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Supplemental Information

Exosomes from *SIRT1*-Overexpressing ADSCs Restore Cardiac Function by Improving Angiogenic Function of EPCs

Hui Huang, Zhenxing Xu, Yuan Qi, Wei Zhang, Chenjun Zhang, Mei Jiang, Shengqiong Deng, and Hairong Wang

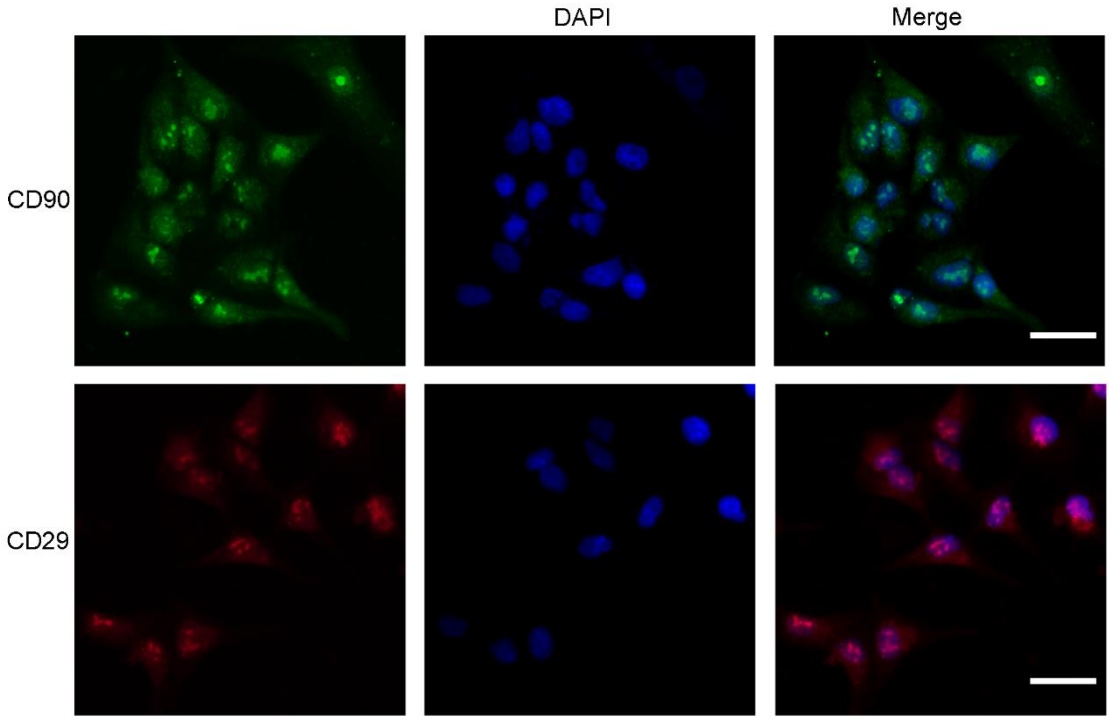

Figure S1 Characteristics of adipose-derived stem cells (ADSCs). Determination of cell surface markers (CD90 and CD29) with immunofluorescence staining. Scale bar = 10 μm.

