

SUPPLEMENTAL MATERIAL

Systems Biology in Chronic Heart Failure – Identification of Potential miRNAs Regulators

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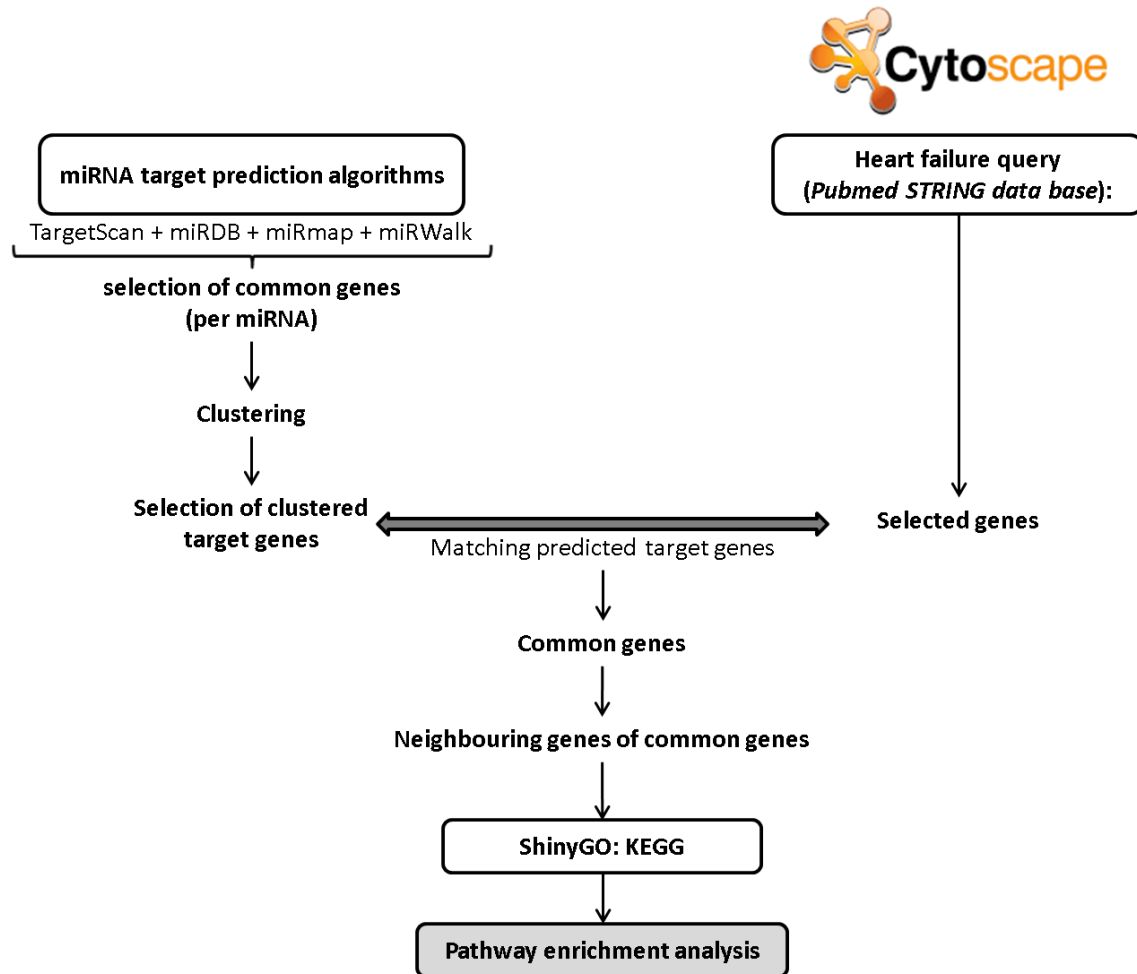
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SUPPLEMENTAL FIGURES



Supplemental Figure S1: Schematic representation of the *in silico* analysis of individual miRNAs.

