Supporting Information

Stapled RGD Peptide Enables Glioma-Targeted Drug Delivery by Overcoming Multiple Barriers

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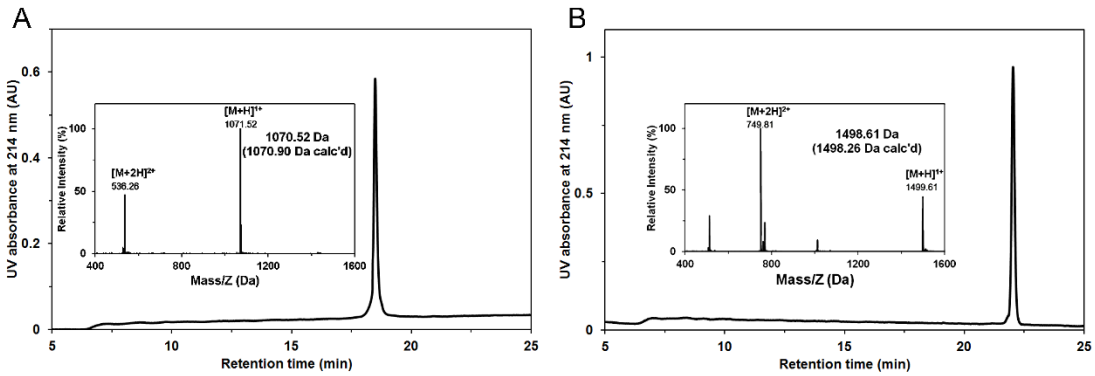

Figure S1. Analysis of sRGD (A) and sRGD-FAM (B) via HPLC and ESI-MS. The conditions of HPLC analysis as follows: temperature: 40℃; C_{18} column: YMC, 3.5 μm, 4.6 × 150 mm; flowing phase: 5-65% acetonitrile in water with 0.1% TFA over 30 min; flow rate: 0.7 mL/min.

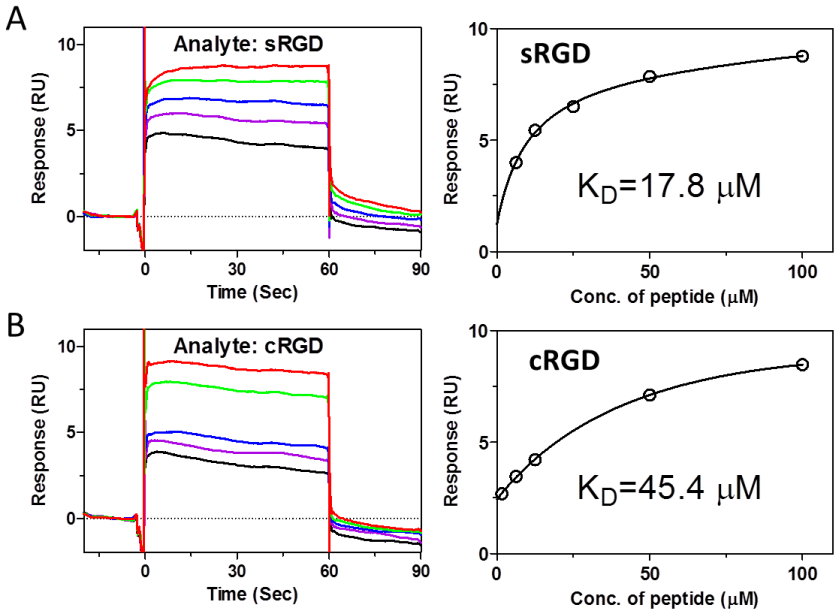

Figure S2. Quantification of the binding affinity of sRGD (A) and cRGD (B) with integrin $\alpha_v\beta_3$ by an SPR-based direct binding assay at 25℃ on a Biacore T200 instrument. The K_D values were determined by the BIA evaluation software.

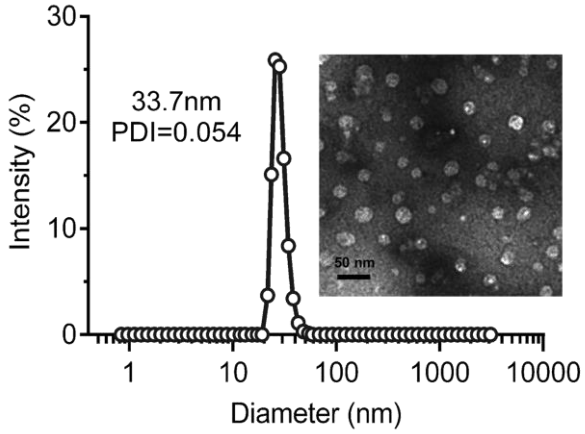

Figure S3. Size distribution of sRGD-PEG-PLA/PTX micelles measured by DLS and TEM.

