Supplementary Data

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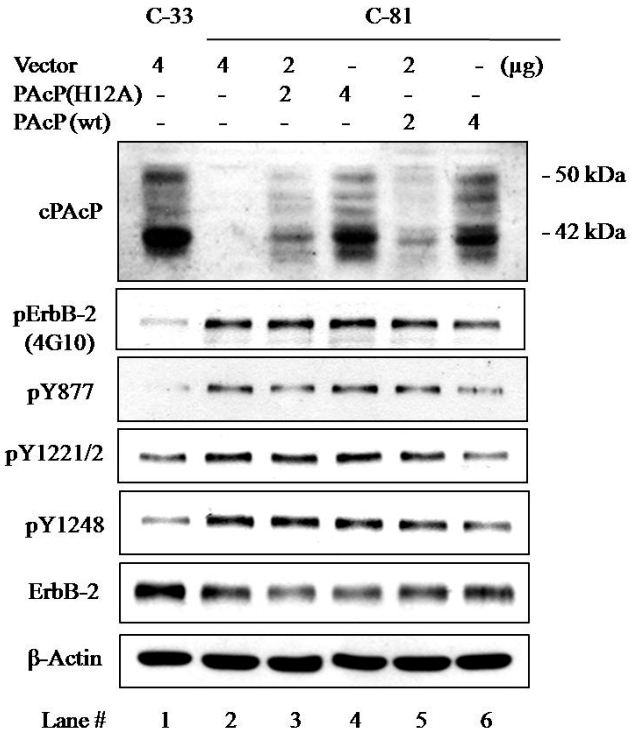
Human prostatic acid phosphatase, an authentic tyrosine phosphatase, dephosphorylates ErbB-2 and regulates prostate cancer cell growth

Supplementary Figure Legends:

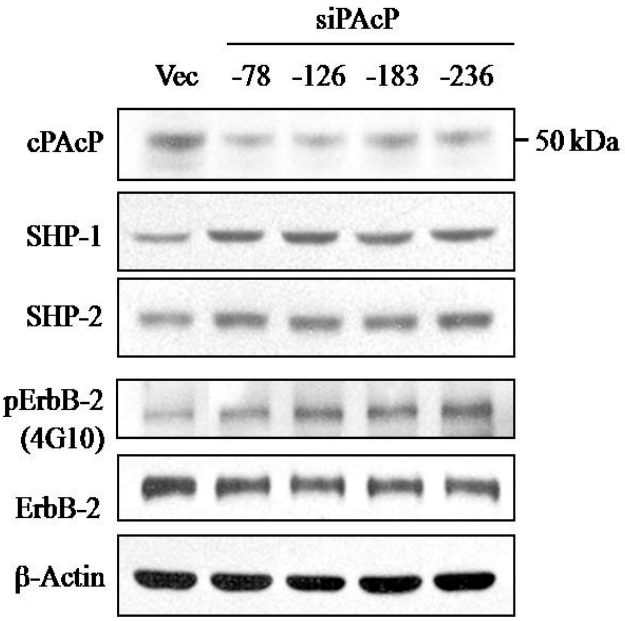
Figure 1. C-81 cells were transiently transfected with the wild-type (wt), mutant PAcP (H12A) plasmids or vector alone. After 48h, cPAcP, the total and the specific tyrosine phosphorylation level of ErbB-2 was respectively analyzed by Western blotting. The protein levels of ErbB-2 and β-actin were examined after the membranes were stripped.

Figure 2. LNCaP C-33 cells were respectively transfected with four pSUPER vector encoding siRNA oligonucleotides (siPAcP) that target to PAcP mRNA at different positions. Control cells were transfected with vector containing scramble oligos (Vec). SHP-1, SHP-2 and the phosphorylation level of ErbB-2 were determined in total cell lysates by western blotting. The protein level of ErbB-2 was analyzed by rehybridizing the same membrane with ErbB-2 Ab after stripping. β-actin level was used as a loading control.

Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Table

Supplementary Table 1. Subfractionations of AcP activity in LNCaP C-33 cells

Fraction	AcP Activity (A_{410})	PAcP Activity (A_{410})
Cytosol	6.90 (37.0%)	4.14 (40%)
Membrane-Associated	0.38 (2.0%)	Not detectable
Membrane-Bound	11.24 (61.0%)	6.15 (60%)
Total	18.52 (100%)	10.29 (100%)

LNCaP C-33 cells were seeded in regular medium for 3 days for its confluency and then maintained in a steroid-reduced medium for 2 days. Confluent cells were harvested and separated into the cytosol fraction, membrane-associated fraction and membrane-bound fraction by ultracentrifugation. The cytosol fraction was the supernatant of 160,000 xg centrifugation as described in Materials and Methods. These three fractions were used to detect total AcP activity and PAcP activity.