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Supplemental Information

Selection of Metastatic Breast Cancer Cell-Specific Aptamers for the Capture of CTCs with a Metastatic Phenotype by Cell-SELEX

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A, Supplemental Figures

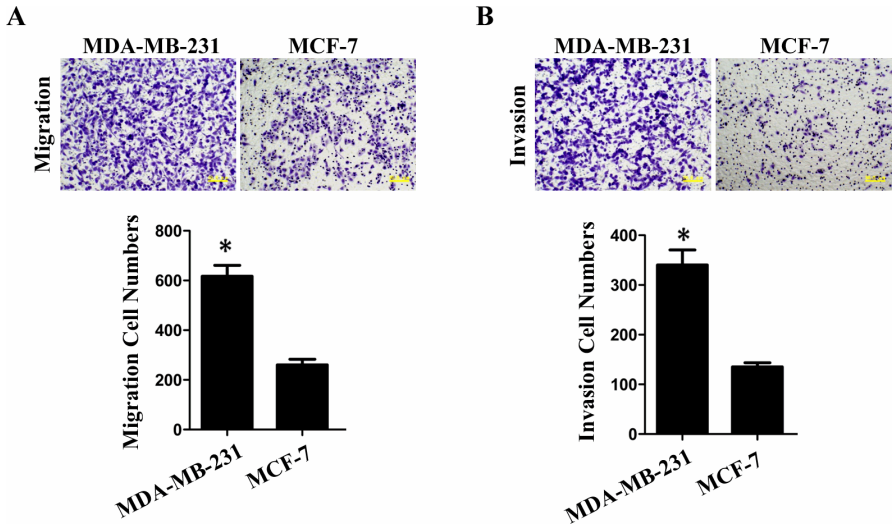

Figure S1. Migration (A) and invasion (B) ability analysis of MDA-MB-231 and MCF-7 cells. Values shown are mean ± SD of three independent experiments. *p<0.05.

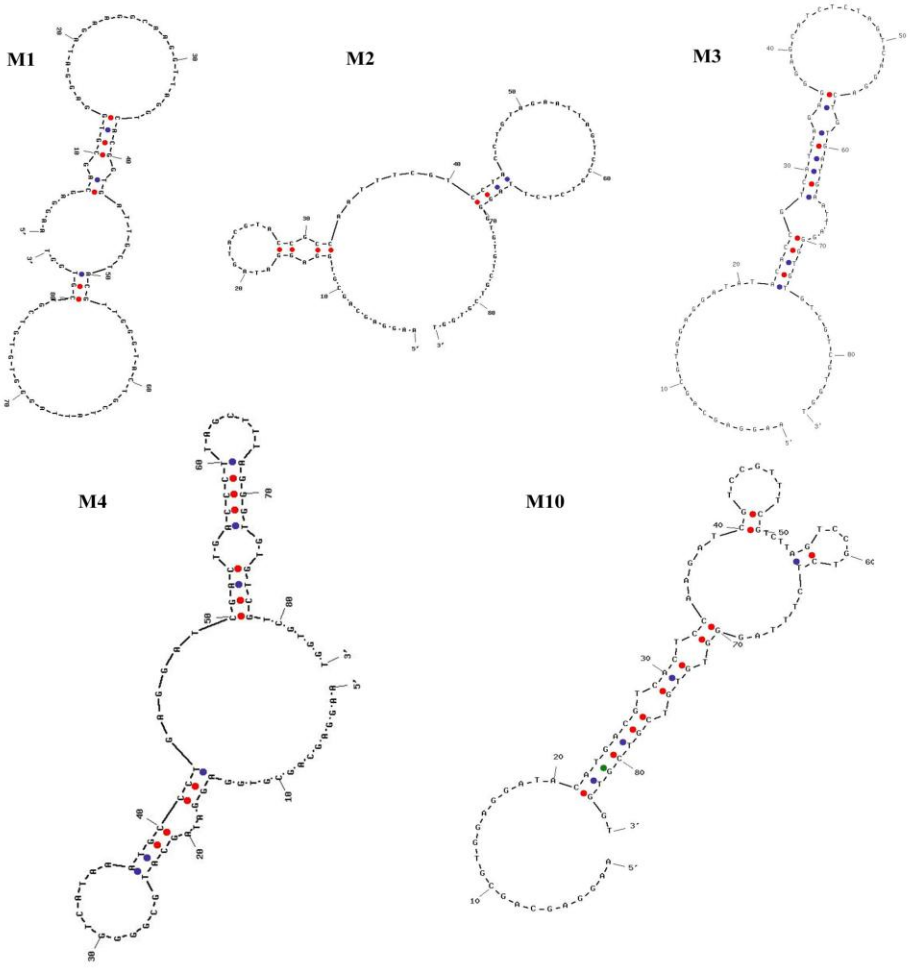

Figure S2. The Secondary structures of aptamers (M1, M2, M3, M4 and M10) were predicted with Oligo Analyser.

