

SUPPLEMENTARY DATA

Supplementary Table S1. Patient characteristics of the miRNA profiling pilot study

	DM +/- early DN (N=9)	DN (N=7)	p-value
Sex, male, N (%)	6 (67%)	4 (57%)	0.719
Age (years)	54 ± 15	44 ± 5	0.107
BMI (kg/m ²)	24.5 ± 2.2	23.3 ± 3.3	0.392
Systolic blood pressure (mmHg)	133 ± 12	135 ± 13	0.754
Diastolic blood pressure)	71 ± 9	83 ± 9	0.019
Hemoglobin (mmol/L)	8.6 ± 1.5	7.5 ± 0.6	0.083
Hematocrit (L/L)	0.41 ± 0.05	0.36 ± 0.04	0.047
HbA1c (%)	6.9 ± 0.8	9.7 ± 3.2	0.026
Glucose (mmol/L)	13.7 ± 4.5	14 ± 6.5	0.928
eGFR (mL/min/1.73m ²)	70 ± 25	22 ± 4	0.001
Median proteinuria (g/24 hr) (IQR)	0.2	0.5	0.460
Duration diabetes (years)	40 ± 10	37 ± 10	0.600
Smoking, N (%)	2 (22%)	0 (0%)	0.179
Acetylsalicylic acid, N (%)	2 (22%)	1 (14%)	0.710
Antihypertensive drug			
Ace inhibitor	4 (44%)	6 (86%)	0.223
Angiotensin-II antagonist	2 (22%)	4 (57%)	0.174
β-blocker	0 (0%)	3 (43%)	0.029
Calcium antagonist	1 (11%)	2 (29%)	0.409
Diuretic	5 (56%)	3 (43%)	0.953
Statin, N (%)	4 (44%)	6 (86%)	0.103

*eGFR, estimated GFR; †IQR, interquartile range; ‡ACE, angiotensin converting enzyme. Parametric data are presented as mean ± SD. Non parametric data are presented as median and IQR. Differences between groups were analysed using the two-tailed unpaired Student's t-test. Nonparametric data are shown as median and interquartile range (IQR) and were analysed using a Kruskal–Wallis test. Categorical data are presented as frequency and percentage.

SUPPLEMENTARY DATA

Supplementary Table S2. HDL-miRNA expression profile of DM +/- early DN and DN patients in the pilot study.

	Mean DM +/- early DN (N=9)	Mean DN (N=7)	Fold change	p-value
HDL-miRNA-152	0,5313	1,6752	3,15	0,037
HDL-miRNA-145	2,3212	4,7325	2,04	0,043
HDL-miRNA-660	0,9412	0,1333	0,14	0,057
HDL-miRNA-126	83,2224	112,9948	1,36	0,122
HDL-miRNA-132	2,1652	4,029	1,86	0,172
HDL-miRNA-214	0,1403	0,977	6,96	0,201
HDL-miRNA-200a	0,0119	0,2604	21,88	0,276
HDL-miRNA-1	0,5187	0,0394	0,08	0,282
HDL-miRNA-221	4,5982	1,9647	0,43	0,31
HDL-miRNA-181a	0,294	0,6572	2,24	0,338
HDL-miRNA-125a_5p	0,548	0,3107	0,57	0,357
HDL-miRNA-223	907,0155	724,8598	0,80	0,368
HDL-miRNA-29a	0,8367	1,4713	1,76	0,468
HDL-miRNA-210	0,7413	0,2579	0,35	0,49
HDL-miRNA-106a	246,0424	188,2239	0,77	0,494
HDL-miRNA-423_5p	5,3455	3,7697	0,71	0,515
HDL-miRNA-133a	13,265	9,952	0,75	0,625
HDL-miRNA-574_3p	6,0457	4,1945	0,69	0,64
HDL-miRNA-25	2,3798	1,9654	0,83	0,642
HDL-miRNA-340	1,4672	1,8132	1,24	0,664
HDL-miRNA-331	1,4524	1,1511	0,79	0,715
HDL-miRNA-130b	0,2366	0,3288	1,39	0,721
HDL-miRNA-34a	1,9885	1,4956	0,75	0,739
HDL-miRNA-320	69,9223	64,951	0,93	0,775
HDL-miRNA-133b	0,777	0,8875	1,14	0,866
HDL-miRNA-103	0,5135	0,595	1,16	0,889
HDL-miRNA-27a	1,8756	1,9766	1,05	0,893
HDL-miRNA-21	16,521	17,1775	1,04	0,93
HDL-miRNA-17	243,8355	238,357	0,98	0,965
HDL-miRNA-150	87,4147	88,8398	1,02	0,968
HDL-miRNA-326		No signal		
HDL-miRNA-146b_3p		No signal		
HDL-miRNA-187		No signal		
HDL-miRNA-374-5p		No signal		
HDL-miRNA-450a		No signal		
HDL-miRNA-548a		No signal		
HDL-miRNA-208		No signal		
HDL-miRNA-520b		No signal		
HDL-miRNA-212		No signal		
HDL-miRNA-422b		No signal		
HDL-miRNA-15a		No signal		
HDL-miRNA-377		No signal		
HDL-miRNA-424		No signal		
HDL-miRNA-497		No signal		

