Supplementary Figures

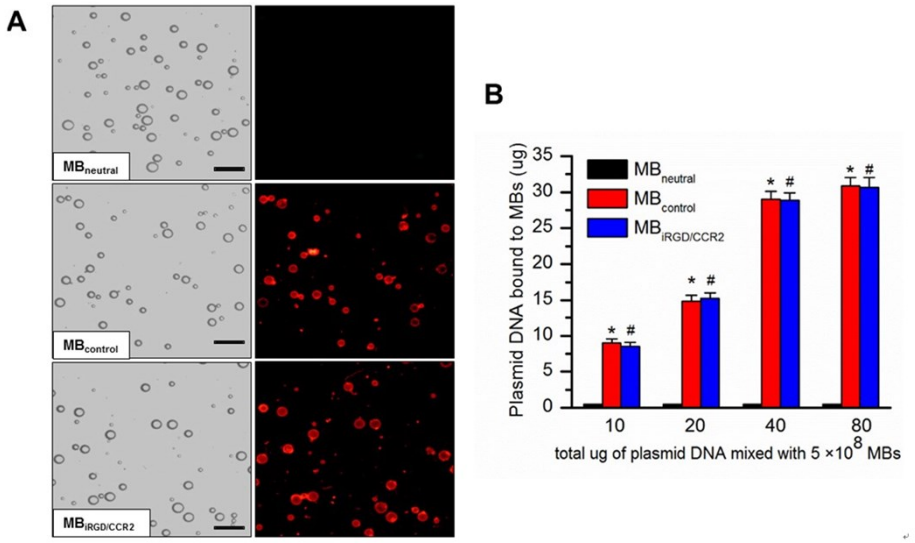


Fig. S1 DNA-loading efficiency of dual-targeted MBs. (**A**) DNA plasmids bound to $MB_{neutral}$, $MB_{control}$, or $MB_{iRGD/CCR2}$ were stained with PI dye (red). (**B**) Quantitative analysis of the loading DNA per 5×10^8 MBs. Scale bar: 5 μm.

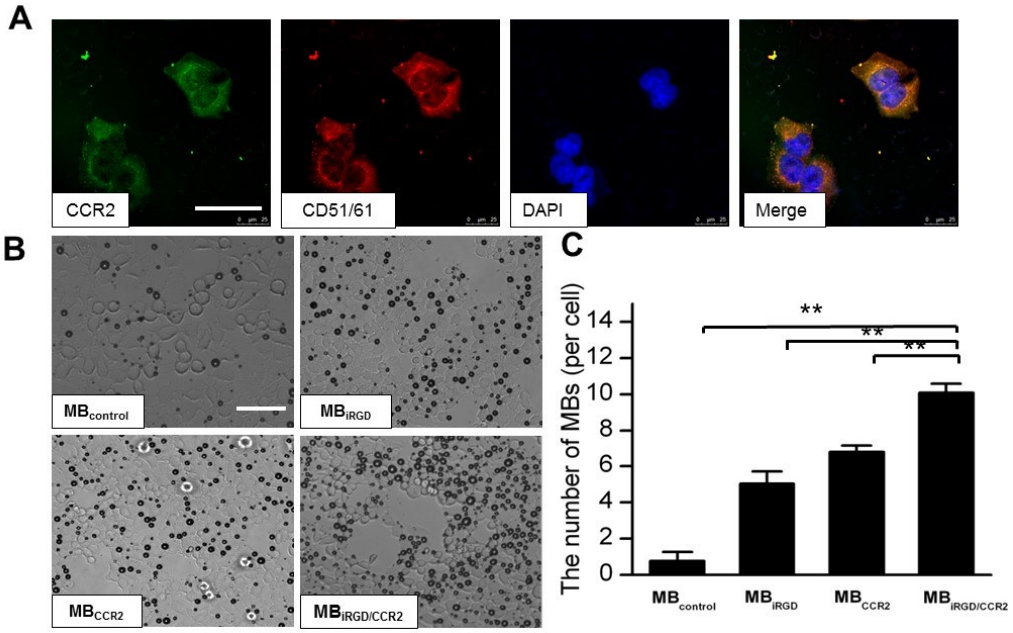


Fig. S2 Protein expression and cell attachment efficiency. (**A**) Immunofluorescence histochemical staining of integrin $\alpha_V\beta_3$ and CCR2 receptors in MCF-7 cells. The expression of CCR2 and integrin $\alpha_V\beta_3$ was confirmed. Scale bar: 10 μm. (**B**) Static binding assay on MCF-7 cells with $MB_{control}$, MB_{iRGD}, MB_{CCR2}, and $MB_{iRGD/CCR2}$. Scale bar: 10 μm. (**C**) Comparison of the adherent bubble number per cell among the groups.

