CircUbe3a from M2 macrophage-derived small extracellular vesicles mediates myocardial fibrosis after acute myocardial infarction

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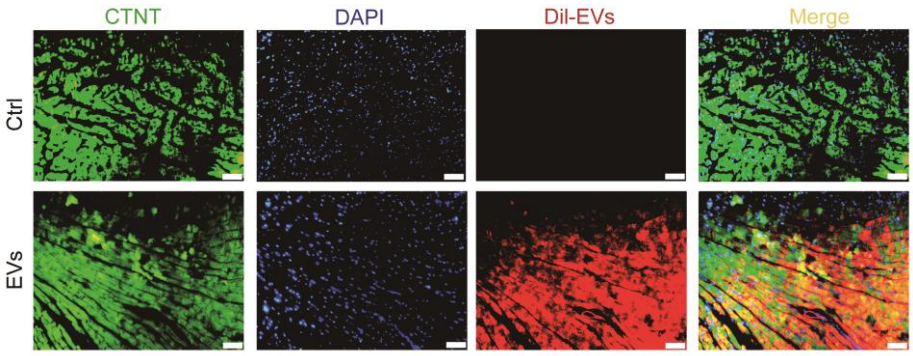

Figure S1. EVs injected via the intramyocardial and tail vein routes are internalized by cardiomyocytes (CMs). DiI-labeled M2M-EVs were injected into mice via the tail vein for 72 h. The relative fluorescence intensity (red) was quantified to identify the uptake of EVs by CMs. Scale bar=50 μm (n=5).

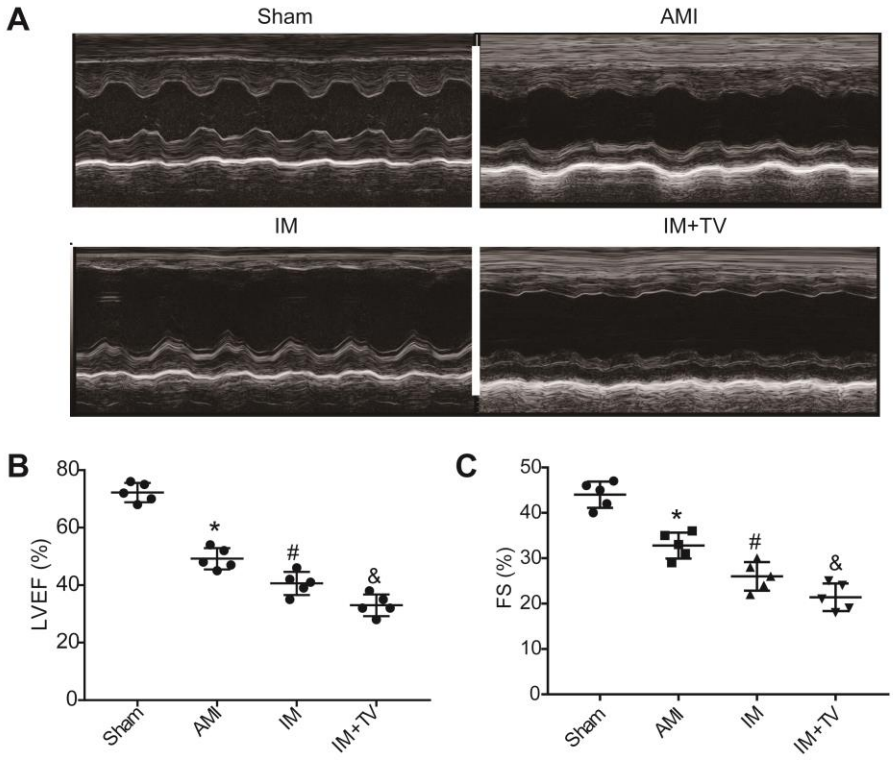

Figure S2. Echocardiography was performed to evaluate the effects of different EV injection methods on cardiac function in mice. A: Representative echocardiographic images. **B and C:** The LVEF (%) and FS values in the indicated groups were calculated (*P<0.05 versus the sham group; $^{\#}P$<0.05 versus the AMI group; $^{\&}P$<0.05 versus the IM group; n=5 per group).

