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Supplemental Information

Systems Genetics Approach Identifies Gene Pathways and Adamts2 as Drivers of Isoproterenol-Induced Cardiac Hypertrophy and Cardiomyopathy in Mice

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Data S1: Edge strengths and Module Memberships for the Control, Treated and Ratio networks. Related to STAR Methods wMICA section.

Available on Mendeley Data doi: 10.17632/ntd85jnwxd.1

Table S1: Top Gene Ontology Enrichments in Modules of the left ventricular Gene Coexpression Network. Related to Figure 1A

Module	GO Term	DAVID Score
1	Cell Morphology	2.18
2	Protein Transport	4.01
3	Intracellular Organelle Lumen	10.88
4	Innate Immune Response	10.51
5	Signaling	17.81
6	RNA binding	2.75
7	Translation	2.64
8	Zinc Binding	5.00
9	Tissue Morphogenesis	4.17
10	Oxidative Phosphorylation	2.22
11	Sarcomere	1.99
12	Mitochondrion	5.48
13	Transcription	1.95
14	Transit Peptide	8.04
15	Cytochrome P450	9.51
16	Immune Response	5.4
17	Lung Development	3.25
18	Zinc Binding	2.14
19	Contractile Fiber	2.09
20	Cytoskeleton	3.07

Table S2: Drivers and reactive genes in module 5. Related to Figure 2C

Probe ID	Gene Symbol	Forward	Reverse	% Forward	Category
ILMN_1216661	COL6A2	19	0	100	Driver
ILMN_2698499	NOX4	1	0	100	Driver
ILMN_1226259	ADAMTS2	23	2	92	Driver
ILMN_2729103	ADAMTS2	24	3	89	Driver
ILMN_1238000	SRPX	7	1	88	Driver
ILMN_2671755	CERCAM	22	4	84	Driver
ILMN_1253741	PCOLCE	16	3	84	Driver
ILMN_1256676	DDAH1	5	1	83	Driver
ILMN_2728985	FBLN1	13	4	76	Driver
ILMN_1217009	RAB15	9	3	75	Driver
ILMN_1249000	1500015O10RIK	6	2	75	Driver
ILMN_1253791	KCNV2	2	1	67	Intermediary
ILMN_2981542	MFAP2	7	4	64	Intermediary
ILMN_2701778	ITGA11	3	2	60	Intermediary
ILMN_2591027	COL14A1	8	6	57	Intermediary
ILMN_2903926	PDGFRL	4	3	57	Intermediary
ILMN_2636424	ITGBL1	1	1	50	Intermediary
ILMN_2862538	COL12A1	2	2	50	Intermediary
ILMN_2926480	DIRAS2	5	5	50	Intermediary
ILMN_3128725	EGFR	2	2	50	Intermediary
ILMN_2463181	TNC	7	8	47	Intermediary
ILMN_2463180	TNC	6	8	43	Intermediary
ILMN_3161547	NPPA	2	3	40	Intermediary
ILMN_1234824	CCDC80	5	8	38	Intermediary
ILMN_1231851	ENPP1	5	9	36	Intermediary
ILMN_2627041	CX3CL1	1	2	33	Intermediary
ILMN_2627566	CAPN5	2	4	33	Intermediary
ILMN_2638114	PTN	2	4	33	Intermediary
ILMN_1259653	GNG8	3	6	33	Intermediary
ILMN_2675697	KDELR3	3	6	33	Intermediary
ILMN_2698728	SRPX2	5	10	33	Intermediary
ILMN_2818294	SRPX2	4	12	25	Reactive

ILMN_2886618	NBL1	1	5	17	Reactive
ILMN_1233455	OLFML3	1	13	7	Reactive
ILMN_2852957	DKK3	0	1	0	Reactive
ILMN_2746556	DKK3	0	2	0	Reactive
ILMN_2904765	SNAI1	0	3	0	Reactive
ILMN_2706268	SCARA3	0	6	0	Reactive
ILMN_2869110	COMP	0	9	0	Reactive
ILMN_1225835	MFAP5	0	13	0	Reactive
ILMN_3103896	TIMP1	0	15	0	Reactive
ILMN_2691951	SVEP1	0	16	0	Reactive
ILMN_2769918	TIMP1	0	18	0	Reactive

