

## **Supplementary Materials**

### **Methods**

#### **Characterization of Microbubbles (MBs).**

MB-CON and MB-cRGD were characterized with a Coulter counter to determine their size distribution and concentration. And the *in vivo* half-life of the MBs were measured as described in a previous study [1].

#### **Cell culture and treatments.**

A human umbilical vein endothelial cell (HUVEC) line, (American Type Culture Collection, Manassas, VA, USA) was maintained in Dulbecco's modified Eagle's Medium (DMEM, Gibco/Life Technologies, Carlsbad, CA, USA) containing 10% fetal calf serum and a mixture of glutamine, penicillin, and streptomycin at 37 °C in a 5% CO<sub>2</sub> humidified incubator. To measure the effect of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) upon von willebrand factor (vWF) expression, HUVECs were washed and incubated in serum-free DMEM for 10h and then stimulated with various concentrations of recombinant TNF- $\alpha$  recombinant protein (ProSpec, East Brunswick, NJ, USA) for 1h, and vWF expression on HUVECs was measured by immunofluorescence labeling.

#### **Preparation of human platelets.**

Human venous blood was obtained from healthy adult volunteers in accordance with a protocol approved by the Southern Medical University Institutional Review Board.

The volunteers did not take any drugs in the 10 days prior to blood collection. Platelets were isolated as previously described [2-4]. Briefly, blood was drawn into syringes containing 1/10th of the final blood volume of sodium citrate anticoagulant (Sigma-Aldrich, St. Louis, MO, USA). The anti-coagulated blood was centrifuged at 200×g for 10 min to obtain platelet-rich plasma (PRP). Platelets were purified from PRP by centrifugation at 1000×g in the presence of prostacyclin (0.1 µg/ml) and washed twice with 10 ml of modified HEPES/Tyrode buffer (129 mM NaCl, 0.34 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.9 mM KCl, 12 mM NaHCO<sub>3</sub>, 20 mM HEPES, 5mM glucose, 1 mM MgCl<sub>2</sub>; pH 7.3) containing 0.1µg/ml prostacyclin. To selectively pellet erythrocytes, platelets were resuspended in the same buffer and centrifuged twice at 120×g for 3 min. Erythrocytes content was 1% as counted using a hemocytometer. The purified platelets were resuspended in the medium described above containing prostacyclin (0.1 µg/ml) and incubated at 37 °C and 5% CO<sub>2</sub>. To measure the effect of vWF on GP IIb/IIIa expression, platelets were washed and incubated in serum-free DMEM for 6 h and then stimulated with various concentrations of recombinant vWF (H00007450-P01; Abnova, Taipei, Taiwan) for 1h, and GP IIb/IIIa expression on platelets was measured by immunofluorescence labeling.

### **Immunocytochemistry.**

Cultured cells or platelets were rinsed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde for 40 min, and then permeabilized with 0.1% Triton X-100 for 5 min at room temperature. They were then blocked with 5% bovine serum

albumin in PBS before incubation with rabbit polyclonal primary antibodies against vWF or GP IIb integrin (ab6994 and ab63983, respectively; Abcam, Cambridge, MA, USA) overnight at 4 °C. The negative control was incubated with PBS instead of primary antibody. After washing with PBS, cells or platelets were incubated with the appropriate Alex Fluor 488-conjugated secondary antibody (Invitrogen) for 60 min in the dark at room temperature. After washing, cell nuclei were counterstained with DAPI. Samples were imaged using a confocal laser microscope (Olympus, Tokyo, Japan). The intensity of vWF and GP IIb/IIIa fluorescence in several randomly selected visual fields was analyzed using Image-Pro Plus software. The average fluorescence intensity was calculated from 40 cells of each sample as previously described [5].

### **Histological analysis of the effect of sonication on tissue structure.**

ApoE<sup>-/-</sup>+HCD mice divided into UMI and control groups (n=6, respectively), and abdominal aorta samples were obtained 1 h after UMI. Hematoxylin and eosin staining was carried out to visualize inflammatory cells. Microvessels were labeled by immunohistochemistry using rabbit polyclonal primary antibodies against CD31 (ab28364, Abcam) and extravasated red blood cells (RBCs) were labeled with purified rat anti-mouse TER-119 (550565, Becton, Dickinson and Company, USA).