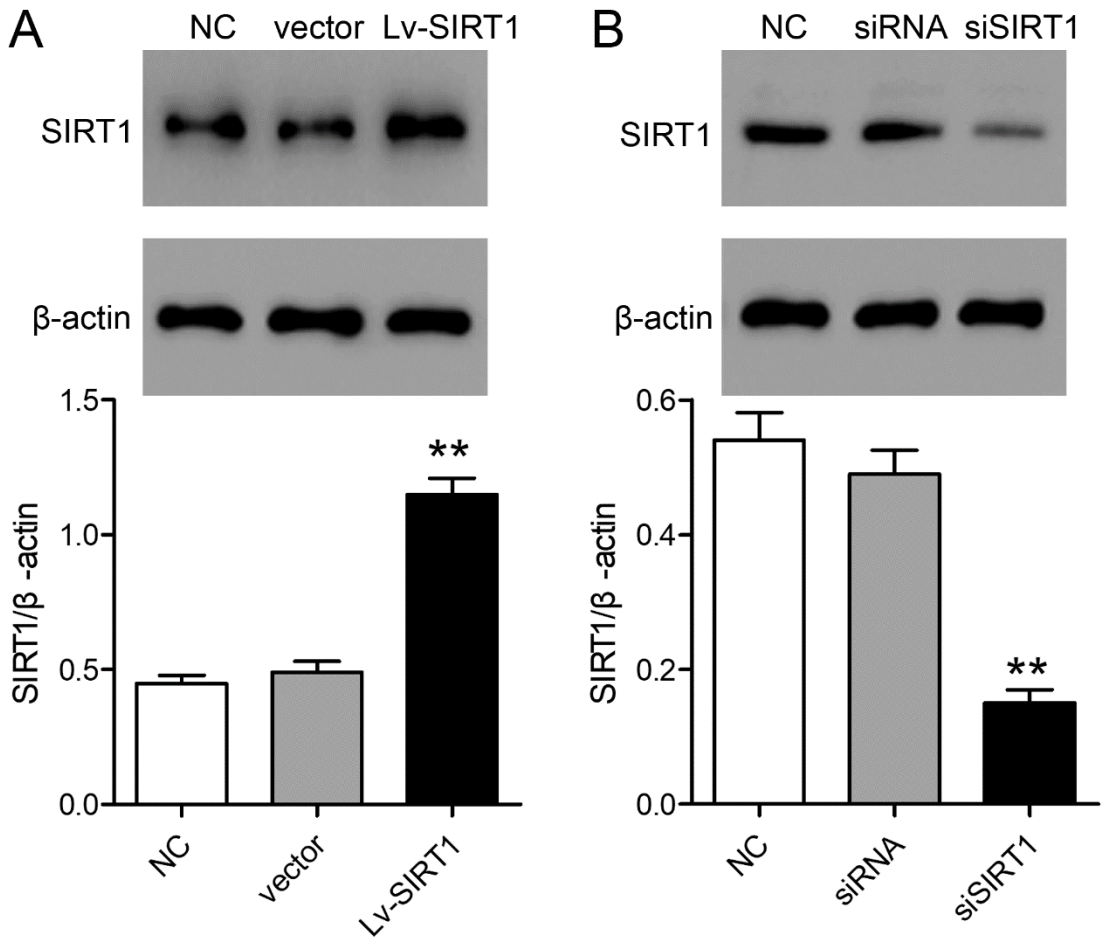

Figure S2. Overexpression and interference efficiency verification. Adipose-derived stem cells were transfected with (A) lentiviral plasmid (vector), lentiviral overexpression of SIRT1 plasmid (Lv-SIRT1), (B) lentiviral interfering control plasmid (siRNA), or lentiviral interfering CXCR7 plasmid (siSIRT1) and cultured for 48 h. Expression of SIRT1 was detected by western blotting. The western blot results were normalized to β-actin. $^{**}p < 0.01$ compared to the non-transfected cells.

