ESM Methods

Blood collection procedures. Serum was obtained using the standardized operation procedure (SOP) adopted by TrialNet in all of its centres. In brief, blood is drawn with a 21-gauge winged-infusion set, by a licensed phlebotomist. Blood is collected in a pre-labelled a 2.5 mL serum separator tube, which is inverted 8-10 times, then allowed to clot for 30 minutes at room temperature. The serum is isolated, within the hour, by centrifugation at 1,000-1,300 g for 10 minutes in a swinging bucket rotor. The liquid phase is collected and aliquoted into pre-labelled 1.8 mL cryovials and stored at -20^{o}/-70^{o} C until distribution.

Control miRNAs and assessment of haemolysis. Preliminary studies of the local cohort utilized Exiqon V2.0 miRNA Ready-to-use PCR Human Panel II V2.M/R - miRBase v.13; these include two plates with an inter-plate calibrator (UniSP6), cellular positive controls (SNORD38B, SNORD49A, U6), an extraction control (UniSP3), and negative control wells. In our custom 93 miRNA panel, we preserved UniSp6 and UniSp3 but excluded cellular positive controls since we studied acellular samples. We assessed haemolysis comparing miR-451a (highly expressed in red blood cells) to miR-23a-3p levels (unaffected by haemolysis) [1]. Delta C_t (miR-23a-3p - miR-451a) values >5 indicate possible erythrocyte miRNA contamination, and values >7–8 indicate high risk of haemolysis. None of the samples studied (190 local cohort and 300 TrialNet samples) had score values greater than 7. The delta C_t scores for all samples studied (control and TrialNet) did not significantly differ (mean ± SD: 5.32 ± 1.47 and 4.48 ± 1.51).

ESM Results

Sample age does not influence serum miRNA detection but only a small proportion of miRNAs are detected in serum in most individuals.

For initial assay validation, we studied serum samples from 40 healthy subjects (20 females and 20 males, mean age 24 ± SD 4.7 years, range 17-32) who had no family history of T1D and tested negative for T1D-associated autoantibodies (not shown). All provided written informed consent under protocol 1995-119, which was approved by the Institutional Review Board of the University of Miami. These samples were used to investigate whether age of the serum samples impacted detection of circulating miRNAs. We used the standard Exiqon panel consisting of primers for 745 miRNAs for the analysis of the 40 control samples.

Importantly, only 36% (mean 274.8 ± SD 61.5) of the 745 miRNAs were detected in serum at the more conservative $\leq$35 C_t threshold. There was individual variability and only 20% (150/745) and 8% (60/745) of the miRNAs were detected in more than 80% of the samples using the 38 and 35 C_t cut-offs, respectively. Therefore, we designed a custom panel that allowed measuring levels of 93 miRNAs (**ESM Table 1**) inclusive of (and/or):

(a) those most commonly expressed in serum;

(b) those with preliminary suggestions of association with T1D and/or islet autoimmunity from the literature and exploratory analyses of T1D patients/relatives from our institution (data not shown): besides the control subjects, this local cohort included 50 patients with T1D, 50 siblings with autoantibodies and 50 siblings without autoantibodies (the data set included 21 family trios composed of a patient and two siblings, one with and the other without autoantibodies); the T1D patients had variable disease duration at the time of the study, ranging from 0.1 to 33 years, yet most (47/50) were still positive for at least 1 autoantibody in the same sample tested for miRNAs; 36 of the T1D patients had $\geq$ 2 autoantibodies. Among the 50

autoantibody-positive relatives, 15 had a single autoantibody and 35 had multiple autoantibodies. We used the data from this pilot study to seek preliminary evidence of associations, estimate variance to appropriately power the study of the TrialNet cohort, and to identify the miRNAs that were more commonly detected in serum across this collection of 190 samples. Thus, selection of miRNAs in the custom panel is representative of miRNAs commonly detected in healthy subjects but also preliminarily found associated with T1D and/or autoantibodies in patients and their siblings. This entire cohort, as shown below in **ESM Table 2**, also included very young children and thus comprises the age range represented in the TrialNet cohort. Of note, no subjects in the local cohorts overlapped with the TrialNet cohort.

(c) those with literature associations with other autoimmune diseases, insulin resistance, hyperglycaemia and beta cell function. Six miRNAs were selected as internal controls.

