Electronic Supplementary Material

Supplementary information

Generally Applicable Circulating Tumor Cell Enrichment and Identification Through the Membrane Glycoprotein-targeted Strategy Combining Magnetic Isolation and Biological Orthogonality Labeling.

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RESULTS AND DISCUSSIONS

characterization of the FITC labeled transferrin

The properties of Tf-FITC were characterized by ultraviolet spectrometer and fluorescence spectrophotometer, as shown in Figure S1c and S1d. The characteristic absorption peaks of transferrin and FITC appeared simultaneously in the ultraviolet-visible absorption spectrum of Tf-FITC, located at ~280 nm and ~488 nm, respectively. the characteristic emission peak (~520 nm) of FITC appeared in the fluorescence emission spectrum, revealing a successful preparation of Tf-FITC.

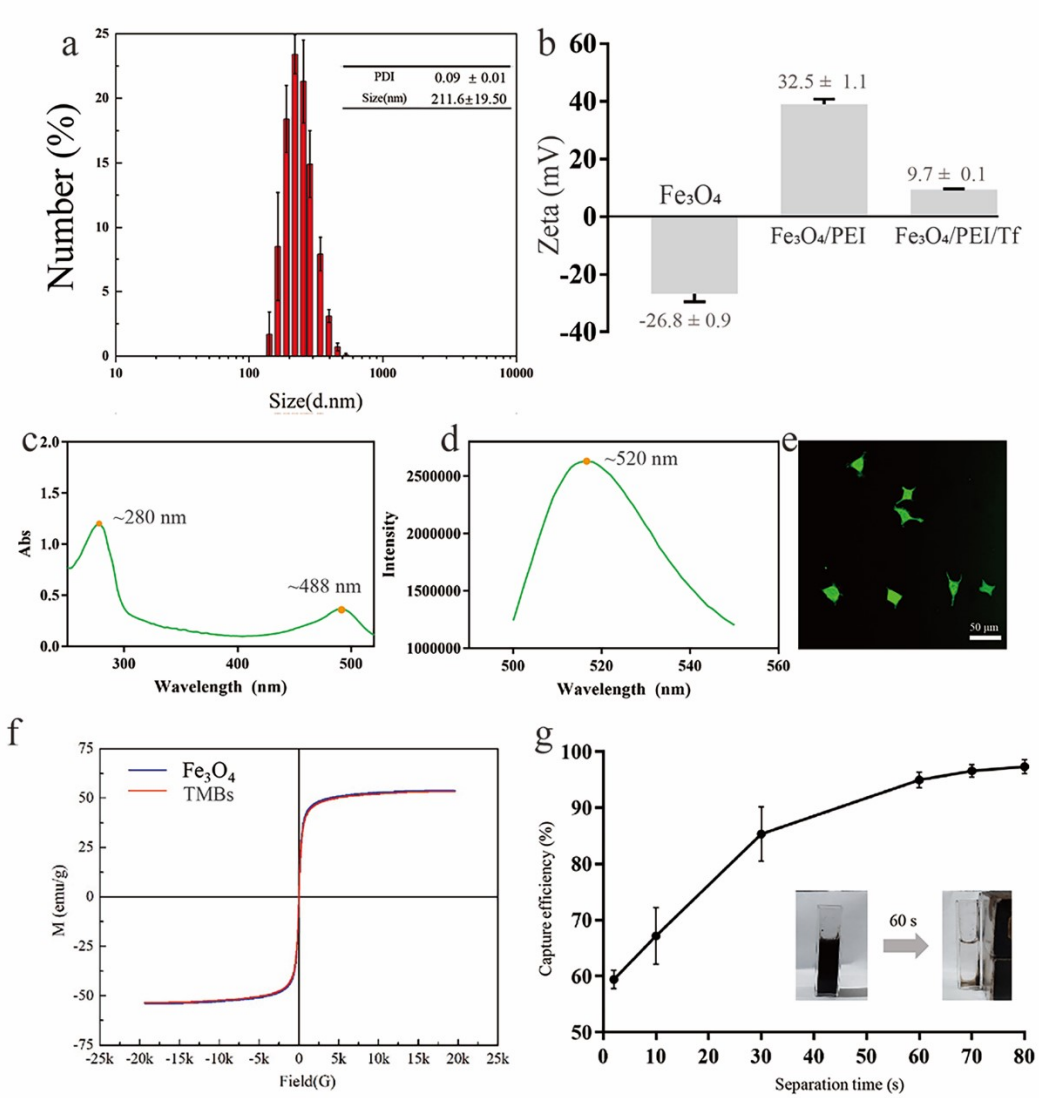

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Figure S1. a) Hydrodynamic size of TMBs. b) Zeta potential of Fe_3O_4 nanoparticles (Fe_3O_4), Fe_3O_4/PEI and Fe_3O_4/PEI/Tf. c) Ultraviolet-visible absorption spectrum and d) fluorescence emission spectrum of Tf-FITC. e) The CLSM image of MCF-7 cells after incubating with Tf-FITC. f) Magnetic hysteresis loops of Fe_3O_4 and TMBs. g) Recovery percentage of TMBs at different separation time with magnetic scaffold, the inset graph showed the magnetic separation process after 60 s.