## **Results**

### **MB characterization.**

The mean diameter of MB-CON and MB-cRGD was approximately 2.5  $\mu\text{m}$ , and the mean concentrations were approximately  $1.1 \times 10^9$  MBs per ml (Table S1). There were no significant differences in the mean diameter or concentration ( $P>0.05$ ) and the half-life of the two MBs was comparable ( $4.17 \pm 0.61$  vs.  $3.80 \pm 0.58$  min).

### **Effect of sonication on tissue structure**

The images of abdominal aortic plaques by hematoxylin-eosin, immunohistochemistry stainings of TER-119 and CD31 in the UMI and control groups were demonstrated in Fig. S1. As compared to the control group, we did not find a significant increase in inflammatory cells and extravasated RBCs in atherosclerotic plaque, and a visual damage in microvessel construction in the UMI group, indicating that sonication with a mechanical index of 1.0 during UMI does not compromise tissue and/or microvessel structures.

### **Reference**

1. Chen CC, Sirsi SR, Homma S, et al. Effect of surface architecture on in vivo ultrasound contrast persistence of targeted size-selected microbubbles. *Ultrasound Med Biol.* 2012; 38: 492-503.
2. Chhatriwala M, Ravi RG, Patel RI, et al. Induction of novel agonist selectivity for the ADP-activated P2Y1 receptor versus the ADP-activated P2Y12 and P2Y13 receptors by conformational constraint of an ADP analog. *J Pharmacol Exp Ther.* 2004; 311: 1038-43.

3. Aslan JE, Tormoen GW, Loren CP, et al. S6K1 and mTOR regulate Rac1-driven platelet activation and aggregation. *Blood*. 2011; 118: 3129-36.
4. Fong KP, Barry C, Tran AN, et al. Deciphering the human platelet sheddome. *Blood*. 2011; 117: e15-26.
5. Zhang F, Wang C, Wang H, et al. Ox-LDL promotes migration and adhesion of bone marrow-derived mesenchymal stem cells via regulation of MCP-1 expression. *Mediators Inflamm*. 2013; 2013: 691023.

## **Figure legends**

**Figure S1: Inflammatory cells, extravasated RBCs, and microvessels in abdominal aortic plaques in UMI and control groups.**

Representative images of abdominal aortic tissue stained with hematoxylin and eosin and immunolabeled for TER-119 and CD31 expression.

**Figure S2: UMI before and after the destruction of MBs in ApoE<sup>-/-</sup>+HCD mice.**

Non-color-coded images of UMI before (A) and after (B) the destruction of MB-cRGD in ApoE<sup>-/-</sup>+HCD mice.

Table S1: The mean diameter, concentration and *in vivo* half-life of MB-CON and MB-cRGD, respectively ( $\bar{x} \pm s$ , N=6).

	<b>MB-CON</b>	<b>MB-cRGD</b>
Mean Diameter(um)	$2.54 \pm 0.30$	$2.52 \pm 0.28$
Concentration( $\times 10^9$ microbubbles/mL)	$1.12 \pm 0.22$	$1.13 \pm 0.19$
Half-life (min)	$4.17 \pm 0.61$	$3.80 \pm 0.58$

