Supplemental Tables and Figures

Supplemental Tables

Supplemental Figures

Supplemental Table 1. Clinical characteristics of RA patients without and with high coronary artery calcium score

	Without high coronary calcium (n=99)	With high coronary calcium (n=62)	P value
Age, years	50 [42, 60]	58 [52, 67]	<0.001
Sex, female	75 (76)	36 (58)	0.03
Race, Caucasian	88 (89)	55 (89)	0.99
DAS28, units	3.46 [2.39, 4.40]	4.16 [2.92, 5.21]	0.01
RF, positive	62 (65)	48 (80)	0.07
hsCRP, mg/L	3 [1, 8]	6 [2, 16]	0.006
Disease duration, years	3 [2, 14]	12 [2, 20]	0.004
Larsen score, points	1 [0, 14]	2 [0, 18]	0.49
Coronary artery disease, #	3 (3)	14 (23)	<0.001
Hypertension, presence	42 (42)	41 (66)	0.006
Systolic BP, mmHg	130 [116, 144]	137 [123, 151]	0.02
Diastolic BP, mmHg	74 [68, 81]	76 [69, 87]	0.34
Waist-hip ratio, units	0.84 [0.79, 0.92]	0.93 [0.86, 0.99]	<0.001
BMI, kg/m^2	28.3 [23.9, 33.3]	28.3 [24.0, 31.5]	0.46

Total cholesterol, mg/dL	183 [155, 205]	188 [157, 216]	0.47
HDL cholesterol, mg/dL	43 [37, 55]	43 [36, 51]	0.43
LDL cholesterol, mg/dL	107 [88, 134]	114 [92, 136]	0.25
Triglycerides, mg/dL	108 [80, 154]	112 [81, 157]	0.75
DMII, presence	6 (6)	12 (19)	0.02
Anti-hypertensive, use	25 (25)	35 (56)	<0.001
Corticosteroid, use	55 (56)	32 (52)	0.74
Methotrexate, use	77 (78)	38 (61)	0.04
Anti-TNF, use	21 (21)	10 (16)	0.55

High coronary artery calcium was defined as ≥300 Agatston units or ≥75th percentile for age, sex and ethnicity. Data are presented as median [interquartile range] for continuous variables and number (percent) for categorical variables. Abbreviations: RA= rheumatoid arthritis, DAS28=disease activity score based on 28 joints, RF=rheumatoid factor, hsCRP=high sensitivity C-reactive protein, ESR=erythrocyte sedimentation rate, BP=blood pressure, BMI=body mass index, DMII= diabetes mellitus type 2, TNF=tumor necrosis factor alpha. Larsen score is available for 94 subjects (54 without high coronary artery calcium and 39 with high coronary artery calcium).

Supplemental Table 2. Logistic regression model characteristics for prediction of presence of coronary calcium in patients with RA

	Odds ratio (95% confidence interval)
Age	8.55 (2.72, 26.89)
Sex (male: female)	5.79 (2.02, 16.54)
DAS28-ESR score	1.78 (0.97, 3.26)
ACC/AHA 10-year risk score	1.45 (0.51, 4.14)
Diabetes	1.88 (0.38, 9.22)
let-7c-5p	1.06 (0.80, 1.40)
miR-30e-5p	1.32 (0.93, 1.87)
miR-4446	1.48 (0.92, 2.37)
miR-126-5p	1.10 (0.73, 1.64)
miR-125a-5p	1.57 (0.77, 3.20)
miR-3168	0.60 (0.35, 1.03)
miR-425-5p	0.74 (0.37, 1.48)
miR-30c-5p	1.12 (0.98, 1.29)

The odds ratio presented is per 1 interquartile range increase for continuous variables (age, DAS28-ESR, ACC/AHA 10-year risk score, and miRNAs) and male versus female for sex and presence versus absence of diabetes. Data from logistic regression model including all listed terms.

Supplemental Table 3. Logistic regression model characteristics for prediction of presence of high coronary calcium in patients with RA

	Odds ratio (95% confidence interval)
Age	2.36 (0.94, 5.90)
Sex	3.06 (1.30, 7.18)
DAS28-ESR	1.46(0.85, 2.50)
ACC/AHA 10-year risk score	1.13 (0.54, 2.37)
Diabetes	2.14 (0.60, 7.67)
let-7c-5p	1.16 (0.85, 1.58)
miR-125a-5p	1.63 (0.85, 3.12)
miR-126-3p	2.19 (0.81, 5.92)
miR-126-5p	0.87 (0.53, 1.44)
miR-30a-5p	0.72 (0.26, 2.02)
miR-30e-5p	0.85 (0.54, 1.35)
miR-3168	0.74 (0.49, 1.12)
miR-425-5p	0.54 (0.24, 1.20)
miR-4446	1.45 (0.94, 2.24)