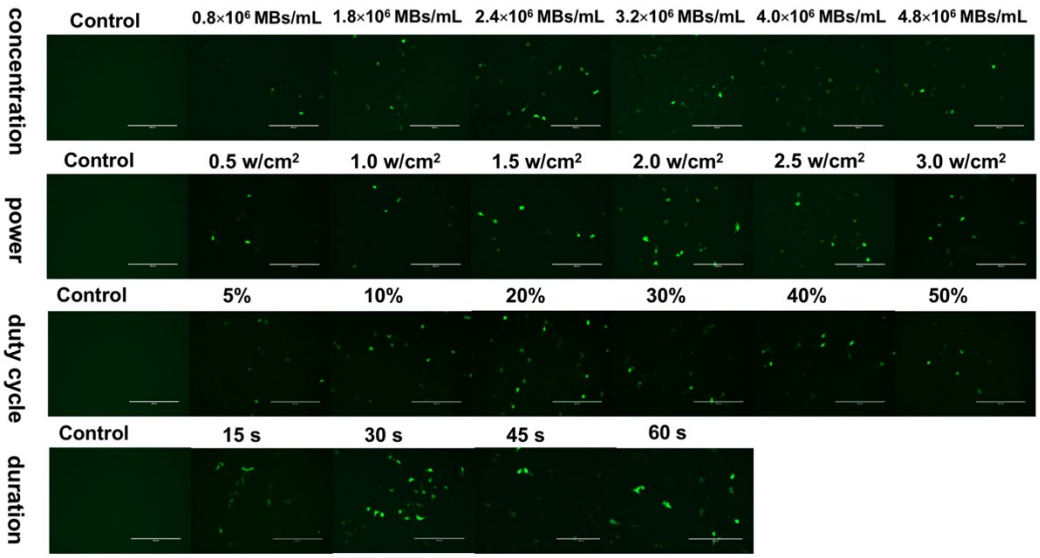


Fig.S3 Cell transfection of dual-targeted cationic microbubbles in various ultrasound irradiation conditions,including: concentration,power,duty cycle and duration.

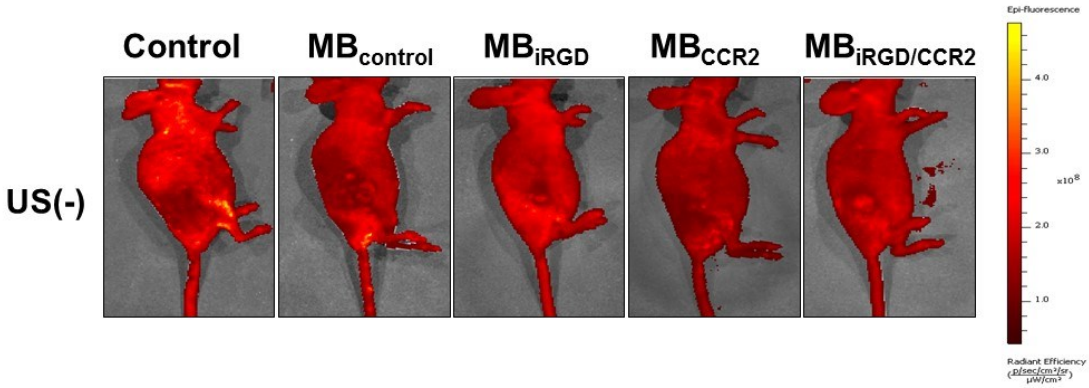


Fig. S4 GFP expression was detected after 72 h transfection with PBS, $MB_{control}$, MB_{iRGD}, MB_{CCR2}, or $MB_{iRGD/CCR2}$ without US.

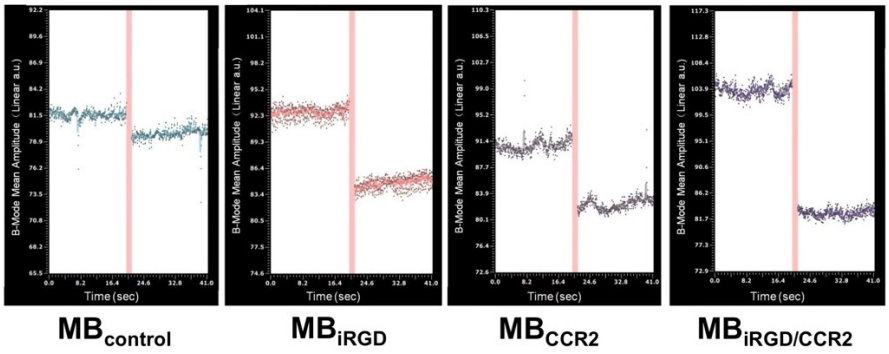


Fig S5 The acoustic intensity of ultrasound image of before-after flash in field of view after intravenous injection of MBcontrol, MBiRGD, MBCCR2, or MBiRGD/CCR2