For both control and TrialNet samples there was no correlation of the global mean of C_t values and of the number of miRNAs detected with the RNA concentration (data not shown). Among the TrialNet subjects, an individual on average expressed 62% of the 93 miRNAs tested with the custom panel (mean n= 58 $\pm$ SD 5) at the <38 C_t threshold. Both in the control (**ESM Fig. 1A-B**) and TrialNet (**ESM Fig. 1C-D**) data there was no correlation of the age of the samples with number of miRNAs detected and with the global mean of C_t values.

References

[1] Blondal T, Jensby Nielsen S, Baker A, et al. (2013) Assessing sample and miRNA profile quality in serum and plasma or other biofluids. Methods (San Diego, Calif) 59: S1-6

ESM TABLES

ESM Table 1. Custom panel of 93 miRNAs used for TrialNet samples.

microRNA Name	Primer	LNA™ PCR primer set, Product No
hsa-miR-103a-3p	AGCAGCAUUGUACAGGGCUAUGA	204063
hsa-miR-106a-5p	AAAAGUGCUUACAGUGCAGGUAG	204563
hsa-miR-124-3p	UAAGGCACGCGGUGAAUGCC	204319
hsa-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG	204227
hsa-miR-141-3p	UAACACUGUCUGGUAAAGAUGG	204504
hsa-miR-143-3p	UGAGAUGAAGCACUGUAGCUC	204190
hsa-miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU	204688
hsa-miR-150-5p	UCUCCCAACCCUUGUACCAGUG	204660
hsa-miR-155-5p	UUAAUGCUAAUCGUGAUAGGGGU	204308
hsa-miR-15a-5p	UAGCAGCACAUAAUGGUUUGUG	204066
hsa-miR-17-5p	CAAAGUGCUUACAGUGCAGGUAG	204771
hsa-miR-192-5p	CUGACCUAUGAAUUGACAGCC	204099
hsa-miR-200c-3p	UAAUACUGCCGGGUAAUGAUGGA	204482
hsa-miR-205-5p	UCCUUCAUUCCACCGGAGUCUG	204487
hsa-miR-20a-5p	UAAAGUGCUUAUAGUGCAGGUAG	204292
hsa-miR-21-3p	CAACACCAGUCGAUGGGCUGU	204302
hsa-miR-214-3p	ACAGCAGGCACAGACAGGCAGU	204510
hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	204230
hsa-miR-221-3p	AGCUACAUUGUCUGCUGGGUUUC	204532
hsa-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA	204256
hsa-miR-224-5p	CAAGUCACUAGUGGUUCCGUU	204641
hsa-miR-23a-3p	AUCACAUUGCCAGGGAUUUCC	204772
hsa-miR-23b-3p	AUCACAUUGCCAGGGAUUACC	204790
hsa-miR-28-3p	CACUAGAUUGUGAGCUCCUGGA	204119
hsa-miR-29b-3p	UAGCACCAUUUGAAAUCAGUGUU	204679
hsa-miR-29c-3p	UAGCACCAUUUGAAAUCGGUUA	204729
hsa-miR-30b-3p	CUGGGAGGUGGAUGUUUACUUC	205933
hsa-miR-30d-5p	UGUAAACAUCCCCGACUGGAAG	204757
hsa-miR-320a	AAAAGCUGGGUUGAGAGGGCGA	204154
hsa-miR-320b	AAAAGCUGGGUUGAGAGGGCAA	205921
hsa-miR-326	CCUCUGGGCCCUUCCUCCAG	204512

ESM Table 1. Custom panel of 93 miRNAs used for TrialNet samples, continued.

