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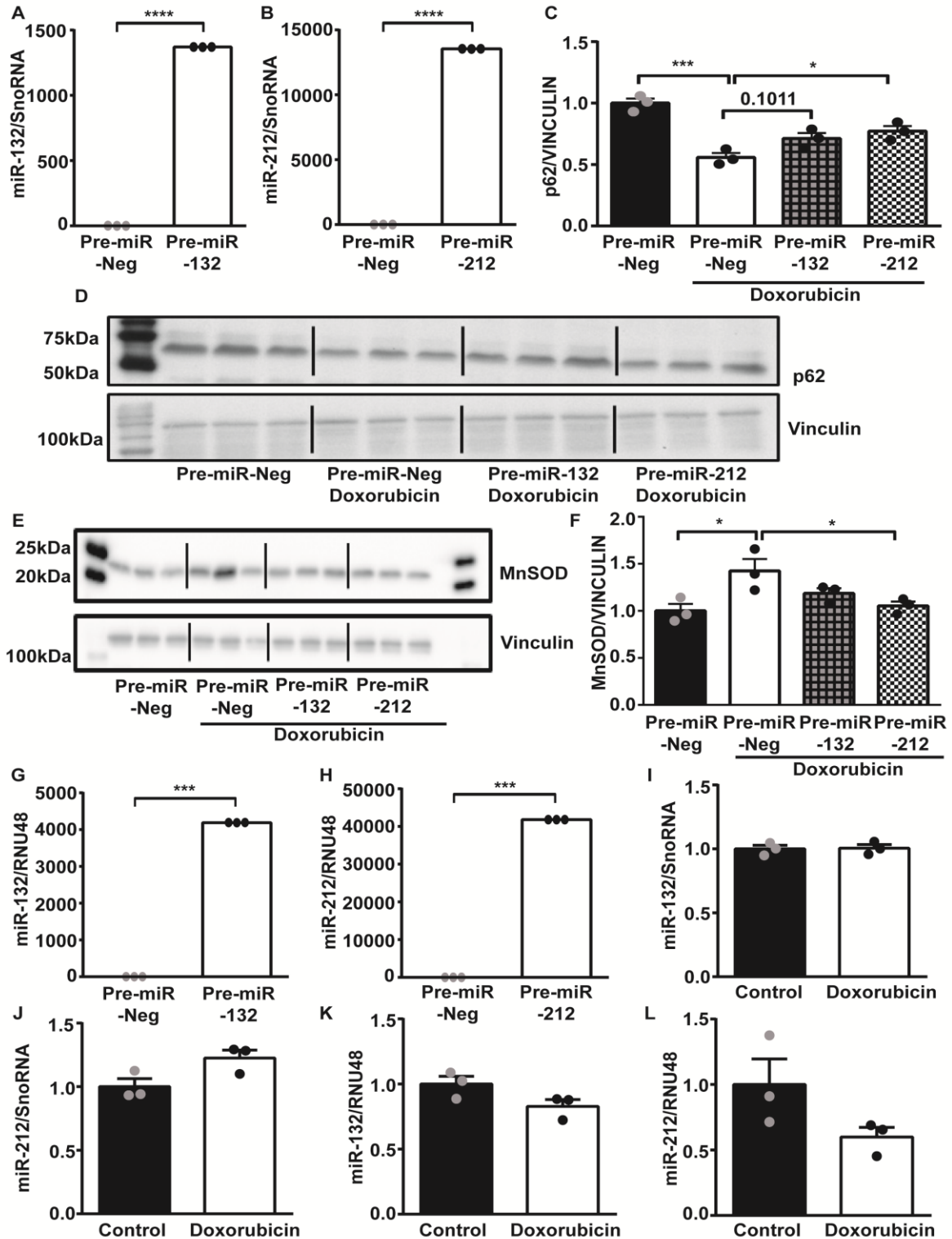
Supplemental Information

