Supplementary Table 1. miRNA transcriptional profiling by RNA_seq in hypertrophic HCM_CsA mice. Total RNA was isolated using miRVana miRNA isolation kit (Ambion) and 1 ug total RNA was used to prepare and sequence miRNA libraries as described (1). Normalized sequencing read counts for each miRNA are expressed as reads per million (RPM). Statistical analysis was performed using unpaired two-tailed t-test and p-value represents comparison with the corresponding WT_CsA group (n=3 mice per group).

miRNA	**WT_CsA (1)**	**WT_CsA (2)**	**WT_CsA (3)**	**WT_CsA Average**	**HCM_CsA (1)**	**HCM_CsA (2)**	**HCM_CsA (3)**	**HCM_CsA Average**	**p-value**	**Fold Change**
mmu-miR-376b	1	0	0	0	5	8	9	7	0.0071315	17.81717
mmu-miR-214	116	84	58	86	787	780	1290	952	0.0069621	11.08839
mmu-miR-299*	0	3	0	1	7	6	9	7	0.0078440	8.42258
mmu-miR-199a-5p	15	17	9	14	83	116	134	111	0.0029131	8.16063
mmu-miR-21*	4	3	2	3	11	20	16	16	0.0088201	5.84328
mmu-miR-382	2	3	2	2	15	8	15	13	0.0144259	5.67214
mmu-miR-574-5p	1	3	2	2	11	6	12	9	0.0129540	5.11304
mmu-miR-146b	2	0	1	1	5	5	6	5	0.0056649	4.79604
mmu-miR-1193	0	4	1	2	6	9	7	7	0.0222100	4.65568
mmu-miR-21	303	327	253	294	866	1229	1769	1288	0.0195016	4.37616
mmu-miR-541	2	5	5	4	13	24	12	16	0.0443837	3.78604
mmu-miR-1981	2	1	5	3	11	9	13	11	0.0090542	3.63257
mmu-miR-674	95	114	96	102	316	280	357	317	0.0007245	3.12597
mmu-miR-423-5p	9	9	11	10	23	25	31	26	0.0026212	2.76970
mmu-miR-433	17	24	10	17	58	28	45	44	0.0468939	2.59123
mmu-let-7i	18144	30455	18292	22297	65688	42740	52837	53755	0.0156472	2.41087
mmu-miR-341	6	10	2	6	14	17	13	14	0.0433316	2.37773
mmu-miR-125a-5p	400	581	390	457	1212	759	1074	1015	0.0194940	2.22085
mmu-miR-1983	7	8	3	6	14	15	13	14	0.0080336	2.20228
mmu-miR-376a	38	67	49	51	100	88	143	110	0.0340381	2.16132
mmu-miR-375	2	4	5	4	7	9	9	8	0.0138494	2.15609
mmu-miR-199a-3p	53	88	70	70	129	126	195	150	0.0324208	2.13589
mmu-miR-351	7	5	7	6	15	10	16	14	0.0179031	2.12537
mmu-miR-99b*	27	34	19	27	45	64	60	56	0.0157450	2.10556
mmu-miR-379	45	93	52	63	104	156	136	132	0.0321569	2.08225
mmu-miR-27b*	9	16	16	13	21	33	29	28	0.0258026	2.05839
mmu-miR-497	53	59	42	51	82	96	135	104	0.0324518	2.04345
mmu-miR-193b	10	18	11	13	30	27	21	26	0.0279232	1.98407
mmu-miR-299	11	18	14	15	25	24	37	29	0.0380456	1.97666
mmu-miR-652	291	412	247	317	531	581	665	593	0.0116797	1.87148
mmu-miR-99b	83	90	55	76	125	128	173	142	0.0248994	1.86535
mmu-miR-365	10	12	13	11	17	18	28	21	0.0485411	1.84975
mmu-let-7e	210	291	215	239	411	401	502	438	0.0087328	1.83477
mmu-miR-598	26	25	24	25	53	31	49	44	0.0480909	1.77167
mmu-miR-23b	8470	11165	7497	9044	15060	13105	19804	15990	0.0377303	1.76802
mmu-miR-1839-5p	121	136	86	114	233	157	215	202	0.0333724	1.76153
mmu-miR-142-3p	540	679	416	545	1061	804	1015	960	0.0195108	1.76051
mmu-miR-487b	11	22	13	15	23	28	28	26	0.0443389	1.72933
mmu-miR-223	73	78	69	74	100	157	119	125	0.0393677	1.70567
mmu-miR-15b	51	55	55	54	71	89	105	88	0.0257010	1.64719
mmu-miR-324-5p	205	259	194	219	343	302	426	357	0.0296910	1.62802
mmu-miR-18a	6	8	5	6	10	12	9	10	0.0280684	1.62775
mmu-miR-671-5p	12	16	17	15	22	24	25	24	0.0054564	1.60335
mmu-miR-374	15	18	16	16	28	23	24	25	0.0085712	1.54268
mmu-miR-425*	170	155	139	155	198	239	275	237	0.0256181	1.53256
mmu-miR-150	172	180	147	166	91	110	134	112	0.0265246	0.66975
mmu-miR-30c-2*	126	119	134	126	60	89	95	81	0.0178079	0.64305
mmu-miR-30e*	955	1048	878	960	496	674	621	597	0.0073273	0.62171
mmu-miR-151-3p	39	51	35	42	19	30	23	24	0.0355757	0.57791
mmu-miR-185	787	704	721	737	387	443	434	422	0.0005105	0.57177
mmu-miR-30e	4141	3303	4700	4048	1283	2657	2684	2208	0.0403558	0.54545
mmu-miR-499	13566	13826	12800	13397	6252	8359	6523	7045	0.0009605	0.52584
mmu-miR-342-3p	24	33	24	27	15	12	12	13	0.0095037	0.47914
mmu-miR-137	6	8	4	6	3	3	1	2	0.0473929	0.41236

Supplementary Table 2. miRNA transcriptional profiling by RNA_seq in hypertrophic HCM_Aged mice. Total RNA was isolated using miRVana miRNA isolation kit (Ambion) and 1 ug total RNA was used to prepare and sequence miRNA libraries as described (1). Normalized sequencing read counts for each miRNA are expressed as reads per million (RPM). Statistical analysis was performed using unpaired two-tailed t-test and p-value represents comparison with the corresponding WT group (n=3 mice per group).

