

Targeted microRNA delivery by lipid nanoparticles and gas vesicles-assisted ultrasound cavitation to treat heart transplant rejection

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Table S1. The characterization of the GVs (mean \pm SD).

	mean diameter (nm)	zeta potential (mV)	PDI
GVs	174.80 \pm 2.20	-24.47 \pm 3.26	0.03 \pm 0.02

PDI, polydispersity index. n=5

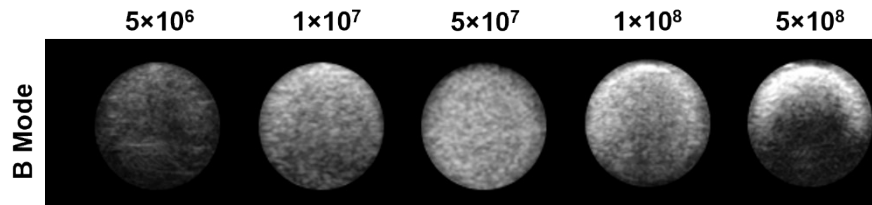


Figure S1. Ultrasound images of a phantom containing GVs at the concentration ranging from 5×10^6 / mL to 5×10^8 / mL.

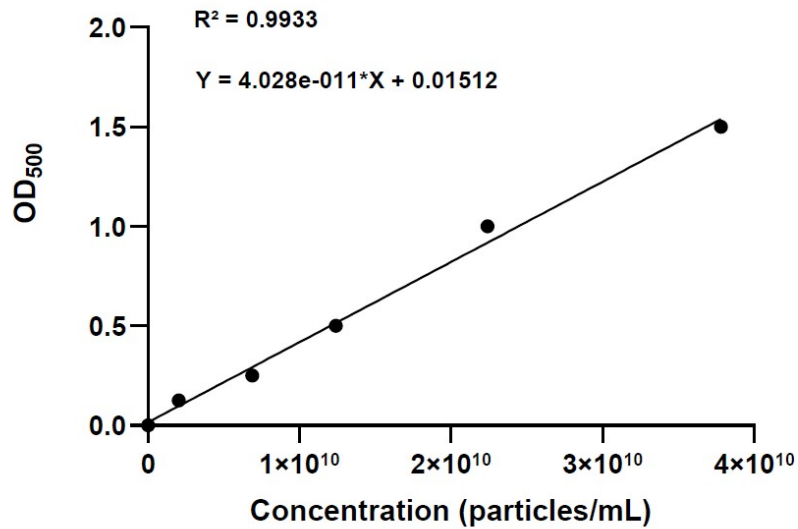


Figure S2. The relationship between concentration and OD₅₀₀ of GVs.

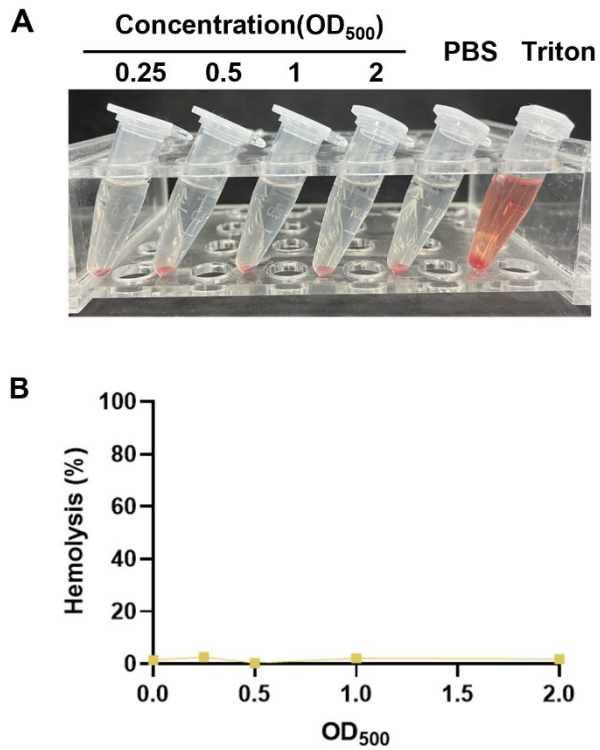


Figure S3. Photograph (A) and hemolysis percentage (B) of RBCs after treated with different concentrations of GVs, Triton and PBS was applied as positive and negative controls respectively.