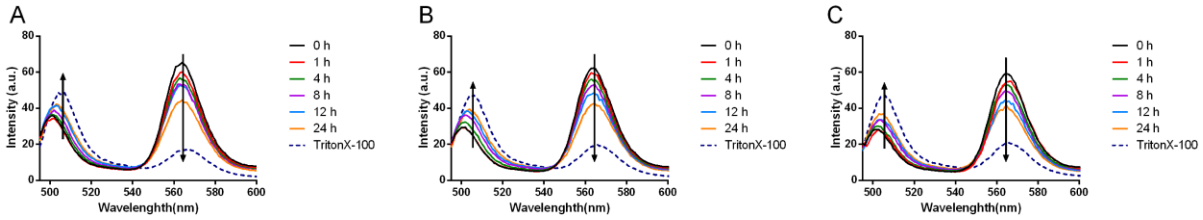

Figure S4. Emission fluorescence spectra overlay for DiO/DiI-loaded PEG-PLA micelles in 20% rat serum: (A) mPEG-PLA micelles, (B) cRGD-PEG-PLA micelles, and (C) sRGD-PEG-PLA micelles. TritonX-100 treated micelles were used as the positive control (complete disassembly of micelle). Ex: 484 nm, Em: 495–600 nm.

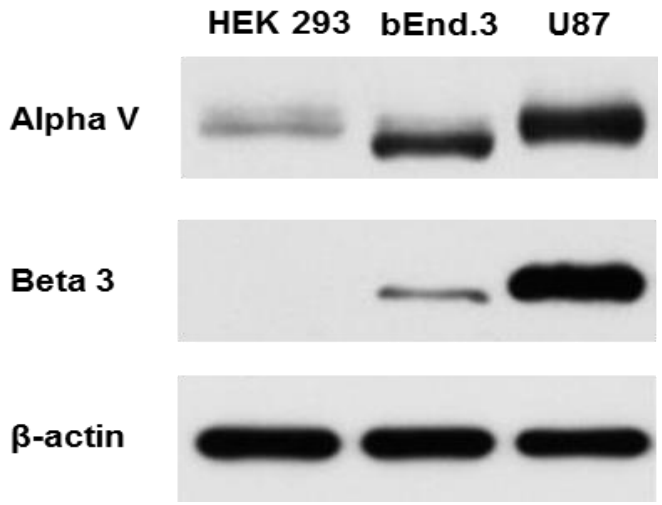

Figure S5. Expression levels of integrin Alpha V and Beta 3 on negative HEK293, bEnd.3 and positive U87 cells analyzed by western blotting.

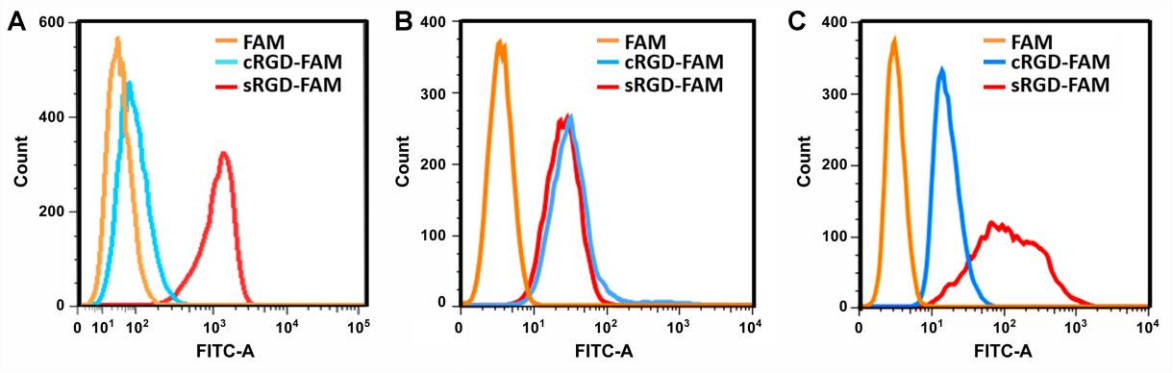

Figure S6. Cellular uptake of 5 μM free FAM, cRGD-FAM or sRGD-FAM on bEnd.3 (A), U87 (B) and HUVEC (C) cells analyzed by flow cytometry.

