

Supplementary Material for

Plasma microRNA signature associated with retinopathy in patients with type 2 diabetes

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Supplementary Table S1. Differentially expressed circulating miRNAs in patients with diabetic retinopathy

<i>microRNA</i>	<i>log₂FC</i>	<i>SE of difference</i>	<i>P value</i>	<i>q value</i>	<i>Significant after FDR?</i>
<i>miR-23a-3p</i>	2.65	0.92	0.0052	0.0243	Yes
<i>miR-25-3p</i>	3.54	1.13	0.0043	0.0243	Yes
<i>miR-92a-3p</i>	2.71	1.18	0.0297	0.0833	No
<i>miR-320b</i>	2.53	0.93	0.0112	0.0392	Yes
<i>miR-346</i>	-2.43	1.11	0.0378	0.0884	No
<i>miR-495-3p</i>	-4.21	0.97	0.0001	0.0025	Yes

Comparisons were assessed by multiple Student's t-test and probability values were corrected for multiple comparisons by using Benjamini-Hochberg approach with a false discovery rate (FDR) set as 5%. Comparisons yielding a q-value < 0.05 were deemed as statistically significant after FDR.

Supplementary Table S2. Summary of multivariate linear regression models**(A) miR-25a-3p**

<i>Variables in the model</i>	<i>Beta-coefficient</i>	<i>P value</i>
<i>Circulating miR-25a-3p</i>	0.48	0.0032*
<i>Age</i>	0.14	0.3544 (n.s.)
<i>Sex</i>	0.41	0.4147 (n.s.)

(B) miR-320b

<i>Variables in the model</i>	<i>Beta-coefficient</i>	<i>P value</i>
<i>Circulating miR-320b</i>	0.52	0.0011*
<i>Age</i>	0.07	0.6348 (n.s.)
<i>Sex</i>	0.08	0.5727 (n.s.)

(C) miR-495-3p

<i>Variables in the model</i>	<i>Beta-coefficient</i>	<i>P value</i>
<i>Circulating miR-495-3p</i>	-0.43	0.0064*
<i>Age</i>	0.16	0.2782 (n.s.)
<i>Sex</i>	0.04	0.8175 (n.s.)

Dependent variable: severity of diabetic retinopathy (DR): grade I, no DR; grade II, mild non-proliferative DR; Grade III, moderate NPDR; Grade IV, severe NPDR; Grade V, proliferative DR. Circulating miRNAs expression are log-transformed. Simultaneous-entry of covariates was employed.

Supplementary Table S3. Summary of multivariate logistic regression models**(A) miR-25a-3p**

<i>Variables in the model</i>	<i>Odds Ratio</i>	<i>95% CI</i>	<i>P value</i>
<i>Circulating miR-25a-3p</i>	2.37	1.16 – 4.85	0.0179*
<i>Age</i>	0.91	0.80 – 1.05	0.1797 (n.s.)
<i>HbA1c</i>	2.70	0.36 – 20.30	0.3336 (n.s.)
<i>Sex</i>	2.71	0.34 – 21.52	0.3446 (n.s.)

(B) miR-320b

<i>Variables in the model</i>	<i>Odds Ratio</i>	<i>95% CI</i>	<i>P value</i>
<i>Circulating miR-320b</i>	1.95	1.03 – 3.70	0.0411*
<i>Age</i>	0.92	0.82 – 1.04	0.1644 (n.s.)
<i>HbA1c</i>	2.45	0.34 – 17.85	0.3738 (n.s.)
<i>Sex</i>	1.65	0.26 – 10.31	0.5943 (n.s.)

(C) miR-495-3p

<i>Variables in the model</i>	<i>Odds Ratio</i>	<i>95% CI</i>	<i>P value</i>
<i>Circulating miR-495-3p</i>	0.46	0.23 – 0.89	0.0220*
<i>Age</i>	0.97	0.87 – 1.09	0.6164 (n.s.)
<i>HbA1c</i>	2.21	0.52 – 9.40	0.2849 (n.s.)
<i>Sex</i>	2.00	0.00 – 13.34	0.4731 (n.s.)

Supplementary Table S4. Association between circulating miRNAs and age

<i>microRNA</i>	<i>Spearman's Rho</i>	<i>P value</i>	<i>Significant?</i>
<i>miR-23a-3p</i>	- 0.052	0.748	No
<i>miR-25-3p</i>	+ 0.147	0.366	No
<i>miR-320b</i>	+ 0.239	0.138	No
<i>miR-495-3p</i>	- 0.021	0.896	No

Bivariate correlation's coefficients were estimated by Spearman's test.

