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Extended Experimental Procedures

Reagents

Antibodies were obtained from the following vendors: anti-MEF2 and anti-HDAC4 from Santa Cruz Biotechnology (Santa Cruz, California, USA), anti-GATA4 and anti-acetyl-lysine from Upstate (Charlottesville, Virginia, USA), anti-actin from Chemicon (Danvers, Massachusetts, USA), anti-HDAC5 and anti–p(S498)-HDAC5 were from Millipore and Abcam respectively. Trichostatin A (TSA) was purchased from Selleck Chemicals and MC1568 was provided by Sigma. The Amersham ECL Western detection system (GE Healthcare Bio-Sciences, Piscataway, New Jersey, USA) was used for chemiluminescence visualization of immunoblots. Reagents for real-time polymerase chain reaction (PCR) including Master Mix® and primers with TaqMan® probes were obtained from Applied Biosystems (Foster City, California, USA). RNA extraction was performed using Trizol (Molecular Research Center, Inc, Cincinnati, Ohio, USA). Rhodamine-conjugated phalloidin and wheat germ agglutinin (WGA) were purchased from Invitrogen (Carlsbad, California, USA).

Myocyte cell culture

Primary neonatal rat ventricular cardiomyocyte cultures were prepared from the hearts of 1-3 day-old neonatal rat pups (Charles River, Wilmington, Massachusetts, USA) as previously described {Bishopric, 1991 #920}, by sequential digestion in a trypsin-containing calcium-free buffer and trituration. Final cultures were >90% myocytes. Cells were allowed to attach overnight in 5% fetal bovine serum-containing minimal essential media (MEM) overnight, and then moved to serum-free MEM supplemented with holotransferrin, insulin and vitamin B12 (MEM-TIB) for at least 48h prior to experiments BrdU (0.1mM) was added to media for the first 48 hours to inhibit fibroblast proliferation. Hypertrophy was induced using FBS (5% final concentration) or norepinephrine (4 μM) in MEM-TIB. MEF2 modulators were dissolved in DMSO and added to cell cultures to varying final concentrations together with hypertrophic agonists. Cardiac myocytes were cotransfected with wt and mutant MEF2D and EP300 expression vectors using a calcium phosphate method as previously described {Bishopric, 1991 #920} and harvested at 96 hr.

Immunofluorescence.

Cells were fixed with 4% paraformaldehyde for 10 minutes at room temperature. Following PBS washes, cells were permeabilized with 0.1% Triton-X in PBS and blocked with 1% bovine serum albumin for 1 hour. Primary antibodies against HDAC-4 and- 5 were incubated in 1% BSA-PBS for 1 hour at room temperature. Cells were washed 2X in PBS and then incubated with an Alexa Fluor® 488-conjugated secondary antibody for 1 hour at room temperature. Cells were then washed in PBS and cured overnight at room temperature in Prolong Gold DAPI. Cells were imaged using a Zeiss LSM 700 confocal microscope. Images were acquired using Zen 2009 Ver. 5.5, SP2.

Western Blot and qPCR Analysis.

Protein samples were collected in RIPA buffer (Sigma), resolved on SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in 0.5% TBS-T for 1 hour at room temperature followed by incubation in primary antibody at appropriate dilutions overnight. The membranes were incubated in HRP-conjugated secondary antibody for 2 hours at room temperature and developed using chemiluminesce. Total RNA was extracted from left ventricular tissue using Trizol Reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized using High Capacity RT-PCR kits (Invitrogen) according to the manufacturer's instructions. cDNA was amplified using TaqMan Universal PCR master mix reagent (Applied Biosystems, Foster City, CA) at the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles: 15 s at 95°C and 1 min at 60°C in an ABI 7900HT thermocycler. mRNA expression levels were normalized to those of the internal reference 18S rRNA. All samples were run in duplicate. Data was analyzed using RQ manager software v. 1.2 (Applied Biosystems).

Immunoprecipitation.
500nG of protein sample collected from heart tissue in RIPA was incubated with antibodies against acetyl-lysine, GATA4 and MEF2 (5µg). Immune complexes were captured using TrueBlot sepharose beads and subjected to Western analysis as above.

Murine surgery and imaging.
All experiments were performed on 2-3 month old wild type C57BL/6 mice under protocols approved by the Institutional Review Board of University of Miami. Cardiac hypertrophy was induced by thoracic aortic constriction (TAC) as described previously, with minor variations (Wei et al., 2008). Pressure gradients induced by TAC were evaluated postoperatively by pulsed-wave Doppler echocardiography to confirm equivalent gradients in all animals (45 ± 5 mmHg). For echocardiography, mice were placed under anesthesia with 40mg/kg ketamine and 5mg/kg xylocaine and secured in a supine position. Mice were evaluated using 40-hertz transducer on a Visual Sonics 770 High Resolution Imaging System. B-mode in the short and long axis view of the ventricle was used to evaluate wall motion defects of ventricle and M-mode in long axis view used for the interventricular septal thickness, posterior wall thickness and the left ventricular dimensions in systole and diastole.
8MI Prevention protocol: 8MI or its vehicle (DMSO) was delivered via intraperitoneal (IP) injection daily for 35 days beginning one week prior to surgical intervention.
8MI Reversal protocol: Daily IP injection of 8MI was initiated beginning 4 weeks post-TAC and continued for 4 more weeks.

