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Supporting Information

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Enhanced and Differential Capture of Circulating Tumor Cells from Lung Cancer Patients by Microfluidic Assays Using Aptamer Cocktail

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Table S1. NSCLC specific aptamers and scramble DNA sequence Rc used in this study.

Aptamer	*Sequence*
Ap1	5'-TTTATGGGTGGGTGGGGGGTTTTT-3’
Ap2	5'-AGGTGGGTGGGTTGGGTGGATTG-3’
Ap3	5'-GGTTGGTTGG GGTTGGGTTG TTTTTGGGGT GATATGGGGG TTGGA-3’
Ap4	5'-CGGTGGGGGTGGGTAGGTAATCGATAGAGTACGGGGGGTTGGATT-3’
Ap5	5'-TAAAGGGCGGGGGGTGGGGTGGTTGGTA-3’
Ap6	5'-CACGTTCCTCTGTCACCGTCGTCCCTTATCATCCGAGCTATCATCCGAG-3’
Ap7	5'-GGTGGGGTTGTATTGGATTTGAATGGTGGGGGATGGTGGTTGGTG-3’
Ap8	5'-GGTTGCATGCCGTGGGGAGGGGGGTGGGTTTTATAGCGTACTCAG-3’
Ap9	5'-GTGGCCAGTCACTCAATTGGGTGTAGGGGTGGGGATTGTGGGTTG-3’
Ap10	5'-GCTATCTTATGGAAATTTCGTGTAGGGTTTGGTGTGGCGGGGCTA-3’
Rc	5'-AATTTTTTAATTATTTATATTAAT-3’