SUPPLEMENTAL TABLES

Supplemental Table S1: miRNA selection strategy

	Fold-change (log ₂)	AUC±SD (95% CI)	P-value	Same family	miRBase reads	Quartile reads
<i>let-7a-5p</i>	-1.777	0.842±0.063 (0.718–0.966)	0.001	1	215,985,765	4
<i>miR-485-5p</i>	-3.245	0.823±0.085 (0.657–0.989)	0.004	0	3,168	1
<i>miR-342-3p</i>	-1.675	0.772±0.079 (0.617–0.928)	0.005	0	484,947	3
<i>miR-30b-5p</i>	-1.058	0.760±0.087 (0.590–0.931)	0.011	0	1,110,974	3
<i>miR-335-5p</i>	-0.995	0.749±0.086 (0.582–0.917)	0.012	0	416,898	3
<i>miR-125a-5p</i>	-0.957	0.748±0.080 (0.591–0.906)	0.012	0	1,146,942	3
<i>miR-150-5p</i>	-1.270	0.747±0.078 (0.594–0.900)	0.010	0	117,587	2
<i>miR-337-5p</i>	-1.548	0.741±0.093 (0.558–0.923)	0.020	0	4,424	1
<i>miR-224-5p</i>	-1.370	0.741±0.095 (0.555–0.926)	0.020	0	74,998	2
<i>miR-494-3p</i>	-1.223	0.740±0.085 (0.574–0.907)	0.018	0	11,622	1
<i>let-7g-5p</i>	-0.791	0.739±0.082 (0.579–0.899)	0.016	1	50,508,337	4
<i>let-7f-5p</i>	-0.973	0.739±0.082 (0.579–0.899)	0.016	1	115,380,455	4
<i>miR-107</i>	-0.968	0.737±0.090 (0.561–0.913)	0.018	0	5,491,348	4
<i>miR-495-3p</i>	-0.894	0.730±0.087 (0.560–0.900)	0.021	0	20,270	1
<i>miR-139-5p</i>	-1.299	0.729±0.088 (0.555–0.902)	0.028	0	85,265	2
<i>miR-301a-3p</i>	-1.168	0.726±0.096 (0.539–0.914)	0.034	0	81,204	2
<i>miR-26a-5p</i>	-0.558	0.726±0.086 (0.557–0.895)	0.024	0	28,542,742	4
<i>miR-328-3p</i>	-0.740	0.722±0.097 (0.532–0.911)	0.032	0	26,824	1
<i>miR-142-3p</i>	-0.840	0.720±0.082 (0.559–0.881)	0.019	0	160,986	3
<i>miR-377-3p</i>	-1.001	0.701±0.087 (0.530–0.872)	0.044	0	4,424	1
<i>miR-374b-5p</i>	-0.593	0.701±0.088 (0.530–0.873)	0.044	0	491,420	3
<i>miR-369-3p</i>	-1.062	0.701±0.095 (0.514–0.887)	0.043	0	11,211	1
<i>miR-574-3p</i>	-0.763	0.700±0.092 (0.521–0.879)	0.050	0	125,985	3
<i>miR-323a-3p</i>	-0.659	0.697±0.091 (0.518–0.875)	0.049	0	603	1
<i>miR-451a</i>	0.421	0.286±0.080 (0.130–0.442)	0.021	0	1,666,621	4
<i>miR-424-5p</i>	0.726	0.269±0.080 (0.113–0.426)	0.016	0	861,807	3
<i>miR-660-5p</i>	0.591	0.263±0.085 (0.096–0.431)	0.017	0	91,879	2
<i>miR-210-3p</i>	1.137	0.253±0.091 (0.075–0.432)	0.023	0	66,359	2

AUC: area under the curve; CI: confidence interval; SD: standard deviation. **In bold:** selected miRNAs.

Selection strategy: 1) Selection of top 15 best miRNAs according to AUC and fold-change. 2) Elimination of miRNAs from the same family (miRNAs with better AUC selected). 3) Elimination of miRNAs with low number of reads (4th quantile).

Supplemental Table S2: Expression values of analysed miRNAs

miRNA	CTL (n=26) median [IQR]	cHF (n=46) median [IQR]	p-value	Fold-change (log₂)
<i>let-7a-5p</i>	0.00045 [0.00008–0.00158]	0.00012 [0.00003–0.0003]	0.004	0.266 (–1.911)
<i>miR-107</i>	0.00004 [0.00002–0.0001]	0.00002 [0.000003–0.00005]	0.044	0.620 (–0.689)
<i>miR-125a-5p</i>	0.00121 [0.00041–0.00167]	0.00031 [0.00012–0.00059]	0.002	0.252 (–1.987)
<i>miR-139-5p</i>	0.00006 [0.00002–0.00013]	0.00003 [0.00001–0.00005]	0.006	0.456 (–1.133)
<i>miR-150-5p</i>	0.00521 [0.00288–0.0088]	0.0021 [0.00083–0.00372]	0.000	0.403 (–1.312)
<i>miR-30b-5p</i>	0.00123 [0.00077–0.0042]	0.00053 [0.00032–0.00136]	0.007	0.430 (–1.217)
<i>miR-335-5p</i>	0.00165 [0.00021–0.00331]	0.00065 [0.00019–0.00107]	0.048	0.397 (–1.334)
<i>miR-342-3p</i>	0.0017 [0.00092–0.00338]	0.00045 [0.00013–0.00087]	0.000	0.264 (–1.921)
<i>miR-224-5p</i>	0.00006 [0.00002–0.00019]	0.00003 [0.00001–0.00007]	0.079	0.486 (–1.040)
<i>miR-16-5p</i>	0.00603 [0.0027–0.00943]	0.00511 [0.00249–0.00843]	0.694	0.847 (–0.240)
<i>miR-223-3p</i>	0.0243 [0.01047–0.03971]	0.01508 [0.00857–0.03479]	0.226	0.621 (–0.688)

cHF: chronic heart failure; **CTL**: controls; **IQR**: interquartile range

Bold indicates statistical significance.

Supplemental Table S3: Pathways identified by enrichment analysis included in Figure 3

