Association of MicroRNAs and YRNAs with Platelet Function

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ABSTRACT

***Rationale*:** Platelets shed microRNAs (miRNAs). Plasma miRNAs change upon platelet inhibition. It is unclear if plasma miRNA levels correlate with platelet function.

***Objective*:** To link small RNAs to platelet reactivity.

Methods and Results: Next-generation sequencing of small RNAs in plasma revealed two peaks at 22-23 and 32-33 nucleotides corresponding to miRNAs and YRNAs, respectively. Among YRNAs, predominantly fragments of RNY4 and RNY5 were detected. Plasma miRNAs and YRNAs were measured in 125 patients with a history of ACS who had undergone detailed assessment of platelet function 30 days after the acute event. Using quantitative real-time polymerase chain reactions, 92 miRNAs were assessed in ACS patients on different anti-platelet therapies. Key platelet-related miRNAs and YRNAs were correlated with platelet function tests. MiR-223 (r_p=0.28, n=121, P=0.002), miR-126 (r_p=0.22, n=121, P=0.016), other abundant platelet miRNAs and YRNAs showed significant positive correlations with the vasodilator-stimulated phosphoprotein phosphorylation assay. YRNAs, miR-126 and miR-223 were also among the small RNAs showing the greatest dependency on platelets, and strongly correlated with plasma levels of P-selectin, platelet factor 4 and platelet basic protein in the population-based Bruneck study (n=669). A single nucleotide polymorphism that facilitates processing of pri-miR-126 to mature miR-126 accounted for a rise in circulating platelet activation markers. Inhibition of miR-126 in mice reduced platelet aggregation. MiR-126 directly and indirectly affects ADAM9 and $P2Y_{12}$ receptor expression.

Conclusions: Levels of platelet-related plasma miRNAs and YRNAs correlate with platelet function tests in ACS patients and platelet activation markers in the general population. Alterations in miR-126 affect platelet reactivity.

Keywords:
Acute coronary syndrome**,** antiplatelet therapy, biomarker, microRNA, platelet.

Nonstandard Abbreviations and Acronyms:

ACS	acute coronary syndrome
ASA	acetylsalicylic acid
Ct	cycle threshold
ELISA	enzyme-linked immunosorbent assay
miRNA	microRNA
PAR4-AP	PAR-4 activating peptide AYPGKF amide
PC	principal component
PF4	platelet factor 4
PPBP	pro-platelet basic protein
PPP	platelet-poor plasma
PRP	platelet-rich plasma
qPCR	real-time polymerase chain reaction
SELP	P-selectin
SNP	single nucleotide polymorphism
STEMI	ST-elevation myocardial infarction
TxA_2	thromboxane A_2

INTRODUCTION

MiRNAs are small non-coding RNAs with cell-type specific expression patterns that are released by cells into the circulation as part of membranous particles or protein complexes.[1] Thus, miRNAs can be readily quantified by real-time polymerase chain reactions (qPCR) in plasma and serum and have generated increasing interest as potential new biomarkers.[2] Our group has previously identified platelet-related miRNA signatures that are predictive of cardiovascular events.[3] Additionally, we measured miRNAs in healthy volunteers and in patients with symptomatic atherosclerosis before and after initiation of dual anti-platelet therapy and demonstrated reduced plasma levels of platelet-related miRNAs upon platelet inhibition.[4]

Dual oral antiplatelet therapy (aspirin (ASA) + a $P2Y_{12}$ inhibitor) is commonly used for the management of non-ST-elevation acute coronary syndromes (ACS) and ST-elevation myocardial infarction (STEMI).[5] ASA irreversibly inhibits cyclooxygenase 1 in platelets, thereby repressing thromboxane A_2 (TxA_2) synthesis and, consequently, platelet activation. Clopidogrel, prasugrel and ticagrelor target the $P2Y_{12}$ receptor for adenosine diphosphate (ADP). However, interindividual variability in the platelet response to clopidogrel has been reported. Prasugrel and ticagrelor exhibit a more consistent anti-platelet effect and have shown benefits over clopidogrel in ACS patients but also increase the risk of bleeding.[6,7] It is currently unclear whether plasma levels of platelet-related miRNAs correlate with the residual platelet activity in ACS patients and how different anti-platelet agents alter miRNAs.

In this study, we used RNA sequencing to characterize small RNAs in plasma. Then, we compared the effect of different anti-platelet agents and explored the association of small RNAs (miRNAs and YRNAs) with platelet function tests in ACS patients. Moreover, we correlated their plasma levels to platelet activation markers in the prospective, population-based Bruneck study[3] and investigated if a single nucleotide polymorphism (SNP) that facilitates miR-126 processing[8] alters circulating miR-126 levels and platelet reactivity. These epidemiological observations were complemented by preclinical studies, assessing platelet function in mice upon treatment with antagomiRs directed against miR-126, and by mechanistic studies measuring miR-126 targets.

METHODS

An expanded Methods section is available in the Online Data Supplement.

Next-generation Sequencing (NGS).
Small RNA libraries were generated from non-normalized RNA (ranging from 375 pg to 1 ng) extracted from equal volumes of platelet-poor (PPP) and platelet-rich plasma (PRP) from healthy human volunteers. Prior to library preparation, RNA was spiked with equal amounts of *C. elegans* miR-39 star (*cel*-miR-39*) to assist in normalization. Libraries were prepared using the small RNA library preparation kit v2.0 (Illumina Cambridge Ltd) according to manufacturer's protocol with limited modifications. In brief, adapters were ligated to the 3' end and 5' end of RNA followed by cDNA preparation through RT. The cDNA was amplified with 12 cycles of PCR. The resulting library was purified using Agencourt Ampure XP beads (Beckman Coulter Inc). Libraries were quantified using Qubit (Life Technologies) and Bioanalyzer (Agilent). Since the total amount of libraries generated was limited, the libraries were mixed together in non-equimolar concentrations and sequenced on HiSeq2000 (Illumina Cambridge Ltd). Traces of the adapter (TGGAATTCTCGGGTGCCAAGG) at the end of the 22 nucleotides for miRNAs were removed using the "Trimgalore" software, allowing for 10% mismatch and discarding reads that, after adapter removal, became shorter than 15 nucleotides. More than 99.5% of the reads were reduced in size after adapter removal.

RNA Isolation, Reverse Transcription (RT) and Pre-amplification.
RNA isolation, RT reaction and pre-amplification as well as individual qPCRs for miRNAs were performed as described previously.[3,4]

Custom-designed qPCR Plates.
The expression profile of 92 miRNAs was assessed using custom-made Exiqon LNA qPCR plates (Exiqon Life Sciences) as described previously.[4]

ACS Study Population and Sample Collection.
Plasma samples were obtained from a patient-based cohort of 125 patients with a history of ACS 30 days previously (STEMI, NSTEMI or unstable angina) who had undergone detailed assessment of platelet function.[5] The Northern General Hospital, Sheffield, UK, is the only center providing PCI and cardiac surgery services to the surrounding population of approximately 1.8 million people and protocols for antiplatelet therapy are standardized and implemented synchronously across this region. This was a prospective observational study intended to phenotype ACS patients, including assessment of the effects of changing patterns of $P2Y_{12}$ inhibitor usage. Treatment algorithms for oral antiplatelet and other secondary prevention therapies in ACS patients are described in Joshi *et al*.[5] All the patients recruited for the study provided informed consent and the study was approved by the local research ethics committee. The exclusion criteria were less than 18 years of age, serious co-morbidities, end-stage renal failure and pregnancy or suspected pregnancy. Patients enrolled in the study attended the Clinical Research Facility, Northern General Hospital, Sheffield, UK, at 30 days after the onset of ACS. Venepuncture with needle and syringe was used to obtain venous blood samples that were anticoagulated with 3.13% trisodium citrate dihydrate ("citrate").

Human Platelet Function Testing.
(1) Light transmittance aggregometry (LTA): Citrate-anticoagulated blood was first centrifuged at 200 RCF for 10 min from which PRP was extracted. The remaining blood was then centrifuged again at 1500 RCF from which PPP was extracted. A platelet count was performed on the PRP and no dilution with PPP was performed unless the platelet count was greater than 400×10^9/L in which case PPP was used to dilute the PRP to a platelet count of 400×10^9/L. Platelet aggregation response to ADP was determined using LTA (BioData PAP-8E optical aggregometer). The maximum platelet aggregation response to ADP 20 μM or

arachidonic acid 2 mM was determined after 6 minutes. *(2) VerifyNow $P2Y_{12}$ assay*: Citrate-anticoagulated blood was analyzed using VerifyNow $P2Y_{12}$ cartridges and VerifyNow analyzer (Accumetrics, USA) according to the manufacturer's instructions. Platelet reaction units (PRU) were recorded. *(3) VASP phosphorylation assay:* Citrate-anticoagulated blood was analyzed using VASP phosphorylation assay kits (BioCytex, France) and platelet reactivity index (PRI) was determined, according to the manufacturer's instructions. Fluorescence of samples was measured using an LSRII flow cytometer (Becton Dickinson).

Bruneck Cohort.

The Bruneck Study is a population-based, prospective survey of the epidemiology and pathogenesis of atherosclerosis and cardiovascular disease.[3,9] The study protocol was reviewed and approved by the Ethics Committees of Verona and Bolzano, and all participants provided their written informed consent before entering the study. At the 1990 baseline evaluation, the study population comprised an age- and sex-stratified random sample of all inhabitants of Bruneck (125 men and 125 women from each of the fifth through eighth decades of age, all white). Samples from the year 2000 follow-up were used for the present study (n=669). As part of the 2000 follow-up, citrate plasma and serum samples were drawn after an overnight fast and 12 hours of abstinence from smoking. Samples were divided into aliquots and immediately stored at −80 °C.

Platelet Spike-in.

Human platelets and plasma were isolated from 4 healthy volunteers. 16 mL of whole blood was drawn onto 4 mL of Acid-Citrate-Dextrose (ACD; stock solution: 2.5 g sodium citrate, 2.0 g glucose, 1.5 g citric acid, in 100 mL H_2O) using a 21-gauge needle and 20 mL syringe and centrifuged at 190 × g for 30 min. All centrifugations were performed at room temperature without brake. The supernatant was transferred into a new tube. To prevent platelet activation, 1 nM prostaglandin E1 (Sigma-Aldrich) and 20 μM indomethacin (Sigma-Aldrich) were added. To deplete leukocytes the supernatant was further centrifuged at 280 × g for 10 min. 100 μL of the leukocyte-depleted PRP was transferred to a 1.5 mL tube for RNA extraction. The remaining volume was transferred to a new tube and centrifuged at 1180 × g for 10 min to pellet platelets. The resulting supernatant was PPP. The platelet pellet was washed twice with modified Tyrode's buffer (134 mM NaCl, 2.9 mM KCl, 0.34 mM Na_2HPO_4, 12 mM $NaHCO_3$, 20 mM HEPES, 1 mM $MgCl_2$, pH 7.4; Glucose (45 mg/50 mL) was added just before use, and the solution was warmed to 37 °C in a water bath). Prostaglandin E1 and indomethacin were added during each wash at the concentrations mentioned above. The solution was centrifuged between washes at 1180 × g for 10 min. The final platelet pellet was resuspended in 1/20 of the volume of original PRP to obtain a 20x stock platelet solution. The 20x platelet solution was then spiked back into the PPP from the same donor to achieve 200%, 100%, 50% and 5% spike-ins. Immediately after spiking PPP, QIAzol lysis reagent was added and RNA was extracted as described previously.[3,4]

Systemic Inhibition of miR-126-3p.

Male C57BL/6J mice (Harlan), aged 8 weeks, were treated with cholesterol-conjugated antagomiR constructs (Fidelity Systems, Gaithersburg, MD) or sterile PBS. Sequences were designed to target miR-126-3p (5`-C*G*CAUUAUUACUCACGGU* A*C*G*A*-Chol*T-3') or to serve as non-targeting control (5`-A*A*GGC AAGCUGACCCUGAA*G*U*U*Chol*T-3'). Intraperitoneal injections were performed on day 0, 1 and 2, in a dose of 25 mg/kg for the platelet function assays in whole blood, and 40 mg/kg for the aggregometry experiments carried out in PRP. On day 7, mice were anesthetized using pentobarbital before collection of blood from the inferior vena cava using syringes containing lepirudin (Refludan, 25 μg/ml; Celgene, Windsor, UK). PRP was isolated as previously described[10]. Briefly, whole blood was diluted 1:1 with HEPES-Tyrode's buffer (137 mM NaCl, 20 mM HEPES, 5.6 mM glucose, 1 g/l BSA, 1 mM $MgCl_2$, 2.7 mM KCl, 3.3 mM NaH_2PO_4) before centrifugation (100 × *g*, 8 min, RT).

Platelet Function in Mice.

Platelet function tests were carried out as described previously.[11] Half-area 96-well microtiter plates (Greiner Bio-One, Stonehouse, UK) were pre-coated with hydrogenated gelatin (0.75% w/v; Sigma, UK) in PBS to block non-specific activation of blood. 4 µl of vehicle or agonist solution was then added to each well: arachidonic acid (AA; 0.03–0.6 mM; Sigma, Poole, UK), Horm collagen (0.1–3 µg/ml; Nycomed, Linz, Austria), the PAR-4 activating peptide AYPGKF amide (PAR4-AP, 50–100 µM; Bachem, Bubendorf, Switzerland), and the stable TxA_2 mimetic U46619 (0.1–10 µM; Cayman Chemical Company, Ann Arbor, USA). To each well, 35 µl of PRP or whole blood was added and the plate was then placed onto a heated plate shaker (Bioshake IQ, Q Instruments, Jena, Germany) at 37 °C for 5 min mixing at 1200 rpm. Where appropriate, light transmission of each well was determined using a 96-well plate reader (SunriseTM, Tecan, Männedorf, Switzerland) at 595 nm. Alternatively, samples were diluted 1:5 with an acid citrate dextrose solution (5 mM glucose, 6.8 mM trisodium citrate, 3.8 mM citric acid) before individual platelet counts of each were determined by flow cytometry. Platelets were labeled with APC-conjugated anti-CD41 (clone eBioMWReg30) for 30 min before further dilution 1:50 in phosphate buffered saline containing 0.1% formalin (Sigma, UK), 0.1% dextrose and 0.2% bovine serum albumin (BSA) and addition of 104 CountBright™ absolute counting beads (Life Technologies). Labeled, diluted blood was then analyzed using a FACSCalibur flow cytometer (BD Biosciences, Oxford, UK).

RESULTS

NGS of plasma miRNAs.

