Supplementary Information

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Table S1. Circulating miRNAs associated with type 1 diabetes by at least 2 independent studies.

Table S1. Circulating miRNAs associated with T1D by at least 2 independent studies published (2012-2020).

			T1D		Control	At-risk				Associated miRNAs (n=30)																																			
First author	Year	Study Design	New Onset (Y/N)	n (mean age)	n (mean age)	n (mean age)	Sample type	Assay type	Number of miRNAs studied	miR-24-3p	miR-375	miR-25-3p	miR-146a-5p	miR-21-5p	let-7g-5p	miR-148a-3p	miR-181a-5p	miR-26b-5p	miR-29a-3p	miR-30e-5p	miR-103a-3p	miR-106a-5p	miR-139-5p	miR-140-5p	miR-144-5p	miR-152-3p	miR-16-5p	miR-342-3p	miR-19a-3p	miR-20a-5p	miR-200a-3p	miR-21-3p	miR-210-3p	miR-222-3p	miR-27b-3p	miR-30c-5p	miR-34a-5p	miR-451a	miR-93-5p
Nielsen et al.	2012	Case-Control, C-peptide levels	Y	275 (12)	151	n/a	serum	Sequencing/qPCR	240/47	X		X		X		X	X	X	X		X					X					X		X		X					
Latreille et al.	2015	Case-Control	N	38 (43.6)	51 (40.8)	n/a	plasma	TLDA qPCR	1		X																													
Marchand et al.	2016	Case-Control	Y	22 (9.8)	10 (9.9)	n/a	serum	TLDA qPCR	1		X																													
Seyhan et al.	2016	Case-Control	N	16 (25.9)	27 (25.3)	n/a	plasma	TLDA qPCR	28	X	X			X		X																						X		
Yin et al.	2016	At-risk relatives	n/a	n/a	n/a	35 (n/a)	serum	TLDA qPCR	754				X																								X			
Erener et al.	2017	Case-Control	Y	38 (8.9)	32 (8.8)	n/a	plasma	Exiqon LNA qPCR	745	X		X			X					X				X	X										X				X
Samandari et al.	2017	C-peptide levels	Y	40 (8.7)	n/a	n/a	plasma	Exiqon LNA qPCR	745	X	X	X	X																											
Snowhite et al.	2017	At-risk relatives	n/a	n/a	n/a	150 (11)	serum	Exiqon LNA qPCR	93			X						X			X						X	X	X	X		X					X		
Akerman et al.	2018	Case-Control, At-risk relatives	Y	8 (11.7)	17 (11.8)	21 (10.2)	serum	Exiqon LNA qPCR	179	X		X			X	X	X	X	X	X		X	X	X	X	X	X	X	X	X			X	X	X	X	X	X
Lakhter et al.	2018	Case-Control	Y	19 (10.5)	16 (10.5)	n/a	serum/exosomes	Digital droplet PCR	1		X			X									X									X						
Grieco et al.	2018	Case-Control	N	15 (32)	14 (28)	n/a	serum	TLDA qPCR	6	X			X																										
Liu et al.	2018	Case-Control	Y	73 (22)	85 (21)	n/a	serum	TLDA qPCR	6									X																					
Assmann et al.	2018	Case-Control	N	33 (19.5)	26 (21.5)	0	plasma	TLDA qPCR	45				X							X										X		X						
Małachowska et al.	2018	Case-Control	Y	9 (n/a)	10 (n/a)	n/a	serum	Exiqon LNA qPCR	752	X					X																								
Samandari et al.	2018	C-peptide levels	Y	40 (8.7)	n/a	n/a	serum/plasma	Exiqon LNA qPCR	179											X																			
Bertoccini et al.	2019	Case-Control, At-risk relatives	Y	49 (16.1)	48 (41.2)	46 (26.8)	serum	TLDA qPCR	1		X																											
Liu et al.	2019	Case-Control	N	29 (24)	19 (30)	n/a	serum	In house qPCR	4			X	X	X			X																					
Garavelli et al.	2020	Case-Control, C-peptide levels	Y	88 (8.9)	47 (8.4)	n/a	plasma	Exiqon LNA qPCR	60				X																										
Garavelli et al.	2020	Case-Control, C-peptide levels	Y	88 (8.9)	47 (8.4)	n/a	plasma	Exiqon LNA qPCR	60	X																													
								TOTAL STUDIES REPORTING		8	6	6	6	4	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

