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Supplemental Information

Therapeutic Delivery of miR-148a Suppresses Ventricular Dilation in Heart Failure

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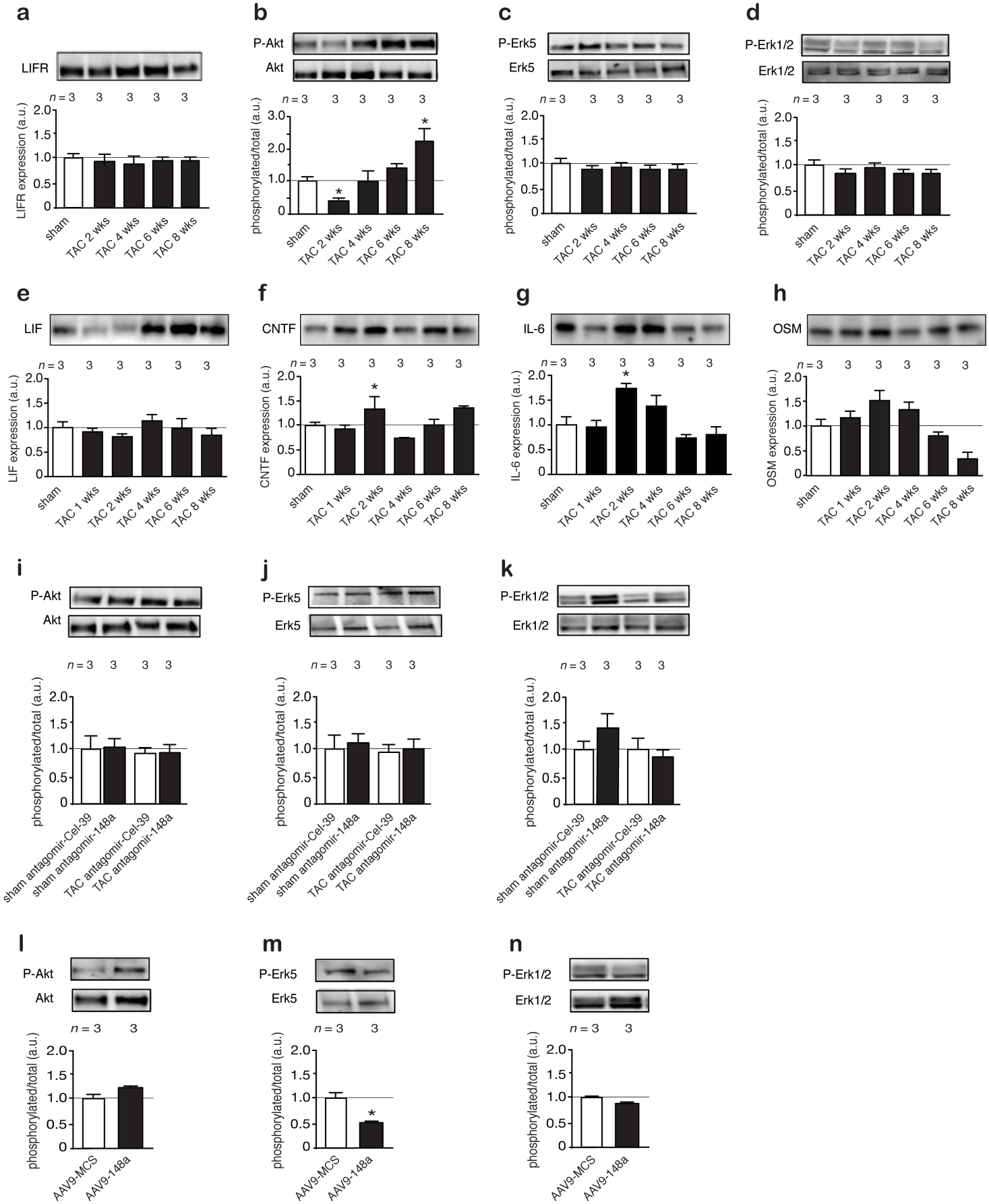
