Supplementary Information

Synthesis of Multi-functionalized DNA Nanosphere Barcode System for Direct Cell Detection

Sangwoo Han[†], *Jae Sung Lee*[†] *and Jong Bum Lee**[†]

[†] Department of Chemical Engineering, University of Seoul, 163 Seoulsiripdaero, Dongdaemungu, Seoul, 02504, Republic of Korea

E-mail: jblee@uos.ac.kr

Supplementary method

Size control of self-assembled DNANS

To adjust the size of DNANSs, each different dNTP concentration was reacted (2.0mM and 0.8mM). Other reactants were used as same concentration for RCA explained in material and method.

Optimization of the ratio between qdot-streptavidin and biotin

To investigate the optimized ration between quantum dots and DNANSs, 1:25, 1:50 and 1:250 molar ratio of qdot-STA and biotin-14-dCTP in DNANSs were mixed and incubated overnight at room temperature. After overnight incubation, the qdots-conjugated DNANSs were washed as the same method mentioned in materials and methods. Each samples were observed by using an Eclipse Ti (Nikon) inverted fluorescent microscope.

Detection limit test

To confirm the detection sensitivity of anti-EGFR Ab-DNANSs, twenty-four hours before the sensitivity test, the Hela cells were trypsinized and prepared with the different density of Hela cells (0, 1, 5, 20, 100 cells μl^{-1}). Each prepared Hela cells were arranged on 96-well plate (100 µl per well). One hour before the treatment of anti-EGFR Ab-DNANSs on Hela cells, the medium was exchanged with fresh serum-free DMEM. Cy5-labeled anti-EGFR Ab-DNANSs were treated at a 50 nM concentration to arranged Hela cells and the cells were incubated at 37 °C with 5% CO_2 for 2 hours. After 2 hours, the anti-EGFR Ab-DNANSs were washed with DPBS and filled with serum-free DMEM. To measure the fluorescence intensity from the anti-EGFR Ab-DNANSs attached to Hela cells, microplate reader (Synergy HT, BioTek) was used and the results were analyzed using Gene5 2.01 software (BioTek).

Optical single cells detection test

Twenty-four hours before the Hela cells detection test, the cells were trypsinized, diluted with fresh medium (1.5×10^5 cells ml^{-1}), and transferred to 8-well plates (200 μl per well) and 24-well plates (500 μl per well). One hour before the treatment of anti-EGFR Ab-DNANSs on Hela cells, the medium was exchanged with fresh serum-free medium. To image and measure the fluorescence intensity of anti-EGFR Ab-DNANS bound to the cell surface, cy5-labeled anti-EGFR Ab-DNANS was treated at a 50 nM concentration to 8-well plates and 24-well plates, and the cells were incubated at 37 °C with 5% CO_2 for 2 hours with gentle agitation. After 2 hours, the anti-EGFR Ab-DNANSs were washed 2 times with DPBS. For imaging, the cells in the 8-well plates were fixed with 4% formaldehyde buffer (MBiotech) and washed with DPBS. The cells were stained with DAPI at a final concentration of 5 μg ml^{-1} to locate the cell nucleus. An Eclipse Ti (Nikon) inverted fluorescent microscope was used to image the cells. To measure the difference in fluorescence intensity of the cells treated with anti-EGFR Ab-DNANS and control group, all the cells were trypsinized from 24-well plates and centrifuged at 2000 r.p.m for 3 min. The medium of each cell was removed and filled with fresh serum-free medium. The cells were stained with Hoechst 33342, and analyzed using a Nucleocounter (NC-3000, Chemometec) and the results were analyzed using NucleoViews NC-300 software (Chemometec) and Flowjo.

Toxicity assay of anti-EGFR Ab-DNANS

Twenty-four hours before the toxicity assay, the HeLa cells were trypsinized and prepared with the different density of Hela cells 40 cells μl^{-1}. HeLa cells were arranged on 96-well plate (100 μl per well). One hour before the treatment of anti-EGFR Ab-DNANSs on Hela cells, the

medium was exchanged with fresh serum-free DMEM. qdots-labeled anti-EGFR Ab-DNANSs were treated at a 50 nM concentration to arranged HeLa cells and the cells were incubated at 37 °C with 5% ^{CO_2} for 2 hours. After 2 hours, the anti-EGFR Ab-DNANSs were washed with DPBS and filled with Cell Counting Kit-8 (CCK-8) (Dojindo, Japan) and incubated for 90 min. The absorbance of the solution was measured at 450 nm on a Synergy HT microplate reader (BioTek), and the results were analysed using Gen5 2.01 software (BioTek).