Swimming stress model: We used a previously established protocol for induction of hypertrophy with swimming exercise, with minor variations {Kaplan, 1994 #11929}. Mice without prior swimming experience underwent a training period of daily swimming in 32-34°C fresh water in a multi-chamber apparatus under constant observation. Swimming twice daily intervals were gradually increased from 10 minutes tp 90 minutes over a 1-week interval. Swimming intervals were separated by 5 hours. On completion of training, a protocol was commenced wherein mice were required to swim for 90 minutes at a time,

twice daily, with occasional interventions (knocks on the side of the apparatus) to prevent resting or holding to the side of the chamber. Control mice were immersed briefly, removed and dried with a towel. Mice were administered DMSO or 8MI (40 mg/kg I.P.) daily, beginning one week prior to the protocol and continued throughout the next 4 weeks of swimming. At the end of 4 weeks of swimming, mice underwent echocardiographic imaging; a subset underwent euthanasia with organ morphologic studies. The remaining mice in the 8MI group were continued on the swimming protocol for an additional 4 weeks, without further administration of the compound.

RNASeq.

Total RNA from mouse left ventricles (n=3-4 per treatment group) was analyzed for quality on a BioAnalyzer Nano 6000 and Illumina TruSeq total strand-specific RNA-Seq library prep was used to prepare libraries for sequencing. Sample llbraries were sequenced as 75 bp single reads, using Illumina NextSeq V2 Flow Cells. Alignment of RNA-Seq reads to the mouse genome (Mus musculus UCSC mm10) was accomplished with TopHat and Cufflinks 2.1.1. Analysis of differential gene expression was performed using DESeq2 software. All applications were accessed within the Illumina BaseSpace genomic analysis environment (http://www.illumina.com/informatics/research/sequencing-data-analysis-management/rna-seq-data-analysis.html).

Differentially regulated transcripts were quantified and ranked for significance, with *p* cutoffs of < 0.01 for transcripts with fold change of > 0.70 or <1.5, and <0.05 for transcripts with fold change of ≤ 0.07 or ≥ 1.5. Gene Set Enrichment Analysis and Hierarchical clustering and heatmapping of expression data were respectively performed using GSEA http://software.broadinstitute.org/gsea/index.jsp
and GENE-E http://www.broadinstitute.org/cancer/software/GENE-E/index.html) accessed via the Broad Institute. Annotations were obtained from the Mouse Genome Informatics (MGI) portal. Gene Ontology and Network analysis was performed using Ingenuity® software (http://www.ingenuity.com).

Figure S1. Wei, Joshi et al.

