

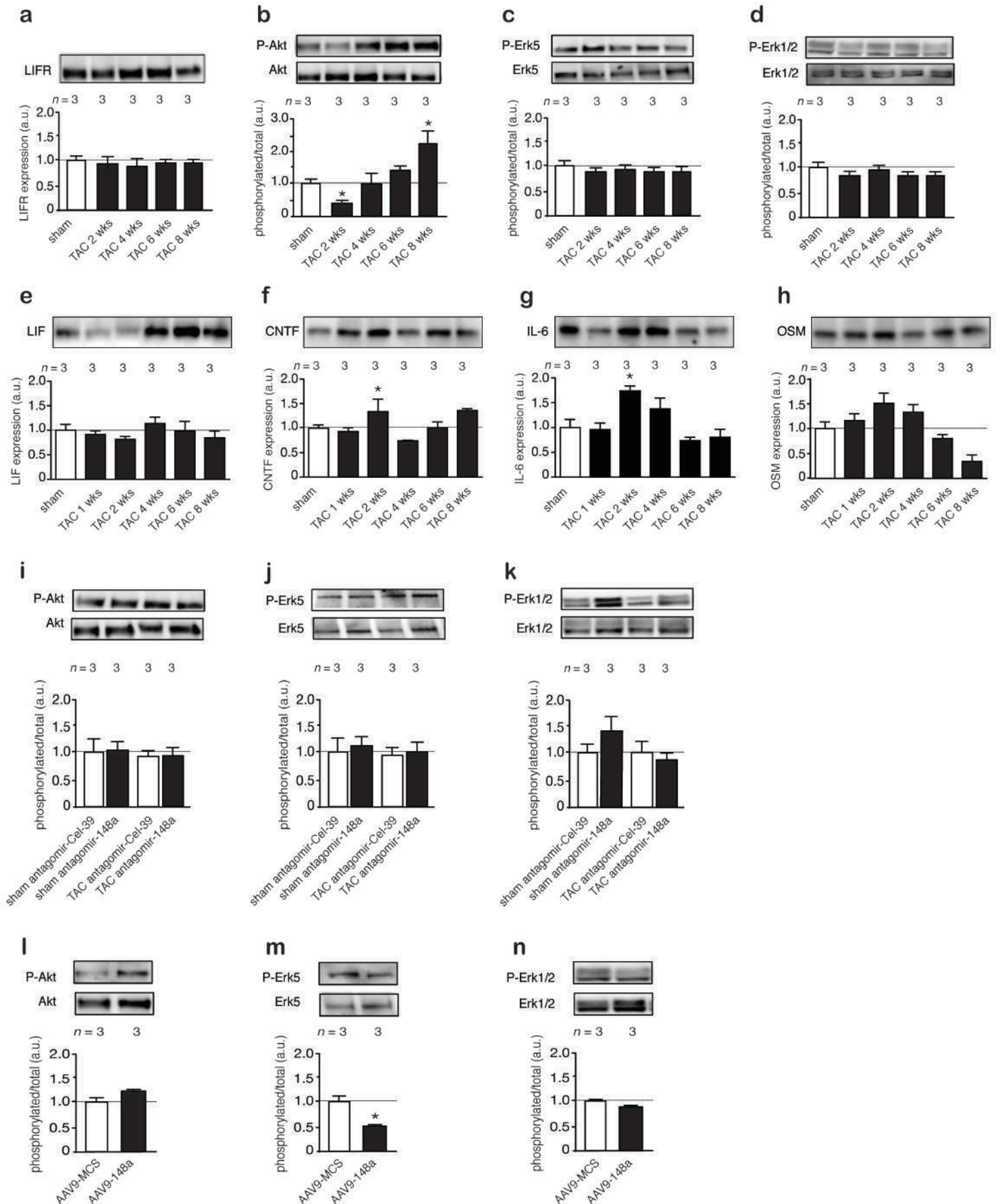
Supplemental Information

Therapeutic Delivery of miR-148a Suppresses

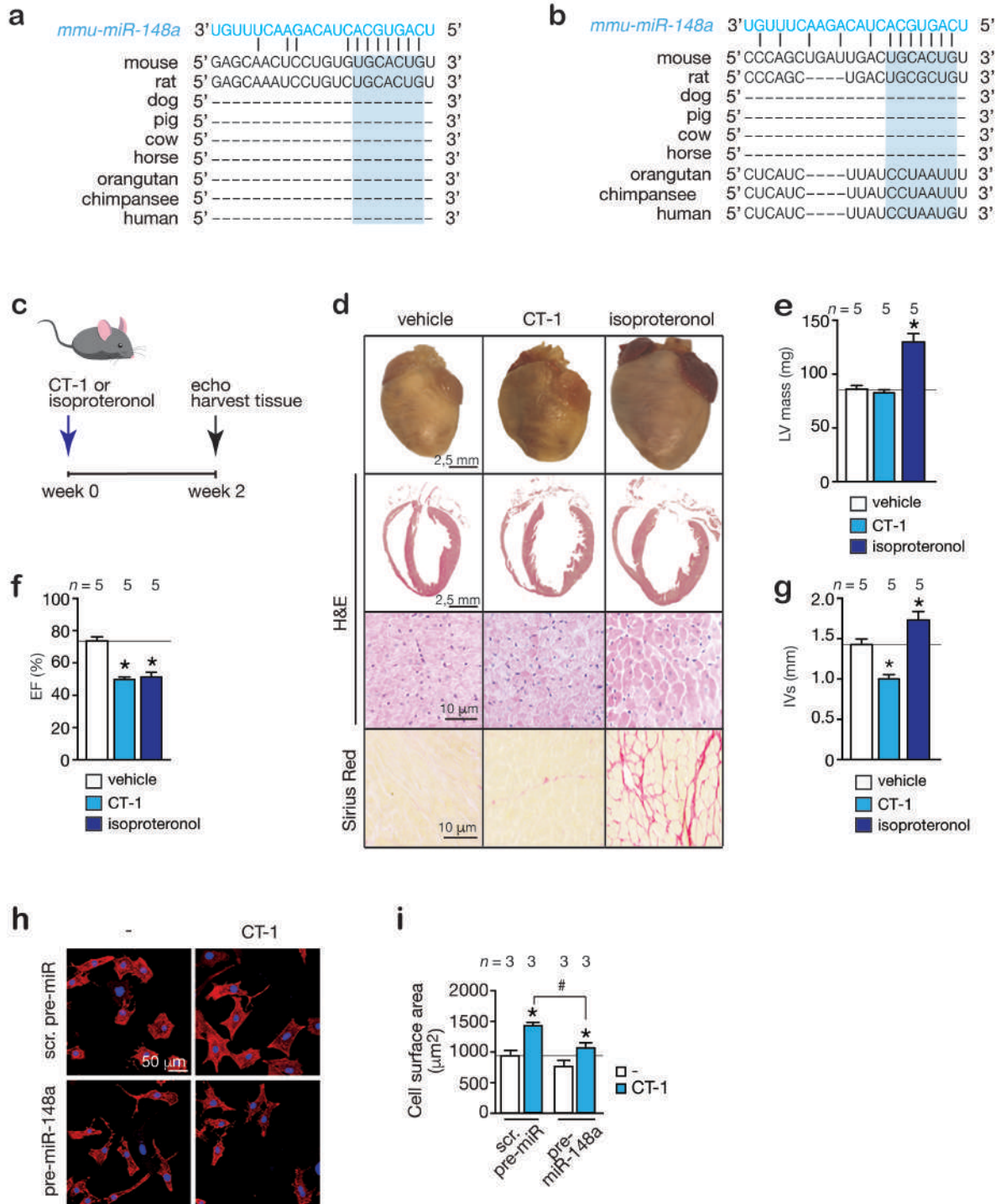
Ventricular Dilation in Heart Failure

Andrea Raso, Ellen Dirx, Leonne E. Philippen, Amaya Fernandez-Celis, Federica De Majo, Vasco Sampaio-Pinto, Marida Sansonetti, Rio Juni, Hamid el Azzouzi, Martina Calore, Nicole Bitsch, Servé Olieslagers, Martinus I.F.J. Oerlemans, Manon M. Huibers, Roel A. de Weger, Yolan J. Reckman, Yigal M. Pinto, Lorena Zentilin, Serena Zacchigna, Mauro Giacca, Paula A. da Costa Martins, Natalia López-Andrés, and Leon J. De Windt