In a review of the published literature last updated on October 29, 2020 we find that circulating levels of 30 miRNAs were associated with type 1 diabetes by at least 2 published studies and 11 miRNAs were reported by at least 3 studies, as indicated in the last row of the table. These 11 miRNAs are: miR-24-3p, miR-375, mir-25-3p, miR-146-3p, miR-21-5p, let-7g-5p, miR-148a-3p, miR-181a-5p, miR-26b-5p, miR-29a-3p, and miR-30e-5p. This Table only includes 19 studies examining association of circulating miRNAs in autoantibody-positive at-risk relatives (4 studies), case-control studies (n=15), or investigations of miRNAs in relation to C-peptide levels after the onset of type 1 diabetes (n=5). Virtually all studies used RT-PCR assays and the number of miRNAs examined ranged between 1 and 754, with 13/19 studies examining fewer than 100 miRNAs, 7<10 miRNAs; Nielsen et al (2012) used sequencing of pooled samples and then RT-PCR to assess levels of 47 miRNAs. All papers listed in this table are referenced in the manuscript.

Table S2. Combinations of miRNAs predict change in C-peptide AUC and Peak between baseline and 12 months.

	Estimate	Std. Error	t value	Pr(>|t|)	
Intercept	-39.5511	20.95	-1.888	0.06551	
BMI	2.6036	1.1169	2.331	0.02429	*
Age at draw	0.3008	0.4802	0.626	0.53419	
Sex	-3.8242	8.0189	-0.477	0.63574	
Baseline C-Peptide AUC	-0.7337	0.1314	-5.583	1.3e-06	***
COMBINATION 103a-3p/3187-3p	37.9503	11.3019	3.358	0.0016	**

Multiple R-squared: 0.4418, Adjusted R-squared: 0.3798
F-statistic: 7.124 on 5 and 45 DF, **p-value: 5.551e-05**
Baseline-12 months C-peptide AUC difference: 37.9 mmol/l

	Estimate	Std. Error	t value	Pr(>|t|)	
Intercept	-0.404444	0.220724	-1.832	0.07352	
BMI	0.024643	0.011931	2.065	0.04467	*
Age at draw	0.00538	0.005116	1.052	0.29854	
Sex	-0.031963	0.084778	-0.377	0.70793	
Baseline C-Peptide Peak	-0.73719	0.130452	-5.651	1.03e-06	***
COMBINATION 103a-3p/3187-3p	0.39958	0.120168	3.325	0.00176	*

Multiple R-squared: 0.4413, Adjusted R-squared: 0.3792
F-statistic: 7.108 on 5 and 45 DF, **p-value: 5.67e-05**
Baseline-12 months C-peptide Peak difference: 0.39 mmol/l

	Estimate	Std. Error	t value	Pr(>|t|)	
Intercept	-38.1881	20.8928	-1.828	0.07421	
BMI	2.6424	1.1132	2.374	0.02194	*
Age at draw	0.3338	0.4759	0.701	0.48666	
Sex	-4.2175	7.9992	-0.527	0.60062	
Baseline C-Peptide AUC	-0.7489	0.1326	-5.65	1.03e-06	***
COMBINATION 3187-3p/4302	44.8617	13.0642	3.434	0.00129	**

Multiple R-squared: 0.4469, Adjusted R-squared: 0.3855
F-statistic: 7.272 on 5 and 45 DF, **p-value: 4.59e-05**
Baseline-12 months C-peptide AUC difference: 44.86 mmol/L

The table reports the detailed results of the analysis for the data shown in Fig. 3.

