Supplementary material

Zhang X, Guan J, Guo M, et al. Rho GTPase-activating protein 1 promotes apoptosis of myocardial cells in an ischemic cardiomyopathy model. Kardiol Pol. 2019; 77: 1163-1169. doi:10.33963/KP.15040

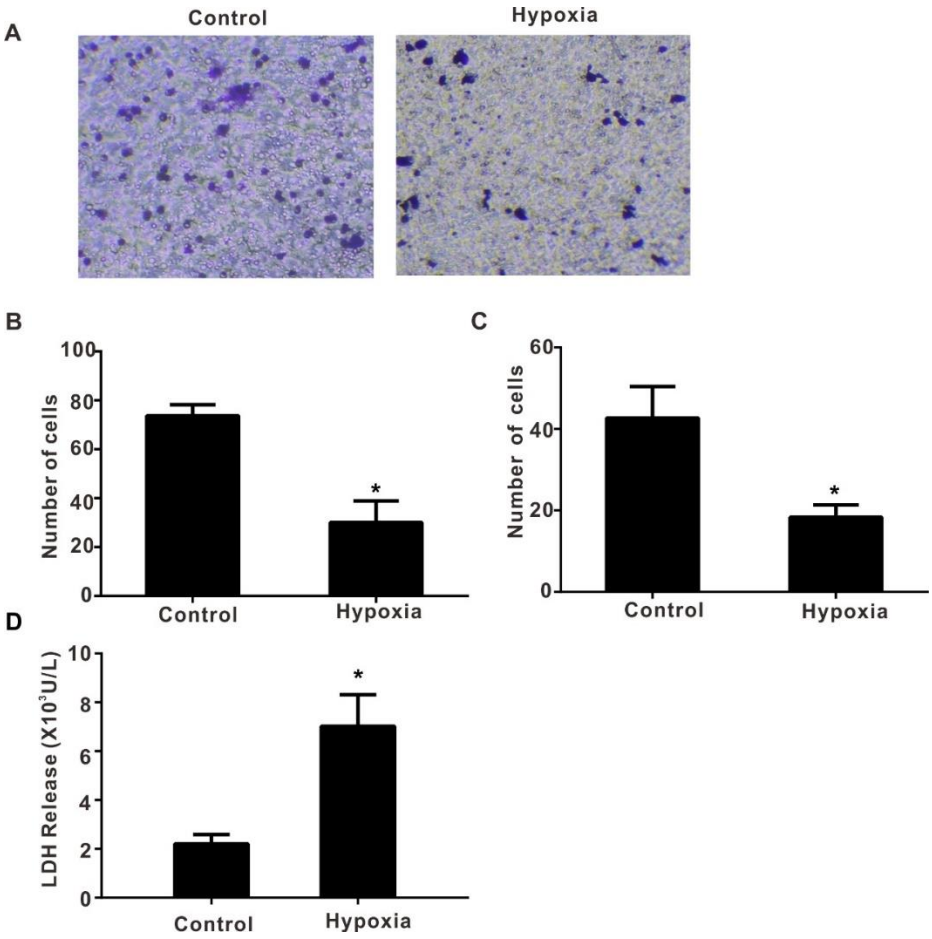

Figure S1. Cell proliferation was arrested and LDH activity was elevated in ischemia myocardial cell model

(A) H9c2 cell invasion under hypoxic and normal conditions was measured based on staining; (B, C) H9c2 cell migration and invasion under hypoxic conditions were quantified; (D) The LDH release in H9c2 cells under hypoxic conditions was increased at 24 h post-transfection.

All the data have been presented as means activity ± SD from at least three independent experiments (n > 3) for each condition. *P < 0.01 versus to control.