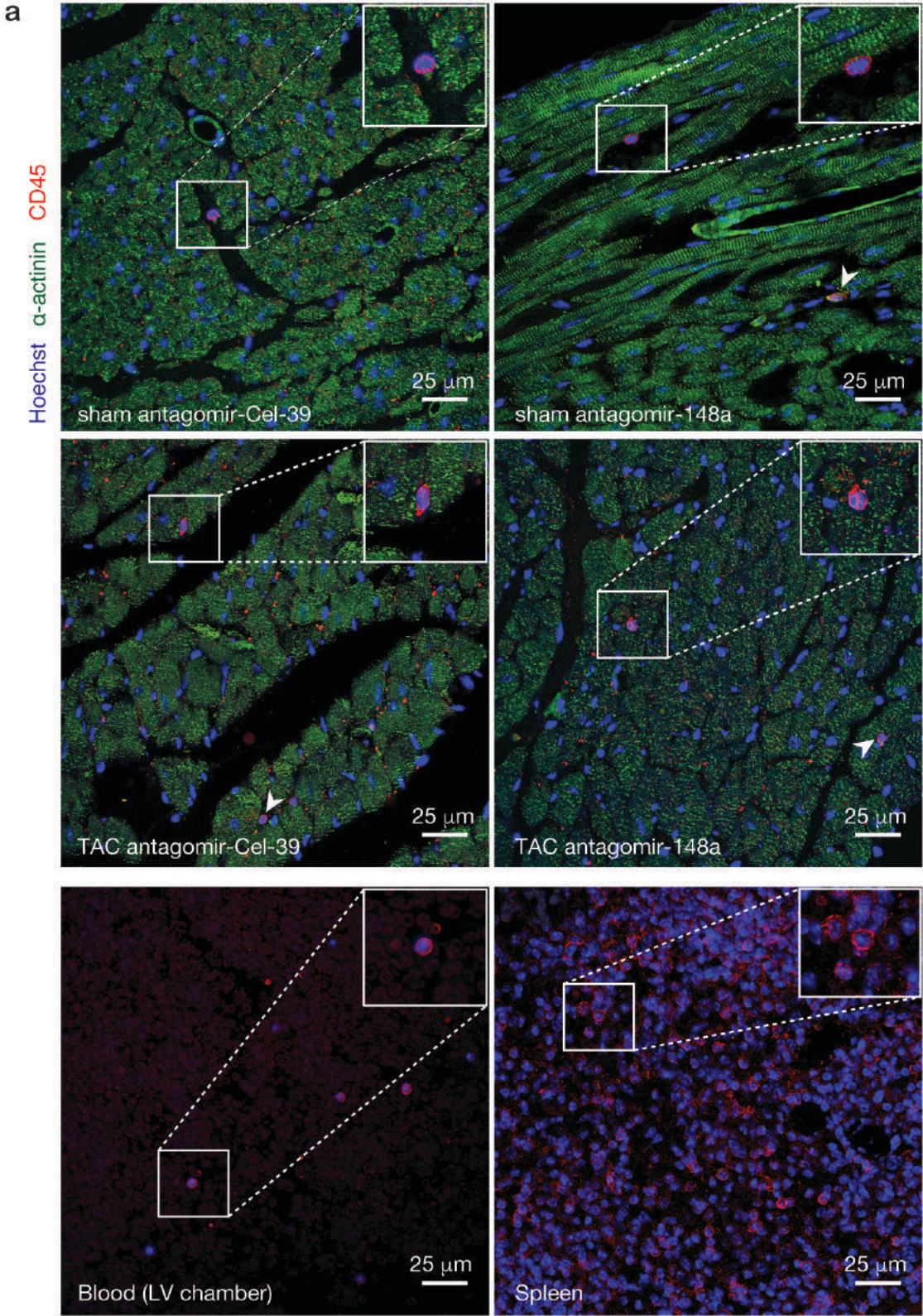
Supplemental Figure 1



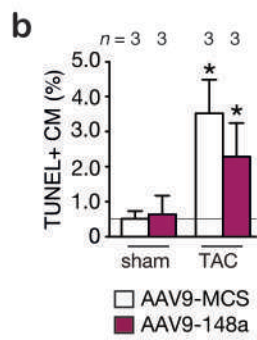
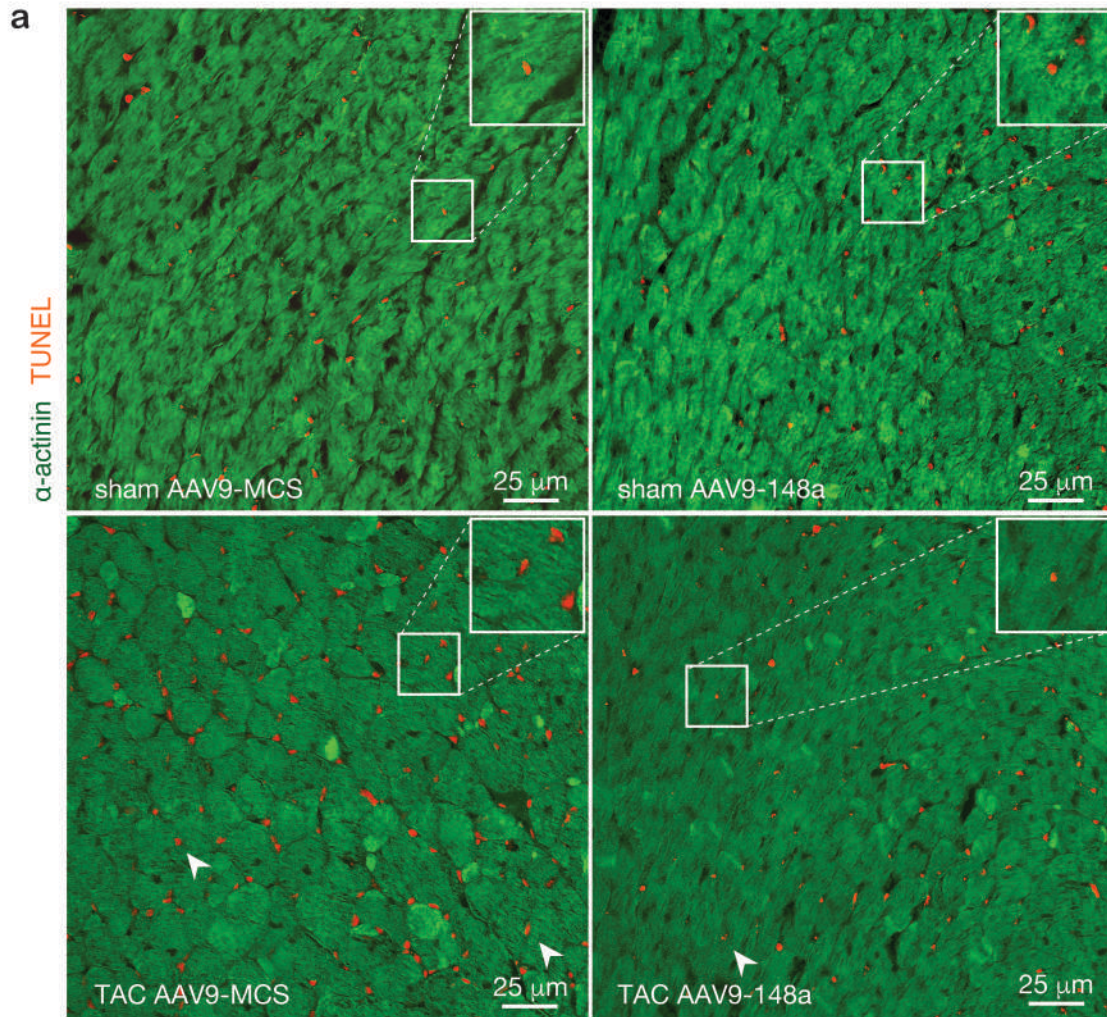
Supplemental Figure 2



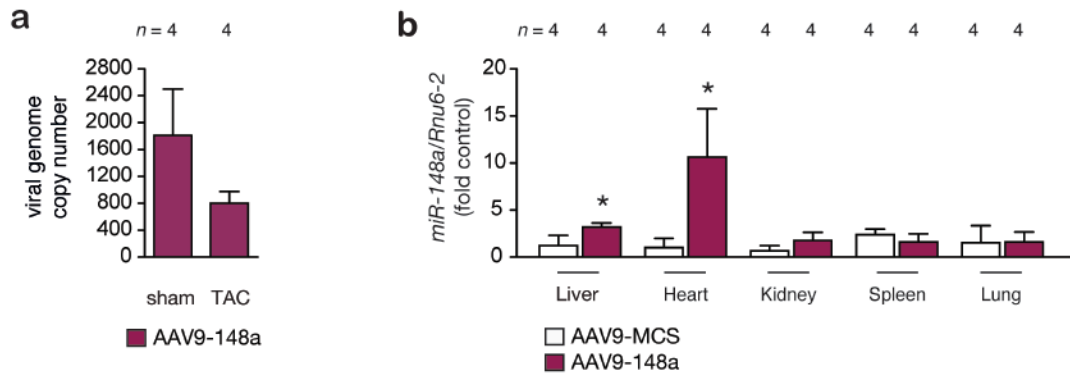
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



1 **Legends to the Supplementary Figures**

2

3 **Supplementary Figure 1 | *miR-148a* differentially regulates ERK5, ERK1/2, Akt**
4 **downstream signaling in concentric and eccentric cardiac remodeling. (a)** Western
5 blot analysis of myocardial LIFR expression and quantification at indicated time points.
6 **(b-d)** Western blot analysis of phosphorylated and unphosphorylated forms of Akt, Erk5
7 or Erk1/2 and quantification at indicated time points. **(e-h)** Western blot analysis of
8 myocardial LIF, CNTF, IL-6 or OSM expression and quantification at indicated time
9 points. **(i-k)** Western blot analysis of phosphorylated and unphosphorylated forms of Akt,
10 Erk5 or Erk1/2 and quantification in hearts from mice subjected to sham or TAC surgery
11 and receiving antagomir-148a or a control antagomir (antagomir-*Cel-39*). **(l-n)** Western
12 blot analysis of myocardial gp130 or phosphorylated and unphosphorylated forms of Akt,
13 Erk5 or Erk1/2 and quantification in hearts from mice receiving AAV9-148a or AAV9-
14 MCS.

