Figure Legends to Supplemental Figures

Supplemental Figure 1. Purity of rat neonatal cardiac fibroblast cell culture. (**A, B**) Immunofluorescence staining of primary rat neonatal cardiac fibroblasts. Cardiac fibroblasts were fixed with 4% PFA and stained with (**A, B**) prolyl 4-Hydroxylase (rFB marker) and either, (**A**) α-smooth muscle actin (smooth muscle marker) or, (**B**) Troponin-I (cardiomyocyte marker) and DAPI (nucleus). (n=3 experiments). (**C-E**) Protein expression of (**C**) fibroblast marker S100A4 and (**D**) DDR2 in neonatal rat cardiac fibroblast lysates and (**E**) protein expression of cardiomyocyte marker Troponin-I in neonatal rat cardiac fibroblast lysates and cardiomyocyte lysate. (**F**) mRNA expression of α-MHC and FSP-1 in neonatal rat cardiac fibroblasts. Data are shown as mean ± SEM. ***P<0.005. (n=3). Scale bar is 50 µm.

Supplemental Figure 2. (**A**) nSmase 2 regulates in part the secretion of miRNAs in cardiac fibroblasts. Cardiac fibroblasts were treated with an inhibitor of nSmase 2 for 48 h and miRNA expression of fibroblast-derived exosomes was measured. miRNA expression is shown as fold change (FC) of control. Control cells were treated with DMSO. miRNA expression was normalized to U1. (**B**) Angiotensin II treatment stimulated the secretion of specific miRNAs. Cardiac fibroblasts were stimulated with angiotensin II (1nM) and IL-1β (4 ng/ml) for 24 h and miRNA expression of fibroblast-derived exosomes was measured. miRNA expression is shown as fold change of control. Control cells were treated with PBS and water/0.1% BSA. miRNA expression was normalized to U1. Data are shown as mean ± SEM. * P<0.05; **P<0.01, (n=3).

Supplemental Figure 3. Depletion of exosomes in cardiac fibroblast conditioned media abolishes cardiomyocyte hypertrophy. Cardiomyocytes were incubated with DMEM + 1% FBS (indicated as control, cardiac fibroblast media), cardiac fibroblast conditioned media containing exosomes (indicated as +exo) or cardiac fibroblast conditioned media depleted of exosomes (indicated as -exo) (ratio 1:3; cardiomyocyte media: cardiac fibroblast conditioned media) for 72 h. Cells were fixed and stained with α-actinin for cell size measurements. Data are shown as mean ± SEM. * P<0.05; **P<0.01. Scale bar is 50 µm.

Supplemental Figure 4. Fibroblast-derived exosomes and miRNAs are taken up by cardiomyocytes. (**A**) Primary rat cardiomyocytes were incubated with PKH26-labeled fibroblast-derived exosomes (red) at 37°C for 30 minutes and 2 h. Cardiomyocytes were fixed and stained for confocal microscopy. The membrane of cardiomyocytes was stained with Wheat Germ Agglutinin Alexa Fluor 488 (WGA, green) and the nucleus was stained with DAPI (blue). Scale bar is 5 µm. (**B**) A co-culture assay was used to study the miRNA transport from cardiac fibroblasts to cardiomyocytes (see Figure 3F). Cardiac fibroblasts were transfected with a Cy3-labeled miRNA (red) or a control precursor miRNA (scrambled, non-labeled), co-cultured with cardiomyocytes for 24 h and confocal microscopy was performed. Cardiomyocytes were stained with alpha-actinin (green) and nucleus with DAPI (blue). Scale bar is 8 µm (scr pre-miR) and 10 µm (Cy3 pre-miR). (**C**) Cardiac fibroblasts were transfected with an inhibitor of miRNA-21* or (**D**) a mimic of cel-miR-39. The expression levels in cardiomyocytes were measured after 72 h (**C** and **D**). Data are shown as mean ± SEM. * $P<0.05$, ($n = 3$).

Supplemental Figure 5. miR-21* associates to Ago2. (**A**) Western blot showing the successful Ago2 pulldown. Total input protein (t.i.) level for Ago2 was monitored. HUVEC lysate was used as positive control (p.c.). Afterwards immunoprecipitation (IP) was conducted with control IgG (cont IP) or Ago2 (Ago2 IP) antibody. Enriched Ago2 in HCF was detected in Ago2 fraction. M indicates marker. (**B**) miR-21* expression was measured after control IP and Ago2 IP. Fold change (FC) to control is shown.

Supplemental Figure 6. Inhibition of miR-21* reduces cardiomyocyte size. (**A**) Cardiomyocytes were transfected with an inhibitor of miR-21 (anti-21), miR-21* (anti-21*) or a control miRNA (scr) for 72 h and cell size was measured. Cardiomyocytes were stained with α-actinin (red) and nucleus was stained with DAPI (blue). Data are shown as mean ± SEM. **$P<0.01$. (**B**) Inhibition of miR-21* attenuates the angiotensin-II induced pro-hypertrophic phenotype in cardiomyocytes. Cardiomyocytes were transfected with an inhibitor of miR-21 (anti-21), an inhibitor of miR-21* (anti-21*) or a control inhibitor (scr) and stimulated with the pro-hypertrophic agent angiotensin II. Control is the incubation with

conditioned media and no angiotensin II. Cardiomyocyte cell size was measured after 72 h. Cardiomyocytes were stained with alpha-actinin (red) and nucleus with DAPI (blue). Scale bar is 50 µm in (**A**) and (**B**). Data are shown as mean ± SEM. *P<0.05 compared to scr, ++P<0.01 compared to anti-21, ###P<0.005 compared to control.

Supplemental Figure 7. Sorbs2 and Pdlim5 are downregulated in cardiomyocytes incubated with miR-21* transfected exosomes. (**A**) mRNA expression of Sorbs2 and (**B**) Pdlim5 in cardiomyocytes either incubated with miR-21* transfected fibroblast exosomes. Cardiomyocytes were incubated with transfected exosomes for 72 h. RNA was isolated and mRNA expression of Sorbs2 and Pdlim5 was measured, (n=3). Fold change (FC) to control is shown.

Supplemental Figure 8. miR-21* is increased in mice with cardiac hypertrophy. In situ PCR was performed in heart tissue sections of Sham-operated and TAC mice showing a higher miR-21* detection signal in cardiomyocytes of TAC mice.

Supplemental Figure 9. Inhibition of miR-21* using antagomir treatment in vivo and in vitro. (**A**) Mice were treated with miR-21* antagomir or scrambled antagomir for 72 h and miR-21* expression was measured in total heart. (**B**) Neonatal cardiomyocytes were either treated with miR-21* or scrambled antagomir for 72 h and miR-21* expression was measured. (n=3).

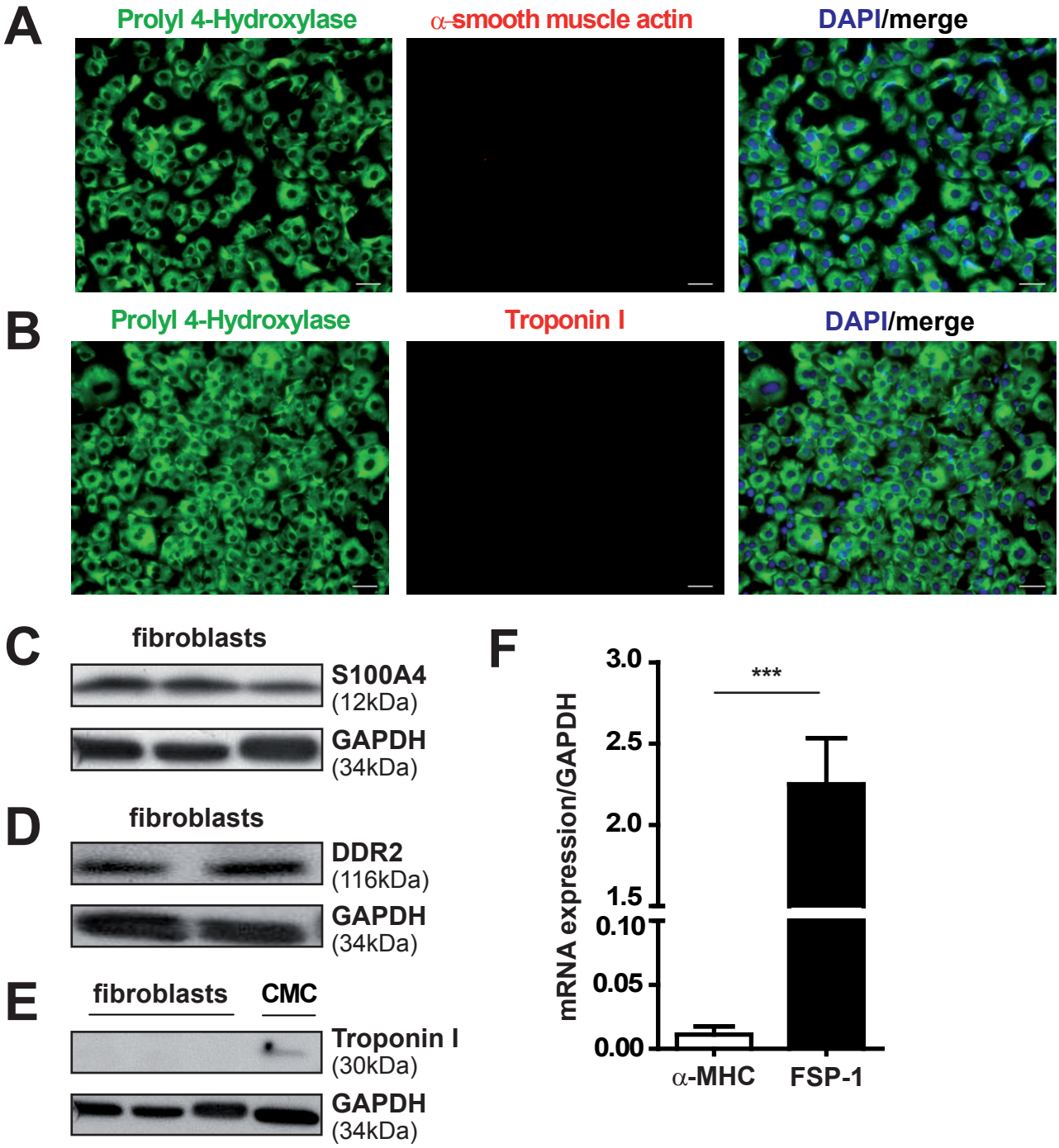
Supplemental Table 1. miRNAs are enriched in fibroblast-derived exosomes. A miRNA-transcriptome profiling assay (388 rat miRNAs) was performed to identify miRNA content in fibroblast-derived exosomes. The mean ratio of exosomes and cells was compared showing 50 miRNAs to be enriched in fibroblast-derived exosomes. Only miRNAs which were detected in all samples and showed a CT<35 are shown.

Supplemental Table 2. De-regulated proteins in cardiomyocytes after transfection of miR-21 and miR-21*. Neonatal cardiomyocytes were transfected with a control precursor miRNA (scr pre-miR), a precursor of miR-21 (pre-miR-21) or a precursor of miR-21* for 72 h. Cardiomyocyte lysates were used for proteome profiling. (n=3 per group). Data are shown as mean.