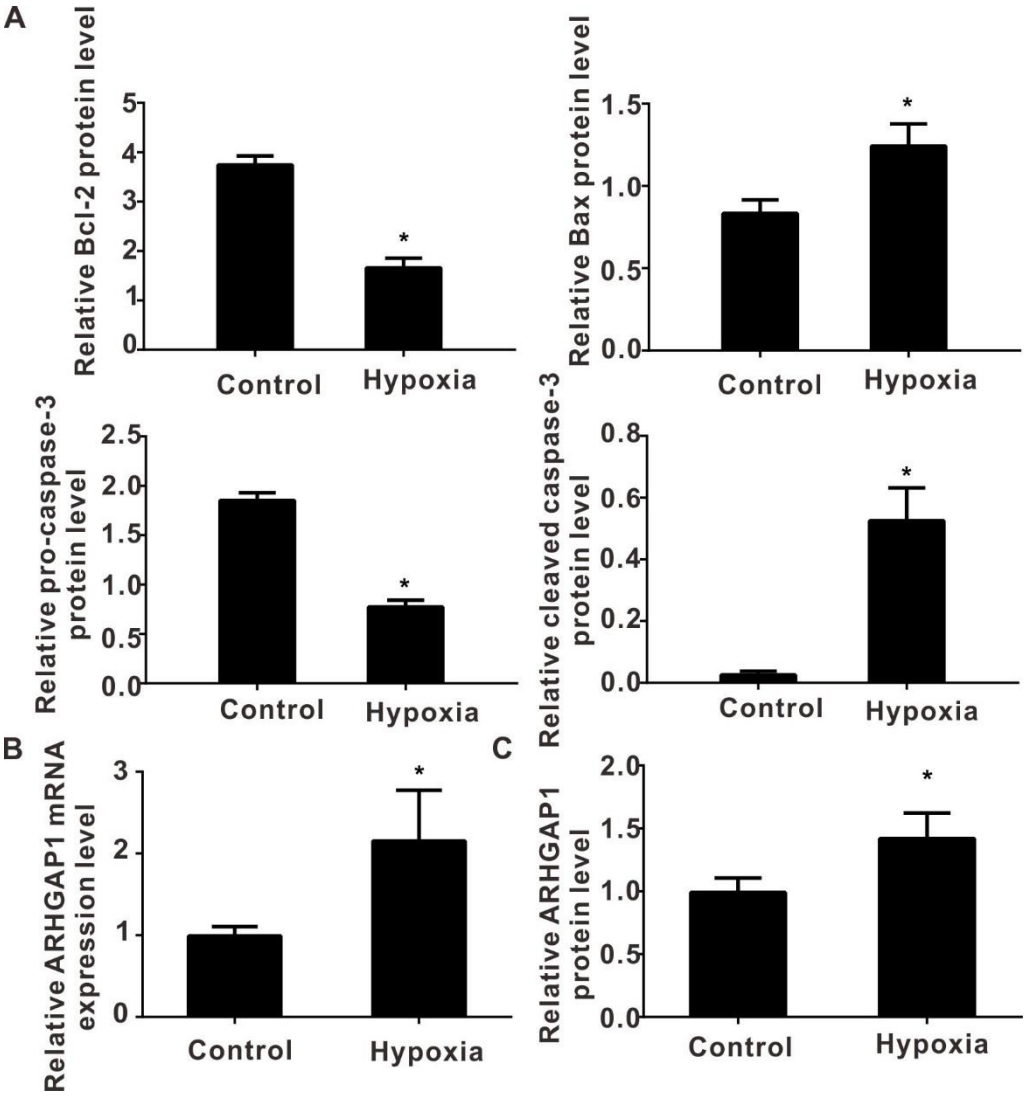

Figure S2. Quantification of apoptosis modulators and ARHGAP1 in H9c2 cells

(A) The expression of modulators was quantified and normalized to tubulin; (B) The relative ARHGAP1 mRNA level was quantified by qPCR for control and hypoxia groups; (C) ARHGAP1 protein level in control and test cells were quantified and normalized to tubulin. Experiments were replicated three times. *P < 0.01 versus to control.