15 Data information: Data are means \pm SEM. One-way ANOVA with Newman-Keuls
16 multiple comparison test was used to compare groups. *n*, number of independent WB
17 experiments; AAV9, adeno-associated virus serotype 9; TAC, transverse aortic
18 constriction. **P* < 0.05 vs corresponding control group.

19

20 **Supplementary Figure 2 | CT-1 infusion results in cardiac dilation. (a-b)**

21 Evolutionary conservation of the other two *mmu-miR-148a* seed regions on gp130
22 3'UTR. In mouse they are respectively located on the nucleotide position 6150-6156 and
23 8532-8538 [ENSMUST00000183663.7]. **(c)** Study design of mice receiving continuous
24 infusion of CT-1 (20 μ g/kg/day) or isoproterenol (60 mg/kg/day). **(d)** Representative
25 image of whole hearts (top panels), H&E-stained cardiac sections (second panels), high
26 magnification H&E-stained sections (third panels), or Sirius Red stained (lower panels)
27 histological sections of hearts from saline, CT-1 infused and isoproterenol-infused mice.
28 Measurements of **(e)** LV mass, **(f)** EF **(g)** IVSs in saline, CT-1-treated or isoproterenol-
29 treated mice. **(h)** Confocal microscopy images of neonatal rat cardiomyocytes treated
30 with scrambled or precursor-148a miR-148a with or without CT-1 stimulation. **(i)** Cell
31 surface measurements from conditions in **(f)**.

32 Data information: Data are means \pm SEM. One-way ANOVA with Bonferonni's multiple
33 comparison test was used to compare groups. *n*, number of mice CT-1, cardiotrophin 1;

1 LV, left ventricular; EF, ejection fraction; IVSs, Interventricular septum in systole. * $P < 0.05$ vs corresponding control group; # $P < 0.05$ vs experimental group.

3
4 **Supplementary Figure 3 | Immune response in hearts from mice with altered *miR-148a* expression.** (a) Representative confocal images of CD45 labeling in hearts after
5 sham-operation or aortic banding in the presence of control antagomir targeting *C. elegans miR-39* (antagomir-*Cel-39*) or antagomir against *mmu-miR-148a-3p* (top and
6 second panel), with CD45 positive cells (red), cardiomyocytes visualized with sarcomeric
7 actin (green) and nuclei visualized with Hoechst (blue). Blood clot into the left ventricular
8 chamber (left-bottom panel) and spleen (right-bottom panel) were used as internal and
9 external staining positive controls.
10
11

12
13 **Supplementary Figure 4 | Apoptotic events in hearts from mice with altered *miR-148a* expression.** (a) Representative confocal images of TUNEL labeling in hearts after
14 sham-operation or aortic banding in the presence of AAV9-MCS or AAV9-148a, with
15 TUNEL positive nuclei (red) and cardiomyocytes visualized with sarcomeric actin
16 (green). (b) Quantification of TUNEL positive cardiomyocytes in experimental groups
17 demonstrates a higher percentage of TUNEL positive myocytes after TAC compared to
18 sham.
19

20 Data information: Data are means \pm SEM. One-way ANOVA with Bonferroni's multiple
21 comparison test was used to compare groups. *n*, number of mice; AAV9, adeno-
22 associated virus; MCS, multiple cloning site; TAC, transverse aortic constriction. * $P < 0.05$ vs corresponding control group.
23
24

25 **Supplementary Figure 5 | Characterization of AAV9 transduction treatment.** (a) RT-
26 PCR analysis of viral genome copy numbers in 20ng of total DNA in hearts from mice
27 receiving AAV9-148a virus. (b) RT-PCR analysis of *miR-148a-3p* expression in diverse
28 organs from mice receiving AAV9-MCS or AAV9-148a virus.

29 Data information: Data are means \pm SEM. One-way ANOVA with Bonferroni's multiple
30 comparison test was used to compare groups. *n*, number of mice; AAV9, adeno-
31 associated virus; MCS, multiple cloning site. * $P < 0.05$ vs corresponding control group.
32

Supplemental Table 1. Morphometric and echocardiographic characteristics of mice subjected to sham or TAC surgery and treated for 3 weeks with control (Ctrl) antagomir or antagomir-148a.