microRNA Name	Primer	LNA™ PCR primer set, Product No
hsa-miR-342-3p	UCUCACACAGAAAUCGCACCCGU	205625
hsa-miR-346	UGUCUGCCCGCAUGCCUGCCUCU	204538
hsa-miR-34a-5p	UGGCAGUGUCUUAGCUGGUUGU	204486
hsa-miR-412	ACUUCACCUGGUCCACUAGCCGU	204460
hsa-miR-423-5p	UGAGGGGCAGAGAGCGAGACUUU	205624
hsa-miR-485-3p	GUCAUACACGGCUCUCCUCUCU	205924
hsa-miR-491-5p	AGUGGGGAACCCUUCCAUGAGG	204695
hsa-miR-523-3p	GAACGCGCUUCCCUAUAGAGGGU	204452
hsa-miR-542-5p	UCGGGGAUCAUCAUGUCACGAGA	204198
hsa-miR-548a-3p	CAAAACUGGCAAUUACUUUUGC	205650
hsa-miR-577	UAGAUAAAAUAUUGGUACCUG	204666
hsa-miR-584-5p	UUAUGGUUUGCCUGGGACUGAG	204568
hsa-miR-593-3p	UGUCUCUGCUGGGGUUUCU	204083
hsa-miR-595	GAAGUGUGCCGUGGUGUGUCU	204070
hsa-miR-671-5p	AGGAAGCCCUGGAGGGGCUGGAG	205649
hsa-miR-760	CGGCUCUGGGUCUGUGGGGA	204549
hsa-miR-922	GCAGCAGAGAAUAGGACUACGUC	204478
hsa-miR-92a-3p	UAUUGCACUUGUCCCGGCCUGU	204258
hsa-miR-93-3p	ACUGCUGAGCUAGCACUUCCCG	204470
hsa-miR-93-5p	CAAAGUGCUGUUCGUGCAGGUAG	204715
hsa-miR-96-5p	UUUGGCACUAGCACAUUUUUGCU	204417
hsa-miR-148a-3p	UCAGUGCACUACAGAACUUUGU	205867
hsa-miR-152	UCAGUGCAUGACAGAACUUGG	204294
hsa-miR-181a-5p	AACAUUCAACGCUGUCGGUGAGU	204566
hsa-miR-200a-3p	UAACACUGUCUGGUAACGAUGU	204707
hsa-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG	204260
hsa-miR-25-3p	CAUUGCACUUGUCUCGGUCUGA	204361
hsa-miR-26a-5p	UUCAAGUAAUCCAGGAUAGGCU	205905
hsa-miR-27a-3p	UUCACAGUGGCUAAGUUCCGC	204764
hsa-miR-27b-3p	UUCACAGUGGCUAAGUUCUGC	205915
hsa-miR-29a-3p	UAGCACCAUCUGAAAUCGGUUA	204698

ESM Table 1. Custom panel of 93 miRNAs used for TrialNet samples, continued.

microRNA Name	Primer	LNA™ PCR primer set, Product No
hsa-let-7g-5p	UGAGGUAGUAGUUUGUACAGUU	204565
hsa-miR-1183	CACUGUAGGUGAUGGUGAGAGUGGGCA	204176
hsa-miR-122-5p	UGGAGUGUGACAAUGGUGUUUG	205664
hsa-miR-134	UGUGACUGGUUGACCAGAGGGG	205896
hsa-miR-141-5p	CAUCUUCCAGUACAGUGUUGGA	204500
hsa-miR-142-3p	UGUAGUGUUUCCUACUUUAUGGA	204291
hsa-miR-142-5p	CAUAAAGUAGAAAGCACUACU	204722
hsa-miR-16-5p	UAGCAGCACGUAAAUAUUGGCG	205702
hsa-miR-183-5p	UAUGGCACUGGUAGAAUUCACU	204652
hsa-miR-185-3p	AGGGGCUGGCUUUCCUCUGGUC	205710
hsa-miR-191-5p	CAACGGAAUCCCAAAAGCAGCUG	204306
hsa-miR-195-5p	UAGCAGCACAGAAAUAUUGGC	205869
hsa-miR-19a-3p	UGUGCAAAUCUAUGCAAAACUGA	205862
hsa-miR-202-3p	AGAGGUAUAGGGCAUGGGAA	204101
hsa-miR-210	CUGUGCGUGUGACAGCGGCUGA	204333
hsa-miR-222-3p	AGCUACAUCUGGCUACUGGGU	204551
hsa-miR-31-5p	AGGCAAGAUGCUGGCAUAGCU	204236
hsa-miR-375	UUUGUUCGUUCGGCUCGCGUGA	204362
hsa-miR-376a-3p	AUCAUAGAGGAAAAUCCACGU	204508
hsa-miR-424-5p	CAGCAGCAAUUCAUGUUUUGAA	204736
hsa-miR-451a	AAACCGUUACCAUUACUGAGUU	204734
hsa-miR-452-3p	CUCAUCUGCAAAGAAGUAAGUG	204201
hsa-miR-452-5p	AACUGUUUGCAGAGGAAACUGA	204301
hsa-miR-490-3p	CAACCUGGAGGACUCCAUGCUG	205875
hsa-miR-509-3p	UGAUUGGUACGUCUGUGGGUAG	204458
hsa-miR-524-5p	CUACAAAGGGAAGCACUUUCUC	204135
hsa-miR-619	GACCUGGACAUGUUUGUGCCCAGU	204120
hsa-miR-624-3p	CACAAGGUAUUGGUAUUACCU	205684
hsa-miR-675-5p	UGGUGCGGAGAGGGCCCACAGUG	205687
hsa-miR-934	UGUCUACUACUGGAGACACUGG	205694
hsa-miR-9-5p	UCUUUGGUUAUCUAGCUGUAUGA	204513

