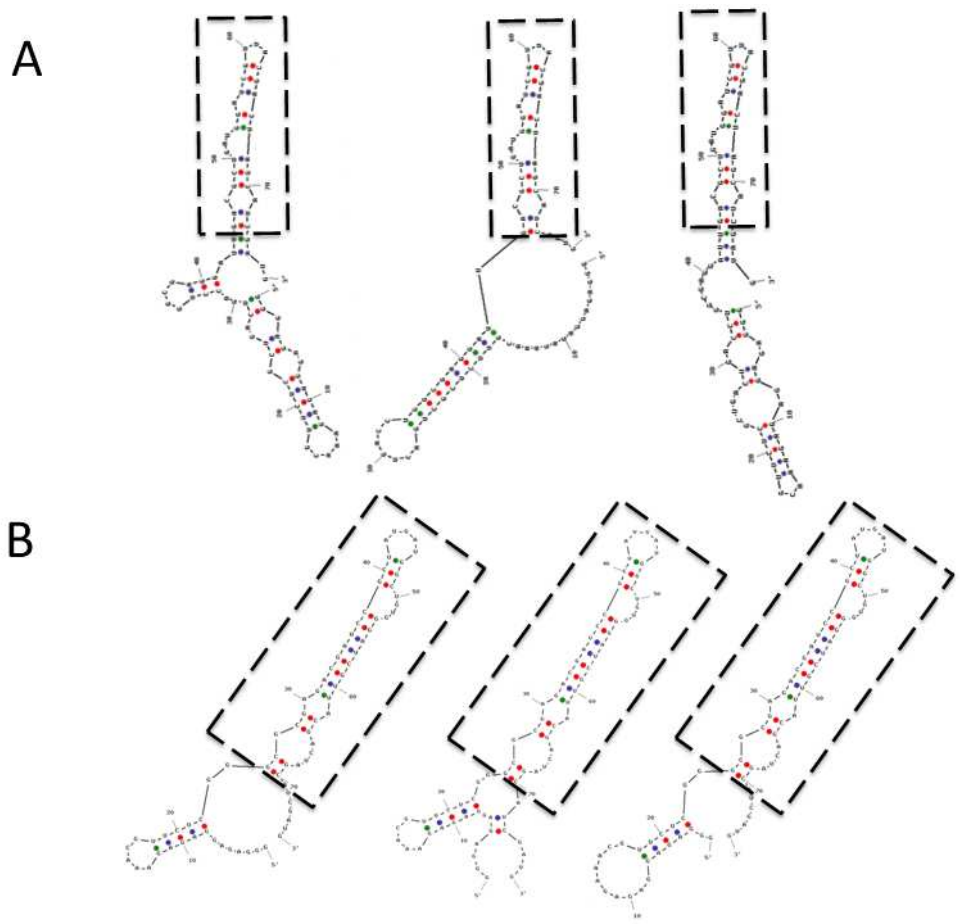

Figure S2. Predicted secondary structures of selected Apt XEO2 (A) and XEO6 (B). The conserved regions that share similar structures in the box are truncated as XEO2mini and XEO6mini.

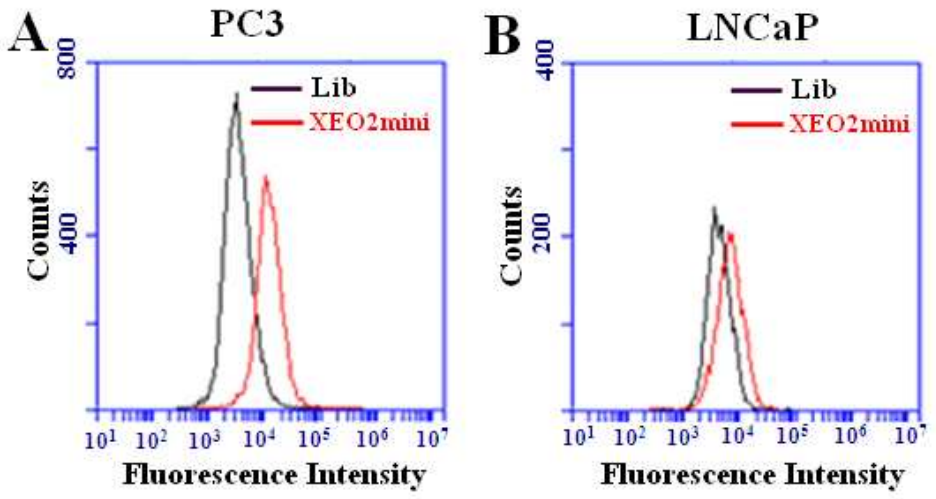

Figure S3. XEO2mini internalization into PC3 (A) and LNCaP (B) by flow cytometry analysis.