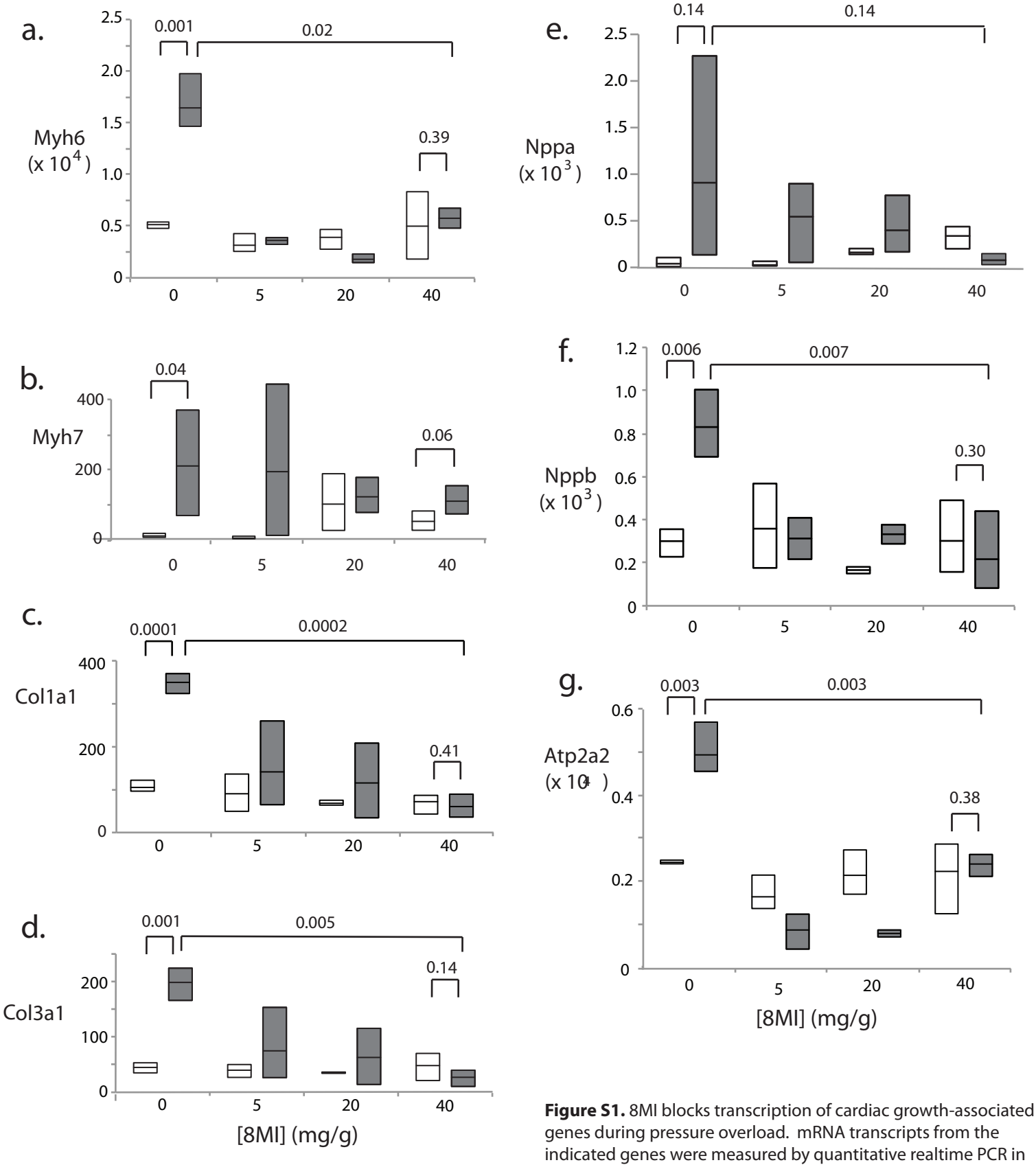

Figure S1. 8MI blocks transcription of cardiac growth-associated genes during pressure overload. mRNA transcripts from the indicated genes were measured by quantitative realtime PCR in myocardial samples from mice treated as in Figure 2. N=3 mice per treatment group. Y axis = normalized transcript units. Bars denote full data range and mean for each replicate set. Open bars: sham-operated mice. Shaded bars: transverse aortic banded mice.

Figure S2. Wei, Joshi et al.

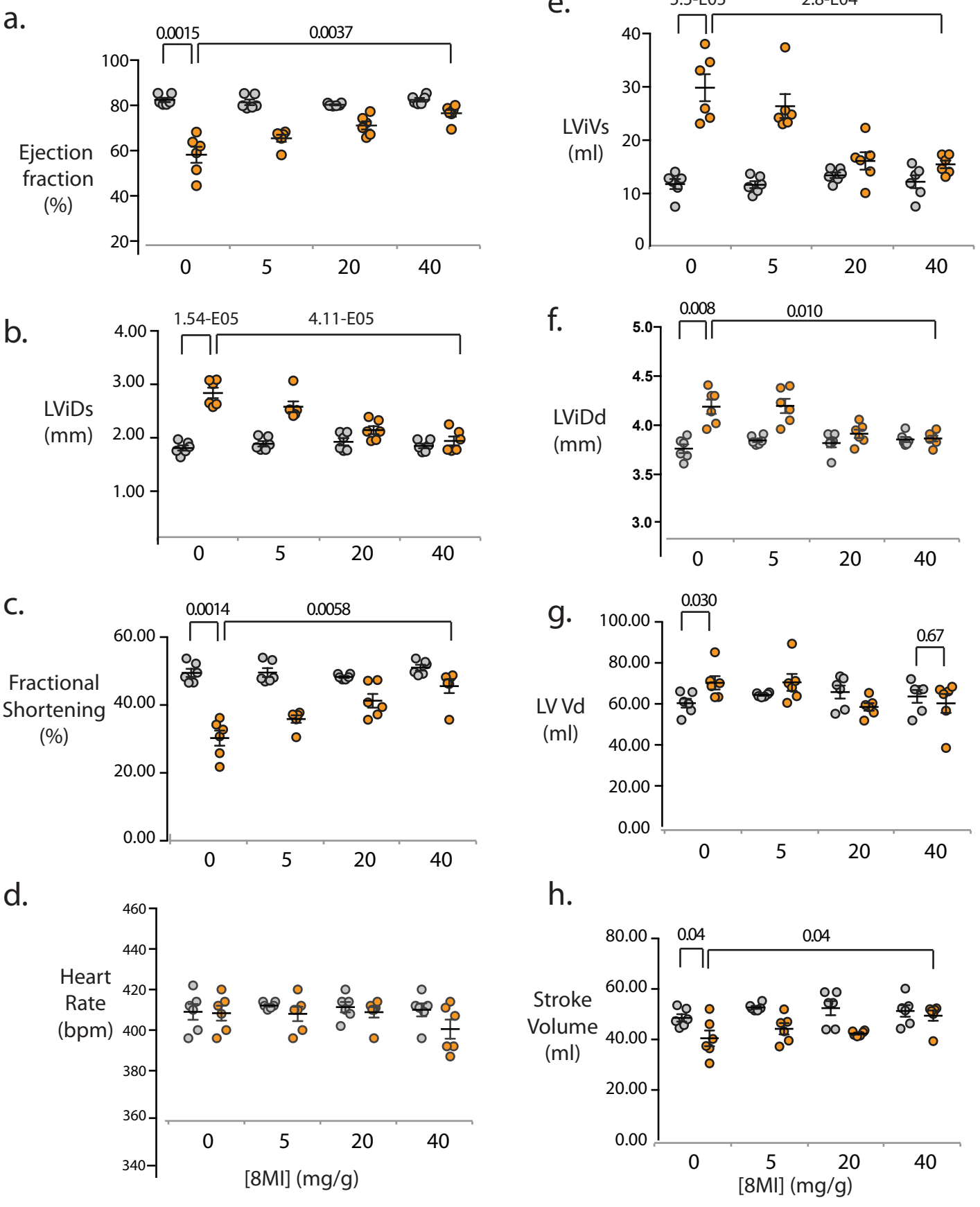

Figure S2. 8MI preserves cardiac function after TAC. Myocardial functional parameters including ejection fraction (EF, a.) and left ventricular internal diameter at end systole (LViDs, b.) were determined by echocardiography was performed 21 days post TAC or sham operation in mice receiving 8MI (5, 20 and 40 mg/kg) or its vehicle. N= 6 per experimental group.

Supplemental Figure 3.

