

## Supporting information

# Localized Delivery of shRNA against PHD2 Protects the Heart from Acute Myocardial Infarction through Ultrasound-Targeted Cationic Microbubble Destruction

Li Zhang<sup>1,\*</sup>, Zhenxing Sun<sup>1,\*</sup>, Pingping Ren<sup>1</sup>, Manjie You<sup>1</sup>, Jing Zhang<sup>1</sup>, Lingyun Fang<sup>1</sup>, Jing Wang<sup>1</sup>, Yihan Chen<sup>1</sup>, Fei Yan<sup>2,✉</sup>, Hairong Zheng<sup>2,✉</sup>, Mingxing Xie<sup>1,✉</sup>

<sup>1</sup> Department of Ultrasound, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei Province Key Laboratory of Molecular Imaging, Wuhan, China

<sup>2</sup> Paul C. Lauterbur Research Center for Biomedical Imaging, Institute of biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

✉ Corresponding Authors:

Mingxing Xie, MD, 1277 Jiefang Avenue, Wuhan 430022, China. E-mail:

[xiemx64@126.com](mailto:xiemx64@126.com); Telephone: +86 27 85726386

Fei Yan and Hairong Zheng, PhD, 1068 Xueyuan Avenue, Shenzhen University Town, Shenzhen 518055, China. E-mails: [fei.yan@siat.ac.cn](mailto:fei.yan@siat.ac.cn) and [hr.zheng@siat.ac.cn](mailto:hr.zheng@siat.ac.cn);

Telephone: +86 755 86392284, Fax: +86 755 96382299.

\*Both authors contributed equally to this manuscript.

## **Supplementary methods:**

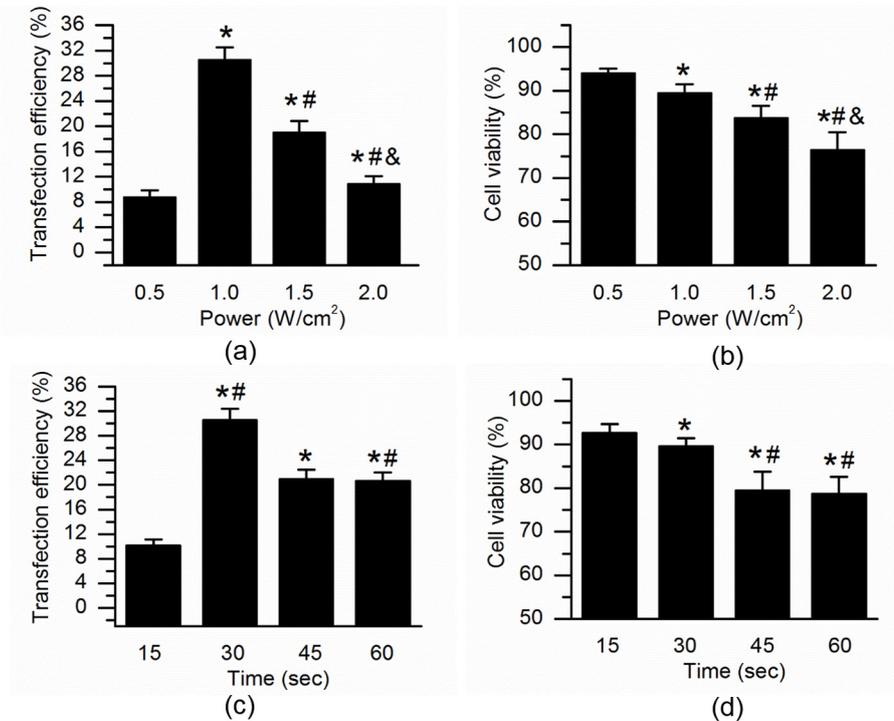
### **S1. Ultrasound-mediated gene transfection with CMBs**

The H9C2 cardiac cells incubated with CMB/DNA complexes were exposed to different ultrasound intensities (1 MHz; duty cycle, 20%; power, 1.0, 1.5 and 2.0 W/cm<sup>2</sup>; duration, 15, 30 and 60 seconds) to identify the optimal transfection parameters for gene delivery. After gene transfection, the H9C2 cells were cultured in a medium containing 10% FBS for further 48 hours and the gene transfection was demonstrated using a FACS caliber flow cytometer (EPICS XL; Beckman Coulter Inc., Fullerton, CA, USA) with 20,000 events per sample and fluorescent microscopy. Cell viability was measured at 48 h after the transfection, using Cell Counting Kit-8 (CCK-8) according to the manufacture's protocol (Dojindo, Japan).