ESM Table 2. Age information for the cohorts examined.

Cohorts	**Mean ± SD, range, in years**
Local Control (n=40)	24.9 ± 4.7, 17-32
Local T1D (n=50)	18.7 ± 11.6, 4.8-49.6
Local AAb- (n=50)	16.4 ± 13.9, 0.05-48.1
Local AAb+ (n=50)	18.8 ± 14.9, 4.5-63.7
Entire Local Cohort (n=190)	19.4 ± 11.8, 0.05-63.7
TrialNet Cohort (n=300)	11.0 ± 3.7, 1.0-18.0

T1D, type 1 diabetes; AAb, autoantibody; –, negative; +, positive

ESM Table 3. OGTT status at the time of miRNA testing for 21 Progressors and 74 non-Progressors, autoantibody-positive subjects.

AGE (Years)	OGTT OUTCOME
Non-Progressors	
11	diabetic range
13	dysglycemia
16	dysglycemia
14	dysglycemia
6	dysglycemia
8	dysglycemia
9	dysglycemia
12	dysglycemia
16	dysglycemia
8	dysglycemia
15	dysglycemia
10	dysglycemia
10	normal
8	normal
14	normal
13	normal
15	normal
16	normal
3	normal
12	normal
5	normal
16	normal
12	normal
14	normal
12	normal
11	normal
15	normal
14	normal
7	normal
15	normal
13	normal
15	normal
10	normal
4	normal
4	normal

AGE (Years)	OGTT OUTCOME	TIME FROM OGTT to T1D (Months)
Progressors		
11	diabetic range	0.0
12	diabetic range	0.0
14	diabetic range	0.0
7	diabetic range	0.0
15	diabetic range	0.0
9	diabetic range	0.1
13	diabetic range	0.1
9	diabetic range	0.4
9	diabetic range	2.1
16	diabetic range	9.3
18	dysglycemia	1.8
15	dysglycemia	2.6
7	dysglycemia	5.1
14	dysglycemia	5.9
13	dysglycemia	9.2
14	dysglycemia	11.0
7	dysglycemia	16.7
12	dysglycemia	25.7
17	dysglycemia	35.7
7	normal	2.2
7	normal	20.2

ESM Table 3. OGTT status at the time of miRNA testing for 21 Progressors and 74 non-Progressors, autoantibody-positive subjects, continued.

AGE (Years)	OGTT OUTCOME
Non-Progressors	
16	normal
6	normal
8	normal
12	normal
18	normal
4	normal
13	normal
9	normal
9	normal
16	normal
17	normal
15	normal
10	normal
5	normal
9	normal
8	normal
14	normal
8	normal
9	normal
15	normal
6	normal
11	normal
11	normal
15	normal
9	normal
8	normal
9	normal
4	normal
13	normal
4	normal
14	normal
10	normal
8	normal
12	normal
13	normal
16	normal