Supplemental Table 3. Hemodynamic analysis of Sham-operated (Sham) and Angiotensin II (Ang II) minipumps implanted mice treated either with control (scr Ant) or miR-21* antagomir (miR-21* Ant) using pressure-volume catheter system. Data are shown as mean ± SEM. n=5 Sham scr Ant; n=6 Ang II scr Ant; n=5 Ang II miR-21* Ant.

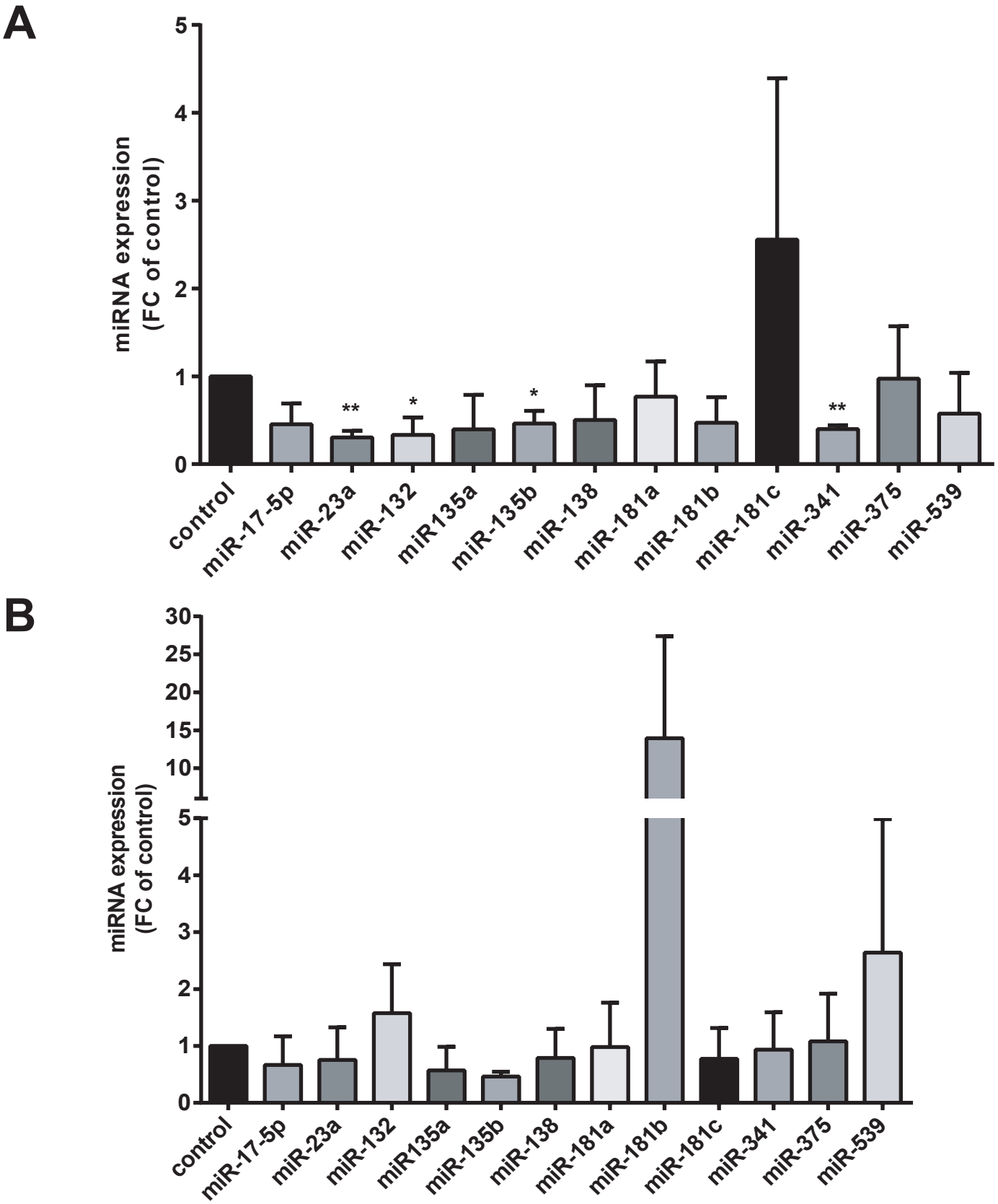
Supplemental Movie 1. Fibroblast-derived exosomes are taken up by cardiomyocytes. Neonatal rat cardiomyocytes were incubated with PKH26-labeled exosomes for 2 h. Cells were fixed, stained and confocal analysis was performed. Cardiomyocyte cell membrane is stained with Wheat Germ Agglutinin Alexa Fluor 488 (green), fibroblast-derived exosomes with PKH26 (red) and nucleus with DAPI (blue). Scale bar as indicated.

Supplemental Figure 1



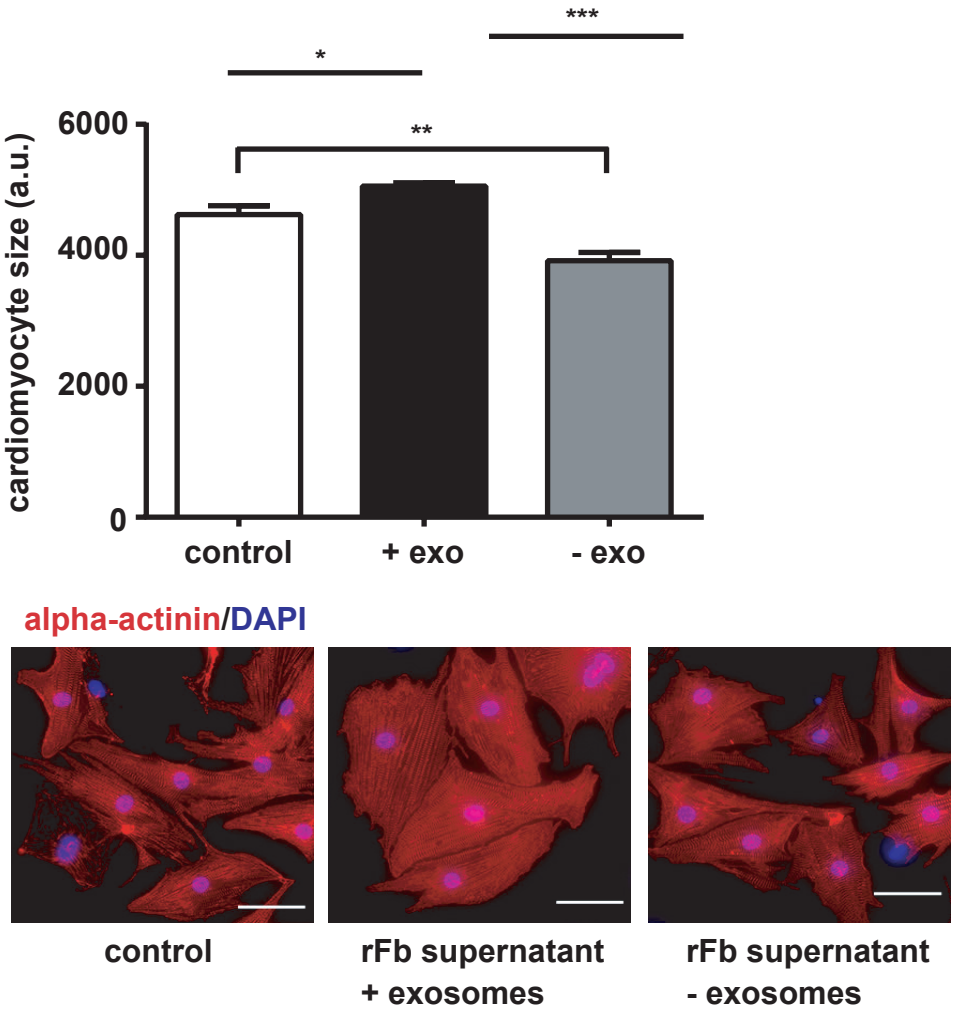
Supplemental Figure 1. Purity of rat neonatal cardiac fibroblast cell culture. (A, B) Immunofluorescence staining of primary rat neonatal cardiac fibroblasts. Cardiac fibroblasts were fixed with 4% PFA and stained with (A, B) prolyl 4-Hydroxylase (rFB marker) and either, (A) alpha-smooth muscle actin (smooth muscle marker) or, (B) Troponin I (cardiomyocyte marker) and DAPI (nucleus). (n=3 experiments). (C-E) Protein expression of (C) fibroblast marker S100A4 and (D) DDR2 in neonatal rat cardiac fibroblast lysates and (E) protein expression of cardiomyocyte marker Troponin I in neonatal rat cardiac fibroblast lysates and cardiomyocyte lysate (CMC). (F) mRNA expression of alpha-MHC and FSP-1 in neonatal rat cardiac fibroblasts. Data are shown as mean ± SEM. ***P<0.005. (n=3). Scale bar is 50 μm.