	Sham		TAC	
	Ctrl antagomir	Antagomir-148a	Ctrl antagomir	Antagomir-148a
n	5	5	8	8
BW (g)	22.8±2.1	25.3±2.0	23.8±1.5	24.3±1.7
LV mass (mg)	73±1	104±18*	120±19*	121±26*
LV mass/BW (mg/g)	3.2±0.2	3.8±0.4	5.2±0.4*	6.4±1.0*
IVSd (mm)	0.87±0.03	0.84±0.06	0.89±0.14	0.87±0.13
IVSs (mm)	1.42±0.01	1.33±0.10	1.03±0.16*	1.17±0.17
LVIDd (mm)	3.39±0.10	3.57±0.25	3.66±0.54	3.62±0.57
LVIDs (mm)	2.06±0.16	2.49±0.26	2.76±0.43*	2.78±0.50*
LVPWd (mm)	0.76±0.06	1.08±0.12*	0.87±0.15	0.86±0.14
LVPWs (mm)	1.12±0.09	1.18±0.15	0.96±0.14	1.07±0.16
EF (%)	77±4	66±5*	57±3*	56±5*
FS (%)	39±3	31±3*	25±2*	25±3*
E/A (mm/s)	1.16±0.04	1.23±0.08	1.65±0.13*	1.57±0.24*

Data are expressed as means ± SEM. BW, body weight; LV, left ventricular; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; EF, ejection fraction; FS, fractional shortening; E/A, Doppler E/A ratio. *, indicates $P < 0.05$ vs sham group subjected to treatment with a control antagomir; #, indicates $P < 0.05$ vs experimental group.

Supplemental Table 2. Morphometric and echocardiographic characteristics of mice subjected to sham or TAC surgery and treated for 6 weeks with control (Ctrl) antagomir or antagomir-148a.

	Sham		TAC	
	Ctrl antagomir	Antagomir-148a	Ctrl antagomir	Antagomir-148a
n	7	8	8	8
BW (g)	22.5±1.6	25.6±1.6	25.6±1.2	24.0±1.6
LV mass (mg)	84±3	77±11	135±26*	135±20
LV mass/BW (mg/g)	3.8±0.3	4.0±0.2	4.8±0.4*	6.1±0.5*
IVSd (mm)	0.87±0.03	0.72±0.05	0.88±0.05	1.00±0.04
IVSs (mm)	1.39±0.05	1.08±0.10*	1.13±0.06	1.22±0.11
LVIDd (mm)	3.44±0.07	3.93±0.34	4.06±0.29	4.47±0.21*
LVIDs (mm)	2.07±0.16	2.90±0.36	2.97±0.33*	3.62±0.25*
LVPWd (mm)	0.89±0.03	0.69±0.04	1.04±0.12	0.85±0.08
LVPWs (mm)	1.28±0.09	0.94±0.09	1.33±0.11	1.00±0.11
EF (%)	78±4	59±6*	61±4*	47±4*
FS (%)	40±4	27±4*	27±2*	20±2*
E/A (mm/s)	1.33±0.13	1.32±0.17	1.52±0.17	1.27±0.04

Data are expressed as means ± SEM. BW, body weight; Ctrl, control; LV, left ventricular; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPwd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; EF, ejection fraction; FS, fractional shortening; E/A, Doppler E/A ratio; TAC, transverse aortic constriction. *, indicates $P < 0.05$ vs sham group subjected to treatment with a control antagomir; #, indicates $P < 0.05$ vs experimental group.

Supplemental Table 3. Morphometric and echocardiographic characteristics of mice subjected to sham or TAC surgery and treated with AAV9-MCS or AAV9-miR-148a for 3 weeks.

	Sham		TAC	
	AAV9-MCS	AAV9-miR148a	AAV9-MCS	AAV9-miR148a
n	8	5	10	14
BW (g)	20.8±0.6	21.6±1.4	20.5±2.3	24.0±0.7
LV mass (mg)	69±6	77±6	125±10*	118±6*
LV mass/BW (mg/g)	3.3±0.03	3.6±1.8	5.7±0.56*	5.0±0.22*
IVSd (mm)	0.75±0.03	0.81±0.04	0.94±0.06*	0.94±0.04*
IVSs (mm)	1.13±0.04	1.19±0.05	1.24±0.07	1.33±0.04*
LVIDd (mm)	3.61±0.10	3.67±0.10	4.07±0.12*	3.98±0.06*
LVIDs (mm)	2.47±0.10	2.54±0.10	3.10±0.17*	2.97±0.11*
LVPWd (mm)	0.68±0.06	0.72±0.03	0.97±0.05	0.96±0.03*
LVPWs (mm)	1.03±0.05	1.03±0.02	1.24±0.05*	1.25±0.03*
EF (%)	67±2	69±1	53±5*	58±3*
FS (%)	31±1	31±1	23±2*	26±2*

Data are expressed as means ± SEM. AAV, adeno-associated virus; BW, body weight; LV, left ventricular; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPwd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; EF, ejection fraction; FS, fractional shortening; MCS, multiple cloning site; TAC, transverse aortic constriction. *, indicates $P < 0.05$ vs AAV-MCS sham group; #, indicates $P < 0.05$ vs experimental group.

