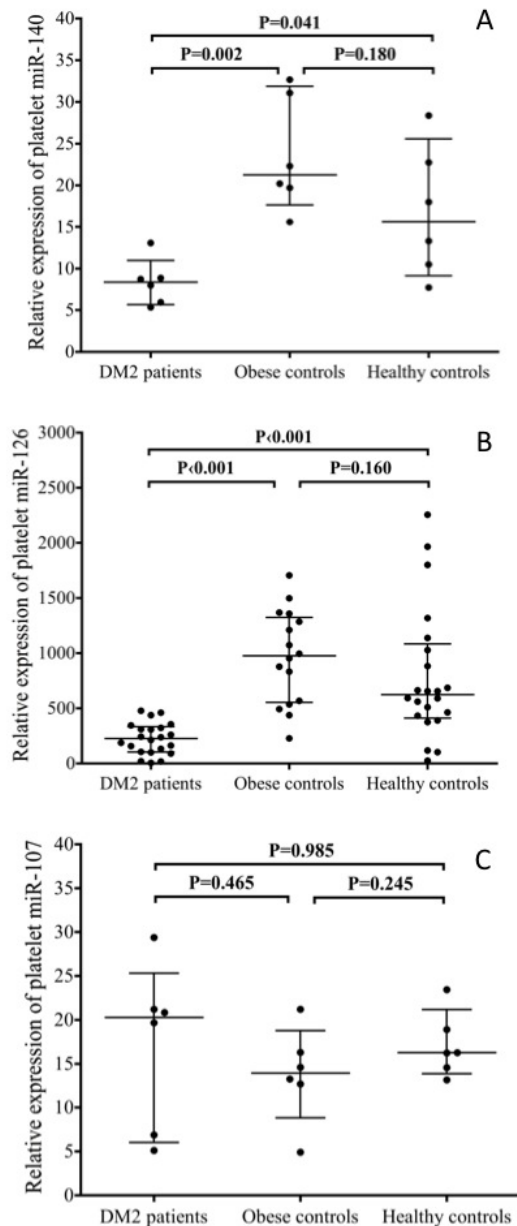
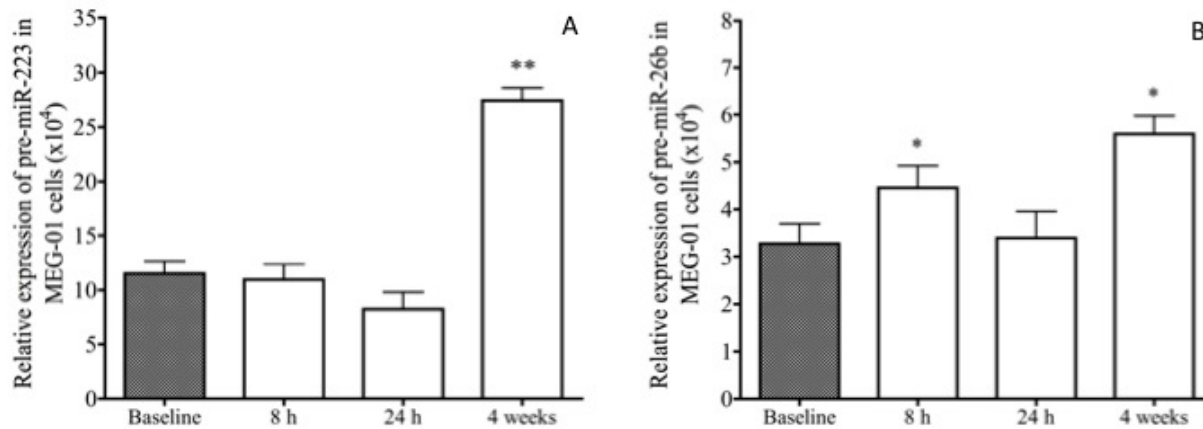


Supplementary Material to Fejes et al. "Hyperglycaemia suppresses miR-223, miR-26b and miR-140 expression in platelets causing elevated P2RY12 and SELP mRNA levels in type 2 diabetes mellitus" (Thromb Haemost 2017; 117.3)