In order to characterize circulating small RNAs, libraries of small RNAs were generated from platelet-rich (PRP) and platelet-poor (PPP) plasma for NGS. The presence of platelets and the absence of leukocyte contamination in PRP samples were verified by qPCR for ITGA2B and CD45, respectively (Online Figure IA). The quality of base-calls is represented by consecutive boxplots for each position on the 100 nucleotide read (Online Figure IB). The total number of digital reads correlated with the amount of RNA obtained from PPP and PRP (r_p=0.94). Two peaks were observed across all four samples, one at 22-23 nucleotides corresponding to miRNAs, and another centered around the 32-33 nucleotide range (Fig. 1A), which aligned predominantly to RNY4, a non-coding RNA, on chromosome 7. The 25 most abundant small RNAs in each sample are shown in Fig. 1B. At a threshold of 0.4 reads per million and without distinguishing between isomiRs, 224 miRNAs were consistently identified in all samples (Online Table I). There was a good correlation for the number of reads per miRNA (Online Figure IC). Coverage of RNY4 was predominantly observed on the 5' end, with some additional fragments from the 3' end. Sequences derived from other RNY genes were also detected; albeit at much lower read counts: For RNY1 and RNY5, like RNY4, mostly 5’ fragments were found; for RNY3 the 3’ fragments were more abundant (Online Figure II). 203 out of 224 miRNAs and both RNY4 fragments were detected at higher levels in PRP than PPP, suggesting that most circulating small RNAs are present in platelets (Online Figure III).

Effect of anti-platelet therapy.

We have previously reported that anti-platelet therapy reduces plasma levels of platelet-related miRNAs, including miR-126 and miR-223.[4] 92 miRNAs were measured using custom-made Exiqon LNA qPCR plates. The plate layout has been published previously[4] and is shown in Online Table II. In a PCA, the interindividual variability in plasma miRNA profiles decreased with prolonged platelet inhibition[4] (Fig. 2A), reinforcing the concept that platelet activity is an important determinant of the plasma miRNA pool. To explore how different anti-platelet agents affect plasma miRNAs, the same 92 miRNAs were screened in a closely matched cohort of ACS patients who were either on ASA only (n=8), ASA+clopidogrel (n=8), ASA+prasugrel (n=8) or ASA+ticagrelor (n=8) for 30 days after the acute event. In the PCA analysis, no

clear separation was obtained between the different anti-platelet agents but the least inter-patient variability was observed in the ASA+prasugrel group (Fig. 2B).

Correlation to platelet function in ACS patients.

Next, platelet-related miRNAs (Online Figure IV) and abundant plasma YRNA fragments were measured by individual qPCR assays in the ACS patient cohort who had undergone detailed assessment of platelet function (n=125, Online Table III). The correlations of small RNAs to measurements of platelet function are shown in Fig. 3. No correlation was found between miRNA and YRNA levels and optical aggregometry in response to arachidonic acid (AA) or ADP. In contrast, significant associations were obtained for the VASP phosphorylation assays, e.g. miR-223 (r_p=0.28, P=0.002), miR-24 (r_p=0.25, P=0.006), miR-191 (r_p=0.24, P=0.009), RNY4 3' (r_p=0.23, P=0.012), miR-126 (r_p=0.22, P=0.016) and RNY4 5' (r_p=0.21, P=0.025). For these miRNAs and YRNA fragments, the correlations with the VerifyNow $P2Y_{12}$ assay were of similar strength (r_p=0.18-0.35), but the statistical power was weaker in view of low numbers and nominal significance was only noted for miR-126 (r_p=0.35, p=0.033). No associations were obtained for miR-93, miR-106a, miR-146b and miR-150.

Evidence for platelet origin.

Although all candidate miRNAs were present in platelets (Online Figure IV), some reflected platelet activation better than others. To determine to what extent platelets may contribute to circulating miRNA and YRNA levels, we reconstituted PPP with washed platelets. Platelets were isolated from PRP and spiked back into PPP corresponding to 5, 50, 100 or 200% of the initial volume (Fig. 4A). For miR-126 and miR-223, a significant linear increase was observed with increasing platelet content (Fig. 4B). As expected, the liver-specific miRNA, miR-122, was not affected by addition of platelets. Notably, miR-126 showed the greatest dependency on platelets in comparison to 21 other miRNAs (Fig. 4C). This spike-in experiment provides further evidence for platelets being a major source of circulating miRNAs, including miR-126, which was previously implicated to be of endothelial origin.[12-14] Levels of RNY4 fragments were also strongly affected by addition of platelets (Fig. 4C). Unlike miRNAs, however, RNY4 was not associated with argonaute-2 complexes in MEG-01 cells, a human megakaryoblastic cell line (Online Figure V).

Mir-126 and platelet function in the general population.

MiRNAs and RNY4 fragments were measured in the Bruneck study (n=669) and correlated to the platelet activation markers[15] platelet factor 4 (PF4), pro-platelet basic protein (PPBP) and P-selectin (SELP) as quantified by ELISA (Fig. 5A). Substantial positive correlations of miRNAs and RNY4 fragments with all three platelet-activation markers were observed in plasma. These were more pronounced for the platelet-specific proteins PF4 and PPBP than for SELP, which is shed from platelets as well as endothelial cells.[16,17] The liver-specific miRNA, miR-122, and other miRNAs, such as miR-150, showed no or considerably weaker correlations with platelet activation markers (Fig. 5A). Accordingly, there was a striking correlation between the platelet dependency of miRNAs in the spike experiment and the correlations of miRNAs with platelet activation markers in the general population (r=0.92 to 0.94, Fig. 5B, Online Fig. VI)**.** RNY4 fragments were strongly correlated with platelet-derived plasma miRNAs (Fig. 5C), suggesting a common platelet origin.

While circulating miRNAs have been shown to be affected by disease state, cardiovascular risk factors and drug treatment, the influence of genetic variability, especially single nucleotide polymorphisms (SNPs), on miRNA expression and function is poorly understood. Thus far, only one functional SNP has been described for miR-126 (Fig. 6A): The primary sequence of human miR-126 contains a SNP (dbSNP: rs4636297) downstream of the pre-miR sequence.[8] The genotype of this SNP has been shown to affect the

processing of pri-miR-126: pri-miR-126 encoded by the major "G" allele is processed to a lesser extent than pri-miR-126 encoded by the minor "A" allele. In the Bruneck cohort (Online Table IV), there was a trend towards higher miR-126 levels in individuals homozygous for the minor allele (AA genotype), that facilitates processing of miR-126, compared to the GA + GG genotype in serum (+4.4%, P=0.050) and in plasma (+6.9%, p=0.099) (Online Fig. VII). Importantly, the AA genotype was associated with higher plasma levels of platelet activation markers: PF4 (p=0.002), PPBP (P<0.001) and SELP (p=0.099) (Fig. 6B). To compare the effect on platelet proteins to other plasma proteins, we measured 219 proteins, utilizing mass spectrometry for detection of high-abundant proteins (n=84) and proximity extension assays for detection of low-abundant proteins (n=132) as well as ELISA (n=3). Out of 62 proteins whose levels are significantly associated with the AA genotype (Online Fig. VIII), PPBP and PF4 showed the 2nd and 3rd highest fold change (mean ratios AA *vs* GA + GG of 1.49 and 1.47, respectively, Online Table V). The proteins associated with the AA genotype also showed an enrichment of the GO term annotation "platelet activation" (p = 0.019, Online Fig. VIII**).**

MiR-126 and platelet function in mice.

The genetic associations of a SNP within miR-126 with parameters of platelet function prompted us to further investigate the role of miR-126 in platelet activation. It has recently been reported that oligonucleotides with a phosphorothioate backbone modification (PS, minimum length of 18 nt) can activate platelets.[18] Thus, we incubated human PRP with fully PS-modified LNAs as well as antagomiRs with 2 and 4 PS-modified nucleotides at the 5' and 3' end, respectively. None of the tested oligonucleotides induced platelet aggregation (Online Fig. IX). Next, C57BL/6J mice were injected with PBS, a control antagomiR or antagomiR-126-3p at 25 mg/kg i.p. for three consecutive days and sacrificed at day 7 (Fig. 7A). The knockdown of miR-126 was confirmed in blood samples by qPCR (Online Fig. X). Whole blood was treated with different concentrations of agonists: arachidonic acid (AA), collagen, PAR4-AP and the TxA_2 analog U46619 (Fig. 7B). The aggregation response to arachidonic acid and U46619 was significantly reduced in platelets from mice treated with antagomiR-126-3p. At antagomiR doses of 25 mg/kg, however, there was no effect on platelet aggregation in response to collagen or PAR4-AP. Thus, we repeated the experiment with a higher antagomiR concentration (40 mg/kg i.p.). Again, the inhibition of miR-126 was confirmed by qPCR (Online Fig. X). To further address the effect of miR-126 in platelets and to minimize a potential influence of other cell types, PRP was used instead of whole blood for platelet aggregation. AntagomiRs against miR-126-3p blocked platelet aggregation induced by 0.6 mM AA (Fig. 7C), and led to a significant reduction of aggregation in response to 50 µM PAR4-AP. The attenuated response to 0.3 µg/ml collagen failed to reach statistical significance. No differences in platelet aggregation were observed at higher agonist concentrations of PAR4-AP (100 µM) and collagen (3 µg/ml) (data not shown).

Targets of mir-126 in platelets.

To explore potential mechanisms, we measured the expression levels of known miR-126 targets with a role in platelet function alongside platelet-related genes (Online Table VI). We observed reduced expression of the $P2Y_{12}$ receptor in whole blood of antagomiR-126-3p treated mice (Fig. 8A). In a human megakaryoblastic cell line (MEG-01), mimics or LNA inhibitors of miR-126-3p (Fig. 8B-C, Online Fig. XI) regulated ADAM9, a confirmed target of miR-126 that has been shown to impact on collagen-induced platelet aggregation (Fig. 8D).[19] Thus, miR-126 affects gene expression in megakaryocytes.

DISCUSSION

In this study, miRNA measurements were performed in 669 subjects of a population-based study as well as in 125 patients with ACS. The study correlates miRNAs with platelet activation markers in the general population and with the residual platelet activity in ACS patients on anti-platelet therapy. Most but not all abundant platelet miRNAs were positively correlated with the VerifyNow $P2Y_{12}$ and VASP assays, which are standardized assays for assessing the effects of $P2Y_{12}$ inhibitors. Additionally, we show an association of plasma YRNAs with platelets and that inhibition of miR-126 attenuates platelet aggregation in response to low but not high agonist concentrations.

Platelet dependency of small RNAs in plasma.

To demonstrate the platelet dependency of our candidate miRNAs, washed platelets were isolated from PRP and spiked back into PPP. This spike-in experiment rules out a cellular contamination, which can hamper direct comparisons between PRP and PPP, and provide further evidence that the selected miRNAs, including miR-126, are genuine platelet miRNAs.[4] Furthermore, we provide evidence that RNY4 fragments in plasma also originate from platelets. YRNA fragments are enriched in exosomes.[20,21] Our NGS data confirm that RNY4 fragments are abundant in plasma.[22] Unlike miRNAs, however, RNY4 fragments were not present in the Ago2 complexes of MEG-01 cells.[23,24] Recently, YRNA fragments were implicated as biomarkers for coronary artery disease.[25] Changes in circulating levels were attributed to apoptotic macrophages, which generate and secrete YRNA fragments.[25] In contrast, our data suggest that platelets are a major source of circulating YRNA fragments, and cellular origin has to be taken into account if YRNA fragments are considered as potential biomarkers for cardiovascular disease. The function of YRNA fragments remains unknown, but it has been suggested that YRNA fragments, including RNY4 5', act as small guide RNAs for tRNase Z^L, forming a tRNA-like duplex with a target RNA, thereby enabling its hydrolysis.[26] This mechanism has been shown for synthetic target RNAs in vitro, and it remains to be seen whether YRNA fragments can act as guide RNAs for tRNase Z^L in vivo.

Platelet miRNAs in patients post ACS.

Plasma was taken 30 days after the acute event when the inflammation associated with the acute injury has receded and anti-platelet drugs had been administered for a month. Importantly, none of the ACS patients had received heparin at the time of sampling.[27,28] Levels of miR-126 and other platelet-related miRNAs showed a positive correlation with the VerifyNow $P2Y_{12}$ and VASP phosphorylation assays. VerifyNow $P2Y_{12}$ is a commercial assay that uses whole blood to monitor $P2Y_{12}$ inhibition.[29] The VASP assay is considered to be among the most specific assays to monitor $P2Y_{12}$ inhibition since it does not rely on co-activation of the $P2Y_1$ receptor by ADP.[30] In contrast, optical aggregometry is performed on isolated platelets. Theoretically, aggregometry responses to ADP and the VASP and Verify Now $P2Y_{12}$ assays assess the same parameter: $P2Y_{12}$ receptor activation. However, the variability of the aggregometry results in isolated platelets being higher compared to tests performed in whole blood may explain the loss of associations with plasma miRNAs. Previous studies have suggested that VerifyNow $P2Y_{12}$ and VASP assays are more discriminating of clopidogrel response and its genetic influences compared to light transmittance aggregometry, and there are only moderate correlations between the different platelet function assays.[30,31] Moreover, aspirin is such a potent inhibitor of the platelet response to AA that any variation between patients is expected to be low. Aspirin inhibits the production of TxA_2. Clopidogrel and prasugrel act by blocking the platelet $P2Y_{12}$ receptor.[5,29] Thus, their mechanisms are complementary. Clopidogrel and prasugrel are both thienopyridine prodrugs that are converted via hepatic CYP isoenzymes to their active metabolite but prasugrel is more efficiently converted to its active form and so achieves more reliable $P2Y_{12}$ inhibition. Ticagrelor is a novel $P2Y_{12}$ receptor antagonist but its clinical profile, both in terms of efficacy and adverse events, differs from that of the thienopyridine prodrugs.[32] Ticagrelor is also supposed to have a dual mode of action as its $P2Y_{12}$ antagonism is complemented by inhibition of adenosine

cell uptake via inhibition of the equilibrative nucleoside transporter 1 thereby increasing extracellular adenosine level and mediating adenosine-receptor activation.[32]

Platelet miRNAs and platelet activation in the general population.

It is currently unclear to what extent platelet miRNAs are mechanistically involved in platelet activation. A SNP (rs4636297) in the miR-126 locus has previously been demonstrated to affect the expression of mature miR-126 in cells overexpressing the different variants of pri-miR-126. An effect on endogenous miR-126 levels has not been investigated thus far.[8] In plasma and serum from the Bruneck cohort, we observed a trend towards higher levels of circulating miR-126 in individuals carrying the minor allele (AA genotype), which facilitates the processing of pri-miR-126. Importantly, the SNP genotype affected the plasma concentrations of three platelet activation markers: PF4 ($p=0.002$), PPBP ($P<0.001$) and SELP ($P=0.099$) were all positively correlated with the AA genotype. Moreover, for proteins showing an association with the AA genotype in a panel of 219 plasma proteins measured in the Bruneck cohort, there was a significant enrichment of proteins linked to platelet activation ($p=0.019$, Online Fig. VIII). These genetic associations, however, await confirmation in independent cohorts. MiR-126 is abundant in endothelial cells and platelets.[3] While miR-126 is known to be an important regulator of endothelial cell function[12,33], its role in platelets is unknown. It is therefore conceivable that higher miR-126 levels in individuals with the AA genotype influence platelet activity either directly by changing platelet function or indirectly by affecting endothelial cells. Recently, the A allele of SNP rs4636297 has been shown to be associated with sight-threatening diabetic retinopathy in patients with type II diabetes mellitus[34], corroborating the relevance of genetic variability of miRNAs for susceptibility to disease.