miRNA	WT_Aged (1)	WT_Aged (2)	WT_Aged (3)	WT_Aged Average	HCM_Aged (1)	HCM_Aged (2)	HCM_Aged (3)	HCM_Aged Average	p-value	Fold Change
mmu-miR-146b	4	6	3	4	8	17	20	15	0.0418726	3.80066
mmu-miR-21*	3	10	9	7	18	26	40	28	0.0369335	3.79503
mmu-miR-214	375	636	360	457	987	895	1679	1187	0.0498859	2.59991
mmu-miR-199a-5p	22	30	21	24	54	51	62	56	0.0020231	2.28738
mmu-miR-147	10	14	7	10	19	25	27	24	0.0161088	2.25118
mmu-miR-34b-5p	10	12	6	9	21	18	15	18	0.0237959	1.91223
mmu-miR-341	6	8	6	7	9	11	14	12	0.0439579	1.75412
mmu-miR-142-5p	22	24	29	25	34	44	50	43	0.0257355	1.70963
mmu-miR-125a-3p	4	7	4	5	8	8	9	8	0.0267334	1.70677
mmu-miR-188-5p	14	18	12	15	21	24	28	24	0.0219152	1.65258
mmu-miR-142-3p	1014	1203	977	1065	1775	1574	1835	1728	0.0032913	1.62320
mmu-miR-10b	71	120	86	92	138	131	174	148	0.0478572	1.59920
mmu-miR-199a-3p	117	168	136	141	201	213	246	220	0.0162898	1.56457
mmu-miR-208b	11031	11794	11541	11455	14522	17711	21523	17919	0.0337176	1.56421
mmu-miR-874	17	14	13	15	11	7	6	8	0.0302834	0.57778
mmu-miR-200c	9	8	13	10	5	6	4	5	0.0459	0.51911
mmu-miR-467d*_	6	4	5	5	4	2	2	3	0.0465471	0.48816
mmu-miR-491	2	3	3	3	1	1	1	1	0.0241559	0.37588
mmu-miR-293	8	5	4	6	2	2	2	2	0.0397184	0.31815
mmu-miR-196a	2	1	1	1	0	0	0	0	0.0390474	0.24882
mmu-miR-708*	2	1	1	1	0	0	0	0	0.0402815	0.13484

Supplementary Table 3. Common differentially regulated miRNAs in hypertrophic HCM_CsA and HCM_Aged mice. Total RNA was isolated using miRVana miRNA isolation kit (Ambion) and 1 ug total RNA was used to prepare and sequence miRNA libraries as described (1). Normalized sequencing read counts for each miRNA are expressed as reads per million (RPM). Statistical analysis was performed using unpaired two-tailed t-test and p-value represents comparison with the corresponding WT group (n=3 mice per group).

miRNA	WT_Aged	HCM_Aged	p-value	Fold Change	WT_CsA	HCM_CsA	p-value	Fold Change
mmu-miR-142-3p	1065	1728	0.003	1.62	545	960	0.020	1.76
mmu-miR-146b	4	15	0.042	3.80	1	5	0.006	4.80
mmu-miR-199a-5p	24	56	0.002	2.29	14	111	0.003	8.16
mmu-miR-199a-3p	141	220	0.016	1.56	70	150	0.032	2.14
mmu-miR-21-3p	7	28	0.037	3.80	3	16	0.009	5.84
mmu-miR-214	457	1187	0.049	2.60	86	952	0.007	11.09
mmu-miR-341	7	12	0.044	1.75	6	14	0.043	2.38

Supplementary Table 4. Ingenuity pathway analysis (IPA, Qiagen) of putative targets of miR-199a-3p (A) Top upstream regulators and **(B)** Top canonical pathways, ratio= no. of predicted target genes mapped/ total no. of genes in the specified canonical pathway.

A. Top Upstream Regulator of Putative miR-199a-3p Targets

Name	**P-value of overlap**
miR-199a-3p (and other miRNAs w/ seed CAGUAGU)	1.34E-06
TGFB1	*1.39E-06*
APP	3.04E-06
FOS	5.34E-06
Sos	6.00E-06

B. Top Canonical Pathways of Putative miR-199a-3p Targets

Name	**P-value**	**Ratio**
Role of Tissue Factor in Cancer	6.59E-05	8/114 (0.07)
CDK5 Signaling	1.06E-04	7/94 (0.074)
Rac Signaling	3.05E-04	7/122 (0.057)
Actin Cytoskeleton Signaling	3.48E-04	10/238 (0.042)
Reelin Signaling in Neurons	4.56E-04	6/82 (0.073)

Supplementary Table 5. Proteomics analysis of conditioned media from cardiac fibroblasts expressing miR-199a-3p mimic or control mimic in basal (unstimulated) or in response to TGFβ1-stimulation. Cardiac fibroblasts were transfected with 50 nM control or miR-199a-3p mimics in serum-free DMEM. 16 hours post-transfection, cells were incubated for 48 hours with DMEM (Unstimulated) or DMEM+TGFβ1 (Stimulated). Conditioned media was collected and processed for mass-spectrometric analysis as described previously (2). Normalized spectral counts identified by liquid chromatography tandem mass spectrometry (LC-MS/MS) are shown. Upregulated proteins are in red, and downregulated proteins are in green.

	Unstimulated		**Stimulated (+TGFbeta)**	
Protein ID	log2(Fold Change)	FDR	log2(Fold Change)	FDR
AEBP1_MOUSE	0.03	1.00	-0.47	0.07
ANXA2_MOUSE	-0.34	1.00	-0.47	0.83
APOE_MOUSE	0.56	0.22	0.96	0.13
ATL2_MOUSE	n/a	n/a	n/a	n/a
ATS5_MOUSE	0.46	0.67	0.52	0.02
BMP1_MOUSE	0.04	1.00	-0.01	1.00
CATB_MOUSE	0.26	0.70	-0.18	0.99
CATD_MOUSE	-0.37	0.53	-0.94	0.01
CATL1_MOUSE	-0.22	1.00	-0.57	0.54
CATZ_MOUSE	0.56	0.44	n/a	n/a
CO1A1_MOUSE	0.28	0.34	0.24	0.12
CO1A2_MOUSE	-0.13	0.84	-0.16	0.70
CO3A1_MOUSE	0.34	0.16	0.30	0.06
CO4A1_MOUSE	-0.53	0.01	-0.37	0.24
CO4A2_MOUSE	-0.42	0.43	-0.41	0.33
CO5A1_MOUSE	0.22	0.71	0.21	0.31
CO5A2_MOUSE	-0.39	0.25	-0.37	0.12
CO6A1_MOUSE	0.36	0.05	0.45	0.02
CO6A2_MOUSE	0.65	1.46E-04	0.37	0.19
CO8A1_MOUSE	-0.20	0.89	n/a	n/a
COBA1_MOUSE	1.23	1.60E-05	0.59	2.81E-03
COCA1_MOUSE	0.00	1.00	0.23	0.40
COEA1_MOUSE	1.07	0.15	n/a	n/a
COFA1_MOUSE	0.73	0.71	0.97	9.00E-07
CSF1_MOUSE	-0.29	0.48	-0.52	0.60
CSPG2_MOUSE	0.29	0.94	0.34	0.34
CTGF_MOUSE	-0.79	0.03	-0.67	0.08
CTHR1_MOUSE	-0.20	1.00	-0.08	1.00
CYC_MOUSE	0.37	0.84	-0.15	0.98
CYTC_MOUSE	0.40	0.76	0.23	0.40
DAG1_MOUSE	-0.11	1.00	0.12	0.95
DERM_MOUSE	0.46	0.59	-0.06	1.00
ECM1_MOUSE	0.07	1.00	0.14	0.53
EMIL1_MOUSE	0.03	1.00	-0.18	1.00
FBLN1_MOUSE	-0.34	0.57	0.36	0.21
FBLN2_MOUSE	-0.23	0.58	-0.16	0.79
FBLN3_MOUSE	0.61	0.02	2.08	0.02
FBLN4_MOUSE	0.22	0.78	0.05	0.97
FBLN5_MOUSE	-0.01	1.00	-0.05	1.00
FBN1_MOUSE	0.08	1.00	-0.06	1.00
FINC_MOUSE	-0.21	0.53	-0.33	0.20
FMOD_MOUSE	0.09	1.00	0.21	0.86
FSTL1_MOUSE	-0.25	0.51	0.01	1.00
FSTL3_MOUSE	n/a	n/a	n/a	n/a
GELS_MOUSE	0.26	0.61	0.96	1.00E-06
GRN_MOUSE	0.63	0.10	0.28	0.36
IBP2_MOUSE	n/a	n/a	-1.17	0.17
IBP4_MOUSE	1.30	2.19E-03	0.57	0.12
IBP6_MOUSE	0.80	0.12	n/a	n/a
IBP7_MOUSE	-0.33	0.36	-0.46	0.06
IC1_MOUSE	-0.07	1.00	0.35	0.15
ITGBL_MOUSE	n/a	n/a	n/a	n/a