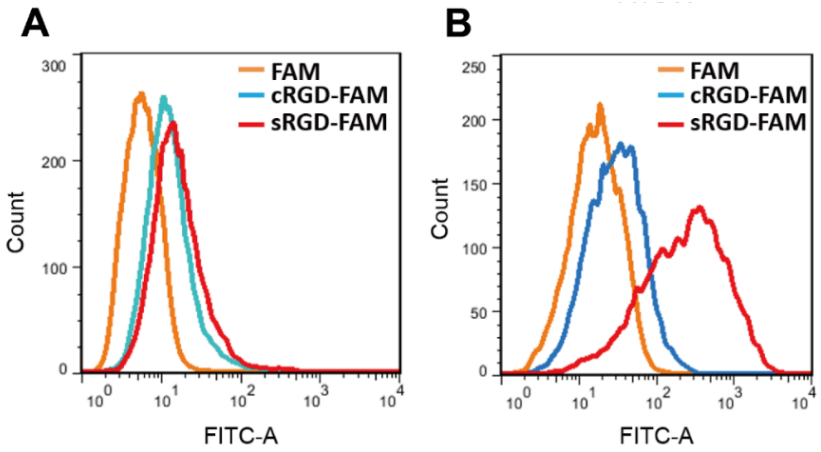

Figure S7. Cellular uptake of 5 μM free FAM, cRGD-FAM or sRGD-FAM on HL-7702 (A) and HEK293 (B) cells analyzed by flow cytometry.

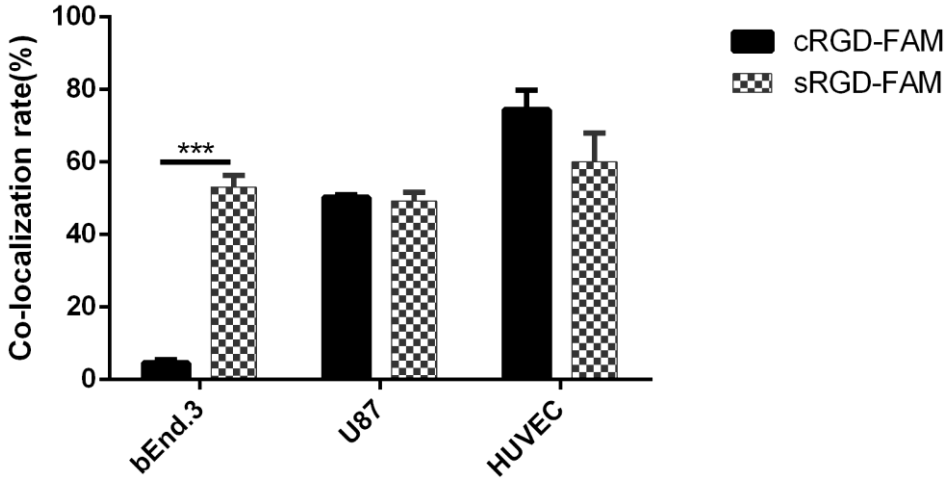

Figure S8. Mander's overlap coefficient analysis of different FAM-labled peptide co-localized with lysosome calculated by Image-Pro Plus software in three different divisions. mean ± SD, n = 3, *** $p < 0.001$.

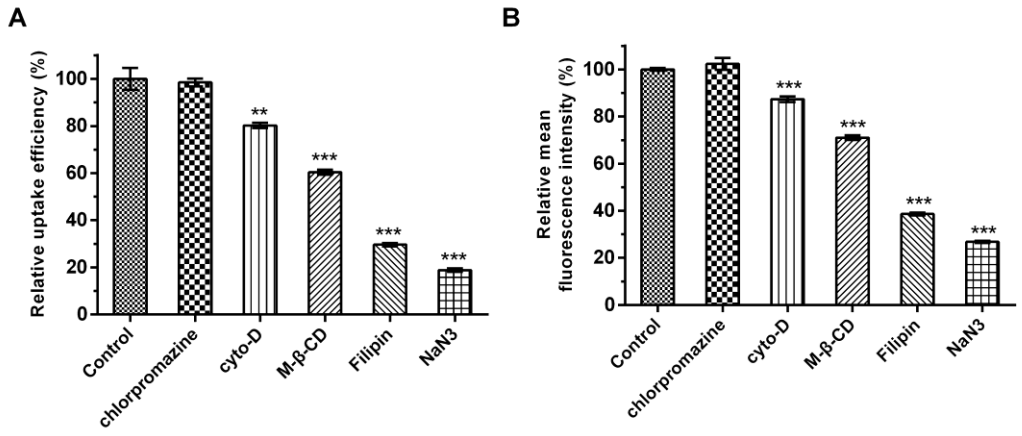

Figure S9. Relative cellular uptake efficiency (A) and relative mean fluorescence intensity (B) of sRGD-FAM by bEnd.3 cells in the presence of different endocytosis inhibitors measured by a flow cytometer. Results were presented as mean ± SD, n = 3. Significance: *** $p < 0.001$, ** $p < 0.01$, versus control group respectively.