MiR-126 and platelet aggregation in mice.

Despite numerous reports on miRNAs, surprisingly little is known about their function in platelets. MiR-223 targets the $P2Y_{12}$ receptor.[35] MiR-223 deficient mice form larger thrombi and have a delayed clot retraction compared to wild type mice.[36] Platelets of miR-223 deficient mice display increased aggregation in response to thrombin and collagen but not to fibronectin and the TxA_2 analog U44619. MiR-96 regulates VAMP8/endobrevin, a protein involved in platelet granule secretion that is upregulated in hyperreactive platelets.[37] Our experiments demonstrate that inhibition of miR-126 in mice attenuates platelet aggregation in response to AA, U44619, and PAR4-AP. Collagen-induced platelet aggregation may also be affected, but we did not obtain statistical significance, probably due to low numbers. Platelet aggregation was reduced in whole blood and in PRP. Thus, the involvement of other cell types is less likely. Moreover, MEG-01 cells express miR-126 in abundance and mimics of miR-126 reduced the expression of ADAM9 while inhibition of miR-126 had the opposite effect. ADAM9 is a predicted and experimentally confirmed target of miR-126 that attenuates the adhesion of platelets to collagen.[19] Given its function as a protease of the ADAM family, it may alter the platelet response by cleaving membrane proteins. In whole blood from antagomiR-treated mice, ADAM9 was not differentially expressed. This is expected because ADAM9 is present in many other blood cells, which lack miR-126 and therefore are not affected. Notably, the expression of the $P2Y_{12}$ receptor was reduced in blood of antagomiR-126-3p treated mice. $P2Y_{12}$ levels are much higher in platelets than in leukocytes[38] and erythrocytes.[39] Thus, the latter finding may, at least in part, explain why platelets from antagomiR-126-3p treated mice display an attenuated aggregation response: The aggregation responses to strong platelet agonists, such as collagen, thrombin receptor-activating peptides and particularly either TxA_2 (generated from AA) or its mimetic U46619, are amplified by ADP, which is released from the platelet dense granules in response to the agonists and activates the $P2Y_{12}$ receptor. On the other hand, higher concentrations of these agonists or less effective $P2Y_{12}$ inhibition or deficiency allows a more robust platelet aggregation response. $P2Y_{12}$ activation may also contribute to the generation of TxA_2 from AA.[40]

Study limitations.

Causality cannot be inferred from associations of miRNAs with platelet function tests in ACS patients. Although recent studies implicated miR-126 in atherogenesis[41,42] and miR-223 has been used for categorization of patients as 'responder' and 'non-responder' to the $P2Y_{12}$ inhibitor clopidogrel[43], larger cohorts with prolonged follow-up are required to determine if miRNA levels are associated with clinical outcomes in ACS patients. Since the $P2Y_{12}$ receptor plays such an important role in platelet reactivity, relationships between the extent of $P2Y_{12}$ receptor inhibition and miRNA levels have to be further explored in future studies. Similarly, the reported association of a SNP for miR-126 with platelet activation markers requires replication in independent cohorts. MiR-126 is also abundant in endothelial cells, and indirect effects on platelets cannot be excluded. On the other hand, gene expression in MEG-01 cells may be subject to different regulation mechanisms than in primary megakaryocytes and platelets, i.e. in undifferentiated MEG-01 cells $P2Y_{12}$ expression is not detectable.

Conclusion.

Numerous studies have demonstrated the importance of platelet reactivity in the risk of clinical events such as stent thrombosis following PCI or recurrent arterial thrombotic events following ACS. Exciting opportunities exist to further pursue platelet miRNAs as potential biomarkers for treatment response in ACS patients.[44] MiRNAs can be measured in frozen samples, which could offer a potential advantage compared to other platelet function tests currently available. Besides their biomarker potential, some miRNAs, such as miR-126 and miR-223,[36] may also regulate platelet reactivity.

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DISCLOSURE
King's College London filed and licensed patent applications related to circulating miRNAs as biomarkers. R.F.S. reports receiving research grants, honoraria and/or consultancy fees from AstraZeneca, Aspen, ThermoFisher Scientific, Eli Lilly/Daiichi Sankyo, Merck, Accumetrics, Novartis, Correvio, PlaqueTec, Sanofi-Aventis, Medscape, Regeneron, Roche and The Medicines Company.

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FIGURE LEGENDS

Figure 1. NGS of small RNAs in plasma. (A) Representative histogram of sequence lengths for PPP after processing for adapter removal and lower bound on length. **(B)** Summary of the top 25 small RNAs (reads per million total reads) in each sample. YRNA fragments are highlighted in bold.

Figure 2. MiRNAs and anti-platelet therapy. PC analyses based on screening of 92 plasma miRNAs using custom-made qPCR plates. **(A)** Decreasing variability of plasma miRNA profiles in healthy volunteers after 2 and 3 weeks of platelet inhibition (n=6 at four time points). **(B)** Effects of different anti-platelet agents in patients with ACS (n=8 per group). The tables show the variances of the two principal components PC1 and PC2. An F-test was used to calculate the differences in variance; P-values reflect difference of variance compared to baseline **(A)** or 75mg ASA **(B)**. "OD" denotes once daily, "BD" twice daily.

Figure 3. Pearson correlation forest plot depicting associations of 11 miRNAs and two YRNA fragments to platelet counts and platelet function tests in ACS patients (n=125). Measurements of RNAs by individual Taqman assays were normalized to exogenous *Cel*-miR-39. Note that fewer samples were measured with the Verify Now (n=40) compared to the VASP aggregation assay (n=121). ADP, denotes adenosine diphosphate; AA, arachidonic acid; PRU, $P2Y_{12}$ Reaction Units; VASP, vasodilator-stimulated phosphoprotein.

Figure 4. Platelet spike-in experiment. (A) Schematic summary of workflow. PPP and PRP denote platelet-poor and platelet-rich plasma. **(B)** Dependence of miR-126, miR-223 and miR-122 levels on platelet spike-in. **(C)** P values for the dependence of levels of 22 microRNAs and two RNY4 fragments on platelet spike-in. RNAs marked in bold were tested in the ACS cohort. The grey line represents the significance threshold for a P value of 0.05 after Bonferroni correction. **(B, C)** Results shown are derived from linear mixed models featuring fixed effects for platelet spike-in in categorical form **(B)** or in continuous form **(C)**, random intercepts for subjects, and a general (unconstrained) covariance structure.

Figure 5. Small RNAs and platelet proteins in the Bruneck cohort. (A) Association of plasma miRNAs and YRNA fragments with three platelet activation markers (PF4, PPBP, SELP) in the population-based Bruneck study (n=669). **(B)** Relationship between the dependency of small RNA levels on platelets in the spike-in experiment (x-axis, see **Fig. 4**) and the correlation of small RNA levels with platelet-derived protein concentrations (PF4, PPBP) in the general population (y-axis, see **A**) for 16 miRNAs and two RNY4 fragments. Lines are Deming regression lines and r denotes Pearson correlation with 95% confidence interval. **(C)** Association of plasma YRNA fragments with plasma miRNAs measured in the Bruneck cohort (n=669). **(A, C)** Tile color codes for direction and magnitude of correlation, while tile text gives its sign and first two decimal digits. PF4, platelet factor 4; PPBP, pro-platelet basic protein; SELP, P-selectin.

Figure 6. Association of rs4636297 with plasma markers of platelet activation. **(A)** Schematic representation of pri-miR-126. Cleavage sites for Drosha/DGCR8 and Dicer are indicated in gray. The SNP rs4636297 is located downstream of the stem-loop (pre-miR-126). pri-miR-126 carrying the major "G" genotype, is less efficiently processed than the minor "A" genotype. **(B)** Plasma levels of platelet activation markers are associated with the rs4636297 genotype in the Bruneck cohort (n=628), P values are shown for one-way ANOVA. PF4, platelet factor 4; PPBP, pro-platelet basic protein; SELP, P-selectin.

Figure 7. Platelet function in mice treated with antagomiR against miR-126-3p. (A) 8 week old male C57BL/6J mice were injected with PBS, control antagomiR or antagomiR-126-3p for three consecutive days and sacrificed on day 7 for platelet function tests. **(B)** Platelet aggregation was measured in whole blood from mice injected i.p. with 25 mg antagomiR/kg in response to different concentrations of the indicated agonists. Asterisks denote significant difference in a two-way ANOVA with Bonferroni post-test

(*, P<0.05; **, P<0.01). **(C)** Platelet aggregation was assessed in PRP in mice injected i.p. with 40 mg antagomiR/kg. Asterisks denote significant difference in a one-way ANOVA with Bonferroni post-test (*, P<0.05). **(B, C)** Data are shown as mean +SEM, n=4 per condition. AA, arachidonic acid.

Figure 8. Effects of miR-126 in mice and MEG-01 cells. (A) Gene expression was analysed in whole blood from mice treated with 25 or 40 mg/kg antagomiR, n=8 per group. Gene expression was normalised to Actb, Gapdh and Ppia. Gene expression was analysed in MEG-01 cells transfected with mimic-126 **(B)** or LNA-126 **(C)** and the respective controls. Gene expression was normalised to GAPDH and SP1. Validated targets of miR-126 are shown in green, predicted targets are shown in blue, platelet-enriched genes are shown in orange. **(B, C)**: n=4 per condition. Graphs represent mean+SEM, asterisks denote statistical significance in a two-way ANOVA with Bonferroni post-test (*, p<0.05; **, p<0.01; ***, p<0.001). **(D)** Schematic illustration of potential miR-126-dependent effects on platelet function.

NOVELTY AND SIGNFICANCE

What Is Known?

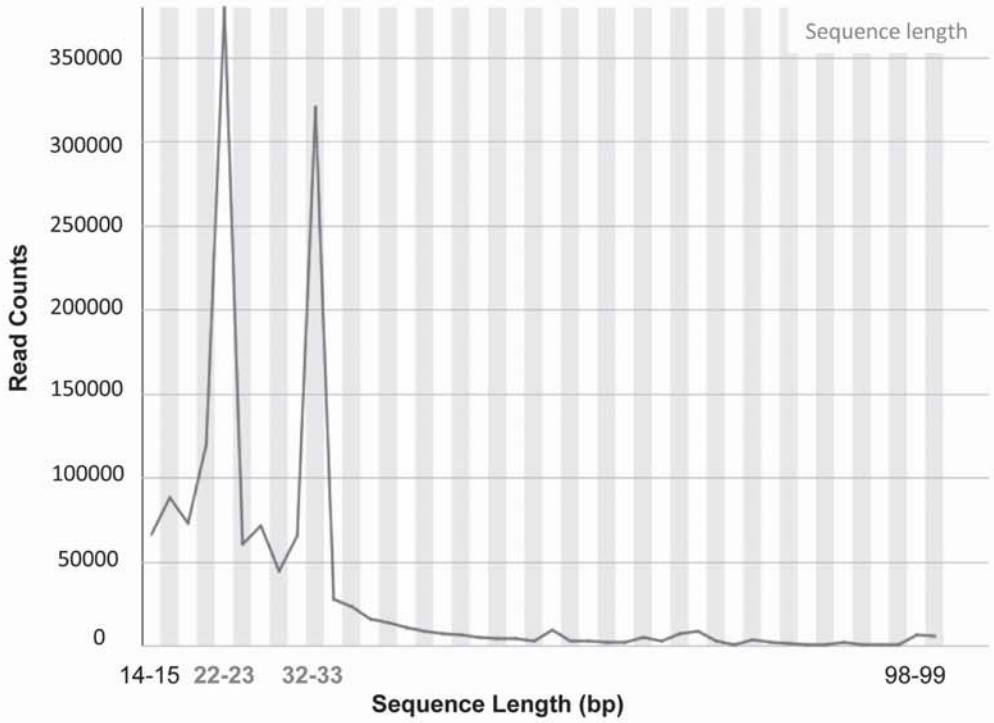
- Plasma microRNAs are highly correlated.
- Platelets contain and release microRNAs.
- Platelet inhibition reduces microRNA levels in platelet-poor plasma.

What New Information Does This Article Contribute?

- Besides microRNAs, YRNA fragments in plasma are also platelet-derived.
- Platelet microRNAs and YRNA fragments correlate with indices of platelet function in patients on dual anti-platelet therapy.
- MicroRNA-126 alters platelet activity.

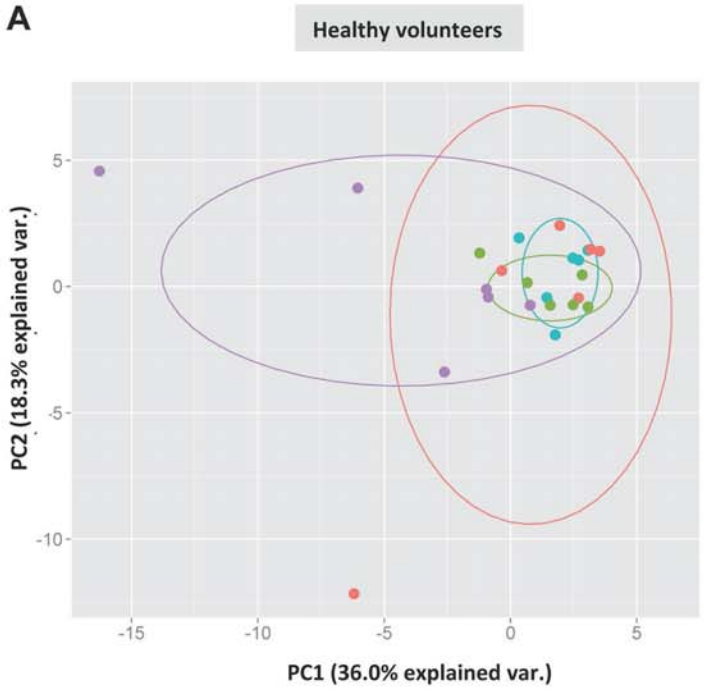
This study provides evidence for platelets being a source of microRNAs and YRNA fragments in plasma. A strong platelet dependency of microRNAs and YRNA fragments was observed in a spike-in experiment. There was also a striking correlation of microRNAs and YRNA fragments with platelet activation markers in the general population. Plasma microRNA and YRNA levels are associated with residual platelet activity in patients on dual anti-platelet therapy. MicroRNA-126, previously considered to be endothelial specific, is present in platelets and in a human megakaryoblastic cell line. A single nucleotide polymorphism that facilitates processing of microRNA-126 increases plasma levels of platelet activation markers. Treatment with antagomiRs to microRNA-126 reduces platelet activation in mice. MicroRNAs may not just be markers of platelet activity but also alter their function, most probably by influencing gene expression in megakaryocytes.