Table S3: Characteristics of Module 5 Adamts2 target genes that were selected for *in vitro* validation using siRNA knockdown in NRVMs. Related to Figure 3

Gene Symbol	Probe ID	Predicted Strength of relationship to Adamts2 by NEO	Number of Predicted Edges in Module 5	Previous Association with cardiovascular disease
Col12a1	ILMN_2862538	0.52919	4	Recessive and dominant mutations in COL12A1 cause a novel EDS/myopathy overlap syndrome in humans and mice
Kcnv2	ILMN_1253791	0.55139	3	
Mfap2	ILMN_2981542	0.54725	13	
Nppa	ILMN_3161547	0.5395	5	Hypertrophic marker
Pcolce	ILMN_1253741	0.75863 0.77621	19	Candidate gene for RV weight (Rau 2015)
Timp1	ILMN_3103896	0.72486 0.69964	15	ECM regulator that is increased during heart failure (Barton 2003)
	ILMN_2769918	0.73708 0.71147	18	
Tnc	ILMN_2463181 ILMN_2463180	N/A 0.62397 0.6225	15 14	Expression of TNC has been suggested to accelerate adverse ventricular remodeling, cardiac failure, and fibrosis in the residual myocardium after MI.

Table S4: Sequence-level Analysis of Driver Genes of Module 5. Related to STAR Methods eQTL Analysis Section

Gene	Sequence Variation	Which Strains	Result	eQTLs
Pcolce	2 missense	Non-core	Tolerated	Significant *cis*-eQTL
Rab15	1 missense	Non-core	Tolerated	Significant *cis*-eQTL
Adamts2	1 missense	Non-core	Tolerated	Significant *cis*-eQTL
	1 splice site	Core	Not predicted to cause splicing	
Srpx	1 missense	Core	Tolerated	Suggestive *cis*-eQTL
	1 splice	Core	Not predicted to cause splicing	
Nox4	1 missense	Core	Tolerated	Suggestive *cis*-eQTL
	1 splice	Core	Not predicted to cause splicing	
1500015O10Rik	1 missense	Core	Wild Type Allele Damaging	Significant *cis*-eQTL
Kcnv2	1 missense	Non-core	Mutant Allele Damaging	Significant *cis*-eQTL
	1 missense	Core	Tolerated	

Table S5: qRT - PCR primers used in NRVM experiments. Related to STAR Methods qPCR Section

Gene	Foward	Reverse
Rpl0	GGCGTCCTCATTAGAGTGACA	GCATCATGGTGTTCTTGCCC
Nppa	GCAAACATCAGATCGTGCCC	GGTGGTCTAGCAGGTTCTTGAAA
Nppb	GTGCTGCCCCAGATGATTCT	GGCGCTGTCTTGAGACCTAA
Adamts2	GGTGTCCCACGTGGTATCTT	CGGCCGAATACTGTGAGGTT
Atp2a2 Isoform A	TCACACCGCTGAATCTGACC	GCACAAAGGGCCAGGAAATG
Atp2a2 Isoform B	GAGACGCTCAAGTTTGTGGC	GGGCTGGAAGATGTGTTGCT
Col12a	CTATCCAGAGCCTTATGTGCCC	AGCACTTGTGGATGAAGCGA
Kcnv2	AGCGCTGGAAGACCTCTCTA	AGATGGGGCAGTCATACCCT
Mfap2	GCGACCAGATAGAGAACGCA	GGGTACTGTTCTTCACGGCA
Pcolce	GGCACTGAGCACCAGTTTTG	AATCCGACTCAGGCCAGTTG
Timp1	ACGCTAGAGCAGATACCACG	AGCGTCGAATCCTTTGAGCA
Tnc	CAGCTACCGACGGGATCTTC	TTCCGGTTCAGCTTCTGTGG

Table S6: Mouse strains used in this study. Related to STAR Methods Animals section

