Ultrasound-responsive nanocarriers with siRNA and Fe_3O_4 regulate macrophage polarization and phagocytosis for augmented non-small cell lung cancer Immunotherapy

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Table S1. Sequences of SIRPα siRNAs

Gene	Sense	Antisense
SIRPα-1	5’-GCCUGACACAGAAAUACAATT-3’	5’-UUGUAUUUCUGUGUCAGGCTT-3’
SIRPα-2	5’-CCGGAUCAAACAGAAGAAATT-3’	5’-UUUCUUCUGUUUGAUCCGGTT-3’
SIRPα-3	5’-CGCCCAAGAAUCUCACAAATT-3’	5’-UUUGUGAGAUUCUUGGGCGTT-3’

Table S2. Information of flow cytometry antibodies used in this study

Antibodies		
CD206 (MMR) Monoclonal Antibody (MR6F3), PE,	Thermo	Cat. 12-2061-82
CD86 (B7-2) Monoclonal Antibody (GL1), FITC	Thermo	Cat. 11-0862-82
FOXP3 Monoclonal Antibody (FJK-16s), PE	Thermo	Cat. 12-5773-82
CD8a Monoclonal Antibody (53-6.7), FITC	Thermo	Cat. 11-0081-82
CD3 Monoclonal Antibody (17A2), PE	Thermo	Cat. 12-0032-82
Ki-67 Monoclonal Antibody (SolA15), PE	Thermo	Cat. 12-5698-82
CD69 Monoclonal Antibody (H1.2F3), PE	Thermo	Cat. 12-0691-82
CD4 Monoclonal Antibody (GK1.5), FITC	Thermo	Cat.11-0041-82
IFN gamma Monoclonal Antibody (XMG1.2), PE	Thermo	Cat. 12-7311-82
Anti-Mouse F4/80, APC	LIANKEBIO	Cat. F21480A03

Table S3. Information of antibodies assays used in Western Blot assay**.**

Antibodies		
Anti-SIRP**α**	Proteintech	Cat. 67337-1-Ig
Anti-GAPDH	Servicebio	Cat. GB15002
Anti-IRF5	Proteintech	Cat.10547-1-AP

Table S4. Sequences of PCR primers used in this study

	PCR primers
TNF-α	Forward: 5'-CCTCTTCTCATTCCTGCTTGTGG-3'
	Reverse: 5'-ATCACCCCGAAGTTCAGTAGACA-3'
IFN-γ	Forward: 5'-ACTATTTTAACTCAAGTGGCAT-3'
	Reverse: 5'-CCATCCTTTTGCCAGTTCC-3'
IL-10	Forward: 5'-CTGCTCTTACTGACTGGCAT-3'
	Reverse: 5'-AGTTATTGTCTTCCCGGCTGT-3'
GAPDH	Forward: 5'-CATCACTGCCACCCAGAAGACTG-3'
	Reverse: 5'-ATGCCAGTGAGCTTCCCGTTCAG-3'
SIRP α	Forward: 5'-TAGTCCTGCTGATGGCTGCTCTC-3'
	Reverse: 5'-TCGTGCAACCGTGTGGAAGATC-3'

Supplementary Figures

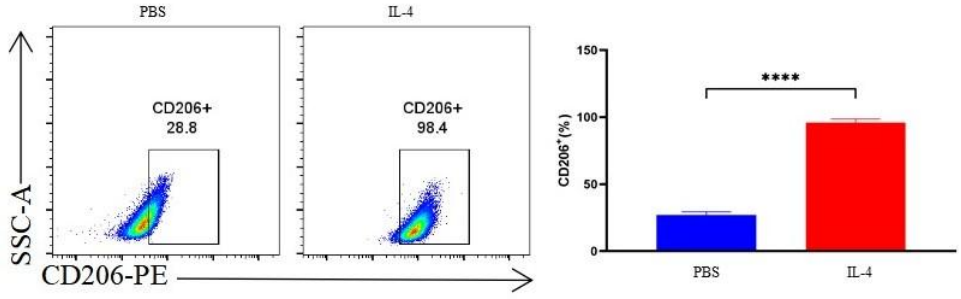

Figure S1. Efficiency of M2 polarization of IL-4 treated RAW 264.7 as analyzed by flow cytometry.

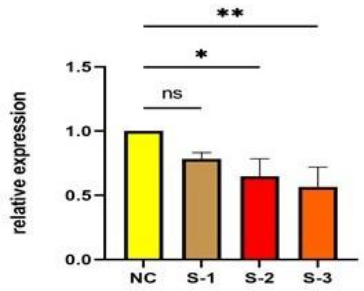

Figure S2. RT-PCR analysis of SIRPα mRNA levels in RAW264.7 cells transfected with different siRNAs (S1: SIRPα-1, S2: SIRPα-2, S3: SIRPα-3) for 24 h, GAPDH served as an internal control. Compared with the control group (RAW264.7), the expression of RAW264.7 decreased in the S3 group and had a significant difference (n = 3) (**$P < 0.005$).

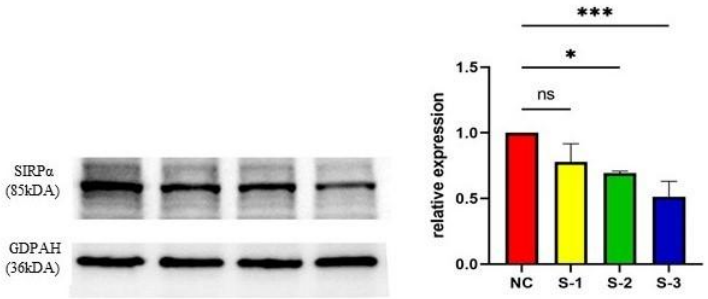

Figure S3. Western blot analysis of SIRPα protein levels in RAW264.7 cells transfected with different siRNAs (S1: SIRPα-1, S2: SIRPα-2, S3: SIRPα-3) for 48 h, GAPDH served as an internal control. Compared with the control group (RAW264.7), the expression of RAW264.7 decreased in the S3 group and had a significant difference (n = 3) (**$P < 0.005$).