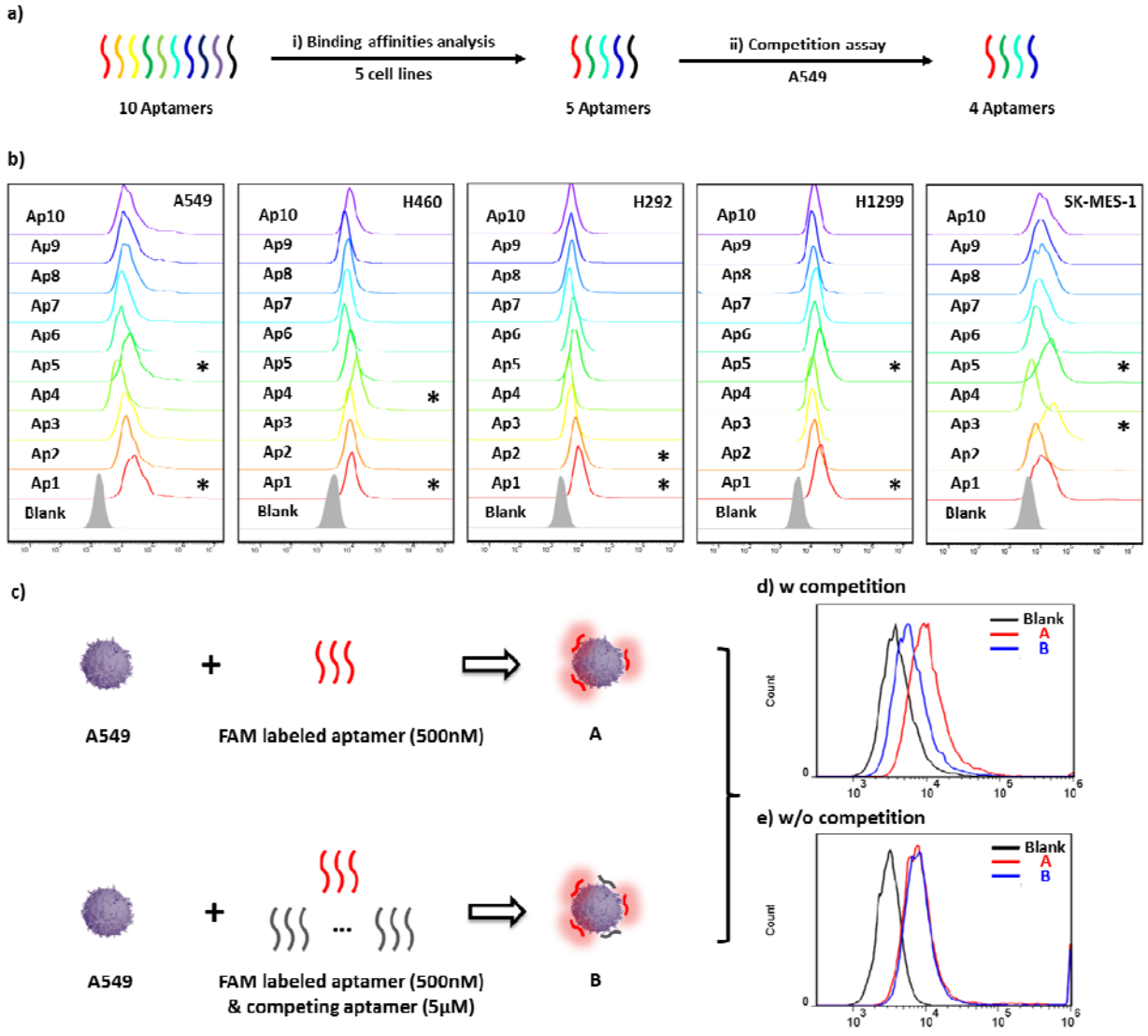

Figure S1. (**a**) The 4 aptamers finally used for formation of different cocktail capture agents (i.e. Ap1, Ap2, Ap3 and Ap4) were obtained following a two-step process: (i) the binding affinities analysis, and (ii) the competition assay. (**b**) Binding affinity analysis.The fluorescent intensity of NSCLC cells after incubation with 500 nM FAM labeled aptamer (ie. Ap1 to Ap10) measured by flow cytomertry. The best two aptamers with the highest binding affinity for each cell line were marked with (*). (**c**) The schematic illustration of competition assay. Harvested A549 cells were divided into two equal aliquots, one aliquot A was incubated with 500 nM FAM labeled aptamer, the other aliquot B was incubated with 500 nM FAM labeled aptamer and 5 μM competing aptamer which has no fluorescence. By comparing the peak shift of fluorescent intensity, it was possible to understand whether competition occurred between these two aptamers. For example, (**d**) Ap1 and Ap5, with competition, and (e) Ap1 and Ap2 without competition.