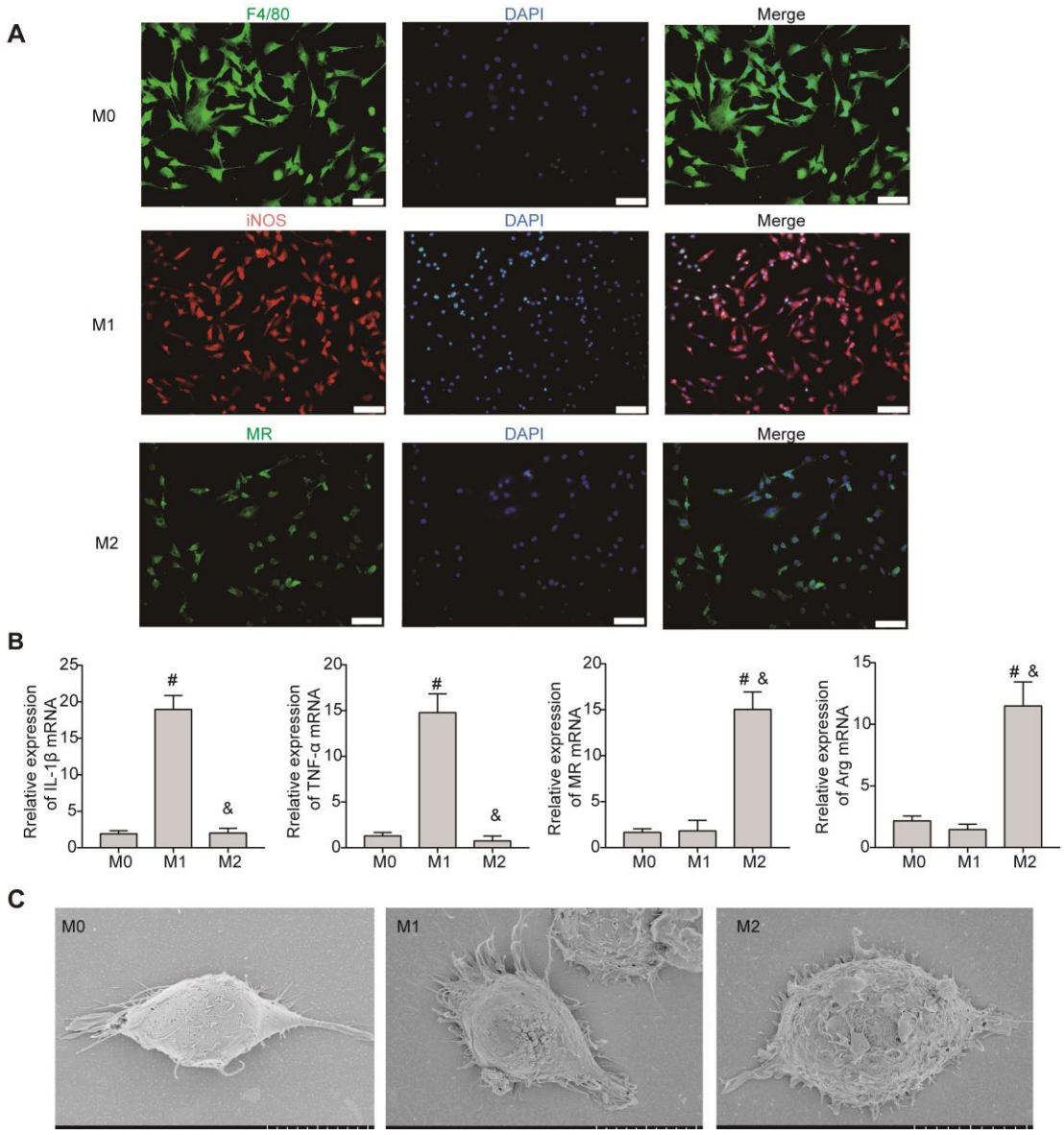

Figure S3. Morphological characteristics and identification of various macrophage types. A: Representative immunofluorescence images of surface markers on various macrophage types. Scale bar=50 μm. **B:** qRT-PCR was used to detect the expression of specific M1- and M2-related genes in various macrophage types. **C.**The morphology of macrophages was visualized by scanning electron microscopy.