LAMA2_MOUSE	0.23	0.93	0.15	0.67
LAMA4_MOUSE	-0.03	1.00	0.05	0.97
LAMB1_MOUSE	-0.11	1.00	-0.14	0.98
LAMB2_MOUSE	0.31	0.37	0.25	0.26
LAMC1_MOUSE	0.14	0.92	0.02	0.99
LEG1_MOUSE	-0.06	1.00	0.37	0.26
LG3BP_MOUSE	0.22	0.99	0.31	0.35
LOXL2_MOUSE	-1.93	9.00E-07	-1.52	0.01
LTBP2_MOUSE	n/a	n/a	n/a	n/a
LTBP4_MOUSE	n/a	n/a	n/a	n/a
LUM_MOUSE	1.00	5.73E-04	1.12	0.16
LYOX_MOUSE	-1.48	3.30E-05	-1.30	0.02
MATN2_MOUSE	0.19	1.00	n/a	n/a
MFAP5_MOUSE	n/a	n/a	0.27	0.55
MFGM_MOUSE	0.92	0.41	n/a	n/a
MIME_MOUSE	1.71	0.02	-0.17	1.00
MMP2_MOUSE	0.93	0.03	0.65	2.00E-06
MMP3_MOUSE	0.14	1.00	0.31	0.72
NID1_MOUSE	-0.02	1.00	-0.01	1.00
NID2_MOUSE	-1.74	9.00E-07	n/a	n/a
PAI1_MOUSE	-0.92	1.00E-06	-0.18	0.76
PCOC1_MOUSE	0.49	0.20	0.33	0.11
PDIA3_MOUSE	-0.33	0.71	-0.11	0.98
PDIA6_MOUSE	-0.02	1.00	-0.30	0.46
PEDF_MOUSE	-0.16	0.88	-0.20	0.68
PGBM_MOUSE	-0.29	0.60	-0.10	1.00
PGS1_MOUSE	-0.52	0.05	-0.55	0.02
PGS2_MOUSE	0.17	0.85	0.07	0.83
PLOD1_MOUSE	0.19	0.99	0.37	0.38
PLOD2_MOUSE	-0.16	1.00	-0.20	0.84
PLOD3_MOUSE	0.14	1.00	0.42	0.19
POSTN_MOUSE	-0.44	0.43	-0.34	0.36
PPIA_MOUSE	-0.10	1.00	0.18	0.87
PPIB_MOUSE	-0.35	0.51	-0.45	0.51
PRDX1_MOUSE	0.08	1.00	-0.12	1.00
PRDX2_MOUSE	-0.11	1.00	-0.12	1.00
PRDX6_MOUSE	0.02	1.00	-0.43	0.71
PRELP_MOUSE	-17.36	0.33	n/a	n/a
PTX3_MOUSE	-0.13	0.99	0.21	0.25
SMOC2_MOUSE	0.09	1.00	n/a	n/a
SODE_MOUSE	-0.19	0.86	-0.62	0.01
SPA3N_MOUSE	0.01	1.00	0.22	0.54
SPON2_MOUSE	0.32	1.00	n/a	n/a
SPRC_MOUSE	0.02	1.00	-0.19	0.71
TENA_MOUSE	-0.79	0.05	-0.37	0.57
TETN_MOUSE	0.79	0.19	5.10	0.06
TFPI1_MOUSE	n/a	n/a	-0.40	0.77
TIMP1_MOUSE	-0.16	0.99	-0.12	1.00
TIMP2_MOUSE	0.44	0.05	0.43	0.09
TIMP3_MOUSE	n/a	n/a	-0.09	1.00
TSP1_MOUSE	-0.92	0.01	-1.42	1.80E-05
TSP2_MOUSE	1.87	1.20E-05	1.60	9.00E-07
VCAM1_MOUSE	-0.36	0.68	-0.12	1.00
VINC_MOUSE	-0.78	0.72	-0.99	0.19

Supplementary Table 6. List of primary and secondary antibodies used.

Antibody name	**Supplier and catalogue code**	**Host species antibody type**	**Dilution**
α-collagen 3α1	Santa Cruz (B-10) CS-271249	Mouse monoclonal	1:1000 in 5% milk/TBST
α-Phospho-AKT (S473)	Cell Signalling 9271S	Rabbit polyclonal	1:1000 in 5% BSA/TBST
α-Phospho-AKT (T308)	Cell Signalling 4056	Rabbit monoclonal	1:1000 in 5% BSA/TBST
α-AKT	Cell Signalling 9272	Rabbit polyclonal	1:1000 in 5% BSA/TBST
α-Periostin	Novus Biologicals NBP1-30042	Rabbit polyclonal	1:1000 in 5% BSA/TBST
α-GAPDH	Cell Signalling 14C10 – 2118	Rabbit monoclonal	1:2000 in 5% milk/TBST
α-rabbit IgG HRP	Cell Signalling 7074	Goat polyclonal	1:2000 in 5%milk/TBST
α-mouse IgG HRP	Dako Agilent P0447	Goat polyclonal	1:2000 in 5%milk/TBST

Supplementary Table 7. Primer sequences for quantification of mRNA expression by qPCR. (A) SYBR-based qPCR primers **(B)** UPL-probe-based (Roche) qPCR primers

(A)

