Supplementary Information

Anti-CD133 Antibody-Targeted Therapeutic Immunomagnetic Albumin Microbeads Loaded with Vincristine-Assisted to Enhance Anti-Glioblastoma Treatment

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Supplementary Information: Preparation of NPs, Stability assay, and analysis of cell viability after treatments in U251 glioblastoma cells

Synthesis of SPIO NPs. SPIO NPs i.e. γ-Fe_2O_3 NPs used in this study were synthetized from magnetite (Fe_3O_4) according to methods proposed elsewhere.[1, 2] First, 4.5 mL $FeCl_3$ (2 M dissolved in 2 M HCl) was added to 15.5 mL DI water, and then 3 mL Na_2SO_3 (1 M) was added dropwise into the mixture within 1 minute with stirring. When the color of the solution changed from red to light yellow, it was added to 120 mL of NH_4OH solution (0.85 M) with vigorous stirring. A black precipitate quickly formed and was allowed to crystallize completely for another 40 min. After washed with deoxygenated water, the black precipitate was diluted to 252 mL (with a mass concentration of 3 mg/mL) and was adjusted to pH 3.0 with HCl (0.1 M). The suspension was then heated to 90 ℃ in 5 min, and was stirred under aeration (with air) for 90 min at 110℃. The color of the suspension slowly changed from black to reddish-brown. After washing with DI water by magnetic decantation, the reddish-brown precipitate was dried to a powder of SPIO NPs.

Preparation of Magnetic Albumin Microbeads. The magnetic albumin microbeads (MAMbs) utilized in this study were prepared by an improved heat-stabilization process according to our previous publication with some modifications.[3] The as-prepared SPIO NPs NPs (90.0 mg) were first dispersed in 1.2 mL of DI water and sonicated for 10 min at room temperature. Then 300.0 mg of HSA and 36.0 mL of cottonseed oil containing 240 μL of sorbitan sesquioleate were added to the SPIO NPs suspension; the mixture was sonicated again until an emulsion was created. The emulsion was then added dropwise over 20 min to 120 mL of cottonseed oil at 140 ℃ with stirring at 500 rpm, and the mixture was stirred for an additional

20 min under the same conditions. Then the microbead suspension was cooled to room temperature and extracted four times with diethyl ether to remove the cottonseed oil and byproducts. After separation with an external magnetic field and washing with PBS (pH 7.4), the MAMbs were stored in a refrigerator at 4 ℃ until use.

RBITC Labeling of the Prepared Immunomagnetic Microbeads. First, 10 mg of MAMbs (or ^{CD133}mAb/MAMbs) was resuspended in 1 mL of PBS solution (pH 9.0) and mixed in a solution of 100 μL of RBITC (1 mg/mL) solution. Subsequently, the suspension was incubated for 6 h under stirring at 400 rpm at RT in the dark. Then, these RBITC modified nanocarriers were washed with PBS (pH 7.4) for three times under a magnetic field, suspended in RPMI-1640 medium, and stored at 4 ℃. The RBITC-labeling fluorescent nanocarriers were observed using an inverted fluorescence microscope (Eclipse TE 2000-U, Nikon, Kyoto, Japan).