miR-212/132 Cluster Modulation Prevents

Doxorubicin-Mediated Atrophy and Cardiotoxicity

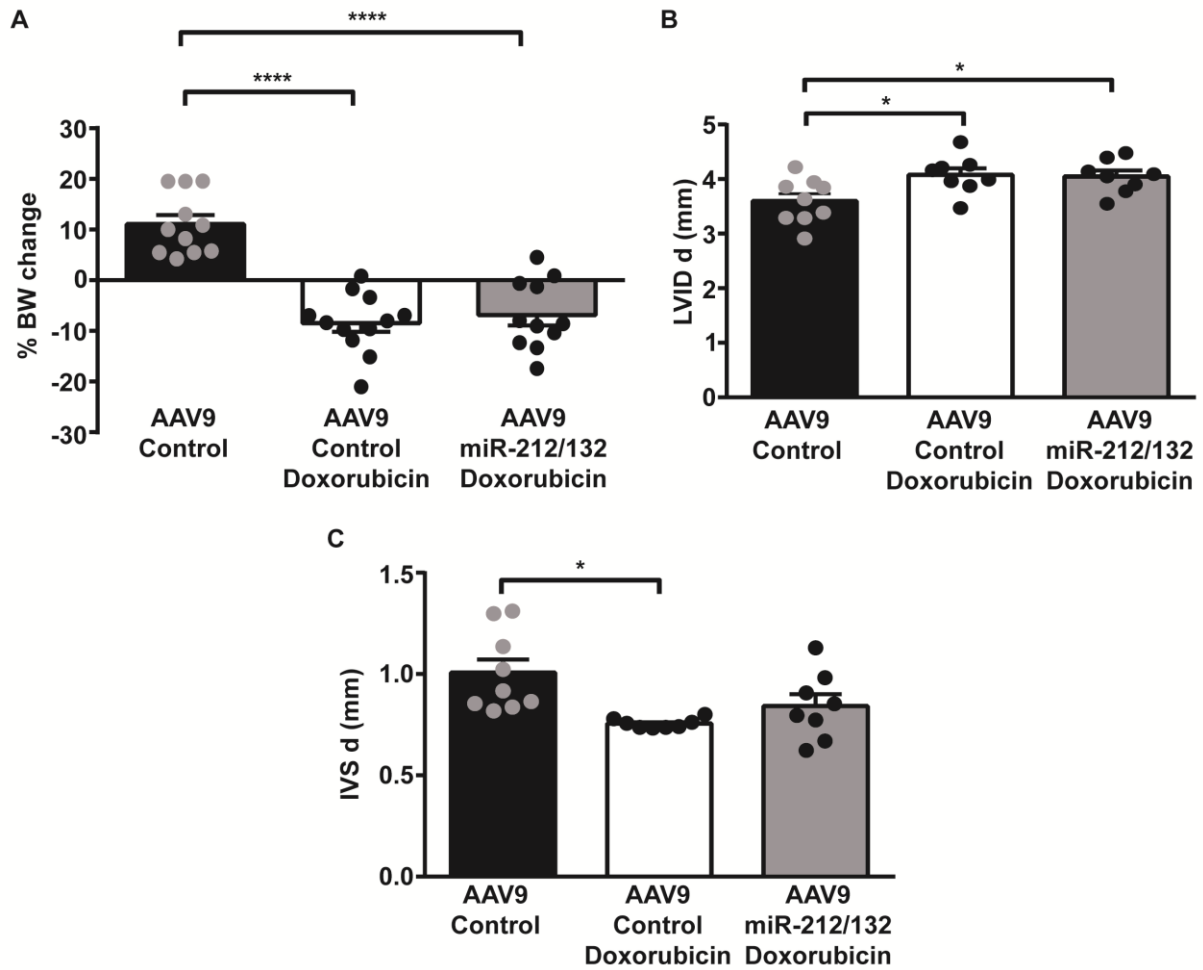
Shashi Kumar Gupta, Ankita Garg, Petros Avramopoulos, Stefan Engelhardt, Katrin Streckfuss-Bömeke, Sandor Batkai, and Thomas Thum