Table S3. Longitudinal comparisons of AUC C-peptide/Glucose ratios.

	miR-3187-3p		miR-3187-3p		miR-3187-3p	
	Q1: Baseline	**Q1: 6-Month**	**Q1: Baseline**	**Q1: 12-Month**	**Q1: 6-Month**	**Q1: 12-Month**
N	13	13	13	13	13	13
Mean ± SD	1.06 ± 0.30	0.09 ± 0.05	1.06 ± 0.30	0.07 ± 0.05	0.09 ± 0.06	0.07 ± 0.05
Paired T-test	p=0.0002		p=0.0002		p=0.0063	
	Q2-4: Baseline	**Q2-4: 6-Month**	**Q2-4: Baseline**	**Q2-4: 12-Month**	**Q2-4: 6-Month**	**Q2-4: 12-Month**
N	35	35	39	39	35	35
Mean ± SD	0.57 ± 0.26	0.04 ± 0.03	0.58 ± 0.26	0.03 ± 0.02	0.04	0.03 + 0.02
Paired T-test	p<0.0001		p<0.0001		p<0.0001	

	miR-103a-3p		miR-103a-3p		miR-103a-3p	
	Q1: Baseline	**Q1: 6-Month**	**Q1: Baseline**	**Q1: 12-Month**	**Q1: 6-Month**	**Q1: 12-Month**
N	12	12	13	13	12	12
Mean ± SD	0.55 ± 0.34	0.03 ± 0.03	0.38 ± 0.34	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Paired T-test	p=0.0005		p=0.0002		p=0.0256	
	Q2-4: Baseline	**Q2-4: 6-Month**	**Q2-4: Baseline**	**Q2-4: 12-Month**	**Q2-4: 6-Month**	**Q2-4: 12-Month**
N	36	36	39	39	35	35
Mean	0.76 ± 0.33	0.06 ± 0.05	0.75 ± 0.32	0.04 ± 0.03	0.06 ± 0.06	0.04 ± 0.04
Paired T-test	p<0.0001		p<0.0001		p=0.0007	

	3187-3p/miR-103a-3p		3187-3p/miR-103a-3p		3187-3p/miR-103a-3p	
	1: Baseline	**1: 6-Month**	**1: Baseline**	**1: 12-Month**	**1: 6-Month**	**1: 12-Month**
N	11	11	11	11	11	11
Mean ± SD	1.05 ± 0.29	0.09 ± 0.06	1.05 ± 0.05	0.10 ± 0.05	0.09 ± 0.06	0.10 ± 0.06
Paired T-test	p=0.0010		p=0.0010		p=0.0264	
	0: Baseline	**0: 6-Month**	**0: Baseline**	**0: 12-Month**	**0: 6-Month**	**0: 12-Month**
N	37	37	41	41	36	36
Mean ± SD	0.60 ± 0.29	0.04 ± 0.03	0.61 ± 0.29	0.03 ± 0.02	0.04 ± 0.03	0.03 ± 0.01
Paired T-test	p<0.0001		p<0.0001		p=0.0052	

	miR-4302		miR-4302		miR-4302	
	Q1: Baseline	**Q1: 6-Month**	**Q1: Baseline**	**Q1: 12-Month**	**Q1: 6-Month**	**Q1: 12-Month**
N	13	13	13	13	13	13
Mean ± SD	0.90 ± 0.37	0.08 ± 0.06	0.90 ± 0.37	0.06 ± 0.04	0.08 ± 0.06	0.06 ± 0.04
Paired T-test	p=0.0002		p=0.0005		p=0.0017	
	Q2-4: Baseline	**Q2-4: 6-Month**	**Q2-4: Baseline**	**Q2-4: 12-Month**	**Q2-4: 6-Month**	**Q2-4: 12-Month**
N	35	35	39	39	35	35
Mean ± SD	0.63 ± 0.30	0.04 ± 0.04	0.63 ± 0.30	0.03 ± 0.03	0.04 ± 0.04	0.03 ± 0.03
Paired T-test	p<0.0001		p<0.0001		p<0.0001	

	miR-3187-3p/4302		miR-3187-3p/4302		miR-3187-3p/4302	
	1: Baseline	**1: 6-Month**	**1: Baseline**	**1: 12-Month**	**1: 6-Month**	**1: 12-Month**
N	8	8	8	8	8	8
Mean ± SD	1.08 ± 0.31	0.10 ± 0.06	1.08 ± 0.31	0.07 ± 0.04	0.10 ± 0.06	0.07 ± 0.04
Paired T-test	p=0.0078		p=0.0078		p=0.5469	
	0: Baseline	**0: 6-Month**	**0: Baseline**	**0: 12-Month**	**0: 6-Month**	**0: 12-Month**
N	40	40	44	44	39	39
Mean ± SD	0.63	0.04	0.64	0.03	0.04	0.03
Std. Deviation	0.30	0.04	0.29	0.03	0.04	0.03
Paired T-test	p<0.0001		p<0.0001		p=0.0122	