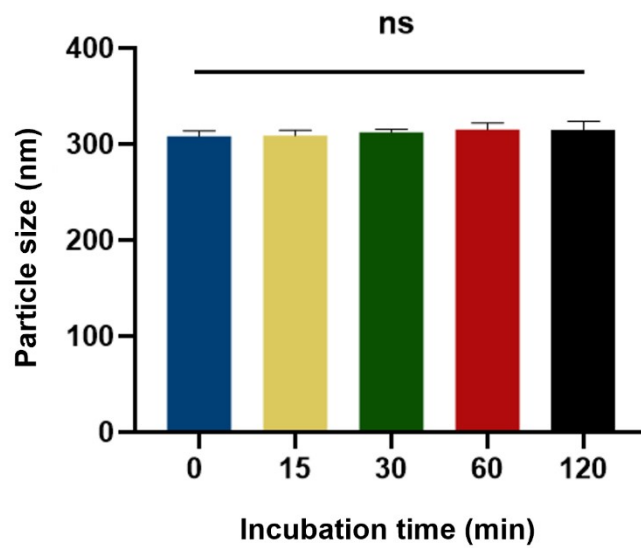


Figure S4. Diameter distributions of GVs exposed to serum for 0 min, 15min, 30 min, 60 min and 120 min. Data were presented as mean \pm SD. n = 3 per group.

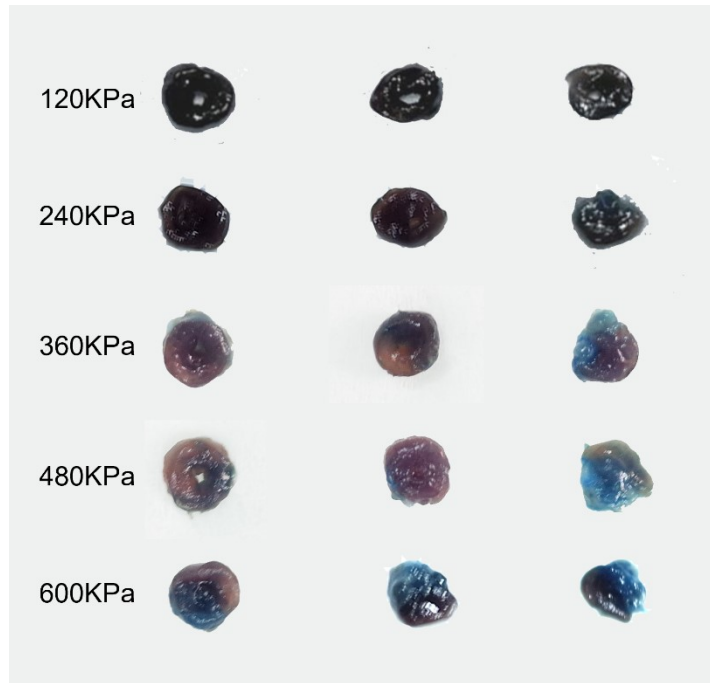


Figure S5. Representative photographs of the hearts and changes in EB extravasation. Exposure time = 2 min.

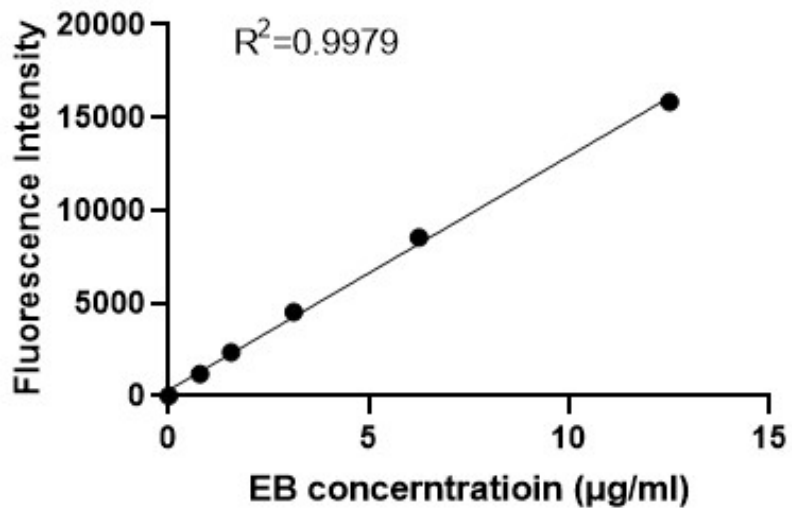


Figure S6. Standard curve of EB optical density at the wavelength of 620 nm.