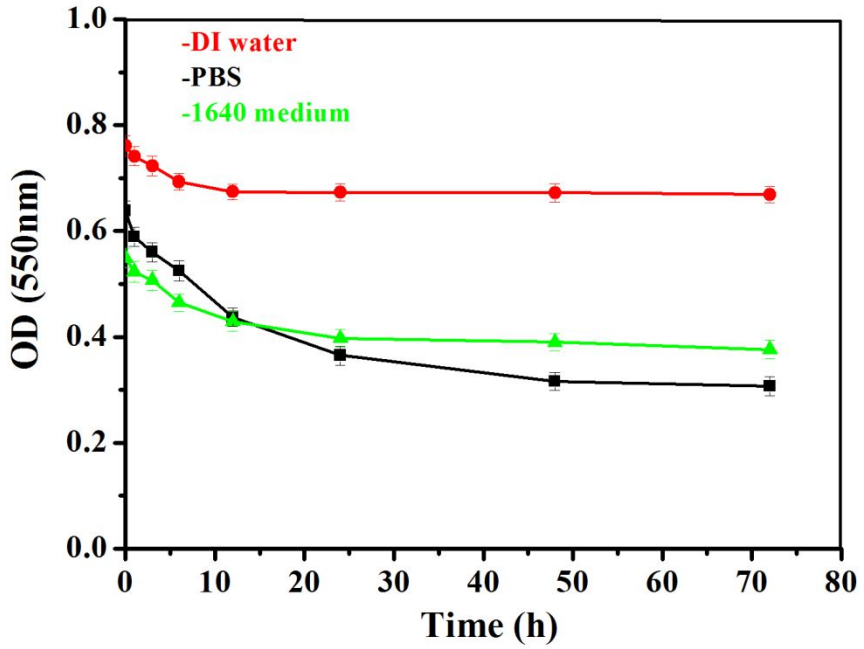

Figure S1. Stability assay of the ^{CD133}mAb/MAMbs in aqueous solution including 1640 medium, PBS (pH 7.4), and DI water.

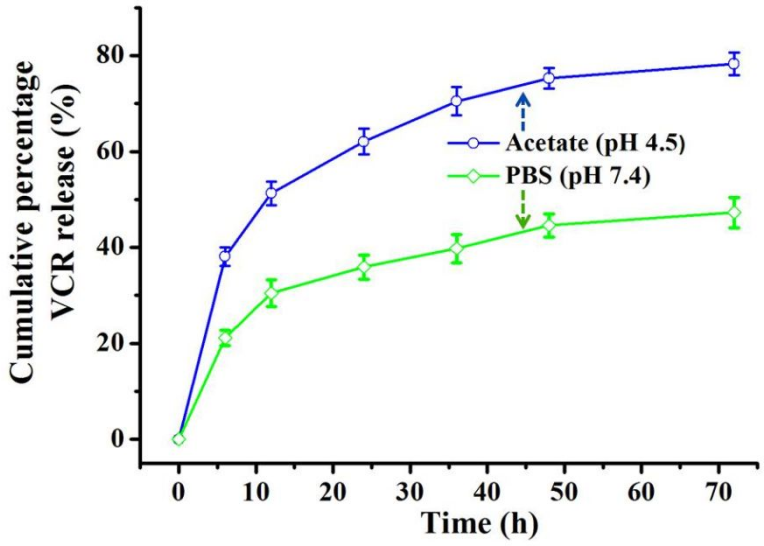

Figure S2. The release of VCR from MAMbs was evaluated in the solution with representative pH including acetate (pH 4.5) and PBS (pH 7.4).

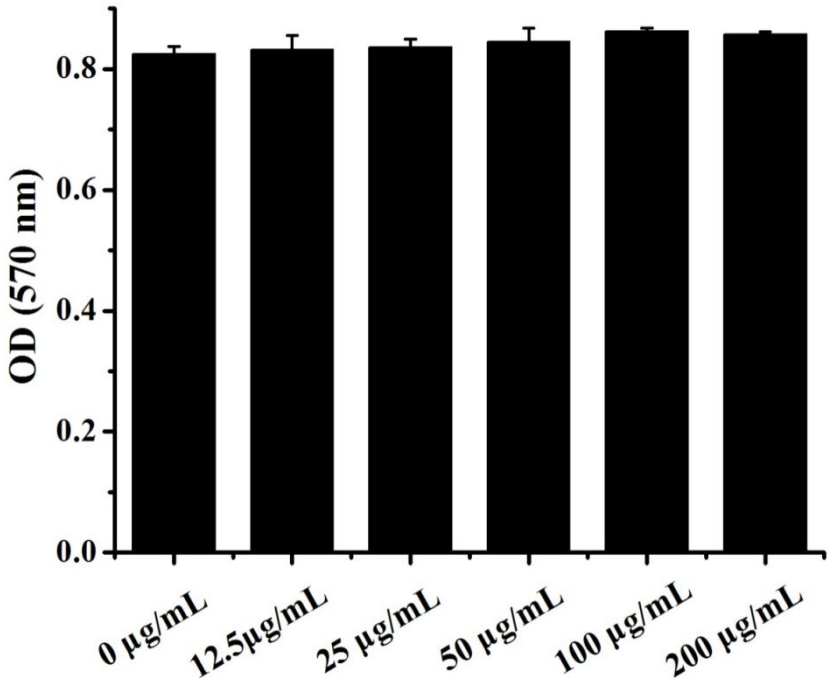

Figure S3. The growth of U251 glioblastoma cells after treated with differerent concentrations of MAMbs (12.5 μg/mL to 200 μg/mL) for 24 h, unlabeled counterparts used as control.

