

**OMTN, Volume 17**

**Supplemental Information**

**MicroRNA-221 Is Cardioprotective  
and Anti-fibrotic in a Rat Model  
of Myocardial Infarction**

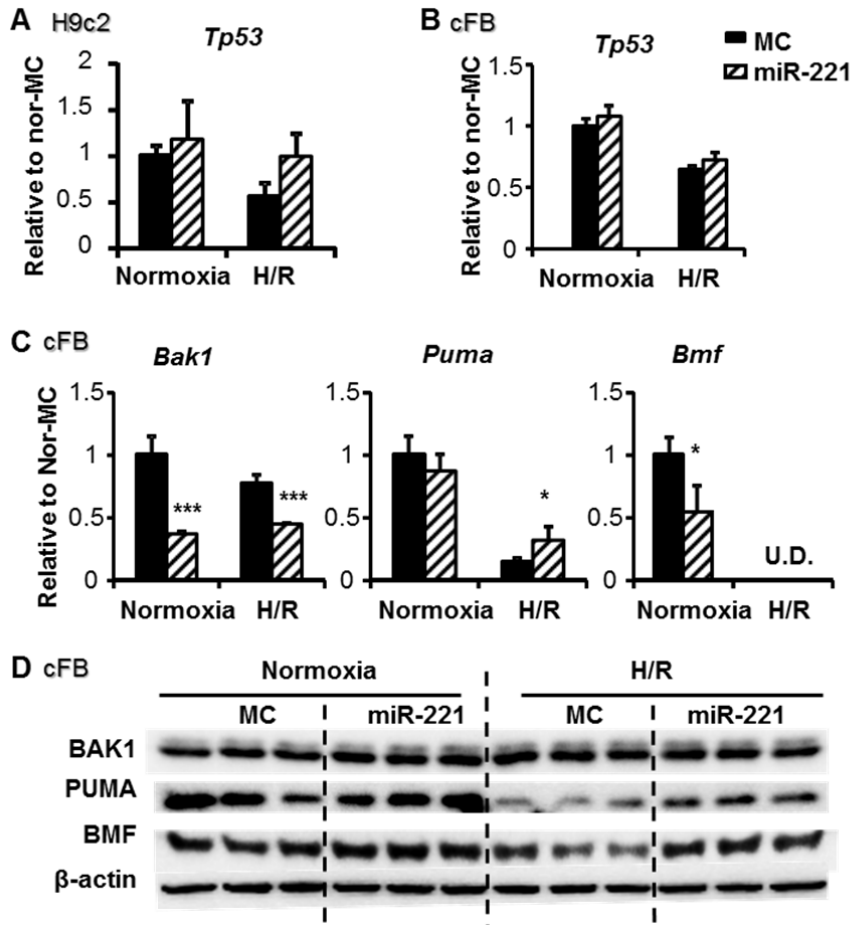
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## Supplemental methods

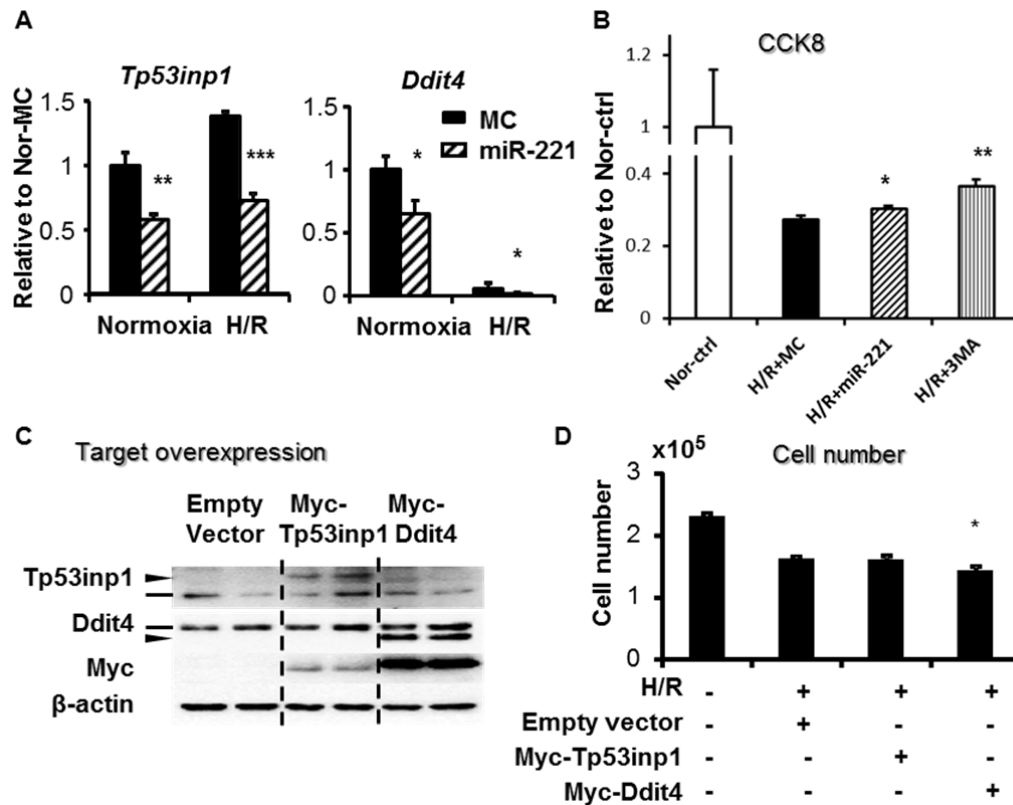
**Supplemental Table 1: Primers used for mRNA RT-qPCR.**