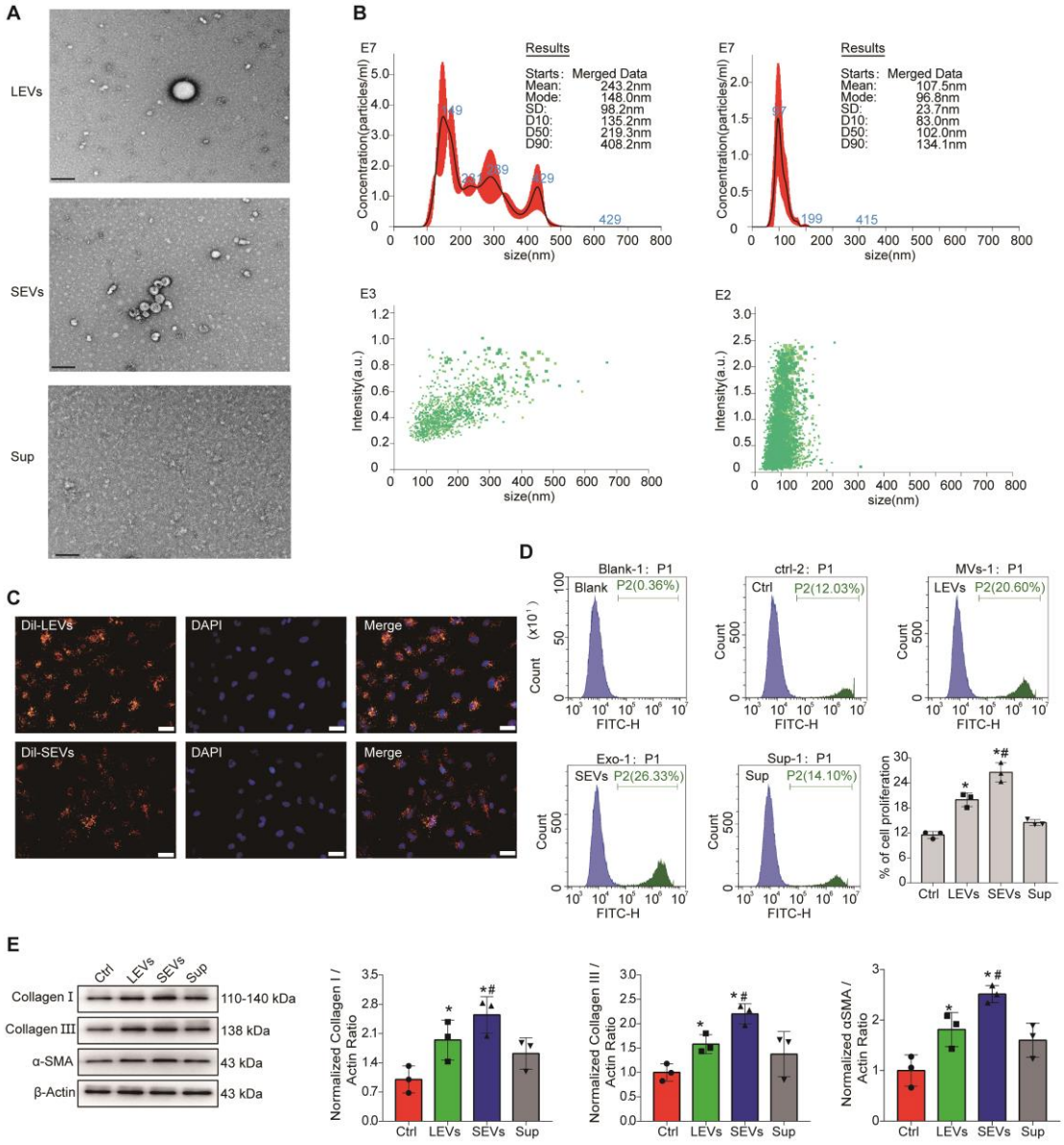

Figure S4. Effects of EVs of different sizes on the proliferation of fibroblasts. A: TEM demonstrated that the LEVs and SEVs secreted by M2Ms were cup-shaped vesicles. **B:** Size distributions of LEVs and SEVs isolated from M2M-conditioned medium, as determined by NTA. **C:** Uptake of LEVs and SEVs by CFs was assessed by immunofluorescence staining. Scale bar=50 μm. **D:** Effects of EVs of different sizes on CF proliferation, as determined by the EdU assay. **E:** Representative Western blot results from three independent experiments and quantification of collagen I, collagen III, and α-SMA expression in CFs treated with LEVs, SEVs and Sup (**P*<0.05 versus the Ctrl group; $^{\#}P$<0.05 versus the LEVs group; n=3 per group).