Supplemental Figure 2



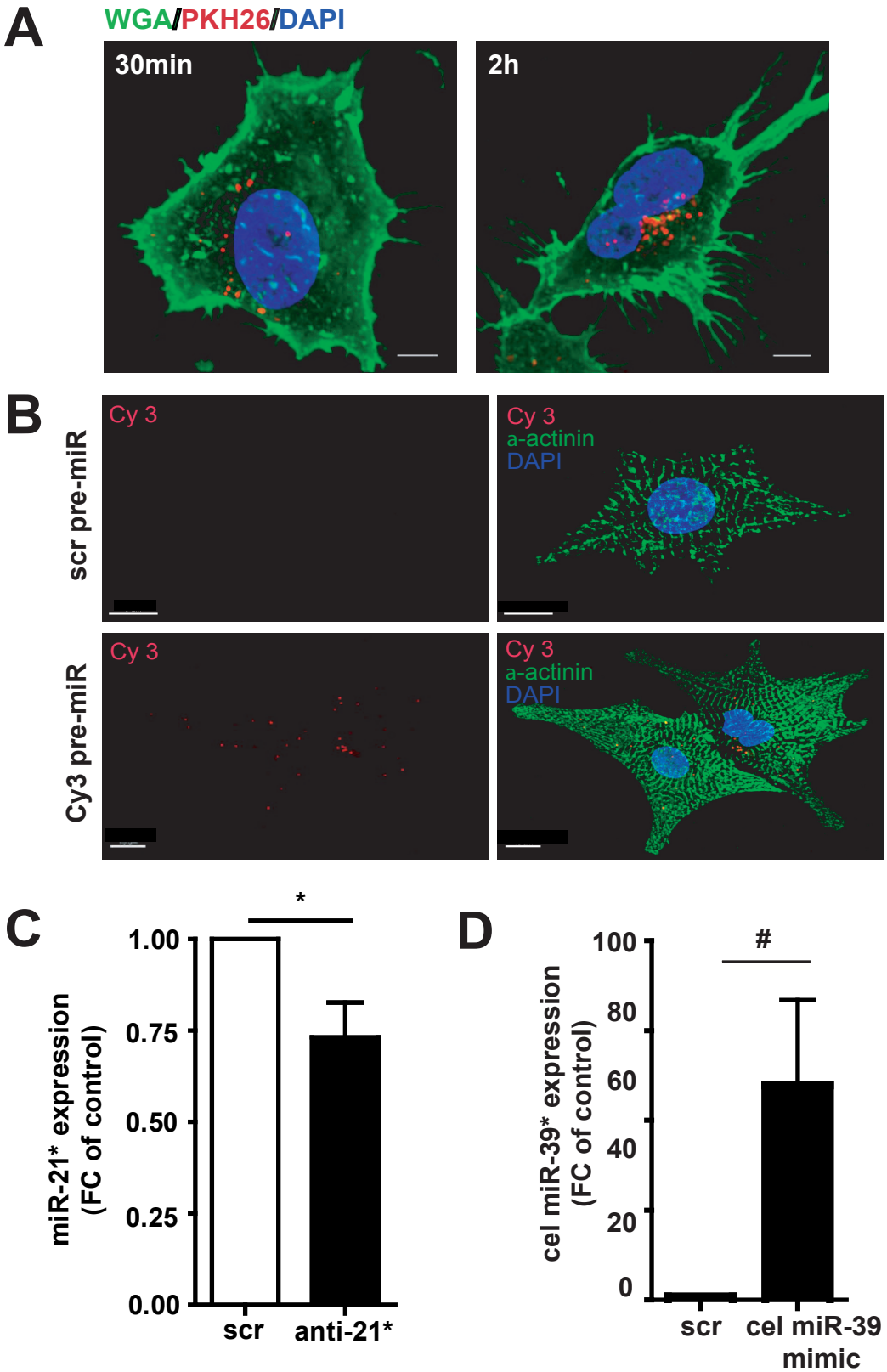
Supplemental Figure 2. (A) nSmase 2 regulates in part the concentration of miRNAs inthe supernatant of cardiac fibroblasts. Cardiac fibroblasts were treated with an inhibitor of nSmase 2 for 48 h and miRNA expression of fibroblast-derived exosomes was measured. miRNA expression is shown as fold change (FC) of control. Control cells were treated with DMSO. miRNA expression was normalized to U1. (B) Angiotensin II treatment stimulated the secretion of specific miRNAs. Cardiac fibroblasts were stimulated with angiotensin II (1nM) and IL-1β (4 ng/ml) for 24 h and miRNA expression of fibroblast-derived exosomes was measured. miRNA expression is shown as fold change of control. Control cells were treated with PBS and water/0.1% BSA. miRNA expression was normalized to U1. Data are shown as mean ± SEM. *P<0.05, **P<0.01, (n=3).

Supplemental Figure 3



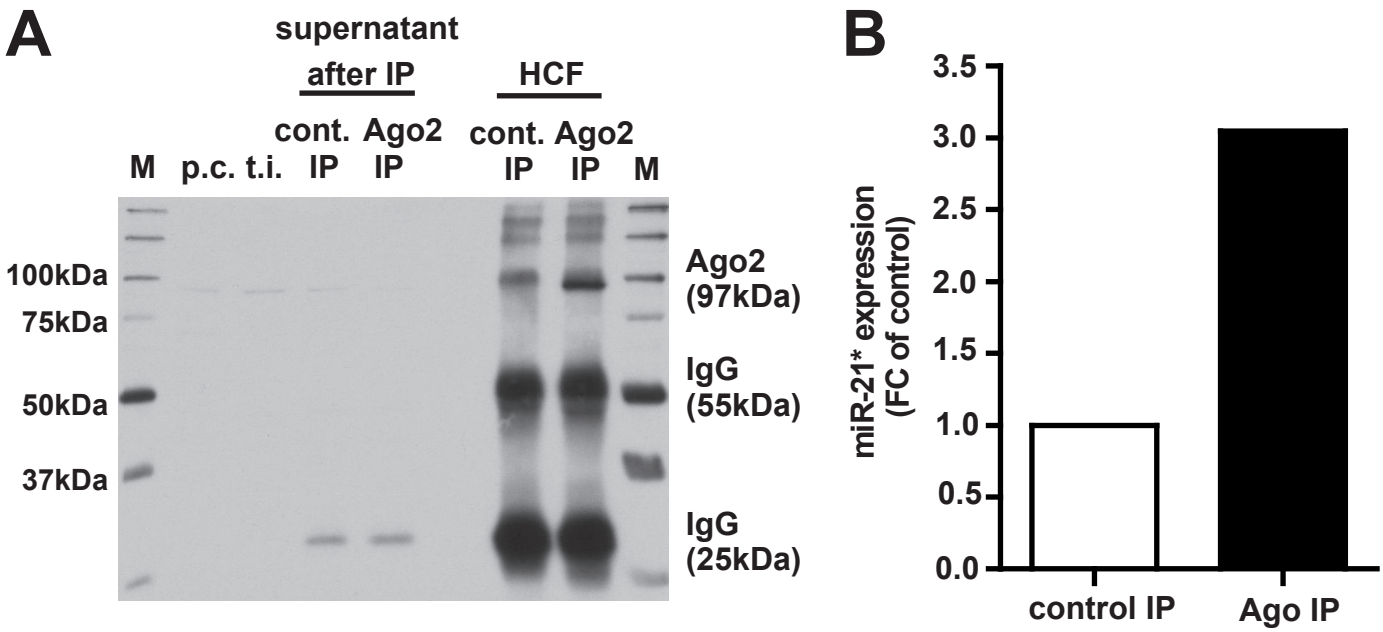
Supplemental Figure 3. Depletion of exosomes in cardiac fibroblast conditioned media abolishes cardiomyocyte hypertrophy. Cardiomyocytes were incubated with DMEM + 1% FBS (indicated as control, cardiac fibroblast media), cardiac fibroblast conditioned media containing exosomes (indicated as +exo) or cardiac fibroblast conditioned media depleted of exosomes (indicated as -exo) (ratio 1:3; cardiomyocyte media: cardiac fibroblast conditioned media) for 72 h. Cells were fixed and stained with alpha-actinin for cell size measurements. Data are shown as mean ± SEM. * P<0.05; **P<0.01; ***P<0.005. rFb= rat fibroblast; Scale bar is 50 µm.

Supplemental Figure 4



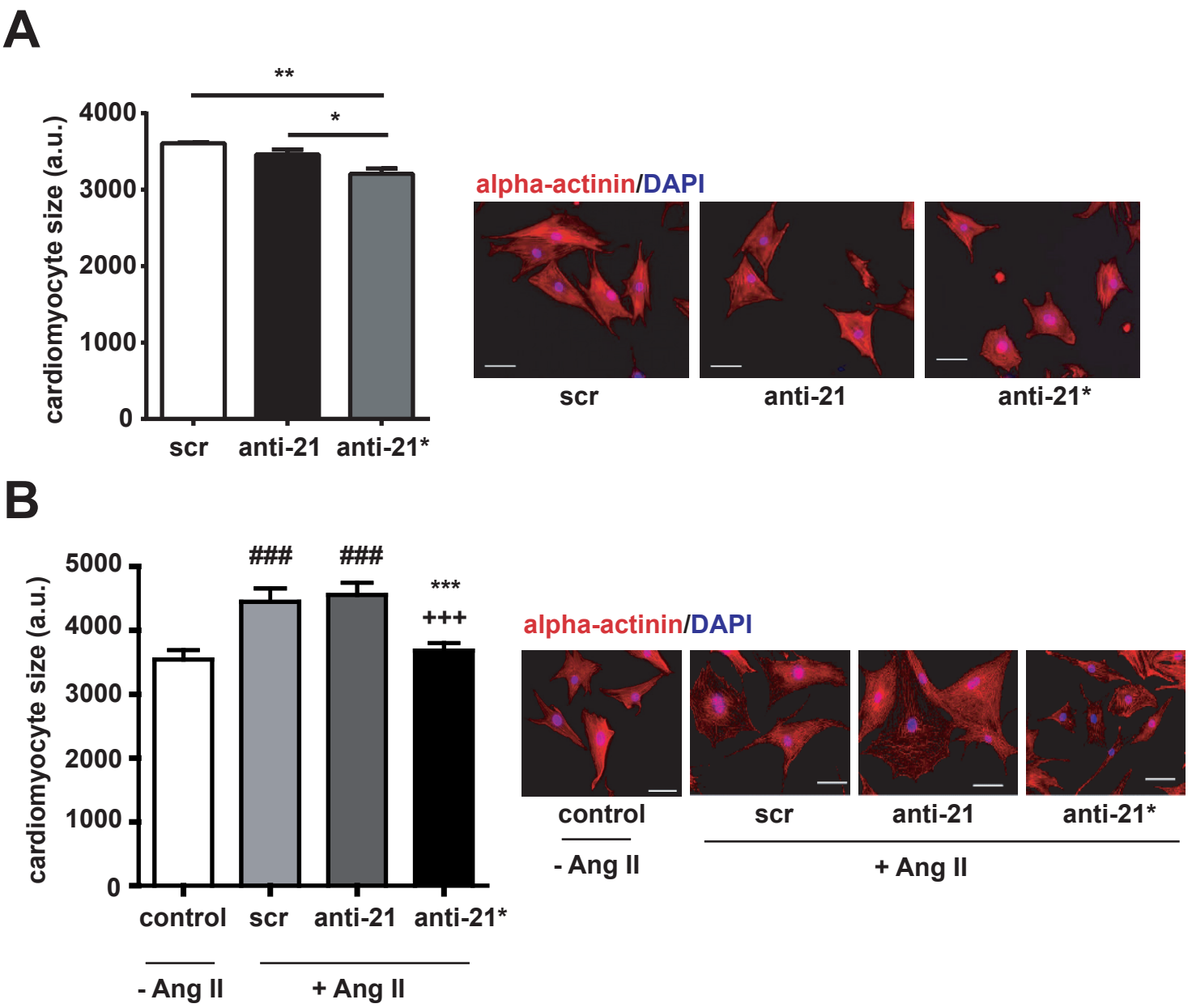
Supplemental Figure 4. Fibroblast-derived exosomes and miRNAs are taken up by cardiomyocytes. (A) Primary rat cardiomyocytes were incubated with PKH26-labeled fibroblast-derived exosomes (red) at 37°C for 30 minutes and 2 h. Cardiomyocytes were fixed and stained for confocal microscopy. The membrane of cardiomyocytes was stained with Wheat Germ Agglutinin Alexa Fluor 488 (WGA, green) and the nucleus was stained with DAPI (blue). Scale bar is 5 µm. (B) A co-culture assay was used to study the miRNA transport from cardiac fibroblasts to cardiomyocytes (see Figure 3F). Cardiac fibroblasts were transfected with a Cy3-labeled miRNA (red) or a control precursor miRNA (scrambled, non-labeled), co-cultured with cardiomyocytes for 24 h and confocal microscopy was performed. Cardiomyocytes were stained with alpha-actinin (green) and nucleus with DAPI (blue). Scale bar is 8 µm (scr pre-miR) and 10 µm (Cy3 pre-miR). (C) Cardiac fibroblasts were transfected with an inhibitor of miRNA-21* or (D) a mimic of cel-miR-39. The expression levels in cardiomyocytes were measured after 72 h (C and D). Data are shown as mean ± SEM. *P<0.05, #P=0.07, (n = 3).