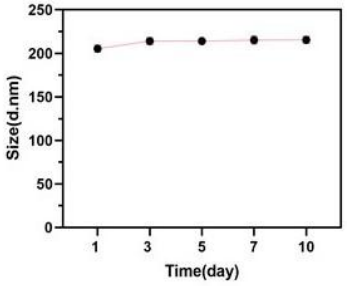

Figure S4. The size changes of FA-PFNB-SIRP**α** siRNA NBs for different days.

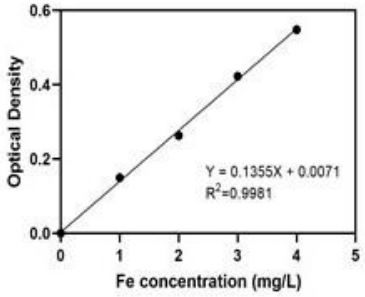

Figure S5. The iron standard curves by atomic absorption spectroscopy.

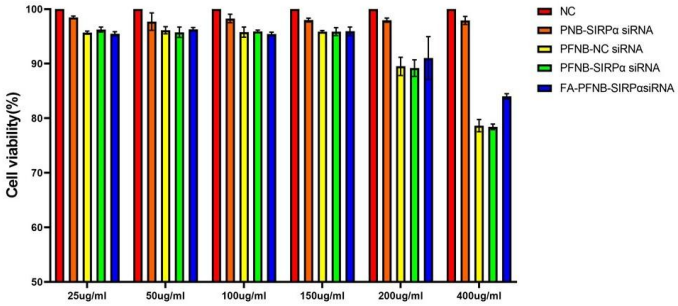

Figure S6. Cytocompatibility assessment for fresh mouse peritoneal macrophages treated with different concentrations of nanomaterials.

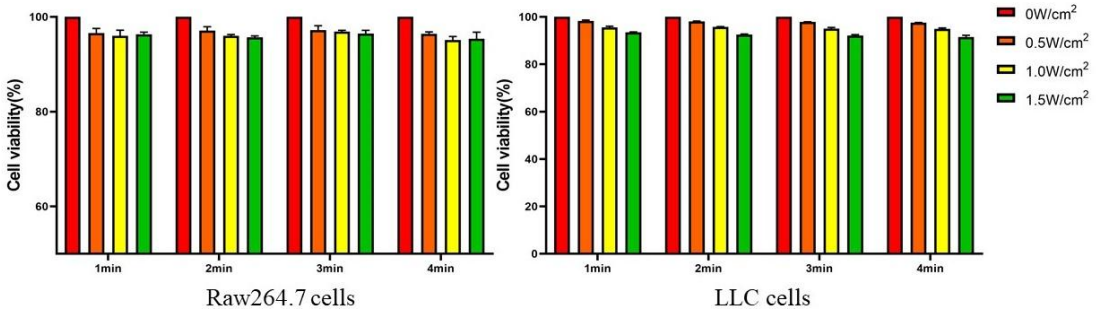

Figure S7. Effects of different ultrasound parameters on the viability of LLC cells and RAW264.7 cells.

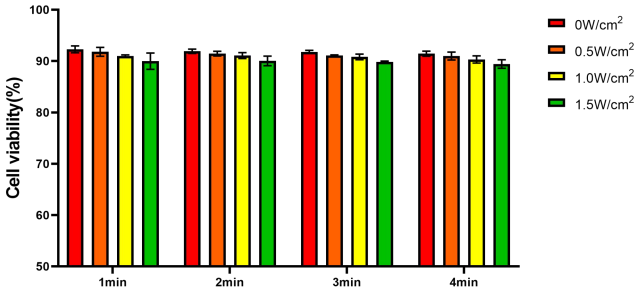

Figure S8. Effects of different ultrasound parameters combined with PNB-SIRPα siRNA (200 μg/mL) on the viability of RAW264.7 cells.

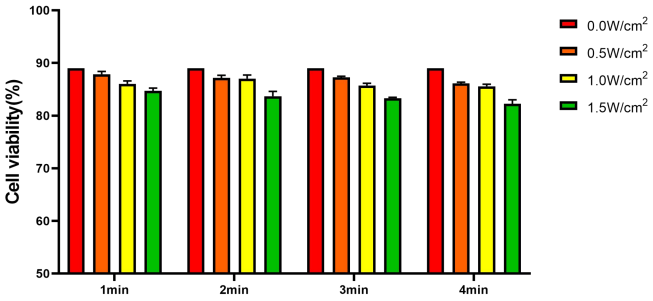

Figure S9. Effects of different ultrasound parameters combined with FA-PFNB-SIRPα siRNA (200 μg/mL) on the viability of RAW264.7 cells.