Gene symbol	Forward primer (5'→3')	Reverse primer (5'→3')
<i>Acta2</i> ( $\alpha$ -SMA)	CTGTTATAGGTGGTTTCGTGGA	AGAGCTACGAACTGCCTGA
<i>Actb</i> ( $\beta$ -actin)	GTACAACCTTCTTGCAGCTCCTC	TGACCCATACCCACCATCAC
<i>Bak1</i>	GCCTACGAACTCTTACCAAG	CACGCTGGTAGACATACAGG
<i>Bbc3</i> (Puma)	GCAGTACGAGCGGCGGAGACAA GAAGAGC	CCCTGGGTAAGGGGAGGAGTCCCA TGAAGAG
<i>Bmf</i>	TTGCAGACCAGTTCATCG	CCCTCCCTGTTTTCTCGTC
<i>Colla1</i>	GAGAACCAGCAGAGCCA	GAACAAGGTGACAGAGGCATA
<i>Colla2</i>	CAGCTCCACTCTCACCTG	CAAGCCGGGAGAAAGGG
<i>Col3a</i>	GAATCACCTTGCCTCCAG	GTCCACAAGGATTACAAGGCA
<i>Ddit4</i>	TTGTCCGCAATCTTCGCT	GAAACGATCCCAAAGGCTAGG
<i>Fn1</i>	CACCCTACCAACCTTAATCC	GAAGCGATGACCTCCAGAT
<i>Fn-EDA</i>	GGCAAGTTTCCAGGTACAGG	GCAAGGCAACCACACTGACT
<i>Smad2</i>	ACCATAAGAATGAGCTTCGTGA	GTTAATACTTTGTCCAACCACTGC
<i>Smad3</i>	CATTACCATCCCCAGGTCAC	TGTTGAAGGCGAACTCACAG
<i>Tp53</i>	TGGCAGAACAGCTTATTGAGG	TGTCATCTCCGTCCCTTCT
<i>Tp53inp1</i>	TCCTGGTCTCAGTGAAGCTA	ACAGCAGTGAATGTGCGT