A



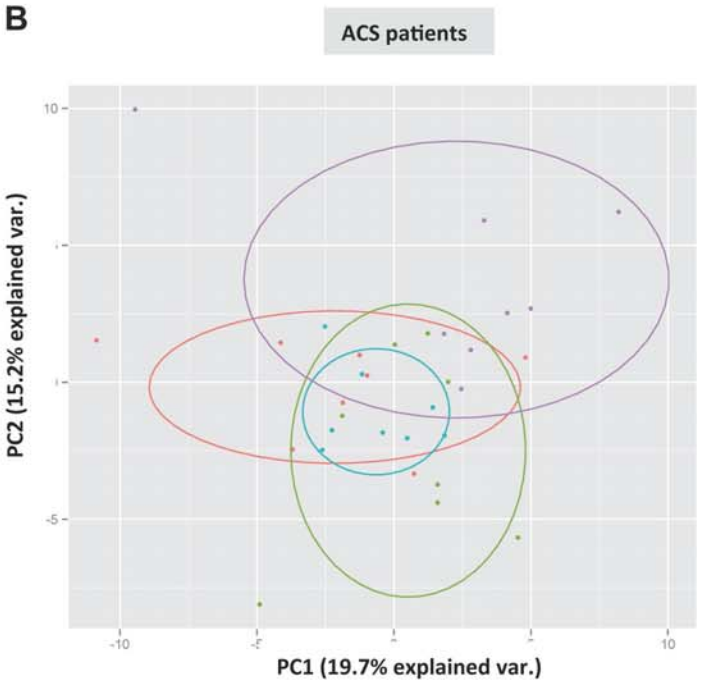
B

	PPP				PRP			
	1		2		3		4	
Rank	Name	Norm. Count	Name	Norm. Count	Name	Norm. Count	Name	Norm. Count
1	**RNY4 5'**	**22308**	miR-486	15392	**RNY4 5'**	**97325**	**RNY4 5'**	**101280**
2	miR-486	20832	**RNY4 5'**	**5957**	miR-486	22977	miR-486	22038
3	miR-92a	2865	miR-92a	2417	miR-26a	15454	miR-191	16986
4	miR-191	1651	miR-22	1168	miR-191	14051	miR-26a	14734
5	miR-423-5p	1428	miR-191	897	miR-92a	10121	miR-92a	9482
6	miR-10b	1336	miR-423-5p	894	let-7f	8248	let-7f	8999
7	**RNY5 5'**	**1213**	miR-16	772	miR-22	8222	miR-22	8306
8	miR-22	1191	miR-26a	689	**RNY4 3'**	**7183**	let-7a	6179
9	let-7f	813	miR-10b	668	let-7a	5986	miR-423-5p	5803
10	miR-142	810	miR-142	586	miR-423-5p	4367	**RNY4 3'**	**5091**
11	miR-26a	808	miR-451a	553	miR-181a	3911	miR-126-5p	4900
12	**RNY4 3'**	**735**	**RNY5 5'**	**521**	miR-30d	2943	miR-181a	3965
13	miR-181a	726	miR-181a	515	miR-151a-5p	2846	miR-21	3658
14	miR-16	528	let-7f	463	miR-16	2813	miR-30d	3526
15	miR-10a	520	miR-25	341	miR-126-5p	2661	miR-16d	3069
16	let-7a	409	miR-126-5p	327	miR-423-3p	2642	miR-142	2659
17	miR-126-5p	397	miR-10a	326	miR-222	2349	miR-151a-5p	2425
18	miR-25	390	let-7a	257	miR-142	2293	miR-30e	2088
19	miR-192	357	miR-27b	241	miR-21	2221	miR-151a-3p	2076
20	miR-27b	331	miR-30e	239	**RNY5 5'**	**1892**	miR-423-3p	1976
21	hsa-miR-30d-5p	293	**RNY4 3'**	**238**	hsa-miR-30e	1845	hsa-miR-27b	1928
22	**RNY1 3'**	**255**	hsa-miR-30d	235	hsa-miR-151a-3p	1724	hsa-miR-222	1837
23	hsa-miR-21-5p	249	hsa-miR-192	210	hsa-miR-409	1438	**RNY5 5'**	**1679**
24	hsa-miR-148a-3p	240	hsa-miR-21-5p	172	hsa-miR-28-3p	1373	hsa-miR-28-3p	1667
25	hsa-miR-423-3p	239	hsa-miR-186	172	hsa-miR-27b	1252	hsa-let-7i	1425



Baseline: Untreated
Week 1: 10mg Prasugrel OD
Week 2: 10mg Prasugrel OD + 75mg ASA
Week 3: 10mg Prasugrel OD + 300mg ASA

Group	Variance PC1	Variance PC2	p-value
Baseline	39.59	9.17	
Week 1	13.65	30.21	0.60
Week 2	0.99	2.06	**9.45e-05**
Week 3	2.65	0.74	**1.95e-04**



75mg ASA (30 days)
75mg ASA + 75mg Clopidogrel OD (30 days)
75mg ASA + 90mg Ticagrelor BD (30 days)
75mg ASA + 10mg Prasugrel OD (30 days)

Group	Variance PC1	Variance PC2	p-value
75mg ASA	20.16	3.39	
75mg ASA + 75mg Clopidogrel	8.06	12.55	0.99
75mg ASA + 90mg Ticagrelor	26.41	11.2	0.44
75mg ASA + 10mg Prasugrel	3.15	2.32	**0.005**

FIGURE 3

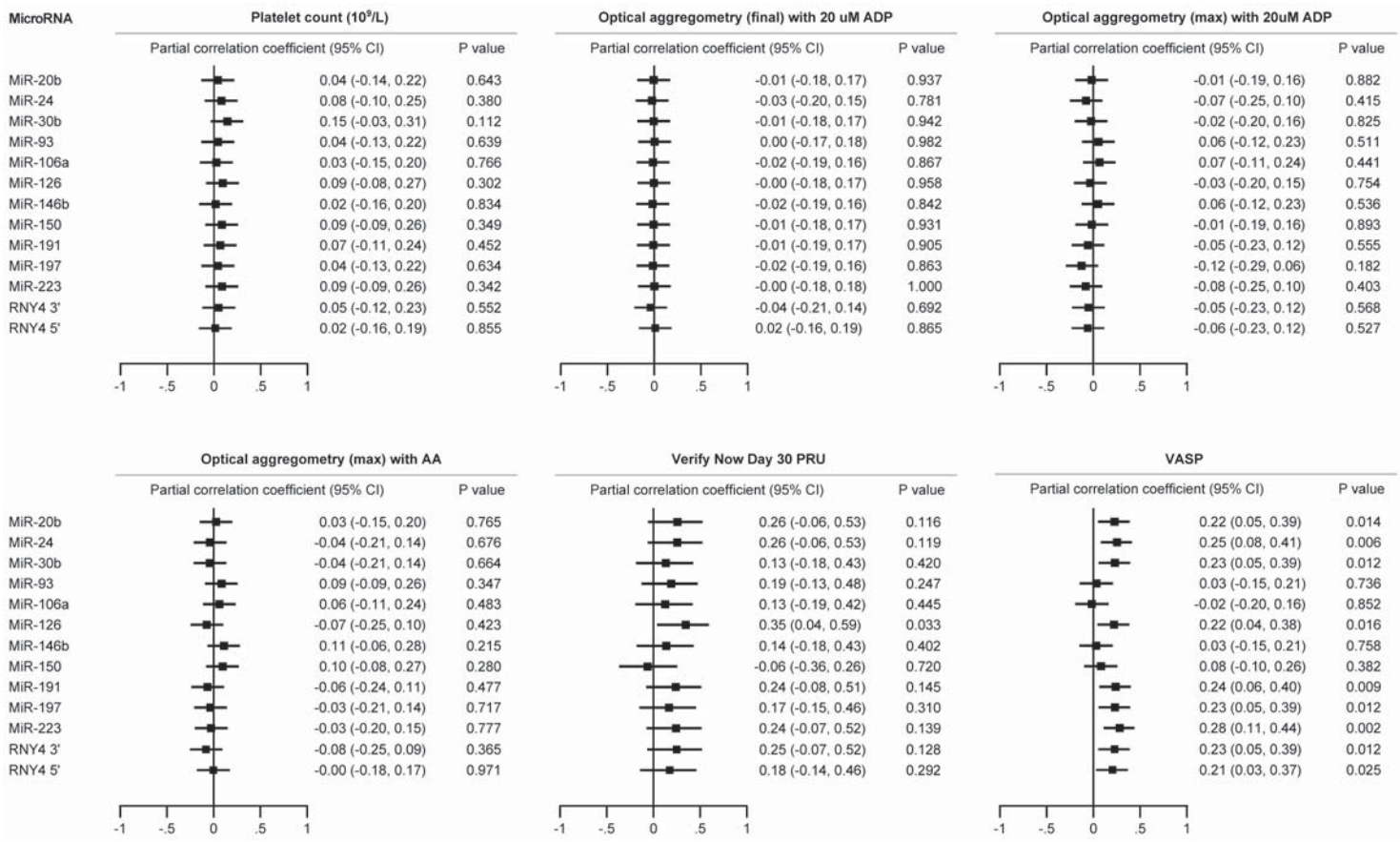
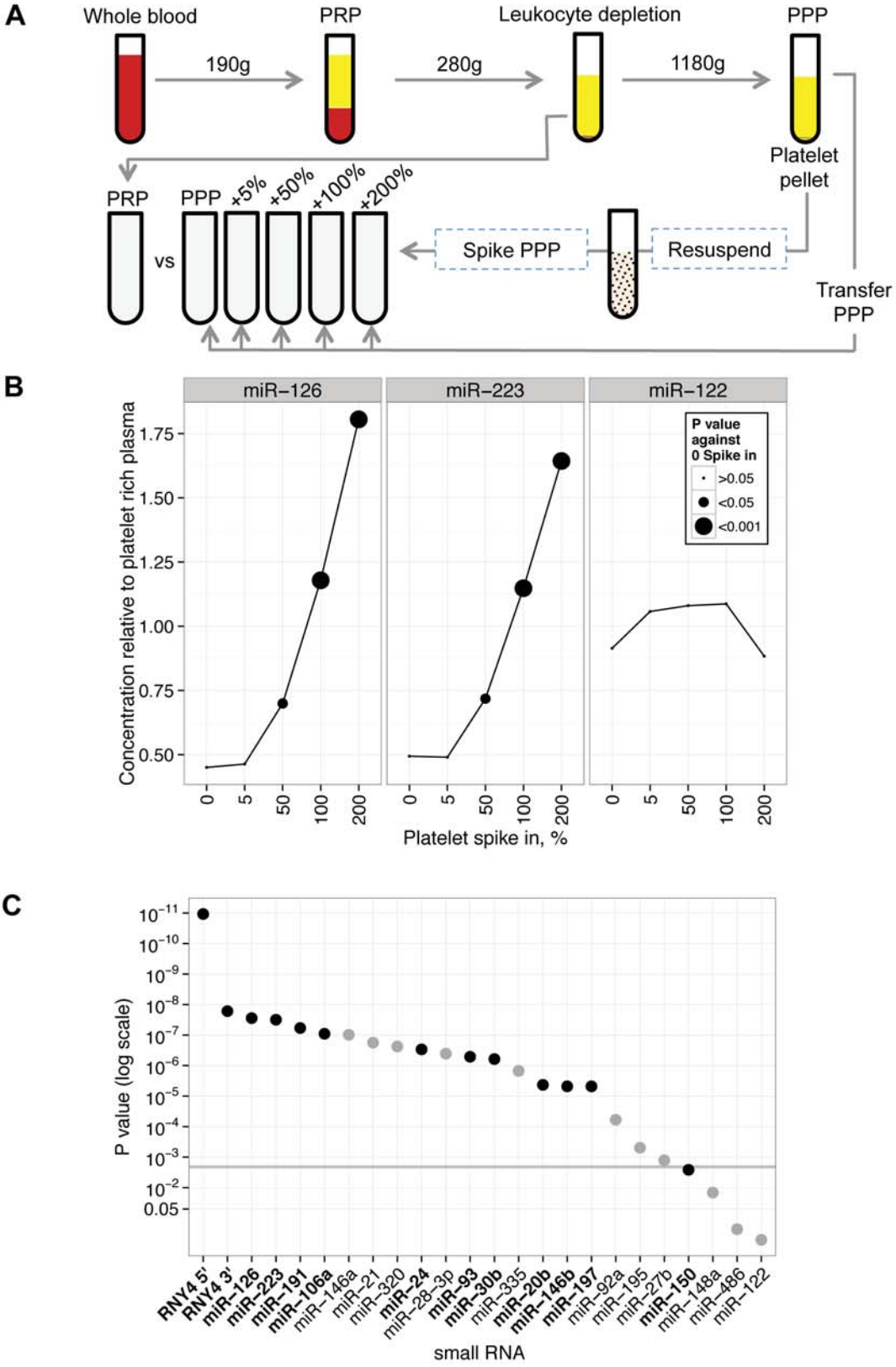