Supplemental Figure 5



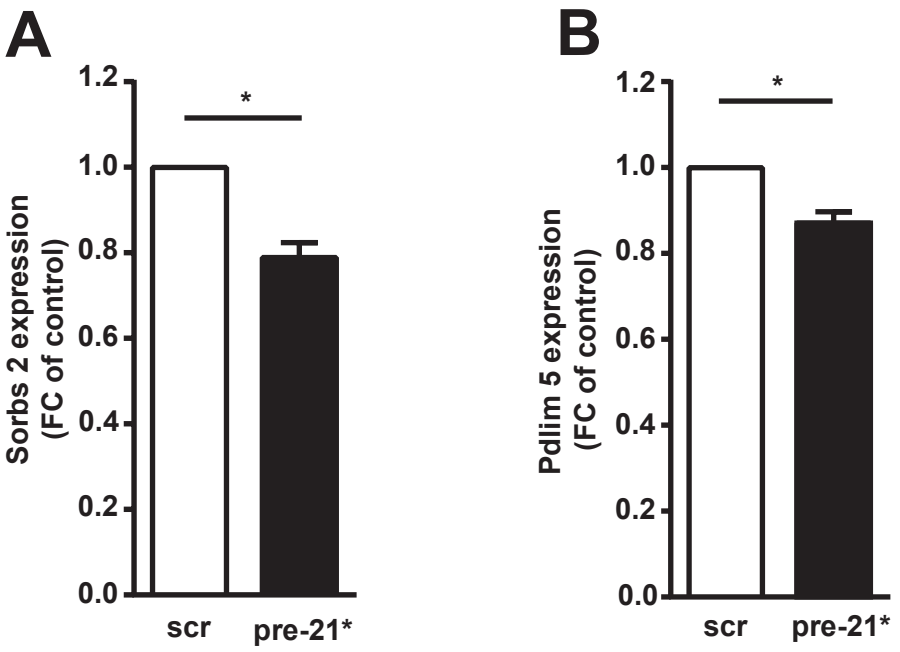
Supplemental Figure 5. miR-21* associates to Ago2. (A) Western blot showing the successful Ago2 pulldown. Total input protein (t.i.) level for Ago2 was monitored. HUVEC lysate was used as positive control (p.c.). Afterwards immunoprecipitation (IP) was conducted with control IgG (cont IP) or Ago2 (Ago2 IP) antibody. Enriched Ago2 in HCF was detected in Ago2 fraction. M indicates marker. (B) miR-21* expression was measured after control IP and Ago2 IP. Fold change (FC) of control is shown. HCF, human cardiac fibroblasts.

Supplemental Figure 6



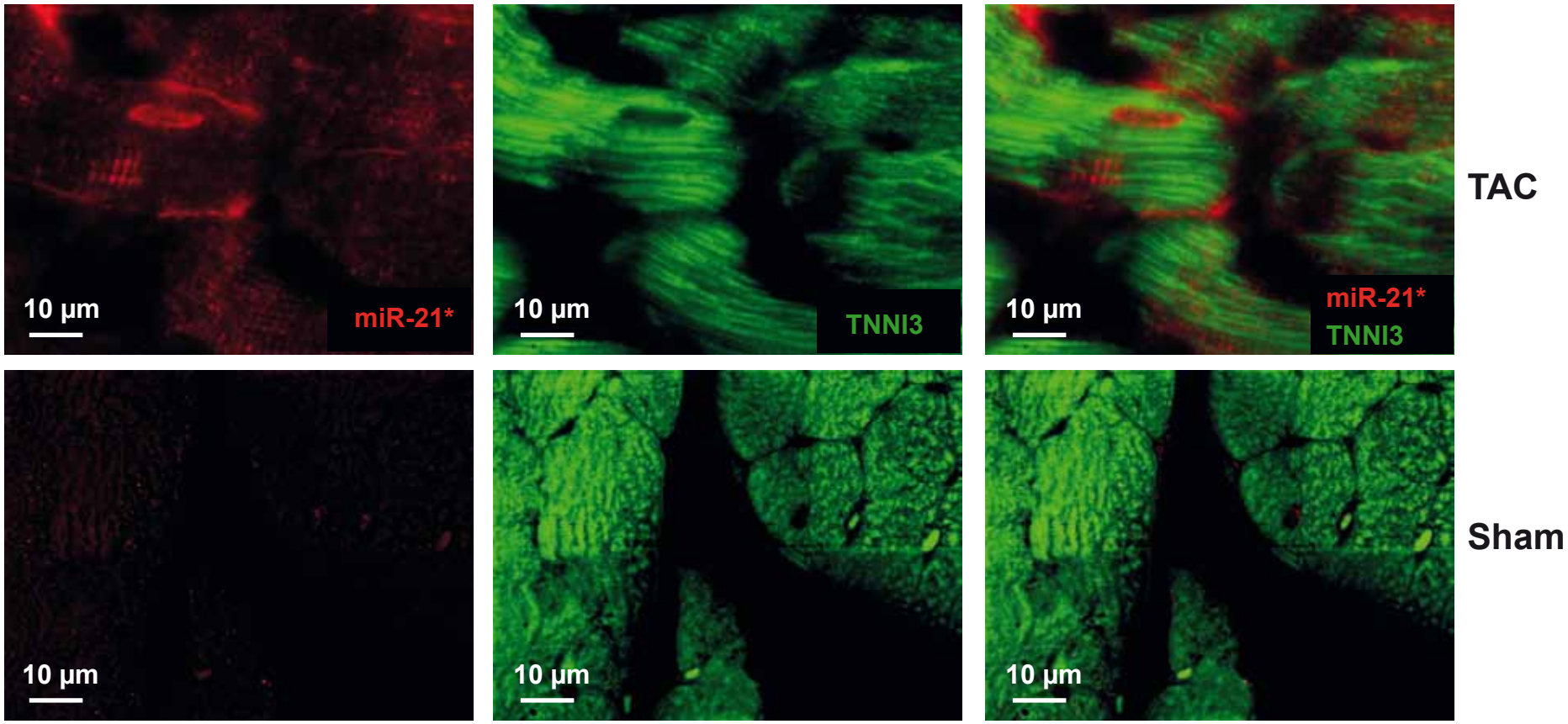
Supplemental Figure 6. Inhibition of miR-21* reduces cardiomyocyte size. (A) Cardiomyocytes were transfected with an inhibitor of miR-21 (anti-21), miR-21* (anti-21*) or a control miRNA (scr) for 72 h and cell size was measured. Cardiomyocytes were stained with alpha-actinin (red) and nucleus was stained with DAPI (blue). (B) Inhibition of miR-21* attenuates the angiotensin-II induced pro-hypertrophic phenotype in cardiomyocytes. Cardiomyocytes were transfected with an inhibitor of miR-21 (anti-21), an inhibitor of miR-21* (anti-21*) or a control inhibitor (scr) for 4h in the presence of the transfection reagent. Afterwards, the transfection reagent was replaced by fresh media (containing 1% FBS) and cardiomyocytes were stimulated with the pro-hypertrophic agent angiotensin II (1 nm) for 72h by adding it directly to the media. Control represents incubation with normal media but no addition of angiotensin II. Cardiomyocyte cell size was measured after 72h. Cardiomyocytes were stained with alpha-actinin (red) and nucleus with DAPI (blue) and cell sizes were measured. Scale bar is 50 µm in (A) and (B). Data are shown as mean ± SEM. *P<0.05, **P<0.01, ***P<0.005 compared to scr, +++P<0.005 compared to anti-21, ###P<0.005 compared to control.

Supplemental Figure 7



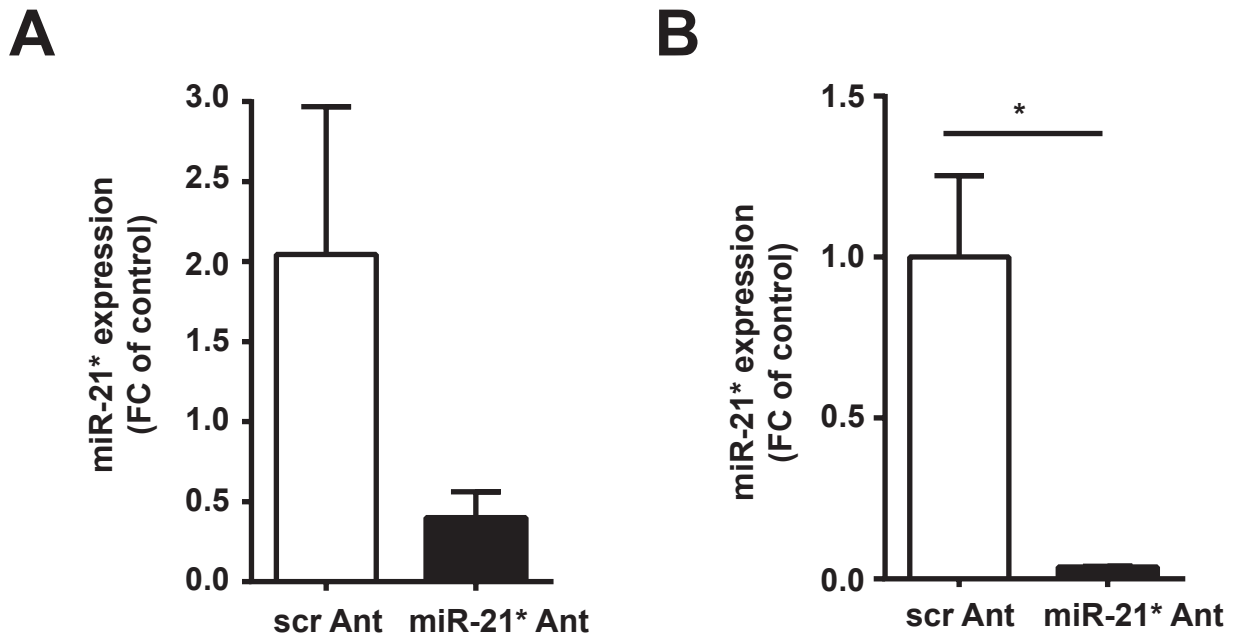
Supplemental Figure 7. Sorbs2 and Pdlim5 are downregulated in cardiomyocytes incubated with miR-21* transfected exosomes. (A) mRNA expression of Sorbs2 and (B) Pdlim5 in cardiomyocytes either incubated with miR-21* transfected fibroblast exosomes. Cardiomyocytes were incubated with transfected exosomes for 72 h. RNA was isolated and mRNA expression of Sorbs2 and Pdlim5 was measured, (n=3). Fold change (FC) to control is shown.

Supplemental Figure 8



Supplemental Figure 8. miR-21* is increased in mice with cardiac hypertrophy. In situ PCR was performed in heart tissue sections of Sham-operated and TAC mice showing a higher miR-21* detection signal in cardiomyocytes of TAC mice. Cardiomyocytes were stained with Troponin I (TNNI3, green).

Supplemental Figure 9



Supplemental Figure 9. Inhibition of miR-21* using antagomir treatment in vivo and in vitro. (A) Mice were treated with miR-21* antagomir or scrambled antagomir for 72 h and miR-21* expression was measured in total heart. (B) Neonatal cardiomyocytes were either treated with miR-21* or scrambled antagomir for 72 h and miR-21* expression was measured. (n=3).

