ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Targeted Delivery of PD-L1-Derived Phosphorylation-Mimicking Peptides by Engineered Biomimetic Nanovesicles to Enhance Osteosarcoma Treatment

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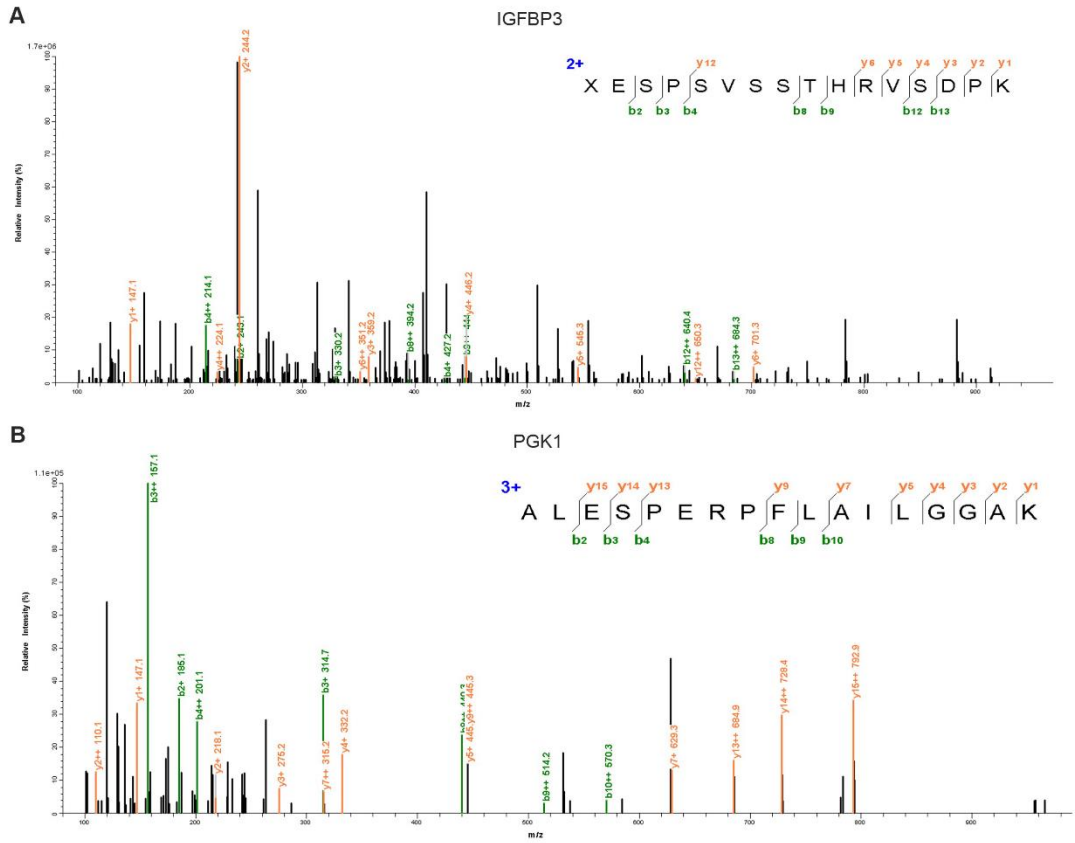
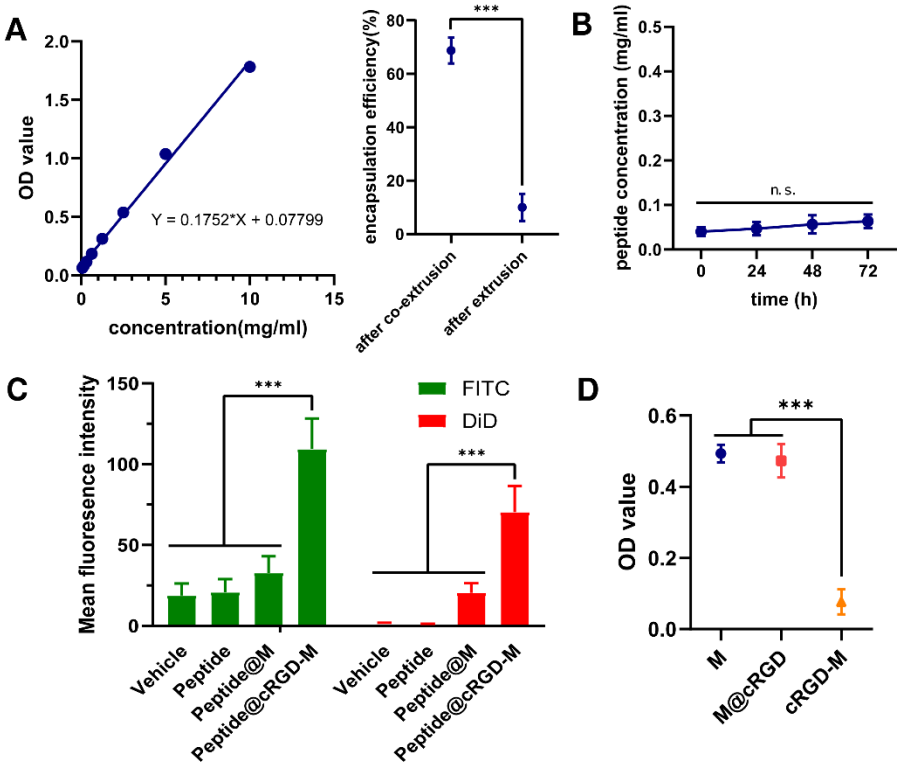