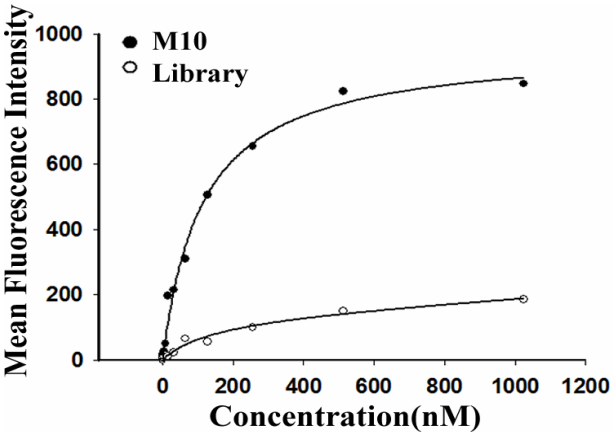

Figure S3. Binding saturation curves of aptamer M10 to target MDA-MB-231 cells. The cells were incubated with increasing concentrations of FITC-labeled aptamer M10 and assessed by flow cytometry. The K_D value of M10 was obtained by Sigma Plot software according to $Y = B_{max} \times X/(K_D + X)$. The ssDNA library was used as negative control. At least three independent experiments were conducted.

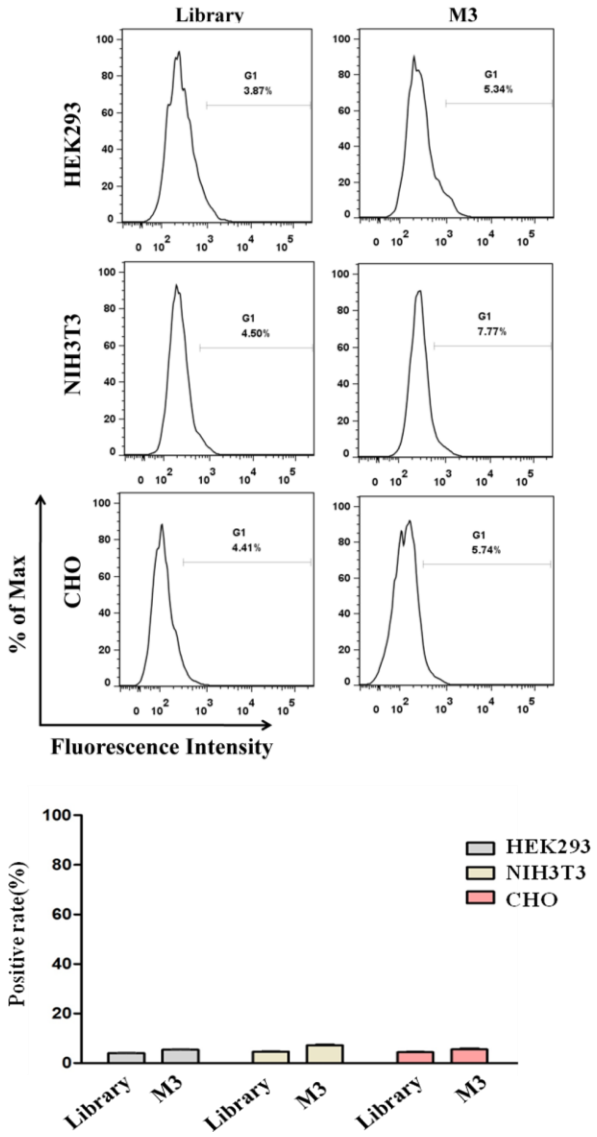

Figure S4. Specificity of M3 to normal cell lines by flow cytometry. The FITC-labeled M3 were incubated with HEK293, NIH3T3, and CHO cells for 30 min at 4 ℃. Then, the fluorescence intensity was determined by flow cytometry. The ssDNA library was used as negative control. At least three independent experiments were conducted. The values represent the mean ± SD of three independent experiments.