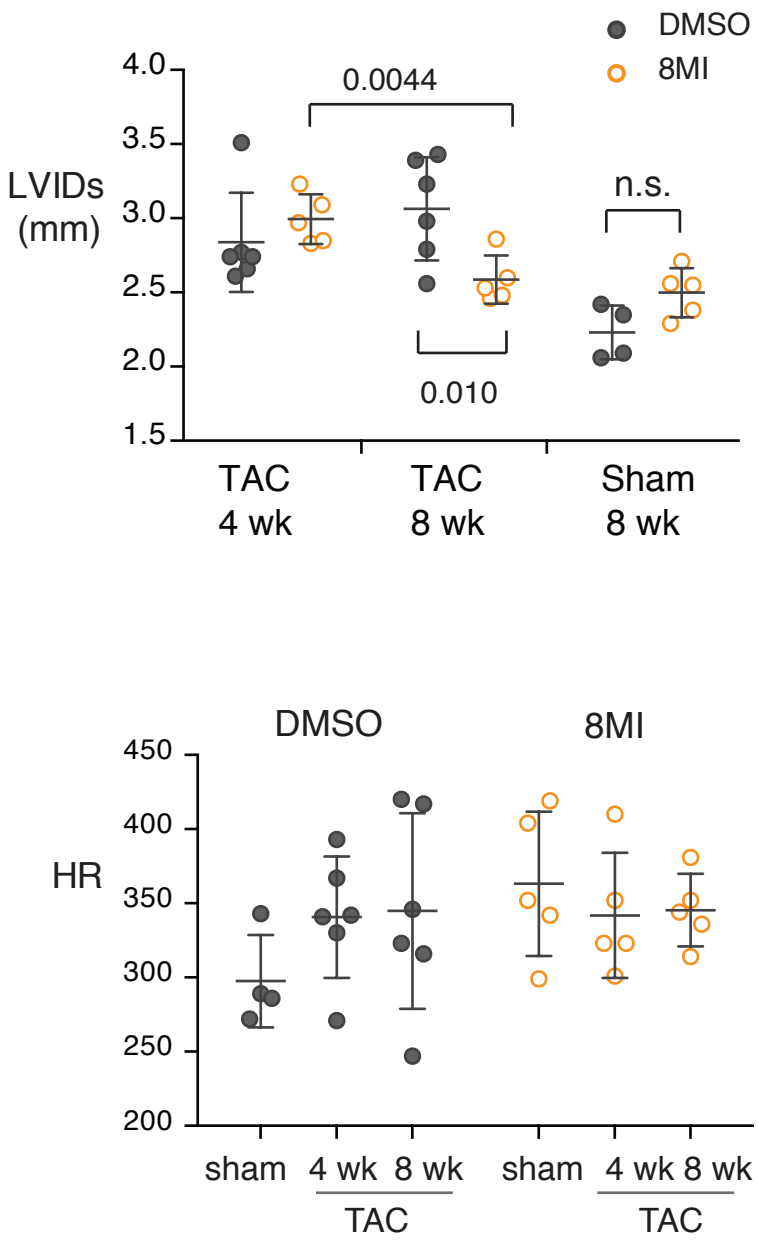

Figure S3. Effect of 8MI on LV systolic dimension and heart rate after TAC. Scatterplots indicate data points from individual mice (N=6 per group) with superimposed mean ±SEM. Exact p values were calculated using ANOVA with post-hoc testing for multiple comparisons.

Supplemental Figure 4. Wei, Joshi et al.