A

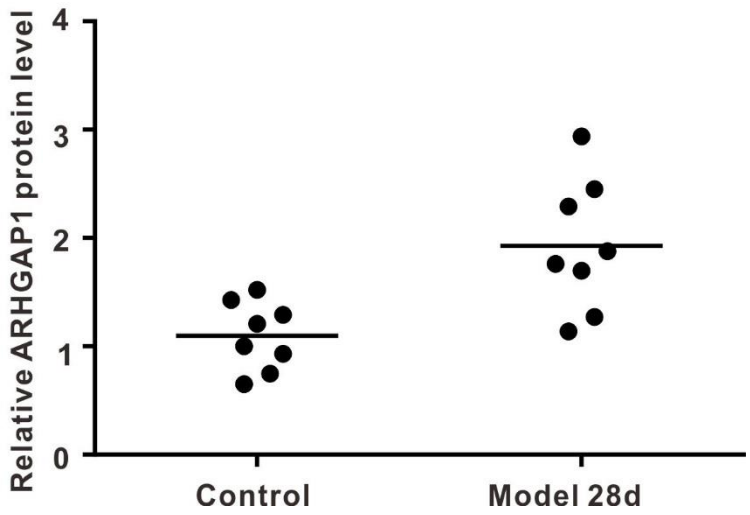

Figure S3. Statistical analysis of ARHGAP1 expression in the ICM rat model

Protein levels of ARHGAP1 in cells of the control and ICM groups were quantified and summarized.