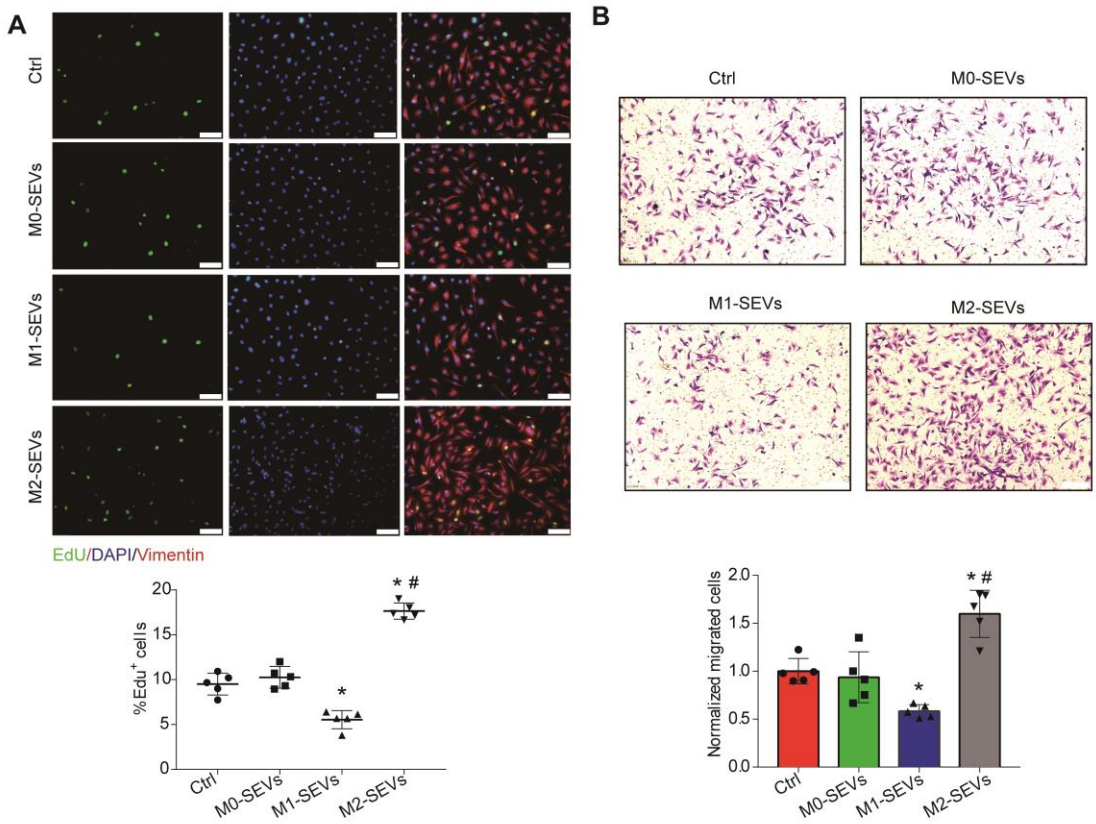

Figure S5. Comparison of the effects of M0M-, M1M-, and M2M-SEVs on the proliferation and migration of CFs. A: Representative immunofluorescence images showing the CF expression levels in different groups. The number of EdU-positive nuclei was used to assess cell proliferation. Scale bar=50 μm. **B:** The transwell assay was performed to evaluate the migration of CFs treated with M0-SEVs, M1-SEVs and M2-SEVs (*P<0.05 versus the Ctrl group; $^{\#}P$<0.05 versus the M0-SEVs group; n=3 per group).