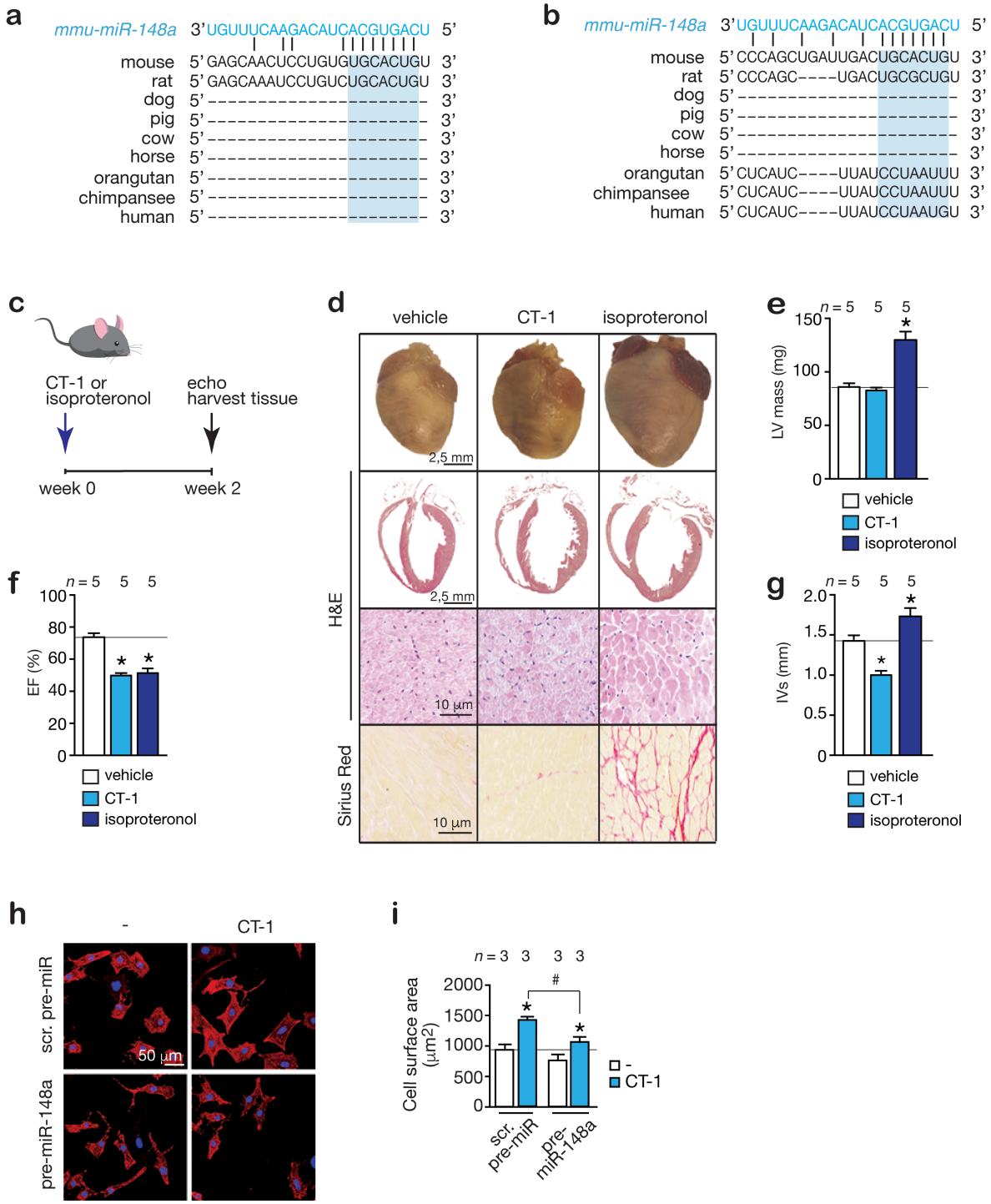
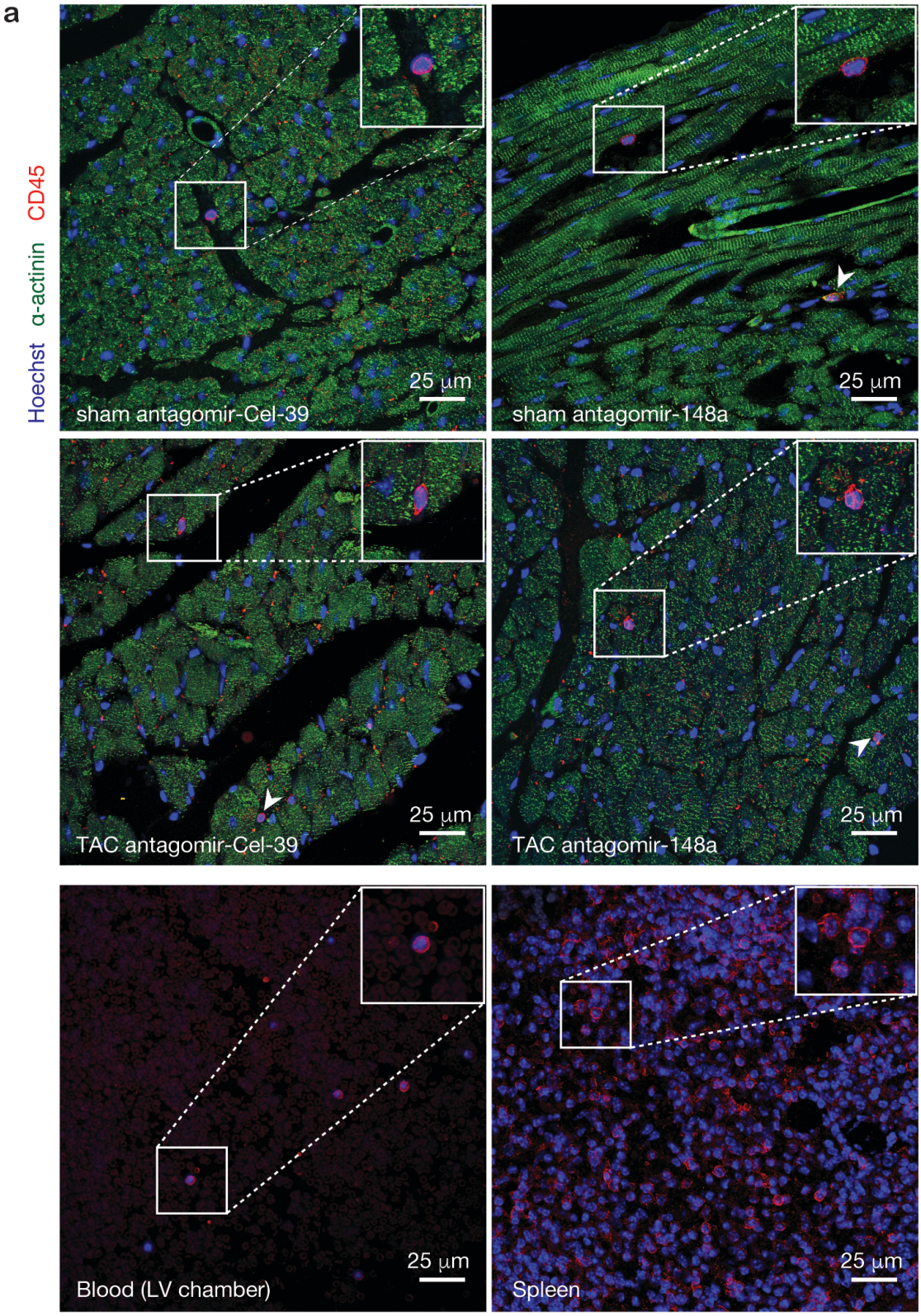
Supplemental Figure 1