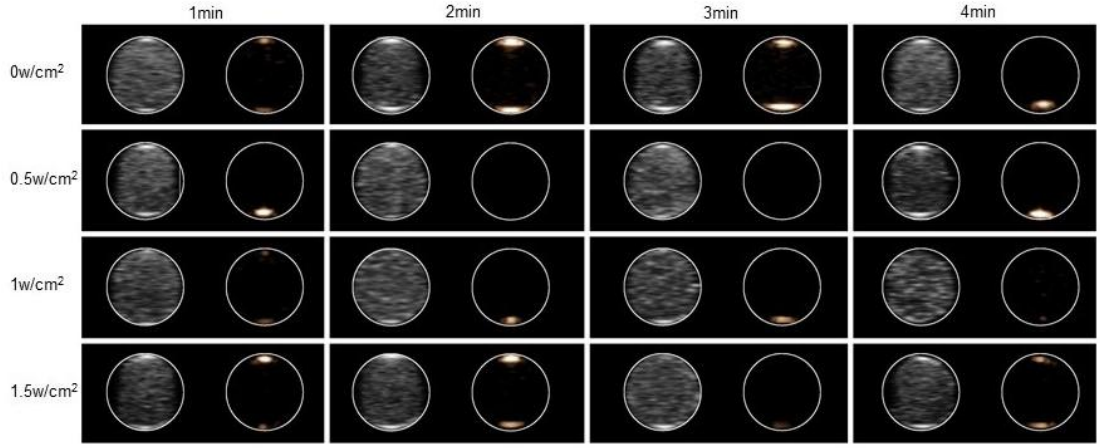

Figure S10. ADV and US imaging of FNB-SIRPα siRNA NBs at different intensities of LIFU irritation and different time in vitro. Echo intensity did not change in all cases.

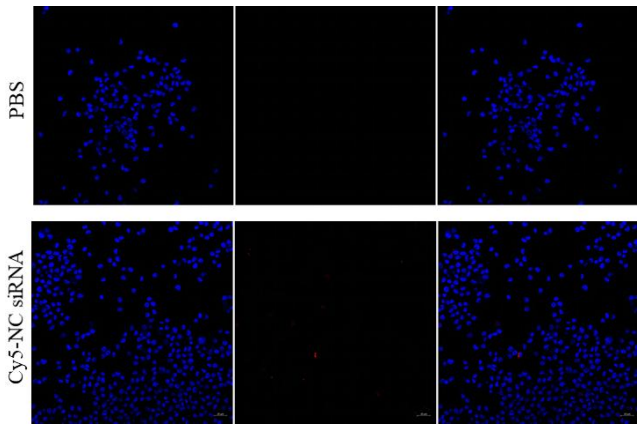

Fig S11. Laser confocal microscope images of the macrophage uptake of Cy5-NC siRNA .

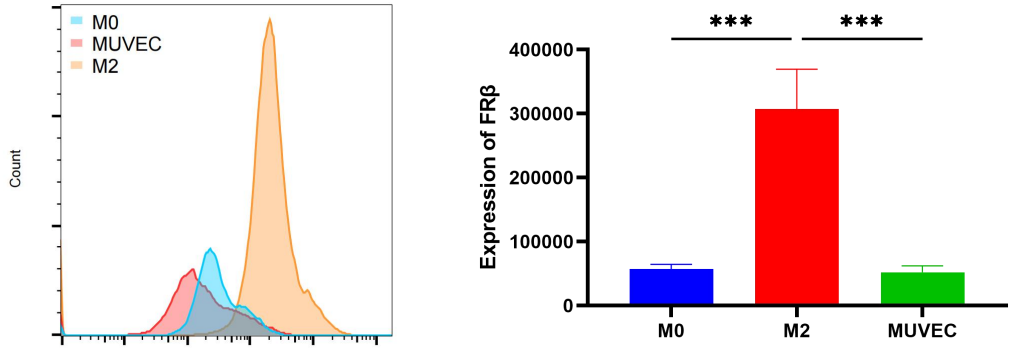

Figure S12. FRβ expression in different cell lines. (n = 3, *p < 0.05; ***p < 0.005).

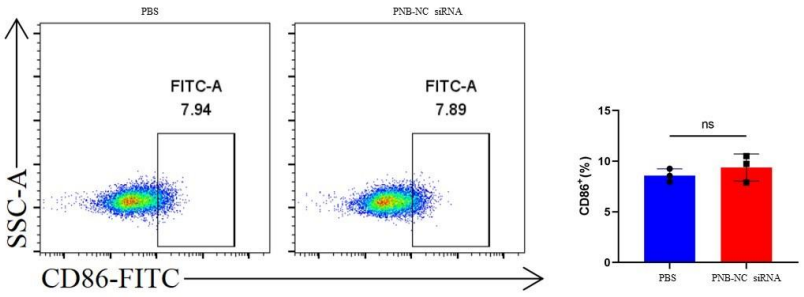

Figure S13. Effect of PNB-NC siRNA at different concentrations on CD86 expression in M2-like macrophages (n = 3).

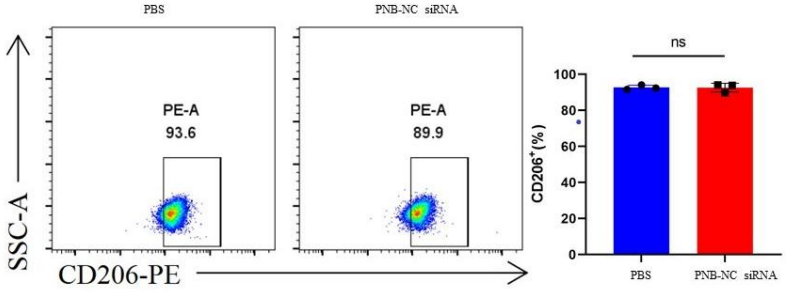

Figure S14. Effect of PNB-NC siRNA at different concentrations on CD206 expression in M2-like macrophages (n = 3).