AGE (Years)	OGTT OUTCOME	TIME FROM OGTT to T1D (Months)
Progressors		

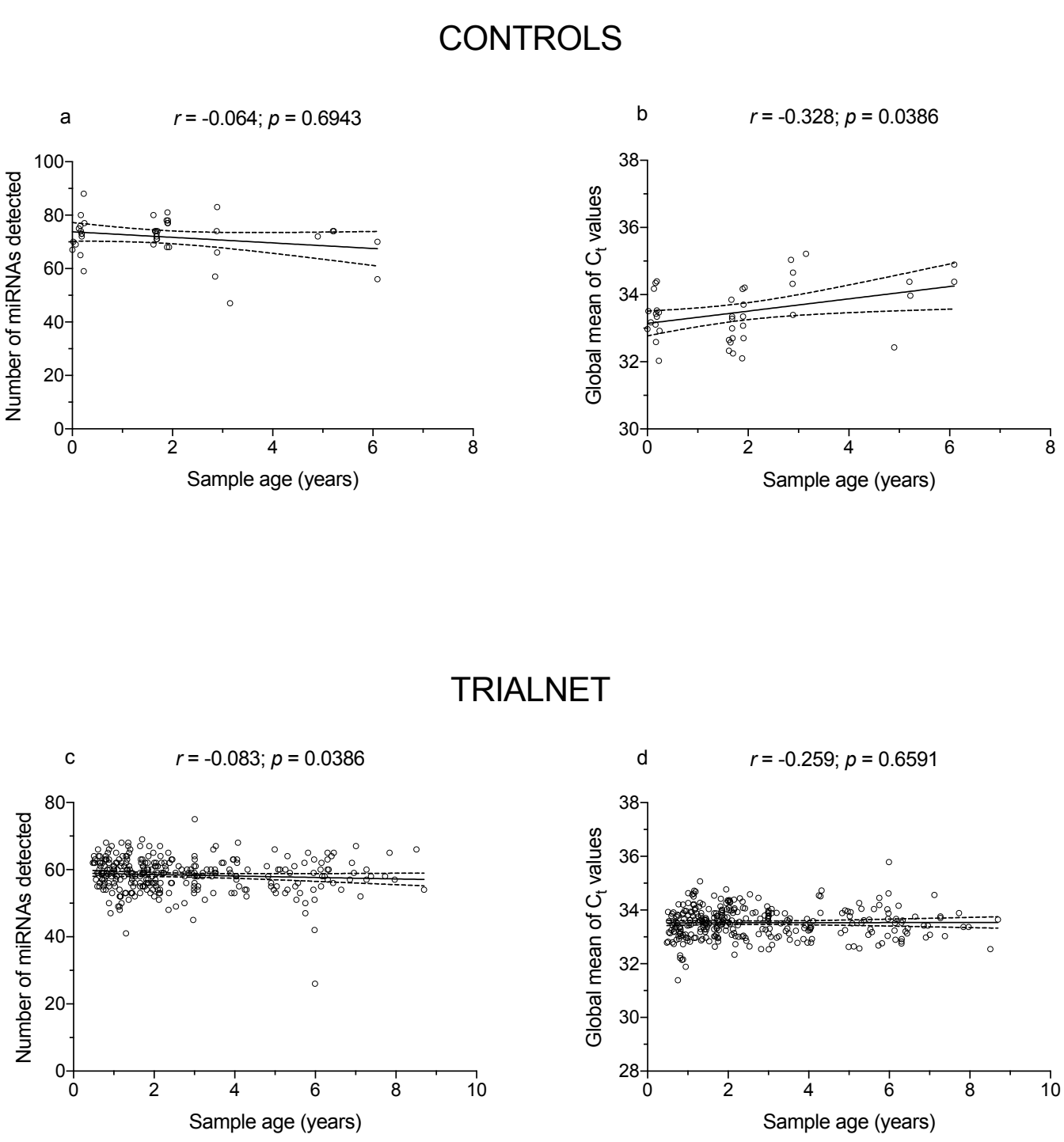
ESM Table 3. OGTT status at the time of miRNA testing for 21 Progressors and 74 non-Progressors, autoantibody-positive subjects, continued.