Gene	GenBank Accession no	Forward Primer (FP) 5'-->3'	Reverse Primer (RP) 5'-->3'	Amplicon Size (bp)	Annealing Temp ℃ (FP, RP)
Pcoc1 (Pcolce)	NM_008788	GCCAGACCCCCAACTACAC	CCGTAATTGTCCAGATGCACTT	132	62, 60.6
Fbln3 (Efemp1)	NM_146015	GCGCTGGTCAAGTCACAGTA	AAGCATCTGGGACAATGTCAC	127	62.1, 60.3
Igfbp4	NM_010517	AGAAGCCCCTGCGTACATTG	TTGTTGGGATGTTCGCTCTCA	118	62.2, 61.7
Col3a1	NM_009930	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC	144	60.4, 61.4
Ctgf (Ccn2)	NM_010217	GGGCCTCTTCTGCGATTTC	ATCCAGGCAAGTGCATTGGTA	151	60.5, 61.8
Tsp2 (Thbs2)	NM_011581	CTGGGCATAGGGCCAAGAG	GTCTTCCGGTTAATGTTGCTGAT	89	61.8, 60.9
Lif	NM_008501	AACCAGATCAAGAATCAACTGGC	TGTTAGGCGCACATAGCTTTT	121	60.8, 60.3
Postn	NM_001198766	CACGGCATGGTTATTCCTTCA	TCAGGACACGGTCAATGACAT	151	60.4, 61.1
Col1a1	NM_007742	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG	103	62.7, 62.3
Myh6	NM_001164171	AACTACCACATCTTCTACC	TAGTCGTATGGGTTGTTG	86	56.6, 56.6
Myh7	NM_080728	GCTGTTATTGCCGCCATTG	GTTGTCATTCCGAACTGTCTTG	137	56.5, 56.5
Anp	NM_008725	GCTTCCAGGCCATATTGGAG	GGGGGCATGACCTCATCTT	126	60.2, 61.1
Tbp	NM_013684	CCTTGTACCCTTCACCAATGAC	ACAGCCAAGATTCACGGTAGA	119	60.6, 60.9
Cd151	NM_001111050	GGCCTATGTCTATTATCAGCAGC	CTTGCCAGTCTTGAGAGTTGTT	167	60.4, 60.4
Itga3	NM_013565	CCTCTTCGGCTACTCGGTC	CCGGTTGGTATAGTCATCACCC	112	61.2, 61.9
Rgs4	NM_009062	GAGTGCAAAGGACATGAAACATC	TTTTCCAACGATTCAGCCCAT	153	60, 60.2
Adamtsl3	NM_001190374	CCAGAAGTCTCTAATCCAGTGGG	AATGAGCTTGAGAACGACCGT	172	61.4, 61.7
Rb1	NM_009029	TGCATCTTTATCGCAGCAGTT	GTTCACACGTCCGTTCTAATTTG	208	60.4, 60
Kdm6a	NM_009483	CTTAATGCAGCAACACCAAATGA	TGTAGGCAGTGATGAGTCAACT	97	60, 60.8
Pnrc1	NM_001033225	TGGGAAAATCGGAGAAAATTGCC	TCCTTTTTGCAGAGGTGATCTTG	135	61.6, 60.7
Dnmt3a	NM_007872	ATGTGGTTCGGAGATGGCAAG	AGATGGCTTTGCGGTACATGG	124	62.4, 62.8
Mkrn1	NM_018810	ACAGTCCATACGGCGTAGTGT	ACTCAGATCAGTAGCAGTCACTT	122	63, 60.5
mTOR	NM_020009	CAGTTCGCCAGTGGACTGAAG	GCTGGTCATAGAAGCGAGTAGAC	130	62.9, 62.1
Egr1	NM_007913	TATGAGCACCTGACCACAGAG	GCTGGGATAACTCGTCTCCA	81	60.9, 60.7
Sbno2	NM_183426	GCCATCACCTATGCCTGCC	GCCCCATCCCCAATCAGAAA	80	62.9, 61.9
Nid2	NM_008695	TGGATTACCCAATGGATTGACCT	GTGGTTTTGGATGACACGTCG	124	61.1, 61.8
Itga8	NM_001001309	GAGCACTCACCTTGTCGAAAC	ACCTTGCCAGTAAAAACTTCCA	132	61.2, 60.0
Dnmt1	NM_001199433	GAGGCGGAAATCAAAGGAGGA	GGGAGTCTCTGGAGCTACCT	178	61.8, 61.6
Hprt	NM_013556	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG	142	60.8, 62.4
Pik3cb	NM_029094	CTATGGCAGACAACCTTGACAT	CTTCCCGAGGTACTTCCAACT	120	60.0, 60.9
Gna12	NM_010302	TTCGAGGGTTCTTGTGGACG	CATCAGAAACATCCCGTGCTT	85	61.8, 60.6
Phlpp2	NM_001122594	GCCACAATCTTCTTACAGAGGTC	TCGAGGGGAATGTGCTCCA	121	60.6, 62.6
Vim	NM_011701	TCCACACGCACCTACAGTCT	CCGAGGACCGGGTCACATA	100	62.7, 62.7
Col6a1	NM_009933	CTGCTGCTACAAGCCTGCT	CCCCATAAGGTTTCAGCCTCA	139	62.3, 61.5
Col6a2	NM_146007	AAGGCCCCATTGGATTCCC	CTCCCTTCCGACCATCCGAT	87	61.7, 63
Col11a1	NM_007729	CCAGCGGGTCTTATGGGTC	TGGTAACATCAGCATGGTTCC	144	61.8, 60.0

(B)

Gene	Genbank Accession No.	Forward Primer (FP) 5'-->3'	Reverse Primer (RP) 5'-->3'	Amplicon Size (bp)	Annealing Temp ℃ (FP, RP)	Roche UPL Probe	Cat No.
Cited2	NM_010828.3	ATCGCAAAGACGGAAGGA	TGCTGCTGGTGATGATGC	149	59, 60	77	4689003001
Hprt	NM_013556.2	CCTCCTCAGACCGCTTTTT	AACCTGGTTCATCATCGCTAA	91	59, 59	95	4692128001

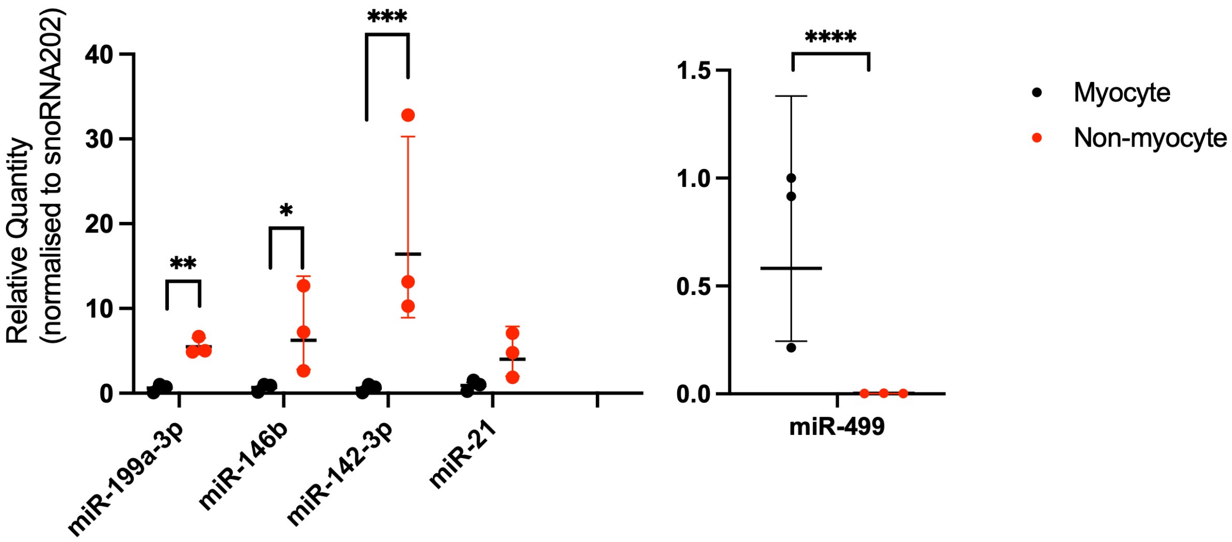
Supplemental Table 8. List of miRNA Taqman Assays (Applied Biosystems) used for quantification of miRNAs

microRNA name	Product no-Assay no
miR-146b	4427975-001097
miR-214	4427975-002306
miR-142-3p	4427975-000464
miR-499	4427975-001352
snoRNA202	4427975-001232
miR-199a-3p	4427975-002304
miR-199a-5p	4427975-000498
miR-21	4427975-000397