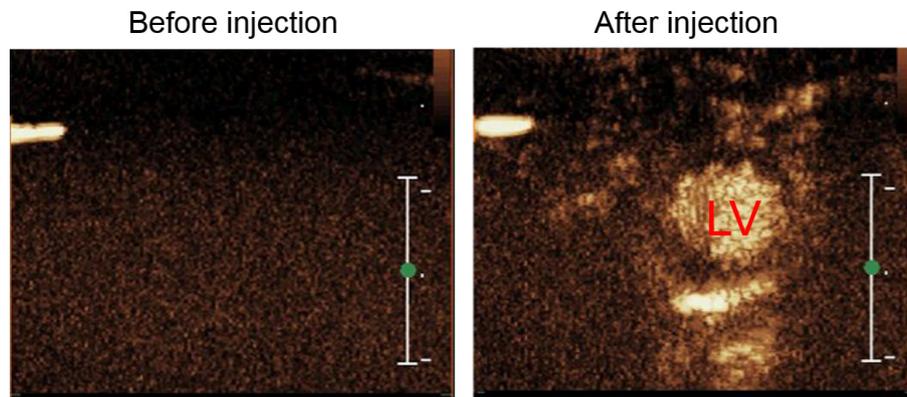
### **S2. Ultrasound contrast imaging**

Ultrasound contrast imaging was performed in vivo on 14 days following gene transfection to assess the infarcted myocardial perfusion. Briefly, the skin in front heart area was shaved before imaging session. After rat were anesthetized, the short axis view at the midpapillary muscle level was acquired to observe the contrast agent perfusion and washout duration. To assess the imaging capability of CMB/DNA complexes, cardiac imaging in normal rats were observed before and after enhanced ultrasonography. To demonstrate that microvascular flow following inhibition of PHD2, thirty rats were divided into three groups: (i) Sham (rat chest opened/closed without ligation, untreated, n = 10 rats), (ii) MI-EGFP (MI, Ultrasonic-targeted destruction of MBs with EGFP, n = 10 rats), and (iii) MI-shPHD2-EGFP (MI, ultrasonic targeted destruction of MBs with shPHD2-EGFP, n = 10 rats). After enhanced ultrasonography, the digital images were analyzed for the assessment of the myocardial perfusion by calculating the contrast agent signal intensity ratio of the anterior wall (infarct area) to the posterior wall (normal area). Ultrasound contrast images analyzed by the QLAB software (Philips Medical Systems HSG).

