

Supplementary Information

Inhibition of *miR-25* Improves Cardiac Contractility in the Failing Heart

Christine Wahlquist*¹, Dongtak Jeong*², Agustin Rojas-Muñoz¹, Changwon Kho², Ahyoung Lee², Shinichi Mitsuyama², Alain van Mil^{1,3}, Woo Jin Park⁴
Joost P. Sluijter³, Pieter A. Doevendans³, Roger J. Hajjar², and Mark Mercola¹

*co-first authors

¹Department of Bioengineering,
University of California, San Diego,
and the Muscle Development and Regeneration Program
Sanford-Burnham Medical Research Institute
10901 North Torrey Pines Road
La Jolla, California 92037
mmercola@sanfordburnham.org

²The Cardiovascular Research Center,
Icahn School of Medicine at Mount Sinai,
New York, NY 10029

³Department of Cardiology, University Medical Center Utrecht, Utrecht, the Netherlands and
ICIN, Netherlands Heart Institute, Utrecht, the Netherlands

⁴Global Research Laboratory, Gwangju Institute of Science and Technology,
Gwangju, Korea

Contents

1. Supplementary Tables 1-3

Supplementary Tables

Supplementary Table 1

microRNAs confirmed to inhibit the eGFP-SERCA2a 3'UTR fusion transcript in the primary screen assay

microRNA	% Inhibition	p-value	conservation
hsa-miR-346	80.99247163	5.32233E-05	1
hsa-miR-376a*	80.67109749	5.91496E-05	1
hsa-miR-132	79.36745878	6.20595E-05	2
hsa-miR-361-3p	78.85458317	6.72732E-05	0
has-miR-365	74.48046024	0.000118857	2
hsa-miR-192	74.13398041	0.000106474	2
hsa-miR-22	73.55894622	0.000128031	2
hsa-miR-655	73.55337769	0.000113881	0
hsa-miR-518f*	70.60204452	0.000186218	0
hsa-miR-641	69.9709797	0.000202443	0
hsa-miR-136	69.20063646	0.000186708	1
hsa-miR-379	67.5394754	0.000208388	1
hsa-miR-146a	67.4320154	0.000296461	2
hsa-miR-1268	64.89555056	0.000299082	0
hsa-miR-510	61.77727977	0.000388732	0
hsa-miR-493*	61.66179919	0.000407322	0
hsa-miR-1181	61.07225296	0.000422212	0
hsa-miR-489	61.05624092	0.000470095	2
hsa-miR-25	60.49249903	0.000435206	2
hsa-miR-1271	60.08764848	0.000412262	2
hsa-miR-1289	59.50868784	0.000557928	0
hsa-miR-532-3p	58.74229446	0.000634729	0
hsa-miR-650	58.11050937	0.000562452	0
hsa-miR-545*	58.10588279	0.000985349	0
hsa-hsa-miR-17	57.83026355	0.000618753	2
hsa-miR-1826	57.67481764	0.000779978	0
hsa-miR-96*	57.52630557	0.000672782	2
hsa-miR-200b	56.7970574	0.000628775	2

hsa-miR-338-5p	56.63809328	0.000630039	0
hsa-miR-760	55.94751228	0.00076837	0
hsa-miR-1228*	55.60390456	0.006898672	0
hsa-miR-1290	54.39392509	0.000915859	0
hsa-miR-511	53.01741968	0.002094018	0
hsa-miR-103-2	52.72457439	0.001540782	2
hsa-miR-657	52.19393814	0.00140793	0
hsa-miR-548d-3p	51.84547609	0.00189473	0
hsa-miR-202	51.41224777	0.002045283	1
hsa-miR-423-3p	51.32883469	0.001316693	0
hsa-miR-539	50.83376221	0.002146518	1
hsa-miR-29b	50.51561473	0.001675845	2
hsa-miR-593	49.41504139	0.001627232	0
hsa-miR-1287	48.84290102	0.00156881	0
hsa-miR-874	48.77629085	0.005761531	1
hsa-miR-632	48.68117424	0.001513586	0
hsa-miR-298	48.11594395	0.002021351	0
hsa-miR-621	46.83251027	0.002414331	0
hsa-miR-24-1*	46.46532287	0.002666724	2
hsa-miR-512-3p	46.33635395	0.002896382	0
hsa-miR-1296	46.30272219	0.003197384	0
hsa-miR-486-5p	45.35176147	0.006869943	1
hsa-miR-888	45.01699329	0.003404262	0
hsa-miR-185	44.03597034	0.003474043	1
hsa-miR-324-5p	43.34089609	0.006605924	1
hsa-miR-627	42.49883801	0.005236739	0
hsa-miR-301b	42.08558824	0.005367971	0
hsa-miR-933	41.81702545	0.004582975	0
hsa-miR-151-5p	41.46036462	0.00469128	0
hsa-miR-1273	40.25804615	0.005667077	0
hsa-miR-200b*	39.5197365	0.005266684	2
hsa-miR-661	39.25728318	0.011202166	0
hsa-miR-483-5p	36.77548192	0.012979638	0
hsa-miR-493	36.64972869	0.007543937	0