SUPPLEMENTARY DATA

Supplementary Table S3. EV-miRNA expression profile of DM +/- early DN and DN patients in the pilot study

	Mean DM +/- early DN (N=9)	Mean DN (N=7)	Fold change	p-value
EV-miRNA-1	0,4612	1,017	2,21	0,095
EV-miRNA-25	17,6565	11,9001	0,67	0,115
EV-miRNA-29b	0,0563	0,0037	0,07	0,166
EV-miRNA-212	0,1101	0,3713	3,37	0,178
EV-miRNA-377	0,0015	0,0078	5,20	0,235
EV-miRNA-497	0,1105	0,0358	0,32	0,262
EV-miRNA-208	0,0787	0,0018	0,02	0,265
EV-miRNA-21	75,3744	56,5088	0,75	0,287
EV-miRNA-660	6,9649	4,8785	0,70	0,309
EV-miRNA-133a	7,9175	12,3056	1,55	0,314
EV-miRNA-574_3p	42,0115	34,4912	0,82	0,362
EV-miRNA-106a	1128,3727	943,4677	0,84	0,381
EV-miRNA-200a	0,3674	0,1927	0,52	0,401
EV-miRNA-424	0,0212	0,0018	0,08	0,401
EV-miRNA-326	0,08	0,1376	1,72	0,407
EV-miRNA-17	873,4237	768,4762	0,88	0,435
EV-miRNA-125a_5p	1,465	1,838	1,25	0,459
EV-miRNA-331	8,8678	7,3971	0,83	0,475
EV-miRNA-130b	5,8494	4,8609	0,83	0,506
EV-miRNA-221	54,1448	47,1898	0,87	0,518
EV-miRNA-214	0,8626	1,3869	1,61	0,527
EV-miRNA-150	248,4152	324,4381	1,31	0,553
EV-miRNA-423_5p	12,5929	10,6945	0,85	0,553
EV-miRNA-29a	6,434	7,4693	1,16	0,605
EV-miRNA-320	627,0214	547,0993	0,87	0,61
EV-miRNA-223	6015,3015	5412,7396	0,90	0,612
EV-miRNA-133b	0,4628	0,6273	1,36	0,621
EV-miRNA-15a	0,318	0,4287	1,35	0,696
EV-miRNA-181a	2,0609	1,8379	0,89	0,7
EV-miRNA-146b_3p	0,0033	0,0025	0,76	0,72
EV-miRNA-34a	0,6763	0,8032	1,19	0,79
EV-miRNA-103	2,3796	2,0111	0,85	0,799
EV-miRNA-27a	21,5305	20,4835	0,95	0,814
EV-miRNA-145	13,8272	12,5755	0,91	0,843
EV-miRNA-126	637,2329	653,25	1,03	0,857
EV-miRNA-152	2,7648	2,9221	1,06	0,87
EV-miRNA-340	15,3121	15,8289	1,03	0,88
EV-miRNA-132	8,6091	8,4494	0,98	0,93
EV-miRNA-210	1,3029	1,3144	1,01	0,98
EV-miRNA-187		No signal		
EV-miRNA-374-5p		No signal		
EV-miRNA-450a		No signal		
EV-miRNA-548a		No signal		
EV-miRNA-520b		No signal		
EV-miRNA-422b		No signal		