Table S2. Hematology parameters in whole blood of HT mice following the treatment of LIPUS for 24 h. All data represent mean \pm SD, n = 3.

Hematologic Parameters	Isograft	Reference
White blood cell ($10^9/L$)	1.53 ± 0.45	0.8-6.8
Lymphocytes ($10^9/L$)	0.83 ± 0.37	0.7-5.7
Monocytes ($10^9/L$)	0.10 ± 0.00	0.0-0.3
Neutrophil($10^9/L$)	0.6 ± 0.08	0.1-1.8
Lymphocytes (%)	69.43 ± 9.55	55.8-90.6
Red blood cell ($10^{12}/L$)	6.63 ± 0.84	6.36-9.42
Hemoglobin (g/L)	123.00 ± 11.23	110-143
Hematocrit (%)	35.13 ± 1.90	34.6-44.6
Mean corpuscular volume (fl)	51.50 ± 1.50	48.2-58.3
Mean corpuscular hemoglobin (pg)	15.97 ± 0.12	15.8-19
Mean corpuscular hemoglobin concentration (g/L)	324.00 ± 6.53	302-353
Red blood cell distribution width (%)	16.7 ± 0.45	13-17

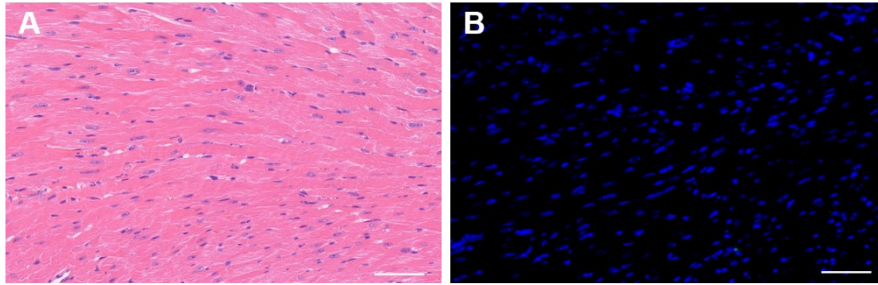


Figure S7. Typical H&E staining image (A) and TUNEL imaging (B) of graft heart after treated with LIPUS. There were almost no TUNEL positive (green) nuclei. Scale bar: 50 μ m.