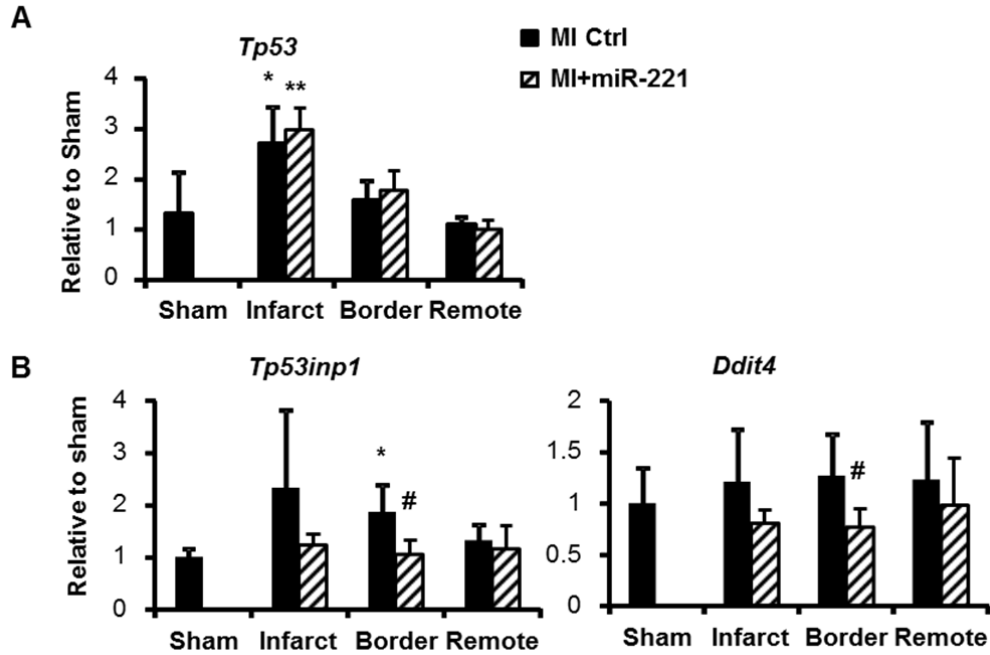
Supplemental Results



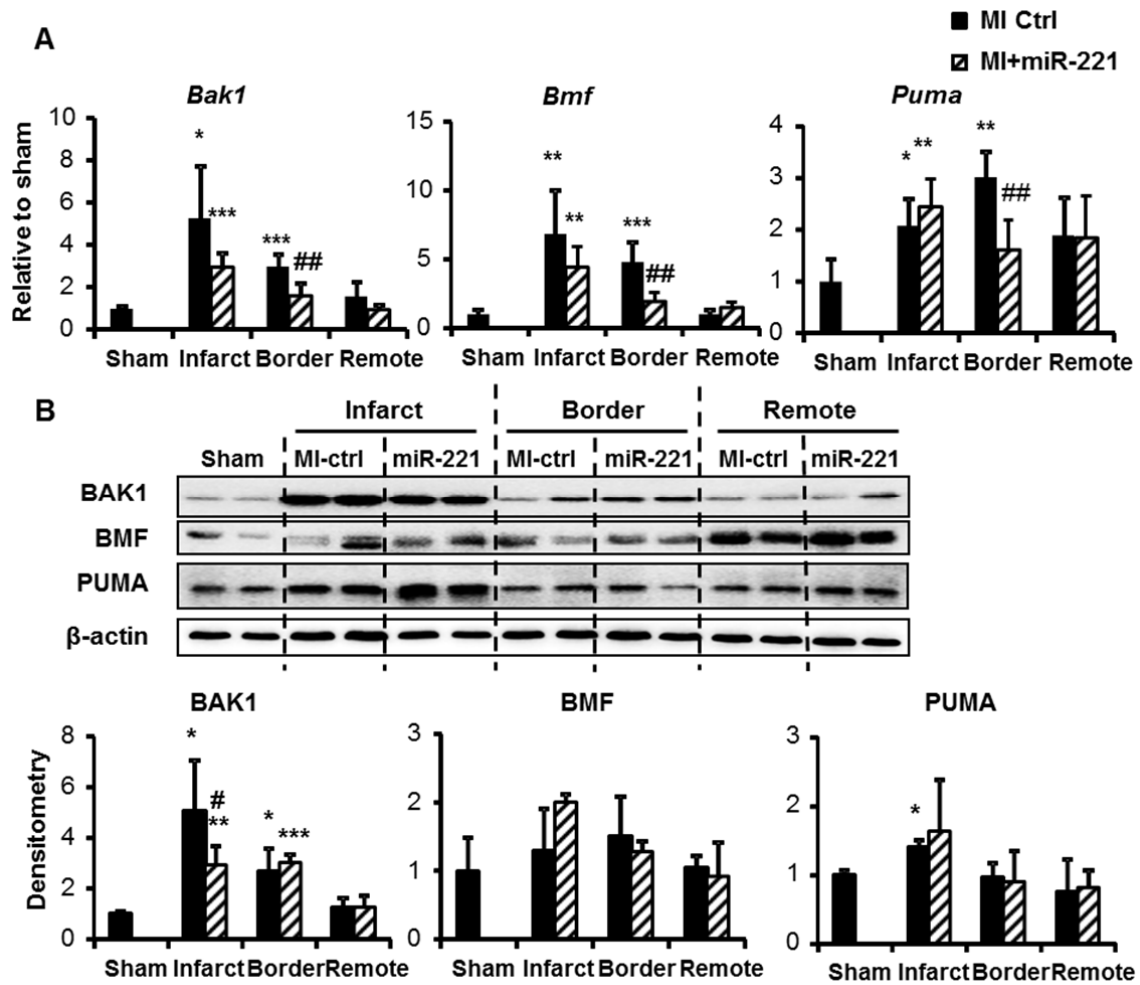
**Suppl. Fig. 1:** miR-221 regulation of apoptosis-related gene targets in myocyte and fibroblast. H9c2 cell line or adult rat cardiac fibroblast (cFB) was transfected with a miR-221 mimic or control (miR-221 and MC) for 24 hr and then subject to hypoxia for 24 h and re-oxygenation of 2 h (H/R). RT-qPCR was used to measure *Tp53* mRNA expression in (A) h9c2 and (B) cFB. Bcl2 family members Bak1, Puma and Bmf were analyzed in cFB using RT-qPCR measurement of mRNA (C) or western blot (D) of protein expression. The miR-221 groups were compared to the matching MC groups, \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .  $n = 3$  in triplicates.