Table 1. DNA sequences used in DNANS assembly.

Name	Sequence (5’ to 3’)	Modification
Linear DNA (92bp)	ACG TAC GGG TGA CGA AAC GAC GTT CCA TCG CTG TTA GAC TCA GAT TGG TTG CAC TTT CAG CAC GGG TTA TTC CGA GTG AAC CGT CCA CCA TC	5’-Phosphate
Primer (20bp)	CCC GTA CGT GAT GGT GGA CG	

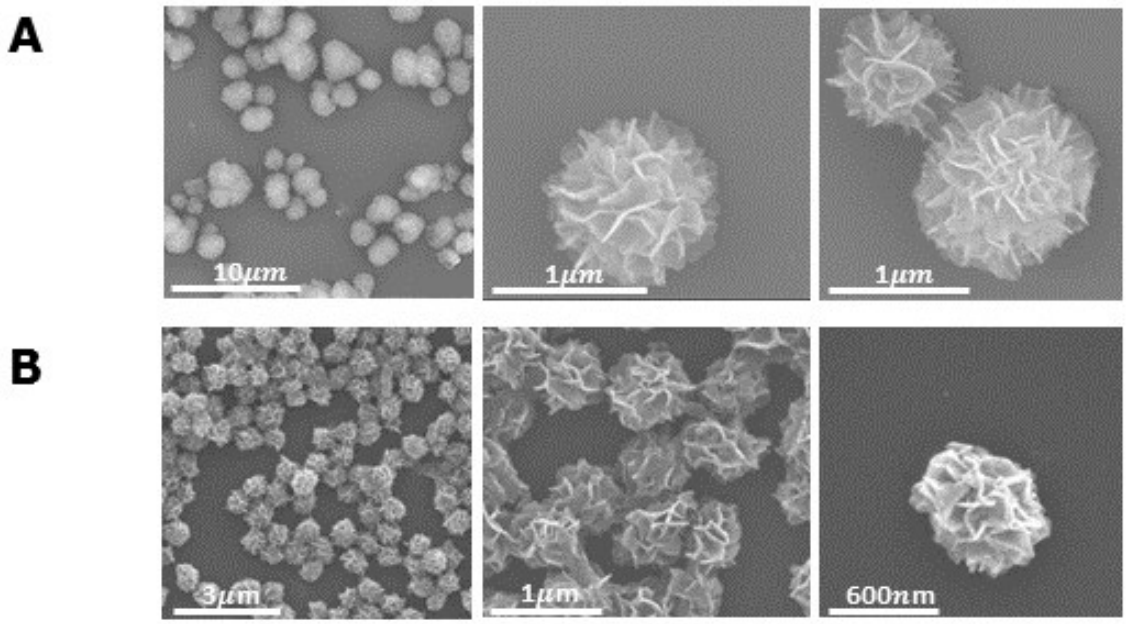

Fig. S1. FE-SEM images of A) 2.0mM dNTP containing DNANS and B) 0.8mM dNTP containing DNANS.