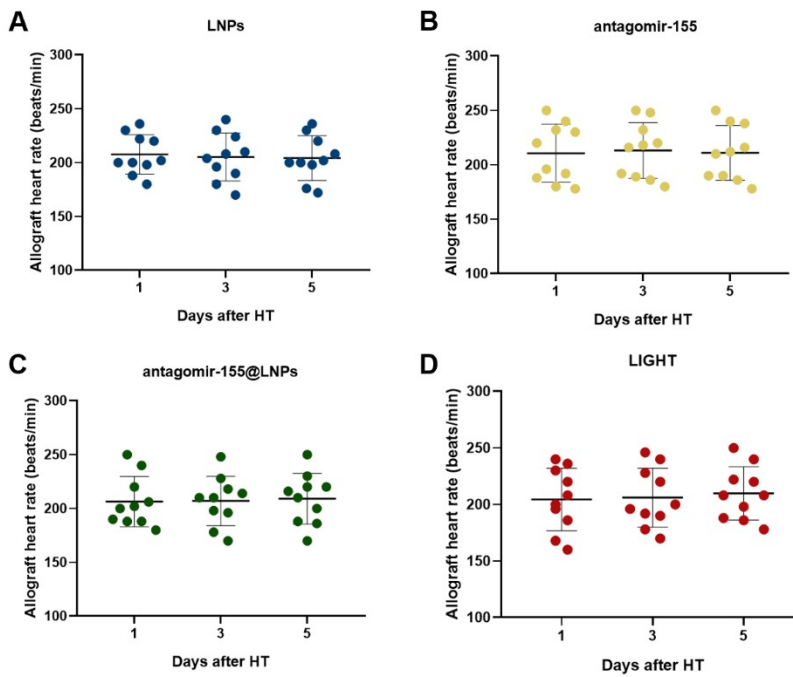


Figure S8. Heart rate of mice before each treatment at 1th, 3th, 5th day after HT, each mouse was measured twice. There was no significant change in each group over time. $p > 0.05$. $n = 5$ per group for all analyses. One way ANOVA test was used.



Figure S9. The prediction results of miR-155 target genes in the miRDB, TargetScan, PicTar and miRmap databases: the four irregular graphs in the image represented the gene prediction results of the four databases, and the middle part represented the intersection of four database prediction results (Socs1, Astn2, Jarid2, Tspan14, Picalm, Zic3, Hivep2, Csnk1g2, Tbr1, Myb, Rps6ka5, Acta1).

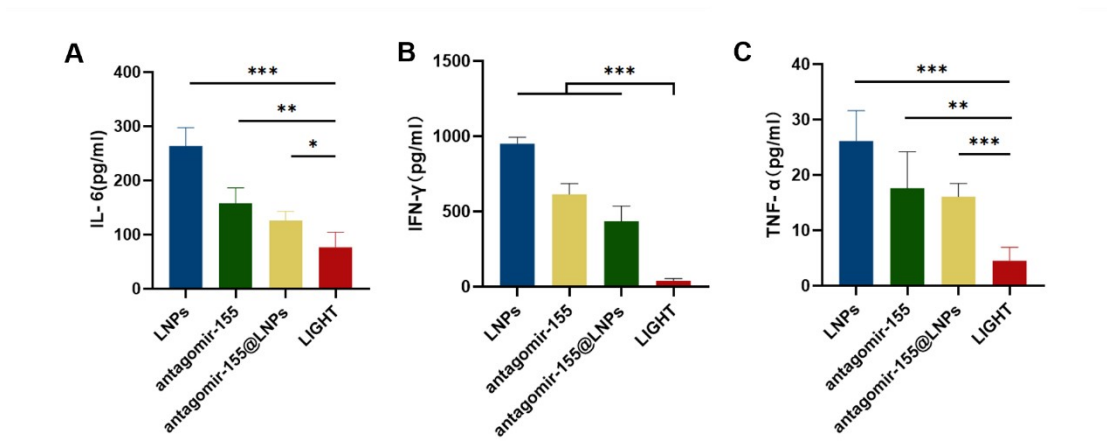


Figure S10. Changes in inflammatory cytokines (IL-6, TNF- α , and IFN- γ) expression at the protein levels analyzed by ELISA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. $n = 4$ per group for all analyses. One-way ANOVA test was used.