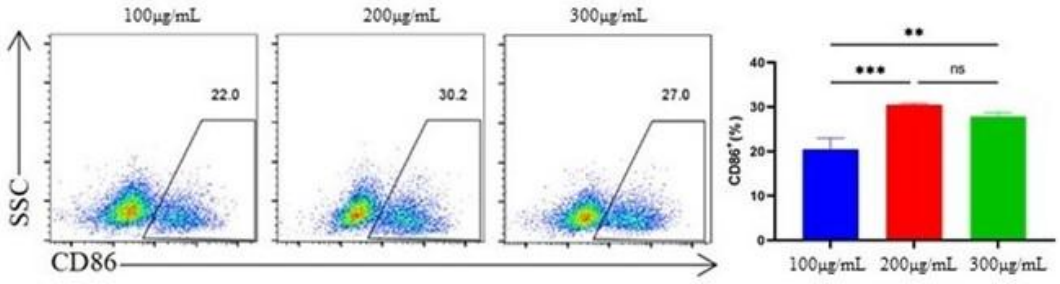

Figure S15. Effect of PFNB-NC siRNA at different concentrations on CD86 expression in M2-like macrophages (n = 3, *p < 0.05; **p < 0.01).

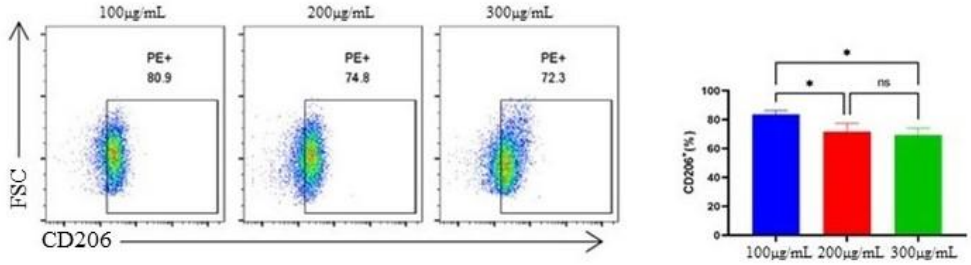

Figure S16. Effect of PFNB-NC siRNA at different concentrations on CD206 expression in M2-like macrophages (n = 3, *p < 0.05).

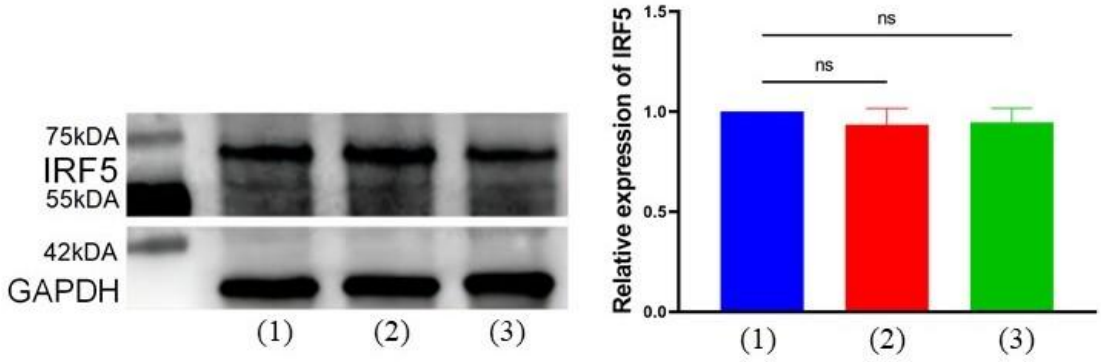

Figure S17. Effect of PNB-NC siRNA, Lipo-SIRPα siRNA on IRF5 transcription factor expression in M2-like macrophages ((1): PBS, (2): PNB-NC siRNA, (3): Lipo-SIRPα siRNA. PNB-NC siRNA:200 μg/mL, Lipo-SIRPα siRNA:120 nM).

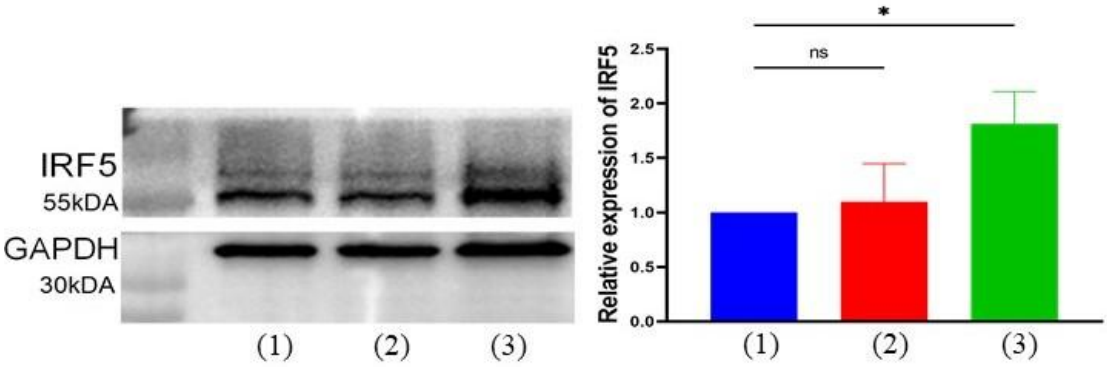

Figure S18. Effect of PNB-NC siRNA, PFNB-NC siRNA on IRF5 transcription factor expression in M2-like macrophages (n = 3, *p < 0.05) ((1): PBS, (2): PNB-NC siRNA, (3): PFNB-NC siRNA. PNB-NC siRNA:200 μg/mL, PFNB-NC siRNA: 200 μg/mL).