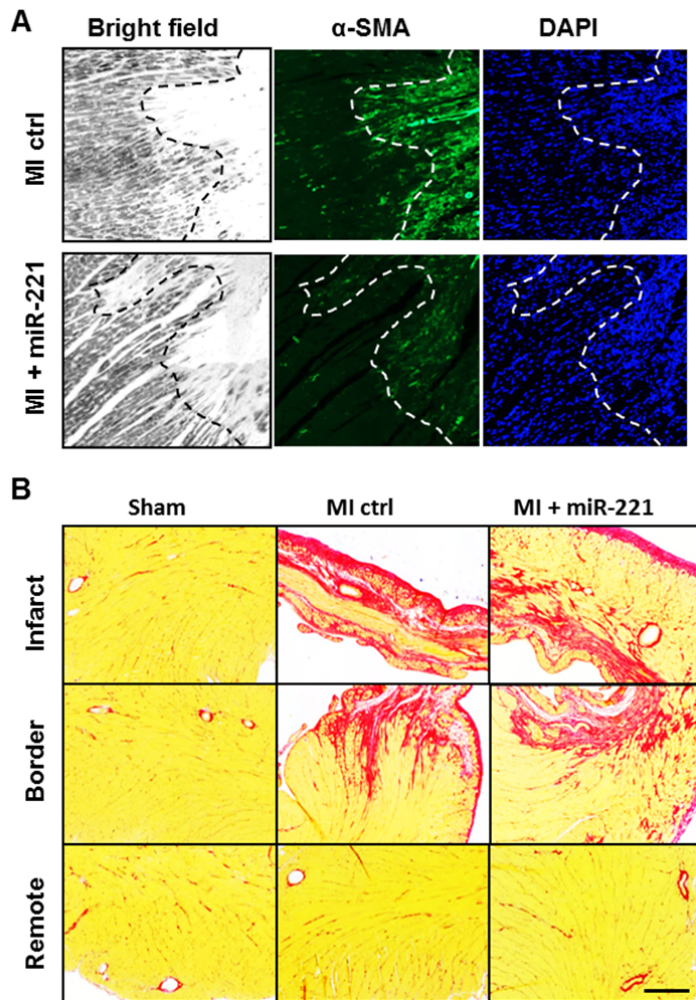
**Suppl. Fig. 2:** MiR-221 protects fibroblasts against hypoxia/re-oxygenation injury through anti-autophagy effects by targeting Ddit4. Adult rat cardiac fibroblast (cFB) was transfected with a miR-221 mimic or control (miR-221 and MC), or treated with 5 mM 3-methyladenine (3MA). The cFB was then subject to hypoxia for 24 h and re-oxygenation of 2 h (H/R). (A) RT-qPCR measurement of *Tp53inp1* and *Ddit4* mRNA expression. (B) Cell viability was then assessed with CCK8 assay. (C) Western blots of cFB lysates 24 h after plasmid transfection to verify overexpression of *Tp53inp1* or *Ddit4*. The straight line indicates the endogenous protein band; arrowhead indicates the overexpressed protein band. (D) Over-expression of *Ddit4*, but not *Tp53inp1*, further reduced cell survival in H/R. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. H/R control.  $n = 3$  in triplicates.