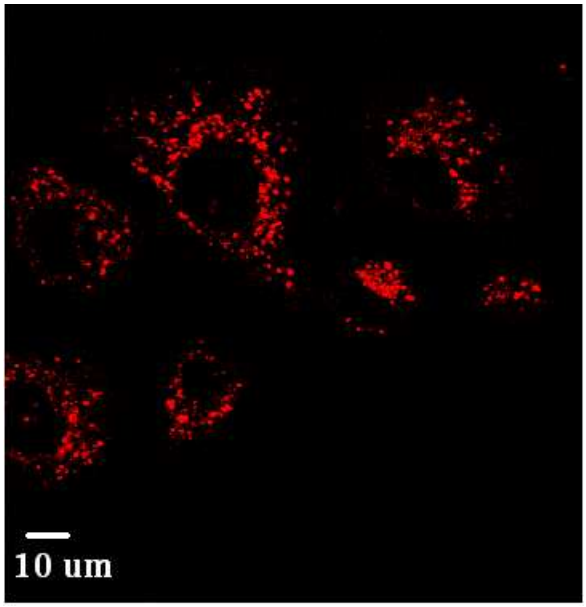

Figure S4. A representative fluorescence image of internalized XEO2mini-Cy3 inside PC3 cells.

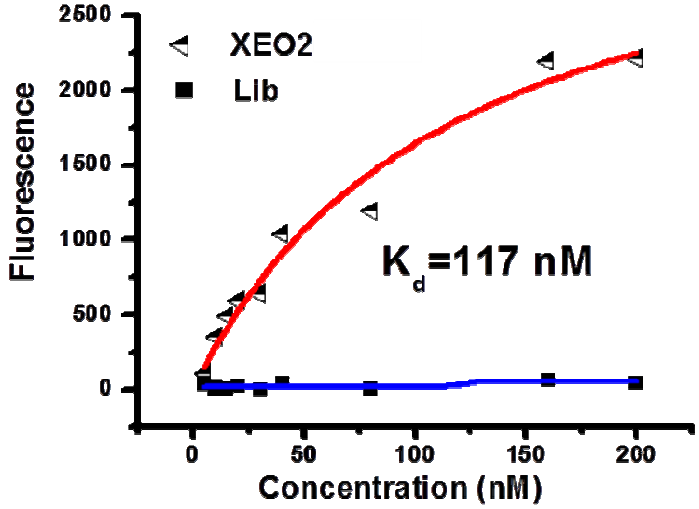

Figure S5. Binding curve of cy3-labeled Apt XEO2 to PC3 cells with a Kd=117 nM. Blue line showed the nonspecific binding by cy3-labeled initial library.

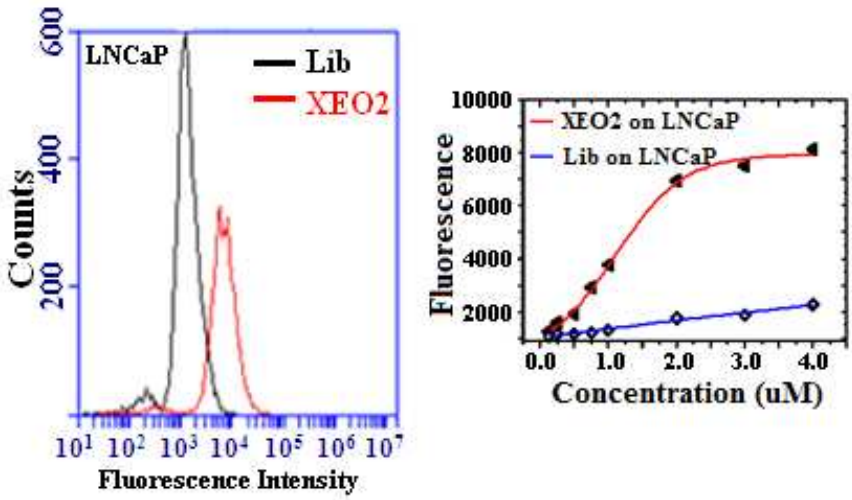

Figure S6. Left: representative flow cytometric profiles showing XEO2 internalization signals in LNCaP cells. The black curve represents the background uptake of unselected initial library. The red curve is the XEO2 profile. Right: Cy3-labeled XEO2 (Library) was incubated with LNCaP cells at different concentrations. Fluorescence signals from inside cells were determined by flow cytometry.