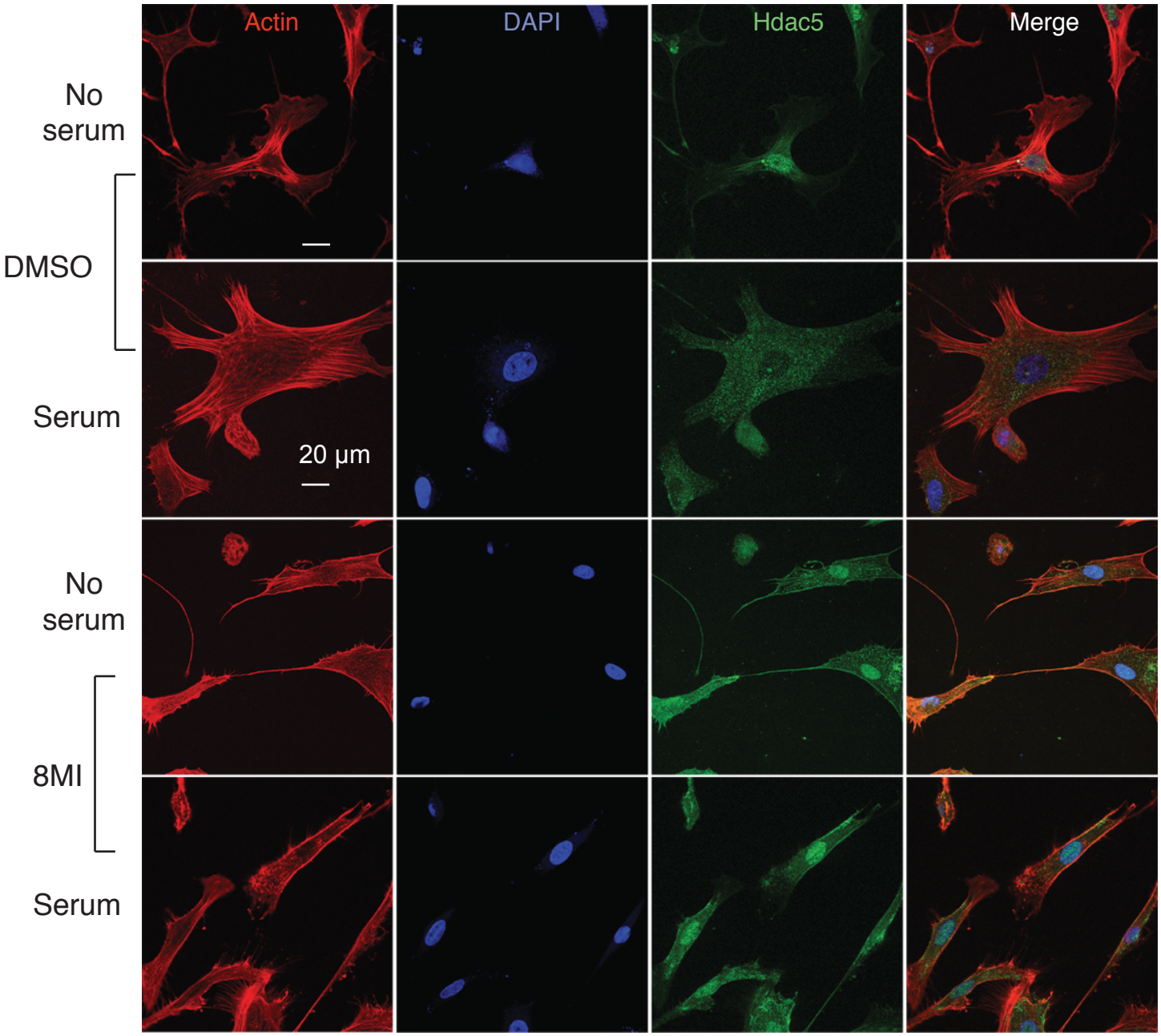

Figure S4. 8MI prevents nuclear export of Hdac5 in response to serum. NRVMs were obtained from 1 day old Sprague-Dawley rat hearts and cultured in serum-free media for 48 hours as described in Methods, and subsequently exposed to 5% FCS-containing media. Figures are representative of 3 independent experiments. Original magnification: 630x

Supplemental Table 1. Characteristics of human subjects analyzed for this study.

ID	Gender	Condition	Heart Weight (g)	Age	Ethnicity	Notes
39306A1D	Male	non-failing	414	55	White	Normal anatomy
322A20D	Male	non-failing	N/A	46	Black	Stent in LAD, apical scarring
51565T_004	Male	non-failing	472	40	White	Triple vessel CAD, septal infarct
1040640A3	Male	ICM	476.6	40	White	Severe concentric hypertrophy and chamber dilatation, ICD in place
1030528A2	Male	ICM	518	42	Black	Biventricular dilatation and hypertrophy. interstitial fibrosis
1050968B3	Male	Hypertrophic, failing	840	51	Unknown	
1040221A2	Female	Hypertrophic, failing	280	33	White	Stent in LAD, aortic valve prosthesis
1050508A3	Female	CHF	unknown	59	White	Posterior infarction, fibrosis
1040319A3	Male	ICM, aortic valve disease	548	67	White	Concentric hypertrophy, patchy fibrosis, ICD in place
1030994A3	Male	ICM	360	39	White	s/p CABG, old anterior MI, concentric hypertrophy
1062110A2	Female	ICM	526	62	Black	Stent in LAD, apical scarring
1030952A3	Male	ICM	547	62	White	Triple vessel CAD, septal infarct

Supplemental Table 2. Serum chemistries in mice subjected to TAC or a sham operation and treated with indicated doses of 8MI. Blood samples were obtained at sacrifice on day 21 after surgery and serum was frozen at 80 deg. C until analysis. Note that animal #5 (administered DMSO only) is in renal failure following TAC.

Animal ID	Treatment [8MI]	Procedure	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Calcium (mg/dL)	Total Protein (g/dL)	ALT (U/L)
1	DMSO	Sham	24	30	0.5	9.7	5.5	69
2	DMSO	Sham	135	26	0.4	10.7	6.6	65
3	DMSO	Sham	295	21	0.5	10.4	5.8	98
4	DMSO	TAC	<10	314	7.5	11.3	6.1	536
5	DMSO	TAC	205	21	0.3	11.5	6.0	73
6	DMSO	TAC	188	27	0.4	12.2	6.3	377
7	5 mg/kg	TAC	375	26	0.2	11.7	5.6	47
8	5 mg/kg	TAC	158	21	0.2	9.7	5.8	53
9	5 mg/kg	TAC	294	25	0.2	11	5.4	58
10	20 mg/kg	TAC	224	21	0.2	9.9	5.3	51
11	20 mg/kg	TAC	177	19	0.2	10.3	5.6	73
12	20 mg/kg	TAC	326	28	0.2	11.1	6.0	57
13	40 mg/kg	TAC	455	21	0.3	10.6	6.2	56
14	40 mg/kg	TAC	140	26	0.6	8.7	5.5	308
15	40 mg/kg	TAC	252	23	0.3	10.1	5.7	60

Supplemental Table 3. Annotations for hypertrophy-regulated genes. Genes in this table appear in Blocks I-IV, Figure 6. Ontologies obtained through Mouse Genome Informatics (http://www.informatics.jax.org) and Gene, PubMed and OMIM databases at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