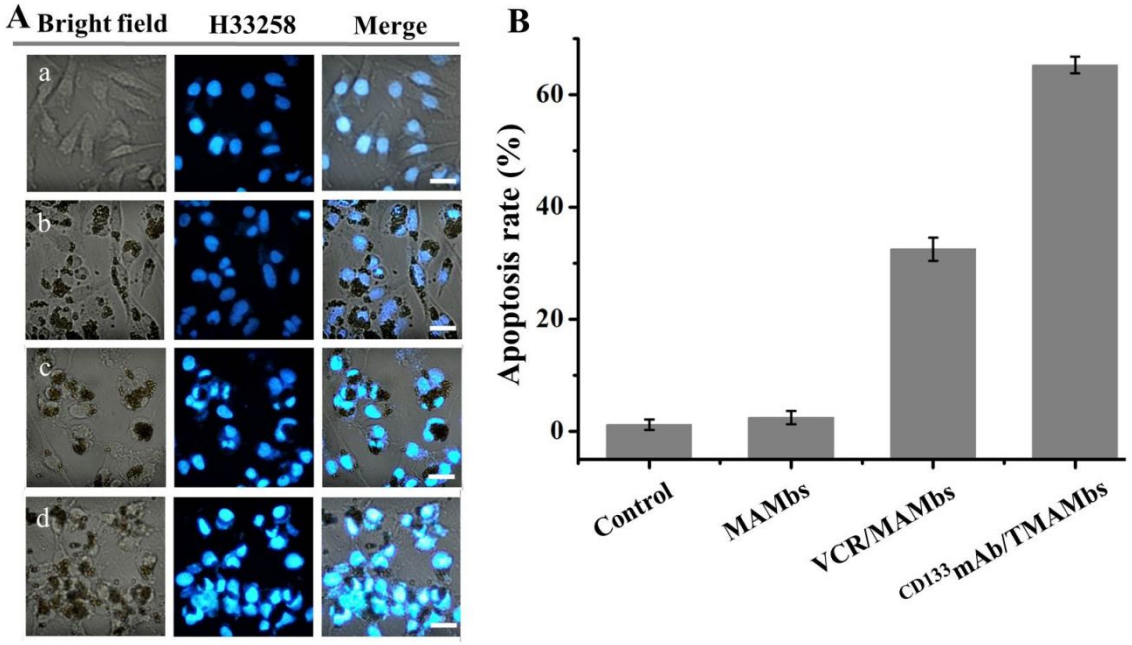

Figure S4. Hoechst H33258 staining of the treated U251 glioblastoma cells. (A) The observed nuclei of the treated U251 glioblastoma cells with different drug loading formulations, and (B) the statistic analysis of apoptotic U251 glioblastoma cells. Scale bar = 50 μm.