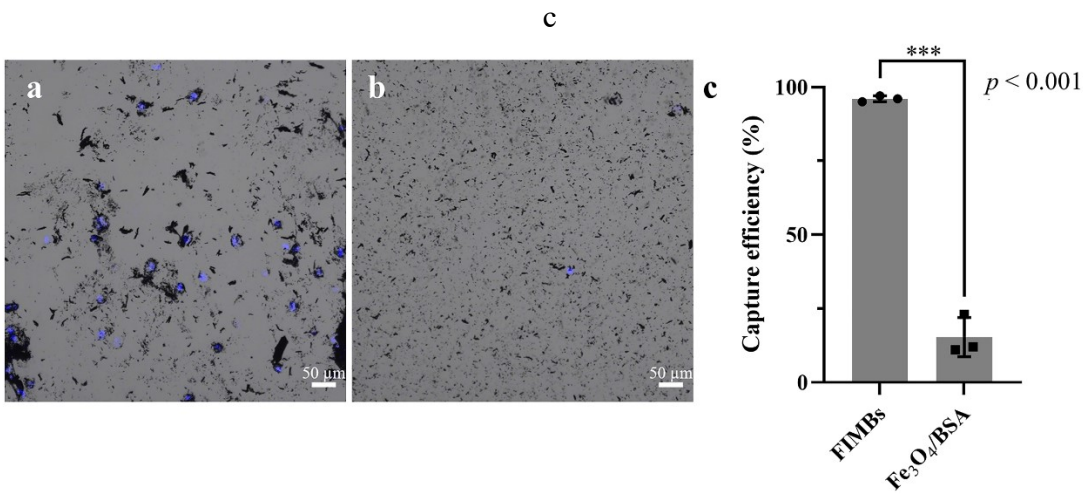

Figure S2. The typical CLSM images of captured CTCs (pre-stained with Hoechst 33342) by a) TMB and b) Fe_3O_4/BSA. c) Captured performance of TMB and Fe_3O_4/BSA. 150 mg functionalized magnetic beads were applied to 2.5×10^5 MCF-7 cell in 1 mL PBS for 120 s.

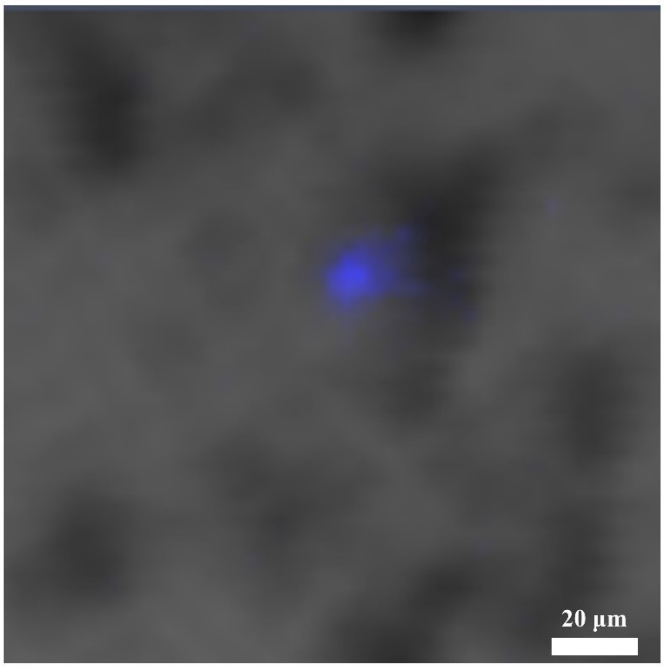

Figure S3. The representative CLSM image of CTCs (pre-stained with Hoechst) captured by TMBs in the sensitivity exploration experiment.