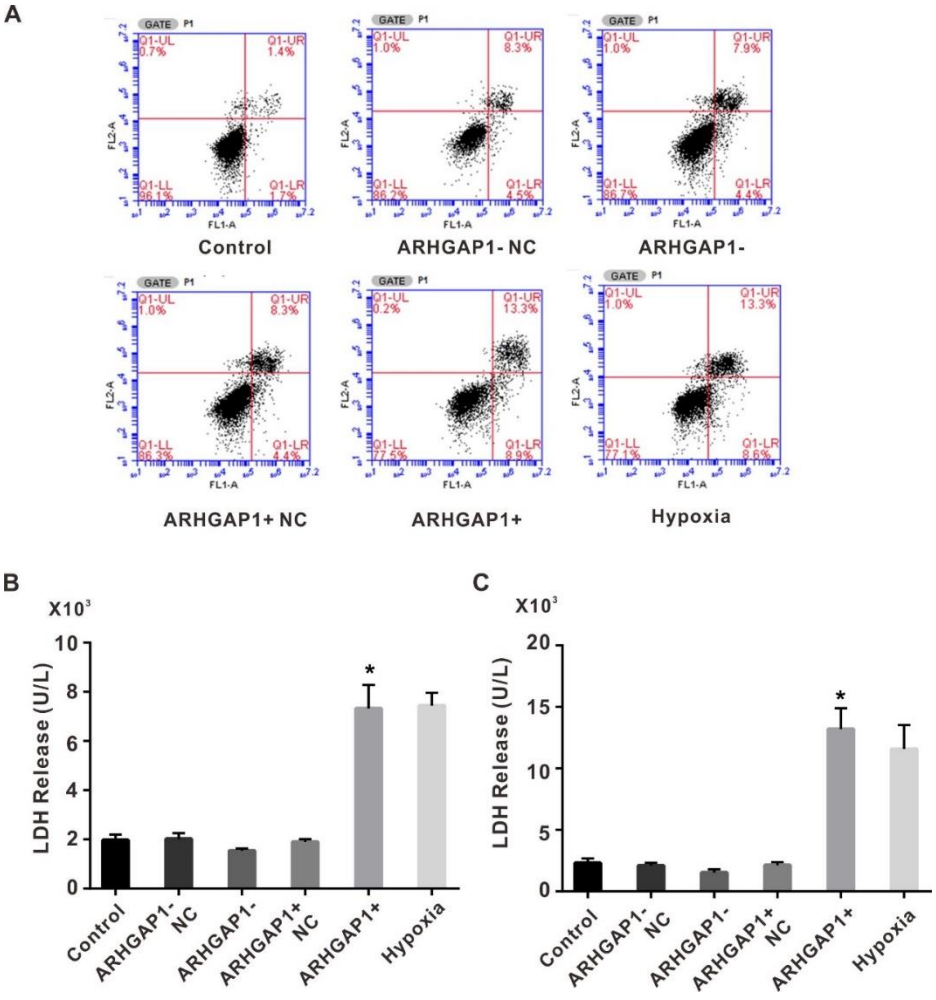

Figure S4. Overexpression ARHGAP1 induced LDH release

There were six groups: untreated H9c2 cells were set as the control, cells transfected with siRNA targeting ARHGAP1 was ARHGAP1-, those transfected with irrelevant siRNA sequence were ARHGAP1-NC, those containing vectors overexpressing ARHGAP1 were ARHGAP1+, and those with only blank vectors were ARHGAP1+NC. Cells cultured in hypoxia were set as positive control. (A) The apoptosis of H9c2 cells was determined by flow cytometry using Annexin V/PI staining; (B) The LDH release in myoblast cell lines was determined in these six groups; (C) The LDH release in primary myoblast cells was determined in these groups. Cells overexpressing ARHGAP1 had the highest LDH activity. All the data have been presented as means ± SD from at least three independent experiments ($n > 3$). *$P < 0.01$ versus to control.

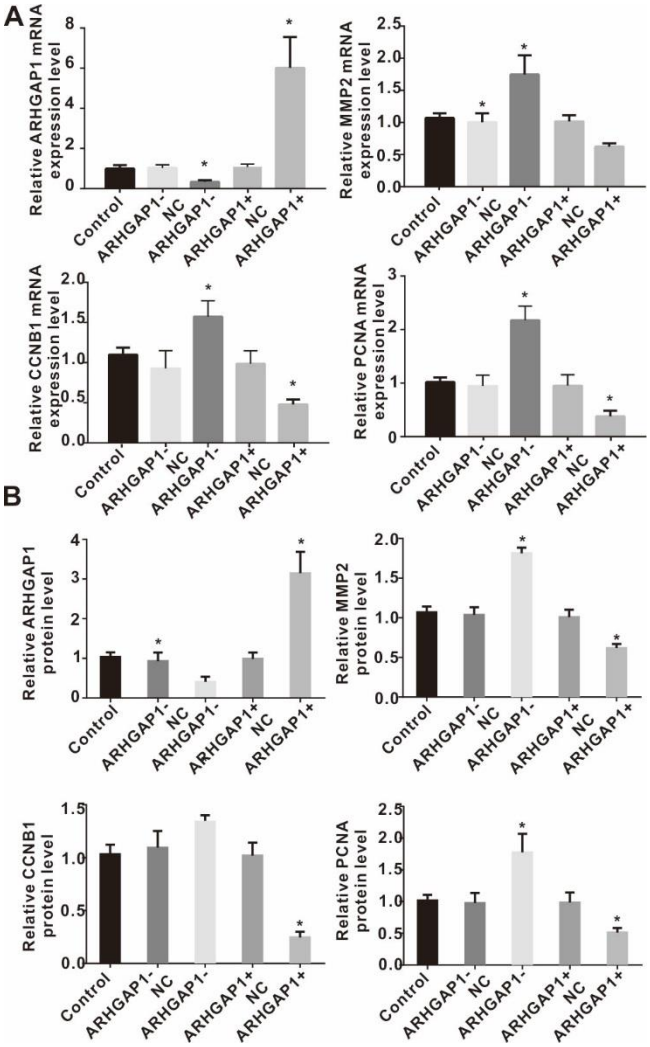

Figure S5. mRNA and protein of cell proliferation effectors were quantified in ARHGAP1 overexpressed myoblast cells

Untreated H9c2 cells were set as control, cells transfected with siRNA targeting ARHGAP1 was ARHGAP1-, those transfected with irrelevant siRNA sequence were ARHGAP1-NC, those containing vectors overexpression ARHGAP1 were ARHGAP1+, and those with only blank vectors were ARHGAP1+NC. (A) The mRNA expression level of ARHGAP1, MMP2, CCNB1, and PCNA were determined by qPCR in these five groups; (B) The protein expression levels of ARHGAP1, MMP2, CCNB1, and PCNA analyzed by western blot were normalized to tubulin and quantified. All the data have been presented as means ± SD from at least three independent experiments ($n > 3$). *$P < 0.01$ versus to control.