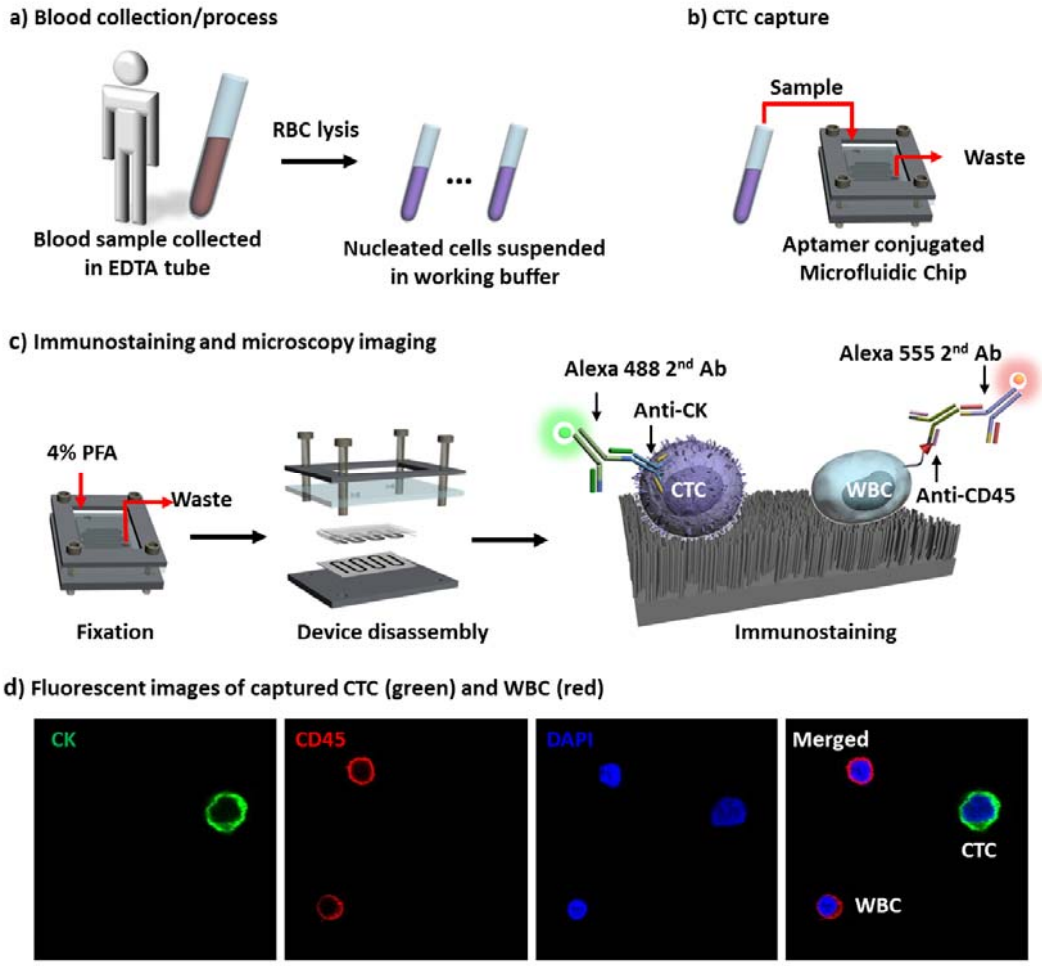

Figure S2. The schematic illustration of the general protocol for the processing of clinic samples. (**a**) Peripheral blood (3 mL-4 mL depending on the number of parallel study) was collected from each individual, subjected to red blood cell (RBC) lysis, and then split into equal aliquots. (**b**) 1-mL cell suspension was then loaded into a syringe and process through an aptamer conjugated NanoVelcro Chip for CTC capture. (**c**) Captured cells were fixed with 4% paraformaldehyde (PFA) prior to device disassembly. A three-color immunocytochemistry method based on Alexa 488 labeled anti-CK (green), Alexa 555 labeled anti-CD45 (red), and DAPI (blue) staining was employed for the identification of CTC from nonspecifically captured white blood cells (WBC). (**d**) Fluorescent images of captured CTC (green) and white blood cell (red).

