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**Electronic Supplementary Information (ESI)**

**Improving Acute Cardiac Transplantation Rejection Therapy by Ultrasound-Targeted FK506-Loaded Microbubbles in Rats**

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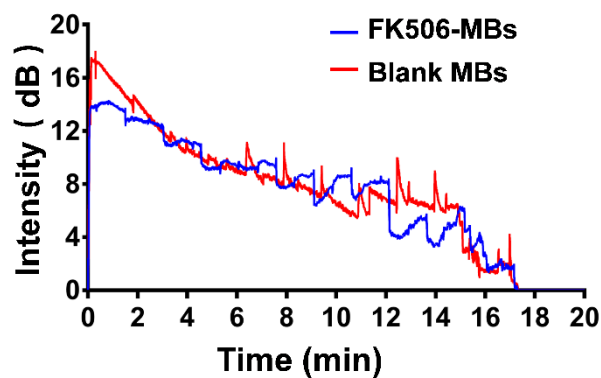
## Supplementary information

### S1. *In vivo* stability of FK506-MBs

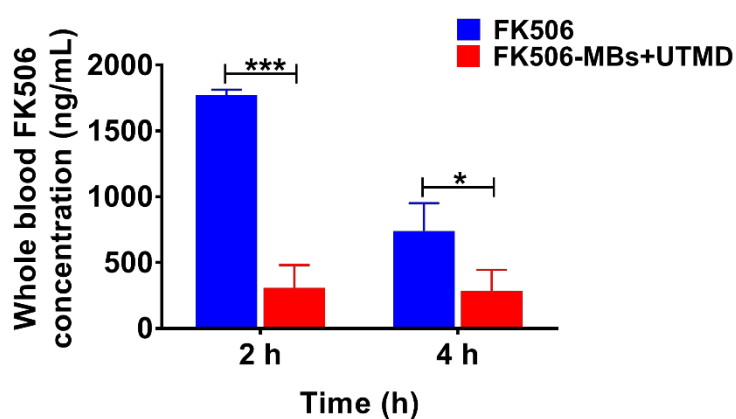
We evaluated the lifetime of the FK506-MBs *in vivo* by evaluating their echo intensity in heart tissues, using a commercial ultrasound system IU22 (Philips Medical Systems, Amsterdam, Netherlands) using a L12-5 linear array transducer. FK506-MBs and blank MBs were diluted to the same concentration ( $5 \times 10^7$  MBs/mL) with 0.9% normal saline. After separately bolus injection of  $5 \times 10^7$  MBs via the tail vein, the time–intensity curve (TIC) was obtained. The lifetime of the FK506-MBs and blank MBs *in vivo* was then estimated by the clearance time. All data were analysed with Q-Lab software (Philips Medical Systems, Amsterdam, Netherlands).

### S2. Safety evaluation of MBs and UTMD

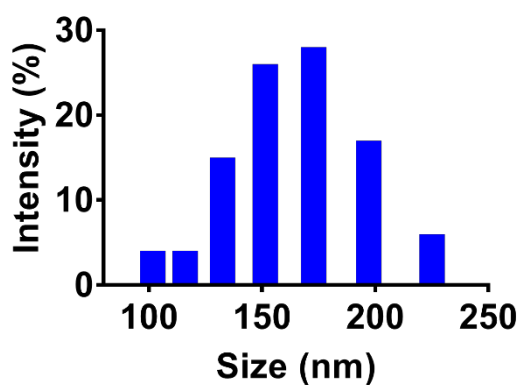
To further illustrate the safety of MBs and UTMD, a total of 27 rats were randomly divided into three groups: (1) PBS group: rats were only injected with PBS; (2) MBs group: rats were only injected with  $5 \times 10^8$  MBs; (3) UTMD group: rats were injected with  $5 \times 10^8$  MBs and combined with ultrasound, the ultrasound probe (Sonitron 2000 V, Japan) was placed at the heart. The parameters of UTMD were ultrasound frequency = 1 MHz; duty cycle = 50%; ultrasound intensity =  $2 \text{ w/cm}^2$ ; irradiation time = 2 min. Blood samples and major organs of three rats in each group were collected after treatment 30 min, 1 day and 7 days. Serum biochemical indexes including lactic dehydrogenase 1 (LDH1), creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr) and blood urea nitrogen (BUN) and complete blood count were measured and analysed. Additionally, major organs were HE stained.



**Fig. S1.** *In vivo* lifetime of FK506-MBs and blank MBs measured from backscattered ultrasound intensity within heart.



**Fig. S2.** The concentration of FK506 in whole blood were evaluated at 2 h and 4 h after administration. ( $n = 6$ ),  $*P < 0.05$ ,  $***P < 0.001$ .