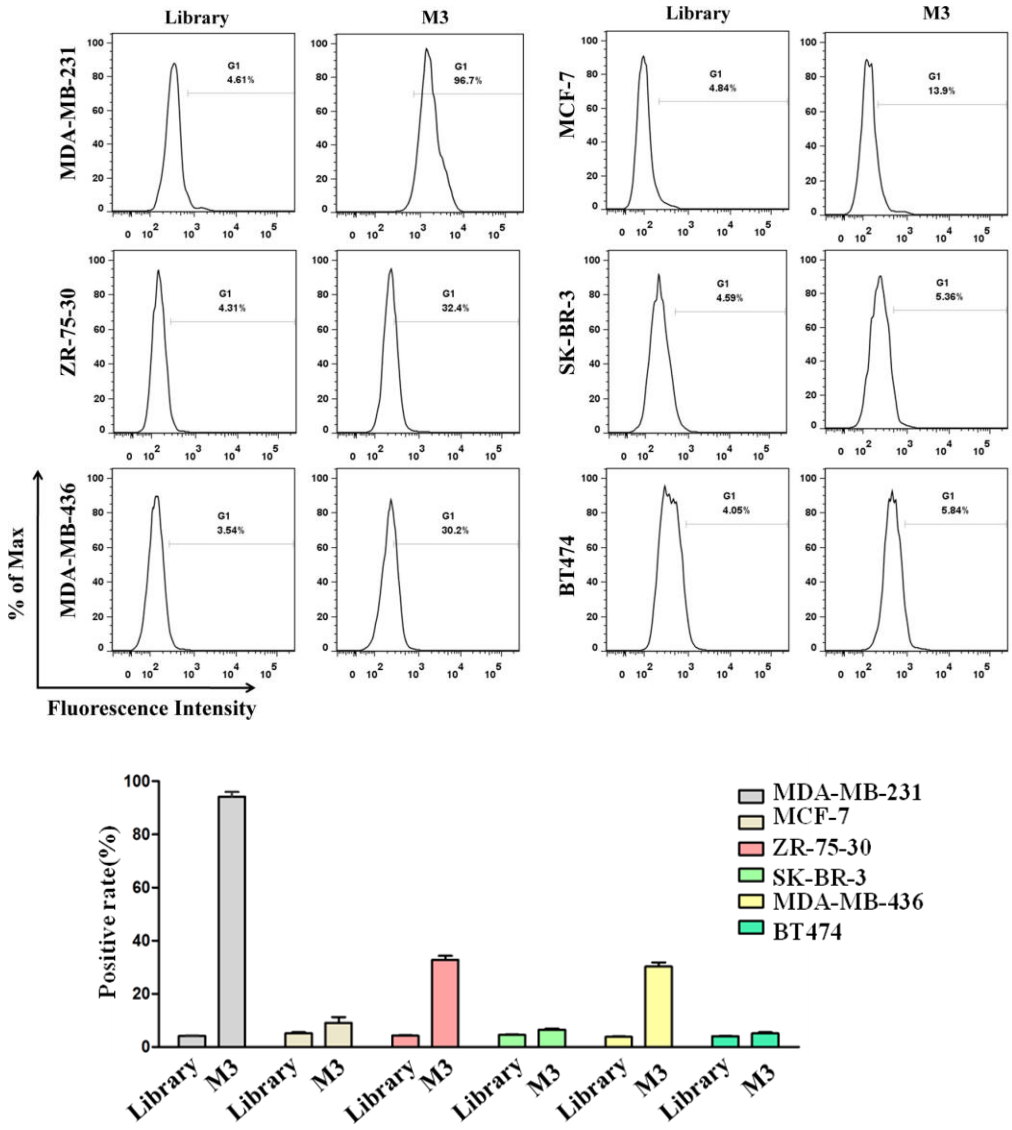

Figure S5. Specificity of M3 to breast cancer cell lines by flow cytometry. The FITC-labeled M3 were incubated with MDA-MB-231, MCF-7, ZR-75-30, SK-BR-3, MDA-MB-436, and BT474 cells for 30 min at 4 ℃. Then, the fluorescence intensity was determined by flow cytometry. The ssDNA library was used as negative control. At least three independent experiments were conducted. The values represent the mean ± SD of three independent experiments.