Supplementary Figure 1



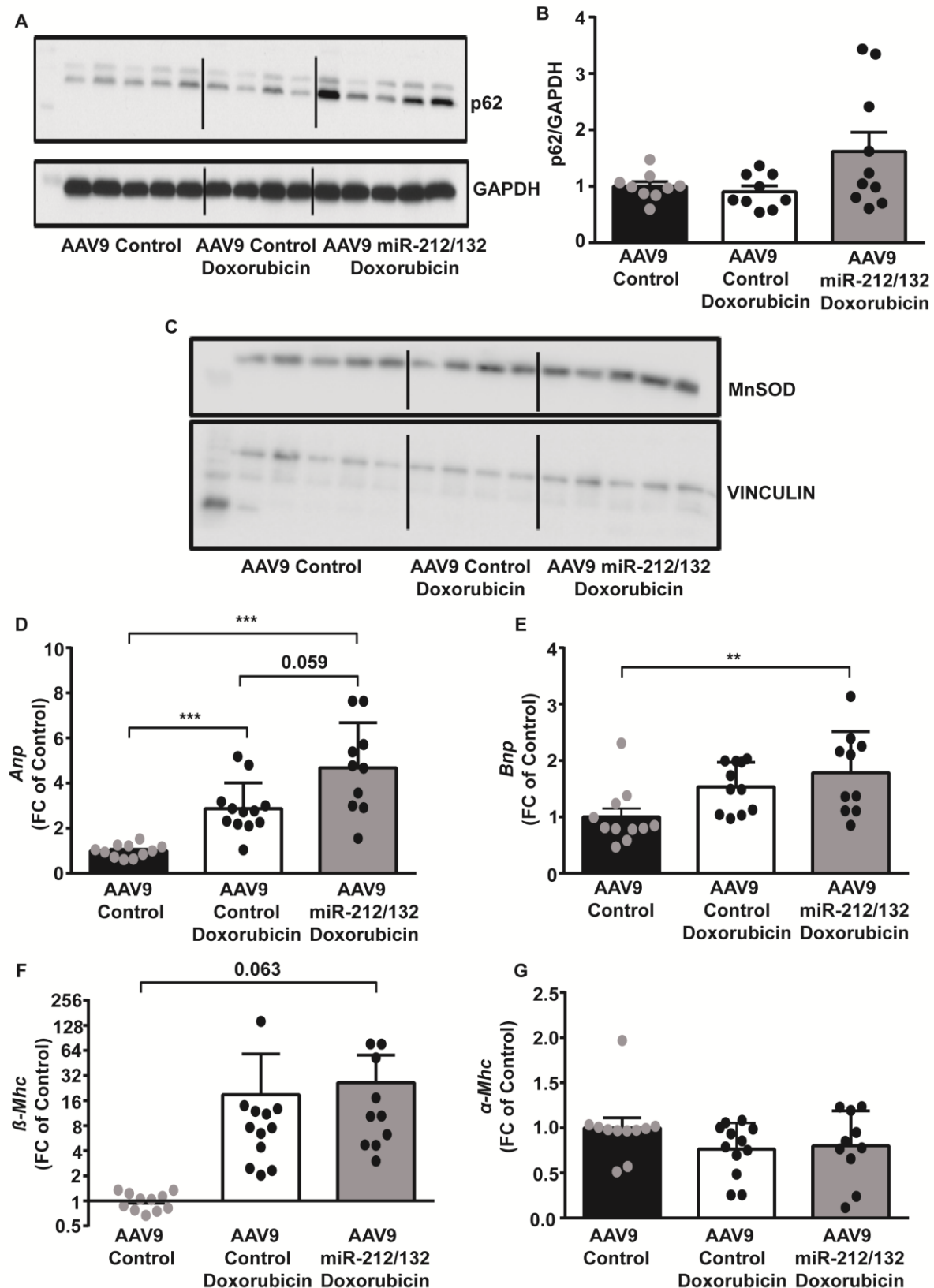
Supplementary Figure 1. Expression levels of miR-132 (A) and miR-212 (B) after Pre-miR transfections in rat primary cardiomyocytes. (C-D) Expression level of autophagic marker p62 in primary rat cardiomyocytes transfected with Pre-miR-212/132 or control. (E-F) Expression level of oxidative stress marker MnSOD in primary rat cardiomyocytes transfected with Pre-miR-212/132 or control. Expression levels of miR-132 (G) and miR-212 (H) after Pre-miR transfections in human iPSC-derived cardiomyocytes. Expression levels of miR-212/132 in response to doxorubicin treatment in primary rat cardiomyocytes (I-J) and hiPSC-derived cardiomyocytes (K-L). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Supplementary Figure 2