Supplemental Table 4. Morphometric and echocardiographic characteristics of mice subjected to sham or TAC surgery and treated with AAV9-MCS or AAV9-miR-148a for 6 weeks.

	Sham		TAC	
	AAV9-MCS	AAV9-miR148a	AAV9-MCS	AAV9-miR148a
n	11	10	10	14
BW (g)	19.1±0.5	23.8±1.0	22.6±1.2	25.1±0.9
LV mass (mg)	82±3	99±8	102±7*	116±5*
LV mass/BW (mg/g)	3.9±0.1	4.1±0.2	4.6±0.4*	4.6±0.2*
IVSd (mm)	0.84±0.02	0.93±0.02*	0.85±0.02	0.91±0.02*#
IVSs (mm)	1.21±0.05	1.39±0.03*	1.17±0.05	1.26±0.03
LVIDd (mm)	3.57±0.07	3.58±0.08	4.06±0.14*	4.09±0.08*
LVIDs (mm)	2.27±0.10	2.22±0.09	3.23±0.19*	3.08±0.11*
LVPWd (mm)	0.82±0.02	0.95±0.10	0.79±0.03*	0.92±0.02#
LVPWs (mm)	1.23±0.03	1.38±0.09	1.00±0.04*	1.22±0.03#
EF (%)	74±2	76±2	46±5*	57±3*#
FS (%)	37±2	38±2	19±2*	26±2*#
E/A (mm/s)	1.24±0.04	1.25±0.05	1.35±0.03	1.19±0.02

Data are expressed as means ± SEM. AAV, adeno-associated virus; BW, body weight; LV, left ventricular; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; EF, ejection fraction; FS, fractional shortening; E/A, Doppler E/A ratio; MCS, multiple cloning site; TAC, transverse aortic constriction. *, indicates $P < 0.05$ vs AAV-MCS sham group; #, indicates $P < 0.05$ vs experimental group.

Supplemental Table 5. Morphometric and echocardiographic characteristics of mice subjected to sham or TAC surgery for 3 weeks, before treatment with AAV9-MCS or AAV9-miR-148a at 4 weeks after surgery.

	Sham		TAC	
	AAV9-MCS	AAV9-miR148a	AAV9-MCS	AAV9-miR148a
n	9	10	10	11
BW (g)	21.1±0.18	21.2±0.3	19.4±0.3	19.5±0.4
LV mass (mg)	79±3	85±4	97±3*	101±5*
LV mass/BW (mg/g)	3.7±0.12	4.0±0.22	5.0±0.17*	5.2±0.25*
IVSd (mm)	0.68±0.02	0.71±0.08	0.90±0.03*	0.94±0.03*
IVSs (mm)	0.99±0.04	1.05±0.10	1.23±0.04*	1.32±0.05*
LVIDd (mm)	3.31±0.06	3.39±0.34	3.69±0.06*	3.72±0.06*
LVIDs (mm)	2.14±0.06	2.15±0.21	2.59±0.05*	2.59±0.11*
LVPWd (mm)	0.72±0.04	0.77±0.09	0.92±0.02*	0.90±0.06*
LVPWs (mm)	1.03±0.02	1.08±0.10	1.22±0.03*	1.20±0.06*
EF (%)	73±2	74±3	65±2*	65±3*
FS (%)	35±1	37±2	30±2*	30±3*

Data are expressed as means ± SEM. AAV, adeno-associated virus; BW, body weight; LV, left ventricular; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; EF, ejection fraction; FS, fractional shortening; MCS, multiple cloning site; TAC, transverse aortic constriction. *, indicates $P < 0.05$ vs AAV-MCS sham group; #, indicates $P < 0.05$ vs experimental group.

Supplemental Table 6. Morphometric and echocardiographic characteristics of 3 weeks sham and TAC surgery followed by a 1 week post-treatment with AAV-MCS or AAV9-miR-148a.