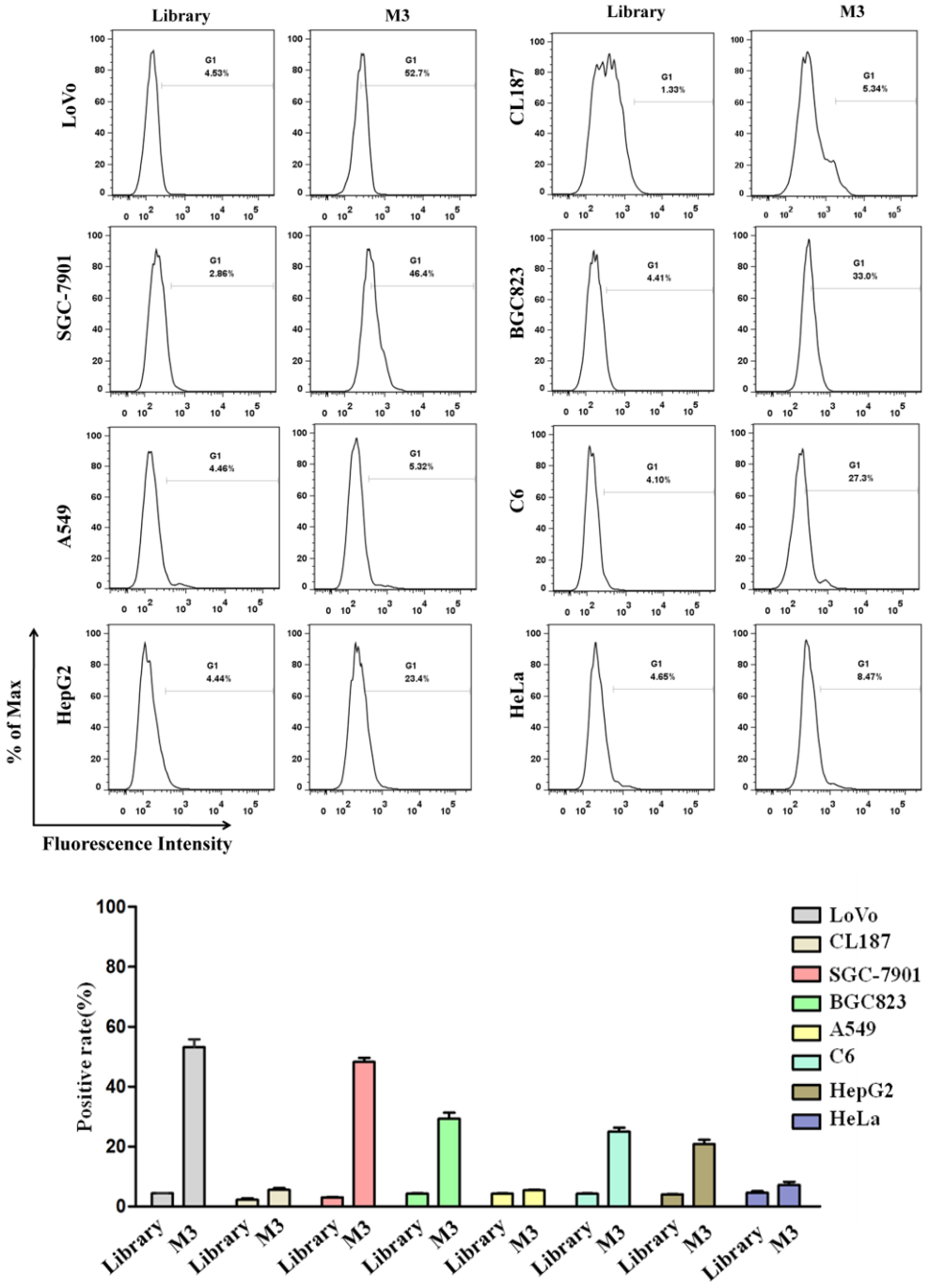

Figure S6. Specificity of M3 to other cancer cell lines by flow cytometry. The FITC-labeled M3 were incubated with LoVo, CL187, SGC-7901, BGC823, A549, C6, HepG2, and HeLa cells for 30 min at 4℃. Then, the fluorescence intensity was determined by flow cytometry. The ssDNA library was used as negative control. At least three independent experiments were conducted. The values represent the mean ± SD of three independent experiments.