Table S3 reports full results of the longitudinal comparisons of the AUC C-peptide/Glucose ratios from the curves shown in Fig. 4, for the indicated quartiles of individual miRNAs, or for combinations of miRNAs, in which case the combined quartiles of expression are indicated as “0” or “1”. For miR-3187-3p/miR-103a-3p, combination 0= Q1 miR-103a-3p + Q2-4 miR-3187-3p, combination 1= Q2-4 miR-103a-3p + Q1 miR-3187-3p; for miR-3187-3p/miR-4302, 0= Q2-4 miR-3187-3p + Q2-4 miR-4302, combination 1= Q1 miR-3187-3p + Q1 miR-4302. Statistically significant changes occur for each of the two groups of patients defined by miRNA levels, consistent with the disease natural history. However, 6-month and 12-month curves of miR-3187-3p/miR-4302 were statistically different from each other for participants in the combination group 0, but not for those in combination group 1, suggesting that the latter did not experience significant worsening in this time interval.

Table S4. Cross-sectional comparisons of AUC C-peptide/Glucose ratios.

	miR-3187-3p		miR-3187-3p		miR-3187-3p	
	Q1: Baseline	**Q2-4: Baseline**	**Q1: 6-Month**	**Q2-4: 6-Month**	**Q1: 12-Month**	**Q2-4: 12-Month**
N	13	40	13	35	13	39
Mean ± SD	1.06 ± 0.30	0.59 ± 0.26	0.09 ± 0.06	0.05 ± 0.03	0.07 ± 0.05	0.03 ± 0.02
Paired T-test	p<0.0001		p=0.0004		p=0.0007	

	miR-103a-3p		miR-103a-3p		miR-103a-3p	
	Q1: Baseline	**Q2-4: Baseline**	**Q1: 6-Month**	**Q2-4: 6-Month**	**Q1: 12-Month**	**Q2-4: 12-Month**
N	13	40	12	36	13	39
Mean ± SD	0.38 ± 0.64	0.60 ± 0.93	0.03 ± 0.02	0.06 ± 0.01	0.02 ± 0.01	0.05 ± 0.04
Paired T-test	p=0.0140		p=0.0303		p=0.0557	

	3187-3p/miR-103a-3p		3187-3p/miR-103a-3p		3187-3p/miR-103a-3p	
	1: Baseline	**0: Baseline**	**1: 6-Month**	**0: 6-Month**	**1: 12-Month**	**0: 12-Month**
N	11	42	11	37	11	41
Mean ± SD	1.05 ± 0.29	0.61 ± 0.28	0.09 ± 0.06	0.04 ± 0.03	0.07 ± 0.05	0.03 ± 0.02
Paired T-test	p=0.0001		p=0.0005		p=0.0004	

	miR-4302		miR-4302		miR-4302	
	Q1: Baseline	**Q2-4: Baseline**	**Q1: 6-Month**	**Q2-4: 6-Month**	**Q1: 12-Month**	**Q2-4: 12-Month**
N	13	40	13	35	13	39
Mean ± SD	0.90 ± 0.37	0.64 ± 0.30	0.08 ± 0.06	0.04 ± 0.04	0.06 ± 0.04	0.03 ± 0.03
Paired T-test	p=0.0268		p=0.0086		p=0.0397	