AGE (Years)	OGTT OUTCOME
Non-Progressors	
14	normal
10	normal
9	normal

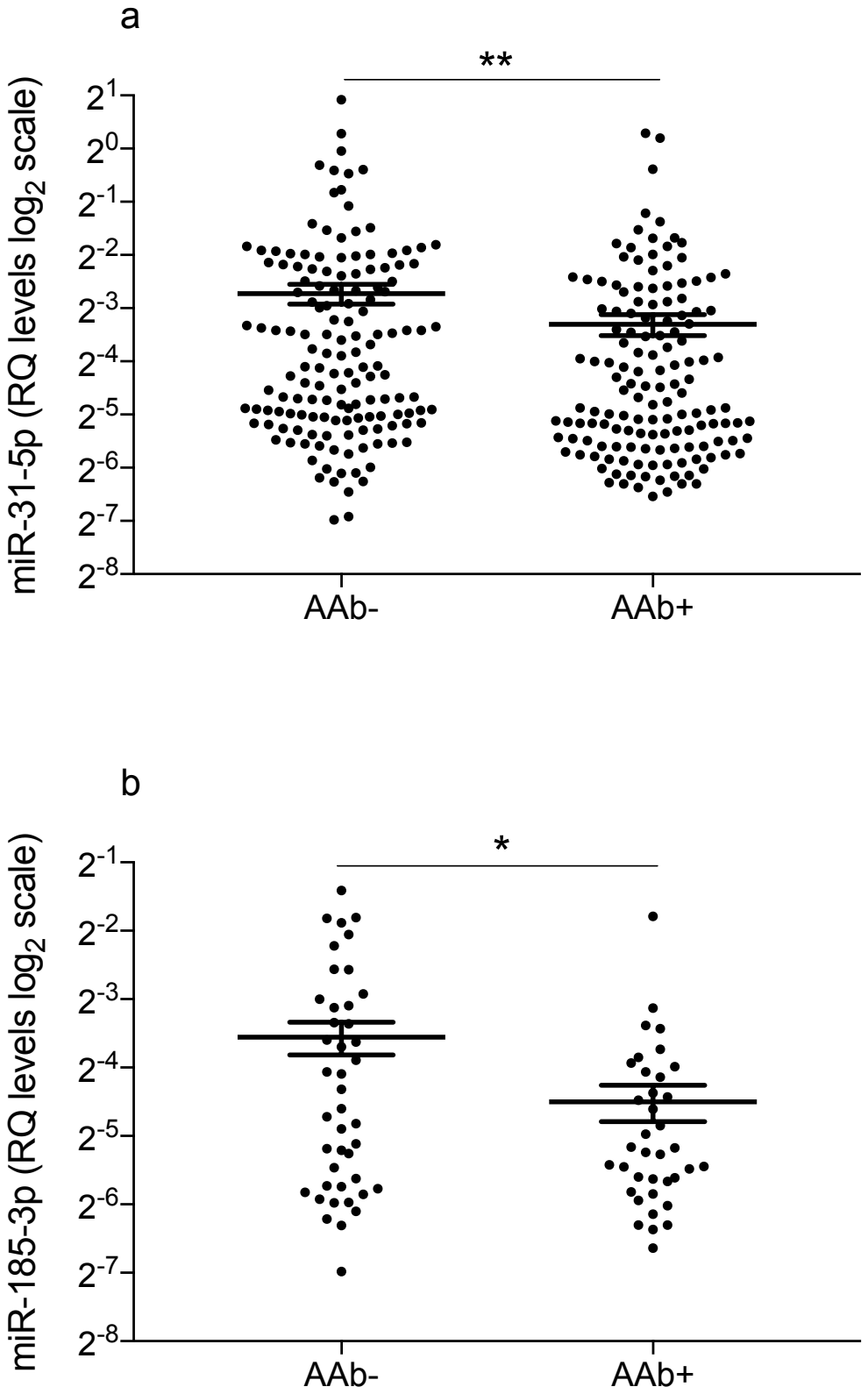
AGE (Years)	OGTT OUTCOME	TIME FROM OGTT to T1D (Months)
Progressors		

ESM Table 4. Additional miRNAs showing correlations with OGTT outcomes.
P values are noted as **p*<0.05, ***p*<0.01, ****p*<0.001.

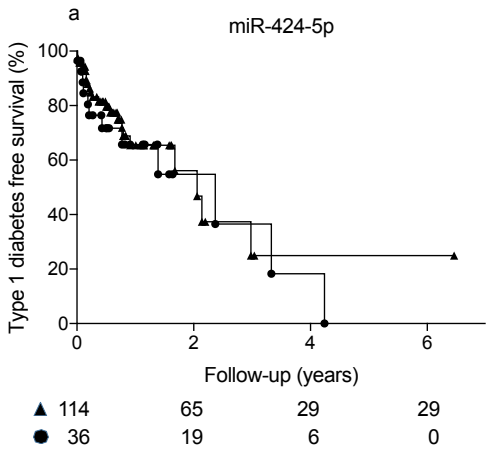
		Index60	AUC Glucose	30-0 C-peptide	AUC C-peptide	Peak C-peptide
miRNAs	N	***r***	***r***	***r***	***r***	***r***
miR-122-5p	94	-0.159	-0.033	0.154	0.204*	0.166
miR-126-3p	94	0.207*	0.158	-0.158	-0.137	-0.111
miR-155-5p	50	-0.247	0.000	0.377**	0.258	0.253
miR-181a-5p	96	0.220*	0.058	-0.122	-0.201*	-0.168
miR-222-3p	94	0.213*	0.027	-0.177	-0.212*	-0.231*
miR-23a-3p	96	0.194	-0.003	-0.179	-0.205*	-0.205*
miR-27a-3p	96	0.221*	0.190	-0.168	-0.143	-0.132
miR-542-5p	73	0.035	0.259*	-0.005	0.034	0.007
miR-548a-3p	84	-0.295**	0.084	0.128	0.281**	0.265*