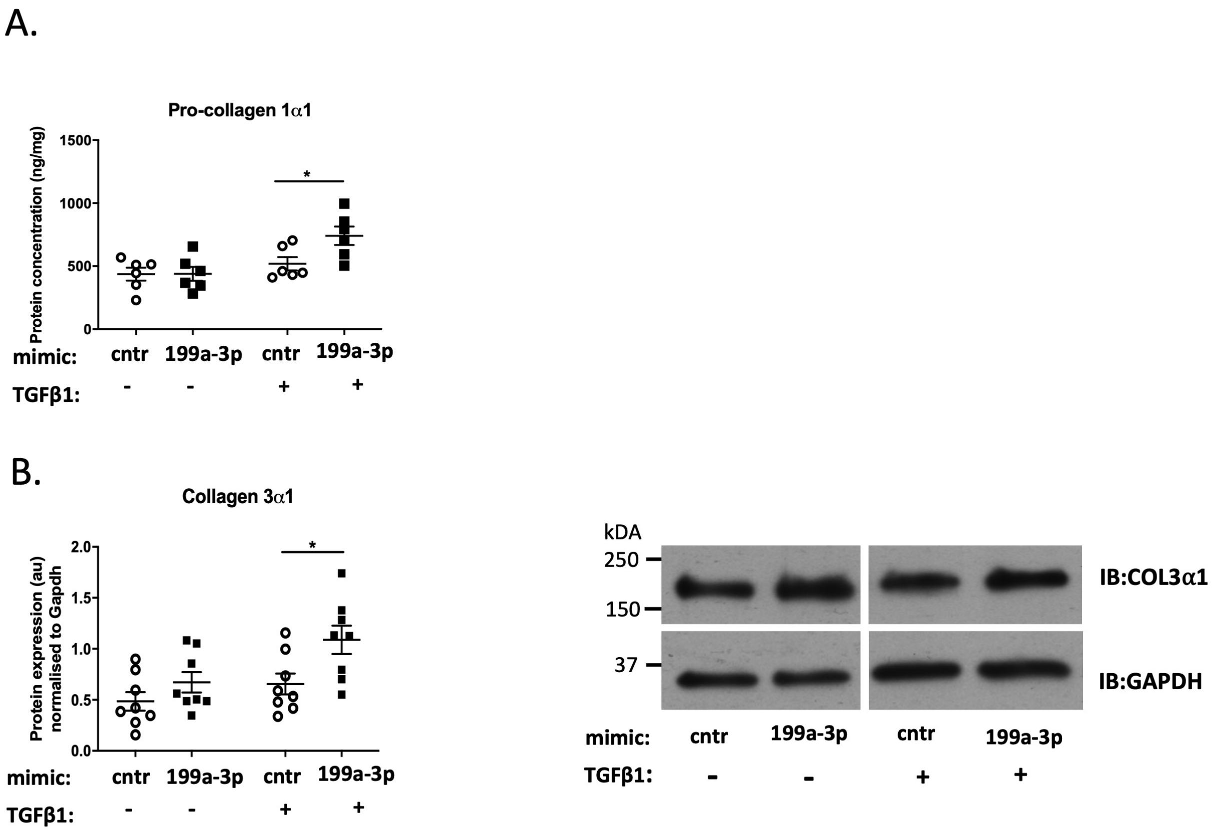
Supplemental Table 9. **Functional echocardiographic parameters of LNA-Control and LNA-antimiR-199a-3p treated mice at end-point.**

LSLAX M-Mode	**LNA Control**	**LNA-antimiR-199a-3p**	**p-value**
Sample n	9	7	
IVS;d (mm)	1.22 ±0.15	1.10 ±0.12	0.088
IVS;s (mm)	1.78 ±0.24	1.62 ±0.19	0.159
LVID;d (mm)	3.42 ±0.22	3.45 ±0.10	0.763
LVID;s (mm)	1.95 ±0.32	1.88 ±0.26	0.611
LVPW;d (mm)	1.20 ±0.16	1.14 ±0.11	0.368
LVPW;s (mm)	1.59 ±0.26	1.69 ±0.17	0.347
CO (mL/min))	15.44 ±1.90	16.16 ±2.73	0.567
EF (%)	74.93 ±7.12	77.54 ±6.99	0.476
FS (%)	43.25 ±6.65	45.66 ±6.47	0.477
Heart Rate (BPM)	413 ±34	407 ±42	0.778

Values are shown as mean ± standard deviation. LNA-Control (n=9) and LNA-antimiR-199a-3p (n=7) treated HCM mice were subjected to echocardiography imaging at end-point. Statistics: Unpaired 2-tailed t-test with Welch's correction. IVS, interventricular septum; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall; CO, cardiac output; EF, ejection fraction; FS, fractional shortening; d, diastole; s, systole

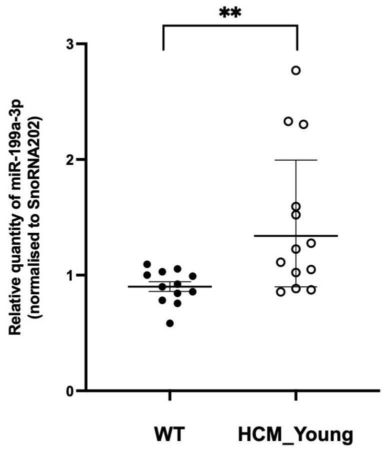


Supplemental Figure 1. Cardiac non-myocyte enrichment of HCM-related miRNAs. RNA isolated from myocytes and non-myocytes (from aged mice, n=3) was analyzed by Taqman qPCR to quantify miRNA-199a-3p, miR-142-3p, miR-146b and miR-21 (left graph) and myocyte-specific miRNA-499 (right graph). Relative quantity of each miRNA was calculated using the $2^{-\Delta\Delta CT}$ method (snoRNA202 used as a normalization control). Data are represented as geometric mean±SD, Statistics: 2way ANOVA with Sidak's multiple comparisons test. Adjusted P value *p=0.01, ** p=0.008, ***p=0.0003, **** p<0.0001