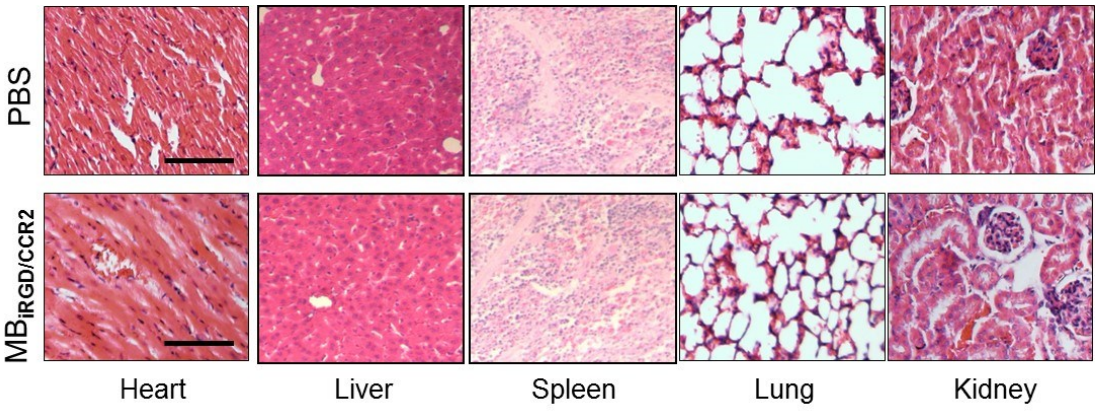
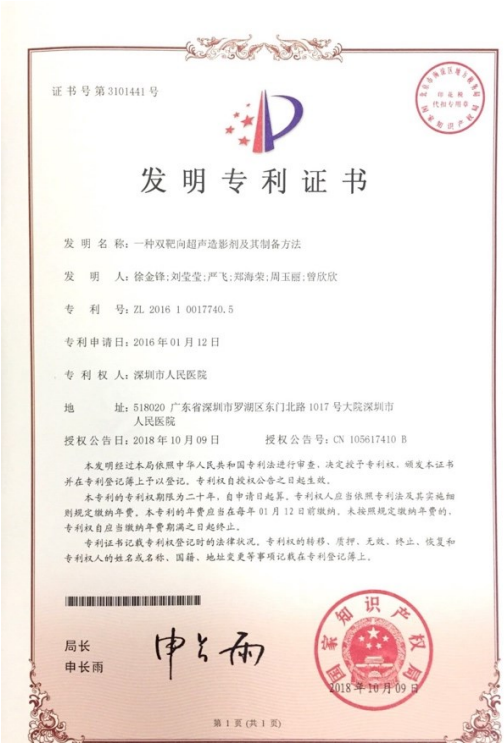


Fig. S6 Hematoxylin/eosin (HE) staining analysis of the histological structure of tissues, including the heart, liver, spleen, lung, and kidney, 72 h after gene transfection with $MB_{iRGD/CCR2}$. PBS treatment was used as a control.



证书号第3101441号

发明专利证书

发明名称：一种双靶向超声造影剂及其制备方法

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专利号：ZL 2016 1 0017740.5

专利申请日：2016年01月12日

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授权公告日：2018年10月09日　　授权公告号：CN 105617410 B

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2018年10月09日

第1页(共1页)

Fig. S7 The patent certificate issued by the state intellectual property office of China. The invention relates to a dual-targeted ultrasound contrast agent and its preparation method.

Supplementary Tables

Table S1. The characteristics of the resulting various microbubbles, including $MB_{neutral}$, $MB_{control}$, MB_{iRGD}, MB_{CCR2}.

MB name	Diameter (μm)	Zeta-potential (mV)	Concentration ($\times 10^9$/ml)
$MB_{neutral}$	1.26±0.10	-11.38±1.06	4.22±0.31
$MB_{control}$	1.21±0.13	25.40±2.26*	4.45±0.25
MB_{iRGD}	1.24±0.14	27.50±2.32*	4.30±0.44
MB_{CCR2}	1.28±0.17	32.64±2.05*	3.96±0.17
$MB_{iRGD/CCR2}$	1.32±0.22	28.45±2.41*	3.98±0.65

* $P<0.001$ VS. $MB_{neutral}$, n = 3 per group