	DESCRIPTION - KEGG	FDR value	Genes (background genes)	Fold enrichment
CLUSTER 1				
hsa04510	Focal adhesion	5.52×10^{-21}	21 (198)	10.61
hsa04062	Chemokine signalling pathway	3.89×10^{-20}	20 (186)	10.75
hsa04014	Ras signalling pathway	4.27×10^{-20}	21 (226)	9.29
hsa04151	PI3K-Akt signalling pathway	4.67×10^{-19}	23 (350)	6.57
hsa04917	Prolactin signalling pathway	1.60×10^{-17}	14 (69)	20.29
hsa01522	Endocrine resistance	2.05×10^{-17}	15 (95)	15.79
hsa04926	Relaxin signalling pathway	3.37×10^{-17}	16 (128)	12.5
hsa04915	Oestrogen signalling pathway	1.66×10^{-15}	15 (133)	11.28
hsa01521	EGFR tyrosine kinase inhibitor resistance	2.11×10^{-15}	13 (78)	16.67
hsa04012	ErbB signalling pathway	4.02×10^{-15}	13 (83)	15.66
hsa04935	Growth hormone synthesis, secretion and action	8.07×10^{-15}	14 (118)	11.86
hsa04015	Rap1 signalling pathway	1.82×10^{-14}	16 (202)	7.92
hsa04810	Regulation of actin cytoskeleton	2.82×10^{-14}	16 (209)	7.66
hsa04722	Neurotrophin signalling pathway	1.24×10^{-13}	13 (114)	11.4
hsa04912	GnRH signalling pathway	2.20×10^{-13}	12 (89)	13.48
hsa04933	AGE-RAGE signalling pathway in diabetic complications	5.44×10^{-13}	12 (98)	12.24
hsa04910	Insulin signalling pathway	6.05×10^{-13}	13 (133)	9.77
hsa04010	MAPK signalling pathway	1.77×10^{-12}	16 (288)	5.56
hsa04370	VEGF signalling pathway	2.44×10^{-12}	10 (57)	17.54
hsa04071	Sphingolipid signalling pathway	2.53×10^{-12}	12 (116)	10.34
hsa04919	Thyroid hormone signalling pathway	3.24×10^{-12}	12 (119)	10.08
hsa04611	Platelet activation	4.01×10^{-12}	12 (122)	9.84
hsa04068	FoxO signalling pathway	6.05×10^{-12}	12 (127)	9.45
hsa04520	Adherens junction	8.53×10^{-12}	10 (67)	14.93
hsa04660	T cell receptor signalling pathway	1.30×10^{-11}	11 (101)	10.89
hsa04550	Signalling pathways regulating pluripotency of stem cells	1.54×10^{-11}	12 (140)	8.57
hsa04921	Oxytocin signalling pathway	2.84×10^{-11}	12 (149)	8.05
hsa04664	Fc epsilon RI signalling pathway	1.92×10^{-10}	9 (66)	13.64
hsa04625	C-type lectin receptor signalling pathway	2.67×10^{-10}	10 (102)	9.8
hsa04072	Phospholipase D signalling pathway	3.70×10^{-10}	11 (147)	7.48
hsa04150	mTOR signalling pathway	4.77×10^{-10}	11 (151)	7.28
hsa04540	Gap junction	1.43×10^{-9}	9 (87)	10.34
hsa05418	Fluid shear stress and atherosclerosis	2.03×10^{-9}	10 (130)	7.69
hsa04371	Apelin signalling pathway	2.14×10^{-9}	10 (131)	7.63
hsa04929	GnRH secretion	3.09×10^{-9}	8 (63)	12.7
hsa04670	Leukocyte transendothelial migration	8.23×10^{-9}	9 (109)	8.26
hsa04662	B cell receptor signalling pathway	1.36×10^{-8}	8 (78)	10.26
hsa04720	Long-term potentiation	8.54×10^{-8}	7 (64)	10.94
hsa04024	cAMP signalling pathway	1.16×10^{-7}	10 (208)	4.81
hsa04650	Natural killer cell mediated cytotoxicity	2.93×10^{-7}	8 (121)	6.61
hsa04210	Apoptosis	5.46×10^{-7}	8 (132)	6.06
hsa04211	Longevity regulating pathway	5.58×10^{-7}	7 (87)	8.05
hsa04270	Vascular smooth muscle contraction	5.64×10^{-7}	8 (133)	6.02
hsa04530	Tight junction	1.76×10^{-6}	8 (156)	5.13
hsa04144	Endocytosis	4.14×10^{-6}	9 (241)	3.73
hsa04666	Fc gamma R-mediated phagocytosis	1.18×10^{-5}	6 (90)	6.67
hsa04723	Retrograde endocannabinoid signalling	1.32×10^{-5}	7 (145)	4.83
hsa04218	Cellular senescence	1.61×10^{-5}	7 (150)	4.67
hsa05142	Chagas disease	1.91×10^{-5}	6 (99)	6.06
hsa04928	Parathyroid hormone synthesis, secretion and action	2.34×10^{-5}	6 (103)	5.83
hsa04022	cGMP-PKG signalling pathway	2.53×10^{-5}	7 (162)	4.32
hsa04066	HIF-1 signalling pathway	2.69×10^{-5}	6 (106)	5.66
hsa04668	TNF signalling pathway	3.52×10^{-5}	6 (112)	5.36
hsa04960	Aldosterone-regulated sodium reabsorption	8.51×10^{-5}	4 (37)	10.81
hsa04261	Adrenergic signalling in cardiomyocytes	1.50×10^{-4}	6 (147)	4.08
hsa04657	IL-17 signalling pathway	1.80×10^{-4}	5 (92)	5.43
hsa04930	Type II diabetes mellitus	1.80×10^{-4}	4 (46)	8.7