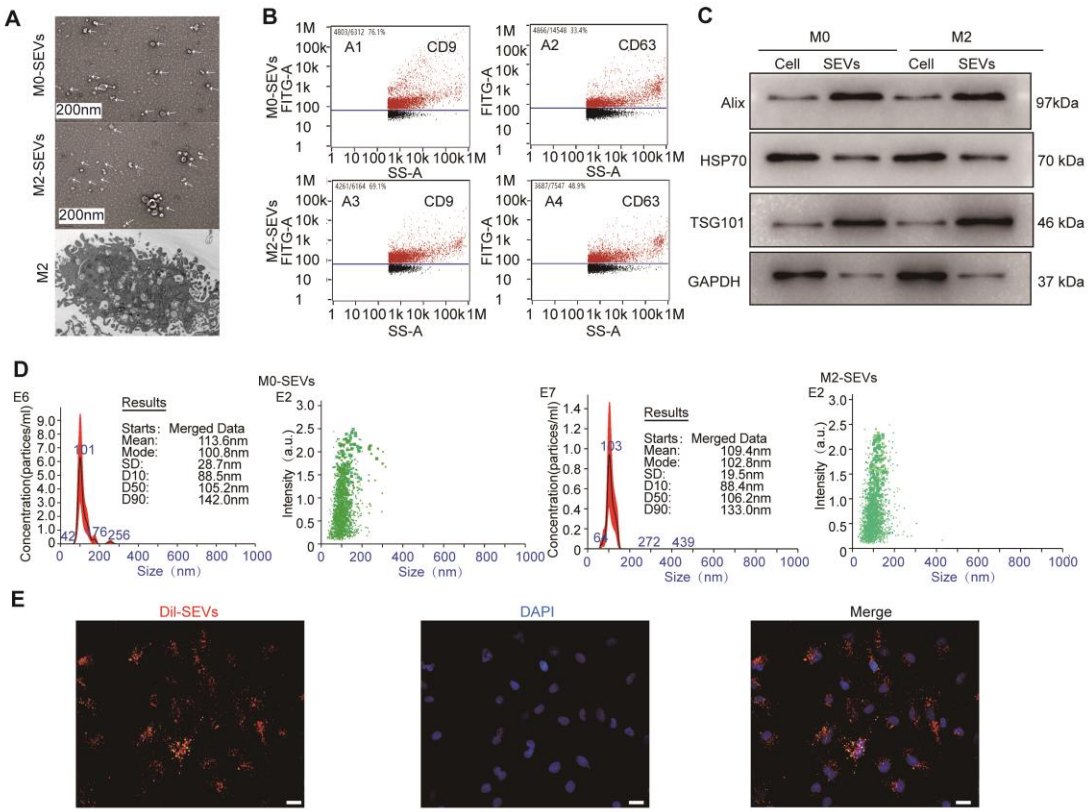

Figure S6. Morphological characteristics and phenotypic identification of M0M- and M2M-SEVs. A: TEM demonstrated that the SEVs secreted by M0Ms or M2Ms were cup-shaped vesicles. **B:** The markers CD63 and CD9 in M0M-SEVs and M2M-SEVs were detected by FCM. **C:** Alix, HSP70, and TSG101 protein expression was detected by Western blot analysis. **D:** Size distributions of SEVs isolated from M0M- or M2M-conditioned medium, as determined by NTA. **E:** The uptake of SEVs by CFs was measured by immunofluorescence staining. Scale bar=50 μm.