The odds ratio presented is per 1 interquartile range increase for continuous variables (age, DAS28-ESR, ACC/AHA 10-year risk score, and miRNAs) and male versus female for sex and presence versus absence of diabetes. Data from logistic regression model including all listed terms. Supplemental Table 4. Plasma miRNA qPCR-based concentrations based on coronary calcium measurements

	Coronary artery calcium			High coronary artery calcium		
	Geometric mean (95% CI), fM		P	Geometric mean (95% CI), fM		P
	Not present	Present		Not present	Present	
let-7c-5p	188 (108, 327)	242 (177, 331)	0.47	198 (127, 310)	242 (163, 360)	0.80
miR-125a-5p	34 (28, 40)	41 (35, 47)	0.31	36 (31, 41)	40 (33, 48)	0.97
miR-126-3p	272 (180, 413)	338 (239, 479)	0.05	291 (207, 408)	327 (208, 512)	0.12
miR-126-5p	553 (325, 942)	694 (469, 1027)	0.17	604 (389, 936)	650 (398, 1059)	0.48
miR-30a-5p	113 (86, 149)	119 (92, 155)	0.55	115 (91, 146)	118 (86, 163)	0.79
miR-30c-5p	455 (387, 535)	420 (350, 502)	0.56	461 (392, 541)	401 (334, 480)	0.27
miR-30e-5p	84 (62, 113)	98 (78, 124)	0.20	88 (67, 114)	96 (76, 122)	0.26
miR-3168	103 (90, 118)	102 (88, 117)	0.97	107 (94, 121)	96 (82, 112)	0.27
miR-425-5p	15 (5, 32)	24 (12, 50)	0.44	15 (7, 32)	24 (10, 56)	0.61
miR-4446-3p	5 (3, 8)	7 (5, 10)	0.64	5 (3, 7)	7 (5, 11)	0.25

CI= confidence interval. fM=femtomolar concentration.

Supplemental Table 5. Univariate relationship between miRNA plasma concentration and cardiovascular risk factors (as dependent variables)

	let-7c-5p	miR-125a-5p	miR-126-3p	miR-126-5p	miR-30a-5p	miR-30c-5p	miR-30e-5p	miR-3168	miR-425-5p	miR-4446
Age, years	-0.06 (-0.56, 0.43)	1.05 (-0.54, 2.63)	-0.02 (-1.53, 1.48)	0.29 (-0.35, 0.93)	-0.83 (-2.58, 0.92)	0.18 (-0.24, 0.6)	0.23 (-0.64, 1.1)	0.5 (-0.77, 1.77)	-1.09 (-2.38, 0.2)	-0.59 (-2.04, 0.86)
Hypertension, presence	0.96 (0.85, 1.07)	1.15 (0.87, 1.53)	0.92 (0.71, 1.19)	0.69 (0.51, 0.94)	0.85 (0.63, 1.16)	1.01 (0.94, 1.09)	0.98 (0.84, 1.14)	1.1 (0.88, 1.37)	0.8 (0.63, 1.02)	1.13 (0.87, 1.46)
Total cholesterol, mg/dl	0.53 (-1.1, 2.16)	1.8 (-3.44, 7.03)	6.45 (1.54, 11.36)	0.46 (-1.66, 2.58)	4.83 (-0.93, 10.6)	0.87 (-0.54, 2.27)	1.36 (-1.5, 4.22)	-0.1 (-4.43, 4.23)	3.59 (-0.67, 7.84)	-1.68 (-6.46, 3.1)
HDL, mg/dl	0.32 (-0.25, 0.9)	-0.29 (-2.14, 1.55)	0.82 (-0.93, 2.57)	0.21 (-0.54, 0.95)	0.63 (-1.41, 2.67)	0.23 (-0.27, 0.72)	-0.26 (-1.27, 0.74)	-0.71 (-2.23, 0.81)	0.82 (-0.68, 2.33)	-0.32 (-2, 1.37)
LDL, mg/dl	-0.21 (-1.61, 1.2)	0.8 (-3.71, 5.32)	4.06 (-0.21, 8.32)	0.23 (-1.59, 2.06)	3.11 (-1.87, 8.09)	0.58 (-0.64, 1.8)	0.95 (-1.52, 3.42)	-0.38 (-4.11, 3.36)	1.23 (-2.46, 4.92)	0.32 (-3.8, 4.45)
Triglycerides, mg/dl	1.01 (0.99, 1.03)	1.06 (0.99, 1.14)	1.07 (1, 1.15)	1 (0.97, 1.03)	1.05 (0.97, 1.14)	1 (0.98, 1.02)	1.03 (0.99, 1.07)	1.04 (0.98, 1.11)	1.05 (0.99, 1.12)	0.95 (0.89, 1.02)
BMI, kg/m^2	0.23 (-0.05, 0.5)	0.07 (-0.81, 0.96)	-0.01 (-0.85, 0.83)	0 (-0.35, 0.36)	0.11 (-0.86, 1.09)	0.08 (-0.16, 0.31)	0.06 (-0.42, 0.55)	0.82 (0.12, 1.52)	-0.06 (-0.78, 0.66)	-0.17 (-0.98, 0.64)
Waist-hip ratio, units	0 (0, 0.01)	0.01 (0, 0.02)	0.01 (0, 0.03)	0 (-0.01, 0.01)	0.02 (0, 0.03)	0 (0, 0.01)	0.01 (0, 0.01)	0.02 (0.01, 0.03)	0 (-0.01, 0.01)	0.01 (0, 0.02)
Diabetes mellitus II, presence	0.97 (0.79, 1.2)	1.34 (0.92, 1.94)	1.2 (0.8, 1.8)	0.98 (0.77, 1.25)	1.17 (0.72, 1.89)	1.02 (0.92, 1.13)	0.98 (0.74, 1.31)	1.28 (0.96, 1.7)	0.67 (0.38, 1.2)	0.95 (0.6, 1.51)
Creatinine, mg/dl	0 (-0.01, 0.01)	0.05 (0.01, 0.08)	0.03 (0, 0.06)	0.01 (0, 0.02)	0.01 (-0.02, 0.05)	0 (-0.01, 0)	0.02 (0, 0.03)	0.02 (-0.01, 0.04)	0 (-0.03, 0.02)	0.01 (-0.02, 0.04)
hsCRP, mg/l	1.02 (0.94, 1.11)	0.98 (0.76, 1.27)	1.38 (1.08, 1.75)	1.06 (0.96, 1.18)	1.3 (0.98, 1.73)	1.01 (0.95, 1.09)	1.02 (0.88, 1.17)	0.9 (0.73, 1.1)	1.1 (0.89, 1.36)	0.93 (0.74, 1.17)

