Supplementary Materials

An aptamer-based probe for molecular subtyping of breast cancer

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Figure S1. Electrophoretogram of PCR results of each selection round. From the first lane to the 25th lane were DNA marker, initial library, the first to 21st selection round, negative control, and DNA marker, respectively.



Figure S2. Confocal microscopic imaging of different breast cancer cells after incubation with FAM-labeled 21^{st} pool. The 21^{st} pool can specifically bind to SK-BR-3 breast cancer cells other than MDA-MB-231, MCF-7 breast cancer cells and MCF-10A human normal mammary epithelial cells. The final concentration of the pool was 250 nM. Scale bar = 10 µm.



Figure S3. The secondary structures of these six aptamers. All the six aptamers are

composed of various stem-loop and hairpin structures.



Figure S4. Confocal microscopic imaging of SK-BR-3 breast cancer cells after incubation with these six FAM-labeled aptamers, respectively. The final concentration of both aptamers and initial library was 250 nM. Scale bar = $20 \mu m$.



Figure S5. Confocal microscopic imaging of MCF-7 breast cancer cells after incubation with these six FAM-labeled aptamers, respectively. The final concentration of both aptamers and initial library was 250 nM. Scale bar = $20 \mu m$.



Figure S6. Confocal microscopic imaging of MDA-MB-231 breast cancer cells after incubation with these six FAM-labeled aptamers, respectively. The final concentration of both aptamers and initial library was 250 nM. Scale bar = $20 \mu m$.



Figure S7. Confocal microscopic imaging of MCF-10A breast cancer cells after incubation with these six FAM-labeled aptamers, respectively. The final concentration of both aptamers and initial library was 250 nM. Scale bar = $20 \mu m$.



Figure S8. FAM-labeled initial library stained breast cancer cells of different molecular subtypes and human normal mammary epithelial cells. The final concentration of the initial library was 250 nM. Scale bar = $20 \mu m$.



Figure S9. *In vivo* fluorescence imaging of SK-BR-3 tumor-bearing mice after being injected with Cy5-labeled sk6Ea or library through tail vein.



Figure S10. In vivo fluorescence imaging of MDA-MB-231 tumor-bearing mice

after being injected with Cy5-labeled sk6Ea or library through tail vein.



Figure S11. In vivo fluorescence imaging of MCF-7 tumor-bearing mice after

being injected with Cy5-labeled sk6Ea or library through tail vein.



Figure S12. The confocal microscopic analysis of the competition between aptamer sk6Ea and anti-HER2 polyclonal antibodies (Poly-anti-HER2) for binding to SK-BR-3 breast cancer cells. From the left column to right, the first is the negative control, the second is SK-BR-3 cells treated with FAM-labeled sk6Ea, the third is SK-BR-3 cells treated with Poly-anti-HER2 for 60 min, followed by being treated with FAM-labeled sk6Ea for 15 min; the fourth is SK-BR-3 cells treated with Poly-anti-HER2 for 60 min. Scale bar = $20 \,\mu$ m.

Aptamers	Sequences
sk6a	ACGACCCGATAAGGGCGATGCCGATCCCTGTGGCCGTAGGGGCAGTC
	CCGCTAG
sk6b	TCACGCCCGATAAGGGCGATGCCGATCCCTGTGGCCGTAGGGCAGTC
	CCCTAGAG
sk6c	TCACGACCCGATAAGGGCGATGCCGATCCCTGTGGCCGTAGGGGCAG
	TCCCGCTAGAG
sk6d	AGCAGAGTTCACGACCCGATAAGGGCGATGCCGATCCCTGTGGCCGT
	AGGGGCAGTCCCGCTAG
sk6e	TCACGACCCGATAAGGGCGATGCCGATCCCTGTGGCCGTAGGGGCAG
	TCCCGCTAG
sk6Ca	TCACGCCCGATAAGGGCGATGCCGATCCCTGTGGCCGTAGGGGGCAGT
	CCCGCTAGAG
sk6Ea	TCACGCCCGATAAGGGCGATGCCGATCCCTGTGGCCGTAGGGCAGTC
	CCCTAG

 Table S1. The truncated aptamers of full-length aptamer sk6.