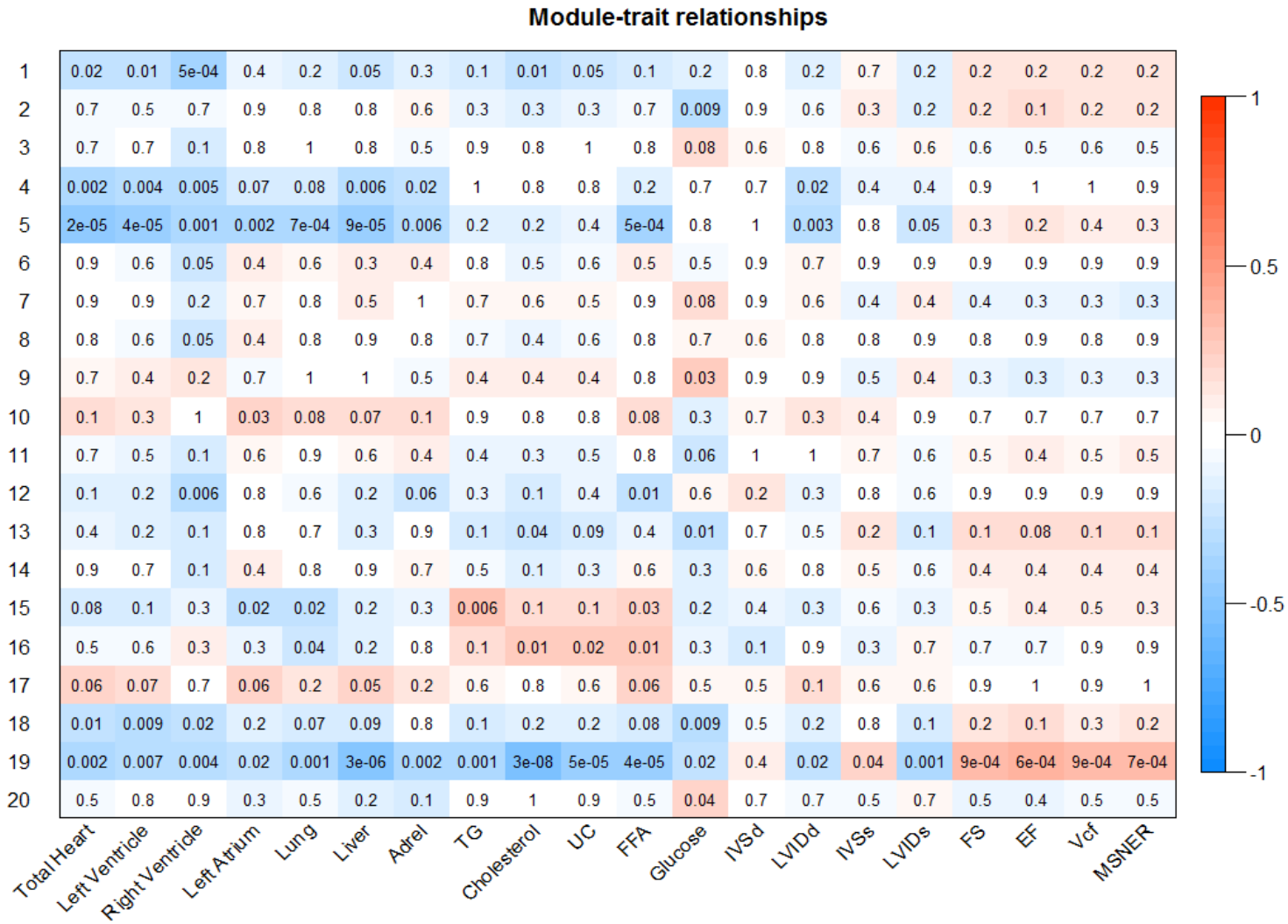

Figure S1. Module-Trait Correlation for Treated Network, Related to Figure 1B. A weighted PCA algorithm was used to calculate the first weighted principle component of each module. These weighted first principle components were then correlated to the HF-related phenotypes. Strength of correlation is indicated by color, while the p-value of the correlation is indicated by the numbers in the table.