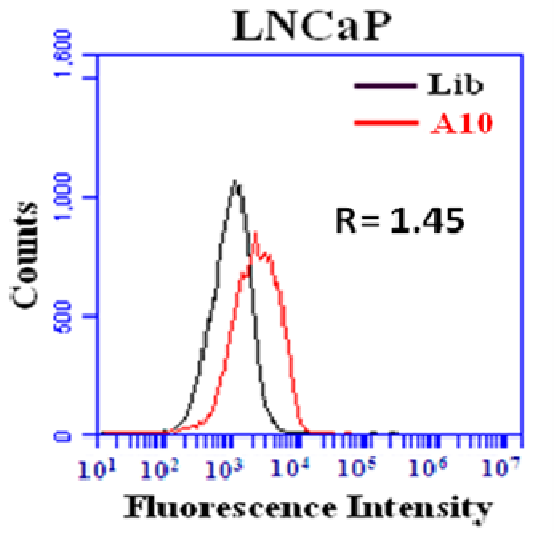

Figure S7. The internalization of A10 Apt (i.e. anti-PSMA Apt) into LNCaP by flow cytometry analysis. The R value is 1.45 by using the same criteria in Table 2.

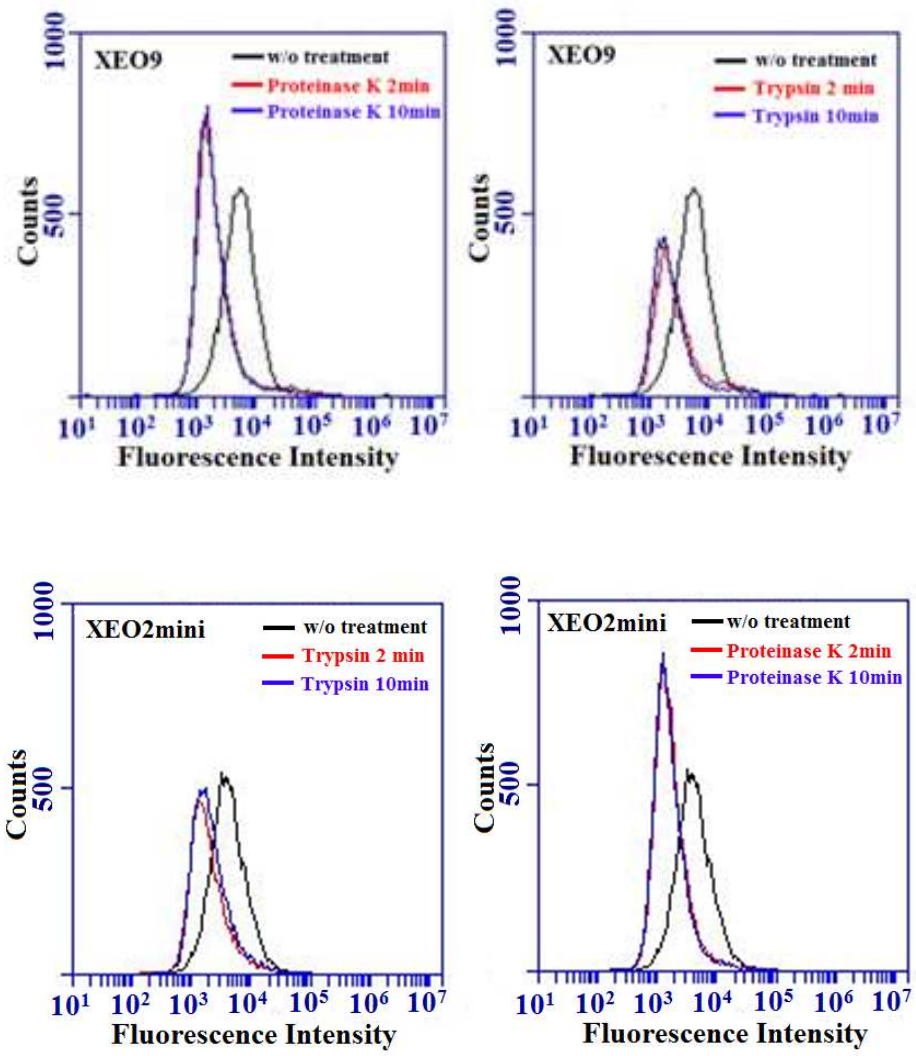

Figure S8. Effects of proteinase K (left) or trypsin (right) treatment on XEO9 (upper) and XEO2mini (bottom) binding profile to PC3. Proteinases were treated for 2 or 10 min before incubation with Apts.