hsa-miR-645	36.42883929	0.011064936	0
hsa-miR-141	35.07166974	0.015614575	2
hsa-miR-1224-5p	34.8810789	0.010524377	0
hsa-miR-626	34.47731879	0.014511279	0
hsa-miR-26a-1*	33.1167919	0.018040856	2
abi-13242	30.04330505	0.027578154	0
hsa-miR-212	29.78331172	0.029586534	2
hsa-miR-1299	29.50034848	0.034501079	0
hsa-miR-29c	28.90602278	0.021812834	2
hsa-miR-1975	28.71812077	0.032634286	0
hsa-miR-188-3p	28.56285993	0.0337377	0
hsa-miR-1303	28.12752016	0.033872202	0
hsa-miR-500	28.01127437	0.02246561	0
hsa-miR-519c-3p	27.99004101	0.030065494	0
hsa-miR-2052	27.07716367	0.037869696	0
hsa-miR-671-5p	26.48213729	0.038303191	0
hsa-miR-767-5p	26.19956964	0.03385671	0
hsa-miR-128	25.95506908	0.046439374	2
hsa-miR-205	25.94412511	0.03911706	2
hsa-miR-194*	25.41378256	0.03534099	2

Inhibitory activity is the area under the curve (A.U.C.) from the 5-point dose response confirmatory assay. Data are expressed as a percentage of the inhibitory activity achieved with SERCA2a siRNA (set to 100% inhibition) and normalized to inert sequence control siRNA (set to 0% inhibition). The assay was performed in triplicate, and only those miRs yielding a statistically significant activity relative to the inert sequence control siRNA ($P < 0.05$, Student's t-test) are listed. Evolutionary conservation is indicated: 0 = poorly-conserved, 1 = conserved across human, mouse, rat and dog species; 2 = highly conserved across human mouse, rat, dog and chicken species (nomenclature from TargetScan v6.2 (<http://www.targetscan.org>)). miRs highlighted in blue are reported to be upregulated in heart failure (see **Detailed Methods**).

Supplementary Table 2**Echocardiographic assessment of cardiac function in cohort groups.**

	Sham		HF + SC		HF + anti- <i>miR-25</i>		AAV9-VLP		AAV9- <i>miR-25</i>		AAV9- <i>miR-92a</i>	
	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev
IVSd (mm)	1.09	0.16	1.06	0.18	1.13	0.16	1.044	0.011	1.064	0.053	1.128	0.080
LVIDd (mm)	2.98	0.61	4.23	0.26	3.51	0.11	3.458	0.215	3.636	0.172	3.488	0.185
LVPWd (mm)	1.08	0.10	1.09	0.16	1.12	0.15	1.044	0.016	1.032	0.067	1.096	0.077
IVSs (mm)	1.73	0.15	1.78	0.35	1.97	0.19	1.944	0.083	1.85	0.073	1.934	0.082
LVIDs (mm)	1.27	0.13	2.37	0.21	1.61	0.15	1.282	0.081	1.628	0.087	1.382	0.103
LVPWs (mm)	1.81	0.06	1.81	0.28	1.76	0.19	1.728	0.067	1.76	0.068	1.84	0.087
EF (%)	93.923	0.518	77.771	5.220	89.620	2.224	94.432	0.503	90.292	1.119	93.192	1.288
FS (%)	61.695	1.121	39.987	5.664	54.308	3.458	62.876	1.153	52.636	1.510	60.364	2.489
HR (BPM)	595.250	21.701	623.571	35.256	622.400	25.560	652.106	45.286	664.346	31.936	667.338	28.679