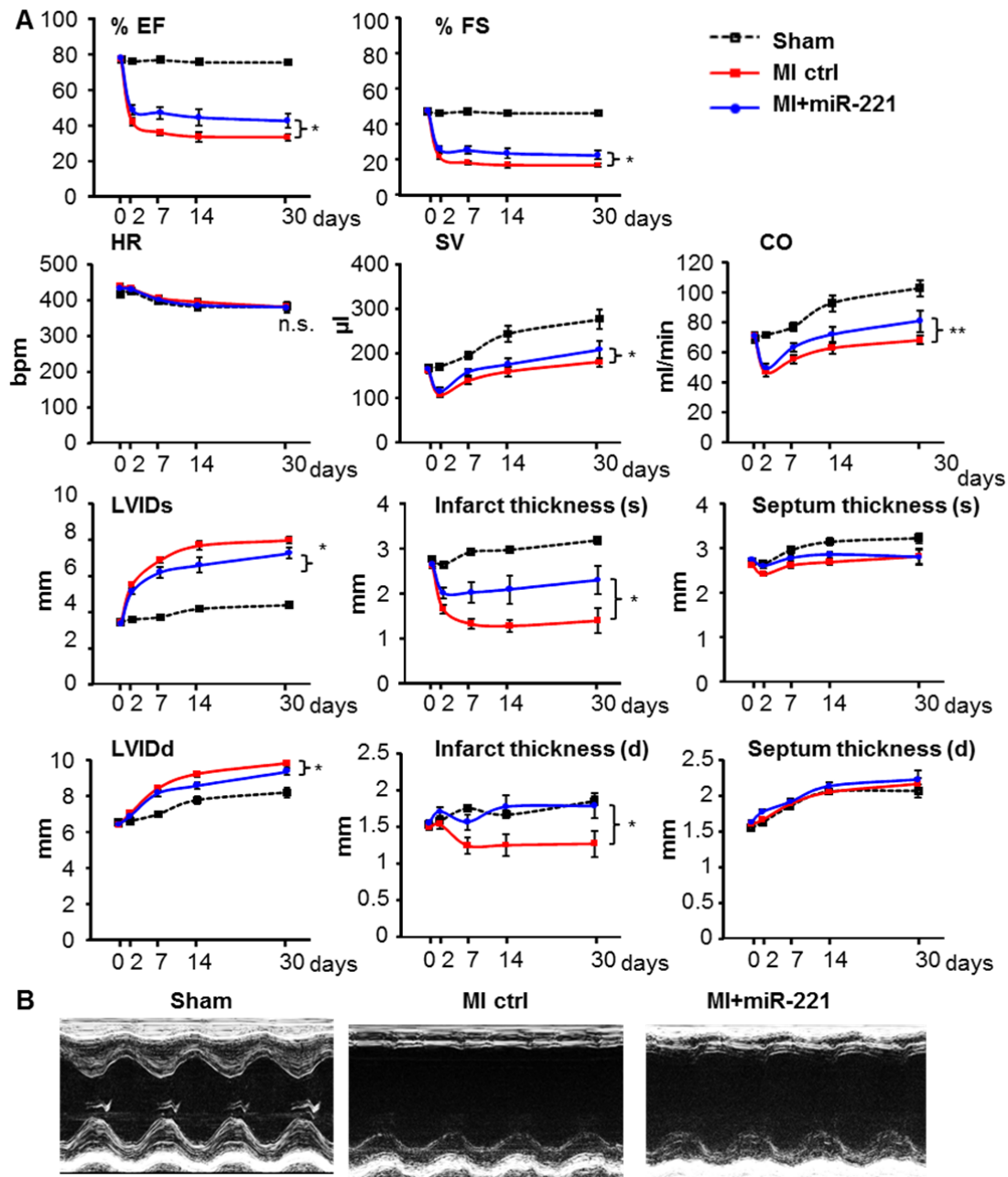
**Suppl. Fig. 3:** MI rats received tail vein injection of 1 mg/kg miR-221 mimic or PBS immediately after LAD ligation (MI +miR-221 and MI-Ctrl). RNA was extracted from heart tissues from day-2 post-MI. RT-qPCR measurement of mRNA expressions for (A) *Tp53*; (B) *Tp53inp1* and *Ddit4*. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. Sham. #  $p < 0.05$  vs. MI-Ctrl.  $n = 4-6$  each group.