	Gene ID	Annotation	MGI locus	Function
I	Agt	Angiotensinogen; serpin 8a (serpin peptidase inhibitor, clade A, member 8)	Chr8: 124556587-124569707 (-)	serine protease inhibitor; maintenance of blood pressure; pathogenesis of essential hypertension
	Tfrc	Transferrin receptor protein 1	Chr16: 32608920-32632794 (+)	cell surface receptor; cellular iron uptake by receptor-mediated endocytosis; required for erythropoiesis
	Gm7120	Transmembrane protein C5orf28 homolog	Chr13: 119487941-119495505 (+)	integral membrane protein; interacts with Alzheimer-associated tubulin polymerization promoting protein TPPP
	Cd79b	B-cell antigen receptor complex-associated protein beta chain	Chr11: 106311341-106314762 (-)	immunoglobulin receptor superfamily; integral membrane protein complex; adaptive immunity; B cell proliferation, differentiation
	Acta1	actin, alpha 1, skeletal muscle	Chr8: 123891767-123894736 (-)	constituent of the contractile apparatus; thin filament protein; associated with cardiac hypertrophy
	Lman1l	Protein ERGIC-53-like	Chr9: 57607085-57620774 (-)	Protein processing in endoplasmic reticulum; ER-Golgi intermediate compartment; vesicle transport
	Dct	dopachrome tautomerase; TRP-2; TYRP2	Chr14:118012792-118052246 (-)	Melanin biosynthesis; oxidase; oxygenase; isomerase
	Mmp3	Stromelysin-1	Chr9:7445822-7455972 (+)	Metalloprotease; wound repair; degrades ECM components fibronectin, laminin, collagens, proteoglycans
	Dkk3	dickkopf WNT signaling pathway inhibitor 3	Chr7:112116019-112159057 (-)	Antagonist of canonical Wnt signalling; negative regulator of aldosterone and cortisol biosynthesis; expressed in heart, brain, and spinal cord
	Tceal7	transcription elongation factor A (SII)-like 7; Bex4	ChrX:136214779-136226100 (+)	Tumor suppressor; negative regulation of c-Myc and NFkB transcription
	Nppb	natriuretic peptide B	Chr4:147985788-147987205 (+)	peptide hormone; associated with cardiac hypertrophy
II	Myh7	myosin, heavy chain 7, cardiac muscle, beta	Chr14:54970688-54994634 (-)	actin binding motor protein; component of contractile apparatus; associated with cardiac hypertrophy
	Nuak1	NUAK family, SNF1-like kinase, 1; AMPK-related kinase, Ark5	Chr10:84370905-84440597 (-)	non-receptor serine/threonine protein kinase; inhibitor of mTORC1 signalling pathway; maintenance of mitochondrial respiratory chain complexes and respiratory capacity
	Nppa	natriuretic peptide A	Chr4:148000722-148002079 (+)	peptide hormone; associated with cardiac hypertrophy

	Apod	apolipoprotein D	Chr16:31296192-31314808 (-)	Lipocalin/cytosolic fatty-acid binding domain Resistance to oxidant stress
	Wisp2	WNT1-inducible-signaling pathway protein 2; Ccn5	Chr2:163820861-163833146 (+)	Mesenchymal cell-derived activator of canonical Wnt signaling; repressor of stemness; adipogenesis of MSCs
	Mybpc2	myosin binding protein C, fast type	Chr7:44501699-44524669 (-)	cytoskeletal protein; non-membrane-bound organelle; component of contractile apparatus
	Cry1	cryptochrome circadian clock 1	Chr10:85131702-85185054 (-)	DNA photolyase; nuclear protein; mitochondrial protein; adenine dinucleotide-binding; key component of circadian core oscillator complex; regulator of circadian clock
	Col5a2	Collagen alpha-2(V) chain	Chr2:27886425-28039514 (+)	extracellular matrix structural protein; Expressed in fetal myocardium and adult heart valves
	Bgn	Biglycan	ChrX:73483602-73495933 (+)	cytokine; extracellular matrix protein; required for normal body weight; targeted by miR466 family
	Abra	Actin-binding Rho-activating protein; STARS	Chr15:41865293-41869720 (-)	required for arteriogenesis after vascular occlusion
	Vim	Vimentin	Chr2:13573927-13582826 (+)	Class III intermediate filament; required for endothelin-NO balance, flow-mediated dilatation
	Mt2	Metallothionein-2	Chr8:94172618-94173567 (+)	Metallothionein, vertebrate, metal binding site, NO signalling, copper detoxification, xenobiotic sensitivity
	Adamtsl 2	ADAMTS-like protein 2	Chr2:27079379-27108981 (+)	Metalloprotease; extracellular matrix glycoprotein; serine protease inhibitor
	Mfap4	Microfibril-associated glycoprotein 4	Chr11:61485431-61488900 (+)	Required for elastinogenesis; oxidative stress defense
	Col1a2	Collagen alpha-2(I) chain	Chr6:4504814-4541543 (+)	Extracellular matrix structural protein; Repair of myocardial damage and prevention of post-MI rupture; myocardial tensile strength
	Fdn3	Endothelin-3	Chr2:174760619-174784042 (+)	Melanocyte development; neural crest-derived neural precursors; peptide hormone
	Fnpp1	Ectonucleotide pyrophosphatase/ phosphodiesterase family member 1	Chr10:24637914-24712159 (-)	Pyrophosphatase; nucleotide phosphatase; Inhibitor of ectopic calcification; negative regulator of FGF-23 and phosphaturia; inhibitor of osteocalcin
	Thbs4	Thrombospondin-4	Chr13:92751590-92794818 (-)	Matrix-myocyte signalling; adaptation to pressure overload; promotion of ER stress response
III	Emp1	Epithelial membrane protein 1	Chr6:135362931-135383173 (+)	Integral tetraspan membrane protein, belonging to the PMP-