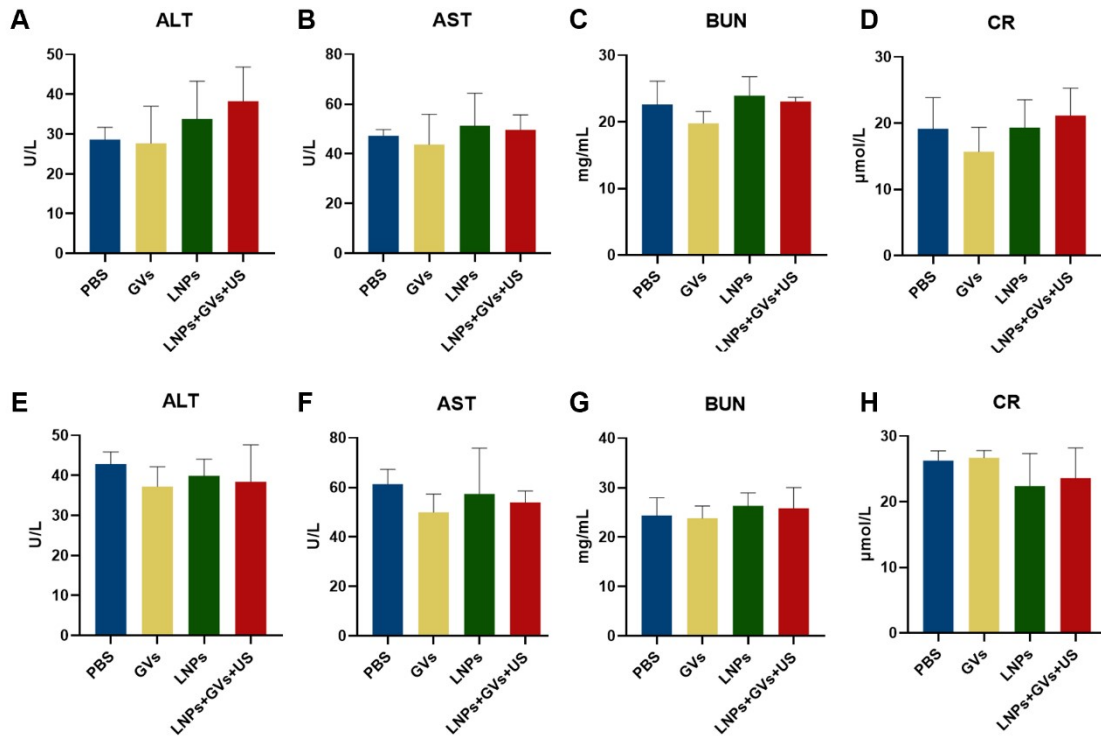


Figure S11. *In vivo* liver and kidney functional markers examined in the peripheral blood of mice treated with PBS, GV, LNP, and LNP + GV + US. Serum levels of ALT (A), AST (B), BUN (C) and CR (D) 1 day and 7 days (E-H) after treatment. Data were presented as mean \pm SD. $n = 5$ per group.

Table S3. Hematology parameters in whole blood of healthy mice following the treatment of PBS, GV, LNP and LNP + GV + US for 7 days. All bars represent mean \pm SD ($n = 6$).

Hematologic Parameters	PBS	GVs	LNPs	LNPs + GVs + US	Reference
White blood cell (10 ⁹ /L)	2.37± 0.26	2.96 ± 1.00	5.53 ± 2.19	5.50 ± 0.69	0.8-6.8
Lymphocytes (10 ⁹ /L)	1.67± 0.28	2.3 ± 0.93	4.63 ± 1.84	4.23 ± 0.72	0.7-5.7
Monocytes (10 ⁹ /L)	0.08 ± 0.04	0.08 ± 0.04	0.15 ± 0.07	0.18 ± 0.07	0.0-0.3
Neutrophil(10 ⁹ /L)	0.63 ± 0.17	0.58 ± 0.16	0.75 ± 0.38	1.08 ± 0.40	0.1-1.8
Lymphocytes (%)	68.65± 8.12	75.6 ± 5.80	81.3 ± 5.69	83.03 ± 3.64	55.8-90.6
Red blood cell (10 ¹² /L)	8.80 ± 0.51	8.14 ± 0.45	7.68 ± 0.65	8.50 ± 0.54	6.36-9.42
Hemoglobin (g/L)	131.50 ± 6.70	126.5 ± 6.99	120.5 ± 12.04	122.17 ± 7.40	110-143
Hematocrit (%)	40.60 ± 3.02	37.78± 2.12	37.5 ± 2.97	41.33 ± 2.60	34.6-44.6
Mean corpuscular volume (fl)	49.15 ± 0.82	49.45 ± 0.66	48.93 ± 0.44	48.7 ± 0.96	48.2-58.3
Mean corpuscular hemoglobin (pg)	15.90 ± 0.28	16.5 ± 0.57	15.17 ± 0.65	16.3 ± 0.23	15.8-19
Mean corpuscular hemoglobin concentration (g/L)	324.00 ± 8.96	334.5 ± 9.07	313.33 ± 14.18	315.00 ± 5.80	302-353
Red blood cell distribution width (%)	16.73 ± 0.27	16.28 ± 0.49	16.17 ± 0.45	16.25 ± 0.20	13-17