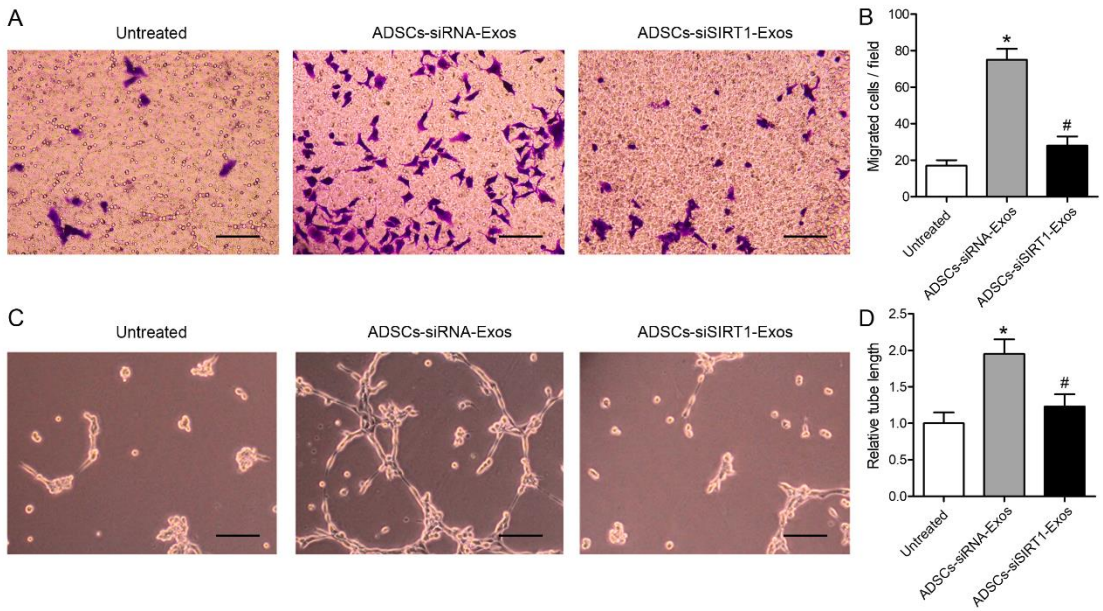

Figure S3. The effect of exosomes from adipose-derived stem cells (ADSCs) interfering with SIRT1 during cell migration and tube formation of AMI-EPCs. (A) Cell migration was measured using Transwell assays. Upper chamber, acute myocardial infarction (AMI) patient endothelial progenitor cells with or without pretreatment with 200 μg/mL ADSCs-siRNA-Exos or ADSCs-siSIRT1-Exos for 24 h; lower chamber, Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum. Scale bar = 100 μm. (B) The number of migrated cells was calculated. $^{*}p < 0.05$ compared with the untreated group; $^{\#}p < 0.05$ compared with the ADSCs-siRNA-Exos treated group. (C) EPCs from AMI patients were pretreated with ADSCs-siRNA-Exos or ADSCs-siSIRT1-Exos for 24 h. The tube formation assay was performed. Scale bar = 100 μm. (D) The tube lengths were measured. The AMI-EPCs without any treatments were normalized to 1. $^{*}p < 0.05$ compared with the untreated group; $^{\#}p < 0.05$ compared with the ADSCs-siRNA-Exos-treated group.