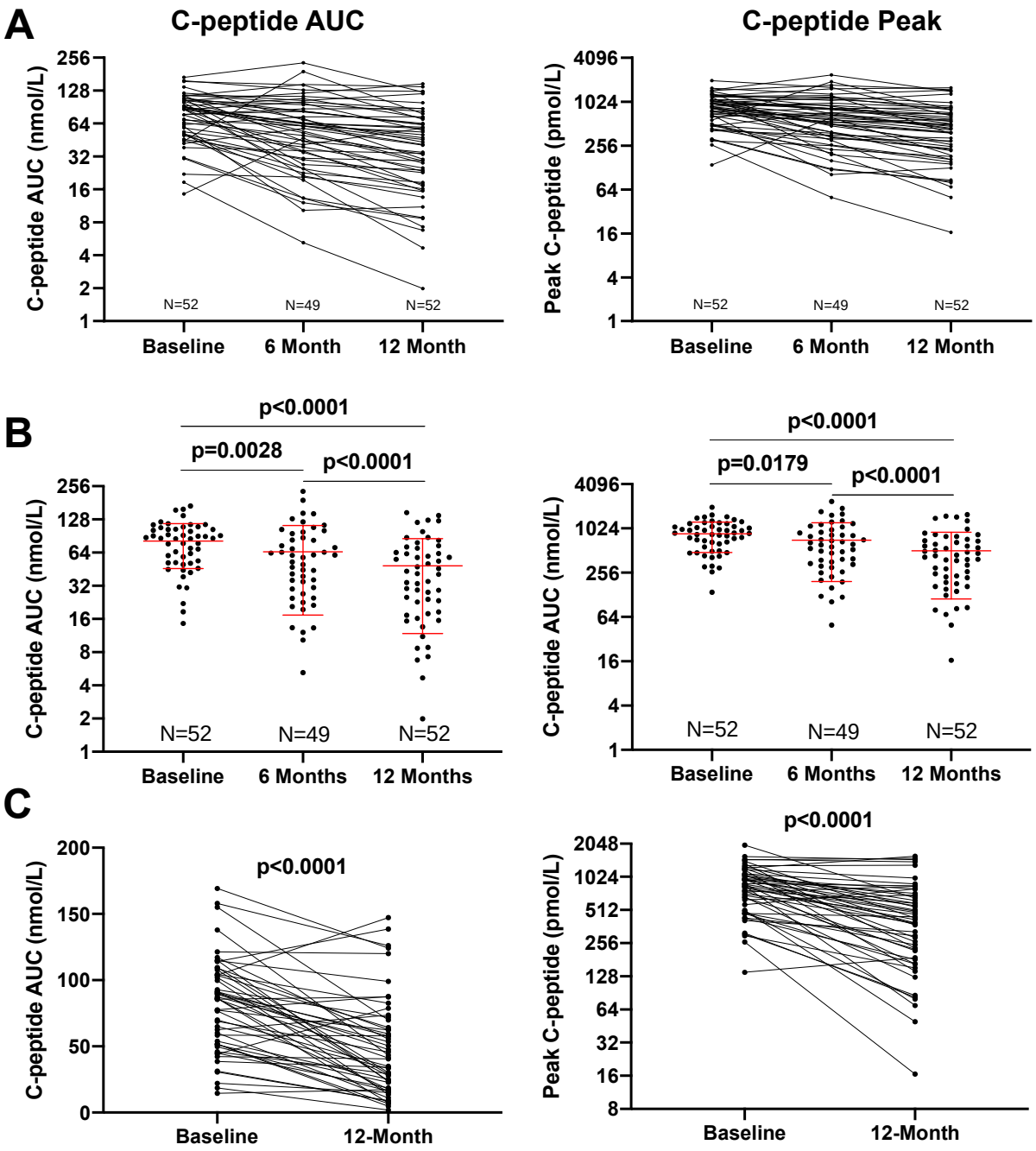
	miR-3187-3p/4302		miR-3187-3p/4302		miR-3187-3p/4302	
	1: Baseline	**0: Baseline**	**1: 6-Month**	**0: 6-Month**	**1: 12-Month**	**0: 12-Month**
N	8	45	8	40	8	44
Mean ± SD	1.08 ± 0.31	0.63 ± 0.29	0.10 ± 0.06	0.04 ± 0.04	0.07 ± 0.04	0.03 ± 0.03
Paired T-test	p=0.0007		P<0.0001		p=0.0003	