Supplemental Table 1. miRNAs enriched in fibroblast-derived exosomes

miRNA	Mean ratio exosomes/cells
let-7b star	19,31
let-7d star	24,53
miR-9 star	302,26
miR-17-5p	19,07
miR-20a	2,71
miR-21 star	14,90
miR-23a	0,39
miR-23b	0,57
miR-25	8,14
miR-30a star	8,66
miR-30c	1,04
miR-30e star	12,00
miR-34c star	3,33
miR-92b	7,00
miR-125a-5p	3,68
miR-129	24,96
miR-132	22,50
miR-133a	70,30
miR-135a	173,22
miR-135b	61,77
miR-138	33,39
miR-139-3p	1,17
miR-139-5p	13,46
miR-140	19,78
miR-181a	57,32
miR-181b	4,63
miR-181c	1098,61
miR-214	1,95
miR-320	2,75
miR-323 star	1,83
miR-330 star	65,01
miR-339-3p	41,31
miR-341	71,97
miR-345-5p	2,73
miR-346	68,14
miR-347	1,40
miR-365	16,29
miR-375	82,96
miR-494	0,43
miR-505	10,52
miR-532-3p	15,63
miR-539	1,96
miR-671	52,33
miR-770	15,94
miR-1224	1,63
miR-547	34153,94
miR-665	71,91
miR-666	1,91
miR-667	2,56
miR-685	15,35

Supplemental Table 2. De-regulated proteins in cardiomyocytes after transfection of miR-21 and miR-21*

Proteins	Accession Number	scr pre-miR	pre-miR-21	pre-miR-21*	ANOVA Test (P-Value)
Leucyl-cystinyl aminopeptidase	LCAP	5,00	1,00	0,00	0,00036
Retinol-binding protein 1	RET1	0,33	2,67	0,00	0,0013
Filamin-A	FLNA	58,00	61,33	35,00	0,0018
40S ribosomal protein S19	RS19	8,67	5,33	8,67	0,0019
Beta-enolase	ENOB	12,33	10,00	17,00	0,0025
Endoplasmic reticulum resident protein 29	ERP29	0,00	1,67	0,00	0,0026
Sarcalumenin	SRCA	13,33	2,00	10,33	0,0029
Eukaryotic initiation factor 4A-II	IF4A2	6,67	8,00	1,00	0,0032
Protein-glutamine gamma-glutamyltransferase 2	TGM2	32,00	27,67	43,00	0,0044
Troponin T, cardiac muscle	TNNT2	44,00	36,67	33,00	0,0049
Ornithine aminotransferase, mitochondrial	OAT	6,67	2,33	8,67	0,0051
Galectin-3-binding protein	LG3BP	1,00	4,00	0,33	0,0053
Sodium/calcium exchanger 1	NAC1	1,33	3,33	6,33	0,0058
Myosin light chain 3	MYL3	22,67	37,00	32,67	0,0065
Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	ACADV	11,67	15,67	22,00	0,0089
60S ribosomal protein L18a	RL18A	3,67	5,67	8,33	0,01
Reticulon-3	RTN3	1,67	6,33	4,67	0,011
Tropomyosin beta chain	TPM2	16,33	13,00	1,67	0,012
GTP-binding nuclear protein Ran	RAN	14,00	8,67	9,00	0,013
Desmin	DESM	115,00	105,00	90,67	0,013
Fibronectin	FINC	4,00	1,33	0,00	0,015
D-dopachrome decarboxylase	DOPD	6,00	2,67	7,00	0,017
Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	SUCB1	5,67	9,00	9,00	0,017
Calpain-2 catalytic subunit	CAN2	0,67	3,00	5,00	0,018
2,4-dienoyl-CoA reductase, mitochondrial	DECR	3,33	5,33	6,33	0,018
PDZ and LIM domain protein 3	PDLI3	2,00	3,67	1,67	0,019
Nucleobindin-1	NUCB1	0,00	0,67	2,67	0,019
Mitochondrial import inner membrane translocase subunit TIM50	TIM50	3,67	1,33	1,33	0,02
Transmembrane protein 65	TMM65	0,67	3,67	2,00	0,023
Hsc70-interacting protein	F10A1	3,00	3,33	5,00	0,025
Nucleoside diphosphate kinase B	NDKB	16,33	26,67	19,33	0,025
Importin-5 OS=Mus musculus	IPO5	16,33	10,67	6,00	0,026
Heat shock cognate 71 kDa protein	HSP7C	129,67	117,00	134,00	0,026
Alpha-enolase	ENOA	47,67	37,00	46,33	0,027
Proteasome subunit alpha type-7	PSA7	2,67	6,67	6,33	0,028
Myotrophin	MTPN	4,33	2,00	5,33	0,028
60S ribosomal protein L7a	RL7A	6,67	12,00	11,00	0,028
Heterogeneous nuclear ribonucleoprotein L	HNRPL	3,00	6,00	2,00	0,029
Chloride intracellular channel protein 4	CLIC4	2,00	4,00	2,67	0,029
Golgi apparatus protein 1	GSLG1	2,00	5,67	4,00	0,03
Hydroxyacylglutathione hydrolase, mitochondrial	GLO2	0,33	3,00	2,00	0,03
Protein NipSnap homolog 2	NIPS2	2,00	6,00	5,33	0,031
Pyruvate kinase isozymes M1/M2	KPYM	68,00	64,33	78,33	0,032
F-actin-capping protein subunit alpha-1	CAZA1	0,00	3,00	0,00	0,033
Protein disulfide-isomerase A6	PDIA6	5,67	6,67	1,67	0,034
Peroxiredoxin-4	PRDX4	0,67	1,67	4,00	0,034
Annexin A7	ANXA7	0,67	2,67	1,00	0,035
Ras-related protein Rab-5C	RAB5C	0,33	0,67	2,33	0,036
Trifunctional enzyme subunit alpha, mitochondrial	ECHA	51,33	58,67	71,33	0,037
ADP-ribosylation factor 1	ARF1	5,33	5,67	7,00	0,037
Myosin-6	MYH6	1131,33	977,67	964,67	0,038
40S ribosomal protein S18	RS18	15,00	22,67	19,67	0,039
Peptidyl-prolyl cis-trans isomerase B	PPIB	12,33	9,00	9,67	0,043
Caprin-1	CAPR1	4,33	2,33	0,67	0,044
GMP reductase 1	GMPR1	2,00	0,67	3,33	0,044
Methylmalonate-semialdehyde dehydrogenase [acylating], mitoch.	MMSA	2,00	0,67	4,00	0,045
Ribonuclease inhibitor	RINI	5,33	4,67	10,67	0,047
Glycogen phosphorylase, brain form	PYGB	1,67	6,00	8,00	0,049
Ubiquitin thioesterase OTUB1	OTUB1	1,00	4,00	2,00	0,05
4-trimethylaminobutyraldehyde dehydrogenase	AL9A1	2,67	5,67	7,33	0,05
Calcium-binding mitochondrial carrier protein Aralar1	CMC1	0,33	4,00	5,00	0,051
40S ribosomal protein S2	RS2	12,67	18,33	11,00	0,052
Galectin-3	LEG3	0,67	2,00	0,00	0,052
60S ribosomal protein L13a	RL13A	3,33	5,00	7,00	0,053
Stress-70 protein, mitochondrial	GRP75	41,00	41,33	30,33	0,054
Desmoplakin	DESP	1,67	2,00	0,33	0,056
Cytochrome b-c1 complex subunit 8	QCR8	5,00	3,00	6,67	0,056
Splicing factor, proline- and glutamine-rich	SFPQ	1,67	3,67	6,67	0,058
Filamin-C OS=Mus musculus	FLNC	190,67	161,00	151,67	0,058
Ribosome-binding protein 1	RRBP1	3,00	6,00	5,00	0,059
Acyl-coenzyme A thioesterase 13	ACO13	1,00	3,33	0,67	0,061
Nuclease-sensitive element-binding protein 1	YBOX1	13,67	20,67	9,33	0,062
Glucosidase 2 subunit beta O	GLU2B	5,67	9,33	8,33	0,062
3-hydroxyacyl-CoA dehydrogenase type-2	HCD2	1,33	5,00	6,33	0,063
Protein transport protein Sec31A	SC31A	2,67	6,00	2,67	0,063
40S ribosomal protein S17	RS17	5,00	6,00	7,67	0,067
Keratin, type II cytoskeletal 1	K2C1	3,67	12,00	14,00	0,068
Proteasome subunit beta type-2	PSB2	2,67	0,33	1,67	0,069
Sorbin and SH3 domain-containing protein 2	SRBS2	13,67	22,33	6,33	0,069
40S ribosomal protein S26	RS26	5,33	8,33	12,00	0,07
Heat shock protein 75 kDa, mitochondrial	TRAP1	3,00	4,67	8,67	0,07
Cytochrome c1, heme protein, mitochondrial	CY1	19,00	13,33	21,33	0,071
26S proteasome non-ATPase regulatory subunit 7	PSD7	1,00	3,00	1,67	0,072
Thioredoxin-dependent peroxide reductase, mitochondrial	PRDX3	2,00	3,00	6,00	0,072
Insulin-degrading enzyme	DIE	1,67	1,33	4,00	0,073
Cytochrome c oxidase subunit 5A, mitochondrial	COX5A	4,00	7,00	8,67	0,073
GMP synthase [glutamine-hydrolyzing]	GUAA	0,00	2,33	2,67	0,073
Trifunctional enzyme subunit beta, mitochondrial	ECHB	27,67	31,00	35,00	0,073
SPARC	SPRC	4,67	3,67	6,33	0,082
Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	IDH3A	14,67	11,33	9,67	0,082
Tubulin beta-5 chain	TBB5	81,00	87,67	98,00	0,084
Protein canopy homolog 2	CNPY2	0,00	2,67	1,67	0,084
Glutathione S-transferase Mu 2	GSTM2	7,67	11,00	12,33	0,087
Collagen alpha-1(I) chain	CO1A1	23,33	22,33	15,67	0,088
Glutathione S-transferase P	GSTP1	9,67	9,33	14,00	0,089
Cytoskeleton-associated protein 4	CKAP4	16,00	14,00	9,67	0,09
Cysteine and glycine-rich protein 1	CSRP1	4,33	11,67	8,67	0,093
Glyceraldehyde-3-phosphate dehydrogenase	G3P	89,67	67,67	77,67	0,093
Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mit.	CHCH3	5,67	8,67	11,33	0,093
40S ribosomal protein S8	RS8	7,67	14,33	8,33	0,094
Histone H1.2	H12	12,67	13,00	8,33	0,094
Myosin-9	MYH9	92,33	98,33	68,33	0,095
GTP:AMP phosphotransferase, mitochondrial	KAD3	2,33	0,67	0,67	0,095
Zyxin	ZYX	1,67	3,67	0,67	0,095
Ubiquitin carboxyl-terminal hydrolase isozyme L1	UCHL1	1,33	3,33	3,33	0,098
Nebulin-related-anchoring protein	NRAP	1,67	4,00	1,33	0,1
PDZ and LIM domain protein 1	PDLI1	10,33	5,33	5,33	0,1
Reticulon-4	RTN4	20,67	24,67	31,00	0,1
NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitoch.	NDUS4	3,33	2,67	6,00	0,1
Rab GDP dissociation inhibitor alpha	GDIA	1,67	5,00	0,33	0,1
Small nuclear ribonucleoprotein Sm D2	SMD2	0,00	1,33	2,33	0,1
Kinesin-1 heavy chain	KINH	6,00	13,33	14,00	0,1
Transmembrane emp24 domain-containing protein 9	TMED9	1,67	4,67	1,00	0,1
Heat shock protein HSP 90-beta	HS90B	141,67	124,00	121,00	0,11
Low molecular weight phosphotyrosine protein phosphatase	PPAC	2,33	1,67	5,33	0,11
60S ribosomal protein L10a	RL10A	7,67	7,67	10,33	0,11
Creatine kinase M-type	KCRM	6,33	10,33	4,67	0,11
EH domain-containing protein 1	EHD1	2,33	1,00	3,00	0,11
6-phosphofructokinase, muscle type	K6PF	1,67	5,00	4,67	0,11
Hemoglobin subunit alpha	HBA	3,67	5,67	8,33	0,11
Branched-chain-amino-acid aminotransferase, mitochondrial	BCAT2	0,33	1,33	0,33	0,11
Carnitine O-palmitoyltransferase 1, muscle isoform	CPT1B	3,33	6,00	8,33	0,11
Calpain small subunit 1	CPNS1	1,00	4,33	6,67	0,11
Guanine nucleotide-binding protein subunit beta-2-like 1	GBLP	10,33	18,00	14,33	0,11
Histone H4	H4	22,67	21,00	27,67	0,12
Voltage-dependent anion-selective channel protein 3	VDAC3	5,67	12,00	6,67	0,12
Alpha-actinin-1	ACTN1	68,33	68,00	54,33	0,12
Pyruvate dehydrogenase E1 component subunit beta, mitochondria	ODPB	11,33	12,00	8,67	0,12
Troponin C, slow skeletal and cardiac muscles	TNNC1	6,67	6,00	10,00	0,12
Spliceosome RNA helicase Ddx39b	DX39B	0,33	1,00	3,67	0,12
Dynein light chain 1, cytoplasmic	DYL1	5,67	4,33	9,00	0,12
Vimentin	VIME	107,00	93,00	82,33	0,12
Adenosylhomocysteinase	SAHH	5,33	10,00	15,67	0,12
60S ribosomal protein L9	RL9	2,33	3,67	4,67	0,13
C-1-tetrahydrofolate synthase, cytoplasmic	C1TC	0,67	1,00	2,67	0,13
Chloride intracellular channel protein 1	CLIC1	1,00	3,33	2,67	0,13
Vinculin OS=Rattus norvegicus	VINC	69,67	74,67	87,00	0,13
60S ribosomal protein L30	RL30	1,67	2,67	5,00	0,13
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7	NDUA7	1,00	3,00	2,33	0,13
Profilin-1	PROF1	6,67	5,00	11,00	0,14
Tubulin beta-4B chain	TBB4B	79,67	83,00	93,67	0,14
14-3-3 protein eta	1433F	4,33	7,67	7,33	0,14
Alpha-soluble NSF attachment protein	SNAA	0,67	2,00	0,33	0,14
Hexokinase-1	HXK1	33,67	42,00	49,00	0,14
PDZ and LIM domain protein 5	PDLI5	25,00	33,67	15,33	0,14
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4	NDUA4	5,00	6,00	6,67	0,14
Myosin-10	MYH10	12,00	23,67	16,67	0,14
Proliferation-associated protein 2G4	PA2G4	2,67	4,67	6,67	0,15
Mitochondrial 2-oxoglutarate/malate carrier protein	M2OM	0,33	2,00	0,00	0,15
Hsp90 co-chaperone Cdc37	CDC37	0,33	1,67	0,00	0,15
Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2	4,67	8,67	10,00	0,15
Aconitate hydratase, mitochondrial	ACON	113,33	104,00	125,67	0,15
Importin subunit beta-1	IMB1	16,00	17,67	13,00	0,15
Brain protein 44	BR44	0,33	2,33	3,33	0,15
Fatty aldehyde dehydrogenase	AL3A2	3,33	2,33	1,67	0,15
40S ribosomal protein S4, X isoform	RS4X	6,00	8,67	10,67	0,15
Annexin A2	ANXA2	19,33	17,00	12,67	0,15
Monocarboxylate transporter 1	MOT1	5,67	7,67	8,67	0,15
26S proteasome non-ATPase regulatory subunit 11	PSD11	3,67	3,67	7,67	0,15