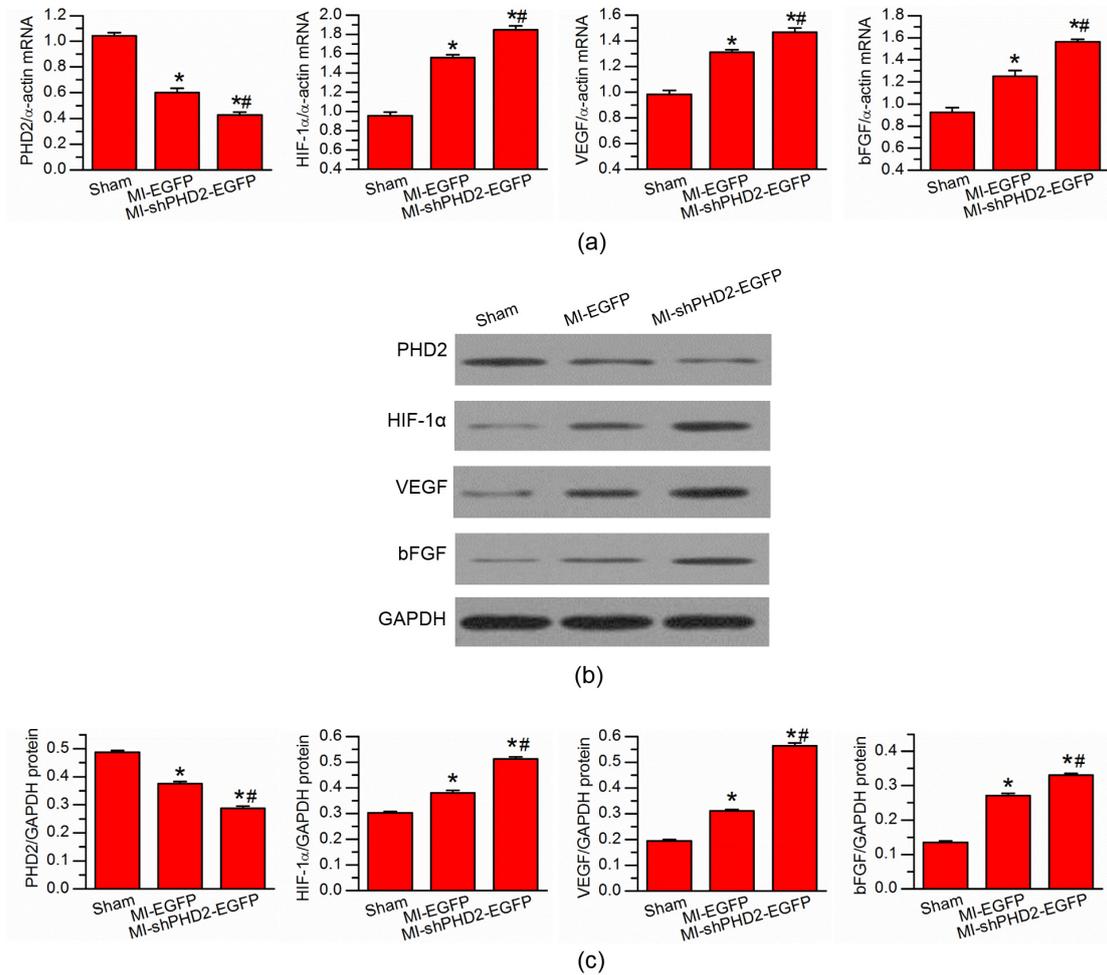
**Supplementary figures:**



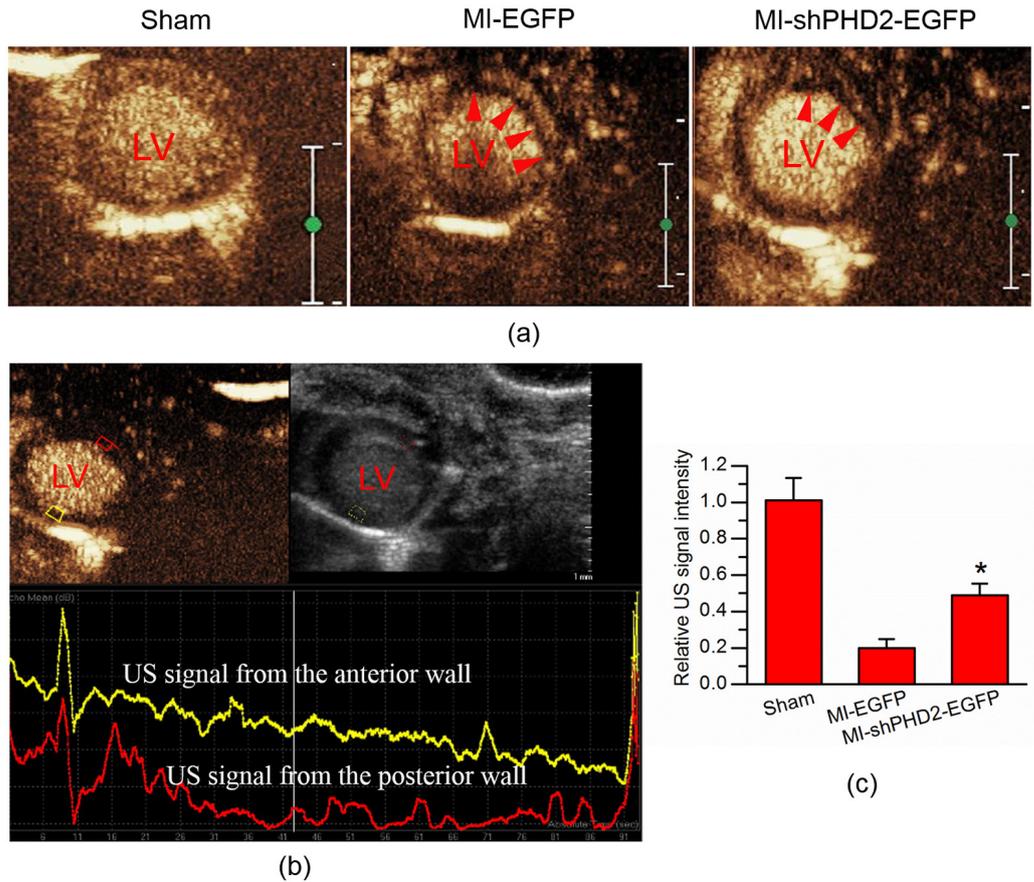
**Figure S1.** UTMD-mediated gene transfection efficiency and cell viability under different conditions. (a) EGFP-positive cells ratios at different US powers, showing significantly higher in the 1.0 W/cm<sup>2</sup> US group than any of the other 3 groups ( $P < 0.05$ ). (b) CCK-8 assay indicated little cell damage observed in the cells treated with 0.5 or 1.0 W/cm<sup>2</sup> US power than these treated with 1.5 or 2.0 W/cm<sup>2</sup> exposure.  $N = 6/\text{group}$ . \*  $P < 0.0125$  vs. 0.5 W/cm<sup>2</sup>; #  $P < 0.0125$  vs. 1.0 W/cm<sup>2</sup>, &  $P < 0.0125$  vs. 1.5 W/cm<sup>2</sup>. (c) EGFP-positive cells ratios at different US exposure times. Treatment with 30 s ultrasound exposure resulted in significantly higher EGFP-positive cells ratios than any of other 3 groups. (d) Cell damage was markedly increased in the cells treated with 45 s or 60 s ultrasound exposure, whereas little cell damage was observed in cells treated with 15 s or 30 s exposure.  $N = 6/\text{group}$ . \*  $P < 0.0125$  vs. 15 s; #  $P < 0.0125$  vs. 30 s, &  $P < 0.0125$  vs. 45 s.