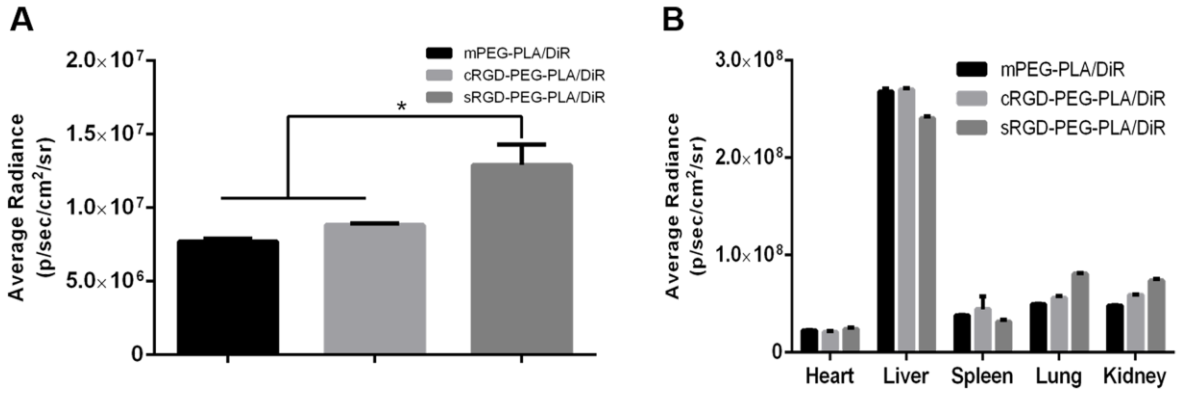

Figure S10. Semi-quantitative ROI analysis of the mean fluorescence intensity of DiR-loaded mPEG-PLA, cRGD-PEG-PLA and sRGD-PEG-PLA in *ex vivo* brain (A) and other major organs (B) 24 h after administration.

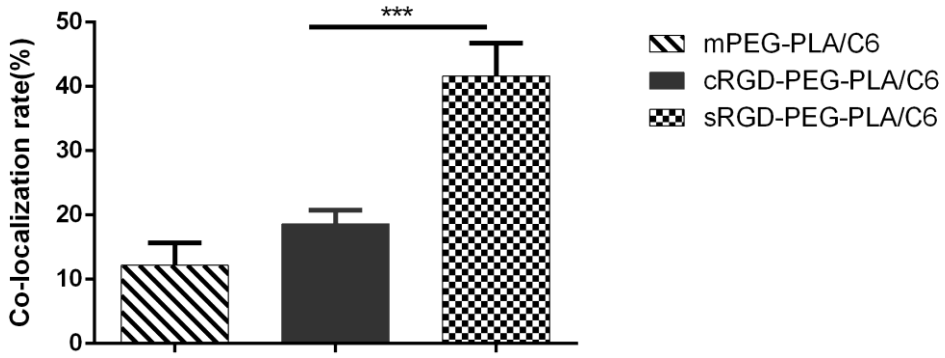

Figure S11. Mander's overlap coefficient analysis of different formulations co-localized with CD31 marker calculated by Image-Pro Plus software. Results were presented as mean ± SD, n = 3, *** $p < 0.001$.

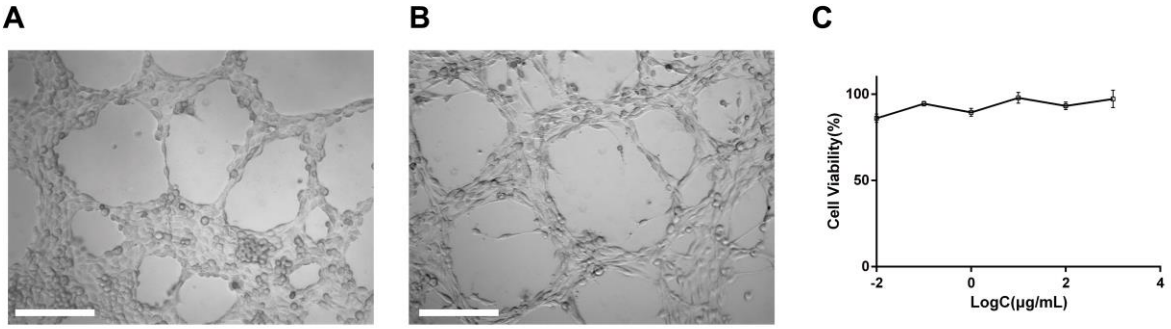

Figure S12. Toxicity evaluation of unloaded sRGD-PEG-PLA micelles *in vitro* anti-neovascularization (A), anti-VM Efficacy (B) and cytotoxicity effect on U87 cells measured by MTT assay (C). Bar = 200 μm.