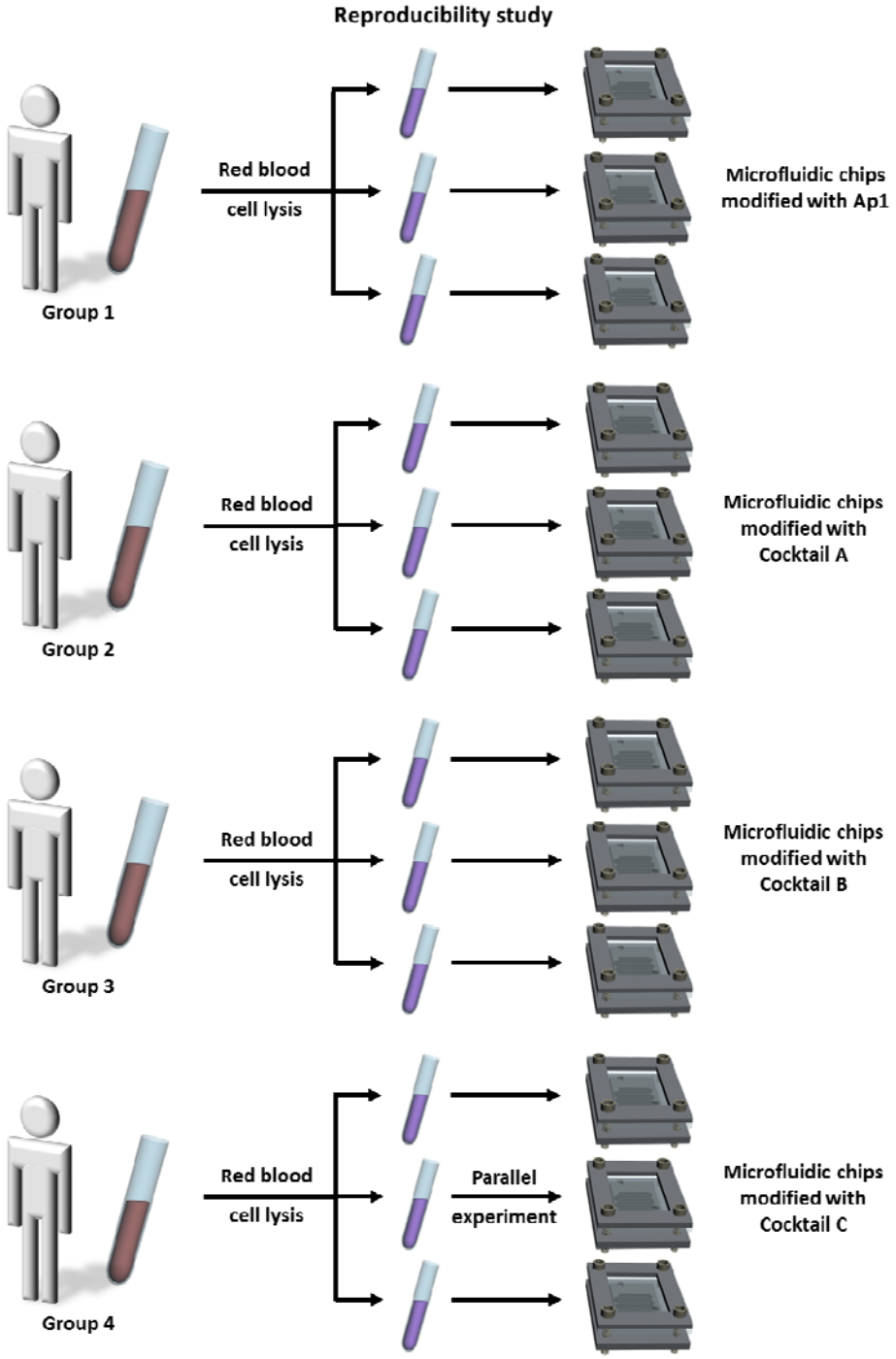

Figure S3. Schematic illustration of the reproducibility study. 12 patients and 12 healthy donors were divided into 4 groups. 3-mL blood was collected from each individual and then subjected to the triplicated CTC enumeration study using microfluidic chips modified with a specific combination of aptamers (i.e. Ap1, Cocktail A, Cocktail B, or Cocktail C). Captured cells were then enumerated under microscope.

Table S2. Detailed clinical information of NSCLC patients enrolled for parallel CTC enumeration studies

Patient	Sex	Age	Smoking Status	Pathology	Clinical Stage	Metastic Site
P13	M	65	No	Adenocarcinoma	Ⅳ	Both lung, bone
P14	M	59	Yes	Adenocarcinoma	Ⅳ	Both lung, brain
P15	F	45	No	Adenocarcinoma	Ⅳ	Bone
P16	M	60	Yes	Adenocarcinoma	Ⅳ	Both lung, liver, adrenal, bone
P17	F	59	No	Adenocarcinoma	Ⅳ	Liver, pleura
P18	M	76	No	Adenocarcinoma	Ⅳ	Pleura
P19	M	45	No	Adenocarcinoma	Ⅳ	Both lung, bone
P20	F	58	No	Adenocarcinoma	Ⅳ	Both lung, liver, brain
P21	M	56	Yes	Squamous cell carcinoma	Ⅳ	Both lung
P22	M	59	Yes	Squamous cell carcinoma	Ⅳ	Lung, bone, brain
P23	M	70	Yes	Squamous cell carcinoma	Ⅳ	Liver, bone

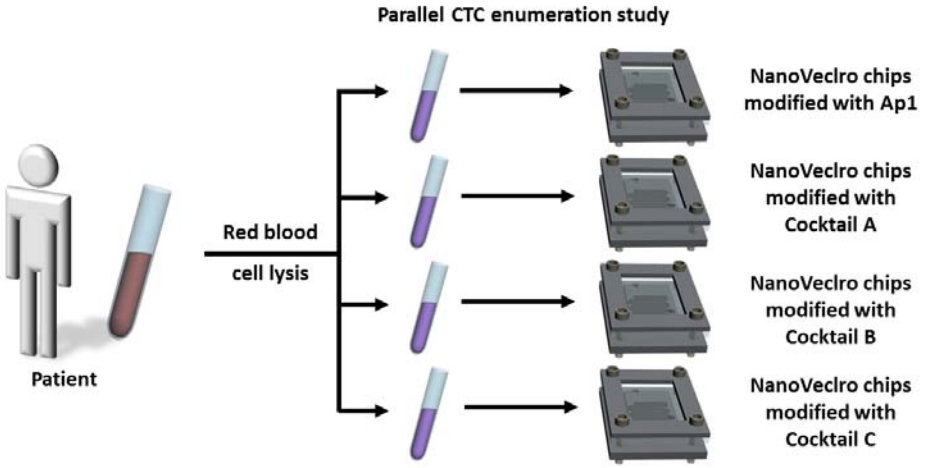

Figure S4. Schematic illustration of the parallel CTC enumeration study. 4 mL blood sample collected from the same patient was divided into 4 equal aliquots and process through microfluidic devices modified with 4 different combinations of aptamers (i.e. Ap1, Cocktail A, Cocktail B, or Cocktail C).

Table S3. Side by side comparision of anti-EpCAM and aptamer(s) in the detection of clincal samples

	Anti-EpCAM	Ap1	Cocktail A	Cocktail B	Cocktail C
P24	0	1	0	1	3
P25	2	1	4	3	6
P26	4	2	2	9	4