	DESCRIPTION - KEGG	FDR value	Genes (background genes)	Fold enrichment
hsa04750	Inflammatory mediator regulation of TRP channels	1.90x10 ⁻⁰⁴	5 (94)	5.32
hsa04630	JAK-STAT signalling pathway	2.20x10 ⁻⁰⁴	6 (160)	3.75
hsa04620	Toll-like receptor signalling pathway	2.60x10 ⁻⁰⁴	5 (101)	4.95
hsa04659	Th17 cell differentiation	2.60x10 ⁻⁰⁴	5 (101)	4.95
hsa01524	Platinum drug resistance	7.90x10 ⁻⁰⁴	4 (70)	5.71
hsa04918	Thyroid hormone synthesis	9.60x10 ⁻⁰⁴	4 (74)	5.41
hsa05412	Arrhythmogenic right ventricular cardiomyopathy	0.001	4 (76)	5.26
hsa04310	Wnt signalling pathway	0.0016	5 (154)	3.25
hsa04350	TGF- β signalling pathway	0.0019	4 (91)	4.4
hsa05414	Dilated cardiomyopathy	0.0022	4 (95)	4.21
hsa04621	NOD-like receptor signalling pathway	0.0026	5 (174)	2.87
hsa04931	Insulin resistance	0.0034	4 (107)	3.74
hsa04961	Endocrine and other factor-regulated calcium reabsorption	0.0045	3 (53)	5.66
hsa04923	Regulation of lipolysis in adipocytes	0.0047	3 (54)	5.56
hsa04152	AMPK signalling pathway	0.0049	4 (120)	3.33
hsa05017	Spinocerebellar ataxia	0.0074	4 (135)	2.96
hsa04714	Thermogenesis	0.008	5 (229)	2.18
hsa04920	Adipocytokine signalling pathway	0.0088	3 (69)	4.35
hsa04141	Protein processing in endoplasmic reticulum	0.0142	4 (165)	2.42
hsa04658	Th1 and Th2 cell differentiation	0.0158	3 (87)	3.45
hsa04512	ECM-receptor interaction	0.0162	3 (88)	3.41
hsa05410	Hypertrophic cardiomyopathy	0.0166	3 (89)	3.37
hsa04922	Glucagon signalling pathway	0.0229	3 (101)	2.97
hsa04973	Carbohydrate digestion and absorption	0.0375	2 (44)	4.55
CLUSTER 2				
hsa04110	Cell cycle	3.13x10 ⁻¹⁴	14 (120)	11.67
hsa04218	Cellular senescence	7.40x10 ⁻¹¹	12 (150)	8
hsa04350	TGF- β signalling pathway	2.47x10 ⁻¹⁰	10 (91)	10.99
hsa04659	Th17 cell differentiation	2.89x10 ⁻⁰⁷	8 (101)	7.92
hsa04550	Signalling pathways regulating pluripotency of stem cells	2.12x10 ⁻⁰⁶	8 (140)	5.71
hsa01522	Endocrine resistance	2.58x10 ⁻⁰⁶	7 (95)	7.37
hsa04310	Wnt signalling pathway	3.76x10 ⁻⁰⁶	8 (154)	5.19
hsa04919	Thyroid hormone signalling pathway	9.76x10 ⁻⁰⁶	7 (119)	5.88
hsa04068	FoxO signalling pathway	1.38x10 ⁻⁰⁵	7 (127)	5.51
hsa04330	Notch signalling pathway	3.56x10 ⁻⁰⁵	5 (52)	9.62
hsa04390	Hippo signalling pathway	3.56x10 ⁻⁰⁵	7 (153)	4.58
hsa04933	AGE-RAGE signalling pathway in diabetic complications	3.56x10 ⁻⁰⁵	6 (98)	6.12
hsa04520	Adherens junction	9.10x10 ⁻⁰⁵	5 (67)	7.46
hsa04371	Apelin signalling pathway	0.0017	5 (131)	3.82
hsa04917	Prolactin signalling pathway	0.0017	4 (69)	5.8
hsa04115	p53 signalling pathway	0.0019	4 (72)	5.56
hsa04630	JAK-STAT signalling pathway	0.0036	5 (160)	3.13
hsa05142	Chagas disease	0.0052	4 (99)	4.04
hsa04926	Relaxin signalling pathway	0.0125	4 (128)	3.13
hsa05418	Fluid shear stress and atherosclerosis	0.0129	4 (130)	3.08
hsa04658	Th1 and Th2 cell differentiation	0.0347	3 (87)	3.45
hsa04657	IL-17 signalling pathway	0.0395	3 (92)	3.26
CLUSTER 3				
hsa03013	RNA transport	9.30x10 ⁻⁰⁵	7 (160)	4.38
CLUSTER 4				
hsa04120	Ubiquitin mediated proteolysis	3.14x10 ⁻⁰⁸	9 (135)	6.67
hsa04310	Wnt signalling pathway	1.22x10 ⁻⁰⁶	8 (154)	5.19
hsa04390	Hippo signalling pathway	0.00015	6 (153)	3.92
hsa04340	Hedgehog signalling pathway	0.0086	3 (47)	6.38
hsa04550	Signalling pathways regulating pluripotency of stem cells	0.0128	4 (140)	2.86

Supplemental Table S4: Pathways identified by enrichment analysis of miRNAs included in Figure 4