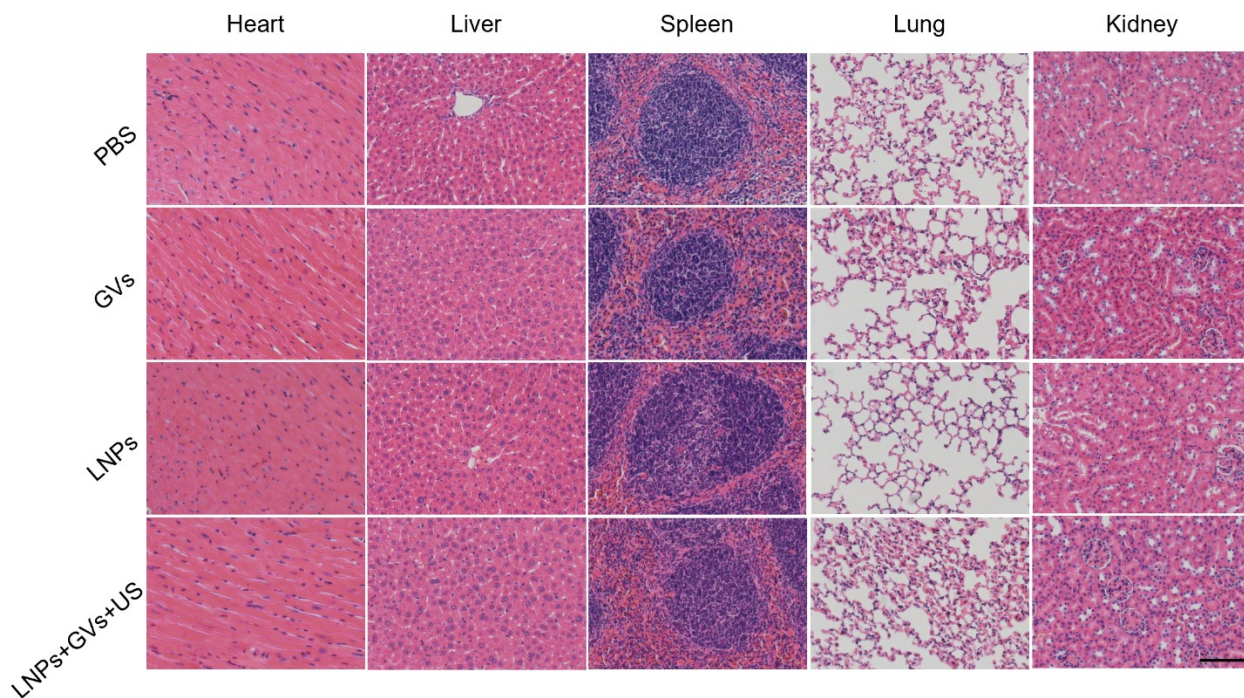


Figure S12. *In vivo* toxicity of LIGHT platform. Healthy mice were treated with PBS, GV_s, LNPs, or LNPs + GV_s + US, respectively. 7 days after treatment, H&E staining images of major organs including heart, liver, spleen, lung, and kidney were acquired. Scale bar: 100 μ m.

Table S4. The premier sequences used for RT-PCR.

Gene	Forward	Reverse
β -actin	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
mmu-miR-155-5p	ACACTCCAGCTGGGTAAATGCT	CTCAACTGGTGTCGTGGAGTCG
IL-6	AATTGTGAT ACACATGTTCTCTGGGAAATCG	GCAATTCAGTTGAGACCCCTAT AAGTGCATCATCGTTGTTTCATC
TNF- α	ACTGGCAGAAGAGGCACTC	CTGGCACCACTAGTTGGTTG

IFN- γ	GGATATCTGGAGCTGGCAA	TGACCTCAAACCTTGGCAATACT C
iNOS	AGCTCGGGTTGAAGTGGTATG	CACAGCCACATTGATCTCCG
IL-1 β	GTAATGAAAGACGGCACACCC	CAGGCTTGTGCTCTGCTTGTG
IL-10	AATAAGCTCCAAGACCAAGGT GT	CATCATGTATGCTTCTATGCAG TTG
Arg1	GGTGGCAGAGGTCCAGAAGAA	CCCATGCAGATTCCCAGAGC
SOCS1	CGAGACCTTCGACTGCCTTT	GGAGTACCGGGTTAAGAGGGA