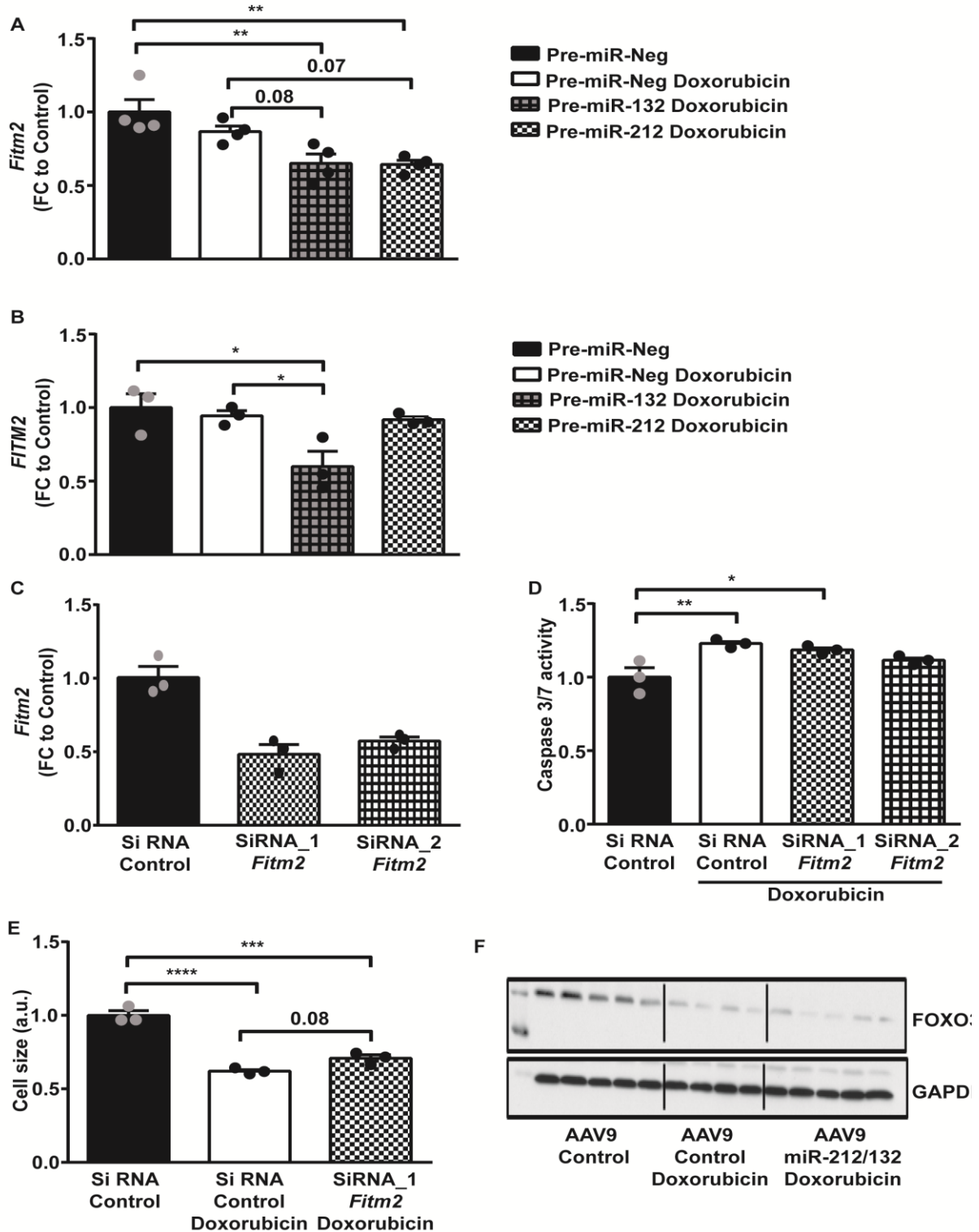
Supplementary Figure 2. (A) Percentage body weight changes, (B) Left ventricular internal diameter diastole (LVID d) and (C) interventricular septal thickness diastole (IVS d) in AAV9-Control healthy animals, AAV9-Control doxorubicin treated animals and AAV9-miR-212/132 doxorubicin treated animals. *p<0.05, ****p<0.0001, BW – body weight, LVID d – Left ventricular internal diameter diastole, IVS d – Interventricular septum diastole