**Suppl. Fig. 4:** MI rats treated with 1 mg/kg miR-221 mimic or PBS through tail vein injection immediately after LAD ligation (MI +miR-221 and MI-Ctrl). Heart tissues of day-2 post-MI were collected for RNA and protein extraction. (A) RT-qPCR measurement of *Bak1*, *Bmf* and *Puma* mRNA expression; (B) Western blot and densitometry measurement of Bak1, Bmf and Puma protein expression. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. Sham; #  $p < 0.05$ , ##  $p < 0.01$  vs. MI-Ctrl.  $n = 4-6$  each group.

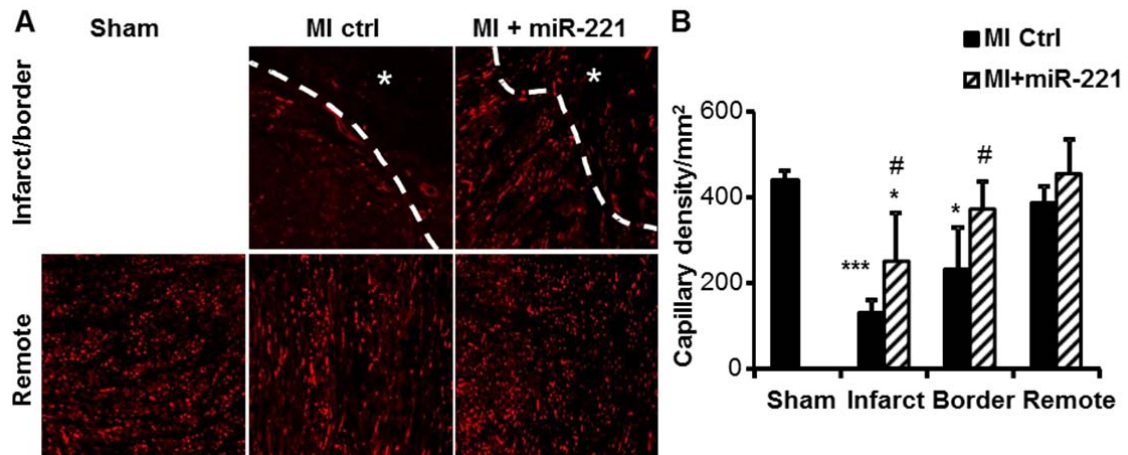


**Suppl. Fig. 5:** The development of myocardium fibrosis and myofibroblast activation at day-7 post-MI. MI rats received tail vein injection of 1 mg/kg miR-221 mimic or PBS immediately and 3-day post-MI (MI +miR-221 and MI-Ctrl). Heart tissues were harvested for histological analysis at day-7 post-MI. (A) Immunohistochemistry staining with  $\alpha$ -SMA antibody (green) and DAPI nuclei stain (blue). Bright field images were used to demarcate the border of the infarcted myocardium. (B) Cardiac fibrosis was assessed by Picro-sirius red staining. LV free wall, septum and the in-between areas from the sham heart were shown in comparison with infarct, remote and border regions of the MI hearts. Scale bar, 200  $\mu$ m. n = 5-8 each group.



**Suppl. Fig. 6:** MI rats were treated with 1 mg/kg miR-221 mimic or PBS through tail vein injection immediately and at day-3 post-MI (MI +miR-221 and MI-Ctrl). LV function was assessed by echocardiography at baseline, 2-, 7-, 14- and 30-day post-MI. (A) Ejection fraction (EF), fraction shortening (FS), heart rate (HR), SV (stroke volume), CO (cardiac output), LV internal dimension (LVID) at systole (s) and diastole (d), infarct and septum thickness at systole (s) and diastole (d). (B) Representative images of M-mode echocardiography at day-30 post-MI. n = 5-14 each group.





**Suppl. Fig. 7:** MiR-221 effects on angiogenesis assessed at day-30 post-MI. (A) Isolectin B4 staining (red fluorescence) was used to visualize capillary density. Auto fluorescence from cardiomyocytes was used to demarcate the border of the infarcted myocardium (dashed line, \* infarct). (B) For each heart section, areas of 2-5 mm<sup>2</sup> were scanned and analysed for infarct and border, areas of 10 mm<sup>2</sup> were analysed for remote and sham. Capillary density was estimated with an arbitrary 100 μm<sup>2</sup> area value per capillary vessel. \*p<0.05, \*\*\* p<0.001 vs. Sham; # p<0.05 vs. MI-Ctrl. n = 4-6 each group.