**Fig. S3.** Size distribution of FK506-MBs after UTMD.



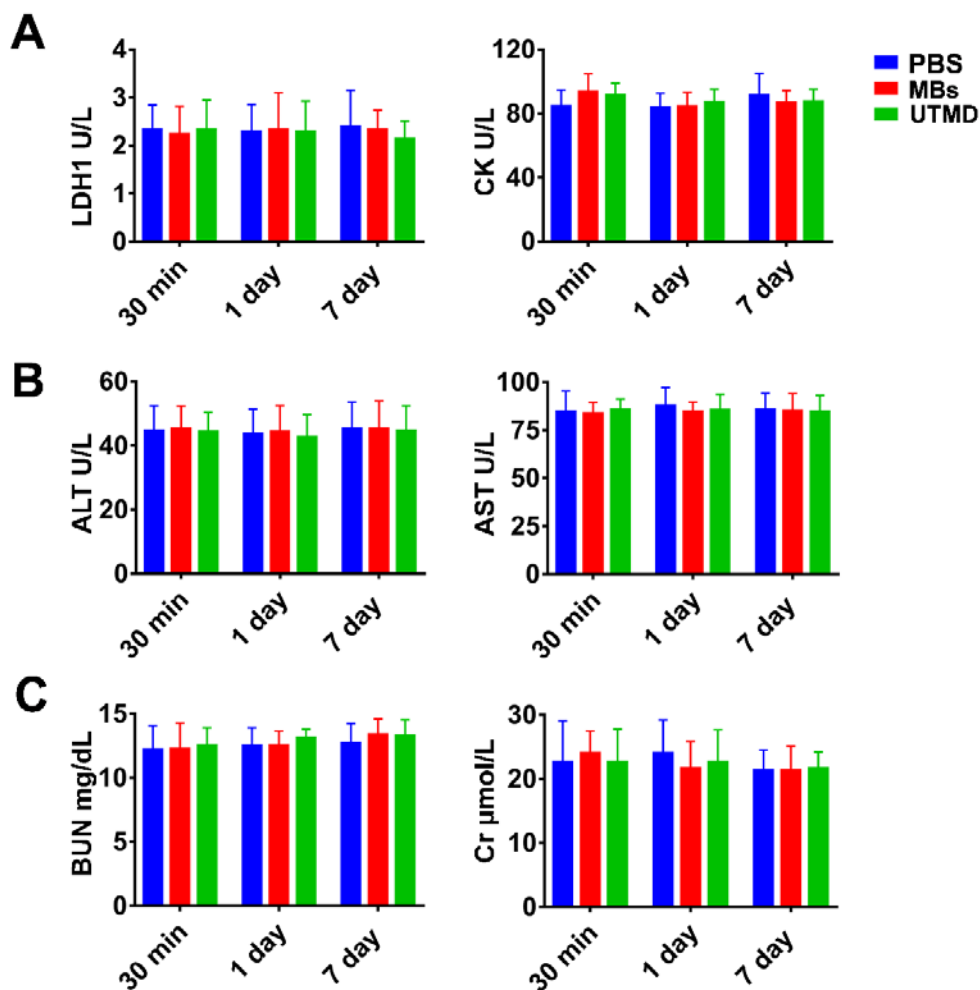
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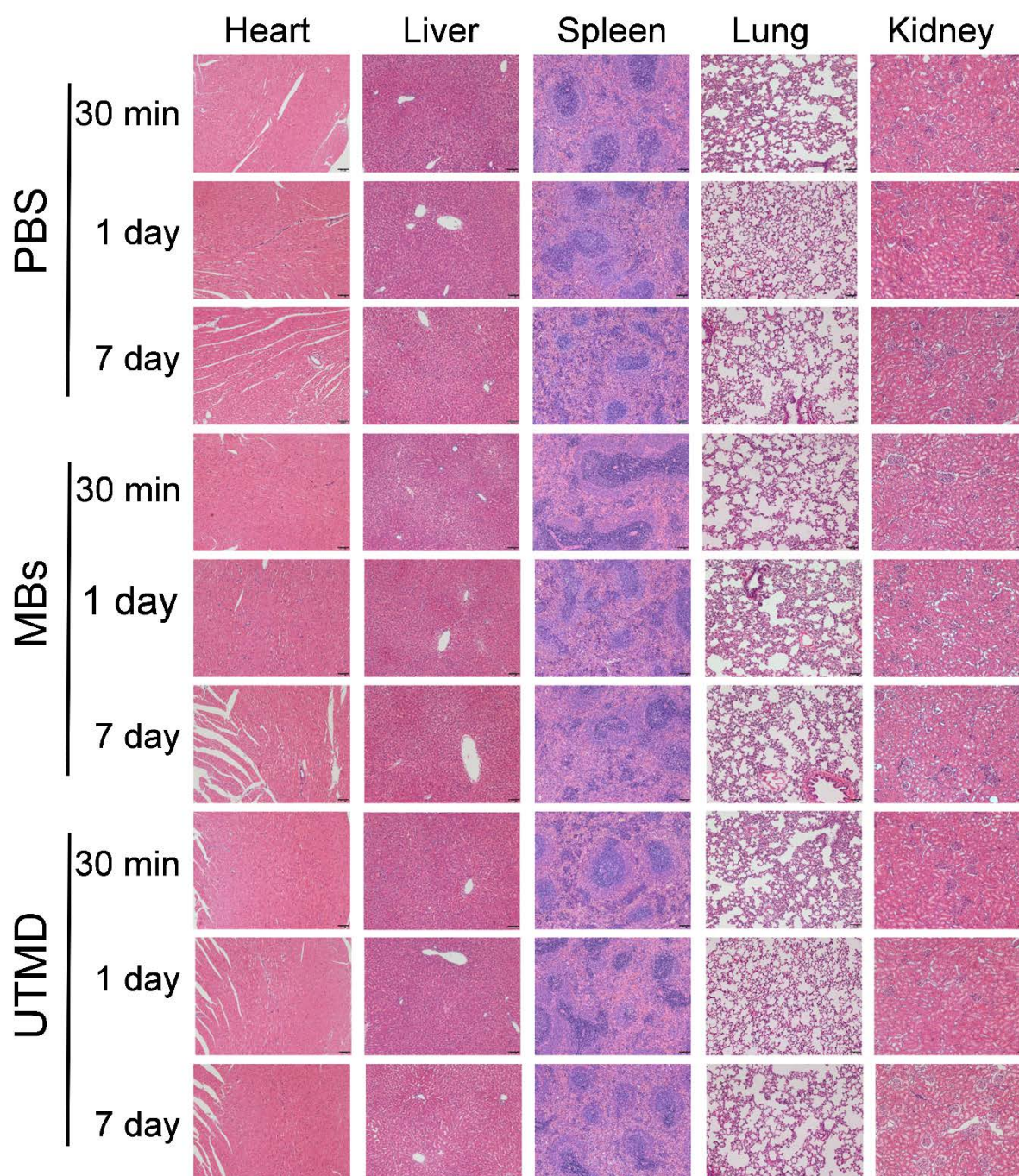
**Table. S1.** Complete blood count analysis (mean  $\pm$  SEM) (n = 3)