Supplementary Figure 1.

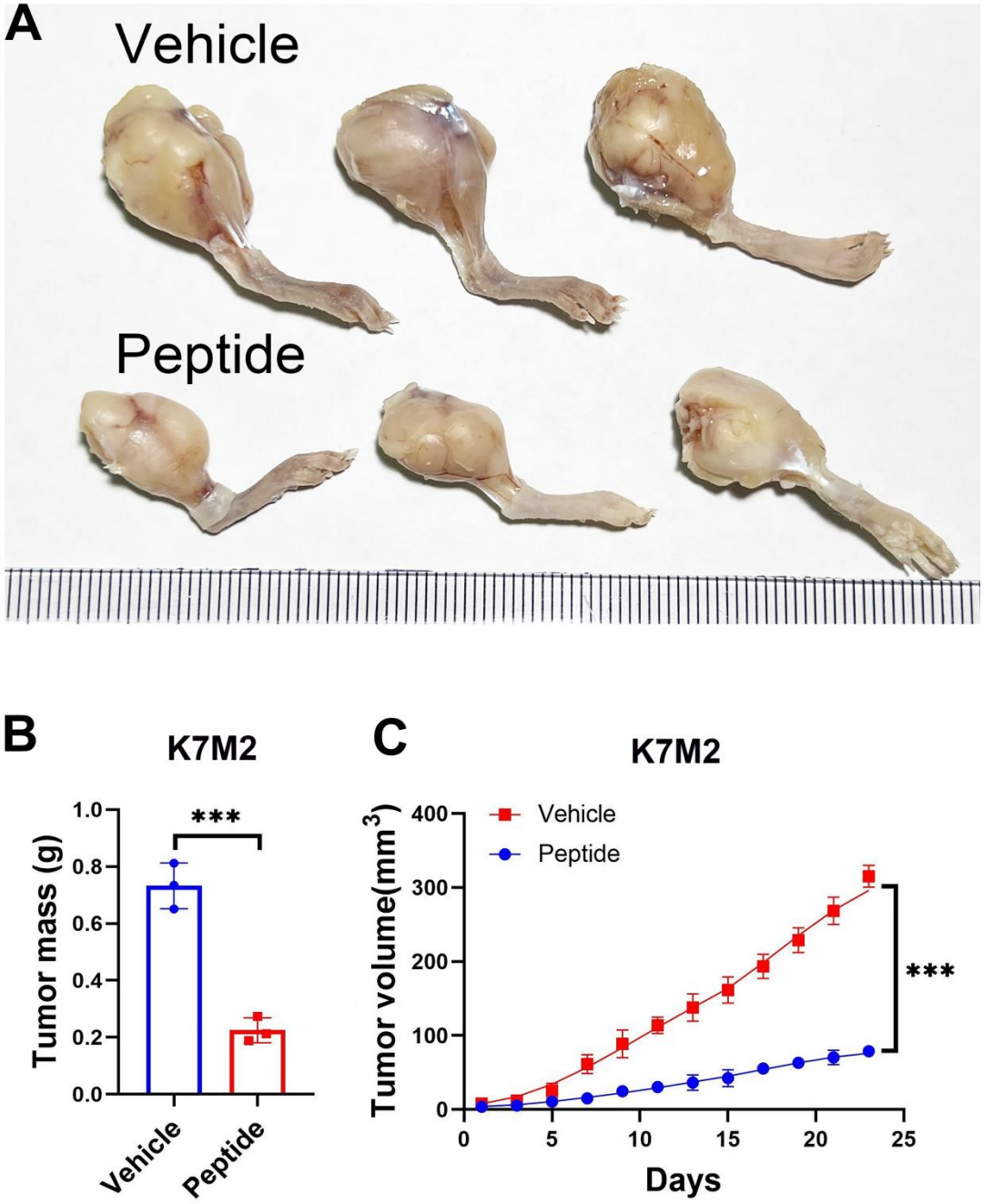
A-B. A model depicting that the plasma membrane of osteosarcoma was removed and the rest of cell compartments were subjected to mass spectrometry analysis by using the IgG or PD-L1 antibody. Based on the detection of the peptide of IGFBP3 and PGK1.