SUPPLEMENTARY DATA

Supplementary Table 4. Comparison of plasma EV-miRs from DM +/- early DN (n=14) and DN (n=19) patients compared to healthy controls (12).

Unique id	Parametric p-value	Average Control Group	Average DM +/- early DN Group	Fold-change
hsa-miR-21	0.0361	1 (0.42)	1.74 (0.86)	1.74
hsa-miR-212	0.1991	1 (1.95)	28.2 (44.5)	28.2
hsa-miR-126	0.2910	1 (0.24)	1.69 (1.28)	1.69
hsa-miR-132	0.3310	1 (0.52)	1.62 (1.95)	1.62
hsa-miR-660	0.6783	1 (0.81)	0.67 (0.43)	0.67
hsa-miR-29a	0.7179	1 (0.64)	1.37 (1.34)	1.37
hsa-miR-1	0.9106	1 (1.11)	1.31 (2.09)	1.31
hsa-miR-145	0.9624	1 (1.07)	1.10 (1.09)	1.10
hsa-miR-223	0.9698	1 (0.72)	0.95 (0.49)	0.95
hsa-miR-152	0.9847	1 (0.73)	1.09 (0.89)	1.09
hsa-miR-574	0.9970	1 (0.67)	1.02 (0.87)	1.02

Unique id	Parametric p-value	Average Control Group	Average DN Group	Fold-change
hsa-miR-21	0.0343	1 (0.42)	1.70 (0.79)	1.70
hsa-miR-126	0.0508	1 (0.24)	2.04 (1.46)	2.04
hsa-miR-29a	0.2400	1 (0.64)	1.73 (1.55)	1.73
hsa-miR-574	0.3483	1 (0.67)	0.68 (0.44)	0.68
hsa-miR-212	0.4340	1 (1.95)	18.5 (50.3)	18.5
hsa-miR-152	0.4456	1 (0.73)	1.50 (1.53)	1.50
hsa-miR-1	0.5409	1 (1.11)	1.73 (2.33)	1.73
hsa-miR-223	0.7760	1 (0.72)	0.88 (0.37)	0.88
hsa-miR-660	0.9153	1 (0.81)	1.15 (1.39)	1.15
hsa-miR-145	0.9921	1 (1.07)	1.04 (0.84)	1.04
hsa-miR-132	0.9974	1 (0.52)	1.03 (0.63)	1.03

Unique id	Parametric p-value	Average DM +/- early DN Group	Average DN Group	Fold-change
hsa-miR-574	0.2953	1.02 (0.87)	0.68 (0.44)	0.67
hsa-miR-132	0.2982	1.62 (1.95)	1.03 (0.63)	0.64
hsa-miR-660	0.3680	0.67 (0.43)	1.15 (1.39)	1.72
hsa-miR-152	0.5411	1.09 (0.89)	1.50 (1.53)	1.38
hsa-miR-126	0.6735	1.69 (1.28)	2.04 (1.46)	1.21
hsa-miR-29a	0.6951	1.37 (1.34)	1.73 (1.55)	1.26
hsa-miR-212	0.7803	28.2 (44.5)	18.5 (50.3)	0.66
hsa-miR-1	0.8142	1.31 (2.09)	1.73 (2.33)	1.32
hsa-miR-223	0.9012	0.95 (0.49)	0.88 (0.37)	0.93
hsa-miR-145	0.9846	1.10 (1.09)	1.04 (0.84)	0.95
hsa-miR-21	0.9887	1.74 (0.86)	1.70 (0.79)	0.98

EV-miRs are represented as mean \pm SEM.