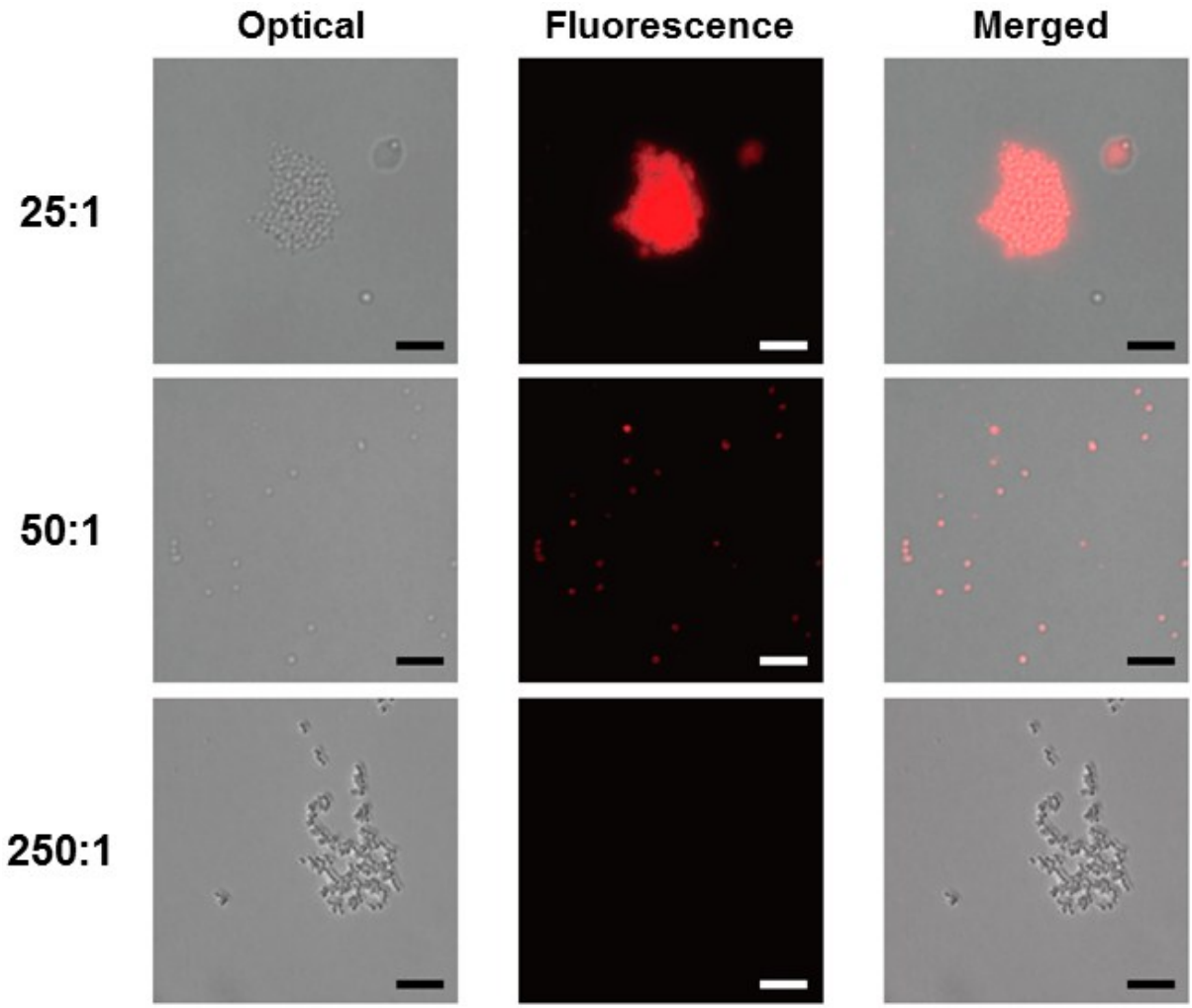

Fig. S2. Biotin : quantum dot ratio-dependent optical, fluorescence and merged microscope images of quantum dots-conjugated DNANSs. Scale bar: 10μm.