	Sham		TAC	
	AAV9-MCS	AAV9-miR148a	AAV9-MCS	AAV9-miR148a
n	9	10	10	11
BW (g)	21.2±0.22	21.3±0.35	20.7±0.47	20.91±0.34
LV mass (mg)	80.5±4	89.2±3	101±7*	102±5*
LV mass/BW (mg/g)	3.7±0.16	4.2±0.13	4.7±0.25*	4.9±0.27*
IVSd (mm)	0.73±0.03	0.78±0.03	0.83±0.04*	0.89±0.04*
IVSs (mm)	1.19±0.04	1.10±0.03	1.14±0.04	1.22±0.03*
LVIDd (mm)	3.83±0.04	3.91±0.09	4.03±0.08*	3.91±0.07*
LVIDs (mm)	2.52±0.06	2.75±0.11	3.14±0.10*	2.88±0.09*^
LVPWd (mm)	0.78±0.04	0.81±0.04	0.85±0.06	0.87±0.05
LVPWs (mm)	1.11±0.03	1.13±0.56	1.02±0.05	1.18±0.04^
EF (%)	72±2	65±9	53±2*	59±3*
FS (%)	34±1	30±2	23±2*	26±2*

Data are expressed as means ± SEM. AAV, adeno-associated virus; BW, body weight; LV, left ventricular; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; EF, ejection fraction; FS, fractional shortening; TAC, transverse aortic constriction. *, indicates $P < 0.05$ vs AAV-MCS sham group; #, indicates $P < 0.05$ vs experimental group.

Supplemental Table 7. Morphometric and echocardiographic characteristics of mice subjected to 7 weeks of sham or TAC surgery and treated AAV9-MCS or AAV9-miR-148a for 3 weeks.

	Sham		TAC	
	AAV9-MCS	AAV9-miR148a	AAV9-MCS	AAV9-miR148a
n	9	10	10	11
BW (g)	22.0±0.3	22.0±0.5	21.2±0.3	22.0±0.5
LV mass (mg)	88±4	86±3	105±6*	116±5*
LV mass/BW (mg/g)	4.0±0.2	3.9±0.1	4.8±0.3*	5.3±0.3*
IVSd (mm)	0.73±0.03	0.79±0.04	0.83±0.05*	0.90±0.03*
IVSs (mm)	1.07±0.03	1.07±0.06	1.15±0.05	1.20±0.04*
LVIDd (mm)	3.85±0.04	3.79±0.06	4.20±0.10*	4.02±0.08*
LVIDs (mm)	2.62±0.04	2.59±0.08	3.31±0.15*	2.90±0.08*#
LVPWd (mm)	0.89±0.08	0.84±0.04	0.80±0.05	0.94±0.05#
LVPWs (mm)	1.17±0.05	1.13±0.04	1.01±0.06	1.23±0.05#
EF (%)	70±2	68±9	50±4*	61±2*#
FS (%)	32±1	31±2	21±2*	27±1#

Data are expressed as means ± SEM. AAV, adeno-associated virus; BW, body weight; LV, left ventricular; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPwd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; EF, ejection fraction; FS, fractional shortening; MCS, multiple cloning site; TAC, transverse aortic constriction. *, indicates $P < 0.05$ vs AAV-MCS sham group; #, indicates $P < 0.05$ vs experimental group.

Supplemental Table 8. Patient and donor characteristics.

	Gender	Age at Htx (yrs)	LVAD	LVEF (%)	LV ED/ES (mm)	LV PWT (mm)
DCM 1	female	48	yes	10	68/65	7
DCM 2	male	55	no	20	69/65	8
DCM 3	male	57	yes	15	47/43	10
DCM 4	male	47	no	25	51/49	10
DCM 5	male	66	no	25	58/54	10
DCM 6	female	21	yes	15	61/54	6
DCM 7	female	58	yes	15	65/57	8
DCM 8	male	36	yes	15	69/63	8
DCM 9	female	58	yes	20	67/62	6
DCM 10	male	61	yes	10	62/60	7
HCM 1	male	57	no	55	43/42	16
HCM 2	male	31	no	15	85/81	13
HCM 3	male	37	no	20	66/54	13
HCM 4	male	36	no	25	47/39	15
HCM 5	female	31	no	50	29/10	18
HCM 6	female	36	no	40	45/20	14
HCM 7	male	25	no	45	40/20	20
HCM 8	female	36	no	45	45/28	15
HCM 9	female	34	no	20	52/40	13
Control 1	-	-	no	-	-	-
Control 2	-	-	no	-	-	-
Control 3	male	65	no	-	-	-
Control 4	female	72	no	-	-	-
Control 5	male	39	no	-	-	-
Control 6	female	48	no	-	-	-
Control 7	male	38	no	-	-	-
Control 8	female	53	no	-	-	-
Control 9	male	32	no	-	-	-
Control 10	male	43	no	-	-	-
Control 11	male	48	no	-	-	-

DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVAD, patient received left ventricular assist device; LVEF, left ventricular ejection fraction; LV ED, left ventricular end-diastolic dimension; LV ES, left ventricular end-systolic dimension; LVPWT, left ventricular posterior wall thickness; yrs, years; mm, millimeter; -, information not available,.