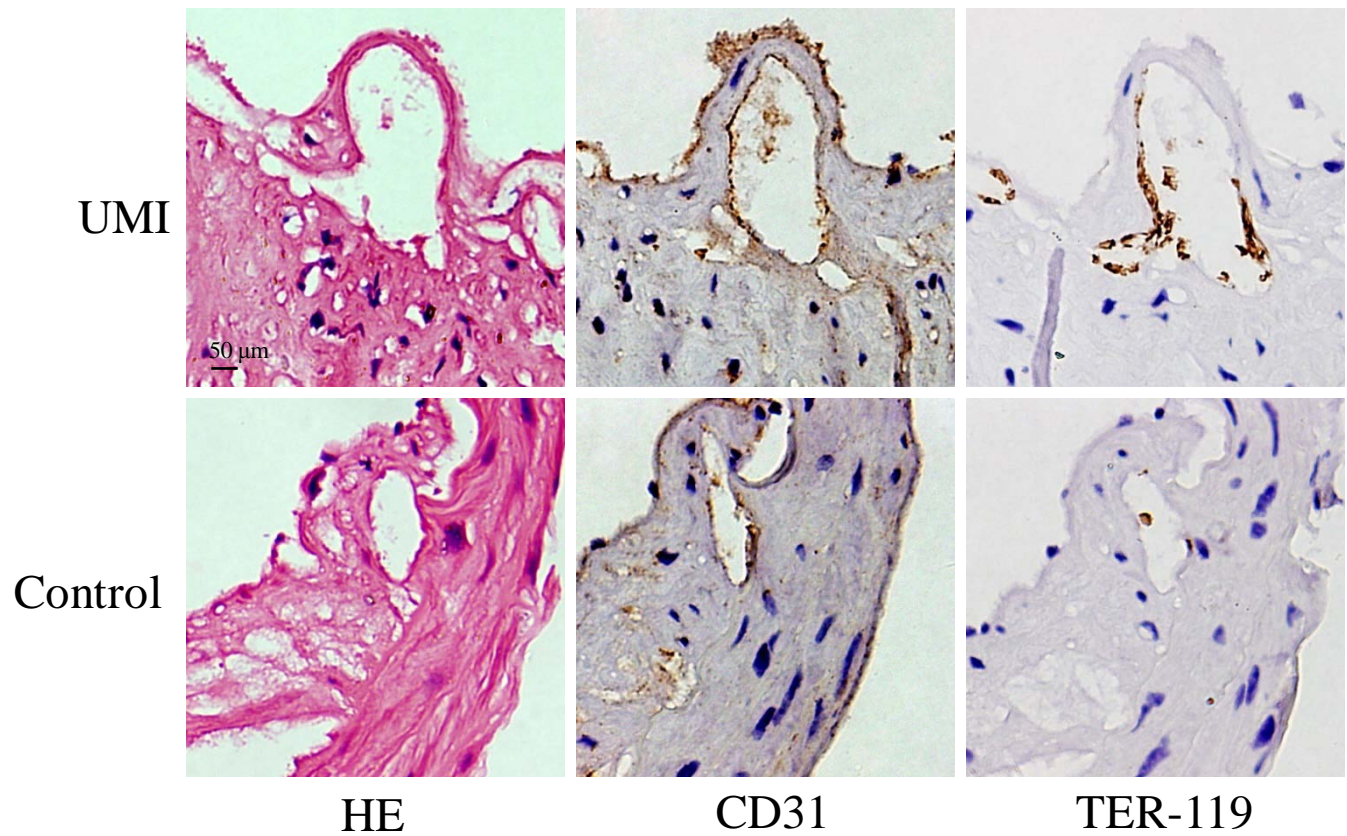


Figure S1

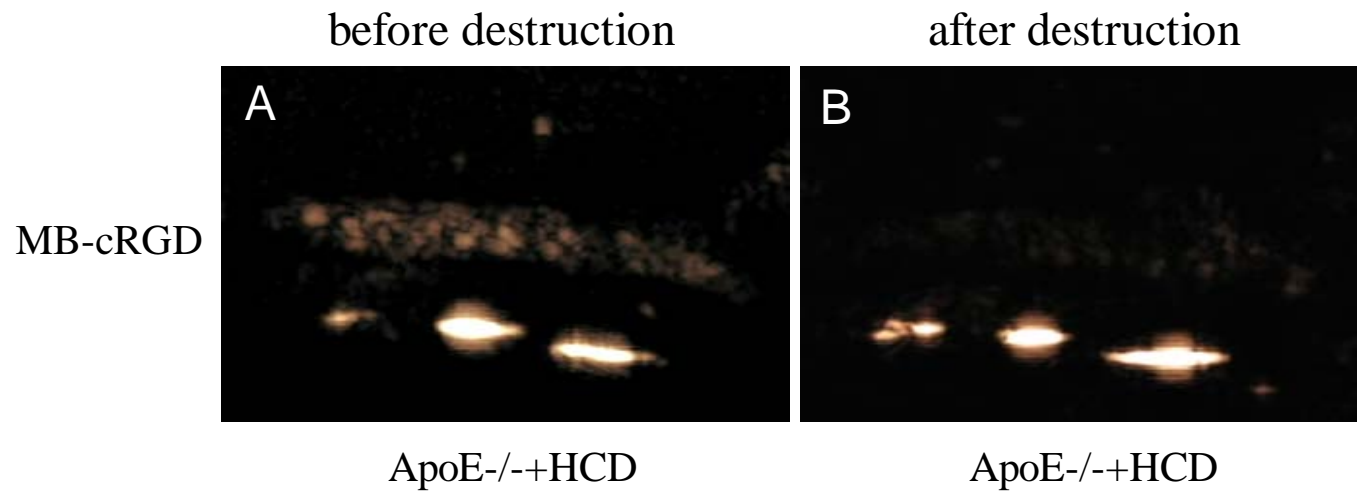


Figure S2