Supplemental Table 3. Hemodynamic analysis of Sham and Angiotensin II minipumps implanted mice treated either with scrambled or miR-21* antagomir using pressure-volume catheter system.

Hemodynamic parameters	**Sham scr Ant (n=5)**	**Ang II scr Ant (n=6)**	**Ang II miR-21*Ant (n=5)**
HR (bpm)	**503.09±18.4**	**464.04±19.4**	**434.0±20.7 ***
Pes (mmHg)	**73.4±4.90**	**107.4±4.4 *****	**108.9±2.7 *****
Ped (mmHg)	**3.7±0.5**	**6.5±2.5**	**7.0±1.8**
dP/dt max (mmHg/s)	**7819.0±569.8**	**10238.8±570.6 ***	**11029.8±1187.4 ***
SV (µL)	**27.3±2.5**	**24.1±2.7**	**22.6±2.3**
Ves (µL)	**14.1±1.6**	**14.8±0.9**	**12.90±1.9**
Ved (µL)	**39.3±3.3**	**36.4±3.4**	**33.2±3.4**

HR: heart rate; SV: stroke volume; Ves/Pes: left ventricular end systolic volume/pressure; Ved/Ped: left ventricular end diastolic volume/pressure; dP/dt: rate of rise of left ventricular pressure. Ang II minipump implanted mice (Ang II), Sham mice (Sham), scrambled antagomir (scr Ant), miR-21* antagomir treatement (miR-21* Ant). Data are shown as mean ± SEM. P all not significant between Ang II scr Ant and Ang II miR-21* Ant. *P<0.05 compared to Sham scr Ant, ***P<0.005 compared to Sham scr Ant.

Supplemental Methods

Cardiomyocyte preparation

Neonatal rat cardiac fibroblasts and rat cardiomyocytes were isolated from newborn rats as described (1). Briefly, hearts were removed from newborn rats (day 0), put into calcium- and bicarbonate-free HEPES buffered Hanks' medium, cut into pieces and digested with trypsin (BD Biosciences) and DNAse I (Roche) under constant stirring. Several digestion steps were performed before the collected primary cells were centrifuged at 700xg for 10 min, passed through a cell strainer (40 mm), seeded onto uncoated plastic dishes and incubated for 90 min at 37°C. The supernatant (containing the cardiomyocytes) was collected and plated in MEM (minimal essential medium; Animed) containing vitamin B12, $NaHCO_3$, L-glutamine, BrdU, Penicillin/Streptomycin and 5% Fetal Bovine Serum (FBS; Invitrogen). The attached primary rat cardiac fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; PAA) supplemented with 10% FBS and 1% Penicillin/Streptomycin at 37°C in 5% CO_2. Immunohistology and protein expression analyses assured highest purity of cell cultures.

Transfection of cells

Cells were transfected with small inhibitory RNAs (siRNAs) or specific miRNAs by a liposomal-based method (Lipofectamine™2000, Invitrogen) according to the manufacturers' instructions. Briefly, specific miRNA or siRNA was mixed separately and incubated for 5 min with Opti-MEM I media (Invitrogen) (complex 1). In a parallel step Lipofectamine™2000 was mixed and incubated for 5 min with Opti-MEM I media (complex 2). Complex 1 and complex 2 were mixed and incubated for 20 min. Cells were incubated for 4 h with the transfection complex before it was discarded and replaced by fresh media. If not otherwise stated protein lysates were prepared or RNA was isolated after 72 h. Details about the used miRNAs are found in Supplemental Table 4; details about the used siRNAs are shown in Supplemental Table 5.

Cells were transfected either with pre-miR™ miRNA Precursor Molecules or anti-miR miRNA Inhibitors from Ambion. Detailed information about the design and chemical modification of

the pre-miRNA and anti-miRNA molecules are provided on the Life Technology website. Pre-miR™ miRNA precursor molecules are small, chemically modified (2'OMe) double-stranded RNA molecules designed to mimic endogenous mature miRNAs. Pre-miR miRNA Precursor Molecules are designed and modified to ensure that the correct strand, representing the desired mature miRNA, is taken up into the RNA-induced silencing-like complex responsible for miRNA activity whereas the other strand is inactivated and is degraded. Pre-miR miRNA Precursors are not hairpin constructs and should not be confused with pre-miRNAs. Ambion® Anti-miR™ miRNA Inhibitors are chemically modified (2'OMe), single stranded nucleic acids designed to specifically bind to and inhibit endogenous microRNA (miRNA) molecules.

For rescue assays, cardiomyocytes were either transfected with scrambled anti-miRNA, anti-miRNA-21 or anti-miR-21* as described above. Cells were incubated for 4 h with the transfection complex before it was discarded and replaced by fresh media (containing 1%FBS). Cardiomyocytes were stimulated with the pro-hypertrophic agent angiotensin II (1 nm) for 72 h by adding it directly to the media. Control represents incubation with normal media but no addition of angiotensin II. Cardiomyocytes were stained with alpha-actinin and cell size was measured. Cell surface area was analyzed from digitally recorded images using the AxioVision (Zeiss) software packages.

RNA isolation

Total RNA from cells, exosomes and pericardial fluid was isolated using TriFast (Peqlab) according to the manufacturer's protocol with minor modifications. For RNA isolation of exosomes and from pericardial fluid a mixture of "spike in" RNAs from *C. elegans* (5fmol) was added before the chloroform step. Further, 1-2 µl glycogen (Roche) was added to the isopropanol precipitation step to increase the RNA yield. Before RNA isolation, exosomes were pre-treated with 0.4 µg/µl RNaseA (Roth) for 30 min at 37°C to eliminate intact exosomes and to assure that isolated small RNAs are derived from inside of the exosomes. The concentration and quality of the isolated RNA was analyzed in a Synergy HT (Biotek).

The Agilent 2100 Analyzer (Agilent) was used to detect the RNA profiles of small and total RNAs in exosomes and cells.

Quantitative Real Time-PCR (qRT-PCR)

To analyze the expression of specific miRNAs in exosomes the QuantiMir™ RT Kit from System Biosciences (SBI) was used. All small, non-coding RNAs were converted into cDNA starting from total RNA samples. A poly-A tail was added to the 3‘ end of small non-coding RNAs. In the presence of poly-A polymerase and by using an oligo-dT adaptor primer the reverse transcription into cDNA was initiated. The ready-to-use cDNA serves as a template for quantitative real time-PCR (qRT-PCR) with a 3’ universal reverse primer, 2x SYBR Green and a specific miRNA assay primer. Two 384-well qRT-PCR primer plates containing 388 rat individual microRNA primers and 3 endogenous reference RNA controls (rno-U6, RNU43 & U1) are included on each plate.