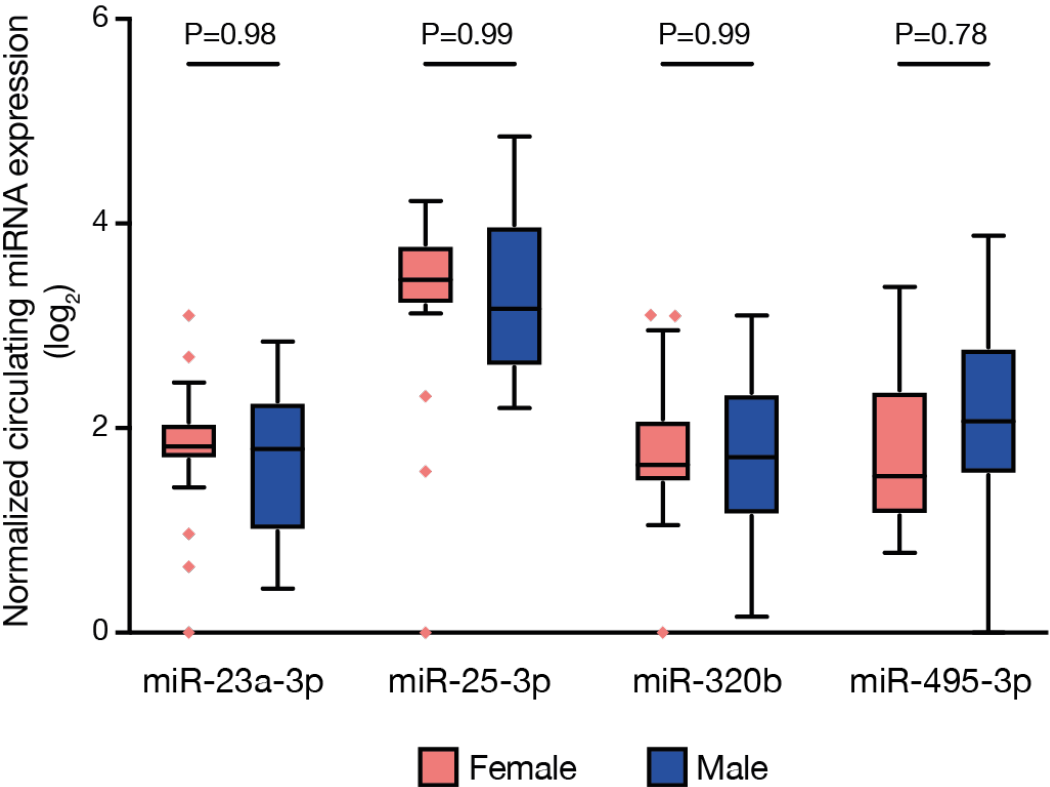
Supplementary table S5. Functionally validated microRNAs' targets

miRNA	Target	Experimental evidence
miR-23a-3p	CXCL12	Luciferase reporter assay; Reporter assay; Other
	HES1	Immunofluorescence; Luciferase reporter assay; Northern blot; Western blot
	POU4F2	Luciferase and GFP reporter assay; Northern blot; qPCR; Western blot
	ATAT1	Luciferase reporter assay
	IL6R	GFP reporter assay; Microarray; qPCR; Western blot
	PPARGC1A	Luciferase reporter assay
	G6PC	Luciferase reporter assay
	FOXO3	Immunoblot; Luciferase reporter assay; qPCR
	FANCG	Immunoblot; Luciferase reporter assay; qPCR; Western blot
	MYH1	Luciferase reporter assay; qPCR; Western blot
	MYH2	Luciferase reporter assay; qPCR; Western blot
	MYH4	Luciferase reporter assay; qPCR; Western blot
	PTEN	Luciferase reporter assay; qPCR; Western blot; Flow cytometry
	PTEN	Luciferase reporter assay
	PTPN11	Luciferase reporter assay
	HMGN2	Luciferase reporter assay
	LDHA	Luciferase reporter assay; qPCR
	CDH1	qPCR; Western blot
	KLF3	Luciferase reporter assay; Western blot
	IRF1	Luciferase and GFP reporter assay; qPCR; Western blot; Immunofluorescence
	FAS	Flow cytometry; Luciferase reporter assay; Microarray; Western blot
	MT2A	Immunofluorescence; Immunohistochemistry; In situ hybridization; Luciferase reporter assay; qPCR; Western blot
	PPP2R5E	GFP reporter assay; qPCR; Western blot
	LDHB	Luciferase reporter assay; qPCR
	CXCL8	Luciferase reporter assay; qPCR; Western blot
	APAF1	Luciferase reporter assay; qPCR; Western blot; Flow cytometry
	NEK6	Luciferase reporter assay
	LPAR1	Luciferase reporter assay; qPCR; Western blot
	FZD5	qPCR; Microarray; Western blot
	TOP1	Luciferase reporter assay; qPCR; Western blot; Flow cytometry
	HOXB4	Luciferase reporter assay; Western blot
	LAMP1	Luciferase reporter assay; qRT-PCR
	HMGB2	Luciferase reporter assay; qPCR; Western blot
	SPRY2	Luciferase reporter assay; qPCR; Western blot; ChIP-seq
	MEF2C	Luciferase reporter assay
	HIP1R	Luciferase reporter assay
	XIAP	Luciferase reporter assay; qPCR; Western blot