FIGURE 4



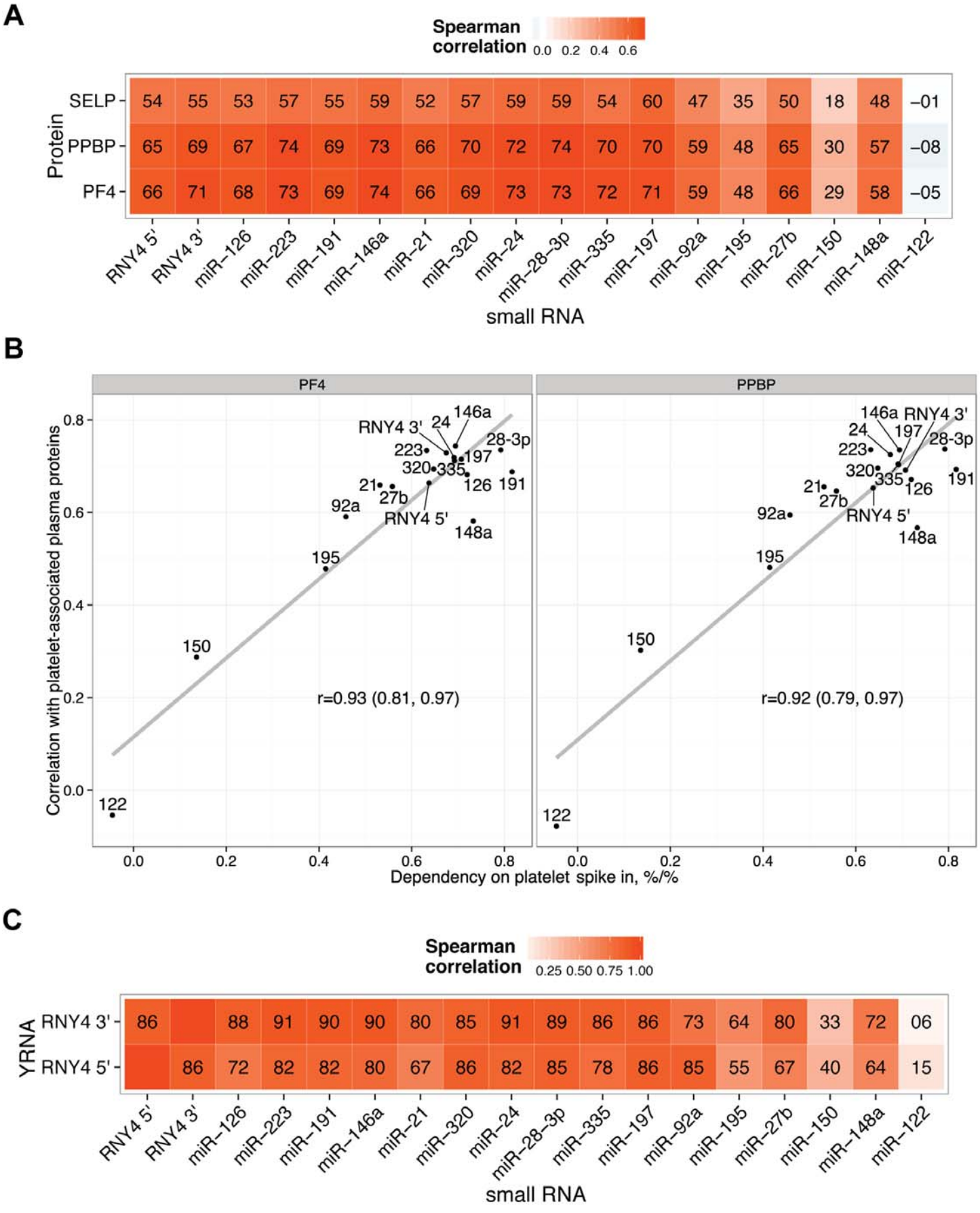
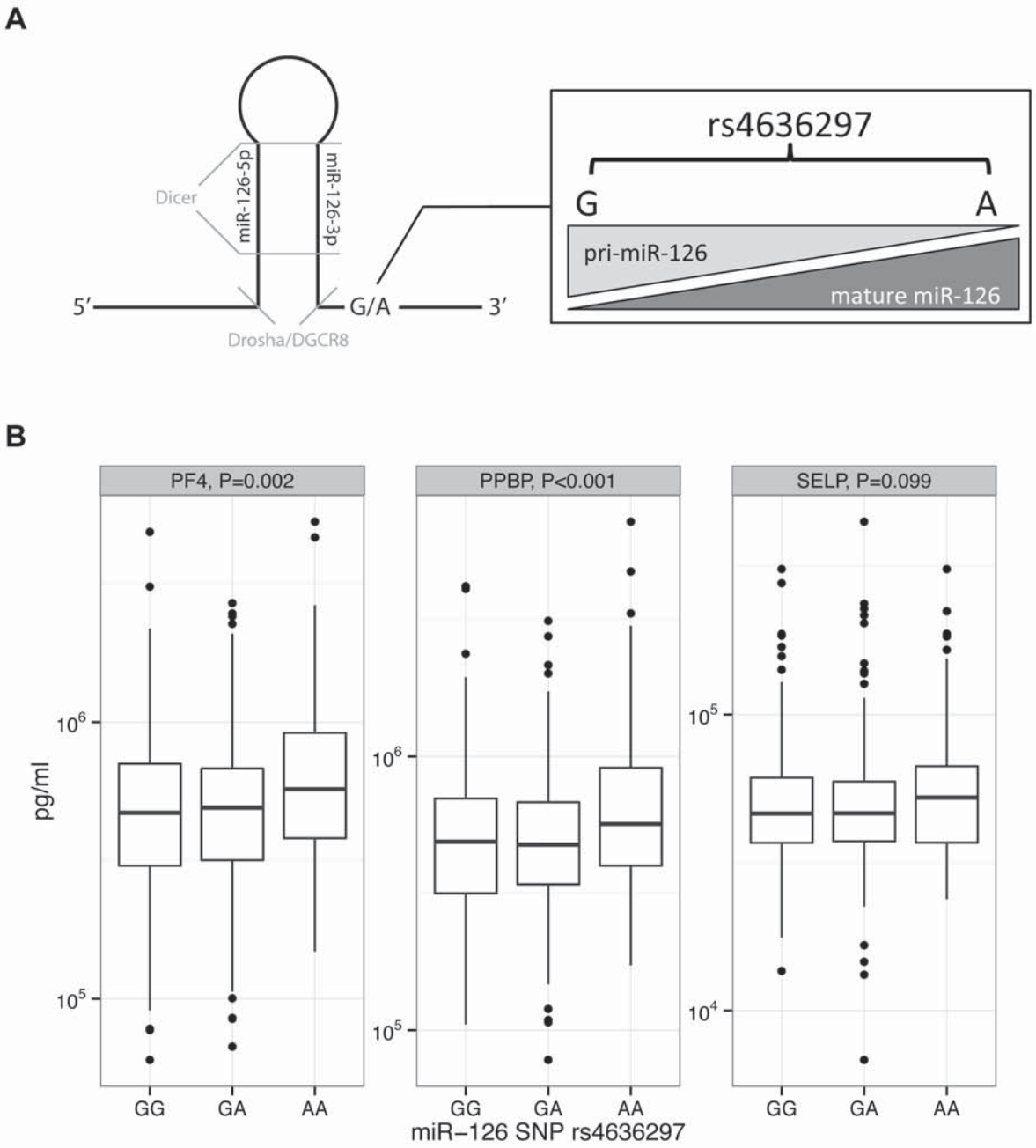
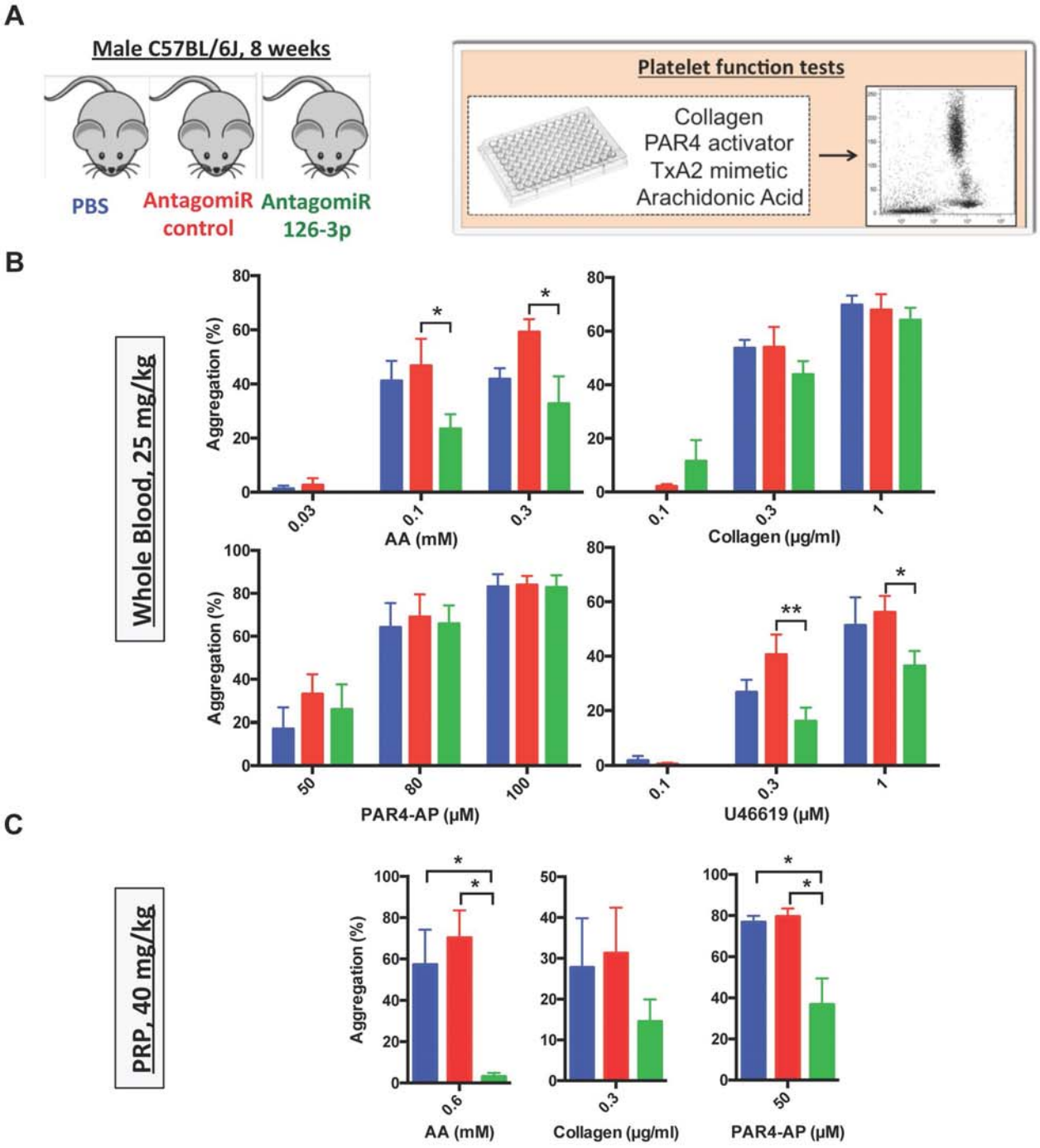
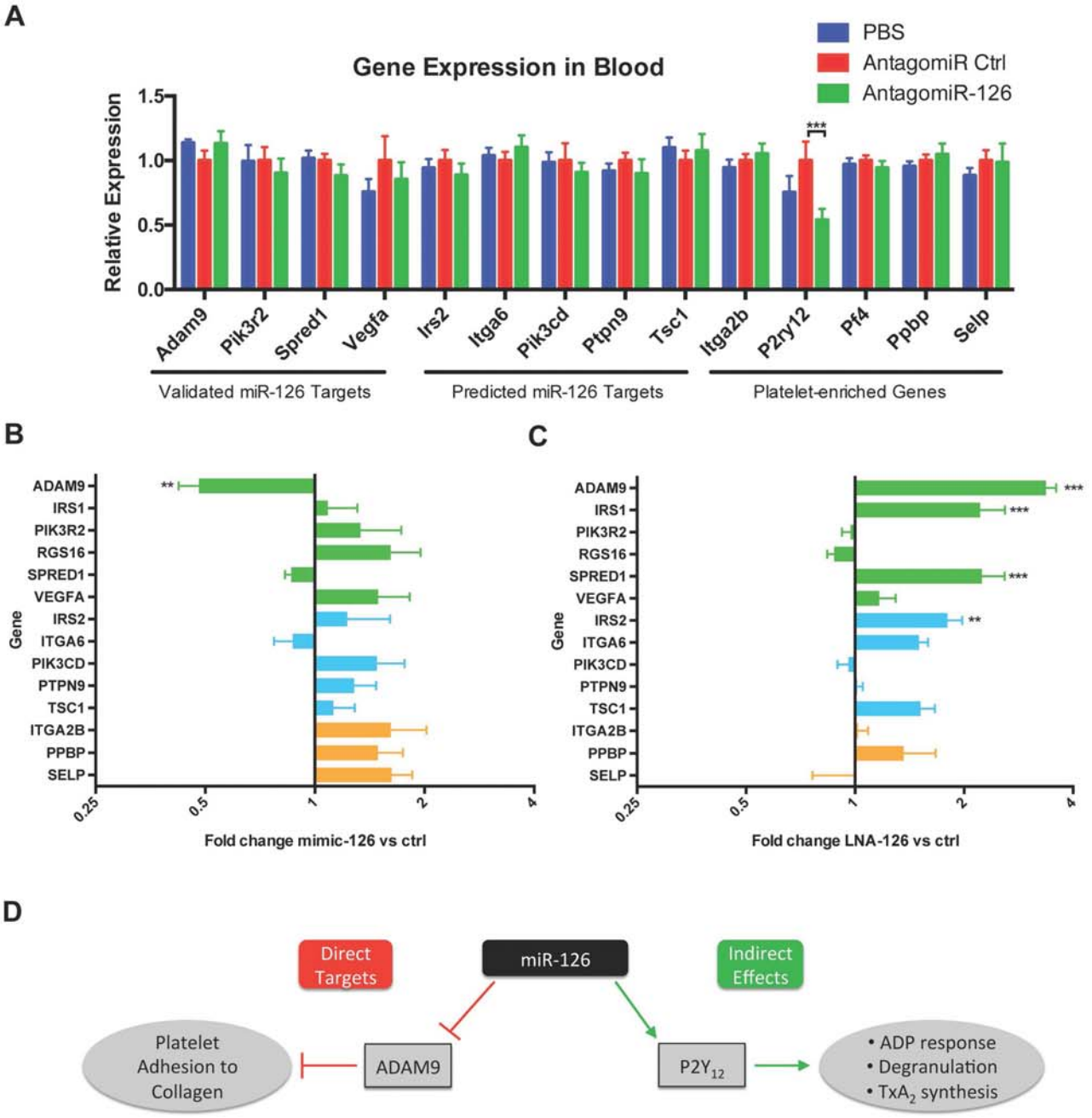


FIGURE 6







Circulation Research
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SUPPLEMENTAL MATERIAL

Association of MicroRNAs and YRNAs with Platelet Function

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Online Figures: 11, Online Tables: 6

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ONLINE METHODS

RNA Isolation, Reverse Transcription (RT) and Pre-amplification. TotalRNA including small RNAs was extracted using the miRNeasy kit (Qiagen).[1,2] For miRNA analysis from plasma samples, a fixed volume of 3 µl of the 25 µl RNA eluate was used as input in each RT reaction. For RNA from tissues or cells, 100 ng RNA were used for the RT reaction. An RT reaction and pre-amplification step were performed as described previously.[1,2] In brief, miRNAs were reversely transcribed using Megaplex Primer Human Pool A v2.1 (Life Technologies). RT reaction products were further amplified using the Megaplex PreAmp Primers A v2.1 (Life Technologies). Both RT and PreAmp products were stored at -80 °C. YRNAs were reversely transcribed using the TaqMan MicroRNA RT Kit (Life Technologies) and specific RT primers provided with custom Taqman assays for the following sequences:

RNY4 3': 5'-CCCCCCCACUGCUAAAUUUGACUGGCU-3'
RNY4 5': 5'-GGCUGGUCCGAUGGUAGUGGGUUAUCAGAACU-3'

For the analysis of gene expression, RNA (100-1000 ng) was reversely transcribed using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies) according to the manufacturer's instructions.