Quantification of mature miRNAs was performed by qRT-PCR using specific TaqMan miRNA assays according to the manufacturer’s protocol (Applied Biosystems). Reverse transcription of RNA was performed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems). For qRT-PCR analysis the samples were pre-diluted 1:3 with nuclease-free water and for each miRNA a standard curve was generated (1:1, 1:5, 1:25, 1:125) using 2µl cDNA as template for qRT-PCR. The small RNA molecule sno202 (for mouse) or sno (for rat) was used as endogenous control in cells. The expression of miRNAs in exosomes and pericardial fluid was normalized to the expression of a “spike in” RNA from *C. elegans*. Details about the specific miRNA assays used for TaqMan qRT-PCR are to be found in Supplemental Table 6.

Western Blotting

Total proteins were extracted as described by Buitrago et al. (2) Western Blot was performed with 7-15 µg of total protein. Protein was separated on polyacrylamide gels, before blotting onto polyvinylidene fluoride membranes over night at 4°C. The membrane

was blocked with 5% milk powder in TBS-Tween for 1h and incubated with primary antibodies at 4°C over night (Specific primary antibodies used for Western blotting are to be found in Supplemental Table 7). The membrane was washed 3x 5 min in TBS-Tween and incubated with a secondary antibody conjugated to horseradish peroxidase for 1 h and subjected to enhanced chemiluminescence.

Stimulation of primary rat cardiac fibroblasts with angiotensin II

Primary rat cardiac fibroblasts were grown to a confluence of 80%. The cells were starved for 24 h in DMEM containing 0.1% pre-depleted FBS before they were stimulated with 1nM angiotensin II and 4ng/ml IL-1β in DMEM + 0.1% pre-depleted FBS for 24 h. The conditioned media of rat cardiac fibroblasts were collected and used for exosome purification by differential ultracentrifugation. As control, cells were cultured in DMEM + 0.1% pre-depleted FBS adding PBS and 0.1% BSA/water. miRNA expression levels of fibroblast-derived exosomes were analyzed using the QuantiMir™ RT Kit from System Biosciences (SBI).

Treatment of rat cardiac fibroblasts with nSmase 2 inhibitor (GW4869)

Primary rat cardiac fibroblasts were treated with 10 µM nSmase 2 inhibitor (GW4869, Sigma) in DMEM containing 10% FCS for 24 h. Thereafter medium was changed to DMEM containing 0.1% pre-exosome-depleted FBS + 10 µM nSmase 2 inhibitor. Conditioned media of control and treated fibroblasts were collected for 24 h and exosomes were purified by differential ultracentrifugation. As control, cells were treated with DMSO in the same concentration as the inhibitor. miRNA expression levels of fibroblast-derived exosomes were analyzed using the QuantiMir™ RT Kit from System Biosciences (SBI). Further, rat cardiac fibroblasts treated with the nSmase 2 inhibitor or a control were fixed in 4% PFA for immunofluorescence staining.

Immunofluorescence staining

Primary rat cardiac fibroblasts or cardiomyocytes were grown/seeded on 24-well plates to a confluence of 70%. Cells were washed twice with PBS, fixed with 4% paraformaldehyde (PFA) for 15 min, washed twice with PBS and permeabilized with 0.1% Triton X-100 for 10 min. Thereafter, cells were washed with PBS and blocked with donkey serum for 30 min, washed again and incubated with a primary antibody in PBS + 5% donkey serum over night. The second day, cells were washed with PBS and incubated with Alexa-conjugated secondary antibodies (1:500) and DAPI (1:1000) for 30 min in PBS + 5% donkey serum. Cells were washed twice with PBS and embedded with Prolong Antifade (Invitrogen). Cell membranes of cells were stained using Wheat germ Agglutinin Alexa Fluor 488 (Invitrogen). Phalloidin-TRITC (Sigma) was used to stain F-actin structures in the cytoplasm of cells.

Fluorescence confocal microscopy

For immunofluorescence confocal microscopy, cardiomyocytes were grown on glass coverslips coated with 0.1% gelatine and cultured in supplemented medium. The labeled exosomes (7.5 µg) were added to cardiomyocytes (100.000 cells) and incubated for the appropriated time points at 37°C. To study the influence of temperature, labeled exosomes were cultured with cardiomyocytes for 2 h at 4°C. To investigate if the cytoskeleton is involved in exosome uptake, cardiomyocytes were treated with an inhibitor of actin polymerization (0.5 µM Cytochalasin D) for 30 min following incubation of labeled exosomes for 2 h. For immunofluorescence staining, cells were fixed in 4% PFA and stained with respective antibodies as described above. The uptake of labeled exosomes by cardiomyocytes was visualized by confocal microscopy. Confocal imaging was prepared with a Zeiss LSM 780 using a Plan-Apochromat 63x/1.40 Oil immersion objective. In all cases z-stacks were taken covering the entire cell volume. Further technical details in brief: lasers: 405 nm / 30mW diode laser @ 1%, 488 nm Ar^{+}-Laser 25 mW @ 6%, 561nm DPSS laser, 50mW @ 1%, Main beam splitters: MBS405 & MBS488/561, 16bit mode, detector gain 600 – 650, Pinhole 1AU, voxel distances according to Nyquist criteria, acquisition protocol: online

fingerprinting mode and lambda mode. For fluorescence signal unmixing single color stains were acquired under identical conditions like in the multicolor stain experiments.

Internalization of labeled exosomes by cardiomyocytes was quantified based on their fluorescence intensity using Imaris Software (version 6.0).

Co-culture assay

Co-cultivation of rat cardiac fibroblasts and cardiomyocytes was investigated in 24-well plate Boyden chambers. Cardiac fibroblasts were seeded onto the 0.4 µm inserts which allow transport of exosomes but no cell compartments. In parallel, cardiomyocytes were seeded in the lower well of the 24-well Boyden plate and cultured in MEM media +5% FBS 24 h before the co-culture experiment. Cardiac fibroblasts were transfected separately with specific miRNA or control miRNA as described. The transfection media was removed after 4 h and fresh media was added to the fibroblasts. The inserts were placed onto the 24-well Boyden plate with cardiomyocytes in the lower chamber. After 72 h the inserts were removed and cardiomyocytes were washed and lysed with Trifast for RNA isolation. The miRNA expression in cardiomyocytes was measured as mentioned in qRT-PCR.

To visualize the transport of miRNAs from cardiac fibroblasts to cardiomyocytes, cells were transfected with a Cy3-labeled miRNA or a respective control miRNA. After 24 h of co-culturing the inserts were removed and cardiomyocytes were fixed with 4% PFA. Cardiomyocytes were stained with alpha-actinin and confocal microscopy analysis was performed.

Electron microscopy

Cell suspension of rat cardiac fibroblasts was spun down by 10000 rpm and fixed by immersion in fixation solution composed of 1.5% glutaraldehyde (Agar Scientific Limited., Essex, UK) and 1.5% formaldehyde freshly prepared from depolymerized paraformaldehyde (PFA) (Merck Chemicals, Darmstadt, Germany) in 0.15 M HEPES buffer (Sigma-Aldrich, Hamburg, Germany, total osmolarity of 800 mosmol/l and a vehicle osmolality of 300

mosm/kg at pH 7.35) for at least 3 h. During each of the following steps of processing, the suspensions were centrifuged at 10000 rpm for 5 min. After repeated rinsing in 0.15M HEPES buffer and in 0.1 mmol/l cacodylate buffer (Plano, Wetzlar, Germany), the suspensions were postfixed in 1% osmium tetroxide (Plano, Wetzlar, Germany) in 0.1 M cacodylate buffer. After a rinsing in 0.1 M cacodylate buffer (2 x 5 min) and twice rinsing in distilled water, specimens were stained and blocked overnight (12-18 h) with a mixture of equal portions of uranyl-acetate and water (half-saturated aqueous uranyl acetate solution (1:1)) (Agar Scientific LTD., Stansted, Essex, UK) at 4-8°C. Cell suspensions were then dehydrated in an ascending series of acetone (J. T. Baker, Deventer, Netherlands) (70%, 90%, 100%) and embedded in epon (SERVA Feinbiochemica GmbH & Co). Ultra-thin sections (70 nm) were cut by an ultra microtome (Reichert Ultracut S, München Germany), collected on nickel grids (Plano, Wetzlar, Germany) and stained with lead citrate (Merck, Darmstadt, Germany) and uranyl acetate.

Exosome pellets purified from neonatal rat cardiac fibroblasts were used for electron microscopic analysis as described previously (3). Briefly, exosome pellets purified by ultracentrifugation were resuspended in 2% PFA and loaded onto formvar carbon-coated grids and dried for 20 min at 40°C. The exosome-loaded grids were washed in PBS and post-fixed in 1% glutaraldehyde (Agar Scientific Limited., Essex, UK) and subsequently rinsed in distilled water several times. The grids were transferred to 4% uranyl-oxalate solution and stained for 5 min and finally embedded in a mixture of uranyl acetate (4%) and methyl cellulose (2%) on ice. To remove excess fluid the grids were deposited on filter paper and dried for 10 minutes before they were stored in a grid storage box.

Ultra-thin sections of cardiac fibroblasts and whole mounted exosomes were examined with an electron microscope (Morgagni 268, FEI, Eindhoven, NL). Micrographs of representative areas were taken by a digital camera (Veleta TEM camera, Olympus Europa Holding GMBH).

Flow cytometry of exosomes

Fibroblast-derived exosomes were coupled to 4 µm diameter latex aldehyde/sulfate beads (Invitrogen) because their diameter is too small for the detection range of the flow cytometer (3). The latex beads (8µl) were incubated with purified exosomes (10µg) overnight at 4°C on a rotator wheel. The next day, the bead-exosome complex was mixed with 1M glycine to block remaining free binding sites on the beads and was washed several times in 0.5% BSA/PBS. To detect CD63, 100 µl of the bead-exosome complex was incubated with mouse anti-rat CD63 antibody (1 µg; BD Biosciences) for 30 min at 4°C following incubation with 200 µl biotin rat anti-mouse IgG1 (0.5 µg; BD Biosciences) and fluorescein-isothiocyanate (FITC)-conjugated Streptavidin (1 µg; BD Biosciences) diluted in 0.5% BSA/PBS for 30 min at 4°C. The samples were analyzed by FACS (Millipore, guava easycyte™ Flow Cytometer) and quantified using FlowJo Software.

Incubation of cardiomyocytes with transfected fibroblast-derived exosomes or fibroblast supernatant

Fibroblast-derived exosomes were purified from the conditioned media of rat cardiac fibroblasts transfected with a control precursor miRNA (scr) or miR-21* precursor (pre-21*). The transfected exosomes were cultured with cardiomyocytes for 72 h and cell size was measured or Sorbs2 and Pdlim5 mRNA expression was determined.

Further, cardiomyocytes were incubated with rat fibroblast (rFb) conditioned medium containing exosomes (+exo) (in a ratio 1:3 cardiomyocyte media + rFb supernatant) or with rFb conditioned medium depleted of exosomes (-exo) by ultracentrifugation. As control, cardiomyocytes were treated with rFb medium (DMEM + 1% FBS). For cell size measurements, cardiomyocytes were fixed in 4% PFA and stained with alpha-actinin.