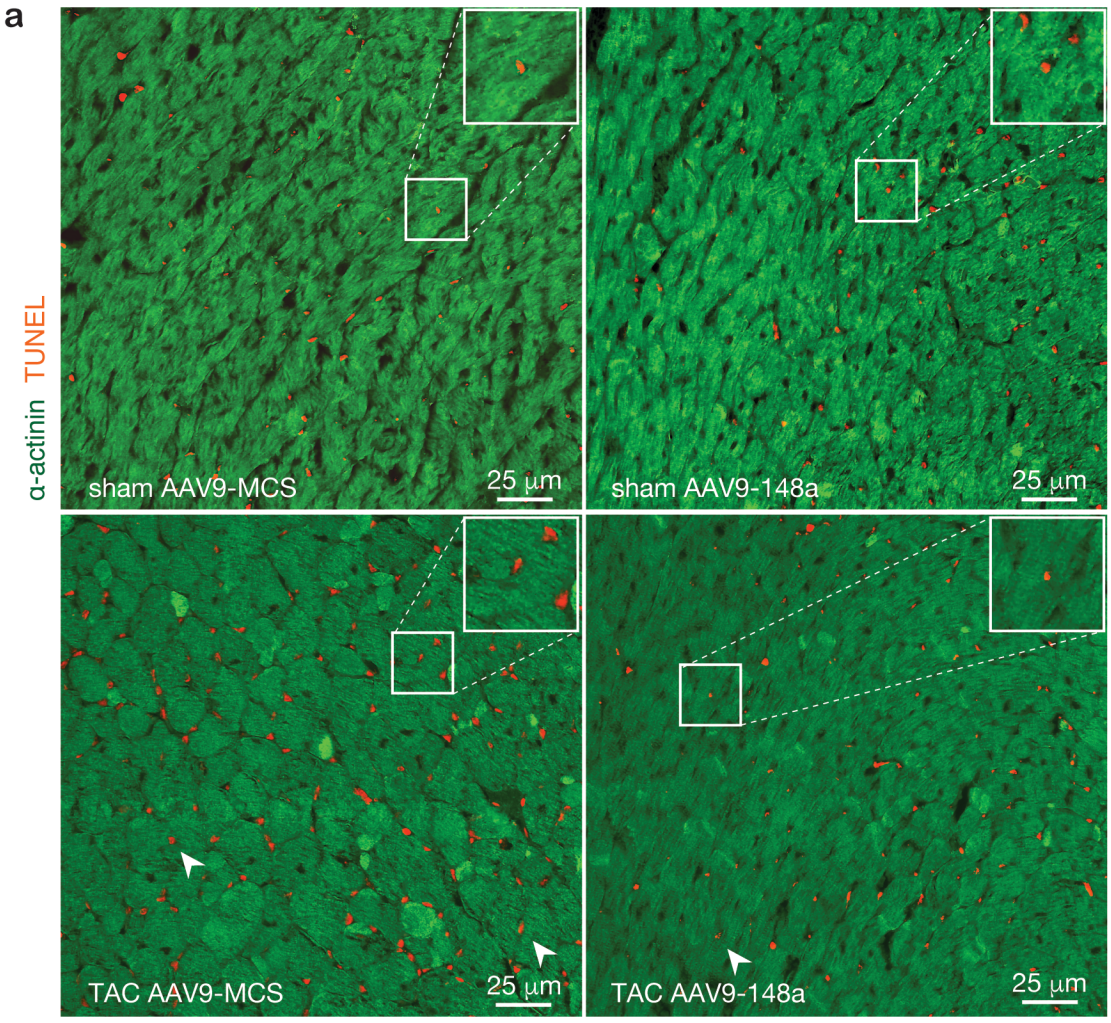
Supplemental Figure 2

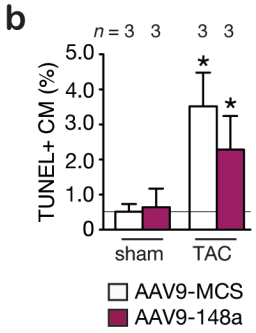


Supplemental Figure 3

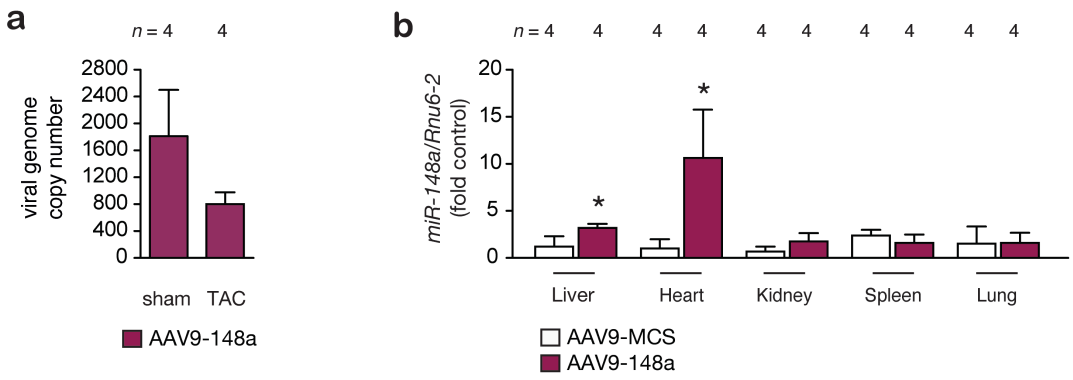


Supplemental Figure 4





Supplemental Figure 5



Legends to the Supplementary Figures

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

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

Supplementary Figure 2 | CT-1 infusion results in cardiac dilation. (a-b) Evolutionary conservation of the other two *mmu-miR-148a* seed regions on gp130 3'UTR. In mouse they are respectively located on the nucleotide position 6150-6156 and 8532-8538 [ENSMUST00000183663.7]. **(c)** Study design of mice receiving continuous infusion of CT-1 (20 µg/kg/day) or isoproterenol (60 mg/kg/day). **(d)** Representative image of whole hearts (top panels), H&E-stained cardiac sections (second panels), high magnification H&E-stained sections (third panels), or Sirius Red stained (lower panels) histological sections of hearts from saline, CT-1 infused and isoproterenol-infused mice. Measurements of **(e)** LV mass, **(f)** EF **(g)** IVSs in saline, CT-1-treated or isoproterenol-treated mice. **(h)** Confocal microscopy images of neonatal rat cardiomyocytes treated with scrambled or precursor-148a miR-148a with or without CT-1 stimulation. **(i)** Cell surface measurements from conditions in (**f**).

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

Supplementary Figure 3 | Immune response in hearts from mice with altered *miR-148a* expression. (a) Representative confocal images of CD45 labeling in hearts after 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 second panel), with CD45 positive cells (red), cardiomyocytes visualized with sarcomeric actin (green) and nuclei visualized with Hoechst (blue). Blood clot into the left ventricular chamber (left-bottom panel) and spleen (right-bottom panel) were used as internal and external staining positive controls.