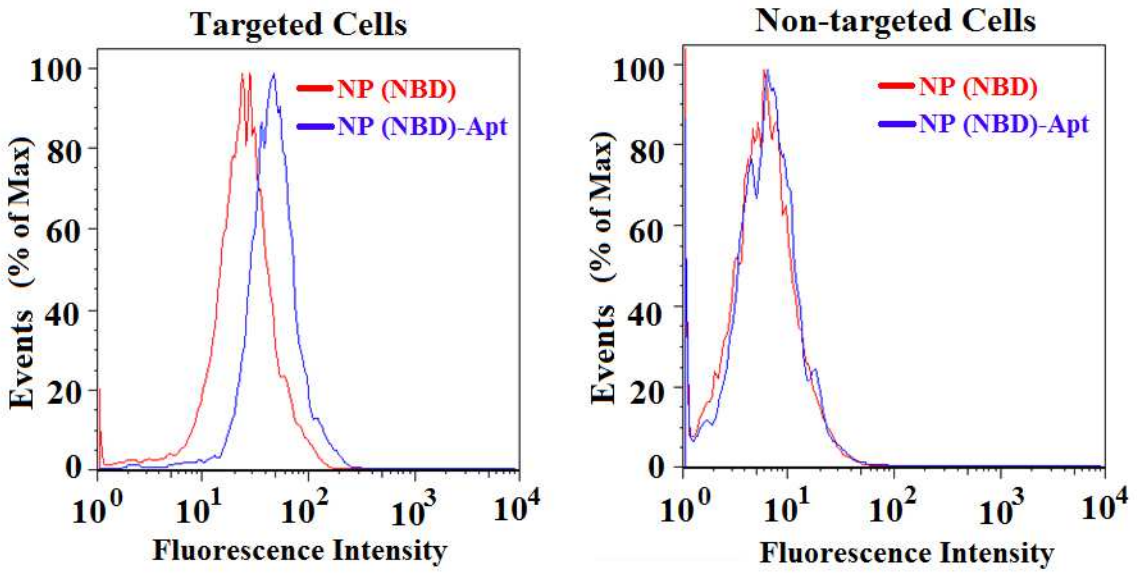

Figure S9. Targeted delivery of NP-XEO2mini (NBD) in PC3 cells and HeLa cells by flow cytometry analysis.

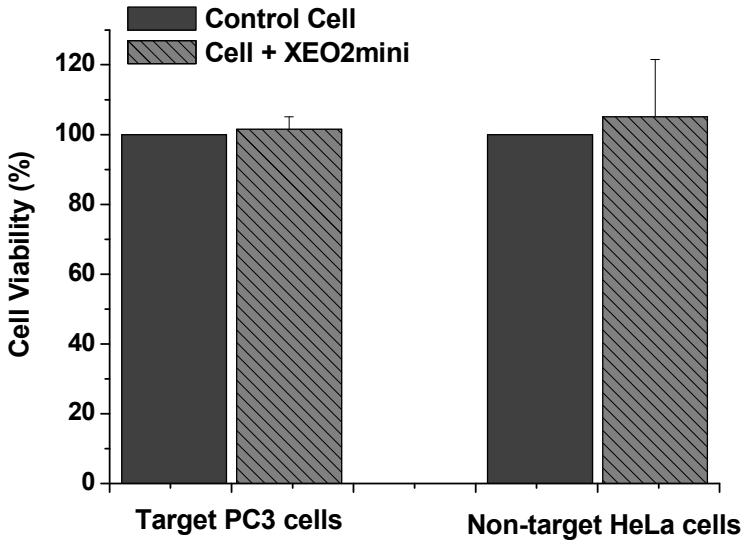

Figure S10. Cytotoxicity study of XEO2mini Apt in target PC3 cells and non-target HeLa Cells.

1. Shangguan, D.; Tang, Z.; Mallikaratchy, P.; Xiao, Z.; Tan, W., Optimization and modifications of aptamers selected from live cancer cell lines. *Chembiochem* **2007,** 8, (6), 603-6.