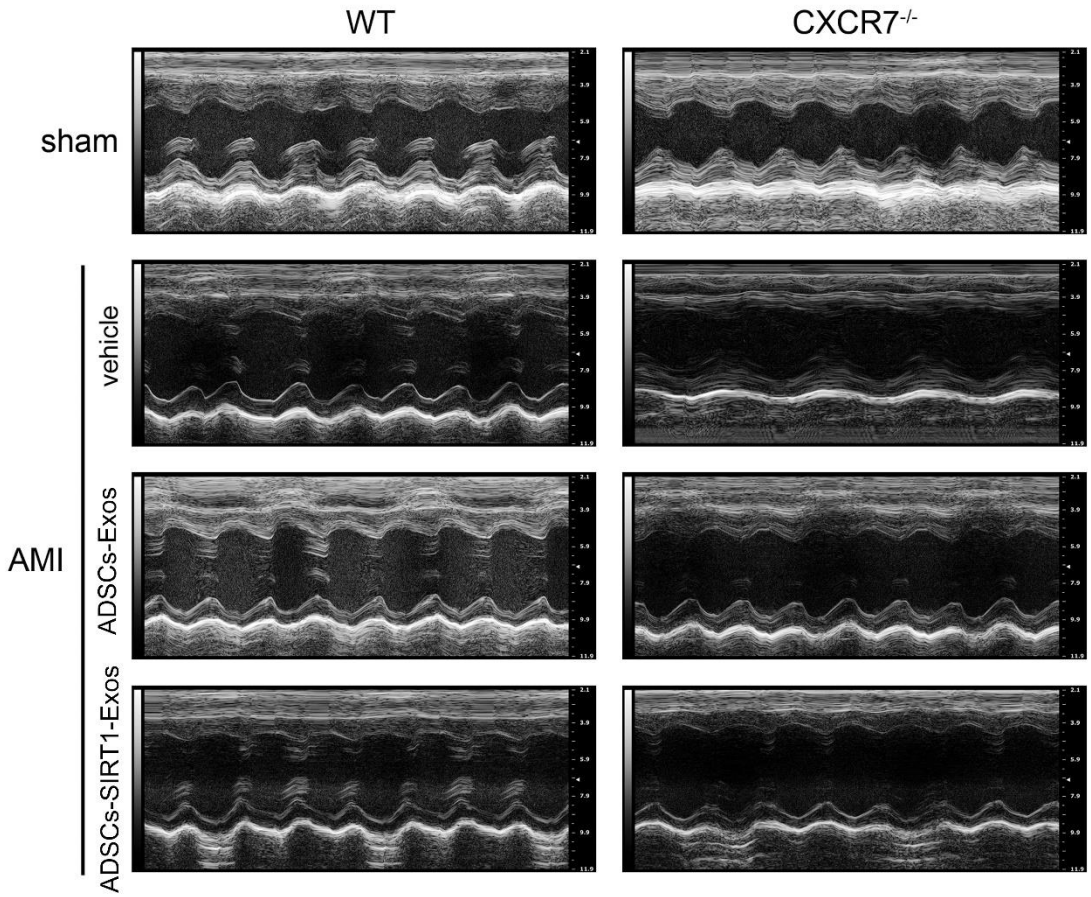

Figure S4. Echocardiogram of mice heart in different groups at 28 days after surgery.

Supplemental Methods

Isolation and culture of human ADSCs

The human tissue and cell processing procedures were approved by the local ethics committee. Human adipose tissues were donated for research purposes with written informed consent. The human adipose tissues used in the experiments were from healthy females undergoing liposuction at Shanghai Gongli Hospital. Adipose-derived stem cells (ADSCs) were isolated and cultured as described previously.[1] Briefly, the adipose tissues were washed twice with PBS, and then digested by 0.075% collagenase I (Sigma-Aldrich, St. Louis, MO, USA) at 37ºC, with shaking for 1 h. After centrifugation for 10 min at 1200 × *g*, the cells were resuspended in DMEM containing 10% FBS and antibiotics, and inoculated in 6-well plates at a density of 5×10^5/cm, and incubated in 37ºC and 5% CO_2 in a saturated humidity incubator. When the primary

cells reached 80%–90% confluency, they were digested and subcultured. The first inoculated primary cells were taken as passage 0. In this study, cells were cultured to passage 3.

To identify cell characteristics, we used passage 3 cells for immunofluorescence staining.[2] After cell slide culture, the cells were fixed with 4% paraformaldehyde for 30 min, and 5% bovine serum albumin (BSA) was added at room temperature for 30 min. Then, antibodies against CD90 or CD29 (1:100; Abcam, Burlingame, CA, USA) were added, and incubated at 4°C overnight. FITC- or PE-labeled rabbit anti-rat secondary antibody (1:200; Abcam) was added and incubated for 3 h at 37°C. DAPI (Sigma-Aldrich) was used to stain nuclei at room temperature for 5 min, and fluorescence microscopy was used to observe and photograph the cells (Olympus, Tokyo, Japan).

References

1. Deng, J, Dai, T, Sun, Y, Zhang, Q, Jiang, Z, Li, S, *et al.* (2017). Overexpression of Prox1 Induces the Differentiation of Human Adipose-Derived Stem Cells into Lymphatic Endothelial-Like Cells In Vitro. *Cellular reprogramming* **19**: 54-63.
2. Luo, Q, Guo, D, Liu, G, Chen, G, Hang, M, and Jin, M (2017). Exosomes from MiR-126-Overexpressing Adscs Are Therapeutic in Relieving Acute Myocardial Ischaemic Injury. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* **44**: 2105-2116.