Supplementary Figure 4 | Apoptotic events in hearts from mice with altered *miR-148a* expression. (a) Representative confocal images of TUNEL labeling in hearts after sham-operation or aortic banding in the presence of AAV9-MCS or AAV9-148a, with TUNEL positive nuclei (red) and cardiomyocytes visualized with sarcomeric actin (green). **(b)** Quantification of TUNEL positive cardiomyocytes in experimental groups demonstrates a higher percentage of TUNEL positive myocytes after TAC compared to sham.
Data information: Data are means ± SEM. One-way ANOVA with Bonferroni's multiple comparison test was used to compare groups. *n*, number of mice; AAV9, adeno-associated virus; MCS, multiple cloning site; TAC, transverse aortic constriction. *$P < 0.05$ vs corresponding control group.

Supplementary Figure 5 | Characterization of AAV9 transduction treatment. (a) RT-PCR analysis of viral genome copy numbers in 20ng of total DNA in hearts from mice receiving AAV9-148a virus. **(b)** RT-PCR analysis of *miR-148a-3p* expression in diverse organs from mice receiving AAV9-MCS or AAV9-148a virus.
Data information: Data are means ± SEM. One-way ANOVA with Bonferroni's multiple comparison test was used to compare groups. *n*, number of mice; AAV9, adeno-associated virus; MCS, multiple cloning site. *$P < 0.05$ vs corresponding control group.

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 dimenstion; LVPWT, left ventricular posterior wall thickness; yrs, years; mm, millimeter; -, information not available,.