Supplementary Figure 3



Supplementary Figure 3. P62 (A-B) and MnSOD (C) expression in myocardium of AAV9-Control, AAV9-Control treated with doxorubicin and AAV9-miR-212/132 treated with doxorubicin. (D-G) expression levels of cardiac remodeling genes *Anp* (D), *Bnp* (E), β -Mhc (F) and α -Mhc (G) in AAV9-Control, AAV9-Control treated with doxorubicin and AAV9-miR-212/132 treated with doxorubicin. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, FC – fold change

Supplementary Figure 4



Supplementary Figure 4. Expression level of *Fitm2* in primary rat cardiomyocytes (A) and hiPSC-derived cardiomyocytes (B) after overexpression of miR-212/132 and control microRNA together with doxorubicin. (C) *Fitm2* levels after siRNA mediated inhibition in rat primary cardiomyocytes. (D) Caspase 3/7 activity in rat cardiomyocytes after *Fitm2* inhibition in presence of doxorubicin. (E) Primary rat cardiomyocyte cell size after *Fitm2* inhibition in response to doxorubicin compared to control. (F) Expression levels of FOXO3 in hearts of AAV9 Control, AAV9 Control doxorubicin and AAV9-miR-212/132 doxorubicin. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, FC – fold change, a.u. – arbitrary unit

Genes	Primers
<i>Rbfox1</i>	Forward: 5'CGGAGTAGTGTATCAAGAGCCA3' Reverse: 5'CGGTACGCAGCATAACCACC3'
<i>Sgk3</i>	Forward: 5'CCAGTGAGCTTTGTTGCCAGT3' Reverse: 5'TCAGAGCTGGGAATGCTTACA3'
<i>Socs2</i>	Forward: 5'GTTGCCGGAGGAACAGTCC3' Reverse:5'ACTCAATCCGCAGGTTAGTCG3'
<i>Fitm2</i>	Forward: 5'AGCTACCTCAGCAACAAGCG3' Reverse:5'GGTGGTAGTTGGTAAGGGCA3'
<i>L3mbtl3</i>	Forward: 5'GCGCGCACCGGGTTA3' Reverse: 5' TCACAAAACCTGAAGCCCGTCT 3'
<i>Anp</i>	Forward: 5' CCTGTGTACAGTGCCGGTGTC 3' Reverse: 5' CCTAGAAGCACTGCCGTCTC 3'
<i>Bnp</i>	Forward: 5' CTGAAGGTGCTGTCCCAGAT 3' Reverse: 5' GTTCTTTTGTGAGGCCTTGG 3'
<i>Alpha-Mhc</i>	Forward: 5' GGTCCACATTCTTCAGGATTCTC 3' Reverse: 5' GCGTTCCTTCTCTGACTTTTCG 3'
<i>Beta-Mhc</i>	Forward: 5' TCTCCTGCTGTTTCCTTACTTGCT 3' Reverse: 5' CAGGCCTGTAGAAGAGCTGTACTC 3'
<i>Mcip1.4</i>	Forward: 5' AGGGACTTTAGCTACAATTT 3' Reverse: 5' TATGTTCTGAAGAGGGATTC 3'