	DESCRIPTION - KEGG	FDR value	Genes (background genes)	Fold enrichment
CLUSTER 1				
hsa04014	Ras signalling pathway	5.82x10 ⁻¹⁷	18 (226)	7.96
hsa04510	Focal adhesion	1.31x10 ⁻¹⁶	17 (198)	8.59
hsa04062	Chemokine signalling pathway	5.14x10 ⁻¹³	14 (186)	7.53
hsa04151	PI3K-Akt signalling pathway	5.14x10 ⁻¹³	17 (350)	4.86
hsa04012	ErbB signalling pathway	1.19x10 ⁻¹²	11 (83)	13.25
hsa04810	Regulation of actin cytoskeleton	1.62x10 ⁻¹²	14 (209)	6.70
hsa04520	Adherens junction	4.71x10 ⁻¹²	10 (67)	14.93
hsa01521	EGFR tyrosine kinase inhibitor resistance	1.72x10 ⁻¹¹	10 (78)	12.82
hsa04015	Rap1 signalling pathway	1.72x10 ⁻¹¹	13 (202)	6.44
hsa04722	Neurotrophin signalling pathway	1.72x10 ⁻¹¹	11 (114)	9.65
hsa01522	Endocrine resistance	7.0x10 ⁻¹¹	10 (95)	10.53
hsa04917	Prolactin signalling pathway	1.31x10 ⁻¹⁰	9 (69)	13.04
hsa04010	MAPK signalling pathway	6.06x10 ⁻¹⁰	13 (288)	4.51
hsa04370	VEGF signalling pathway	9.97x10 ⁻¹⁰	8 (57)	14.04
hsa04625	C-type lectin receptor signalling pathway	2.4x10 ⁻⁹	9 (102)	8.82
hsa04670	Leukocyte transendothelial migration	3.89x10 ⁻⁹	9 (109)	8.26
hsa04071	Sphingolipid signalling pathway	5.46x10 ⁻⁹	9 (116)	7.76
hsa04935	Growth hormone synthesis, secretion and action	6.13x10 ⁻⁹	9 (118)	7.63
hsa04068	FoxO signalling pathway	1.1x10 ⁻⁸	9 (127)	7.09
hsa04926	Relaxin signalling pathway	1.14x10 ⁻⁸	9 (128)	7.03
hsa05418	Fluid shear stress and atherosclerosis	1.27x10 ⁻⁸	9 (130)	6.92
hsa04910	Insulin signalling pathway	1.5x10 ⁻⁸	9 (133)	6.77
hsa04912	GnRH signalling pathway	1.5x10 ⁻⁸	8 (89)	8.99
hsa04915	Oestrogen signalling pathway	1.5x10 ⁻⁸	9 (133)	6.77
hsa04550	Signalling pathways regulating pluripotency of stem cells	2.14x10 ⁻⁸	9 (140)	6.43
hsa04933	AGE-RAGE signalling pathway in diabetic complications	2.88x10 ⁻⁸	8 (98)	8.16
hsa04072	Phospholipase D signalling pathway	3.07x10 ⁻⁸	9 (147)	6.12
hsa04921	Oxytocin signalling pathway	3.37x10 ⁻⁸	9 (149)	6.04
hsa04660	T cell receptor signalling pathway	3.38x10 ⁻⁸	8 (101)	7.92
hsa04919	Thyroid hormone signalling pathway	1.03x10 ⁻⁷	8 (119)	6.72
hsa04611	Platelet activation	1.21x10 ⁻⁷	8 (122)	6.56
hsa04150	mTOR signalling pathway	5.35x10 ⁻⁷	8 (151)	5.30
hsa04664	Fc epsilon RI signalling pathway	1.16x10 ⁻⁶	6 (66)	9.09
hsa04144	Endocytosis	1.32x10 ⁻⁶	9 (241)	3.73
hsa04024	cAMP signalling pathway	4.82x10 ⁻⁶	8 (208)	3.85
hsa04530	Tight junction	8.49x10 ⁻⁶	7 (156)	4.49
hsa04929	GnRH secretion	1.93x10 ⁻⁵	5 (63)	7.94
hsa04650	Natural killer cell mediated cytotoxicity	2.62x10 ⁻⁵	6 (121)	4.96
hsa04371	Apelin signalling pathway	3.94x10 ⁻⁵	6 (131)	4.58
hsa04210	Apoptosis	4.06x10 ⁻⁵	6 (132)	4.55
hsa04270	Vascular smooth muscle contraction	4.18x10 ⁻⁵	6 (133)	4.51
hsa04662	B cell receptor signalling pathway	4.72x10 ⁻⁵	5 (78)	6.41
hsa04540	Gap junction	7.7x10 ⁻⁵	5 (87)	5.75
hsa04218	Cellular senescence	7.78x10 ⁻⁵	6 (150)	4.00
hsa04666	Fc gamma R-mediated phagocytosis	8.79x10 ⁻⁵	5 (90)	5.56
hsa04657	IL-17 signalling pathway	9.61x10 ⁻⁵	5 (92)	5.43
hsa04022	cGMP-PKG signalling pathway	1.1x10 ⁻⁴	6 (162)	3.70
hsa04659	Th17 cell differentiation	1.4x10 ⁻⁴	5 (101)	4.95
hsa04066	HIF-1 signalling pathway	1.7x10 ⁻⁴	5 (106)	4.72
hsa04668	TNF signalling pathway	2.2x10 ⁻⁴	5 (112)	4.46
hsa04720	Long-term potentiation	3.4x10 ⁻⁴	4 (64)	6.25
hsa04920	Adipocytokine signalling pathway	4.5x10 ⁻⁴	4 (69)	5.80
hsa05412	Arrhythmogenic right ventricular cardiomyopathy	6.2x10 ⁻⁴	4 (76)	5.26
hsa04211	Longevity regulating pathway	0.001	4 (87)	4.60
hsa04350	TGF-β signalling pathway	0.0012	4 (91)	4.40
hsa04960	Aldosterone-regulated sodium reabsorption	0.0012	3 (37)	8.11
hsa04621	NOD-like receptor signalling pathway	0.0014	5 (174)	2.87