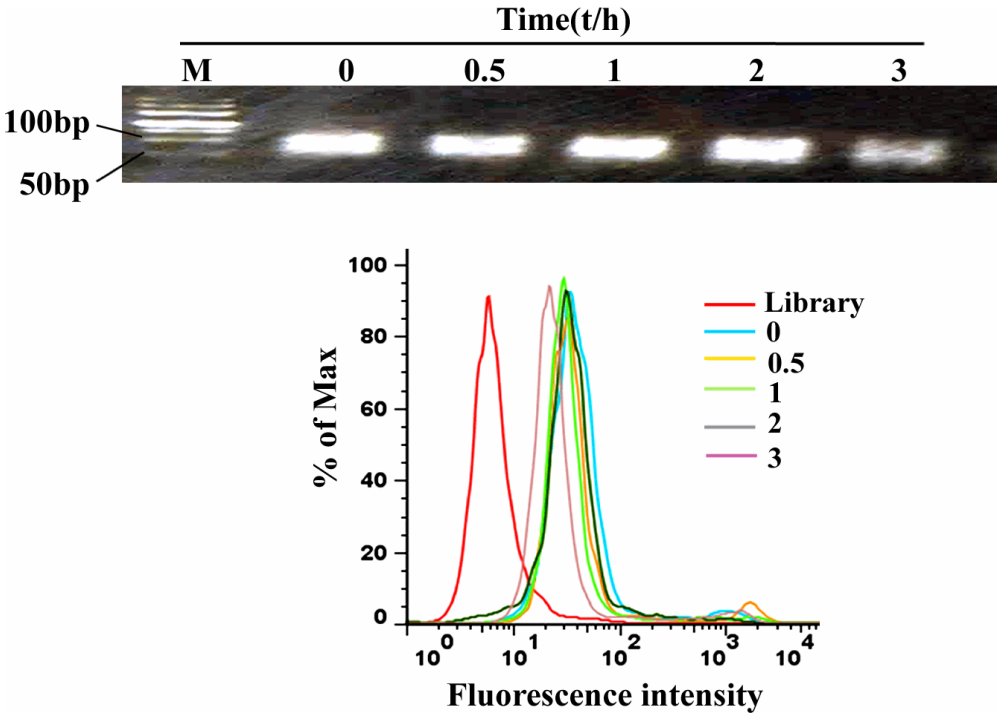

Figure S7. Stability of M3 in plasma at different times. The aptamer M3 was added to the plasma at 37 ℃ for 0.5, 1, 2, or 3 h. Finally, the M3 in plasma was separated by 3% agarose gel electrophoresis, and the DNA integrity was observed under UV spectrophotometry. The binding capacity of M3 to MDA-MB-231 cells after different time incubation in plasma was analyzed by flow cytometry. At least three independent experiments were conducted.