Supplementary Table 1. List of primers used for mRNA quantification

Downregulated genes	Upregulated genes
Arntl	Gm8649
Btnl9	Gm7816
Tbc1d4	Gm13141
Itgb3	Elovl1
ENSMUST00000139210	NAP063041-1
Sgk3	NAP121332-001
Socs2	Bzw1
Alpk2	Cinp
Chd2	Cox16
Smim3	Setd5
Mmd	Gm5093
Sf3b1	Rpl35a-ps7
Aqp4	Gm6621
Lrrc41	Rimkla
C230085N15Rik	AK079655
Angpt1	TC1725193
Sema6c	Gm11449
Nos1ap	Gm16490
Galnt18	A830080D01Rik
Cd3eap	Ankrd29
Akap13	Gm7867
Tom112	ENSMUST00000141235
BQ939345	Gm7634
Gm13251	Rpl17
Fam78b	Sdf4
Chd6	4930506C21Rik
Kat6b	Pcsk1n
Fitm2	Golga7b
Ceacam2	2700060E02Rik
N4bp2	Ccnd2
Rabgap11	Cdk7
Rcor3	BC051226
Satb1	Cflar
Orai3	NAP111477-1
Slc38a1	Rpl17-ps8
Atl2	Dst
Thns11	Gmcl1
Ncam1	Rpl14
Zfp646	Cyb5r4
LOC100862466	Lcorl
Ablim2	Amot
Erc1	Mir99ahg
Bad	BC017158
3110002H16Rik	Gm31105
Crem	NAP111293-1
Amph	Gm6272
Edn1	Serp1
Btnl9	Odc1
Prkeq	4930512M02Rik
Smco1	Otub2
Fam175b	Gm15843
Lrp3	NAP017864-001
Fzd2	Bex1

Neurl4	Icosl
Prune	Gm13194
Ica1 variant 2	Mapt
Smad4	Hsp90aa1
H1f0	Bcl2
Kitl	Gng12
Tbc1d16	Irf2bpl
Zfp932	Khdrbs1
Npepps	Smim8
Pddc1	Gm14231
4933431K23Rik	A730020M07Rik
Nudt7	Kif1c
Mx2	Gm10161
Aqr	NAP102462-1
L3mbtl3	ENSMUST00000154604
Pigh	NAP069232-1
Med24	Tmem251
1700025G04Rik	Lmo2
Fam19a5	Ehd4
TC1601230	3110001N23Rik
Exoc3l4	Lamtor3
Cdc27	Gm5105
D3Ert254e	Inafm2
Zmynd8	5730419F03Rik
Lats2	D7Wsu130e
ENSMUST00000094300	LOC102639982
Acsl1	Dkk3
Ica1 variant 1	Gm12988
Garem	Rarb
Mief2	Meig1
Gnpda2	Gm14816
Slc25a37	Lonrf1
Dnajc28	NAP026464-1
Mtmr2	Gm8075
Eln	Taf15
Synpo2	Nrn1
Ilf2	Gm14115
Zfp868	TC1779162
H2afy	Gm8317
Atp2a2	Cep112
Fam122b	Terc
Zfp945	Papln
Hnrnpul1	Tk1
A630031M23Rik	4930517G19Rik
Dnmt3b	Cyp26b1
Tjp2	Itgam
Stxbp2	2700022O18Rik
Gys1	Agmat
Det1	Fbxo24
Gstm7	Cpb2
Twistnb	Neurod2
Ammecr1	Figl1
Car2	Gm16982
Arid3b	Oxct2b

Nudt14	Gm14322
Nhej1	Erv3
Psme4	Kctd11
Atf7ip	Wfdc6a
Tcap	
Zfp275	
Stk19	
Tmem183a	
Map1lc3a	
B630005N14Rik	
Pla2g6	
Ablim2	
Shisa5	
Rbfox1	
Tmem63b	
Mbtd1	
Depdc5	
Kctd5	
Mecr	
Gm8675	
Flywch1	
Igfbp4	
Tbc1d20	
Cyp4f17	
Zfp763	
Maob	

Supplementary Table 2. List of all the differentially regulated genes in myocardium of AAV9-miR-212/132 treated hearts with doxorubicin compared to control.