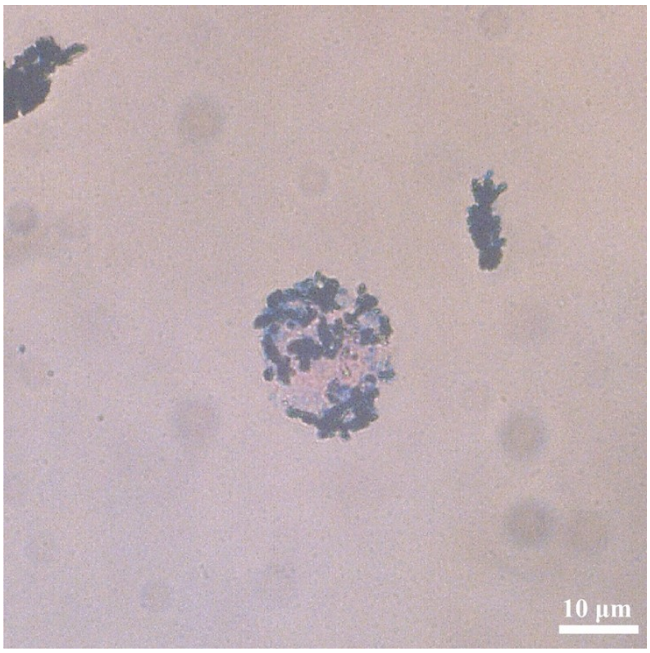

Figure S4. Prussian blue staining of one captured CTC.

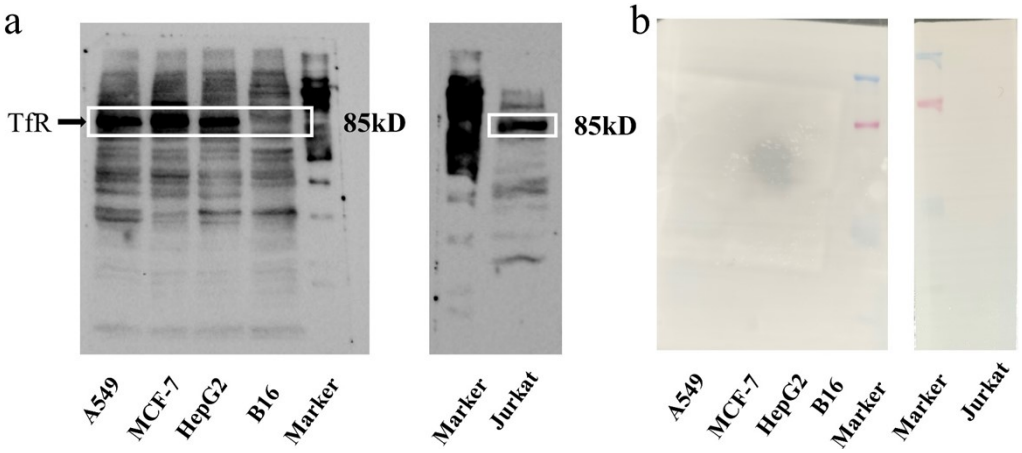

Figure S5. (A)Western blot (WB) analyses of transferrin receptor expression in different cell lines, and (B) corresponding raw data of the WB studies.

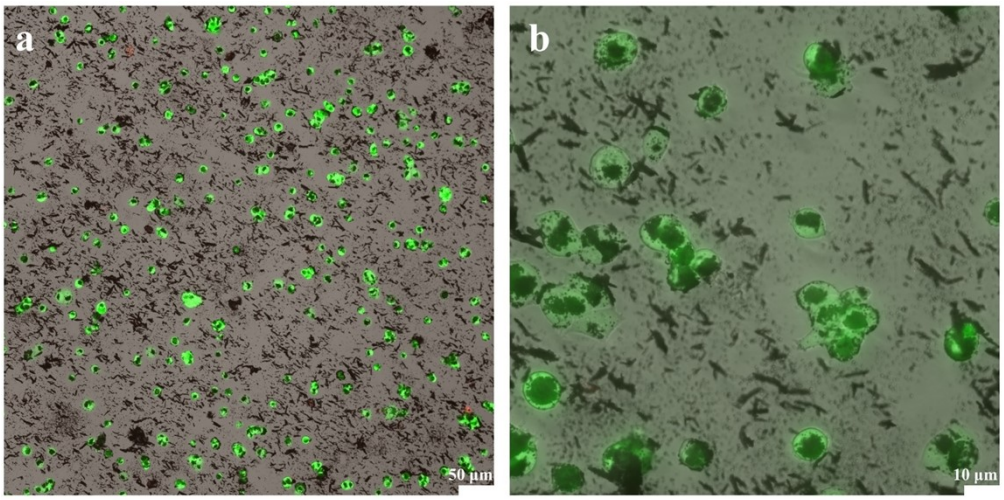

Figure S6. Captured cells stained with AM (green) and PI (red) at different magnifications. Acridine orange (AM) produced green fluorescence in live cells, while propidium iodide (PI) produced red signals in dead cells.