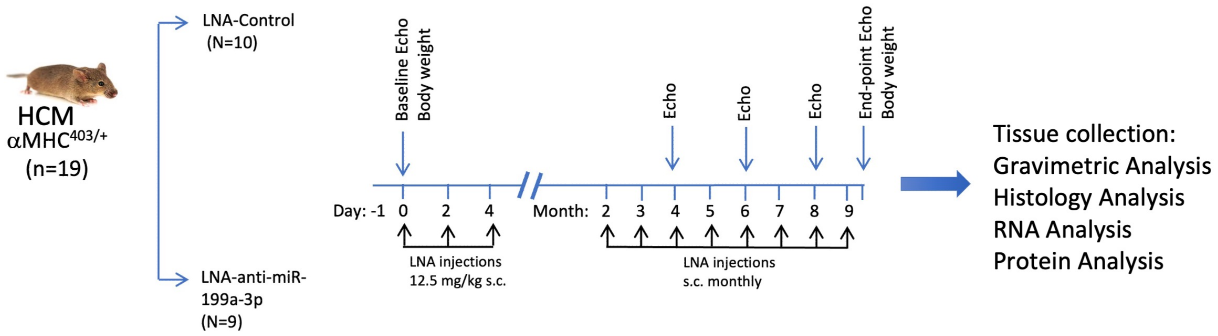


Supplemental Figure 2. miR-199a-3p mimic expression in cardiac fibroblasts induces collagen protein expression. Cardiac fibroblasts transfected with control (cntr) or miR-199a-3p mimic (199a-3p) were cultured either in DMEM only (-) or DMEM+TGFβ1 (+). **(A)** Quantification of pro-Collagen 1a1 by ELISA in conditioned media collected from transfected cells (n=6 per group). **(B)** Quantification of COL3A1 protein levels normalized to GAPDH (left, graph, n=8 per group) and representative immunoblots show COL3A1 and GAPDH (right, panels). Data are presented as mean ± SEM. Statistical analysis was performed by one-way ANOVA with Tukey's multiple comparisons test, * $p < 0.05$.

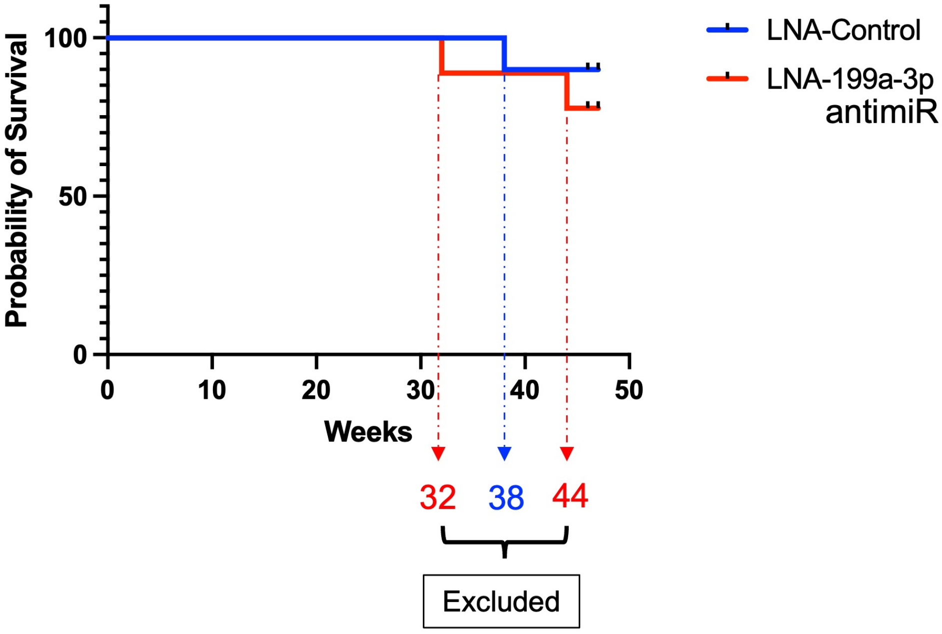
A.


**
Relative quantity of miR-199a-3p
(normalised to SnoRNA202)
0
1
2
3
WT
HCM_Young

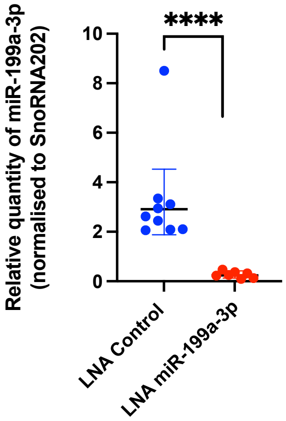
B.


HCM
αMHC403/+
(n=19)
LNA-Control
(N=10)
LNA-anti-miR-
199a-3p
(N=9)
Baseline Echo
Body weight
Echo
Echo
Echo
End-point Echo
Body weight
Day: -1
0
2
4
Month:
2
3
4
5
6
7
8
9
LNA injections
12.5 mg/kg s.c.
LNA injections
s.c. monthly
Tissue collection:
Gravimetric Analysis
Histology Analysis
RNA Analysis
Protein Analysis

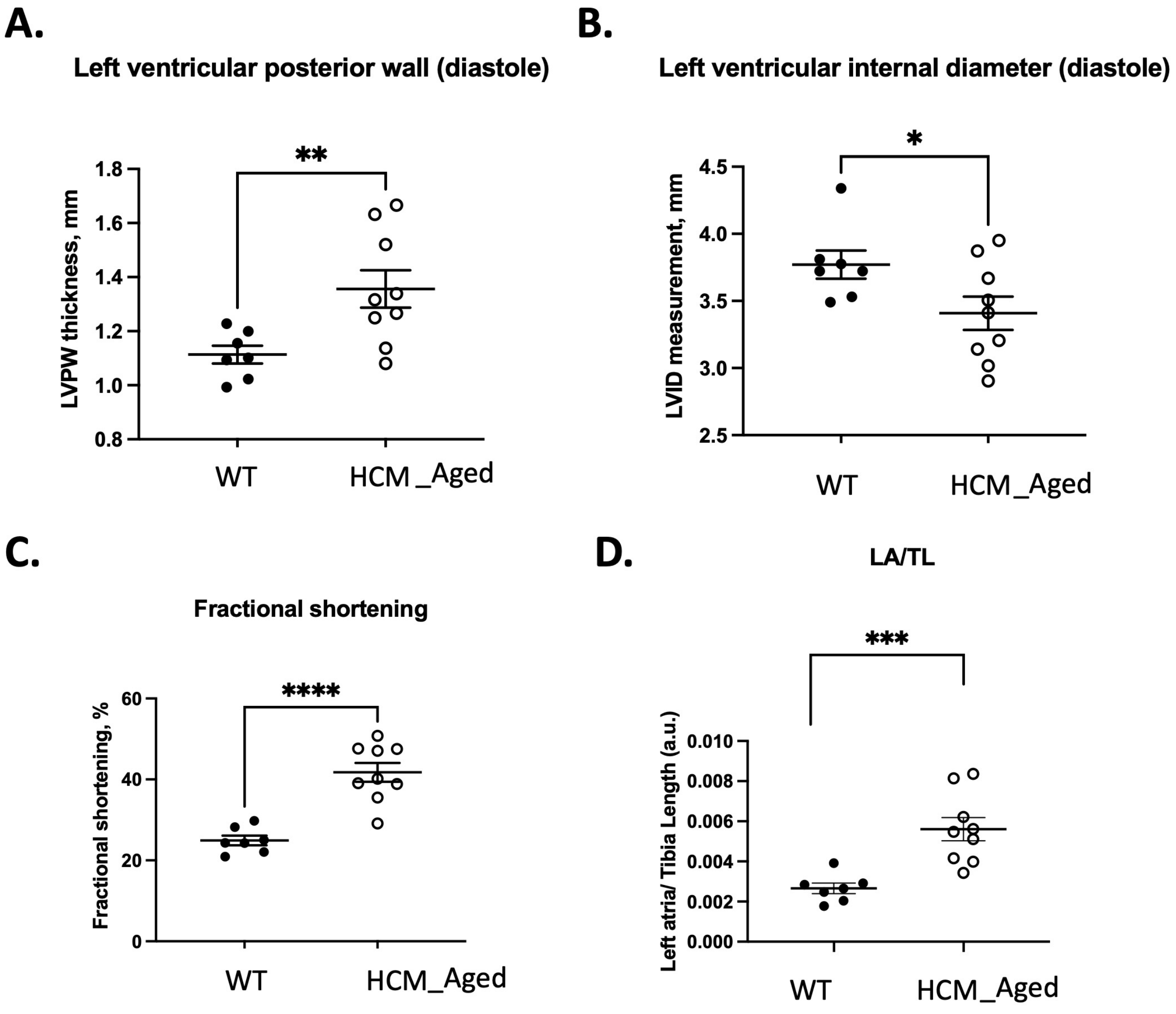
C.