SUPPLEMENTARY DATA

Supplementary Table 5. Comparison of plasma HDL-miRs from DM +/- early DN (n=14) and DN (n=19) patients compared to healthy controls (12).

Unique id	Parametric p-value	Average Control group	Average DM +/- early DN group	Fold-change
hsa-miR-132	0.1284	1 (0.80)	0.56 (0.49)	0.56
hsa-miR-660	0.5317	1 (2.10)	0.54 (0.34)	0.54
hsa-miR-574	0.6691	1 (1.48)	0.69 (0.88)	0.69
hsa-miR-145	0.7129	1 (0.77)	1.21 (0.68)	1.21
hsa-miR-126	0.8165	1 (0.39)	0.89 (0.49)	0.89
hsa-miR-212	0.8532	1 (2.37)	0.66 (0.87)	0.66
hsa-miR-29a	0.8949	1 (1.08)	0.84 (0.56)	0.84
hsa-miR-21	0.9203	1 (0.75)	1.17 (0.64)	1.17
hsa-miR-223	0.9630	1 (0.63)	1.06 (0.71)	1.06
hsa-miR-152	0.9938	1 (0.90)	0.96 (0.68)	0.96
hsa-miR-1	0.9976	1 (2.51)	1.04 (1.20)	1.04

Unique id	Parametric p-value	Average Control group	Average DN group	Fold-change
hsa-miR-132	0.0412	1 (0.89)	0.47 (0.46)	0.47
hsa-miR-152	0.3046	1 (0.90)	1.59 (1.40)	1.59
hsa-miR-126	0.5109	1 (0.39)	1.20 (0.54)	1.20
hsa-miR-21	0.5251	1 (0.75)	1.45 (1.53)	1.45
hsa-miR-660	0.5493	1 (2.10)	0.57 (0.61)	0.57
hsa-miR-574	0.7026	1 (1.48)	0.72 (0.50)	0.72
hsa-miR-29a	0.7684	1 (1.08)	1.24 (1.12)	1.24
hsa-miR-1	0.8603	1 (2.51)	0.70 (0.99)	0.70
hsa-miR-212	0.8839	1 (2.37)	0.70 (1.67)	0.70
hsa-miR-223	0.9745	1 (0.63)	1.05 (0.58)	1.05
hsa-miR-145	0.9999	1 (0.77)	1.00 (0.70)	1.00

Unique id	Parametric p-value	Average DM +/- early DN group	Average DN group	Fold-change
hsa-miR-126	0.1492	0.89 (0.49)	1.20 (0.54)	1.35
hsa-miR-152	0.2002	0.96 (0.68)	1.59 (1.40)	1.66
hsa-miR-29a	0.4210	0.84 (0.56)	1.24 (1.12)	1.48
hsa-miR-145	0.6398	1.21 (0.68)	1.00 (0.70)	0.83
hsa-miR-21	0.7386	1.17 (0.64)	1.45 (1.53)	1.24
hsa-miR-1	0.7965	1.04 (1.20)	0.70 (0.99)	0.67
hsa-miR-132	0.8751	0.56 (0.49)	0.47 (0.46)	0.84
hsa-miR-574	0.9943	0.69 (0.88)	0.72 (0.50)	1.04
hsa-miR-660	0.9960	0.54 (0.34)	0.57 (0.61)	1.06
hsa-miR-212	0.9961	0.66 (0.87)	0.70 (1.67)	1.06
hsa-miR-223	0.9979	1.06 (0.71)	1.05 (0.58)	0.99

HDL-miRs are represented as mean ± SEM.

