

Supplementary Information for

Cardio-renal Exosomes in Myocardial Infarction Serum Regulate Proangiogenic Paracrine Signaling in Adipose Mesenchymal Stem Cells

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Table S1 Sequences of miR-1956 mimic and inhibitor.

miRNA		Sense (5' – 3')	Antisense (5' – 3')
microFFTM inhibitor		CAGUACUUUUGUGUAG	
Negative Control		UACAAA	/
microONTM	mimic	UUUGUACUACACAAAA	CAGUACUUUUGUGUAG
Negative Control		GUACUG	UACAAA
microONTM	mmu-	AGUCCAGGGCUGAGUC	UCCGCUGACUCAGCCCU
miR-1956 mimic		AGCGGA	GGACU
microFFTM	mmu-	UCCGCUGACUCAGCCC	/
miR-1956 inhibitor		UGGACU	

Table S2 Primers for real-time PCR analysis.

Gene	Forward	Reverse
<i>Cd31</i>	AGTTGCTGCCCATTTCATCAC	CTGGTGCTCTATGCAAGCCT
<i>Vegf</i>	AATGCTTTCTCCGCTCTGAA	GCTTCCTACAGCACAGCAGA
<i>Notch-1</i>	GATGGCCTCAATGGGTACAAG	TCGTTGTTGTTGATGTCACAGT
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA

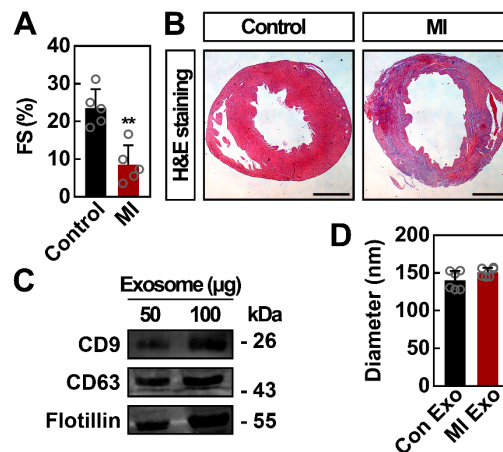


Figure S1. Cardiac function assessment after myocardial infarction. (A) Echocardiographic measurements of FS of control mice and mice with MI for 3 days. MI, myocardial infarction; FS, fractional Shortening. $n = 5$ mice per group. Data are shown as mean \pm SD. *, significantly different from control; **, $p < 0.01$. (B) Representative images of H&E staining of control mice and mice with MI for 3 days. Scale bar, 1 mm. $n = 3$ mice per group. (C) Western blot analysis of exosomal marker proteins including CD9, CD63, and Flotillin. The total protein amount of exosomal lysate was 50 μ g and 100 μ g, respectively. Representative images of two independent experiments are shown. (D) Quantification of the average diameter of Con Exo and MI Exo isolated from an equal volume of serum. $n = 6$ mice per group. Data are shown as mean \pm SD. Unpaired Student's t-test was performed (A and D).