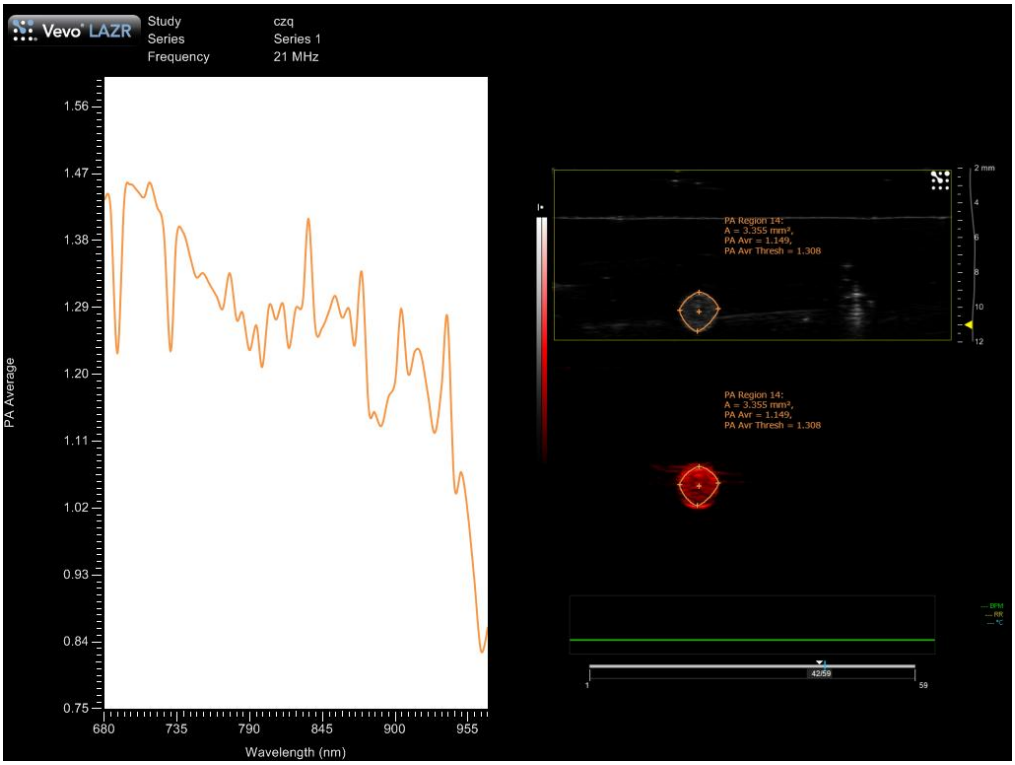

Figure S19. The PA signal changes of PFNB-NC siRNA irradiated by a laser at full spectrum ranging from 680 to 950 nm, and the peak appeared at 690 nm.

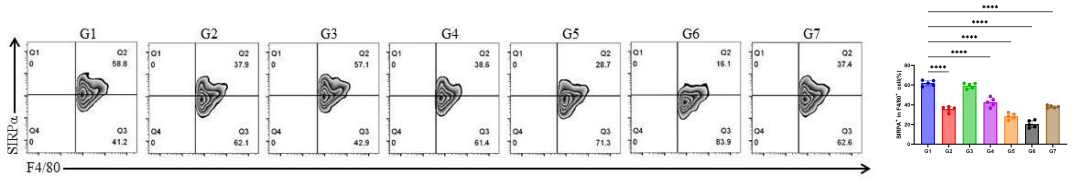

Figure S20. Flow cytometric analysis of the percentages of SIRPα^{+} in F4/80^{+} macrophages in the tumor of tumor-bearing mice of different groups.

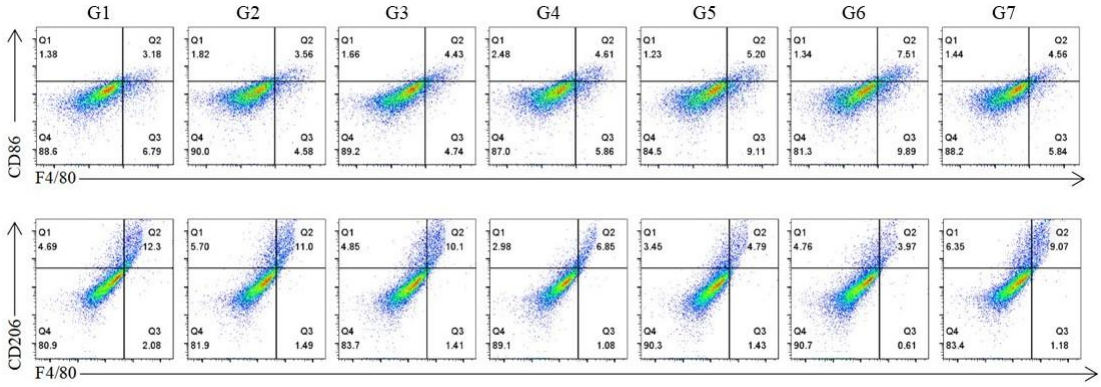

Figure S21. FACs plotting of F4/80^{+}CD86^{+}, F4/80^{+}CD206^{+}macrophages cells in the tumor of tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

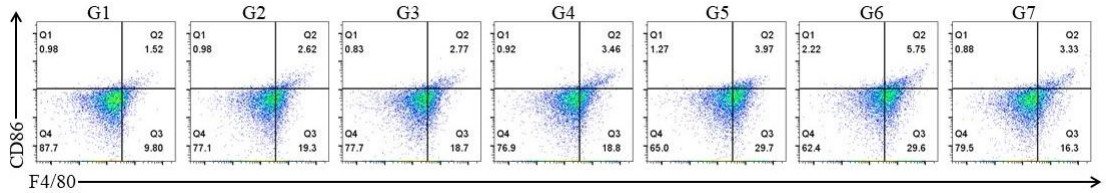

Figure S22. FACs plotting of F4/80^{+}CD86^{+}macrophages cells in the TDLN of tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