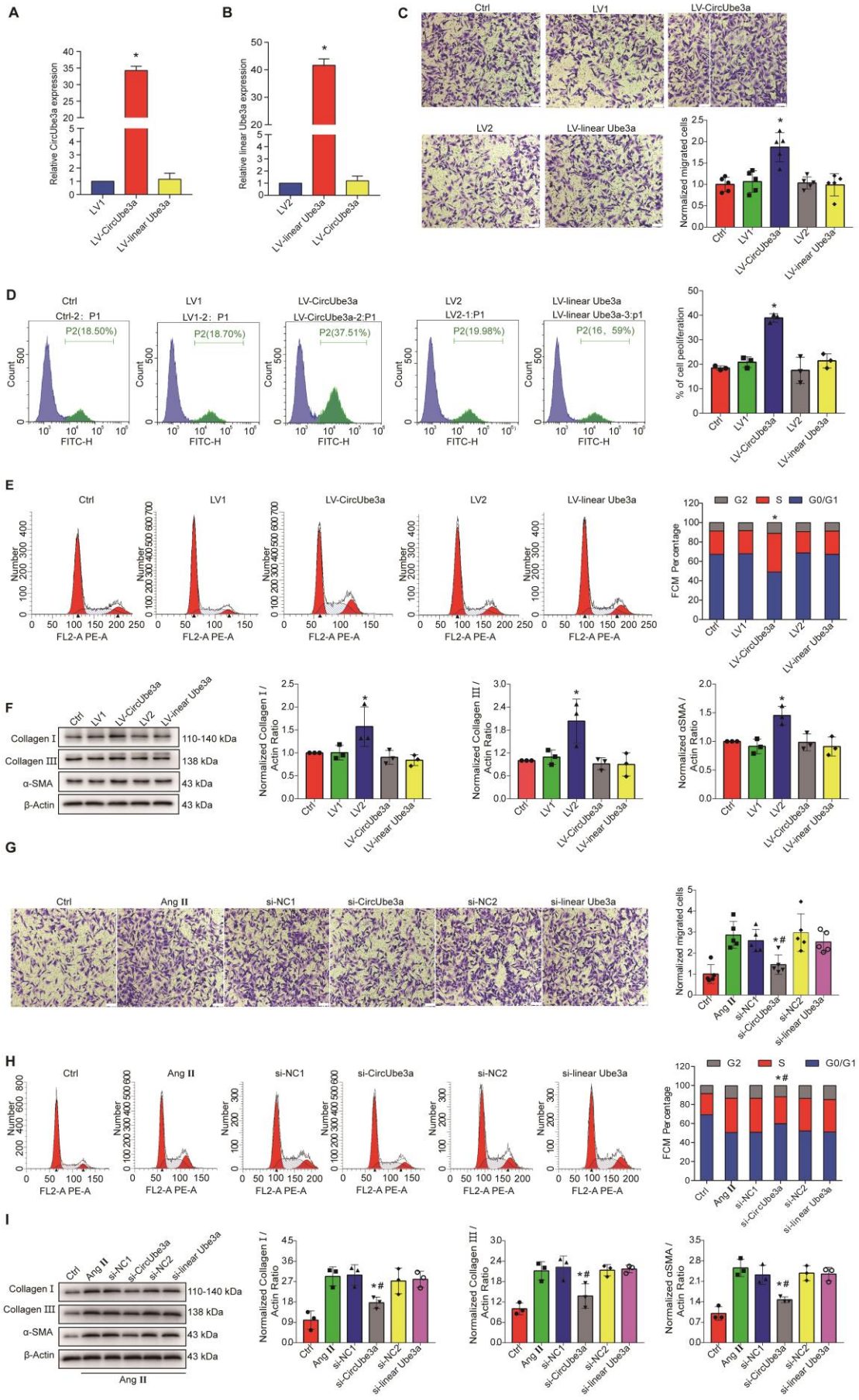
A
Relative CircUbe3a expression
LV1
LV-CircUbe3a
LV-linear Ube3a
B
Relative linear Ube3a expression
LV2
LV-linear Ube3a
LV-CircUbe3a
C
Ctrl
LV1
LV-CircUbe3a
LV2
LV-linear Ube3a
Normalized migrated cells
D
Ctrl
Ctrl-2：P1
P2(18.50%)
LV1
LV1-2：P1
P2(18.70%)
LV-CircUbe3a
LV-CircUbe3a-2:P1
P2(37.51%)
LV2
LV2-1:P1
P2(19.98%)
LV-linear Ube3a
LV-linear Ube3a-3:p1
P2(16，59%)
Count
FITC-H
% of cell peoliferation
E
Number
FL2-A PE-A
G2
S
G0/G1
FCM Percentage
F
Collagen I
Collagen III
α-SMA
β-Actin
110-140 kDa
138 kDa
43 kDa
43 kDa
Normalized Collagen I / Actin Ratio
Normalized Collagen III / Actin Ratio
Normalized αSMA / Actin Ratio
G
Ang II
si-NC1
si-CircUbe3a
si-NC2
si-linear Ube3a
Normalized migrated cells
H
FCM Percentage
I
Collagen I
Collagen III
α-SMA
β-Actin
110-140 kDa
138 kDa
43 kDa
43 kDa
Ang II
Normalized Collagen I / Actin Ratio
Normalized Collagen III / Actin Ratio
Normalized αSMA / Actin Ratio