SUPPLEMENTARY DATA

Supplementary Table 6. Comparison of plasma Ago-2 miRs from DM +/- early DN (n=14) and DN (n=19) patients compared to healthy controls (12).

Unique id	Parametric p-value	Average Control Group	Average DM +/- early DN Group	Fold-change
hsa-miR-145	0.6833	1 (0.72)	2.35 (1.56)	2.35
hsa-miR-126	0.7655	1 (0.32)	1.40 (0.74)	1.40
hsa-miR-660	0.7704	1 (0.35)	1.68 (0.90)	1.68
hsa-miR-574	0.8231	1 (0.55)	4.41 (12.01)	4.41
hsa-miR-223	0.8476	1 (0.55)	0.85 (0.38)	0.85
hsa-miR-132	0.8987	1 (0.48)	1.27 (0.75)	1.27
hsa-mir-21	0.9344	1 (1)	5.60 (13.08)	5.60
hsa-miR-152	0.9719	1 (1.22)	4.11 (7.36)	4.11
hsa-miR-1	0.9987	1 (1.40)	1.23 (1.81)	1.23
hsa-miR-29a	0.9991	1 (0.47)	1.02 (0.71)	1.02
hsa-miR-212	0.9994	1 (1.18)	1.70 (3.93)	1.70

Unique id	Parametric p-value	Average Control Group	Average DN Group	Fold-change
hsa-miR-660	0.0003	1 (0.35)	4.95 (3.57)	4.95
hsa-miR-145	0.0033	1 (0.72)	6.25 (5.8)	6.25
hsa-miR-126	0.0054	1 (0.32)	2.75 (1.99)	2.75
hsa-miR-152	0.0792	1 (1.22)	29.07 (49.28)	29.07
hsa-miR-1	0.1190	1 (1.18)	9.55 (16.23)	9.55
hsa-mir-21	0.1650	1 (1)	23.28 (45.85)	23.28
hsa-miR-29a	0.1705	1 (0.47)	1.93 (1.88)	1.93
hsa-miR-212	0.1749	1 (1.18)	36.83 (76.85)	36.83
hsa-miR-132	0.2962	1 (0.48)	1.87 (2.16)	1.87
hsa-miR-574	0.3691	1 (0.55)	8.14 (18.13)	8.14
hsa-miR-223	0.9875	1 (0.55)	1.04 (0.84)	1.04

Unique id	Parametric p-value	Average DM +/- early DN Group	Average DN Group	Fold-change
hsa-miR-660	0.0012	1.68 (0.90)	4.95 (3.57)	2.95
hsa-miR-145	0.0213	2.35 (1.56)	6.25 (5.8)	2.66
hsa-miR-126	0.0231	1.40 (0.74)	2.75 (1.99)	1.96
hsa-miR-152	0.1063	4.11 (7.36)	29.07 (49.28)	7.07
hsa-miR-1	0.1189	1.23 (1.81)	9.55 (16.23)	7.76
hsa-miR-29a	0.1428	1.02 (0.71)	1.93 (1.88)	1.89
hsa-miR-212	0.1565	1.70 (3.93)	36.83 (76.85)	21.66
hsa-mir-21	0.2786	5.60 (13.08)	23.28 (45.85)	4.16
hsa-miR-132	0.5086	1.27 (0.75)	1.87 (2.16)	1.47
hsa-miR-223	0.7067	0.85 (0.38)	1.04 (0.84)	1.22
hsa-miR-574	0.7344	4.41 (12.01)	8.14 (18.13)	1.85

Ago-2 miRs are represented as mean ± SEM.

SUPPLEMENTARY DATA

Supplementary Table 7. Comparison of miR levels in plasma HDL from DM +/- early DN (n=14) and DN (n=19) patients compared to healthy controls (12).