	PBS			MB			UTMD		
	30 min	1 day	7 day	30 min	1 day	7 day	30 min	1 day	7day
WBC ( $10^9/L$ )	6.63 $\pm$ 0.21	6.43 $\pm$ 0.21	6.43 $\pm$ 0.20	6.36 $\pm$ 0.17	6.56 $\pm$ 0.18	6.66 $\pm$ 0.12	6.7 $\pm$ 0.11	6.63 $\pm$ 0.08	6.66 $\pm$ 0.14
Lymphocyte ( $10^9/L$ )	4.80 $\pm$ 0.37	4.80 $\pm$ 0.20	4.86 $\pm$ 0.46	4.76 $\pm$ 0.29	5.00 $\pm$ 0.05	4.76 $\pm$ 0.16	4.73 $\pm$ 0.14	4.90 $\pm$ 0.15	4.70 $\pm$ 0.17
Monocyte ( $10^9/L$ )	0.2 $\pm$ 0.01	0.21 $\pm$ 0.02	0.22 $\pm$ 0.01	0.196 $\pm$ 0.01	0.19 $\pm$ 0.01	0.19 $\pm$ 0.01	0.18 $\pm$ 0.01	0.21 $\pm$ 0.01	0.20 $\pm$ 0.02
Neutrophils ( $10^9/L$ )	1.8 $\pm$ 0.37	1.83 $\pm$ 0.31	1.9 $\pm$ 0.35	2.26 $\pm$ 0.31	2.30 $\pm$ 0.25	2.03 $\pm$ 0.31	2.33 $\pm$ 0.29	1.93 $\pm$ 0.29	2.10 $\pm$ 0.26
RBC ( $10^{12}/L$ )	5.67 $\pm$ 0.26	5.79 $\pm$ 0.43	5.84 $\pm$ 0.29	5.71 $\pm$ 0.34	5.72 $\pm$ 0.35	5.77 $\pm$ 0.29	5.84 $\pm$ 0.19	5.67 $\pm$ 0.35	5.89 $\pm$ 0.32
PLT ( $10^9/L$ )	1232 $\pm$ 140.5	1297 $\pm$ 89.87	1263 $\pm$ 109.7	1341 $\pm$ 98.97	1269 $\pm$ 83.73	1300 $\pm$ 118.4	1410 $\pm$ 77	1390 $\pm$ 98	1352 $\pm$ 132

WBC, white blood cell; RBC, red blood cell; PLT, platelets



**Fig. S4.** Serum biochemical indexes analysis. (A) Serum biochemical indexes of LDH1 and CK. (B) Serum biochemical indexes of ALT and AST. (C) Serum biochemical indexes of BUN and Cr.



**Fig. S5.** Representative H&E stained sections of major organs obtained at 30 min, day 1 and day 7 after injection of PBS, MBs and treatment with UTMD respectively. Scale bar = 100  $\mu$ m.