DESCRIPTION - KEGG		FDR value	Genes (background genes)	Fold enrichment
hsa05142	Chagas disease	0.0015	4 (99)	4.04
hsa04620	Toll-like receptor signalling pathway	0.0016	4 (101)	3.96
hsa04928	Parathyroid hormone synthesis, secretion and action	0.0018	4 (103)	3.88
hsa04931	Insulin resistance	0.002	4 (107)	3.74
hsa04930	Type II diabetes mellitus	0.0021	3 (46)	6.52
hsa04723	Retrograde endocannabinoid signalling	0.0058	4 (145)	2.76
hsa04261	Adrenergic signalling in cardiomyocytes	0.006	4 (147)	2.72
hsa01524	Platinum drug resistance	0.0065	3 (70)	4.29
hsa04310	Wnt signalling pathway	0.0069	4 (154)	2.60
hsa04630	JAK-STAT signalling pathway	0.0078	4 (160)	2.50
hsa04141	Protein processing in endoplasmic reticulum	0.0087	4 (165)	2.42
hsa04658	Th1 and Th2 cell differentiation	0.0112	3 (87)	3.45
hsa05410	Hypertrophic cardiomyopathy	0.0118	3 (89)	3.37
hsa05414	Dilated cardiomyopathy	0.0137	3 (95)	3.16
hsa04110	Cell cycle	0.025	3 (120)	2.50
hsa04152	AMPK signalling pathway	0.025	3 (120)	2.50
hsa04217	Necroptosis	0.0428	3 (149)	2.01
hsa05416	Viral myocarditis	0.0439	2 (55)	3.64
hsa04390	Hippo signalling pathway	0.0451	3 (153)	1.96
CLUSTER 2				
hsa04330	Notch signalling pathway	1.44x10 ⁻⁶	6 (52)	11.54
hsa04350	TGF- β signalling pathway	1.44x10 ⁻⁶	7 (91)	7.69
hsa04110	Cell cycle	5.51x10 ⁻⁶	7 (120)	5.83
hsa04919	Thyroid hormone signalling pathway	5.51x10 ⁻⁶	7 (119)	5.88
hsa04218	Cellular senescence	2.2x10 ⁻⁴	6 (150)	4.00
hsa04310	Wnt signalling pathway	2.4x10 ⁻⁴	6 (154)	3.90
hsa01522	Endocrine resistance	3.2x10 ⁻⁴	5 (95)	5.26
hsa04659	Th17 cell differentiation	3.7x10 ⁻⁴	5 (101)	4.95
hsa04520	Adherens junction	0.0012	4 (67)	5.97
hsa04550	Signalling pathways regulating pluripotency of stem cells	0.0013	5 (140)	3.57
hsa04068	FoxO signalling pathway	0.0095	4 (127)	3.15
hsa05418	Fluid shear stress and atherosclerosis	0.0099	4 (130)	3.08
hsa04917	Prolactin signalling pathway	0.0159	3 (69)	4.35
hsa04658	Th1 and Th2 cell differentiation	0.0273	3 (87)	3.45
hsa04933	AGE-RAGE signalling pathway in diabetic complications	0.0355	3 (98)	3.06
hsa04024	cAMP signalling pathway	0.0374	4 (208)	1.92
hsa04066	HIF-1 signalling pathway	0.0415	3 (106)	2.83
CLUSTER 3				
hsa04120	Ubiquitin mediated proteolysis	2.34x10 ⁻¹²	11 (135)	8.15
hsa04310	Wnt signalling pathway	6.6x10 ⁻⁶	7 (154)	4.55
hsa04390	Hippo signalling pathway	6.18x10 ⁻⁵	6 (153)	3.92
hsa04340	Hedgehog signalling pathway	0.0057	3 (47)	6.38
hsa04550	Signalling pathways regulating pluripotency of stem cells	0.007	4 (140)	2.86
CLUSTER 4				
hsa03013	RNA transport	8.56x10 ⁻⁵	6 (160)	3.75
hsa03040	Spliceosome	0.0115	4 (132)	3.03
CLUSTER 5				
hsa04742	Taste transduction	1.38x10 ⁻⁵	4 (81)	4.94

Supplemental Table S5: Pathways identified by enrichment analysis of miRNAs included in Figure 5