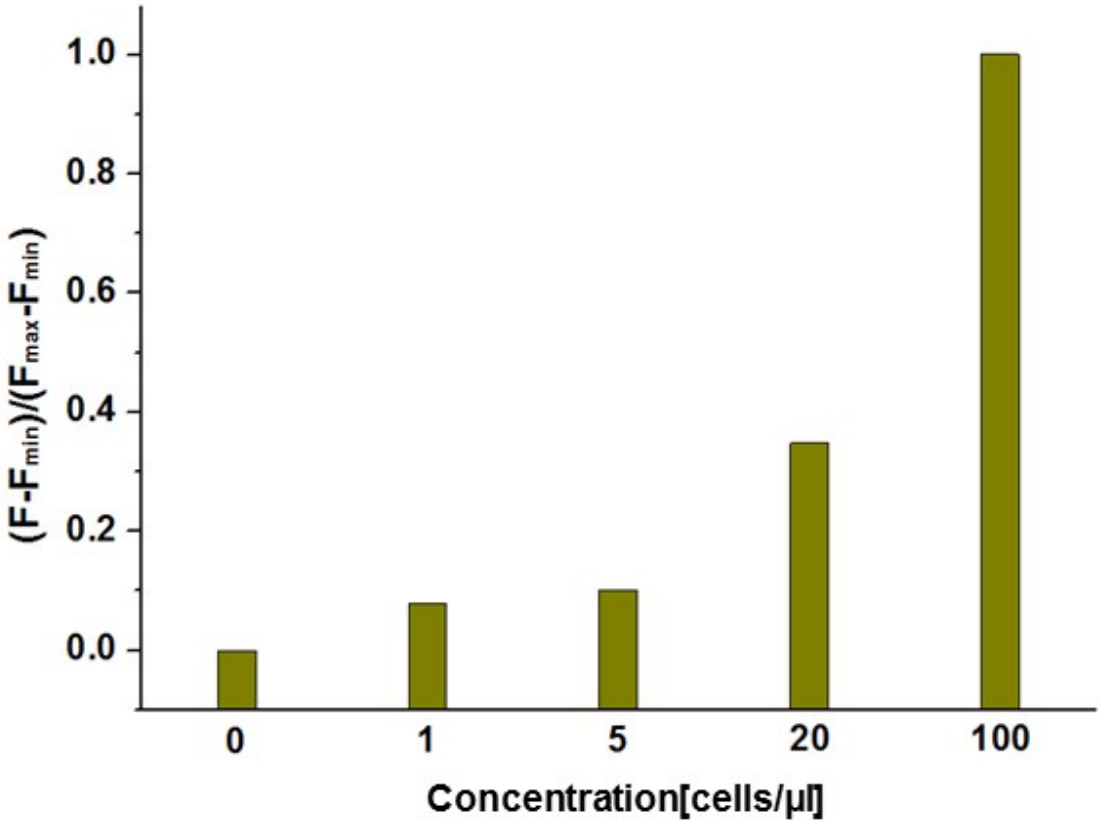

Fig. S3. Detection limit of anti-EGFR Ab-DNANS.