The data is presented as estimated effect size (95% confidence interval) per interquartile range increase in each of miRNA plasma concentration (based on qPCR) for continuous dependent variables or odds ratio (95% confidence interval) per interquartile range increase in the miRNA. Triglycerides and hsCRP were analyzed on a log scale and the effect size with 95% confidence intervals were back-transformed to the original scale. Abbreviations: HDL=high density lipoprotein, LDL=low density lipoprotein, BMI=body mass index, hsCRP=high sensitivity C-reactive protein.

Supplemental Table 6. Top canonical pathways containing upstream regulators of the miRNA panel

Canonical Pathway	# molecules	Members
HOTAIR Regulatory Pathway	13	AGO1, AGO2, AGO3, AGO4, ARGONAUTE, DNA-methyltransferase, Hdac, Histone h3, let-7, MET, NFKB1, TGFB1, YY1
EIF2 Signaling	12	AGO1, AGO2, AGO3, AGO4, ARGONAUTE, HRAS, RPL13, RPLP0, RPLP1, RPLP2, RPS15, Ubiquitin
Senescence Pathway	10	ETS1, ETS2, HRAS, JUN, NFKB1, Rb, SERPINE1, SMAD3, TGFB1, TP53
Glucocorticoid Receptor Signaling	8	Histone h3, HRAS, JUN, NFKB1, SERPINE1, SMAD3, SMARCA4, TGFB1
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	8	APC, F2RL1, HRAS, JUN, MYD88, NFKB1, TGFB1, TNFRSF1B

Supplemental Table 7. Top functions of upstream regulators of the atherosclerosis panel miRNAs

Top functions	P-value	# molecules
Expression of RNA	8.6E-28	58
Fibrosis	7.22 E-26	33
Transcription	4.07 E-23	52
Transcription of RNA	4.88 E-22	48
Cell proliferation of tumor cell lines	9.31 E-22	50
Apoptosis	3.48 E-21	62
Expression of protein	9.65 E-21	26
Morphology of cardiovascular system	2.46 E-20	33

