Supplemental Data:

A Hybrid Approach to Overcome Defects of CE-SELEX and Cell-SELEX in Developing Aptamers against Aspartate β-hydroxylase

Running title: Hybrid approach to improve aptamer selection

Hadi Bakhtiari[1, 2], Hamed Naghoosi[3*], Sina Sattari[4], Mahmoud Vahidi[5], Mehdi Shakouri Khomartash[5], Ali Faridfar[1], Mohsen Rajaeinejad[1], Mohsen Nikandish[1]

[1] *Cancer Epidemiology Research Center, Aja University of Medical Sciences, Tehran, Iran.*

[2] *Department of Medical Laboratory Sciences, Faculty of Medicine, Islamic Azad University, the branch of Arak, Arak, Iran.*

[3] *Infectious Diseases Research Center, Aja University of Medical Sciences, Tehran, Iran.*

[4] *Health Research Center, Shahid Chamran Hospital, Tehran, Iran.*

[5] *Medical Biotechnology Research Center, Aja University of Medical Sciences, Tehran, Iran.*

***Corresponding author:** Hamed Naghoosi,

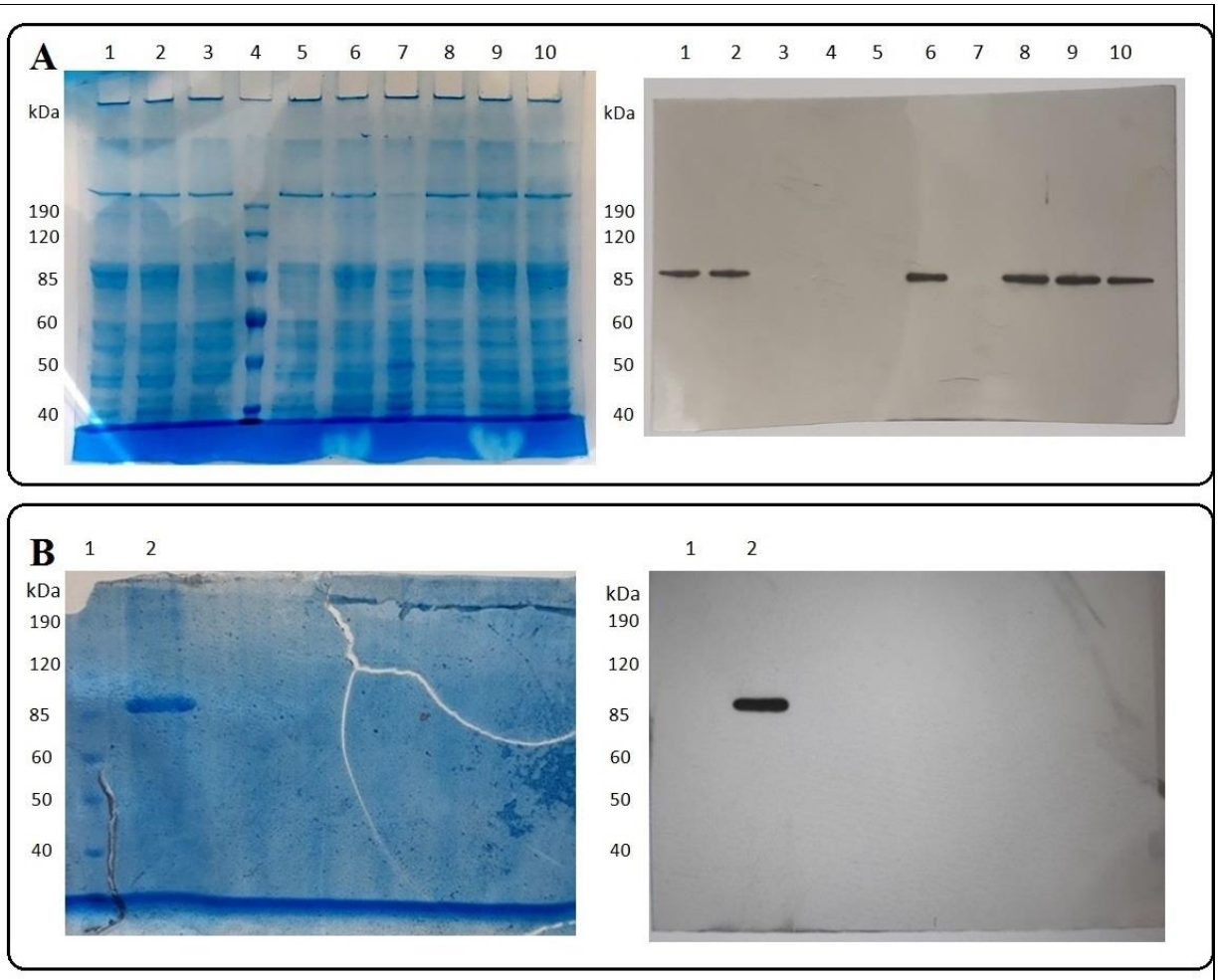

Fig. S1: SDS-PAGE and Western-blot analyses of recombinant ASPH.