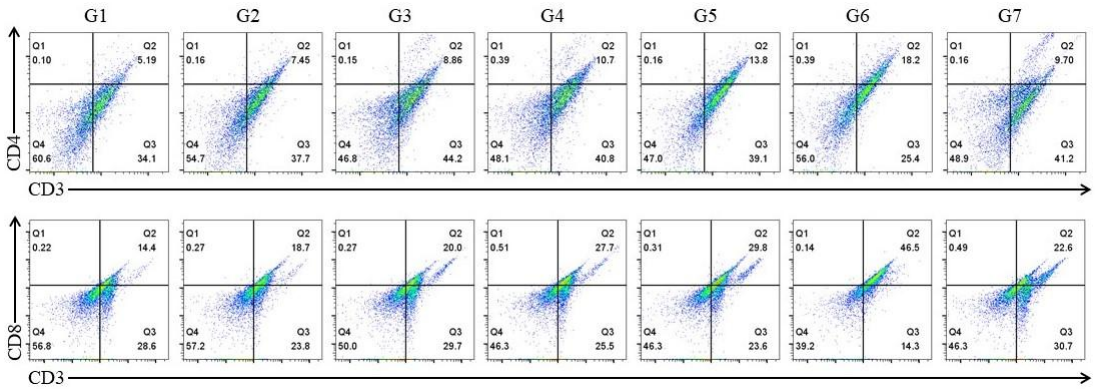

Figure S23. FACs plotting of $CD3^{+}CD4^{+}$, $CD3^{+}CD8^{+}$ T cells in the TDLN of tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

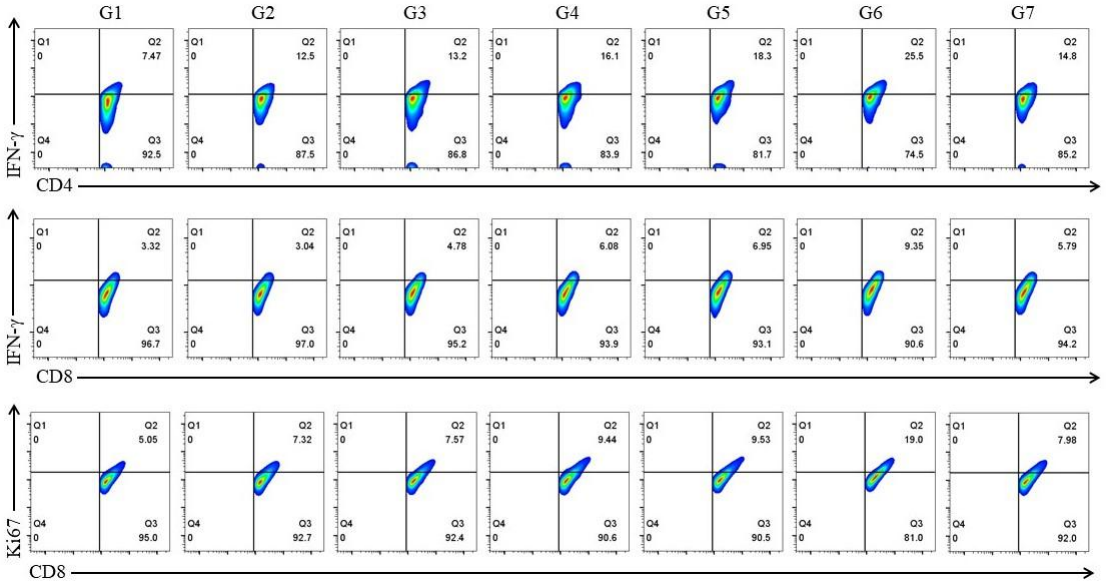

Figure S24. FACs plotting of IFN-γ^{+} in $CD4^{+}$, IFN-γ^{+} in $CD8^{+}$, $Ki67^{+}$ in $CD8^{+}$ T cells in the TDLN of tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

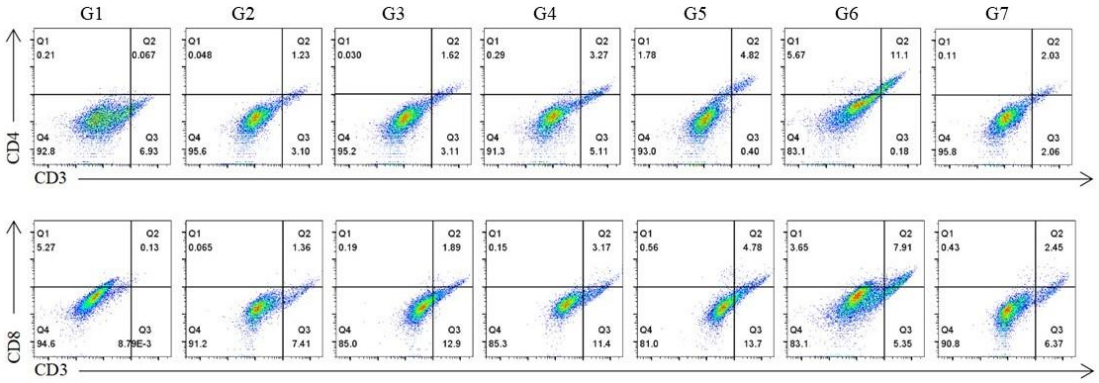

Figure S25. FACs plotting of $CD3^+CD4^+$, $CD3^+CD8^+$ T cells in the tumor of tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