Custom-designed qPCR Plates. The expression profile of miRNAs in ACS patients treated with ASA, ASA + clopidogrel, ASA + ticagrelor or ASA + prasugrel (n=8 for each treatment) was assessed using custom-made Exiqon LNA qPCR plates (Exiqon Life Sciences). A total of 92 miRNAs was measured using the miRCURY LNA Universal RT microRNA PCR protocol which is a two-part protocol consisting of first-strand cDNA synthesis followed by qPCR amplification. For cDNA synthesis, 2 µl of 5x Reaction buffer were combined with 1 µl of 10x Enzyme mix and 2 µl of RNA to a final volume of 10 µl. RT was performed by incubating the samples at 42 °C for 1 h and subsequent heat inactivation of the enzyme at 95 °C for 5 min. RT products were stored at -20 °C. For PCR amplification, 2x SYBR® green master mix (Exiqon Life Sciences) was used for cDNA with the reaction conditions being identical as described for TaqMan qPCR assays. MiRNAs with a Ct higher than 35 in all samples were considered undetectable and omitted from the analysis. The remaining miRNAs were normalized to the average of all endogenous miRNAs.

Individual qPCR Assays. Individual miRNAs were measured by TaqMan miRNA assays as described previously.[1,2] 2.25 µl diluted pre-amplification product (plasma samples) or 2.25 diluted RT product (equivalent to 1 ng/µl RNA) were combined with 0.25 µl TaqMan miRNA Assay (20x) (Life Technologies) and 2.5 µl TaqMan Universal PCR Master Mix No AmpErase UNG (2x) to a final volume of 5 µl. YRNA fragments were measured in the RT product (without preamplification) using custom-made small RNA Taqman assays. QPCR was performed on a ViiA7 thermocycler (Life Technologies) at 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method. Exogenous miRNA (*cel*-miR-39) was used as a spike-in

normalization control for plasma samples. MiRNAs in tissue and cell samples were normalized to endogenous U6 RNA.

Enzyme-linked Immunosorbent Assays (ELISA). The levels of P-selectin, pro-platelet basic protein and platelet factor 4 were measured in plasma samples from the Bruneck cohort using DuoSet ELISA Development kits (catalogue numbers DY137, DY393, DY795) and the DuoSet Ancillary Reagent Kit 2 (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. Absorbance at 450 nm was measured on a Tecan Infinite 200 Pro plate reader (Tecan Group Ltd., Männedorf, Switzerland) using 570 nm as a reference wavelength. Results were calculated using a four-parameter logistic fit.

Cell Culture and transfection. Cells from the megakaryoblastic leukemia cell line MEG-01 (ATCC) were cultured in RPMI-1640 (Life Technologies) supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air / 5% CO_2. For transfection, $1.5x10^5$ cells were washed twice and then seeded in 6-well plates in blank RPMI-1640. MicroRNA mirVana™ mimics were synthesized by Life Technologies and MiRcury™ LNA-antimiRs by Exiqon. The following sequences were used:

LNA-126-3p: CGCATTATTACTCACGGTA
LNA-control: TAACACGTCTATACGCCCA
hsa-miR-126-3p mimic: UCGUACCGUGAGUAAUAAUGCG
Control mimic: sequence not specified.

LNA inhibitors and miRNA mimics were transfected at a final concentration of 20 nmol/L and 50 nmol/L respectively, using Lipofectamine RMAiMAX (Invitrogen) according to the manufacturer's recommendations. 48 h after transfection, cells were lysed in QIAzol for RNA extraction.

Argonaute-2 Immunoprecipitation. Immunoprecipitation of ribonucleo-protein was carried out, as previously described[3], in MEG-01 cells. Briefly, 50-100 million cells were lysed in 500 µl ice cold polysome lysis buffer (5 mM $MgCl_2$, 100 mM KCl, 10 mM Hepes, pH 7.0, 0.5% Nonidet P-40) with freshly added 1 mM DTT, 100 U/ml Rnase inhibitor (Life Technologies) and 1× complete mini EDTA-free protease inhibitor cocktail (Roche) for 15 min. Centrifugation was performed at 14,000 × g at 4 °C for 10 min. The supernatant was mixed with 500 µl of ice-cold NT2 buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$, 0.05% Nonidet P-40) containing freshly added 200 U/ml Rnase inhibitor, 0.5% vanadyl ribonucleoside, 1 mM DTT, 15 mM EDTA and 50 µl mouse anti-human Ago2 (Abcam) coated sepharose G beads (Life Technologies). Incubation was carried out overnight at 4 °C on a rocking platform. On the following day, the beads were washed five times with ice-cold NT2 buffer and beads were used for RNA isolation.

SNP Genotyping. The Bruneck cohort (follow-up 1995) was genotyped for SNP rs4636297 using the KASPar method (Competitive Allele Specific PCR SNP genotyping system) (LGC Ltd., Teddington, UK).

Multiple Reaction Monitoring (MRM). PlasmaDive kits (Biognosys AG) were used to profile plasma proteins by mass spectrometry in the Bruneck Study. Plasma samples were processed according to the manufacturer's instructions with one exception: peptide standards were spiked in before and not after tryptic digestion and C18 clean-up. Briefly, 10 µl of plasma samples were denatured, reduced and alkylated. 20 µg of proteins were spiked with 100 authentic heavy peptide standards. Seven proteins were below the limit of detection. An in-solution digestion was performed overnight. After solid phase extraction with C18 spin columns (96-well format, Harvard apparatus), the eluted peptides were dried using a SpeedVac (Thermo) and resuspended in 40 µl of LC solution. The samples were analyzed on an Agilent 1290 LC system interfaced to an Agilent 6495 Triple Quadrupole MS. 10 µl samples were directly injected onto a 25 cm column (AdvanceBio Peptide Map 2.1 x 250 mm) and separated over a 23 min gradient at 300 µl/min. The data were analyzed using Skyline software version 3.1 (MacCross Lab) and protein concentrations were calculated using the heavy/light (H/L) ratio.

Proximity Extension Assays. Low abundant plasma proteins were analyzed in plasma samples of the Bruneck study using Olink Proseek proximity extension assays (Proseek Multiplex CVD I and Inflammation I panels) as previously published.[4]

Effect of MiRNA Inhibitors. Citrate-anticoagulated human blood was centrifuged for 15 min at 175 × g, from which PRP was extracted. The remaining blood was then centrifuged at 10000 × g for 2 min to obtain PPP. 25 µl of PBS, ADP (50 µM), collagen (10 mg/ml), LNA (10 µM) or antagomiR (10 µM) was added to 225 µl of PRP. Platelet aggregation response was determined using light transmittance aggregometry (LTA). The maximum platelet aggregation response was determined after 40 minutes. LNAs were purchased from Exiqon Life Sciences and consisted of nucleotide sequences with fully Locked Nucleic Acid and phosphorothioate-modified backbones. The used sequences were 5'-ATGTGCAGACTACTG-3' (i-hsa-miR-199a-3p) and 5'-ACGTCTATACGCCCA-3' (non-targeting control) for 15 nucleotides, 5'-CCAATATTTCTGTGCTGCT/CholT-3' for 19 nucleotides (i-miR-195a), 5'-AACACTGATTTCAAATGGTGC/CholT-3' for 21 nucleotides (i-hsa-miR-29b) and 5'-AAGGCAAGCTGACCCTGAAGTTT/CholT-3' for 23 nucleotides (non-targeting control). AntagomiR consisted of a 22 nucleotide sequence with 2'OMe-modified backbone, obtained from Fidelity Systems, Inc. The non-targeting sequence was 5`- A*A*GGCAAGCUGACCCUG-AA*G*U*U* Chol*T-3', with an asterisk denoting a phosphorothiate modification in the preceding nucleotide.

Statistics. Differences in the miRNA profile were assessed by principal component analysis (PCA).[2] The PCA plots were generated in R using the ggbiplot and ggplot2 packages.[5] MiRNA and platelet protein concentrations were log-transformed for analysis. Dependencies of miRNA concentrations on platelet spike-in were analyzed using linear mixed models featuring fixed effects in categorical form or in continuous form, random intercepts for subjects, and a general (unconstrained) covariance structure. Relationships

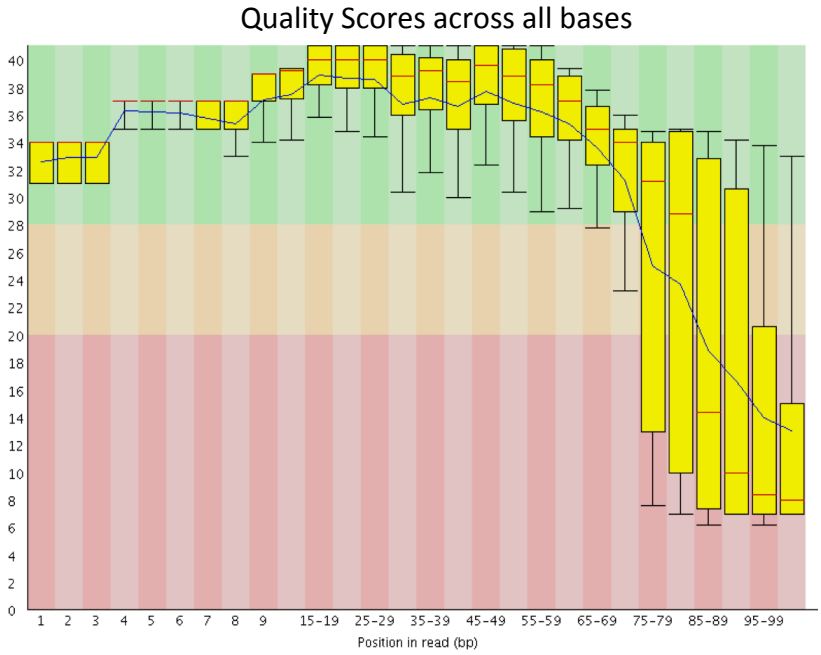
between the concentrations of platelet proteins and of miRNAs were investigated using Spearman's rank correlation coefficient. Differences in platelet protein concentrations by the rs4636297 miR-126 polymorphism were tested by analysis of variance. Associations between the SNP and 219 plasma proteins were assessed by Kruskal-Wallis test, proteins were ordered by significance of association. A Kolmogorov-Smirnov test was used to determine the enrichment of the GO term “platelet activation” (ID GO:0030168). Stata version 12.0 MP was used for statistical analysis.

Online Fig. I. NGS of plasma miRNAs. (A) qPCR for integrin alpha IIb (ITGA2B), CD45 and beta-actin (Actb) in PPP and PRP. Ct, cycle threshold. **(B)** Base-call qualities for NGS (sample 1, PPP). The sequence length (bp, x-axis) is plotted against the quality score (y-axis) on a scale from 1 to 40 with 40 being the highest quality. The shown base-call qualities and sequence distribution are representative for all samples analyzed. **(C)** MiRNA relation between plasma samples. Each point on the plot corresponds to a particular miRNA; shown is the amount of that miRNA in sample 3 divided by the total number of miRNA reads in that sample (x-axis), and the amount of the same miRNA in sample 4 divided by the total number of miRNA reads in that sample (y-axis). The dashed red line represents the perfect diagonal, and the blue line represents the best linear fit across the data.

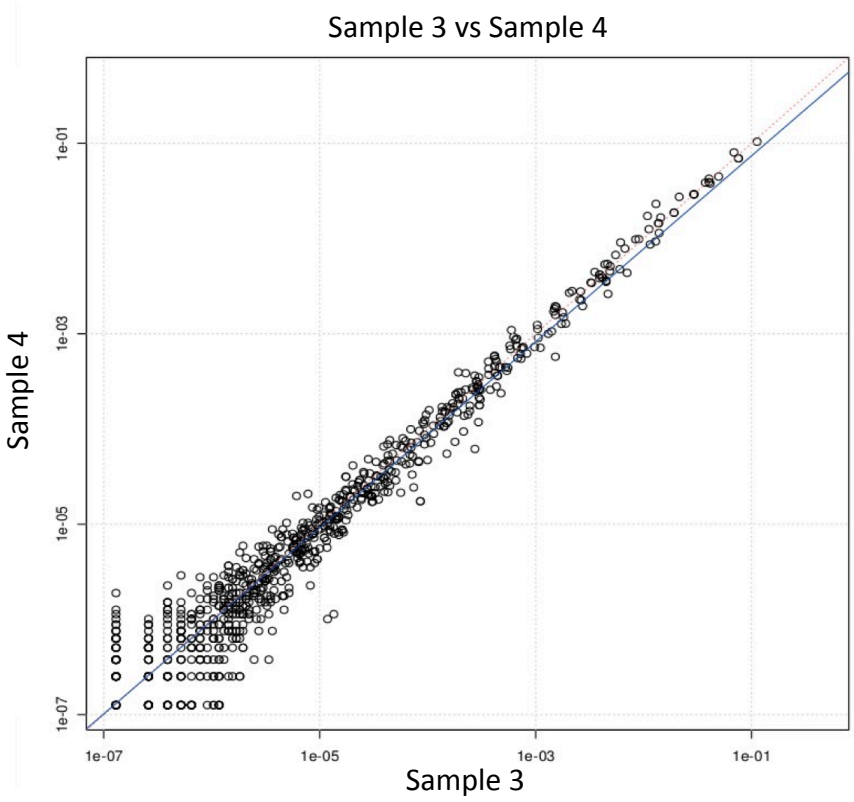
A

Sample	Ct ITGA2B	Ct CD45	Ct Actb
1 (PPP)	Undetermined	Undetermined	37.208
2 (PPP)	Undetermined	Undetermined	37.397
3 (PRP)	33.557	Undetermined	32.807
4 (PRP)	36.892	Undetermined	33.856
Whole Blood	27.67	22.746	24.600