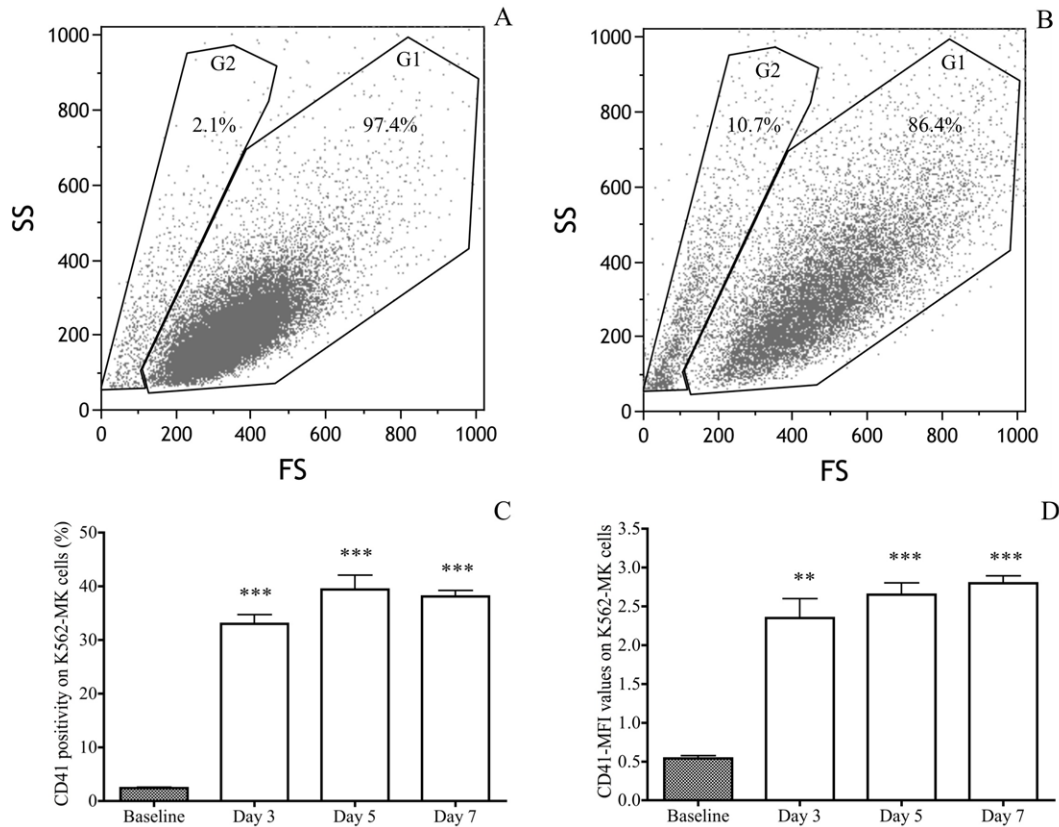
Suppl. Figure 1. DM2 platelets demonstrated decreased level of miR-140 (A) and that of miR-126 (B) versus obese and healthy subjects, while platelet miR-107 was normal in DM2 (C). These measurements were performed only in a selected population. Dots represent single expression values. Median and interquartile values are depicted. Mann-Whitney U test was performed for the comparison.