Table S4 reports full results of the cross-sectional comparison of the AUC C-peptide/Glucose ratios from the curves shown in Fig. 4, for the indicated quartiles of individual miRNAs, or for combinations of miRNAs, in which case the combined quartiles of expression are indicated as “0” or “1”. For miR-3187-3p/miR-103a-3p, combination 0= Q1 miR-103a-3p + Q2-4 miR-3187-3p, combination 1= Q2-4 miR-103a-3p + Q1 miR-3187-3p; for miR-3187-3p/miR-4302, 0= Q2-4 miR-3187-3p + Q2-4 miR-4302, combination 1= Q1 miR-3187-3p + Q1 miR-4302. The findings suggest significant differences in disease progression identified by stratification in groups defined by baseline miRNA levels.

Table S5. Total raw counts, raw CPM, and normalized CPM (log_2) observed for the 31 miRNAs associated with C-peptide AUC and or peak in the primary analysis.

	Total Raw Counts			Raw CPM			Normalized CPM (log_2)		
miRNA	**Average**	**Median**	**SD**	**Average**	**Median**	**SD**	**Average**	**Median**	**SD**
miR-103a-3p	708.89	552	601.22	216.09	194.99	125.07	7.43	7.54	0.84
miR-1208	455.15	235	649.40	129.18	80.12	119.64	6.17	6.31	1.65
miR-127-3p	80.19	54	108.08	23.36	18.58	17.55	4.04	4.17	1.06
miR-1292-5p	203.58	113	266.23	59.30	42.46	52.42	5.13	5.34	1.47
miR-193b-5p	272.943	183	311.91	80.59	69.30	54.68	5.85	5.87	0.99
miR-197-3p	563.91	477	386.48	174.93	163.45	72.95	7.23	7.29	0.65
miR-215-5p	400.75	204	555.05	115.18	78.34	105.67	6.07	6.26	1.41
miR-2355-3p	417.66	227	618.02	119.82	85.13	116.27	6.11	6.13	1.35
miR-3187-3p	255.96	126	367.77	72.39	49.84	65.36	5.43	5.62	1.36
miR-3191-3p	276.79	173	342.07	82.02	55.49	73.73	5.61	5.59	1.35
miR-342-3p	484.98	414	497.72	144.52	126.64	93.03	6.69	6.89	1.09
miR-3678-3p	362.25	199	525.68	102.85	66.30	96.02	5.92	6.00	1.36
miR-4302	390.58	222	499.95	113.51	71.15	93.65	6.23	6.08	1.15
miR-4304	443.13	272	503.33	131.40	92.54	97.79	6.51	6.38	1.09
miR-4669	404.08	222	505.62	118.02	69.82	96.81	6.32	6.02	1.10
miR-4701-3p	434.49	279	588.86	124.72	92.29	109.44	6.29	6.29	1.20
miR-4723-5p	247.15	124	361.32	70.25	44.24	67.29	5.34	5.22	1.39
miR-494-5p	323.17	173	480.73	91.69	59.38	86.41	5.74	5.84	1.42
miR-568	435.66	181	653.15	125.12	74.24	127.33	5.92	5.99	1.69
miR-5682	366.94	182	545.68	105.19	62.00	103.12	5.75	5.82	1.69
miR-589-5p	423.66	238	557.65	123.11	83.55	107.92	6.24	6.16	1.30
miR-6073	404.34	176	608.82	116.03	71.13	118.42	5.83	5.85	1.85
miR-622	397.32	222	522.23	114.30	81.30	95.62	6.24	6.23	1.14
miR-6506-5p	395.55	186	563.44	112.87	71.10	104.45	6.04	6.07	1.35
miR-6748-3p	509.98	390	474.48	155.39	125.36	92.58	6.88	6.94	0.99
miR-7154-3p	472.23	240	646.18	137.49	89.10	124.78	6.36	6.36	1.35
miR-8058	342.83	154	499.62	98.79	63.17	96.36	5.70	5.84	1.58
miR-8079	379.72	213	504.70	110.03	72.06	92.16	6.17	6.10	1.22
miR-934	270.96	113	426.06	76.84	41.68	81.73	5.25	5.33	1.66
miR-98-3p	191.42	94	267.90	56.39	35.23	54.12	4.95	4.95	1.51
miR-99a-5p	535.47	383	530.25	159.66	131.07	94.54	6.96	7.00	0.89
Average	382.3	226.5	482.9	111.6	79.4	91.5	6.0	6.0	1.3
Median	397.3	204.0	504.7	114.3	71.1	95.6	6.1	6.1	1.3
SD	122.8	112.0	128.5	37.8	36.8	25.0	0.7	0.7	0.3
Quartile 25%	300.0	173.0	406.3	86.9	60.7	77.7	5.7	5.8	1.1
Quartile 75%	439.4	239.0	560.5	127.2	87.1	106.8	6.3	6.3	1.4