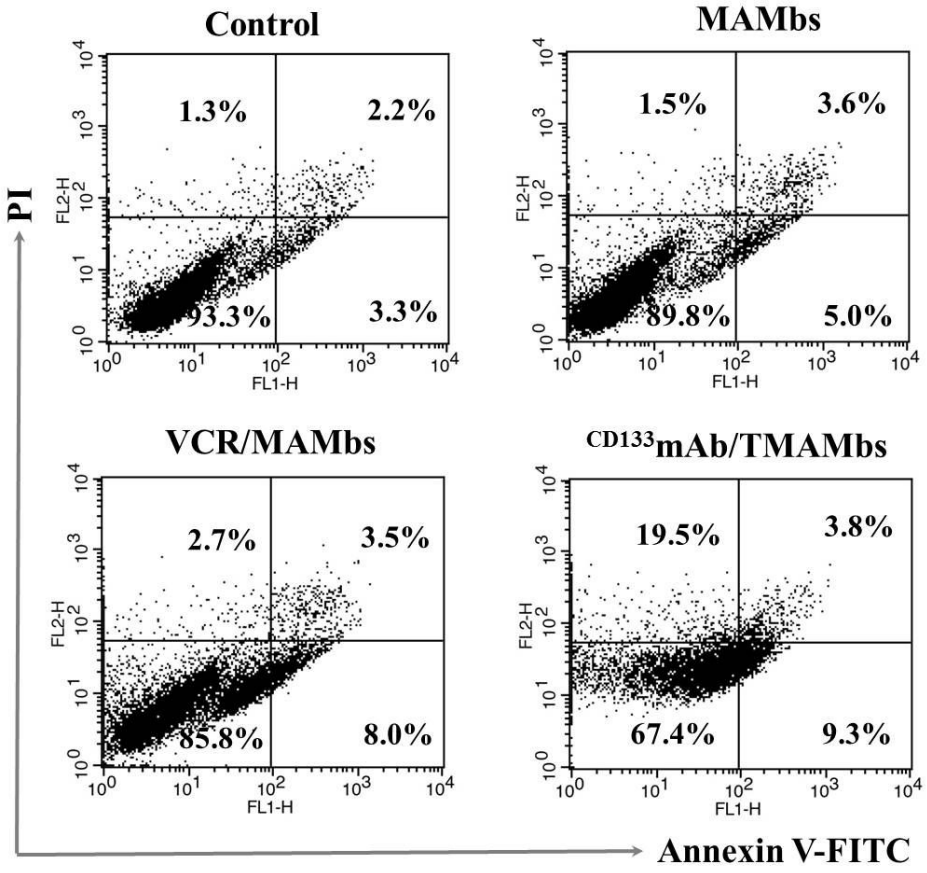

Figure S5. Fluorescein-annexin V and PI staining assays of U251 glioblastoma cells treated for 24h with different formulations.

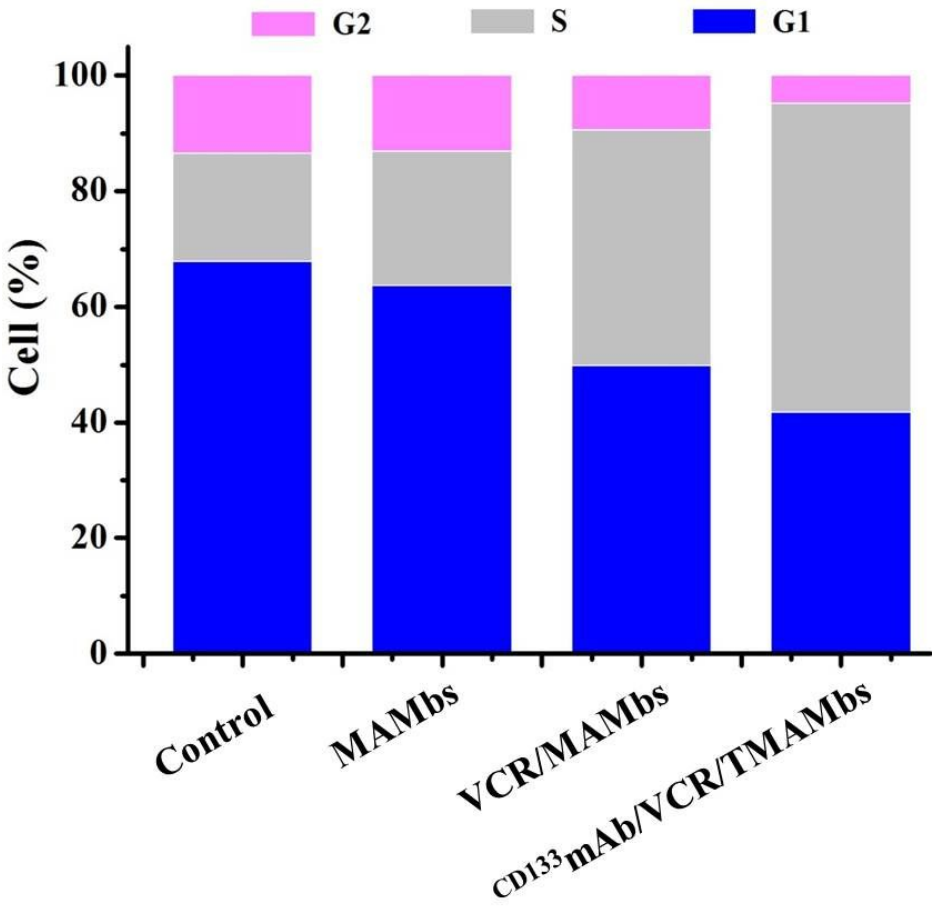

Figure S6. Cell cycle distribution assay of the treated U251 glioblastoma cells for 48h with different formulations: Controls, MAMbs, VCR/MAMbs, and ^{CD133}mAb/TMAMbs.