Supplementary Figure 2.

A. (Left) The measured absorbance rates (OD value at 495 nm) of the peptide solution (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml and 0.1563 mg/ml). The fitted working curves of the peptide concentration and the absorbance rate were obtained after the linear regression analysis of these data. The equation of the fitting curve is Y=0.1752*X+0.07799. (Right) The encapsulation efficiency of cRGD-modified membrane vehicles (cRGD-M) after the extrusion of peptides with or without cRGD-M. 0.5 mg/mL peptide solution was prepared before extrusion with (co-extrusion group) or without (extrusion group) 0.5 mg/mL cRGD-M. The co-extrusion group and extrusion group were then centrifuged to precipitate the membrane vehicles, and the OD values of the supernatant fluids were determined

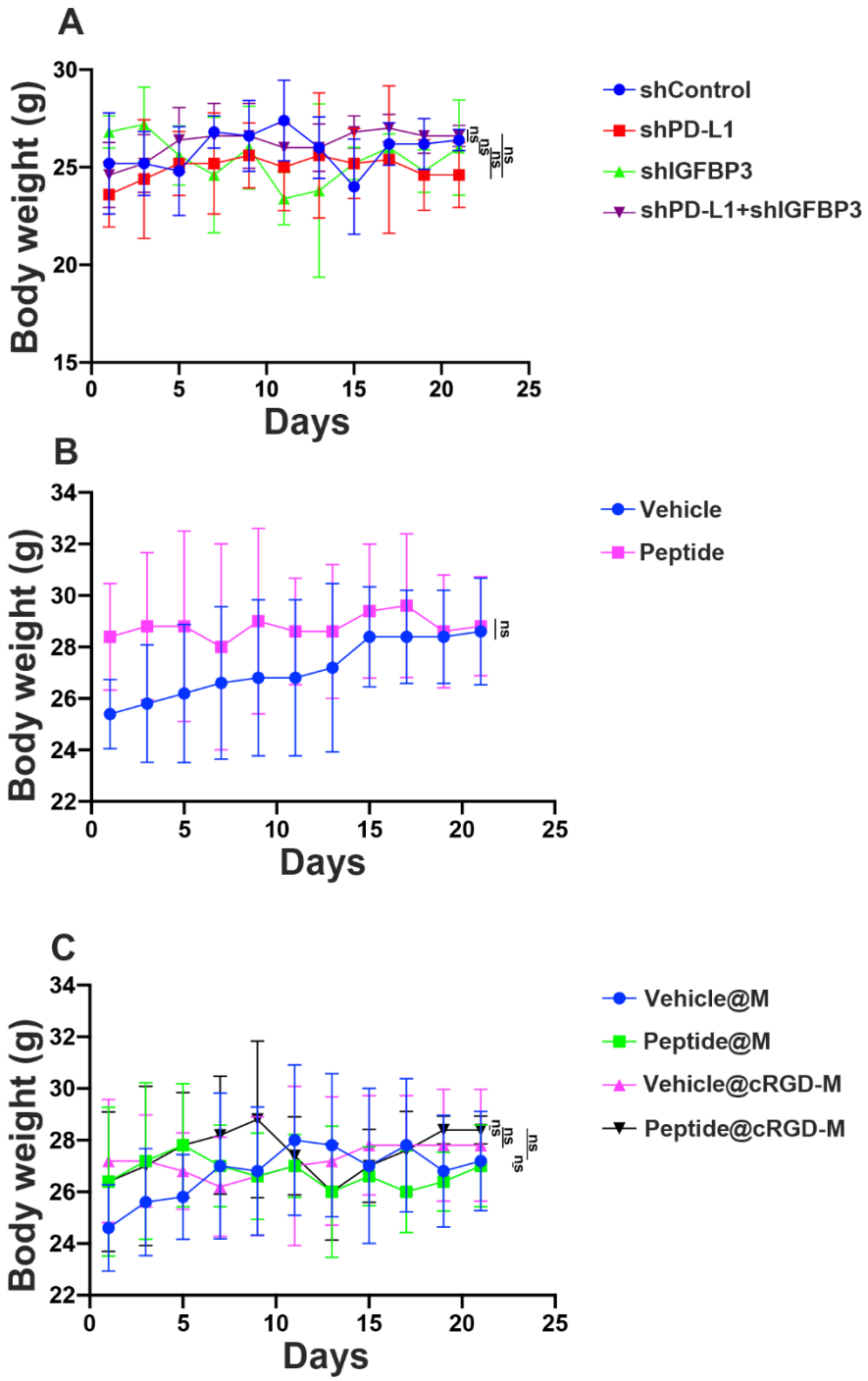
at 495 nm and converted to concentrations (Cs). The encapsulation efficiency was obtained by (1-Cs)/0.5*100%. **B.** The stability of peptide@cRGD-M in PBS at 0 h, 24 h, 48 h and 72 h. 5 mg of peptide@cRGD-M was prepared and dissolved in 5 mL of PBS at 37 ℃. The prepared solution was centrifuged at 0 h, 24 h, 48 h and 72 h, and the concentrations of the free peptide in the supernatant fluids were measured to estimate the stability of peptide@cRGD-M. **C.** The quantitative analysis of mean intracellular FITC and the DiD fluorescence intensity of each group after 3 h of incubation. **D.** The OD values of the FITC-labeled αvβ3 receptors solution treated with membrane vehicles (M), membrane-coated cRGD (M@cRGD), or cRGD-modified membrane vehicles (cRGD-M) in similar amounts. Equal quantities of FITC-labeled αvβ3 receptors were incubated with equivalent amounts of membrane vehicles (M), membrane-coated cRGD (M@cRGD), or cRGD-modified membrane vehicles (cRGD-M) for 3 hours. The resulting solutions were then centrifuged to separate the membrane vehicles, and the OD values of the supernatant fluids were determined at 495 nm to obtain the concentrations of the remaining FITC-labeled αvβ3 receptors. (***: $p < 0.001$). Data are presented as the mean ± SD (n = 3).



Supplementary Figure 3.

A-C. Tumor tissues acquired from BALB/c mice (n = 3/group) in the orthotopic models of tibial injection. A vehicle or peptide was injected into the tumor on days 1, 3, 5, and 7 (1.2 mg/kg every 2 h for 8 h). The tumor image is shown in Supplementary figure 3A. The tumor mass is displayed in panel B and the tumor volume in panel C.