Fig. S1. C-peptide AUC Decline in the Study Cohort



C-peptide AUC and peak levels for individual study participants (panel A) and as means ± SD (panel B) at the baseline, 6-month and 12-month MMTT. In panel B, statistical differences among the time points demonstrate significant C-peptide decline (Mann-Whitney test). Panel C illustrates the statistically significant decline of C-peptide AUC and peak observed in the cohort from baseline to the 12-month MMTT; significance was estimated using the Wilcoxon matched pairs signed rank test. Data are shown for 52/53 participants since a single subject did not have 12-month MMTT data; at 6 months, 4 subjects did not have MMTT data. Data are plotted on a Log^2 scale.

Supplementary material

Blood Processing. Serum samples used in this study were provided by the Type 1 Diabetes TrialNet. Samples were obtained from participants at various TrialNet sites and were uniformly collected and processed according to the TrialNet processing SOPs. Ot obtain serum, blood was collected in 2.5 mL red top SST gel tube. The tube was gently inverted 5 times and placed upright in a tube rack. The blood was allowed to clot for 20-30 minutes at room temperature, then it was centrifuged for 15 minutes. The serum was transferred into a 1.8 mL cryovial, placed upright in a 2" partitioned freezer storage box, then frozen at -70°C.

Assessment of Hemolysis. Only visually apparent hemolysis interferes with hybrid-capture based assays and this was never observed at visual checks performed before sample submission and before processing. In miRNA RT-PCR assays hemolysis is present when the miR-23a-3p/miR-451a ΔCq ratio of is >7 (1) or >9 (2), as reported in different studies. In our data, this translates to differential expression levels of miR-23a-3p/miR-451 greater than 128 or 512-fold, respectively, due to the binary logarithmic nature of ΔCq-values. The observed difference was much lower (mean 13.6 ± SD 18.5) than 128-fold. Thus, hemolysis levels were satisfactorily low in all samples.

Assessment of platelet contribution. Applicable to all studies of circulating miRNAs, the study of serum or plasma samples has the limitation that circulating miRNAs reflect a variety of cellular sources, including platelets. Serum typically contains fewer platelets than plasma when processed by standard clinical collection protocols with normal CBC platelet counts of ~200,000/mL in whole blood, ~28,000/mL plasma, and <1000/mL serum. Thus, since we used serum, the possible contribution from platelets would be much reduced compared to studies that used plasma. We are not aware of reports of truly platelet-specific miRNAs. However, several miRNAs have been linked to platelet activation or are expressed also by platelets.

Figure S2 shows median and interquartile ranges of raw CPM values, in the Log_2 scale, for the 31 miRNAs associated with C-peptide in this study and 50 miRNAs that could be commonly contributed by platelets (albeit not exclusively) (3). Overall, the miRNAs associated with C-peptide had significantly lower expression levels than platelet miRNAs ($p<0.0001$). Moreover, miRNAs associated with platelets were not a key contributor to the main associations reported in this study because only 3 of the miRNAs associated with C-peptide overlapped with those linked to platelets: these are miR-103a-3p, miR-342-3p, and miR-197-3p. As described in the main text, these three miRNAs have been linked to type 1 diabetes by multiple studies.

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Fig. S2. Median and interquartile ranges for 31 miRNAs associated with C-peptide in this study compared to miRNAs associated with platelets in the literature.