Probability of Survival
0
50
100
0
10
20
30
40
50
Weeks
LNA-Control
LNA-199a-3p
antimiR
32
38
44
Excluded

D.

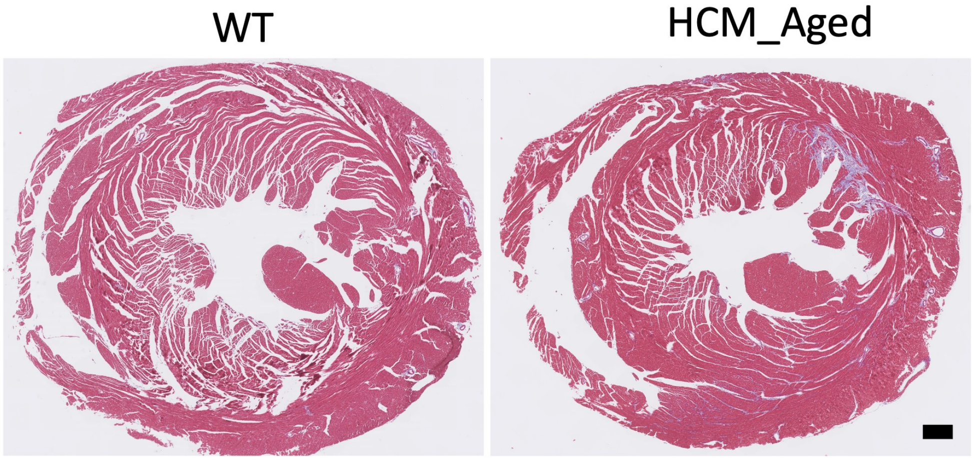


Supplemental Figure 3. ***In vivo* miRNA inhibition study design in HCM mouse model. (A)** Quantification of miR-199a-3p expression by real-time quantitative PCR in young (4–8-week-old) WT (n=12) and age-matched HCM (n=13) mice shows significant upregulation of miR-199a-3p in young HCM mice. Relative quantity of miR-199a-3p was calculated using the $2^{-\Delta\Delta CT}$ method (snoRNA202 used as a normalization control). Data are presented as geometric mean± SD. Statistics: Unpaired two-tailed t-test with Welch's correction, **p=0.004. **(B)** Schematic representation of *in vivo* study design. **(C)** Survival curve of HCM mice treated with LNA-control (blue line) or LNA-antimiR-199a-3p (red line) over 47 weeks. **(D)** Quantification of miR-199a-3p expression by real-time quantitative PCR shows significant inhibition of miR-199a-3p levels in LNA-miR-199a-3p treated mice (n=7) vs LNA-control mice (n=9). Relative quantity of miR-199a-3p was calculated using the $2^{-\Delta\Delta CT}$ method (snoRNA202 used as a normalization control). Data are presented as geometric mean± SD. Statistics: Unpaired two-tailed t-test, ****p<0.0001.

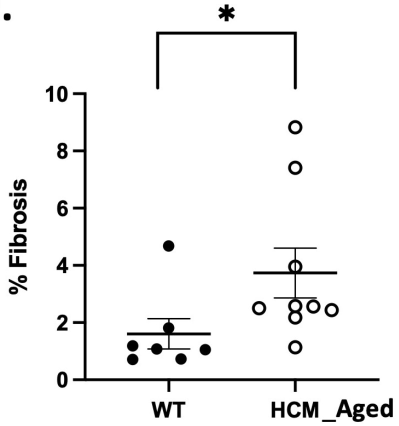


Supplemental Figure 4. Echocardiographic and gravimetric analysis of aged WT and HCM mice. (A) Diastolic left ventricular posterior wall thickness (LVPW; d), mm **(B)** diastolic left ventricular internal dimension (LVID; d), mm **(C)** fractional shortening (FS) % **(D)** Gravimetric analysis of left atrial /tibia length ratio (LA/TL). Data are expressed as mean±SEM, n=7 (WT) and n=9 (HCM) mice. Statistics: Unpaired two-tailed t-test with Welch's correction. *p= 0.04, **=0.01, ***p=0.0007, ****p<0.0001.

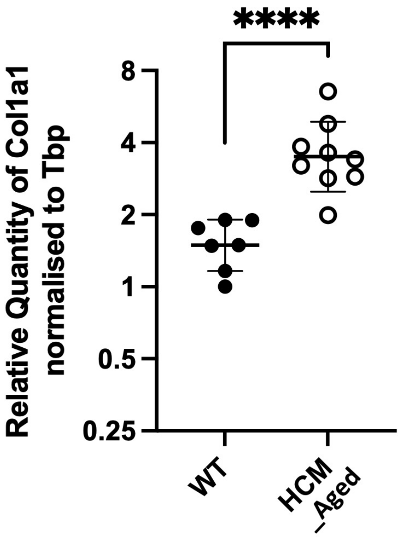
A.


WT
HCM_Aged

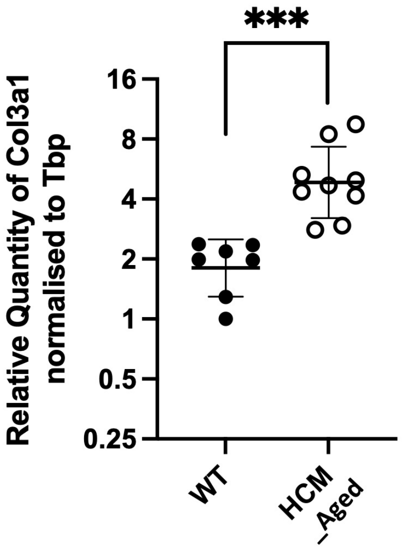
B.


*
% Fibrosis
10
8
6
4
2
0
WT
HCM_Aged

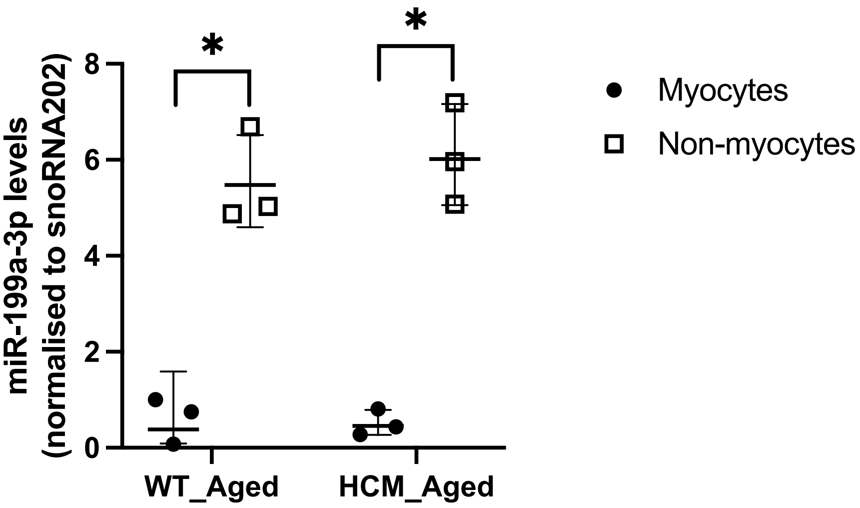
C.