	Sham + SC		Sham + anti- <i>miR-25</i>		S2a KO + SCS		S2a KO + anti- <i>miR-25</i>	
	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev
IVSd (mm)	0.965	0.033	1.048	0.119	0.882	0.052	0.954	0.169
LVIDd (mm)	2.918	0.135	3.078	0.189	3.375	0.185	3.903	0.277
LVPWd (mm)	0.905	0.047	0.970	0.067	0.893	0.042	0.968	0.124
IVSs (mm)	1.435	0.510	1.830	0.135	1.618	0.111	1.715	0.137
LVIDs (mm)	1.020	0.042	1.220	0.109	1.690	0.380	2.149	0.278
LVPWs (mm)	1.540	0.040	1.780	0.163	1.513	0.105	1.683	0.130
EF (%)	94.410	0.231	94.040	0.701	82.388	1.455	81.951	4.529
FS (%)	61.377	0.592	61.943	1.413	44.687	1.307	43.655	3.967
HR (BPM)	593.613	24.302	604.398	28.143	514.195	13.864	508.098	55.448

Supplementary Table 3***In vivo* hemodynamic assessment of cardiac function in cohort groups.**

	Sham		HF + SC		HF + anti- <i>miR-25</i>		AAV9-VLP		AAV9- <i>miR25</i>		AAV9- <i>miR92a</i>	
	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev
dPdtMAX (mmhg/s)	3953	321	3917	524	5327	702	3916	588	3496	399	4061	309
dPdtMin (mmhg/s)	-2953	-214	-2543	-462	-4015	-553	-3098	-414	-3543	-462	-3952	-412
EDV (ul)	39	5	92	14	57	12	29	4	55	14	36	12
ESV (ul)	11	4	82	18	29	14	5	3	38	16	10	14
SV (ul)	29	3	10	5	28	6	24	6	18	3	25	5
EF (%)	73	5	17	17	49	16	73	5	47	18	79	11
HR (BPM)	414	11	368	31	392	25	416	63	453	11	396	30

	Sham + SC		Sham + anti- <i>miR25</i>		S2a KO + SCS		S2a KO + anti- <i>miR25</i>	
	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev
dPdtMAX (mmhg/s)	4366	513	4689	616	560	513	1112	616
dPdtMin (mmhg/s)	-3953	-364	-4132	-412	-512	-434	-973	-570
EDV (ul)	39	5	36	7	110	22	118	31
ESV (ul)	11	4	10	5	93	26	103	32
SV (ul)	29	3	30	3	17	7	14	10
EF (%)	73	5	75	6	17	21	19	26
HR (BPM)	472	33	481	28	220	55	286	49

Table 2 lists echocardiographic parameters at 5.5 months for sham, TAC-operated (heart failure, HF) + scrambled sequence control anti-miR (SC) and TAC-operated (HF) + anti-*miR-25*. Methods: Briefly, at 10-12 weeks post-TAC surgery, when echocardiographic monitoring showed <50% LVEF and evidence of LV dilation, animals were intravenously injected with anti-

miR-25 or control (scrambled sequence) anti-miR formulated with *in vivo*-jetPEI™ reagent mixture for 3 consecutive days followed by an additional anti-*miR-25* injections every week for next 3 weeks. At 5.5 months, animals were sacrificed. See Detailed Methods.

Table 2 also lists echocardiographic parameters at 1 month after control or anti-*miR-25* injection in Sham-operated and *Serca2a* knockout (S2a KO) mice, and at 6 weeks after AAV9-VLP (control), AAV9-*miR25* and AAV9-*miR-92a* infection of WT mice. Anti-miR injections, AAV transduction and description of S2a KO mice are in Detailed Methods.

Table 3 lists *in vivo* hemodynamic parameters at the same time points as for the echocardiographic data in Table 2.