	LRP5	Luciferase reporter assay; qPCR; Western blot
	TMEM64	Luciferase reporter assay; Western blot
	HSP90AA1	Luciferase reporter assay; qPCR; Immunofluorescence
	HNF1B	qRT-PCR//Microarray//Western blot
	TSC1	Luciferase reporter assay//qRT-PCR//Western blot//Microarray
	SMAD3	Luciferase reporter assay; qPCR; Western blot; Immunofluorescence
	STAT3	Luciferase reporter assay; Immunofluorescence; Western blot
	GJA1	Illumina Expression Arrays; Luciferase reporter assay; qPCR; Western blot
	FOXA1	Luciferase reporter assay; qPCR; Western blot
	SMAD5	Luciferase reporter assay; qPCR; Western blot
	CHUK	Luciferase reporter assay
	ST7L	Luciferase reporter assay
	TERF2	Luciferase reporter assay
	RGS5	Luciferase reporter assay
	GLS	Luciferase reporter assay; qPCR; Western blot
miR-25-3p	PRMT5	Western blot
	BCL2L11	Microarray; Luciferase reporter assay; qPCR; Western blot
	KLF4	Luciferase reporter assay; Western blot
	CDKN1C	Luciferase reporter assay; qPCR; Western blot
	KAT2B	Luciferase reporter assay; Western blot
	TP53	Luciferase reporter assay; qPCR; Western blot
	WDR4	Luciferase reporter assay; qPCR; Western blot
	CDH1	Luciferase reporter assay; qPCR; Western blot
	CCL26	Luciferase reporter assay; qPCR; Western blot
	MDM2	Luciferase reporter assay; qPCR; Western blot
	PTEN	Luciferase reporter assay
	EZH2	Luciferase reporter assay; qPCR; Western blot
	SMAD7	Luciferase reporter assay; qPCR; Western blot
	MAP2K4	Western blot
	DSC2	Luciferase reporter assay; Western blot
	ATP2A2	In situ hybridization; Luciferase reporter assay; qPCR; Western blot
	RECK	Luciferase reporter assay; qPCR; Western blot
	TCEAL1	Luciferase reporter assay; qPCR; Western blot; Flow cytometry
	LATS2	Luciferase reporter assay; qPCR; Western blot
	REV3L	Luciferase reporter assay
	FBXW7	Luciferase reporter assay; qPCR; Western blot; Immunohistochemistry
	HAND2	Luciferase reporter assay
	CPEB1	Luciferase reporter assay
	TNFSF10	Western blot
	CYP2B6	Luciferase reporter assay; qPCR; Western blot; EMSA

	RGS3	Luciferase reporter assay; qPCR; Western blot
	DHFR	Luciferase reporter assay; qPCR; Western blot
	SEMA4C	Luciferase reporter assay; qPCR; Western blot
miR-320b	MYC	Luciferase reporter assay; qPCR; Western blot
	DLX5	Luciferase reporter assay; qPCR; Western blot
	NOD2	Luciferase reporter assay; qPCR; Western blot
miR-495-3p	PBX3	Luciferase reporter assay; qPCR; Western blot; Microarray; Immunohistochemistry
	MEIS1	Luciferase reporter assay; qPCR; Western blot; Microarray; Immunohistochemistry
	MTA3	Luciferase reporter assay; qPCR; Western blot;
	SOX9	Luciferase reporter assay; Microarray; qPCR; Western blot; safranin O staining/GAGs contents assay
	PTP4A3	qPCR; Western blot
	BMI1	Luciferase reporter assay; qPCR; Western blot; Microarray; Immunohistochemistry
	SMR3B	qPCR; Western blot
	ATP7A	GFP reporter assay; qPCR; Western blot
	TBC1D9	Luciferase reporter assay; qPCR
	MAT1A	Luciferase reporter assay
	HSPA5	Luciferase reporter assay; qPCR
	FOXC1	GFP reporter assay; qPCR; Western blot
	CCL2	ELISA; qPCR; Luciferase reporter assay; Western blot
	RUNX3	LacZ reporter assay; Microarray; qPCR; Western blot; Luciferase reporter assay
	AKT1	Luciferase reporter assay; qPCR; Western blot
	HMGA2	Luciferase reporter assay; qPCR; Western blot
	ABCB1	Luciferase reporter assay; qPCR; Western blot

Supplementary Figure S1. Circulating miRNAs expression per sex category



Expression of the circulating miRNAs showing a significant dysregulation in patients with diabetic retinopathy was evaluated by qPCR. Data have been stratified by sex and P-values were computed by multiple *t*-tests. No statistically significant differences were observed.