****
Relative Quantity of Col1a1 normalised to Tbp
8
4
2
1
0.5
0.25
WT
HCM_Aged

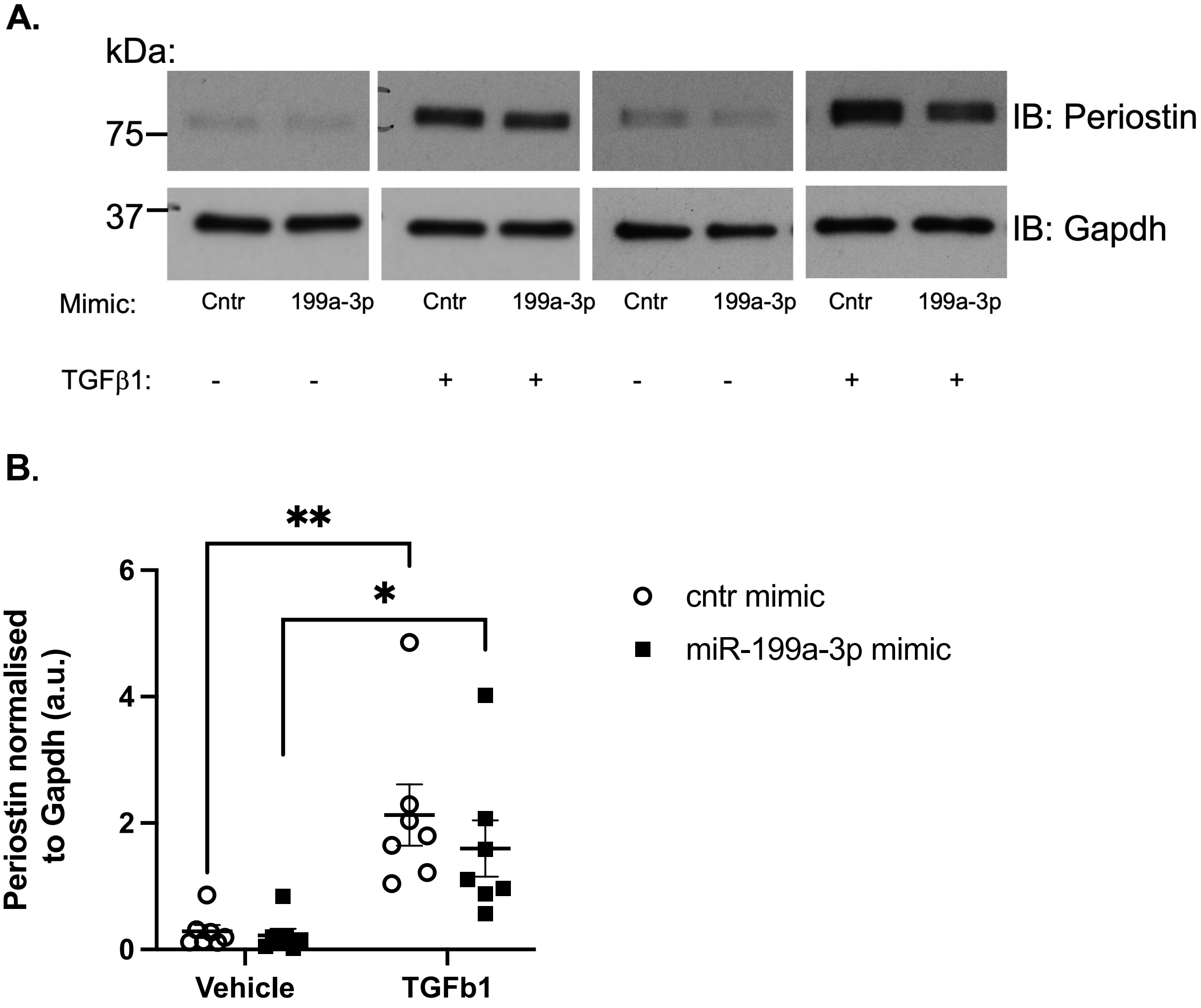
D.


***
Relative Quantity of Col3a1 normalised to Tbp
16
8
4
2
1
0.5
0.25
WT
HCM_Aged

Supplemental Figure 5. Cardiac remodeling in aged HCM mice. (A) Representative micrographs depict 5 μm paraffin-embedded heart sections from WT and HCM mice stained with Masson's Trichrome (MT). Scale bar = 0.5 mm. Blue staining depicts collagen deposition areas. **(B)** Graph shows quantification of fibrosis in heart sections using NIH Image J Software in WT (black-filled circles) and HCM (empty circles) mice hearts. Data were analyzed by Mann-Whitney test (due to lognormal distribution), *p-value<0.016. mRNA levels of collagens **(C)** *Col1a1* and **(D)** *Col3a1* were quantified by real time-quantitative PCR in left ventricular heart tissue obtained from WT (n=7) or HCM mice (n=9). Relative quantity of each gene was calculated using the 2^{-DDCT} method (*Tbp* was used as a normalization control) Statistics: Unpaired two-tailed t-test with Welch's correction *** p=0.0001, ****p<0.0001



Supplemental Figure 6. Relative expression levels of miR-199a-3p in myocytes and non-myocytes isolated from WT_Aged and HCM_Aged mouse hearts (n=3). Relative quantity was calculated using the $2\wedge^{-\Delta\Delta CT}$ method (snoRNA202 used as a normalization control). Data are presented as geometric mean±SD. Statistics: 2way ANOVA, Tukey's multiple comparison test. * p < 0.05



Supplemental Figure 7. Cardiac fibroblast response to TGFβ1. Cardiac fibroblasts transfected with control (cntr) or miR-199a-3p mimic (199a-3p) were cultured either in DMEM only (-) or DMEM+TGFβ1 (+). **(A)** Representative immunoblots depicting Periostin and Gapdh expression **(B)** Quantification of Periostin protein levels normalized to Gapdh (n=7 per group) Data are presented as mean ± SEM. Statistical analysis was performed by 2way ANOVA with Tukey's multiple comparisons test, * $p < 0.05$, ** $p < 0.01$

References

1. Eminaga S, Christodoulou DC, Vigneault F, Church GM, and Seidman JG. Quantification of microRNA expression with next-generation sequencing. *Curr Protoc Mol Biol.* 2013;Chapter 4:Unit 4 17.
2. Barwari T, Eminaga S, Mayr U, Lu R, Armstrong PC, Chan MV, et al. Inhibition of profibrotic microRNA-21 affects platelets and their releasate. *JCI Insight.* 2018;3(21).