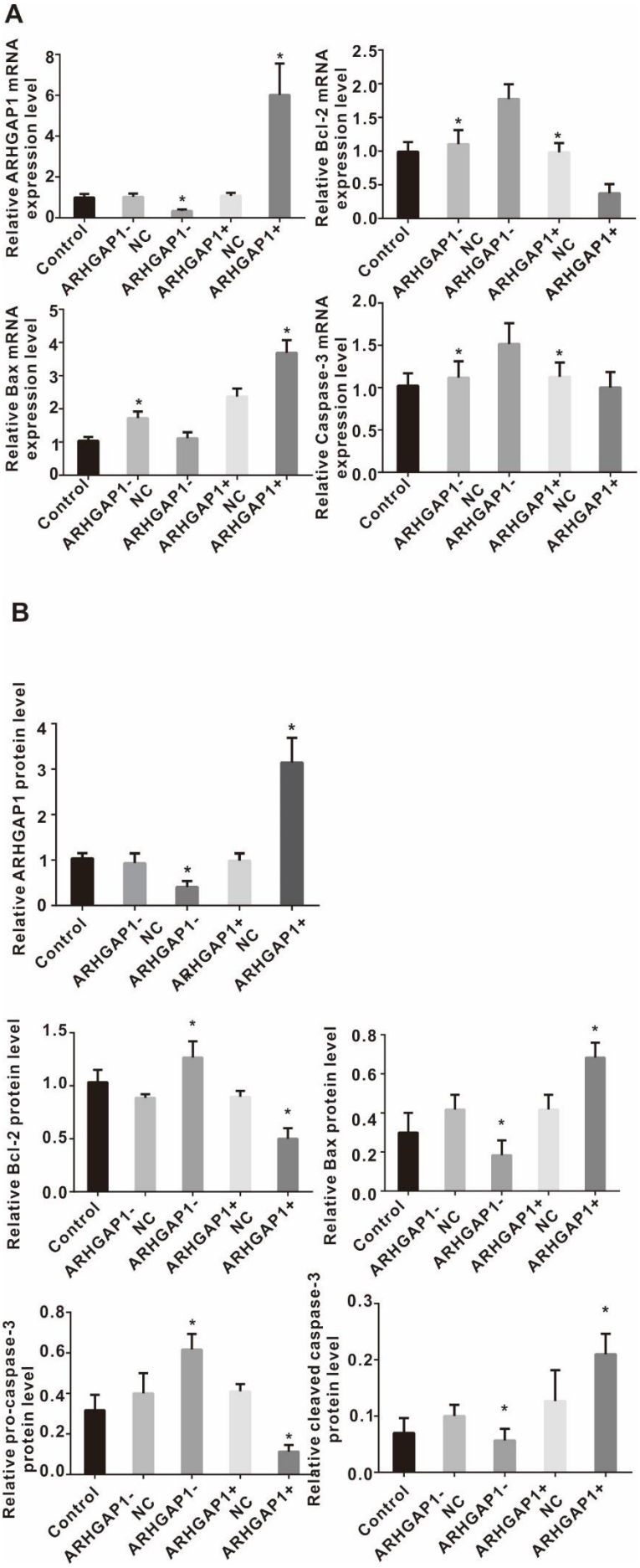
A
Relative ARHGAP1 mRNA expression level
Relative Bcl-2 mRNA expression level
Relative Bax mRNA expression level
Relative Caspase-3 mRNA expression level
Control
ARHGAP1-
NC
ARHGAP1-
ARHGAP1+
NC
ARHGAP1+
B
Relative ARHGAP1 protein level
Relative Bcl-2 protein level
Relative Bax protein level
Relative pro-caspase-3 protein level
Relative cleaved caspase-3 protein level

Figure S6. mRNA and protein of cell apoptotic modulators were quantified in ARHGAP1 overexpressed myoblast cells

Untreated H9c2 cells were set as the control, cells transfected with siRNA targeting ARHGAP1 was ARHGAP1-, those transfected with irrelevant siRNA sequence were ARHGAP1-NC, those containing vectors overexpressing ARHGAP1 were ARHGAP1+, and those with only blank vectors were ARHGAP1+NC. (A) The mRNA expression levels of ARHGAP1, Bcl-2, Bax, and Caspase-3 were determined by qPCR in these five groups; (B) The relative protein expression levels of ARHGAP1, Bcl-2, Bax, pro-caspase-3, and cleaved caspase-3 analyzed by western blot were quantified by normalizing to Tubulin. Experiments were replicated three times. $*P < 0.01$ versus to control.