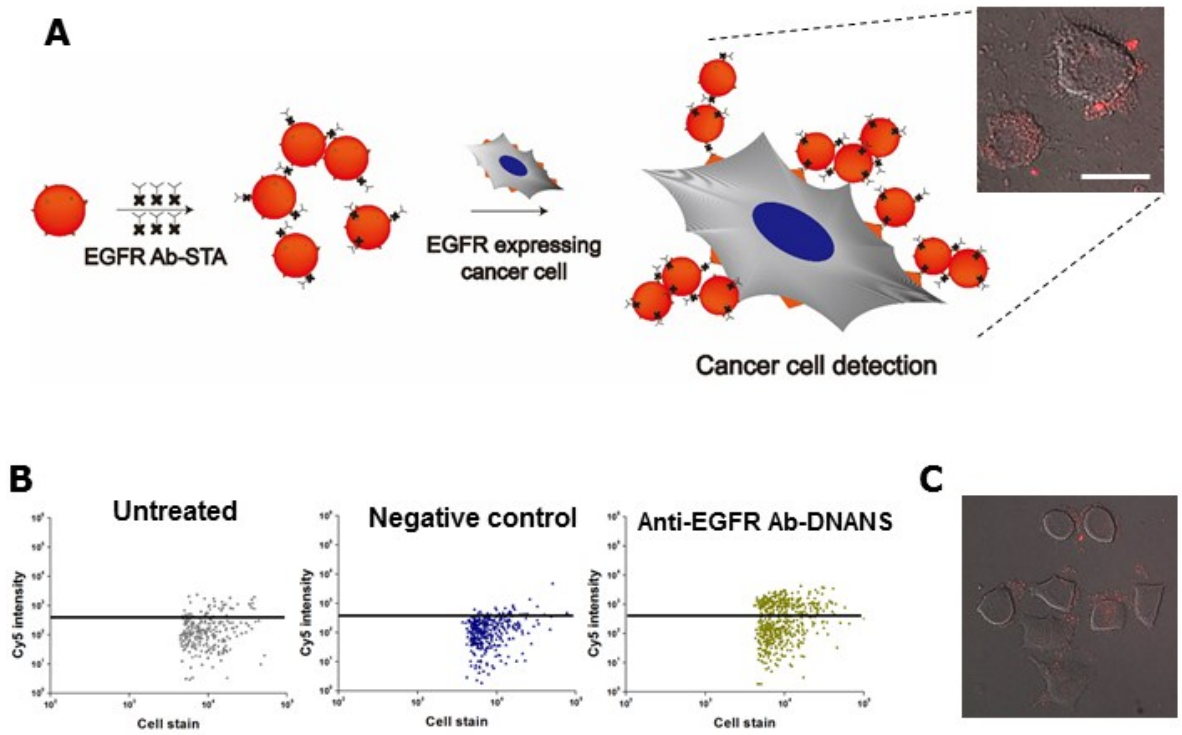

Fig. S4. A) Schematic illustration of the fabrication of anti-EGFR Ab-DNANSs and binding process on the surfaces of cancer cells and B) Image cytometry data after cell surface binding. C) Microscopy images of Hela cells-bound with anti-EGFR Ab-DNANSs. Scale bar: $20\mu m$.

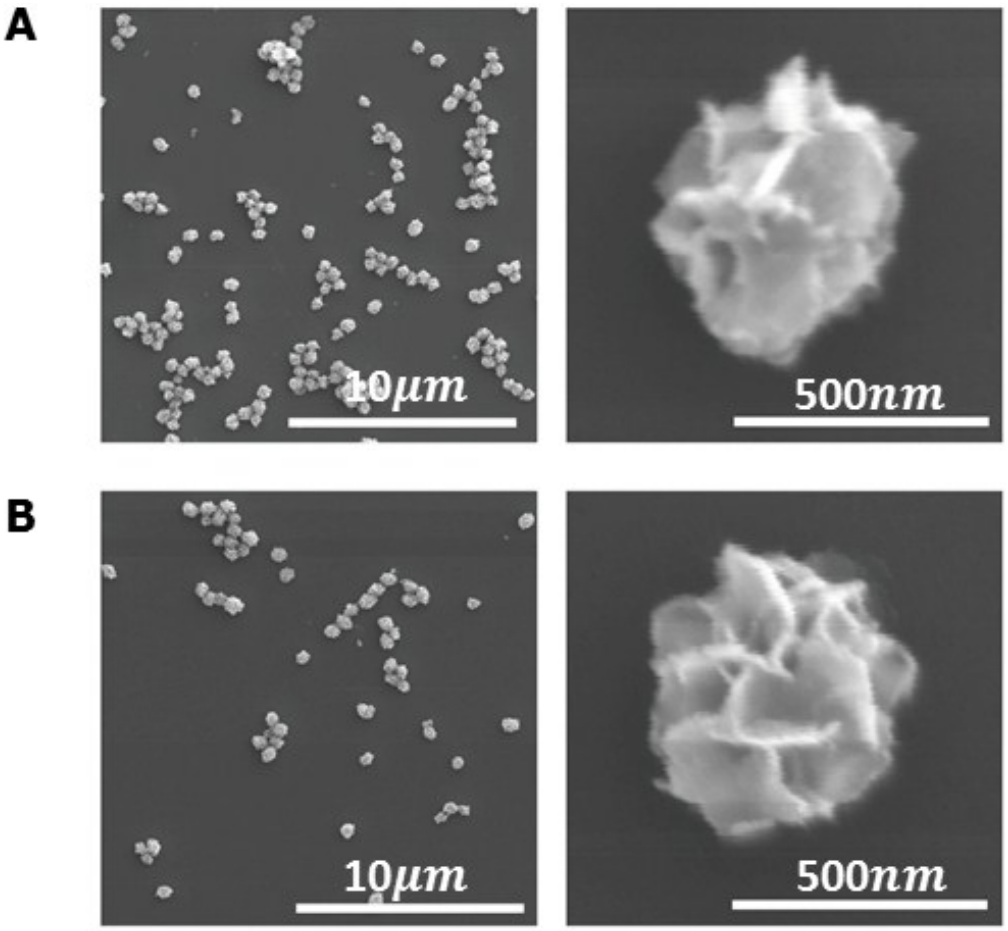

Fig. S5. Structural stability of DNANSs by FE-SEM after storing for 150 days in water. A) DNANS stored for 150 days. B) Newly fabricated DNANS.