				22/EMP/MP20/Claudin family; regulator of adherens and apical tight junction formation
	Cysltr1	Cysteinyl leukotriene receptor 1	ChrX:106574346-106603679 (-)	G-protein coupled receptor; Regulator of vascular permeability during inflammation; eosinophilogenesis; Th2 immunity
	Fhl1	Four and a half LIM domains protein 1	ChrX:56731787-56793346 (+)	Homeobox transcription factor; zinc finger transcription factor; RNA binding protein; actin family cytoskeletal protein; promotes NFATc transcription and hypertrophy; negative regulator of titin phosphorylation and mechanics
	Fuca2	Plasma alpha-L-fucosidase	Chr10:13499540-13519035 (+)	Alpha-L-fucosidase in plasma and fibroblasts; may also have transglycosylation properties
	Tbx15	T-box transcription factor TBX15	Chr3:99240381-99354259 (+)	Transcription factor; regulates glycolytic fiber; promotes oxygen consumption, spontaneous activity, and glucose consumption.
	Fstl1	Follistatin-related protein 1	Chr16:37776873-37836514 (+)	BMP4 antagonist; secreted from adult heart; repressor of hypertrophy; activator of AMPK; pneumocyte differentiation and maturation
	Col14a1	Collagen alpha-1(XIV) chain	Chr15:55307750-55520803 (+)	Formation of cardiac interstitium; promotion of collagen network supporting myocardial cell survival and function
	Col8a1	Collagen alpha-1(VIII) chain	Chr16:57624258-57754737 (-)	extracellular matrix structural protein; cell adhesion molecule
	Fibin	Fin bud initiation factor homolog	Chr2:110360917-110363183 (-)	Essential for pectoral fin bud initiation in fish; control of tbx5 expression
	Mfap5	Microfibrillar-associated protein 5; MAGP2	Chr6:122505845-122529290 (+)	Extracellular matrix glycoprotein; microfibril component; regulates aortic elastogenesis; binds active TGFβ1, TGFβ2, and BMP2
	Myot	Myotilin	Chr18:44334074-44355724 (+)	Protein phosphatase; immunoglobulin receptor superfamily; muscle-specific Z-disc protein; assembly and structural upkeep of the sarcomere in fast fibers
IV	Aldob	Fructose-bisphosphate aldolase B;	Chr4:49535995-49549546 (-)	Gluconeogenesis, glycolysis; fructose metabolism; hereditary fructose intolerance;
	Ucp3	Mitochondrial uncoupling protein 3	Chr7:100472990-100486432 (+)	Mitochondrial carrier protein; mitochondrial inner membrane protein; adaptation to reduced cellular energy balance via increased FAO and reduced oxidative stress; mitochondrial uncoupling and reduced cardiac caloric efficiency

Gm1078	Sbk3, SH3 domain binding kinase family, member 3	Chr7:4965260-4971168 (-)	ATP binding, serine-threonine kinase activity
Angpt1	Angiopoietin-1; Angpt1	Chr15:42424727-42676977 (-)	regulation of ischemic damage; coronary vessel formation; endothelial survival; protection against diabetic nephropathy
Tmem56	Transmembrane protein 56; Tmem56	Chr3:121201761-121283098 (-)	Extensively conserved transmembrane protein (also in Aradopsis and rice), 276 aa protein
Gm10635	Noncoding RNA	Chr9:79444037-79519302 (-)	Non-coding RNA, 3 exons
Kcnv2	Potassium voltage-gated channel subfamily V member 2	Chr19:27322588-27337179 (+)	Encodes Kv8.2, Kv11.1,; Ikr, delayed rectifier K+ channel; upregulated from fetal to adult life
Gm6416	lncRNA	Chr13:117130025-117135884 (+)	Non-coding RNA, 4 exons; expressed in postnatal and adult aorta and vein; induced in hypertensive aorta and myocardium, downregulated in hypotension
Cngb3	Cyclic nucleotide-gated cation channel beta-3; Cngb3	Chr4:19280850-19510623 (+)	cyclic nucleotide-gated ion channel; voltage-gated potassium channel; CAMP signalling, cGMP binding, response to stimulus; color vision
Gm19689	lncRNA	Chr17:83033592-83078225 (-)	Non-coding RNA, 4 exons; high expression in heart and brown fat
Cenpf	Centromere protein F, LEK1	Chr1:189640606-189688086 (-)	Rb binding, nuclear localization, active cell division, cardiomyocyte division, myoblast growth arrest
Ccl11	Eotaxin; Ccl11; Chemokine (C-C-motif) ligand 11	Chr11:82057823-82062955 (+)	chemokine(PC00207); smooth muscle cell migration; eosinophil chemotaxis; inflammation; IL-6 production

Supplemental Table 4. Upstream regulators and networks of hypertrophy-responsive genes. Ingenuity® software was used to identify common upstream regulators and network members of genes induced (+) or repressed (-) by TAC and normalized by 8MI.