Figure S7. CircUbe3a promotes the proliferation, migration and myofibroblastic transformation of CFs. CFs were treated with LV-circUbe3a, LV-linear Ube3a, si-circUbe3a, si-linear Ube3a or their corresponding NCs to assess the effect of circUbe3a. **A and B:** qPCR analysis of circUbe3a and Ube3a mRNA expression in CFs ($^{*}P$<0.05 versus the LV1 group). **C:** Transwell assays were performed to assess CF migration after treatment with LV-circUbe3a or LV-linear Ube3a. **D:** The EdU assay was performed to evaluate CF proliferation after treatment with LV-circUbe3a or LV-linear Ube3a. **E:** FCM after the transduction of LV-circUbe3a or LV-linear Ube3a indicated that circUbe3a is positively associated with cell cycle progression in CFs. **F:** Western blot analysis of the protein expression of collagen I, collagen III, and α-SMA in cells treated with LV-circUbe3a or LV-linear Ube3a ($^{*}P$<0.05 versus the Ctrl group; n=3 per group). **G:** The transwell assay was performed to assess CF migration after treatment with si-circUbe3a or si-linear Ube3a. **H:** FCM after treatment with si-circUbe3a or si-linear Ube3a indicated that circUbe3a is positively associated with cell cycle progression in CFs. **I:** Western blot analysis of the protein expression of collagen I, collagen III, and α-SMA in cells treated with si-circUbe3a or si-linear Ube3a (*P<0.05 versus the Ctrl group; $^{\#}P$<0.05 versus the Ang II group; n=3 per group).

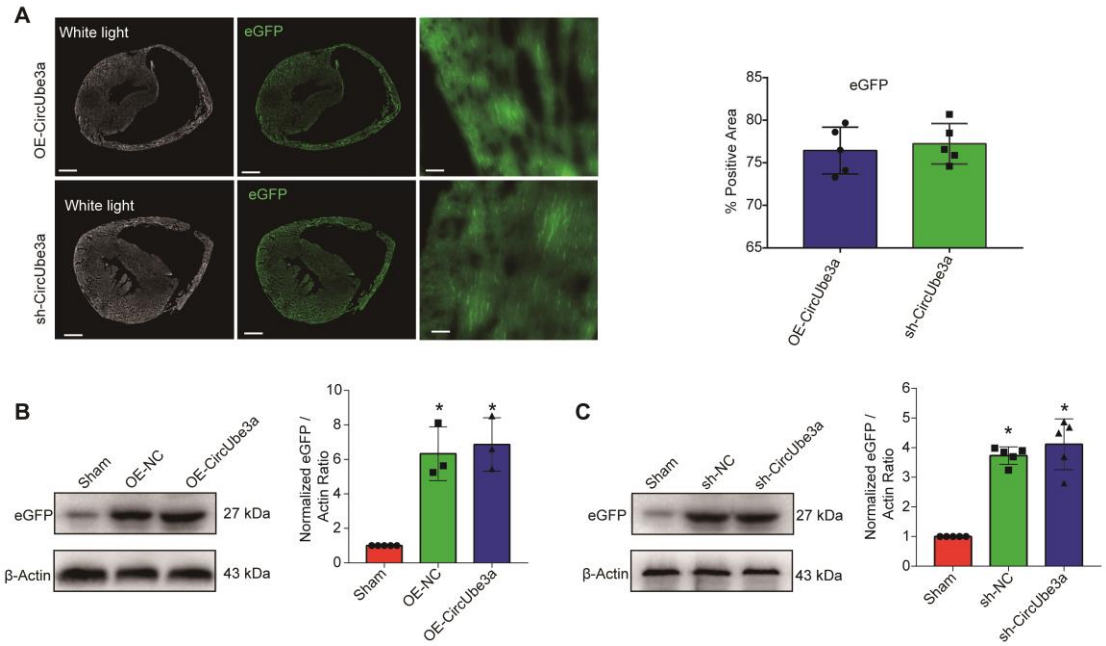

Figure S8. Assessment of the cardiac-specific circUbe3a overexpression and inhibition efficiencies via rAAV transduction. A: Immunofluorescence was used to detect the rAAV-mediated circUbe3a transduction efficiency. Scale bars=1000 μm. **B:**

Representative Western blot results from three independent experiments and quantification of eGFP expression in cardiac muscle tissues treated with rAAV-circUbe3a, rAAV-shcircUbe3a or its NC (*P<0.05 versus the sham group; n=6 per group).