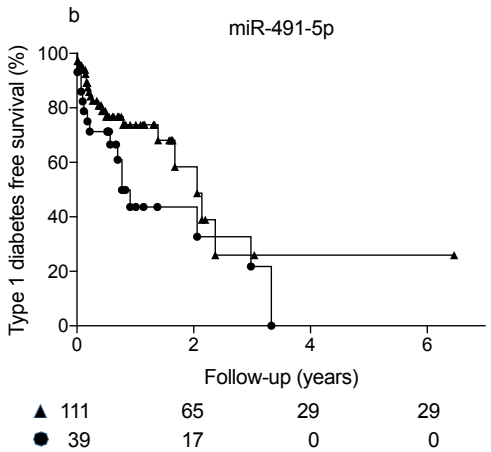


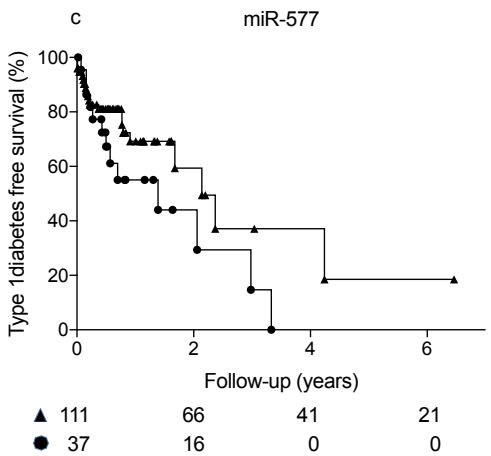
ESM Fig. 1. Correlations of sample age with number of miRNAs detected and global mean of C_t values.



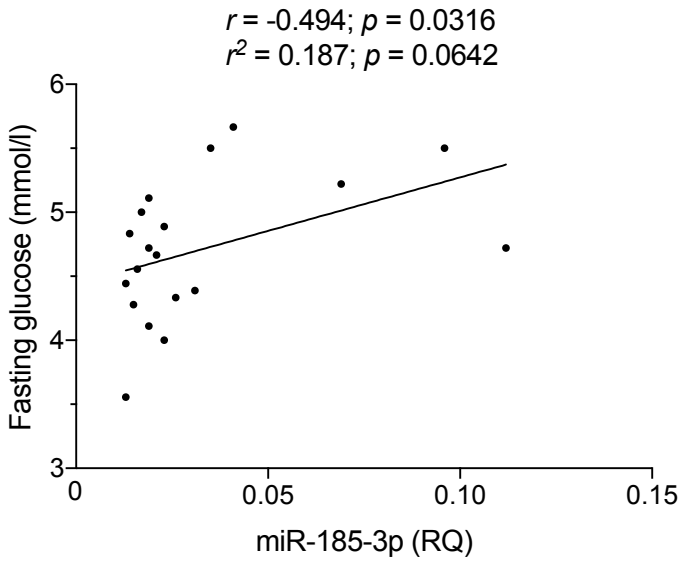
ESM Fig. 2. Levels of miR-31-5p and miR-185-3p in autoantibody-discordant sibling pairs. Significance is noted as *p<0.05, **p<0.01, ***p<0.001.







ESM Fig. 3.; **Kaplan–Maier survival curves for the development of type 1 diabetes among autoantibody-positive participants based on ROC cut-off levels of miRNAs associated with disease progression.** Circles indicate subjects with levels above the ROC cut-off, triangles identify subjects with levels below cut-off. Number of subjects at different time points for each group are shown below the X axis. No statistically significant differences were observed.



ESM Fig. 4. Correlation of miR-185-5p levels with fasting glucose values in autoantibody-positive participants.