**Figure S2.** The ultrasound imaging before or after injection 500  $\mu$ l of CMB/DNA complexes by echocardiography. LV: left ventricular.



**Figure S3. In vivo gene expression analysis.** (a) The mRNA levels of PHD2, HIF-1 $\alpha$ , VEGF and bFGF in the myocardial tissues after UTMD-mediated EGFP or shPHD2 delivery. (b) Western blotting analysis was utilized for the detection the PHD2, HIF-1 $\alpha$ , VEGF and bFGF protein levels. (c) The signal intensities from the western blots were quantified by an AlphaView Software. N = 10/group. \* P < 0.017 vs. Sham, # P < 0.017 vs. MI-EGFP.



**Figure S4.** Ultrasound contrast imaging of the infarct area in the Sham and gene-transfected rats. (a) Ultrasound contrast imaging demonstrated a significant increased myocardial perfusion in the infarction area (arrows) in the MI-shPHD2-EGFP group. LV: left ventricular. (b) The contrast ultrasound image (left: a contrast M-mode image; right: B-mode image) showed the contrast agent signal intensity of the anterior wall and posterior wall. (c) The quantitative analysis of the contrast agent signal intensity ratio of the anterior wall to the posterior wall. N = 10/group.\*P < 0.05 vs. MI-EGFP.