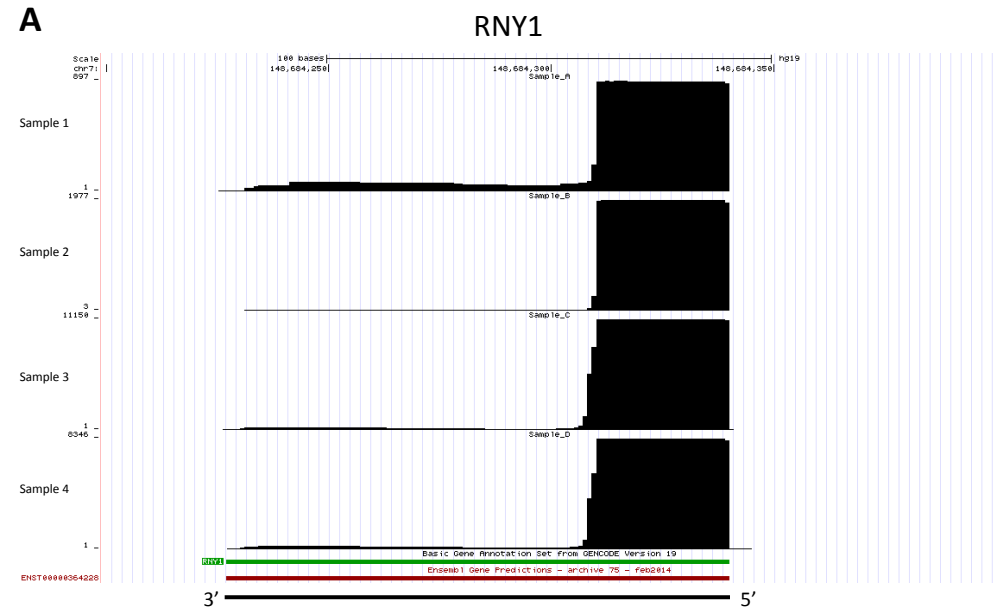
B

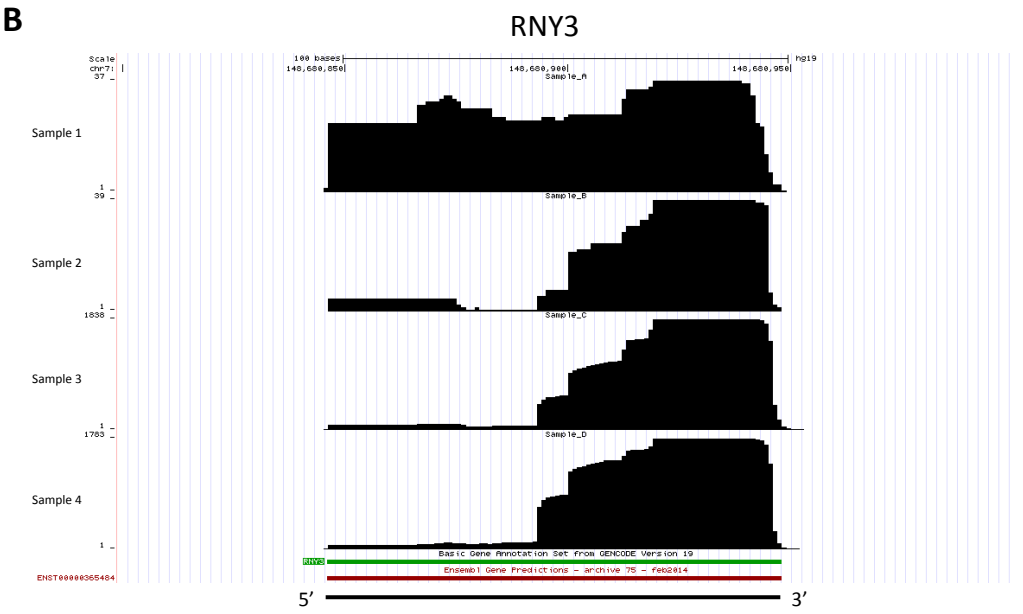


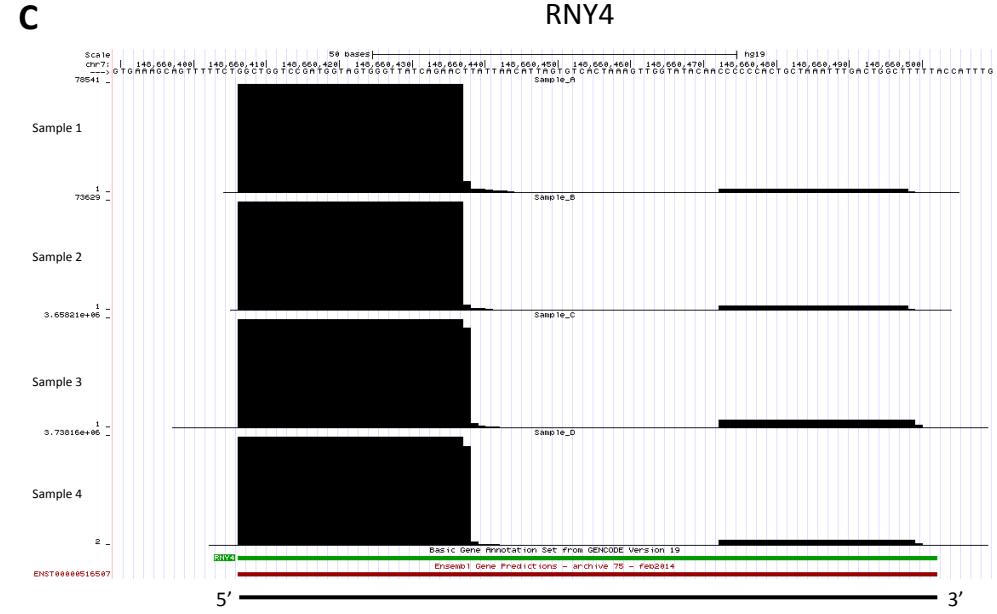
C

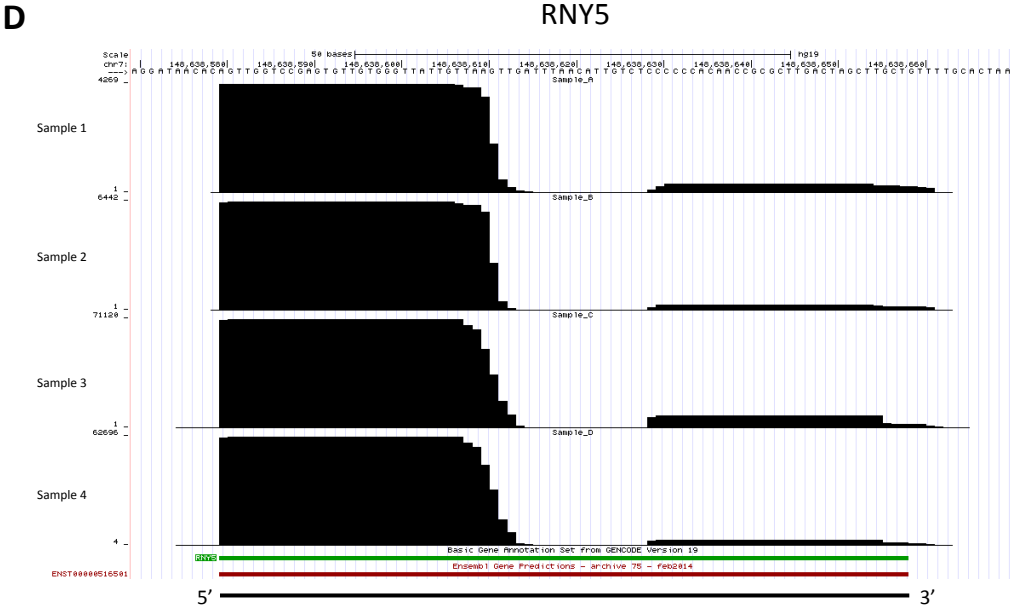


Online Fig. II. YRNA in NGS. Sequencing reads from the 4 samples (1,2: PPP, 3,4: PRP) were aligned to the RNY1 **(A)**, RNY3 **(B)**, RNY4 **(C)** and RNY5 **(D)** genes using the UCSC genome browser. Most reads map to the 5’ end of RNY4.



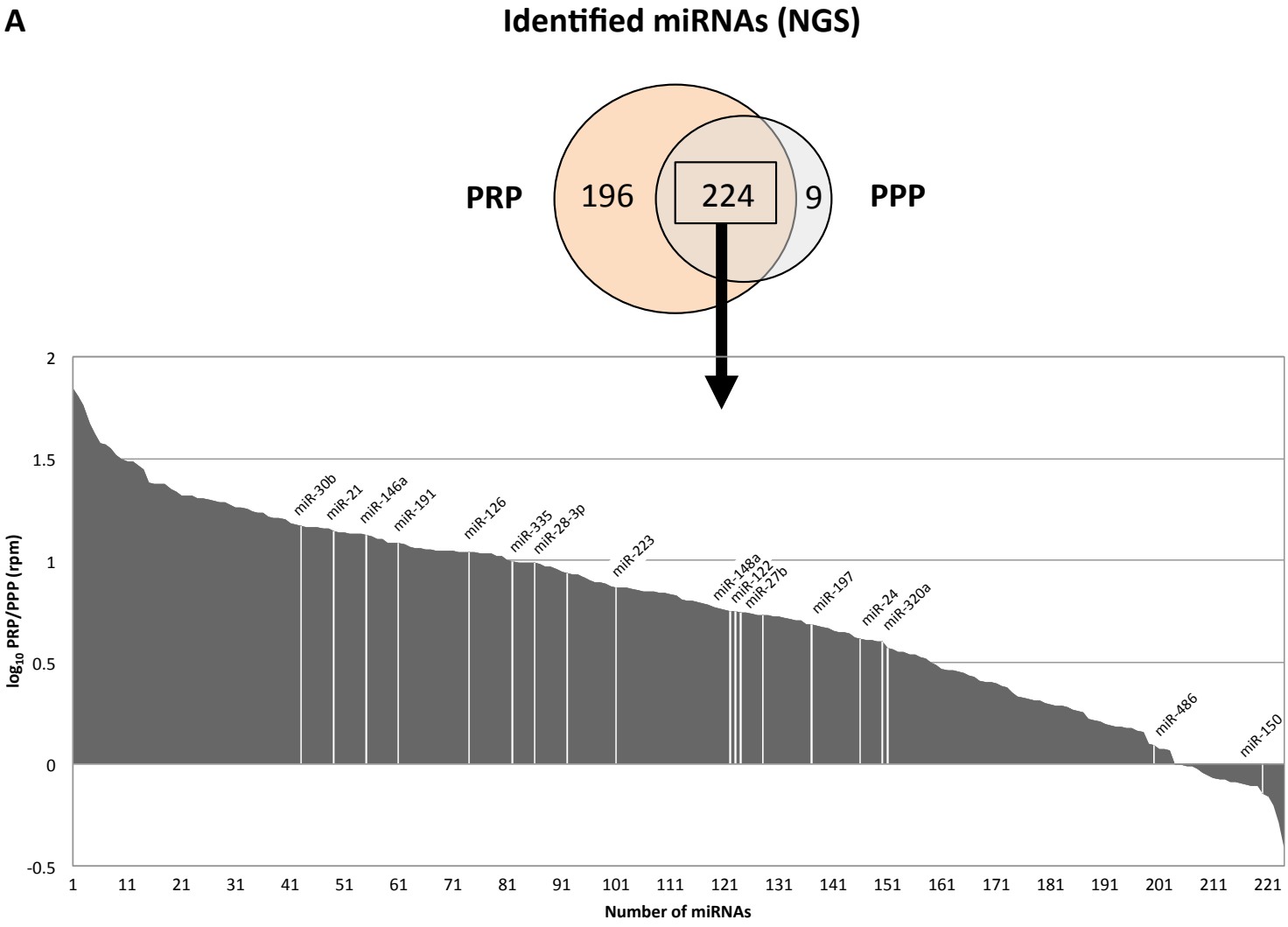






Online Fig. III. MiRNAs and RNY4 fragments are enriched in PRP. (A) For the comparison of PPP and PRP, only miRNAs detected with more than 0.4 reads per million in each sample were taken into consideration (n=224). Out of these 224 miRNAs, 203 had a higher number of relative reads in PRP compared to PPP. Shown in the graph is the ratio of miRNA reads per million in PRP to PPP (logarithmic scale). Individual miRNAs validated in further experiments are highlighted. **(B)** Average reads per million for RNY4 5' and 3' fragments in PPP and PRP (n=2 per group, mean+SD).

A



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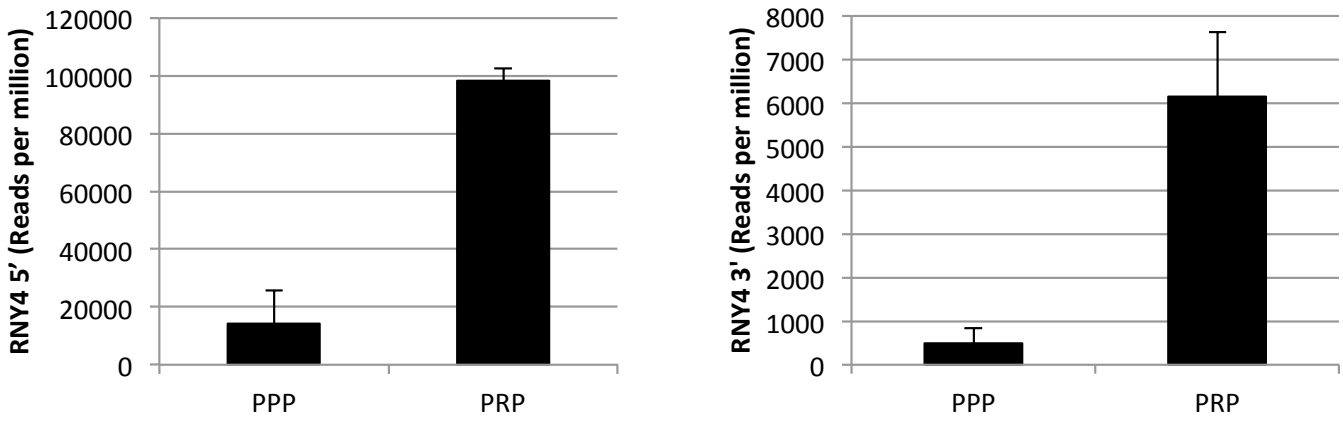
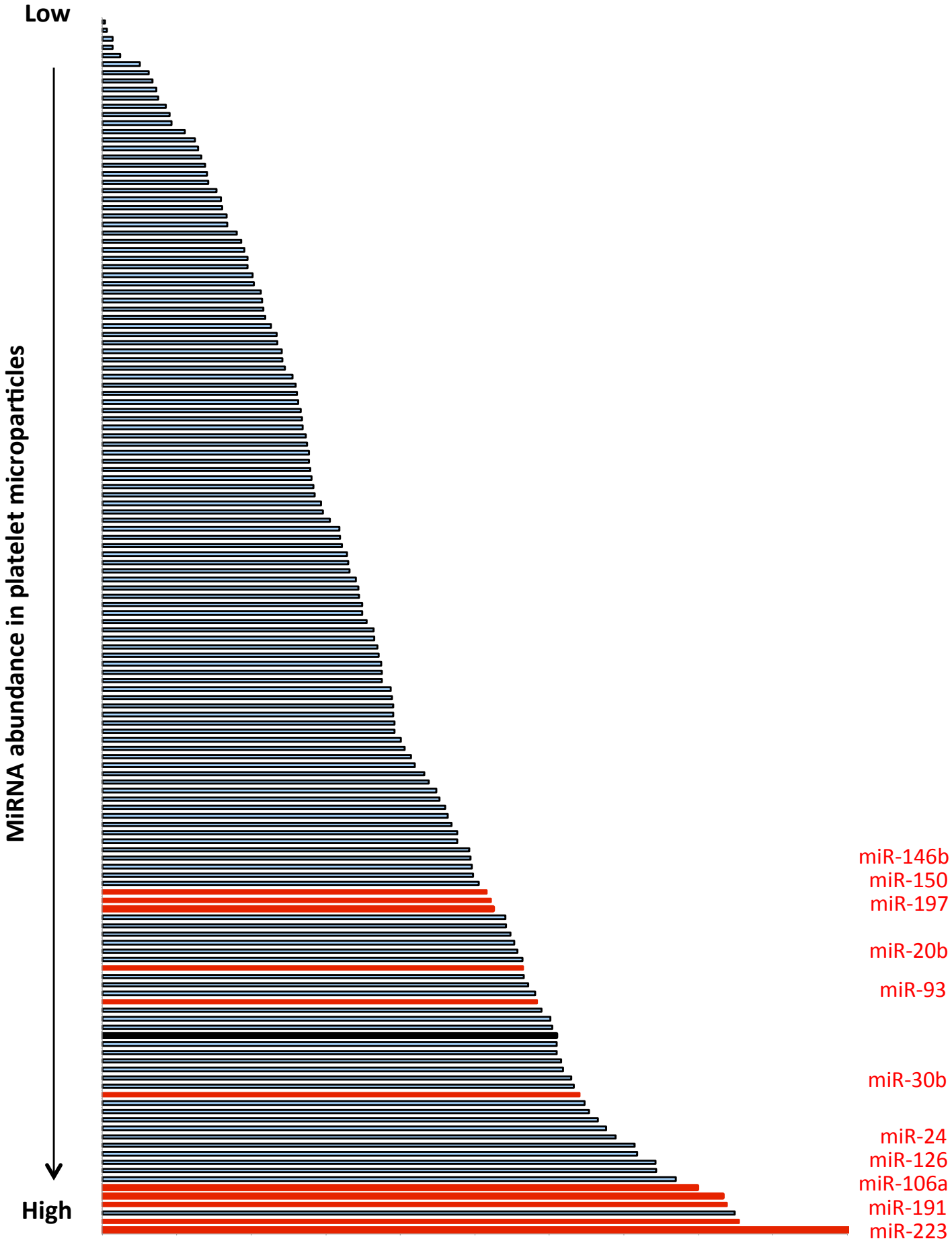
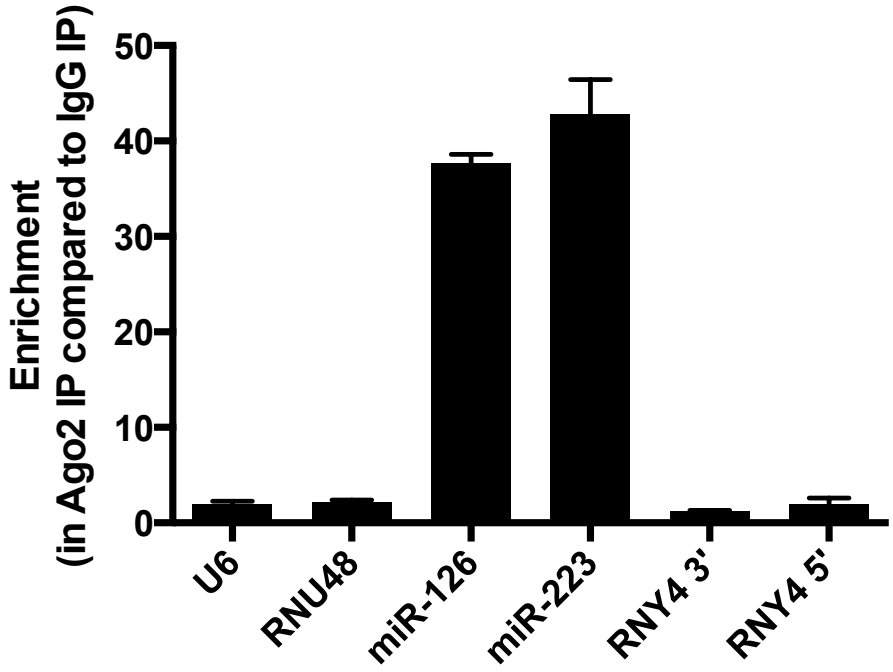


