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Development of a CD63 Aptamer for Efficient Cancer Immunochemistry and Immunoaffinity-Based Exosome Isolation

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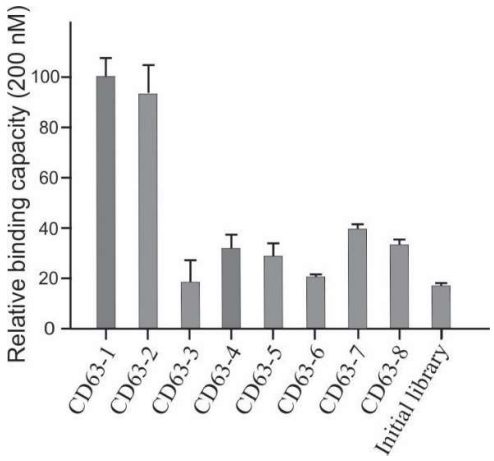
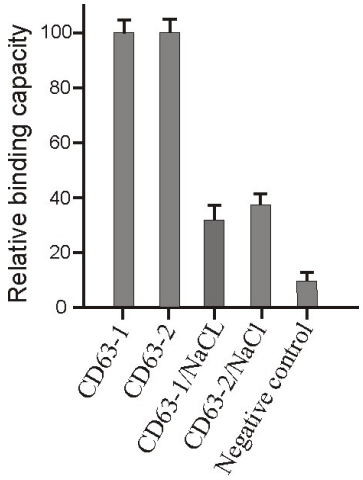
Supplementary Table 1. Sequence information of the top 8 aptamer candidates.

No.	Sequence (without primer binding site)	Frequency
CD63-1	TAACACGACAGACGTTCGGAGGTCGAACCCTGACAGCGTGGG	8.37%
CD63-2	TAACCACCCCACCTCGCTCCCGTGACACTAATGCTAATTCCAA	9.64%
CD63-3	CGACATGCCTGTGCTAGCCGAACCATGCTGCAATTCACTCGTG	4.17%
CD63-4	AACACGACCCTTGTTCGCGAGGCACAGTCCATGCTGGTCGTGA	3.87%
CD63-5	GGCTACGACGTACCTGTGAAAGCCGAACATATTGCGAGTGGGC	1.99%
CD63-6	TACCTACGACATGCCTGTGCTAGCCGAACCATGCTGAGGTCGT	1.87%
CD63-7	ACCCAGACTAGGGTTCGATCATCACAGAACGAGGGCGTCTCCG	1.42%
CD63-8	CGACCGGCAAAGTTCGTCCACCGAACAGATCGGCTGTCGTGCC	0.97%

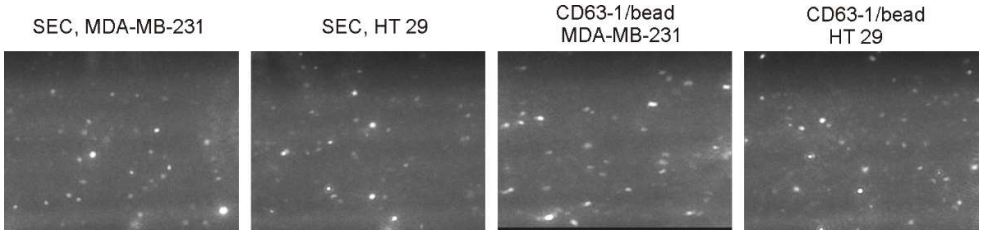
Note: the sequences meeting the criterial (89nt total length with the eprimer binding sites) was 242,342 in total.



Supplementary Figure 1. Evaluation of the relative binding capacity of eight aptamer candidates to CD63 protein. The binding capacity was assessed by ELISA assay. After a one-hour blocking step using salmon sperm DNA (10μg/100μL/test), 200 nM of the indicated aptamer candidates (biotinylated) were incubated with the immobilized CD63 protein for 0.5 h, followed by HRP conjugated anti-biotin antibody incubation. The fluorescence intensity was measured by a plate reader (using the QuantaBlu Fluorogenic Peroxidase Substrate).



Supplementary Figure 2. NaCl incubation reduces the binding of CD63-1 and CD63-2 aptamers to CD63 protein. Biotinylated CD63-1 and CD63-2 aptamers (two groups for each aptamer) were incubated with CD63 protein in a 96-well nickel plate for 30 min at a concentration of 200 nM. After the 30 min incubation, one group of each aptamer were further incubated with NaCl (0.5 mM) for 10 min. After thorough washing and incubating with anti-biotin antibody for 1 h, the fluorescence intensity was measured by a plate reader (using the QuantaBlu Fluorogenic Peroxidase Substrate). The binding of each group was calculated after subtracting the mean fluorescent intensity of the binding of the protein only group. The original library (biotinylated) served as control *, $P \leq 0.05$.



Supplementary Figure 3. Screen capture of the NTA video showing the isolated exosomes by SEC and aptamer/bead methods.