	DESCRIPTION - KEGG	FDR value	Genes (background genes)	Fold enrichment
CLUSTER 1				
hsa04917	Prolactin signalling pathway	1.02x10 ⁻¹³	9 (69)	13.04
hsa01522	Endocrine resistance	4.11x10 ⁻¹¹	8 (95)	8.42
hsa01521	EGFR tyrosine kinase inhibitor resistance	5.55x10 ⁻¹⁰	7 (78)	8.97
hsa04151	PI3K-Akt signalling pathway	5.55x10 ⁻¹⁰	10 (350)	2.86
hsa04012	ErbB signalling pathway	6.43x10 ⁻¹⁰	7 (83)	8.43
hsa04630	JAK-STAT signalling pathway	8.82x10 ⁻¹⁰	8 (160)	5.00
hsa04933	AGE-RAGE signalling pathway in diabetic complications	1.55x10 ⁻⁹	7 (98)	7.14
hsa04062	Chemokine signalling pathway	2.29x10 ⁻⁹	8 (186)	4.30
hsa04935	Growth hormone synthesis, secretion and action	4.45x10 ⁻⁹	7 (118)	5.93
hsa04068	FoxO signalling pathway	6.54x10 ⁻⁹	7 (127)	5.51
hsa04926	Relaxin signalling pathway	6.54x10 ⁻⁹	7 (128)	5.47
hsa04915	Oestrogen signalling pathway	7.42x10 ⁻⁹	7 (133)	5.26
hsa04659	Th17 cell differentiation	6.3x10 ⁻⁸	6 (101)	5.94
hsa04625	C-type lectin receptor signalling pathway	6.35x10 ⁻⁸	6 (102)	5.88
hsa05418	Fluid shear stress and atherosclerosis	1.98x10 ⁻⁷	6 (130)	4.62
hsa04210	Apoptosis	2.08x10 ⁻⁷	6 (132)	4.55
hsa04550	Signalling pathways regulating pluripotency of stem cells	2.82x10 ⁻⁷	6 (140)	4.29
hsa04912	GnRH signalling pathway	9.96x10 ⁻⁷	5 (89)	5.62
hsa04510	Focal adhesion	1.42x10 ⁻⁶	6 (198)	3.03
hsa05142	Chagas disease	1.46x10 ⁻⁶	5 (99)	5.05
hsa04015	Rap1 signalling pathway	1.52x10 ⁻⁶	6 (202)	2.97
hsa04660	T cell receptor signalling pathway	1.53x10 ⁻⁶	5 (101)	4.95
hsa04066	HIF-1 signalling pathway	1.89x10 ⁻⁶	5 (106)	4.72
hsa04722	Neurotrophin signalling pathway	2.62x10 ⁻⁶	5 (114)	4.39
hsa04014	Ras signalling pathway	2.65x10 ⁻⁶	6 (226)	2.65
hsa04071	Sphingolipid signalling pathway	2.73x10 ⁻⁶	5 (116)	4.31
hsa04910	Insulin signalling pathway	4.94x10 ⁻⁶	5 (133)	3.76
hsa04370	VEGF signalling pathway	6.37x10 ⁻⁶	4 (57)	7.02
hsa04921	Oxytocin signalling pathway	7.9x10 ⁻⁶	5 (149)	3.36
hsa04929	GnRH secretion	8.67x10 ⁻⁶	4 (63)	6.35
hsa04664	Fc epsilon RI signalling pathway	9.84x10 ⁻⁶	4 (66)	6.06
hsa04920	Adipocytokine signalling pathway	1.11x10 ⁻⁵	4 (69)	5.80
hsa04662	B cell receptor signalling pathway	1.72x10 ⁻⁵	4 (78)	5.13
hsa04211	Longevity regulating pathway	2.56x10 ⁻⁵	4 (87)	4.60
hsa04657	IL-17 signalling pathway	3.13x10 ⁻⁵	4 (92)	4.35
hsa04750	Inflammatory mediator regulation of TRP channels	3.35x10 ⁻⁵	4 (94)	4.26
hsa04620	Toll-like receptor signalling pathway	4.28x10 ⁻⁵	4 (101)	3.96
hsa04931	Insulin resistance	5.18x10 ⁻⁵	4 (107)	3.74
hsa04668	TNF signalling pathway	6.09x10 ⁻⁵	4 (112)	3.57
hsa04919	Thyroid hormone signalling pathway	7.57x10 ⁻⁵	4 (119)	3.36
hsa04611	Platelet activation	8.22x10 ⁻⁵	4 (122)	3.28
hsa04371	Apelin signalling pathway	1.0x10 ⁻⁴	4 (131)	3.05
hsa04010	MAPK signalling pathway	1.2x10 ⁻⁴	5 (288)	1.74
hsa04930	Type II diabetes mellitus	1.3x10 ⁻⁴	3 (46)	6.52
hsa04150	mTOR signalling pathway	1.6x10 ⁻⁴	4 (151)	2.65
hsa04217	Necroptosis	1.6x10 ⁻⁴	4 (149)	2.68
hsa04218	Cellular senescence	1.6x10 ⁻⁴	4 (150)	2.67
hsa01524	Platinum drug resistance	3.9x10 ⁻⁴	3 (70)	4.29
hsa04024	cAMP signalling pathway	5.0x10 ⁻⁴	4 (208)	1.92
hsa04540	Gap junction	7.0x10 ⁻⁴	3 (87)	3.45
hsa04658	Th1 and Th2 cell differentiation	7.0x10 ⁻⁴	3 (87)	3.45
hsa04152	AMPK signalling pathway	0.0017	3 (120)	2.50
hsa04723	Retrograde endocannabinoid signalling	0.0028	3 (145)	2.07
hsa04072	Phospholipase D signalling pathway	0.0029	3 (147)	2.04
hsa04530	Tight junction	0.0034	3 (156)	1.92
hsa04621	NOD-like receptor signalling pathway	0.0044	3 (174)	1.72
hsa04973	Carbohydrate digestion and absorption	0.0047	2 (44)	4.55

DESCRIPTION - KEGG		FDR value	Genes (background genes)	Fold enrichment
hsa04923	Regulation of lipolysis in adipocytes	0.0068	2 (54)	3.70
hsa04810	Regulation of actin cytoskeleton	0.007	3 (209)	1.44
hsa04720	Long-term potentiation	0.0092	2 (64)	3.13
hsa04520	Adherens junction	0.0098	2 (67)	2.99
hsa04666	Fc gamma R-mediated phagocytosis	0.0165	2 (90)	2.22
hsa04922	Glucagon signalling pathway	0.0199	2 (101)	1.98
hsa04928	Parathyroid hormone synthesis, secretion and action	0.0205	2 (103)	1.94
hsa04650	Natural killer cell mediated cytotoxicity	0.0274	2 (121)	1.65
hsa04261	Adrenergic signalling in cardiomyocytes	0.0391	2 (147)	1.36
hsa04310	Wnt signalling pathway	0.042	2 (154)	1.30
hsa04022	cGMP-PKG signalling pathway	0.0458	2 (162)	1.23
hsa04141	Protein processing in endoplasmic reticulum	0.0471	2 (165)	1.21
CLUSTER 2				
hsa04218	Cellular senescence	1.85x10 ⁻⁵	5 (150)	3.33
hsa04350	TGF- β signalling pathway	4.21x10 ⁻⁵	4 (91)	4.40
hsa04144	Endocytosis	8.0x10 ⁻⁴	4 (241)	1.66
hsa04659	Th17 cell differentiation	0.0013	3 (101)	2.97
hsa04933	AGE-RAGE signalling pathway in diabetic complications	0.0013	3 (98)	3.06
hsa05142	Chagas disease	0.0013	3 (99)	3.03
hsa04110	Cell cycle	0.0019	3 (120)	2.50
hsa04371	Apelin signalling pathway	0.0023	3 (131)	2.29
hsa04550	Signalling pathways regulating pluripotency of stem cells	0.0026	3 (140)	2.14
hsa04390	Hippo signalling pathway	0.0032	3 (153)	1.96
hsa04010	MAPK signalling pathway	0.0168	3 (288)	1.04
hsa04520	Adherens junction	0.0168	2 (67)	2.99
hsa04722	Neurotrophin signalling pathway	0.0423	2 (114)	1.75
hsa04926	Relaxin signalling pathway	0.0481	2 (128)	1.56