Pathway/ regulator	Genes affected	+/-	P value for overlap	Network
Gata4	Acta1, Ankrd1, Dio2, Ltbp2, Myh7, Nppa, Timp1	+	4.59E-09	Gata4, Nkx2-5
Tgfb1	Acta1, Ankrd1, Col8a1, Dusp4, F2rl1, Fhl1, Fstl3, Gdf6, Ltbp2, Myh7, Nppa, Scd, Timp1, Tnc, Wisp2	+	6.68E-09	Crebbp, Egfr, Ep300, Mkl1, Myc, Sp1, Srf, Tgfb1, Tnf
Raf1	Ankrd1, Dio2, Dusp4, Itgb4, Nppa, Timp1, Wisp2	+	3.24E-08	Erk1/2, Fos, Jun, Myc, P38 Mapk, Raf1
Camk2d	Acta1, Myh7, Nppa	+	1.26E-06	Camk2d, Gata4, Hdac5, Mef2c, Nkx2.5
Fos	Agt, Dio2, Itgb4, Ltbp2, Nppa, Scd, Timp1, Tll2	+	1.27E-06	Fos, Fosl2, Jun
Hdac5	Acta1, Myh7, Nppa, Scd	+	1.50E-06	Gata4, Hdac5, Mef2c, Mef2d
Egfr	Acta1, Col8a1, Dusp4, F2rl1, Nppa, Tnc,	+	6.73E-06	Egfr, Ep300, Myc, Sp1
Srf	Acta1, Ankrd1, Fhl1, Myh7, Nppa, Tnc,	+	8.40E-06	Mkl1, Ptk2, Srf, Stat3
Mef2d	Acta1, Myh7, Nppa	+	1.38E-05	Gata4, Mef2d, Nkx2-5
Stat5b	Ankrd1, Myh7, Nppa, Scd	+	1.25E-04	Akt1, Nfkbia, Stat5b
Hdac9	Myh7, Nppa	+	1.29E-04	Gata4, Hdac5, Hdac9, Mef2c, Mef2d
Map3k7	Acta1, Myh7, Nppa,	+	1.43E-04	Atf2, Gata4, Hdac5, Map2k3, Map2k4, Map2k6, Map3k7, Mef2a, Mef2c, Rcan1
Nfkb	Agt, Ankrd1, F2rl1, Hspa1l, Nppa, Timp1	+	2.75E-04	
Cabin1	Myh7, Nppa,	+	2.81E-04	Cabin1, Calcineurin, Gata4, Hdac9, Mef2c, Mef2d, Nfatc3
Crebbp	Agt, Dusp4, Myh7, Nppa, Tnc	+	2.97E-04	Crebbp, Ep300, Klf4, Myc, Sp1, Stat, Stat3
Smad4	Fstl3, Scd, Timp1, Tnc	+	3.25E-04	Ctnnb1, Jun, Smad3, Smad4
Ppargc1a	Dio2, Myh7, Nppa, Scd	+	3.43E-04	Ppara, Ppargc1a
Mef2c	Acta1, Myh7, Nppa	+	3.99E-04	Gata4, Hdac5, Mef2c, Mef2d, Nkx2-5
Map2k3	Atp2a2, Maob, Ppargc1a, Rgs7	-	7.61E-05	
Ppargc1a	Ccbl2, Cyp1a1, Maob, Ppargc1a, Uqcrfs1	-	6.84E-04	
Mapk8	Cyp1a1, Gstm1, Maob, Ppargc1a	-	1.50E-03	
Map2k6	Atp2a2, Ppargc1a, Rgs7	-	1.75E-03	Map2k6, Mef2a
Nfe2l2	Creg1, Entpd5, Gstm1, Ppargc1a, Ptprd, Ryr3	-	1.81E-03	
Ppara	Acot1, Aldob, Ccbl2, Cyp1a1, Ppargc1a, Sord	-	2.27E-03	
Calcineurin A	ATP2A2, PPARGC1A	-	2.53E-03	Calcineurin A, Mef2a

Supplemental Table 5. Promoter features of genes repressed by 8MI during pressure overload. The set of genes induced by pressure overload and downregulated by 8MI (Repressed by 8MI) were compared with the motif gene set collection in MSigDB using Gene Set Enrichment Analysis {Subramanian, 2005 #10355}. Similar analysis was done on the set of genes upregulated in the presence of 8MI (Induced by 8MI). (K = # genes in comparator gene set. k = # genes overlapping in test set.

Repressed by 8MI

Motif/Gene Set Name	K	Promoter Element	k	FDR q-value
TGGAAA_V$NFAT_Q4_01	1896	NFAT, NFATC	23	3.92E-09
TGGNNNNNNKCCAR_UNKNOWN	424	Transcription factor unknown	9	4.73E-05
GCANCTGNY_V$MYOD_Q6	924	MYOD1: myogenic differentiation 1	12	8.10E-05
TGANTCA_V$AP1_C	1121	JUN: jun oncogene	12	4.60E-04
GGGAGGRR_V$MAZ_Q6	2274	MAZ: MYC-associated zinc finger protein (purine-binding transcription factor)	17	5.04E-04
GRRATG V$ TEF1_Q6	226	Similar to TEF-1	6	7.00E-04
NNNNNNKCTAWAAATAGMNNNN V$MEF2_02	228	Myocyte enhancer factor-2; MEF2	6	7.00E-04
WGGAATGY_V$TEF1_Q6	378	TEAD1: TEA domain family member 1 (SV40 transcriptional enhancer factor)	7	1.13E-03
RYTTCCTG_V$ETS2_B	1085	ETS2: v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	10	4.87E-03
CTAWWWATA_V$RSRFC4_Q2	361	MEF2A: MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)	6	5.14E-03

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NWADTAAWTANN V$NKX62_Q2	241	NKX6-2: NK6 transcription factor related, locus 2 (Drosophila)	8	4.76E-06
TTATGYTAAT V$POU3F2_02	260	POU3F2: POU domain, class 3, transcription factor 2	7	6.01E-05
NNNNATGCAAATNAN V$OCT1_Q6	270	POU2F1: POU domain, class 2, transcription factor 1	7	6.01E-05
NNNWAAAYAAAYANNNNN V$FOXJ2_01	184	FOXJ2: forkhead box J2	6	7.82E-05
TGACCTY_V$ERR1_Q2	1043	ESRRA: estrogen-related receptor alpha	11	9.54E-05
AWKTGTTTGTTTA V$HFH4_01	200	FOXJ1: forkhead box J1	6	9.54E-05
NNNGATWANN V$GATA6_01	265	GATA6: GATA binding protein 6	6	2.47E-04
TGGAAA_V$NFAT_Q4_01	1896	NFAT. NFATC	13	4.46E-04