A) solubilizing recombinant ASPH from inclusion bodies (IBs); Coomassie-stained SDS-PAGE and Western blot film (from equivalent gel), bands were visualized using Coomassie-stain (SDS-PAGE) and FB50 anti-ASPH antibody (Western blot): Lane 1: solubilized IB with NaCl 500mM; Lane 2: solubilized IB with Triton X100; Lane 3: before induction with IPTG; Lane 4: BenchMark Pre-stained protein ladder (Thermo Fisher Scientific, USA); Lane 5: solubilized IB with NaCl 500mM before induction with IPTG; Lane 6: solubilized IB with sodium deoxycholate; Lane 7: soluble proteins in supernatant; Lane 8: solubilized IB with Tween 20; Lane 9: solubilized IB with Tween 80; Lane 10: solubilized IB with SDS. B) SDS-PAGE and Western-blot analyses of purified and concentrated ASPH via FPLC and Amicon® centrifugal filter 50K: Lane 1: BenchMark Pre-stained protein ladder; Lane 2: purified ASPH.

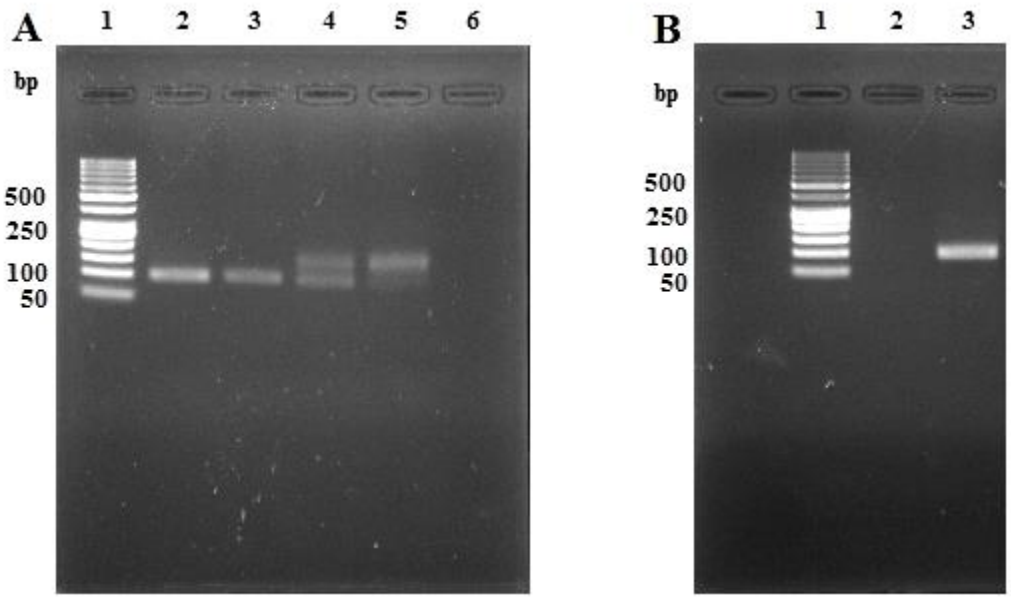

Fig. S2: An example of an agarose gel electrophoresis picture that shows the results of a preparative PCR optimization cycle. A) Lane 1 = 50bp ladder; lane 2 = 6 cycles; lane 3 = 8 cycles; lane 4 = 10 cycles; lane 5 = 12 cycles; lane 6 = negative control. B) Lane 1 = 50bp DNA ladder, lane 2 = negative control, and lane 3 = 6 cycles