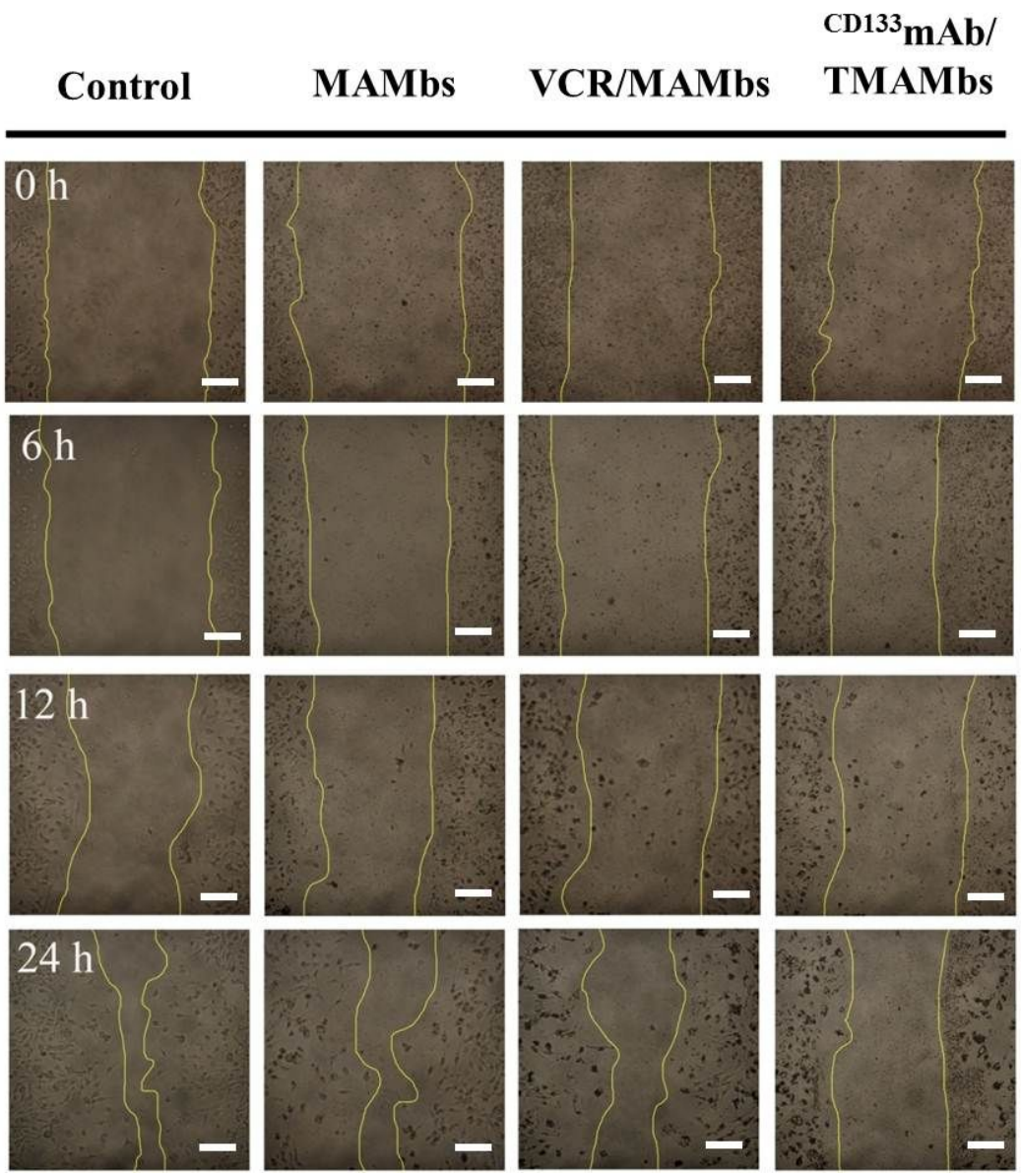

Figure S7. Transwell migration activity assay of the U251 glioblastoma cells treated with different formulations. Scratch wound closure of the U251 glioblastoma cells incubated with different formulations after treatment for 6h, 12h, and 24 h, which displayed arrested closing of the scratch. Scale bar = 200 μm.

REFERENCES

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(2) Sun, Y. K.; Ma, M.; Zhang, Y.; Gu, N. Synthesis of nanometer-size maghemite particles from magnetite. *Colloids Surf. A Physicochem Eng. Aspects* **2004**, *245*, 15–19.

(3) Wang, J.; Wang, X.; Ren, L.; Wang, Q.; Li, L.; Liu, W.; Wan, Z.; Yang, L.; Sun, P.; Ren, L.; Li, M.; Wu, H.; Wang, J.; Zhang, L. Conjugation of biomolecules with magnetic protein microspheres for the assay of early biomarkers associated with acute myocardial infarction. *Anal. Chem.* **2009**, *81* (15), 6210–6217.