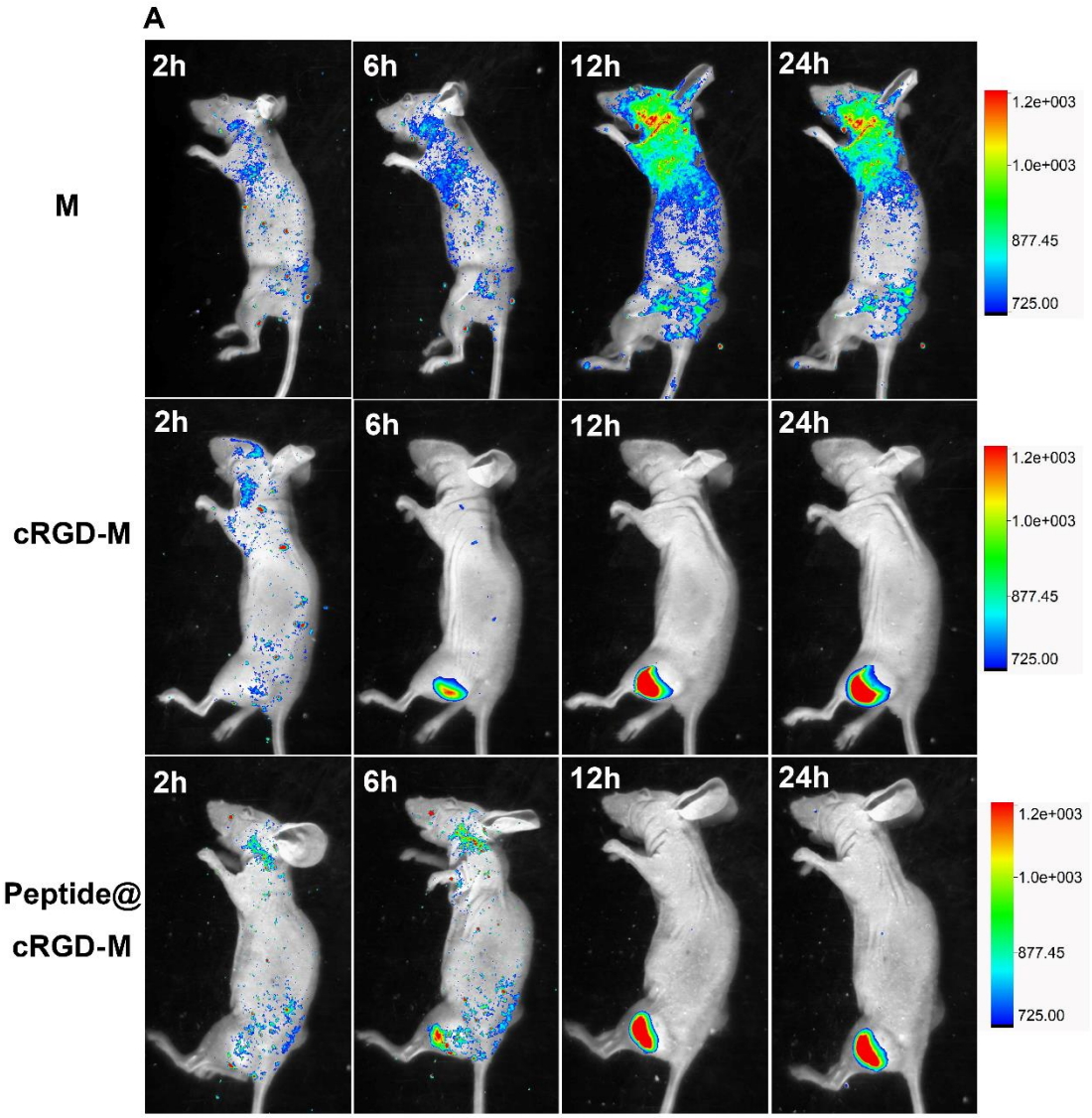
Data are presented as the mean ± SD, with three replicates. ***, P< 0.001.



Supplementary Figure 4.