Unique id	Parametric p-value	Average Control Group	Average DM +/- early DN Group	Fold-change
hsa-miR-126	0.0001	1 (0.27)	2.78 (0.88)	2.78
hsa-miR-132	0.0001	1 (0.41)	1.78 (0.44)	1.78
hsa-miR-660	0.0003	1 (0.15)	2.72 (0.55)	2.72
hsa-miR-223	0.0012	1 (0.48)	1.87 (0.93)	1.87
hsa-miR-152	0.0259	1 (0.36)	2.35 (0.85)	2.35
hsa-mir-21	0.1781	1 (0.73)	1.77 (1.24)	1.77
hsa-miR-1	0.6690	1 (0.87)	4.29 (7.38)	4.29
hsa-miR-574	0.8108	1 (0.47)	1.08 (0.34)	1.08
hsa-miR-212	0.8535	1 (0.80)	2.16 (4.14)	2.16
hsa-miR-145	0.8990	1 (0.72)	0.87 (0.58)	0.87
hsa-miR-29a	0.9991	1 (0.47)	1.02 (0.71)	1.02

Unique id	Parametric p-value	Average Control Group	Average DN Group	Fold-change
hsa-miR-660	0.0001	1 (0.15)	3.60 (1.48)	3.60
hsa-miR-152	0.0001	1 (0.36)	3.19 (1.75)	3.19
hsa-miR-574	0.0016	1 (0.47)	0.53 (0.24)	0.53
hsa-miR-223	0.0164	1 (0.48)	0.39 (0.15)	0.39
hsa-miR-132	0.0233	1 (0.41)	1.43 (0.42)	1.43
hsa-miR-126	0.0801	1 (0.27)	1.52 (0.59)	1.52
hsa-mir-21	0.1433	1 (0.73)	1.77 (1.06)	1.77
hsa-miR-29a	0.1705	1 (0.47)	1.93 (1.88)	1.93
hsa-miR-212	0.3817	1 (0.80)	3.71 (6.85)	3.71
hsa-miR-1	0.9424	1 (0.87)	2.26 (4.82)	2.26
hsa-miR-145	0.9510	1 (0.72)	1.09 (0.88)	6.25

Unique id	Parametric p-value	Average DM +/- early DN Group	Average DN Group	Fold-change
hsa-miR-126	0.0001	2.78 (0.88)	1.52 (0.59)	0.55
hsa-miR-223	0.0001	1.87 (0.93)	0.39 (0.15)	0.21
hsa-miR-574	0.0001	1.08 (0.34)	0.53 (0.24)	0.49
hsa-miR-660	0.0484	2.72 (0.55)	3.60 (1.48)	1.32
hsa-miR-132	0.0617	1.78 (0.44)	1.43 (0.42)	0.80
hsa-miR-29a	0.1428	1.02 (0.71)	1.93 (1.88)	1.89
hsa-miR-152	0.1517	2.35 (0.85)	3.19 (1.75)	1.36
hsa-miR-1	0.6473	4.29 (7.38)	2.26 (4.82)	0.53
hsa-miR-212	0.6738	2.16 (4.14)	3.71 (6.85)	1.72
hsa-miR-145	0.6917	0.87 (0.58)	1.09 (0.88)	1.25
hsa-mir-21	0.9999	1.77 (1.24)	1.77 (1.06)	1.0

Relative expression of plasma miRs is represented as mean ± SEM

SUPPLEMENTARY DATA

Supplementary Figure 1. Correlations of carrier miRs with microvascular injury markers. (A-B) Correlation of EV-miR-21 and EV-miR-126 with MTI. (C) Correlation of HDL-miR-132 with Ang2. (D-E) Correlation of Ago-miR-660 with MTI and Ang2. Microvascular injury markers were adjusted for age, kidney function and diabetes parameters with multivariate analysis by partial correlation. (Multiple testing through FDR with Benjamini Hochberg correction can be found in Table 2.) MTI= mean tortuosity index; Ang2= Angiotensin-2; R= Spearman Correlation coefficient.