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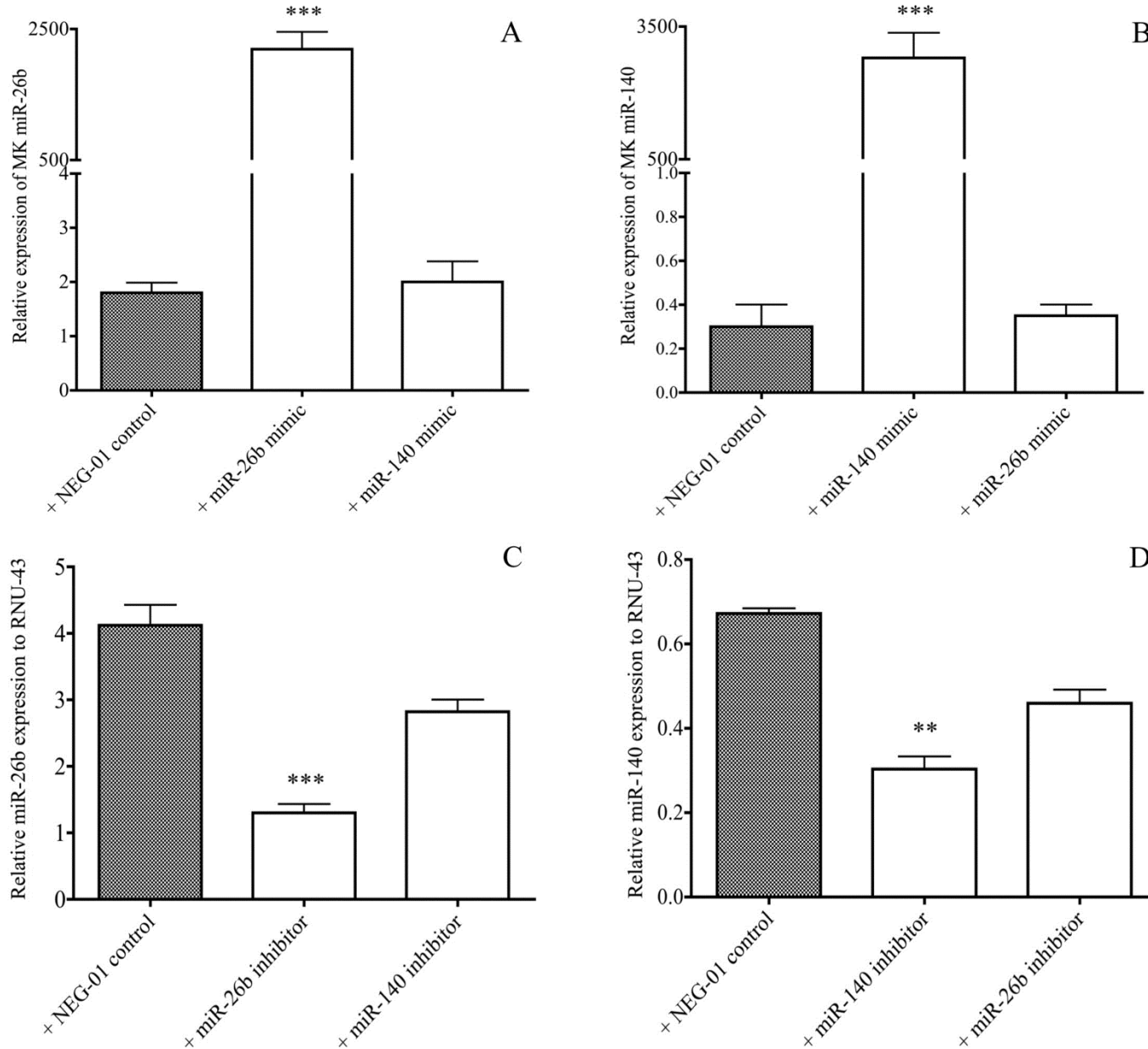
Suppl. Figure 2. The relative expression of pre-miR-223 (A) and pre-miR-26b (B) in MEG-01 MK cells after hyperglycemic treatment. No significant decrease of pre-forms was observed in the first 24 hours as seen in mature miRNAs, while these expressions were upregulated by week 4. Mean±SEM was depicted, * P<0.05, ** P<0.01 based on unpaired t test, (n=6–8/experiment).

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Suppl. Figure 3. Analysis of CD41 positivity on differentiated MK cells from K562 cells. K562-MK cells were cultured with PMA (20 ng/mL) for 7 days, and CD41 expression was followed by flow cytometry. Dot plots represent the gating of viable (G1) and dead cells (G2) based on forward scatter (FS) - side scatter (SS) characteristics before (A) and after PMA treatment by day 7 (B). CD41 percent positivities (C) and their MFI values (D) are depicted as mean±SEM, **P<0.01, ***P<0.001 based on unpaired t test (n=3–4/experiment).

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Suppl. Figure 4. miR-26b and miR-140 were overexpressed or suppressed by specific miRNA mimics or inhibitors. Neither mimics nor anti-miRNAs affected the level of the other miRNA (A-D). Mean±SEM was depicted, ** P<0.01, ***P<0.001 based on statistical analyses.

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Suppl. Table 1. Some of predicted targets of the investigated miRNAs according to the Targetscan and the Microrna.org prediction programs. Minus (-) indicates if the association is not predicted according to that database. These miRNAs may be important in the modulation of other proteins potentially involved in the cellular activation during atherothrombotic diseases. Abbreviations: CDK14: cyclin-dependent kinase 14, CLOCK: major regulator of cell circadian rhythm, ICAM1: intercellular adhesion molecule 1, IRS1: insulin receptor substrate 1, MSTN: myostatin, MYH9: myosin, heavy chain 9, P2RY12: purinergic receptor P2Y, G-protein coupled, 12, PDGFRA: platelet-derived growth factor receptor, alpha polypeptide, PRKCB: protein kinase C, beta, PRKCD: protein kinase C, delta, PTEN: phosphatase and tensin homolog, PTPN9: protein tyrosine phosphatase, non-receptor type 9, RAP1B: member of RAS oncogene family, SELP: selectin P, SPRED1: sprouty-related, EVH1 domain containing 1, STK3: serine/threonine kinase 3, TLR4: toll-like receptor 4, TRAF7: TNF receptor-associated factor 7, VAMP2: vesicle-associated membrane protein 2, VAMP8: vesicle-associated membrane protein 8, VASP: vasodilator-stimulated phosphoprotein, VEGFA: vascular endothelial growth factor A.