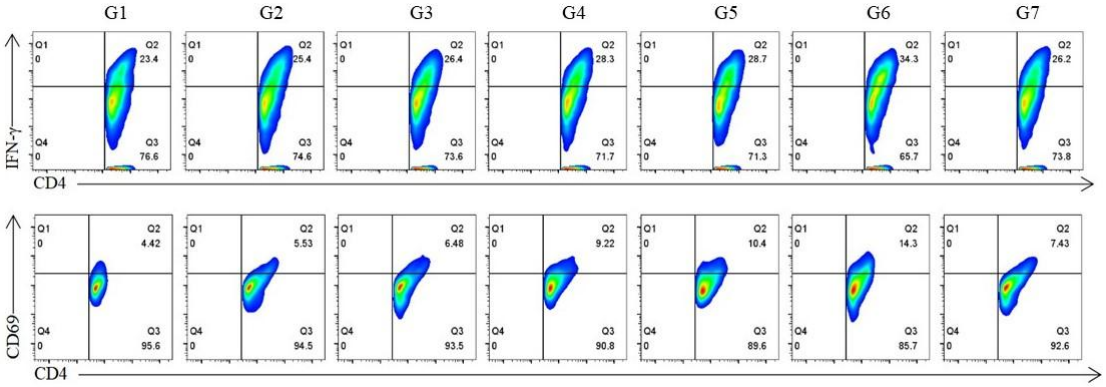

Figure S26. FACs plotting of IFN-γ^+CD4^+, $CD69^+CD4^+$ T cells in the tumor of tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

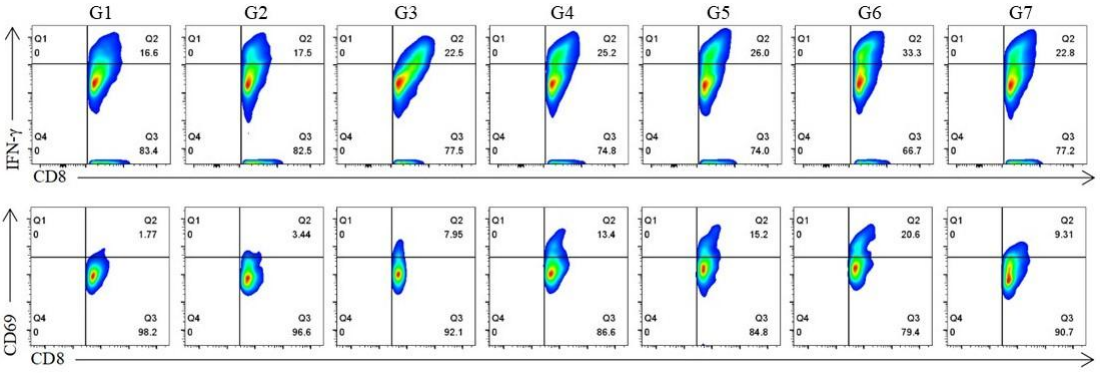

Figure S27. FACs plotting of IFN-γ^+CD8^+, $CD69^+CD8^+$ T cells in the tumor of

tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

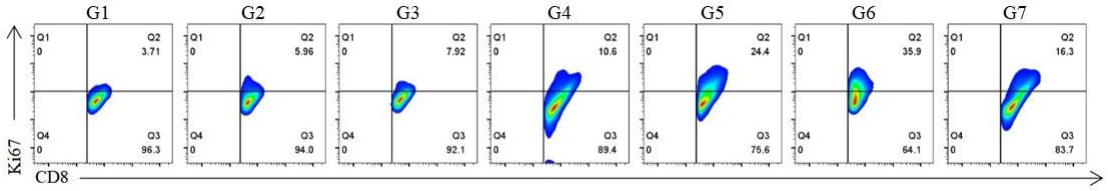

Figure S28. FACs plotting of $Ki67^{+}CD8^{+}$ T cells in the tumor of tumor-bearing mice. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

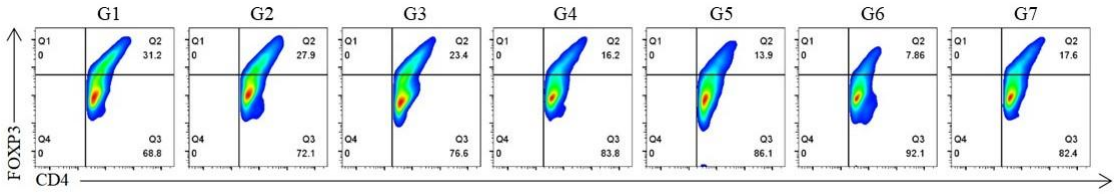

Figure S29. FACs plotting of $FOXP3^{+}CD4^{+}$ T cells in the tumor of tumor-bearing. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