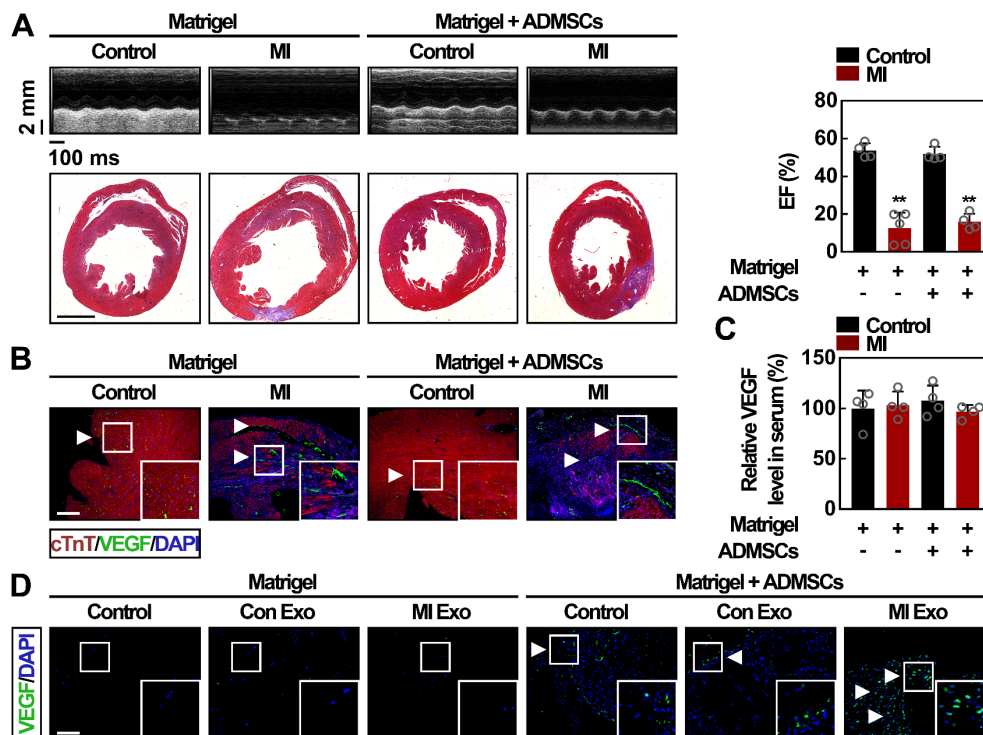


Figure S2. Circulating exosomes after MI and MI Exo promote the expression of VEGF in the Matrigel plugs. (A) Representative images of echocardiography and echocardiographic measurements of EF at Day 14. EF, ejection fraction. $n = 4$ mice per group at least. Data are shown as mean \pm SD. *, significantly different from the control group bearing with cell-free Matrigel; **, $p < 0.01$. Representative images of Masson's trichrome-stained sections were exhibited. Scale bar, 1 mm. $n = 4$ mice per group. (B) Representative images of cTnT (red) and VEGF (green) expression in left ventricular free-wall sections. Nuclei were labeled with DAPI (blue). Scale bar, 200 μ m; arrows indicate VEGF positive area. $n = 4$ mice per group. (C) The level of VEGF in the serum was assessed by ELISA. $n = 4$ mice per group. Data are shown as mean \pm SD. *, significantly different from the control group bearing with cell-free Matrigel. (D) Representative images of VEGF (green) expression in Matrigel plug sections. Nuclei were labeled with DAPI (blue). Scale bar, 100 μ m; arrows indicate VEGF positive area. $n = 4$ mice per group. One-way ANOVA followed by Tukey's post-test was performed (A and C).

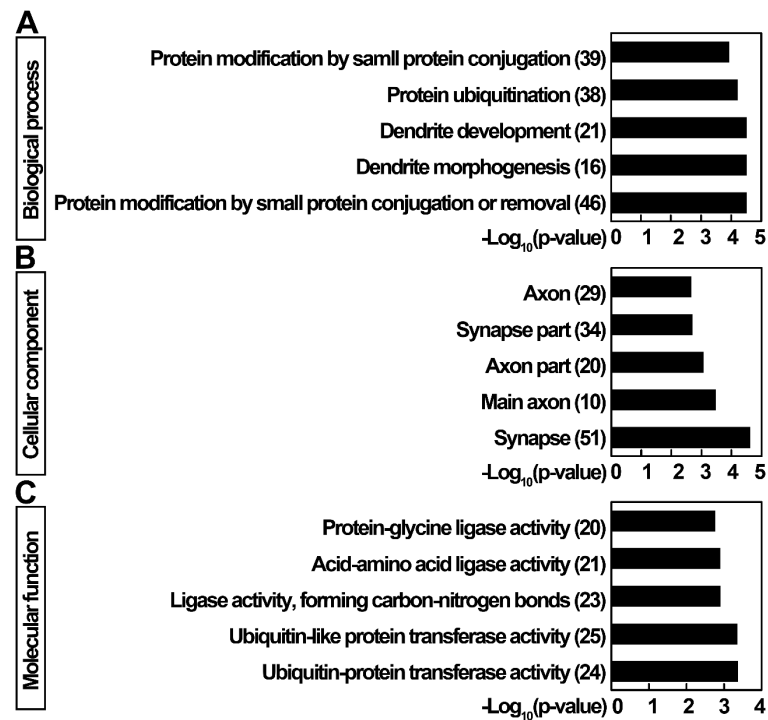


Figure S3. GO analysis of predicted target genes for the 8 significantly upregulated miRNAs in MI Exo. Top 5 enriched GO categories under molecular function (A), cellular component (B), and biological process (C) were shown.

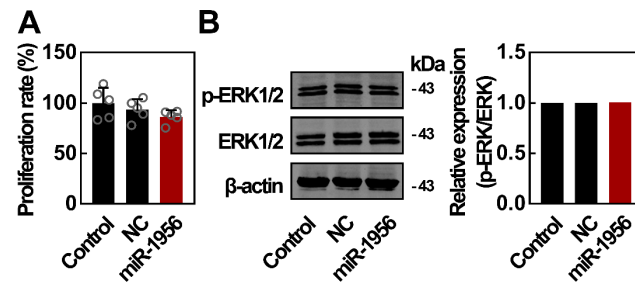


Figure S4. Overexpression of miR-1956 does not affect the cell viability of ADMSCs. After transfection with 50 nmol/L miR-1956 mimic for 3 days, the proliferation rate of ADMSCs was measured by CCK-8 assay (**A**), and the expression of intracellular p-ERK1/2 and ERK1/2 was determined by western blotting (**B**). Data are shown as mean \pm SD. One-way ANOVA followed by Tukey's post-test was performed (**A**).