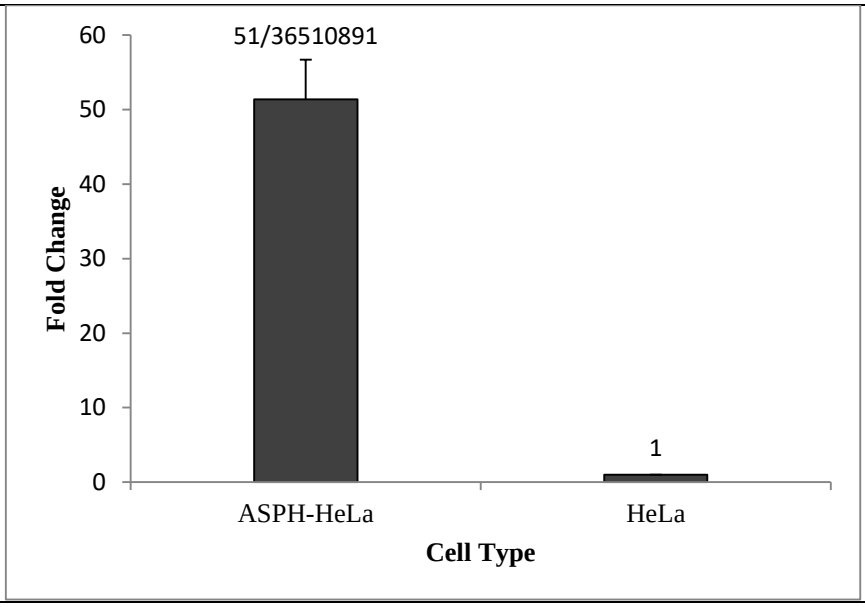

Fig. S3: Comparison of the relative expression of mRNA in HeLa and ASPH-HeLa using Q-PCR. ASPH protein was clearly overexpressed in transfected HeLa cells according to the results of quantitative real-time PCR using the 2-ΔΔCt method of analysis. Statistical significance between groups is calculated by one-sample t-test, * $p < 0.001$.

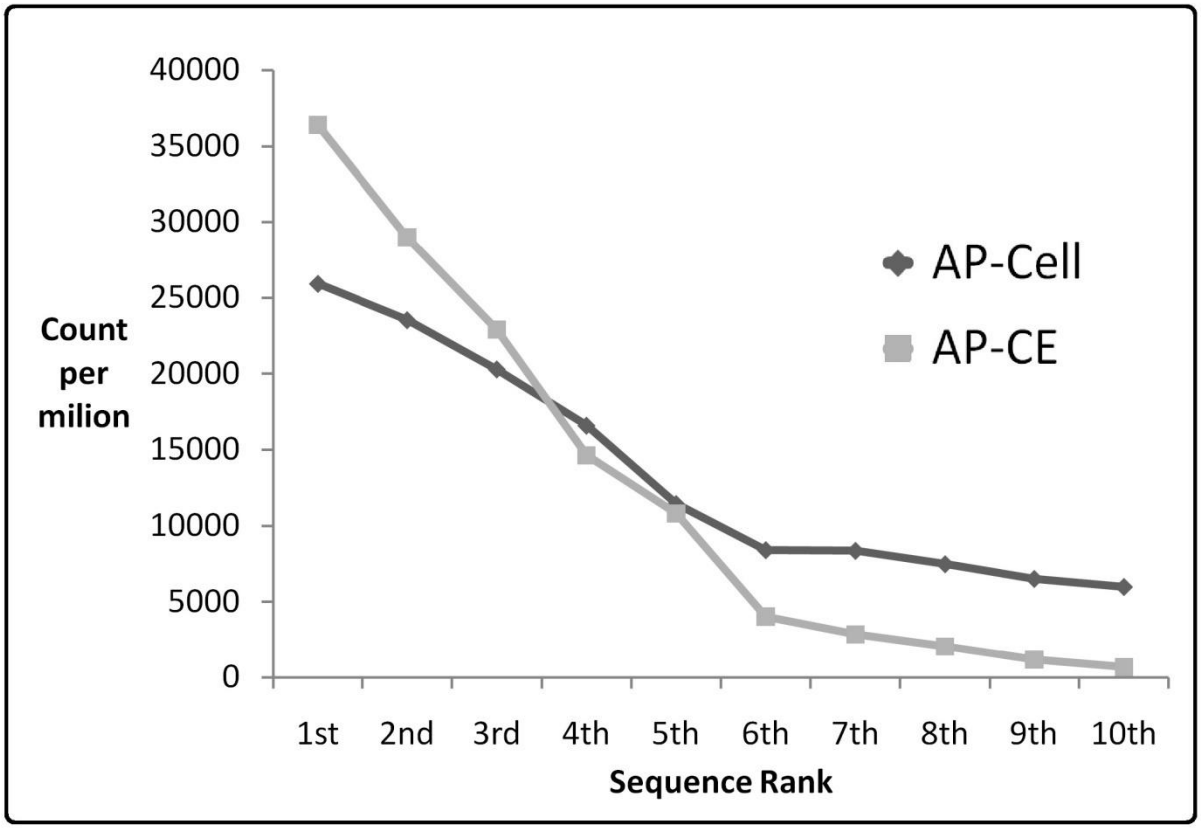

Fig. S4: Comparison of top sequences' frequency between Cell-SELEX and CE-SELEX. A sharp drop in the frequency of top oligomers from CE-SELEX was detected in comparison with the gradual decrement in the case of Cell-SELEX