No.	miRNAs	Predicted target genes									
		TargetScan Score	mirSVR Score	TargetScan Score	mirSVR Score	TargetScan Score	mirSVR Score	TargetScan Score	mirSVR Score	TargetScan Score	mirSVR Score
1	miR-223-3p	P2RY12 (-0.29)	P2RY12 (-1.0376)	-	PRKCB (-0.3231)	SPRED1 (-0.26)	SPRED1 (-0.4188)	ICAM1 (-0.18)	ICAM1 (-0.5660)	VAMP2 (-0.34)	VAMP2 (-0.1360)
2	miR-26b-5p	SELP (-0.22)	SELP (-1.0011)	-	VASP (-0.2734)	PRKCD (-0.41)	PRKCD (-1.3114)	PTEN (-0.15)	PTEN (-0.1517)	MSTN (-0.24)	MSTN (-0.4412)
3	miR-140-5p	SELP (-0.14)	-	PDGFRA (-0.20)	PDGFRA (-0.3169)	VEGFA (-0.38)	VEGFA (-0.9525)	TLR4 (-0.38)	-	STK3 (-0.47)	STK3 (-0.8010)
4	miR-126-3p	SPRED1 (-0.41)	-	-	VEGFA (-0.1029)	PTPN9 (-0.09)	PTPN9 (-1.0357)	IRS1 (-0.35)	-	TRAF7 (-0.20)	-
5	miR-107	CLOCK (-0.16)	CLOCK (-0.1756)	RAB1B (-0.37)	RAB1B (-0.3621)	MYH9 (-0.34)	MYH9 (-1.2106)	CDK14 (-0.34)	-	VAMP8 (-0.38)	VAMP8 (-0.3891)

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Suppl. Table 2. Sequences of primers used in the RT-qPCR analyses. During the investigation of all miRNAs, we applied UPL probes #21, while * and ** denote UPL probe #78 and #75, respectively.

Mature miRNAs	Stem Loop Primers for reverse transcription	Forward Primers for RT-qPCR	Universal Reverse Primer for RT-qPCR
hsa-miR-223-3p	5' - GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC TGGGGT - 3'	5' - GTTGGGTGTCAGTTTGTCAAAT - 3'	5' - GTGCAGGGTCCGAGGT - 3'
hsa-miR-26b-5p	5' - GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC ACCTAT - 3'	5' - GTTTGGGTTCAAGTAATTCAGG - 3'	5' - GTGCAGGGTCCGAGGT - 3'
hsa-miR-140-5p	5' - GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC CTACCA - 3'	5' - GGGCAGTGGTTTTACCCTA - 3'	5' - GTGCAGGGTCCGAGGT - 3'
hsa-miR-107	5' - GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC TGATAG - 3'	5' - GTAGCAGCATTGTACAGGG - 3'	5' - GTGCAGGGTCCGAGGT - 3'
hsa-miR-126-3p	5' - GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC CGCATT - 3'	5' - GGGTCGTACCGTGAGTAAT - 3'	5' - GTGCAGGGTCCGAGGT - 3'
RNU-43	5' - GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC AATCAG - 3'	5' - GTGAACTTATTGACGGGCG - 3'	5' - GTGCAGGGTCCGAGGT - 3'
pre-miRNAs, mRNAs	Forward primers for RT-qPCR	Reverse primers for RT-qPCR	Amplicon length
hsa-pre-miR-223	5' - CGCTCCGTGTATTTGACAAG - 3'	5' - CGCACTTGGGGTATTTGACA - 3'	110 nt
hsa-pre-miR-26b	5' - CGGGACCCAGTTCAAGTAAT - 3'	5' - CCGAGCCAAGTAATGGAGAA - 3'	77 nt
SELP mRNA	5' - CCATTGTCTAGAGGGCCAGT - 3'	5' - GGGCTTCCTGGATAGTCAATG - 3'	119 nt
P2RY12 mRNA	5' - TTTGCCTAACATGATTCTGACC - 3'	5' - GGAAAGAGCATTCTTCACATTCT - 3'	65 nt
RPLP0 (36B4) mRNA	5' - ATGCAGCAGATCCGCATGT - 3'	5' - TCATGGTGTCTTGCCCATCA - 3'	64 nt
PTPRC (CD45) mRNA *	5' - GGCTGAATTCCAGAGACTTCC - 3'	5' - TTCTTGATTCCAATGTGCTGT - 3'	64 nt
ITGA2B (CD41) mRNA **	5' - AGCCTCAATGTGTCCCTACC - 3'	5' - AGTCCAGGACGATTTCGTGTC - 3'	103 nt