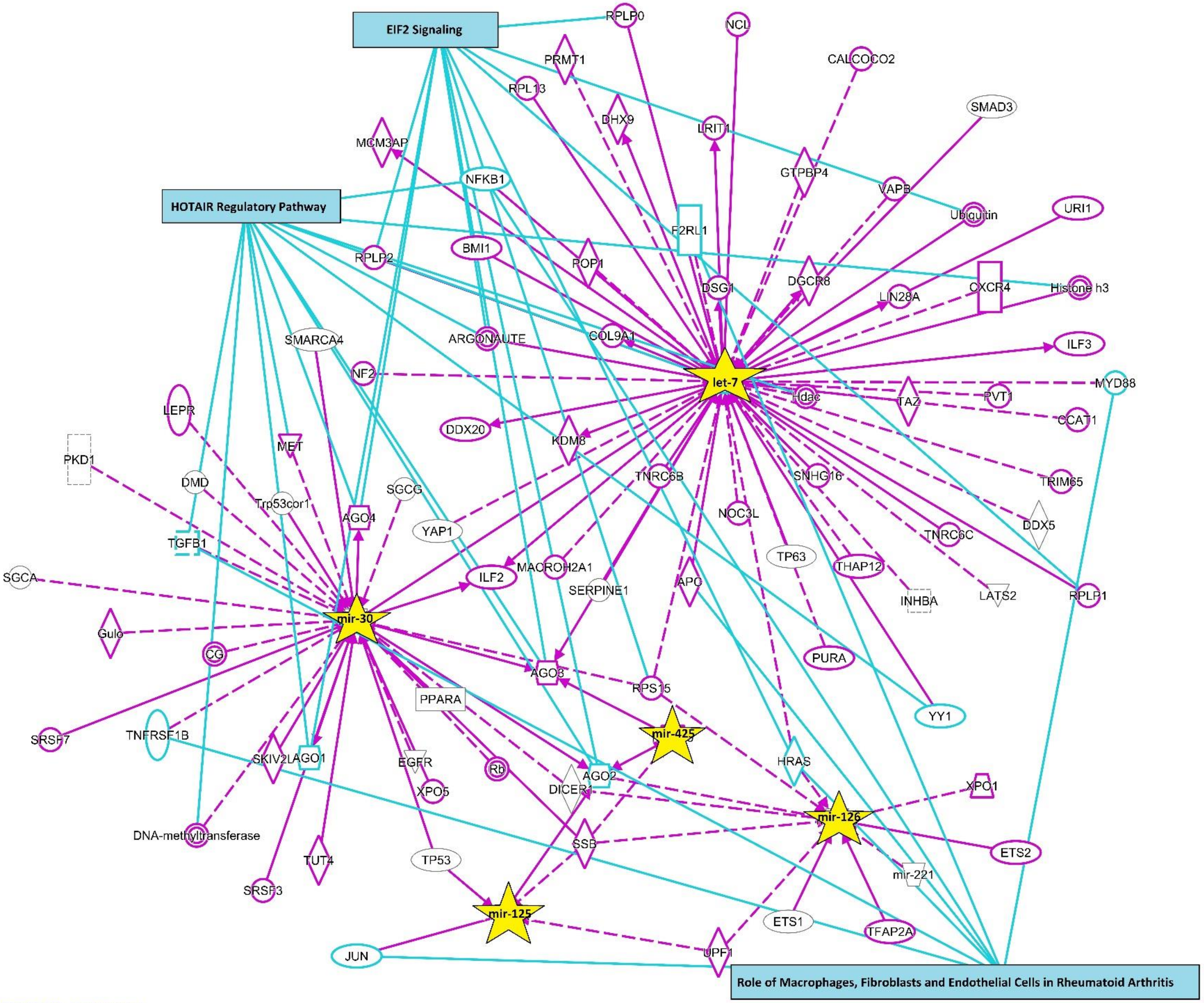
Supplemental Table 8. Top predicted canonical pathway targets of the atherosclerosis panel miRNAs

Canonical Pathway	# molecules	Members
Colorectal Cancer Metastasis Signaling	10	Akt, BCL2L1, BIRC5, CCND1, IL6, JUN, MMP7, PIK3R2, RAS, TNF
Molecular Mechanisms of Cancer	8	Akt, BCL2L1, CCND1, E2F3, IRS1, JUN, PIK3R2, RAS
Systemic Lupus Erythematosus In B Cell Signaling Pathway	8	Akt, BCL2L1, CCND1, IL6, JUN, PIK3R2, RAS, TNF
Glucocorticoid Receptor Signaling	7	Akt, BCL2L1, IL6, JUN, PIK3R2, RAS, TNF
T Cell Exhaustion Signaling Pathway	7	Akt, BCL6, IL6, JUN, PIK3R2, PRDM1, RAS

Supplemental Table 9. Top predicted function targets of atherosclerosis panel miRNAs

Top functions	P-value	# molecules
Invasion of cells	4.50 E-30	30
Invasion of tumor cell lines	2.98 E-27	27
Cell movement	2.46 E-22	32
Benign pelvic disease	4.01E-22	19
Migration of cells	4.26E-22	31
Fibrosis	1.97E-21	20
Atherosclerosis	6.56 E-9	11

Supplemental Figure 1. Upstream regulators of the atherosclerosis miRNA panel



Supplemental Figure 2. Panel miRNAs are predicted to regulate atherosclerosis