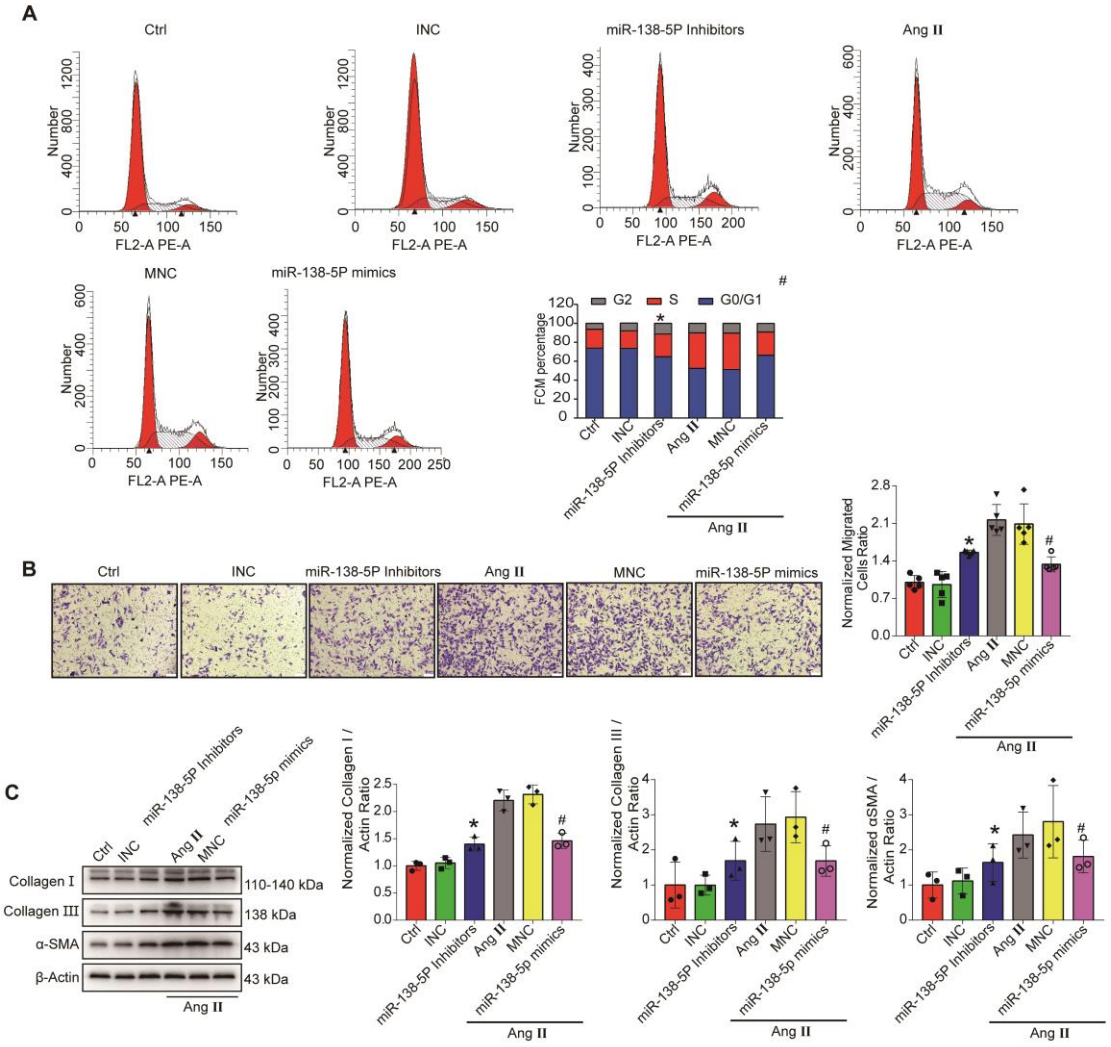

Figure S9. miR-138-5p regulates the proliferation, migration and myofibroblastic transformation of CFs. miR-138-5p mimics, inhibitors, and their corresponding NCs were transfected into CFs to assess the effect of miR-138-5p in CFs. **A:** FCM indicated that miR-138-5p is negatively associated with cell cycle progression in CFs. **B:** The transwell assay was performed to assess CF migration after treatment with miR-138-5p mimics or miR-138-5p inhibitors. **C:** Representative Western blot results from three independent experiments and quantification of collagen I, collagen III, and α-SMA expression in CFs treated with miR-138-5p mimics, miR-138-5p inhibitors or their

corresponding NCs (*P<0.05 versus the Ctrl group; $^{\#}P$<0.05 versus the miR-138-5p inhibitor group; n=3 per group).