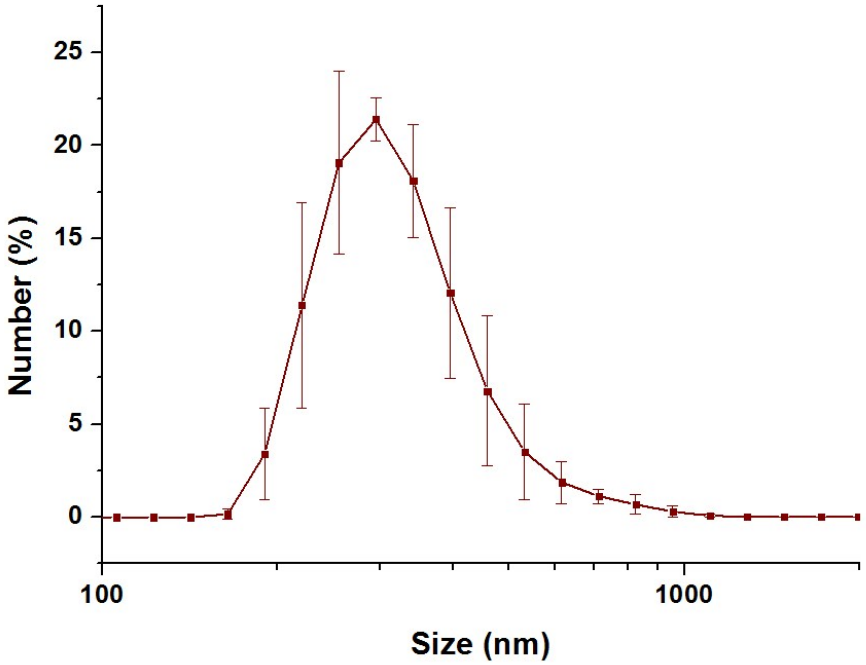

Fig. S6. Size distribution of DNANSs stored in water solution for 6 months.

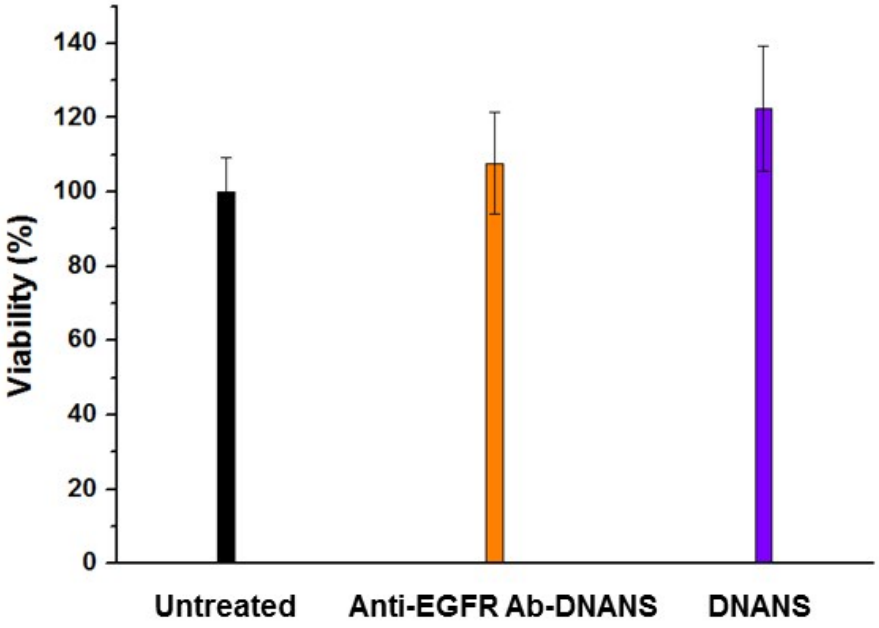

Fig. S7. Toxicity assay of anti-EGFR Ab-DNANS.