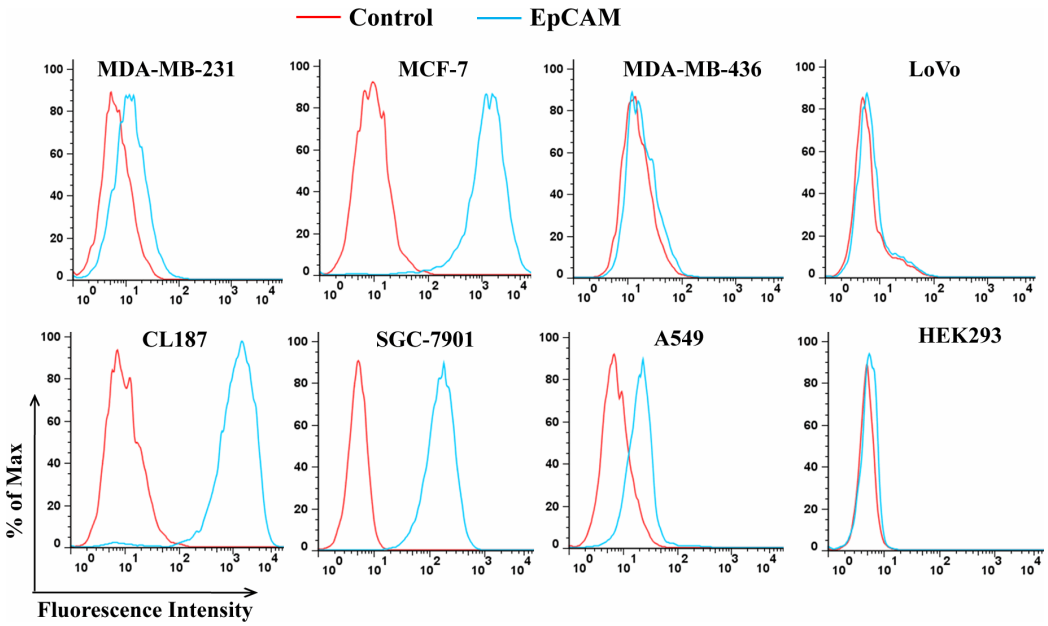

Figure S8. EpCAM expression in various cell lines by flow cytometry. The PE-anti-EpCAM was incubated with MDA-MB-231, MCF-7, MDA-MB-436, LoVo, CL187, SGC-7901, A549 and HEK293 cells for 1 h at 4 ℃. Then, the fluorescence intensity was determined by flow cytometry. At least three independent experiments were conducted.

B, Supplemental Tables

Table S1. Aptamer sequences and frequency

Name	Sequence	Frequency (50 clones)
M1	AAGGAGCAGCGTGGAGGATAGAAGGCAAGGTTAGGTCACGGTGATTGCT ACGTTGGGTACTGCTATTAGGGTGTGTCGTCGTGGT	**4**
M2	AAGGAGCAGCGTGGAGGATAGTACGTACCGCCAATTTCGTCCTACCTGTA GAATTAGTCCGTCTCTTAGGGTGTGTCGTCGTGGT	**7**
M3	AAGGAGCAGCGTGGAGGATATACACCGTCATCAGAGGGAGCATCTCTAGT CAGGACTGTGATGAATTAGGGTGTGTCGTCGTGGT	**4**
M4	AAGGAGCAGCGTGGAGGATACGATGCGGGGTCATAAATGCCCTGGGGATC GACTGACCCTTAGCTTTAGGGTGTGTCGTCGTGGT	**4**
M10	AAGGAGCAGCGTGGAGGATACATGACGTCACTCTAAGATCGTCCGTTTCG TCTTAGTCCGTCTCTTTAGGGTGTGTCGTCGTGGT	**10**

Table S2. CTC numbers and tumor metastasis in patients

Patient number	Numbers of M3 positive CTC	Metastasis characteristics
001	2	Distal metastasis(liver, bone), lymph node metastasis
002	2	Lymph node metastasis
003	7	Distal metastasis(lung), lymph node metastasis
004	0	Lymph node metastasis
005	1	No
006	3	Distal metastasis(bone, lung), lymph node metastasis
007	0	Lymph node metastasis
008	2	Distal metastasis(brain), lymph node metastasis
009	0	No
010	0	No
011	3	Distal metastasis(bone), lymph node metastasis
012	0	No
013	0	No
014	0	No
015	0	No
016	4	Distal metastasis(bone, lung), lymph node metastasis
017	0	Lymph node metastasis
018	3	Lymph node metastasis
019	0	No
020	0	No
021	0	Lymph node metastasis
022	5	Distal metastasis(liver, bone, lung)
023	0	No
024	0	No
025	8	Distal metastasis(lung, liver, bone), lymph node metastasis

Table S3. Comparison of binding assays of M3 and anti-EpCAM to different cell lines

	MDA-MB-231	MCF-7	MDA-MB-436	LoVo	CL187	SGC-7901	A549	HEK293
M3	++++	-	+	++	-	++	-	-
EpCAM	+	++++	-	-	++++	++++	++	-

- ,<14%; +, 15-35%; ++, 36-60%; ++++, >85%.

C, Supplemental Materials and Methods

Transwell assays. MDA-MB-231 or MCF-7 cells (1×10^5 cells in 100 μL free FBS DMEM) were placed in the top chamber of transwell chambers (8 μm BioCoat Control Inserts, Becton Dickinson Labware, Bedford, MA). The lower chamber was filled with 600 μL DMEM containing 10% FBS. After 24 h, un-migrated cells were removed from the upper surface of the transwell membrane with a cotton swab, and migrated cells on the lower membrane surface were fixed, stained, photographed, and counted under a light microscope. To detect invasiveness, diluted Matrigel was loaded on the membranes of the transwell chambers and performed as above.