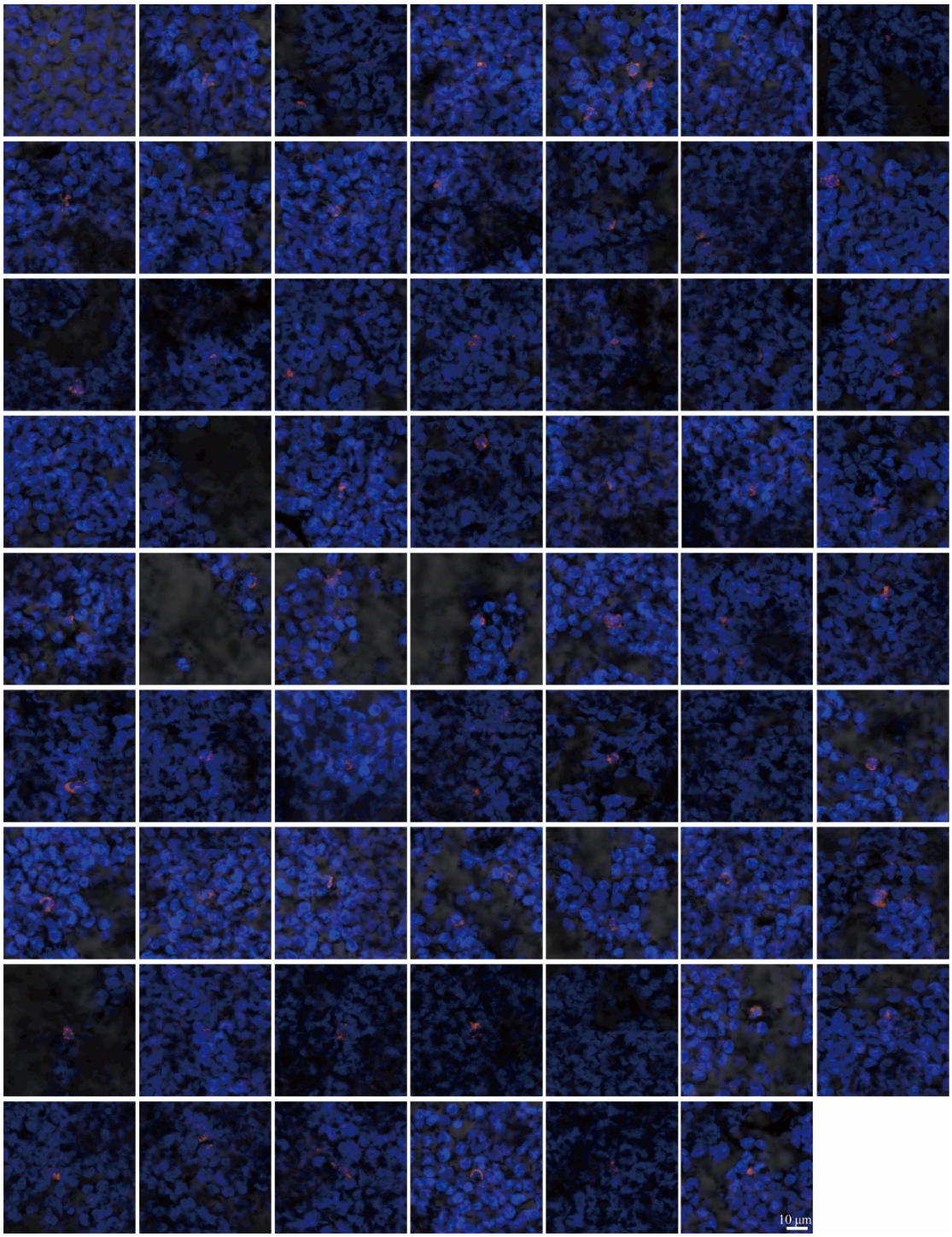

Figure S7. The CLSM images of all captured CTCs from a lymphoma patient through the membrane glycoprotein-targeted strategy.

Table S1. Information of cancer patients.

Patient No.	Gender	Age	Cancer Type	The stage of cancer	Treatment situation
#1	Female	77	Esophageal squamous cell carcinoma	IV	Radiochemotherapy
#2	Male	74	Lung adenocarcinoma	IV	Drug targeted therapy
#3	Male	64	Esophageal squamous cell carcinoma	IV	Radiochemotherapy and immunotherapy
#4	Female	58	Esophageal squamous cell carcinoma	II	Surgical treatment
#5	Female	49	Clear cell sarcoma	IV	Surgical treatment
#6	Female	51	Diffuse large B-cell lymphoma	IV	/

Table S2. Enumeration of captured CTCs that identified by the two methods.

Patient No.	The number of Captured CTCs identified by ICC/Membrane glycoprotein-targeted strategy (per mL)
#1	54/54
#2	154/152
#3	132/223
#4	127/153
#5	0/33
#6	0/63