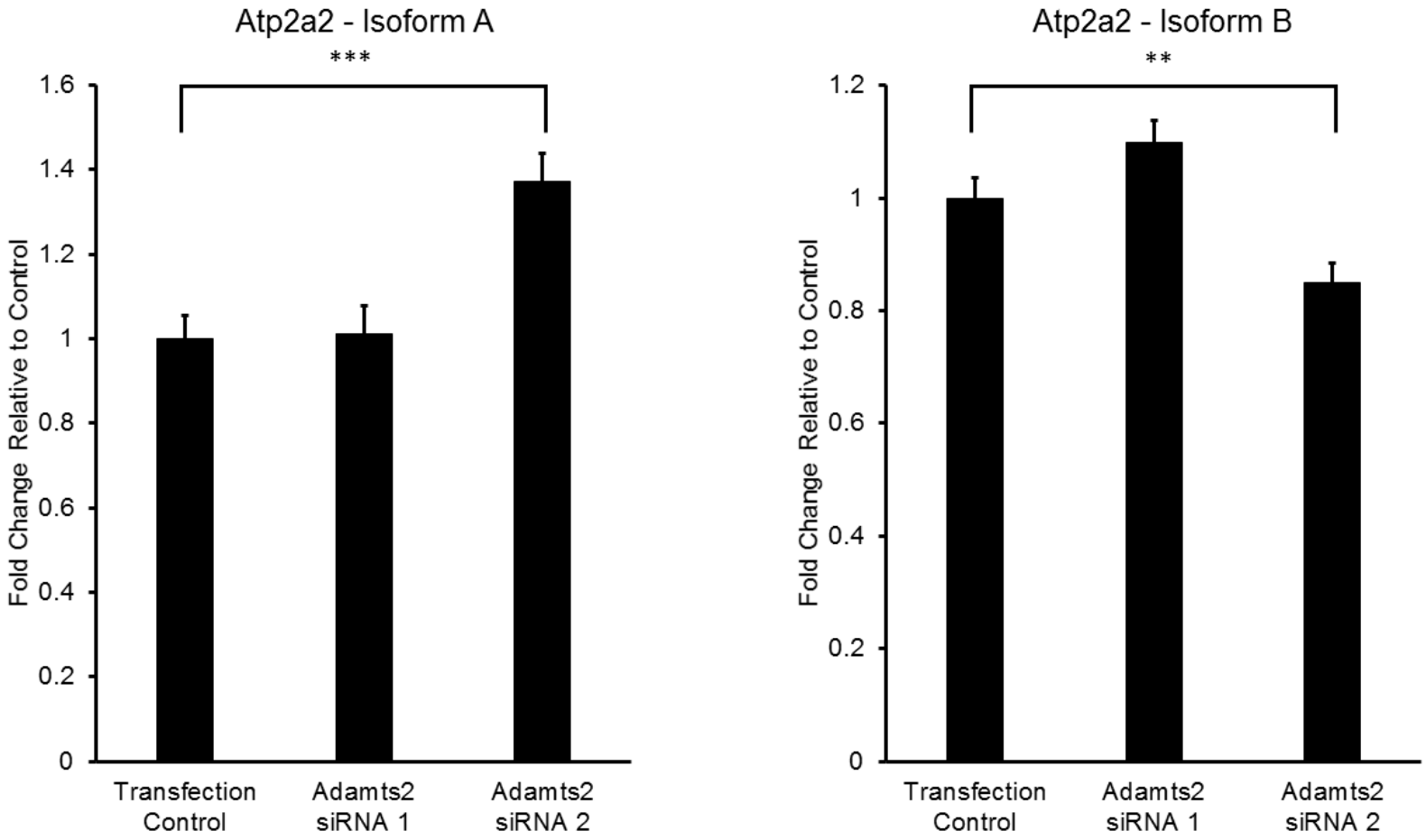

Figure S2. Expression of *Atp2a2* is not altered by Adamts2 knockdown, related to Figure 3. NRVMs were transfected with either control siRNA or *Adamts2* siRNA. Following overnight transfection NRVMs were treated with isoproterenol (60μM) containing media for 48 hours. mRNA levels were quantified using RT-qPCR. Plots show average +/- SEM. N = 8-9