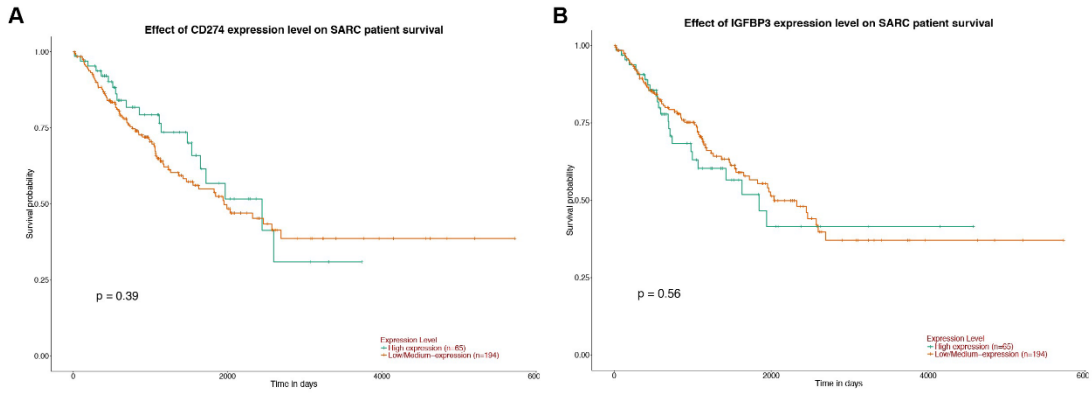
A. The infection of MNNG/HOS cells with corresponding shRNAs for 72 h. After

puromycin selection, cells were subcutaneously injected into nude mice. The body weight of the nude mice (ns, not significant). **B.** The subcutaneous injection of MNNG/HOS cells into nude mice. A vehicle or peptide was injected into the tumor on days 1, 3, 5, and 7 (1.2 mg/kg every 2 h for 8 h). The body weight of the nude mice (ns, not significant). **C.** The subcutaneous injection of MNNG/HOS cells into nude mice. Representative photographs of the treatment with pure vehicles@M, pure peptides@M, vehicle@cRGD-M, or peptide@cRGD-M. The body weight of the nude mice (ns, not significant). Data are presented as the mean ± SD (n = 5).



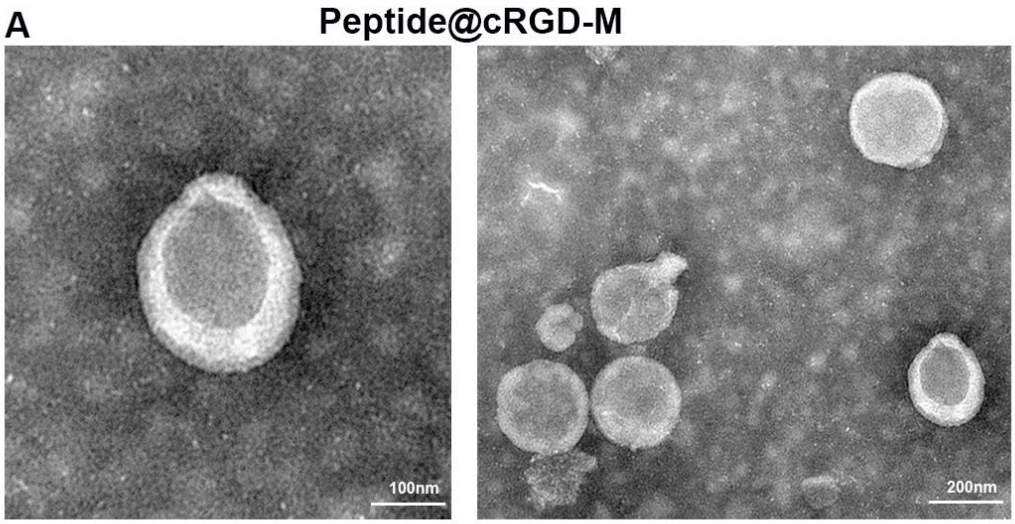
Supplementary Figure 5.

A. The *in vivo* distribution of cRGD-M and peptide@cRGD-M in tumor-bearing nude mice over time (2 h, 6 h, 12 h, 24 h) as determined with fluorescence imaging. RBC membranes (M) were labeled with DiD (red), and the relative membrane amount was determined by measuring the relative intensity of the red fluorescence.



Supplementary Figure 6.

A-B. The analysis of the survival probability in low/high CD274 and IGFBP3 groups using the GEPIA web tool.



Supplementary Figure 7.

A. The TEM images of peptide@cRGD-M. (Scale bar = 100 nm and 200 nm)