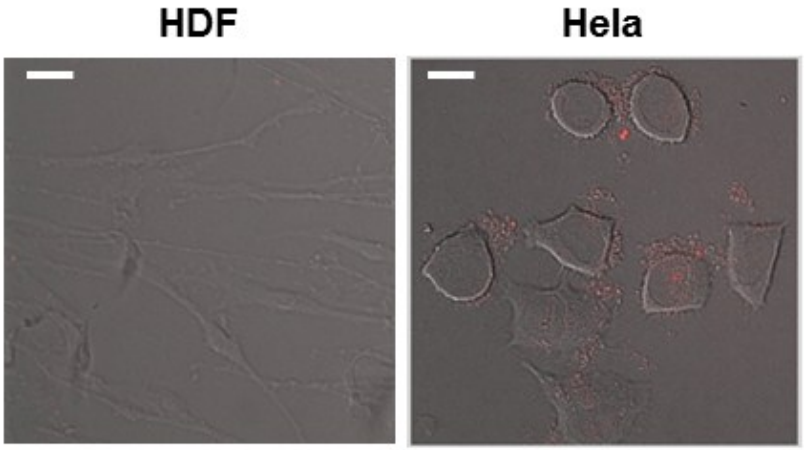

Fig. S8. Binding capability assays of anti-EGFR Ab-DNANS on human dermal fibroblast (HDF) cells as a control group (left) and Hela cells (right). Scale bar: $10\mu m$.

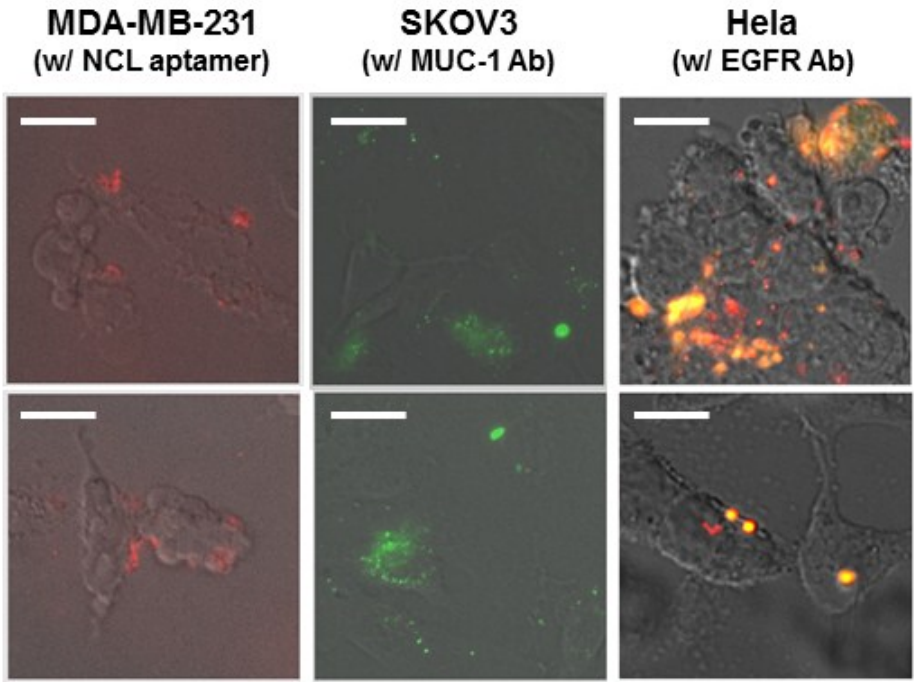

Fig. S9. Three kinds of cancer cells targeted by each different target methods with NCL aptamer for MDA-MB-231 (left), MUC-1 antibody for SKOV3 (middle) and EGFR antibody for Hela (right) cells detection. Scale bar: 20μm.