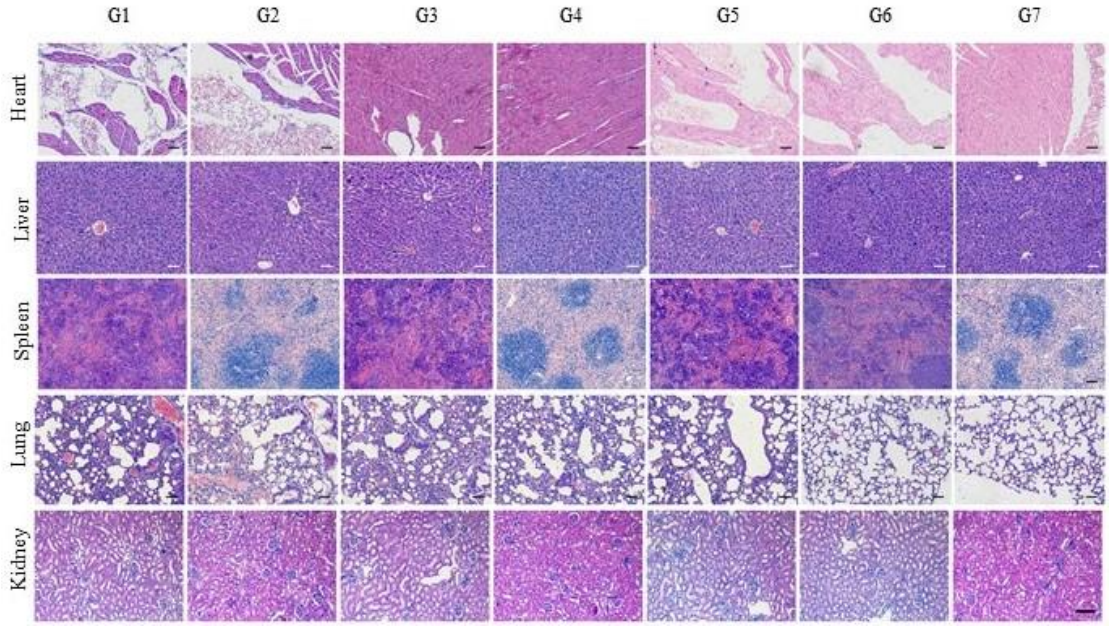

Figure S30. Main organs of heart, liver, spleen, lung, and kidney were collected from mice after treatment and stained with H&E staining for histological examination. (G1)

PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA.

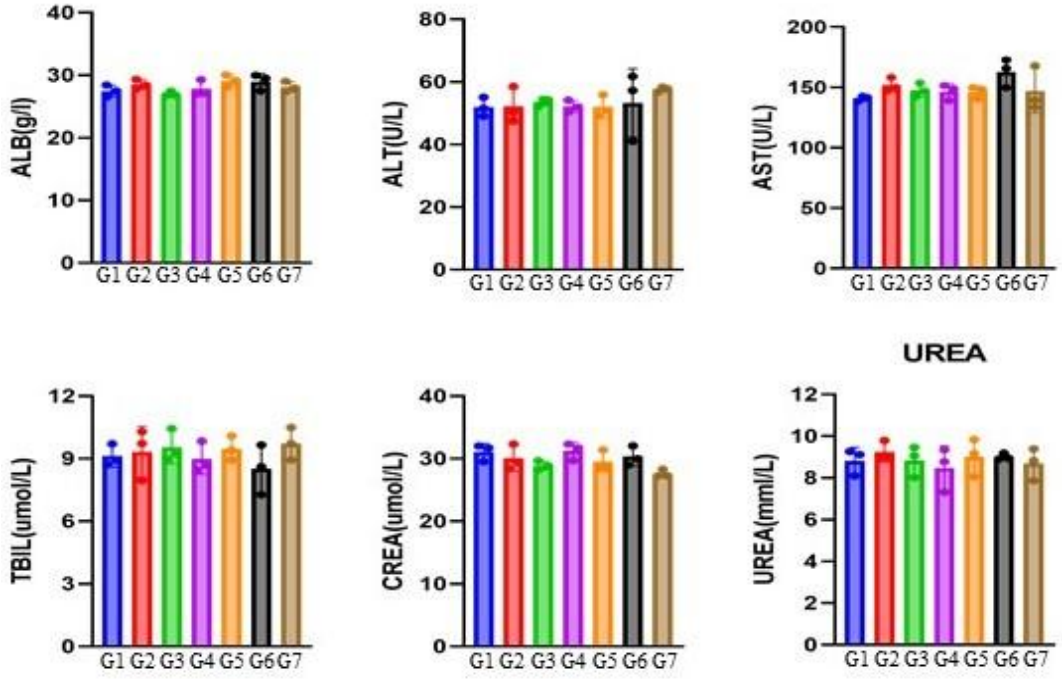

Figure S31. Serological biochemical analysis. Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; CREA, creatinine; UREA, urea. The reference ranges for ALB, ALT, AST, TBIL, CREA and UREA were 21.22-39.15g/L,10.06-96.47U/L,36.31-235.48U/L, 6.09-53.06 μmol/L, 10.91-85.09 μmol/L and 44.42-224.77 μmol/L, (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5) FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA (n = 3).

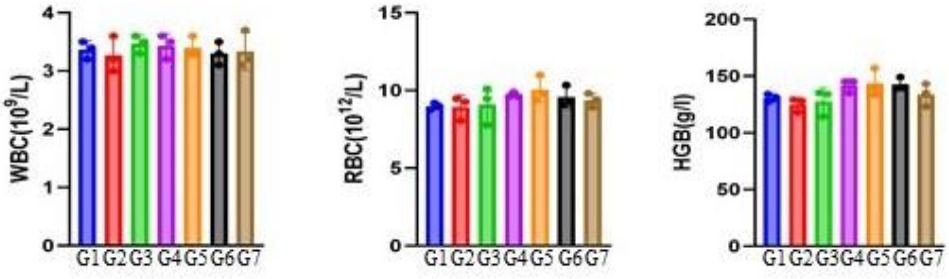

Figure S32. Blood routine examination. Abbreviations: WBC, white blood cell; RBC, red blood cell; and HGB, hemoglobin. The horizontal lines indicate the reference range for each analyte. The reference ranges for WBC, RBC and HGB were 0.8–10.6×10^9/L, 6.5–11.5×10^{12}/L and 110–165 g/L, respectively. (G1) PBS group, (G2) PNB-SIRPα siRNA, (G3) PFNB-NC siRNA, (G4) PFNB-SIRPα siRNA, (G5)

FA-PFNB-SIRPα siRNA, (G6) FA-PFNB-SIRPα siRNA + US, (G7) PNB-SIRPα siRNA+ PFNB-NC siRNA (n = 3).