Proteome profiling of transfected cardiomyocytes

Neonatal cardiomyocytes were either transfected with a control precursor miRNA, a precursor of miR-21 or a precursor of miR-21* for 72 h. Cardiomyocyte pellets (n=3 for each

group) were mixed with 2x sample buffer (Invitrogen) and heated at 97°C for 5 min. The proteins were separated on 4%-12% Bis-Tris gel and stained using a PlusOne silver staining kit (GE Healthcare) (4). Each lane was diced into 10 gel pieces and digested with trypsin (Promega) (5, 6) on an Investigator ProGest digestion robot (DIGILab). Tryptic peptides were separated on a nano-flow HPLC system (RSLC 3000, PepMap100 C18 column, 25-cm length, 75-µm internal diameter, 3-µm particle size, Thermo Fisher Scientific) and eluted with a 40 min gradient (10-25% B in 35 min, 25-40% B in 5 min, 90% B for 10 min and 2% B for 30min where A=0.1% formic acid in HPLC H_2O and B = 80% ACN, 0.1% formic acid in HPLC H_2O). The eluted peptides were directly analyzed by a high mass accuracy tandem mass spectrometer (LTQ Orbitrap XL, Thermo Fisher Scientific) using full MS scan mode over the range of m/z 400-1600. MS/MS was performed on the 6 most abundant ions in each MS scan with dynamic exclusion (7). Raw files were searched against a rodent database (UniProt/Swiss-Prot 2012_03, 25897 entries) using Mascot 2.3.01 (Matrix sciences) (8). Carboxyamidomethylation of cysteine was chosen as fixed modification and oxidation of methionine as variable modification. The mass tolerance was set at 10ppm for the precursor ions and at 0.8 Da for fragment ions. Two missed cleavages were allowed. Scaffold (version 3.6.5, Proteome Software) was used to calculate the spectral counts and to validate MS/MS based peptide and protein identifications. The following peptide thresholds were applied: peptide probability > 95.0%, protein probability > 99.0% with at least 2 unique peptides (9, 10).

Luciferase Reporter Assay

SORBS2 3'UTR (1261 bp) harbouring two potential binding sites for miR-21* was cloned into SpeI and HindIII cloning site of pMIR-REPORT vector (Ambion). The resulting construct (20 ng) was co-transfected with scr-miR or miR-21* (each 100 nM) and 20 ng of β-galactosidase control plasmid (Promega) into 48 well-plated HEK293 reporter cells by the use of Lipofectamine 2000 (Invitrogen). Cells were incubated for 24 h before detecting luciferase and β-galactosidase activity applying different substrates (Promega).

Ago2 immunoprecipitation

Briefly, $10*10^6$ human cardiac fibroblasts (HCFs) were used for Argonaute 2 (Ago2) immunoprecipitation. Cell lysates were subjected to incubation with dynabeads (Invitrogen) coated with 5-10 µg of control IgG (Cell Signaling) or Ago2 IgG (Chromotek). After pulldown, magnetic dynabeads were separated for western blot analysis and Trifast-based (Peqlab) RNA isolation procedure. Isolated RNA underwent qPCR analysis to determine miR-21* enrichment in the Ago2 fraction.

miR-21* in situ PCR

Sections (4 µm in thickness) from 4% PFA-fixed and paraffin-embedded heart tissue were cooked in citrate buffer and treated with DNase I (Roche) after deparaffinization. After DNase digestion, the tissue section was covered with the miRNA PCR solution consisted of miR-21* primers (RT: 5'- GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC GACAGC; forward: 5'- CGCAACAGCAGTCGATG; reverse: 5'- GTGCAGGGTCCGAGGT), digoxigenin dUTP (Roche), RNase inhibitor (Roche) and SuperScript One-Step RT-PCR System with Platinum Taq DNA Polymerase (life technology). The corresponding primer for cel-miR-39 was used as negative control. cDNA synthesis was performed by incubating slides for 30 min at 55°C. Then cDNA was amplified after denaturing at 94°C for 4 min with 26 cycles at 94°C denaturation (15 s), 56°C annealing (30 s) and 72°C extension (20 s) followed with final extension at 72°C for 5 min. The digoxigenin-labelled miRNA cDNA was detected with a digoxigenin antibody (Roche).

Collecting pericardial fluid from mice

Animal studies were performed according to the relevant guidelines and regulations of the responsible authorities. TAC surgery was performed on male C57BL/6 mice (10–12 weeks old) from Charles River Laboratories as described (11). To collect pericardial fluid from 4 week old Sham and TAC-operated mice the pericardium was incised and carefully washed with 350 µl PBS. To remove blood cells, the diluted fluid was centrifuged at 7800xg for 5 min.

For RNA isolation, 150 µl pericardial fluid was used. A “spike in” RNA from *C. elegans* (5fmol) was added during RNA isolation to normalize the miRNA expression.

Antagomir treatment

The miR-21* Antagomir was designed and provided by Integrated DNA Technology (IDT, Belgium). The oligonucleotide was fully methylated and had a 3’ Cholesterol modification as well as phosphorothiorates at certain position (indicated by asterisks). The 3’ Cholesterol modification improves cell uptake of the oligonucleotide. The phosphorothioate are added to antisense oligos to protect them from nuclease degradation. Methyl-modifications increase both nuclease stability and affinity (Tm) of the antisense oligo to the target mRNA. Antagomirs were diluted in nuclease-free water and 100 µl at concentrations of 80 mg/kg were applied to mice via retroorbital injection.

Hemodynamic measurements

Cardiac function was assessed in anesthetized (2% isoflurane) and artificially ventilated (MiniVent respirator Harvard Apparatus, Holliston, MA) mice using a 1F microtip pressure-volume catheter (PVR 1045; Millar Instruments, Houston, TX) coupled with a Powerlab/4SP acquisition system (AD Instruments Ltd, Oxford, UK), as previously described (12, 13) (Nature Protocols 2008, Diabetes 2012). Beat-by-beat P-V loop data were analyzed using Labchart 7 AD Instruments Ltd, Oxford, UK) and LV endsystolic and enddiastolic pressure and volume (Pes, Ped, Ves, Ved), stroke volume (SV), maximal and minimal slope of the systolic pressure increment (+/-dP/dt), ejection fraction (EF), cardiac output (CO), heart rate (HR) were computed. All hemodynamic parameters were calculated and corrected according to in vitro and in vivo volume calibrations.

Supplemental Table 4: miRNA Assays used for transfection

miRNA Assays	Reference
miR-21a-3p	Assay ID PM13039, Ambion
miR-21-5p	Assay ID PM10206, Ambion
miR-132	Assay ID PM10166, Ambion
pre-miR Negative Control #2	Catalog number AM17111, Ambion
Anti-miR Negative Control #1	Catalog number AM17010, Ambion

Supplemental Table 5: siRNAs used for transfection

siRNA	Reference
Sorbs2 Stealth siRNA	Invitrogen, RSS300347
Pdlim5 Stealth siRNA	Invitrogen, RSS329877
Stealth RNAi™ siRNA Negative Control Med GC	Invitrogen, ordering number: 12935-30

Supplemental Table 6: miRNA TaqMan assays used for qRT-PCR

miRNA	Reference
hsa-miR-21	Assay ID 000397, Applied Biosystems
mmu-miR-21*	Assay ID 002493, Applied Biosystems
snoRNA202	Assay ID 001232, Applied Biosystems
snoRNA	Assay ID 001718, Applied Biosystems
cel-miR-39	Assay ID 000200, Applied Biosystems
cel-miR-54	Assay ID 001361, Applied Biosystems

Supplemental Table 7: Primary antibodies used for Western blot and immunofluorescence staining

Primary antibody	Reference
Rabbit anti-nSmase 2	Santa Cruz, sc-67305
Mouse anti-GAPDH	Abcam, ab8245
Mouse anti-rat CD63 antibody	BD, 551458, Clone AD1
Goat anti-DDR2	Santa Cruz, sc-7555 (N-20)
Goat-anti-Troponin I-C	Santa Cruz, sc-31655 (D-12)
Rabbit anti-S100A4	Abcam, ab27957
Rabbit anti-PDLIM5	Avivasysbio. (ARP38796_T100)
Mouse anti-Sorbs 2	Sigma, SAB4200183 (Clone S5C)
Mouse anti-α-Actinin	Sigma, a 7811
Mouse anti- Prolyl-4-Hydrolase	Acris, AF 5110-1
Rabbit anti-smooth-muscle-Actin	Abcam, ab15734

References

1. Thum, T., Gross, C., Fiedler, J., Fischer, T., Kissler, S., Bussen, M., Galuppo, P., Just, S., Rottbauer, W., Frantz, S. et al. 2008. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* **456**:980-984.

2. Buitrago, M., Lorenz, K., Maass, A.H., Oberdorf-Maass, S., Keller, U., Schmitteckert, E.M., Ivashchenko, Y., Lohse, M.J., and Engelhardt, S. 2005. The transcriptional repressor Nab1 is a specific regulator of pathological cardiac hypertrophy. *Nat. Med.* **11**:837-844.

3. Thery, C., Amigorena, S., Raposo, G., and Clayton, A. 2006. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr. Protoc. Cell. Biol.* **Chapter 3**:Unit 3.22.

4. Yan, J.X., Wait, R., Berkelman, T., Harry, R.A., Westbrook, J.A., Wheeler, C.H., and Dunn, M.J. 2000. A modified silver staining protocol for visualization of proteins compatible with matrix-assisted laser desorption/ionization and electrospray ionization-mass spectrometry. *Electrophoresis* **21**:3666-3672.

5. Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S., Schweigerer, L., Fotsis, T., and Mann, M. 1996. Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* **379**:466-469.

6. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal. Chem.* **68**:850-858.

7. Yin, X., Cuello, F., Mayr, U., Hao, Z., Hornshaw, M., Ehler, E., Avkiran, M., and Mayr, M. 2010. Proteomics analysis of the cardiac myofilament subproteome reveals dynamic alterations in phosphatase subunit distribution. *Mol. Cell. Proteomics* **9**:497-509.

8. Perkins, D.N., Pappin, D.J., Creasy, D.M., and Cottrell, J.S. 1999. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **20**:3551-3567.

9. Keller, A., Nesvizhskii, A.I., Kolker, E., and Aebersold, R. 2002. Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. *Anal. Chem.* **74**:5383-5392.

10. Nesvizhskii, A.I., Keller, A., Kolker, E., and Aebersold, R. 2003. A statistical model for identifying proteins by tandem mass spectrometry. *Anal. Chem.* **75**:4646-4658.

11. Rockman, H.A., Ross, R.S., Harris, A.N., Knowlton, K.U., Steinhelper, M.E., Field, L.J., Ross, J.,Jr, and Chien, K.R. 1991. Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in an in vivo murine model of cardiac hypertrophy. *Proc. Natl. Acad. Sci. U. S. A.* **88**:8277-8281.

12. Pacher, P., Nagayama, T., Mukhopadhyay, P., Batkai, S., and Kass, D.A. 2008. Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. *Nat. Protoc.* **3**:1422-1434.

13. Rajesh, M., Batkai, S., Kechrid, M., Mukhopadhyay, P., Lee, W.S., Horvath, B., Holovac, E., Cinar, R., Liaudet, L., Mackie, K. et al. 2012. Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. *Diabetes* **61**:716-727.