Supplemental Table S6: Expression values of analysed miRNAs according to underlying aetiology of CHF

miRNA	Ischaemic CHF (n=14) median [IQR]	Non-ischaemic CHF (n=32) median [IQR]	p-value	Fold-change (log ₂)
<i>let-7a-5p</i>	0.0001 [0.00006–0.00019]	0.00012 [0.00001–0.00043]	0.821	0.847 (–0.239)
<i>miR-107</i>	0.00002 [0.000002–0.00007]	0.00002 [0.000004–0.00005]	0.808	0.804 (–0.315)
<i>miR-125a-5p</i>	0.00012 [0.00006–0.00037]	0.00032 [0.00015–0.00061]	0.084	0.354 (–1.497)
<i>miR-139-5p</i>	0.00005 [0.00004–0.00006]	0.00002 [0.00001–0.00004]	0.022	1.929 (0.948)
<i>miR-150-5p</i>	0.0026 [0.00098–0.00357]	0.00173 [0.00054–0.00407]	0.412	1.503 (0.588)
<i>miR-30b-5p</i>	0.00054 [0.00018–0.00121]	0.00053 [0.00035–0.00136]	0.473	1.016 (0.023)
<i>miR-335-5p</i>	0.00083 [0.00008–0.00156]	0.00064 [0.00023–0.00098]	0.741	1.289 (0.367)
<i>miR-342-3p</i>	0.00028 [0.00007–0.00059]	0.00046 [0.00022–0.0009]	0.169	0.611 (–0.710)

CHF: chronic heart failure; IQR: interquartile range
 Bold indicates statistical significance.

Supplemental Table S7: Expression values of analysed miRNAs according to CHF ejection fraction

miRNA	HFpEF (n=22) median [IQR]	HFrfEF (n=24) median [IQR]	p-value	Fold-change (log₂)
<i>let-7a-5p</i>	0.00014 [0.00002–0.0003]	0.00011 [0.00004–0.00024]	0.947	1.209 (0.274)
<i>miR-107</i>	0.00001 [0.000002–0.00004]	0.00003 [0.00001–0.00009]	0.185	0.492 (–1.023)
<i>miR-125a-5p</i>	0.00031 [0.00015–0.00048]	0.00028 [0.00011–0.00062]	0.952	1.086 (0.119)
<i>miR-139-5p</i>	0.00002 [0.000004–0.00005]	0.00004 [0.00001–0.00005]	0.264	0.624 (–0.681)
<i>miR-150-5p</i>	0.00151 [0.00068–0.00293]	0.00278 [0.00098–0.00407]	0.130	0.546 (–0.874)
<i>miR-30b-5p</i>	0.00036 [0.00021–0.00092]	0.00107 [0.00038–0.00156]	0.075	0.336 (–1.572)
<i>miR-335-5p</i>	0.00065 [0.00032–0.00099]	0.00059 [0.00006–0.00144]	0.726	1.102 (0.140)
<i>miR-342-3p</i>	0.0004 [0.00006–0.00087]	0.00047 [0.00013–0.0009]	0.601	0.849 (–0.236)

CHF: chronic heart failure; **HFpEF:** heart failure with preserved ejection fraction; **HFrfEF:** heart failure with reduced ejection fraction; **IQR:** interquartile range

Supplemental Table S8: Selected miRNAs

microRNA	miRbase ID	ThermoFisher ID	MiRbase sequence
<i>let-7a-5p</i>	MIMAT0000062	478575_mir	ugagguaguagguuguauaguu
<i>miR-16-5p</i>	MIMAT0000069	477860_mir	uagcagcacguaaaauuggcg
<i>miR-107</i>	MIMAT0000104	478254_mir	agcagcauuguacagggcuauc
<i>miR-125a-5p</i>	MIMAT0000443	477884_mir	ucccugagacccuuuaaccuguga
<i>miR-139-5p</i>	MIMAT0000250	478312_mir	ucuacagugcacguguccagug
<i>miR-150-5p</i>	MIMAT0000451	477918_mir	ucucccaaccuuguaccagug
<i>miR-223-3p</i>	MIMAT0000280	477983_mir	ugucaguuugucaaaauacccca
<i>miR-224-5p</i>	MIMAT0000281	477986_mir	ucaagucacuagugguuccguuuag
<i>miR-30b-5p</i>	MIMAT0000420	478007_mir	uguaaacauccuacacucagcu
<i>miR-335-5p</i>	MIMAT0000765	478324_mir	ucaagagcaauaacgaaaaaugu
<i>miR-342-3p</i>	MIMAT0000753	478044_mir	ucucacacagaaucgcacccgu
<i>cel-miR-39-3p*</i>	MIMAT0000010	478293_mir	ucacggguguaaaucagcuug

*miRNA used for normalisation