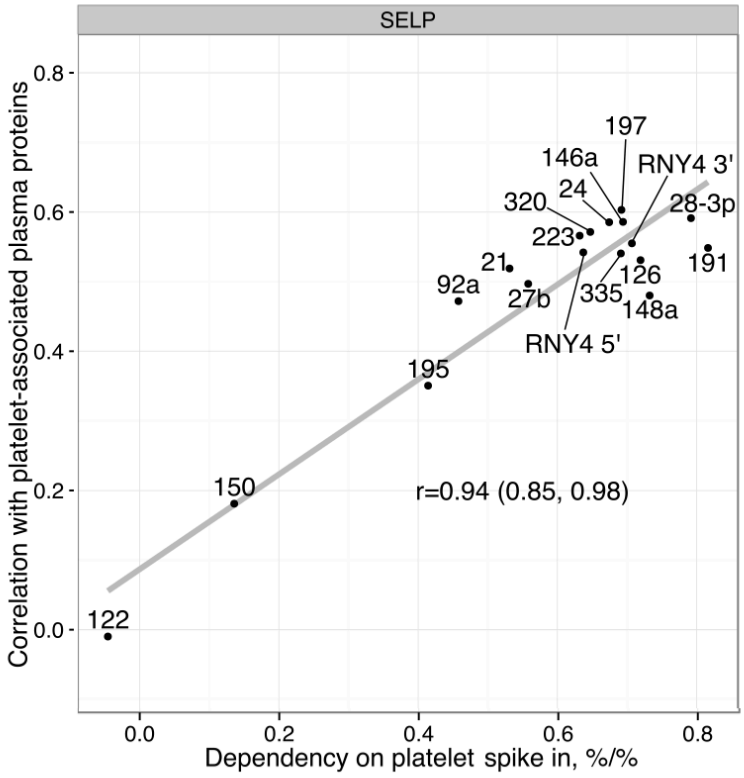
Online Figure IV. MiRNA profile of platelet microparticles. Platelet microparticles were generated by stimulating isolated human platelets with thrombin. MiRNAs were determined by microarray screening. The average Ct value was used as a normalization control. Expression levels ($2^{-\Delta Ct}$) were log transformed. The 11 selected miRNAs (highlighted in red font) are among the most abundant miRNAs in platelet microparticles.



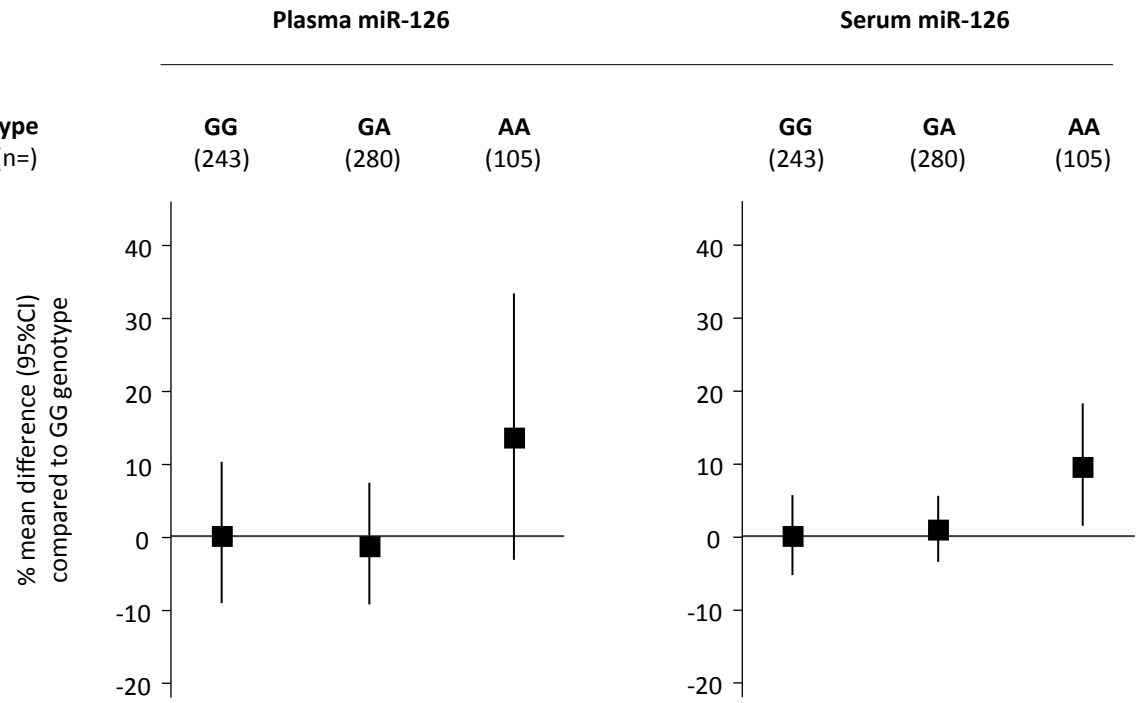
Online Fig. V. Argonaute2 immunoprecipitation. Ago2 complexes were precipitated from MEG-01 cell lysates using an Ago2 antibody or non-specific IgG as a control. As expected, miR-126 and miR-223 were enriched in Ago2 IPs (data shown as mean+SD, n=2 per condition) but we failed to detect RNY 3' and 5' fragments . Other small RNAs (U6, RNU48) were used as negative controls.



Online Figure VI. Dependency on platelets. Relationship between the dependency of small RNA levels on platelets in the spike-in experiment (x-axis) and the correlation of small RNA levels with platelet protein concentrations (SELP) in the general population (y-axis) for 16 miRNAs and two RNY4 fragments. *Ex vivo* dependency of small RNA levels on platelets (see **Fig. 4**) was determined using increasing amounts of spike-in into the PPP of n=4 volunteers each. *In vivo* correlation between small RNAs and platelet-associated plasma proteins were determined in the population-based Bruneck study (n=669, see **Fig. 5A**). Lines are Deming regression lines and r denotes Pearson correlation with 95% confidence interval. SELP, P-selectin.

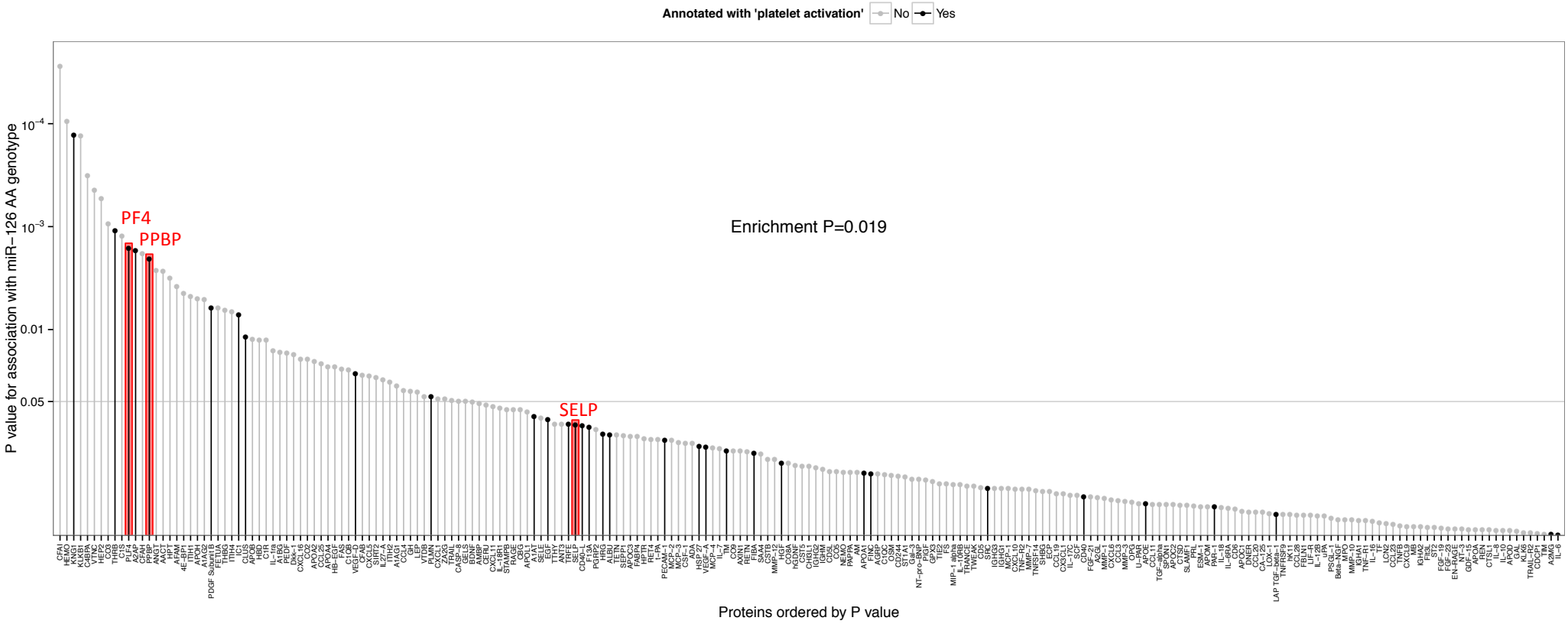


Online Figure VII. Associations of SNP rs4636297 with miR-126 levels in plasma and serum. Circulating miR-126 levels and the genotype of rs4636297 were determined in the Bruneck cohort (n=628, **Online Table IV**).

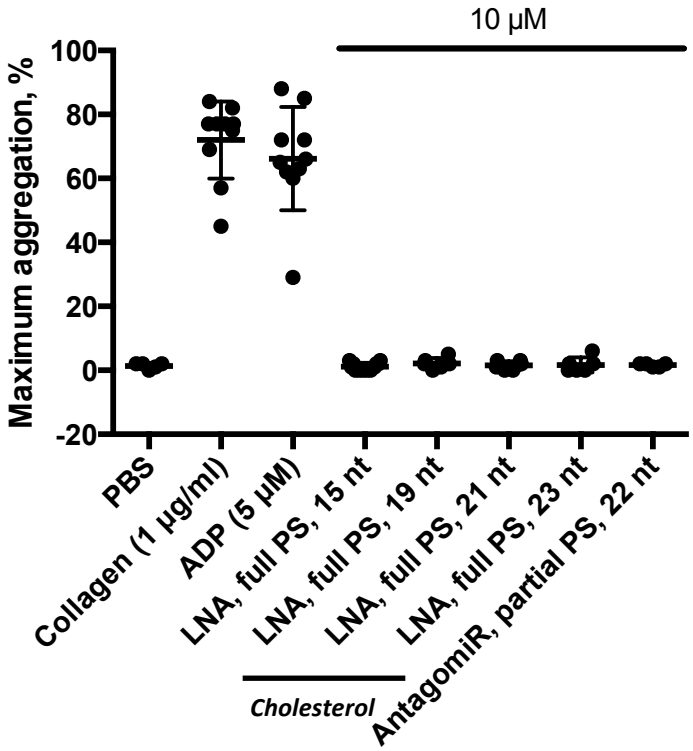


Model		
Additive (per A-allele)	4.9% (-3.5, 14.1%), P=0.258	3.9% (-0.7, 8.6%), P=0.097
Dominant (AA+GA vs. GG)	1.2% (-4.8, 7.6%), P=0.697	1.6% (-1.7, 4.9%), P=0.356
Recessive (AA vs. GA+GG)	6.9% (-1.3, 15.8%), P=0.099	4.4% (-0.0, 8.9%), P=0.050

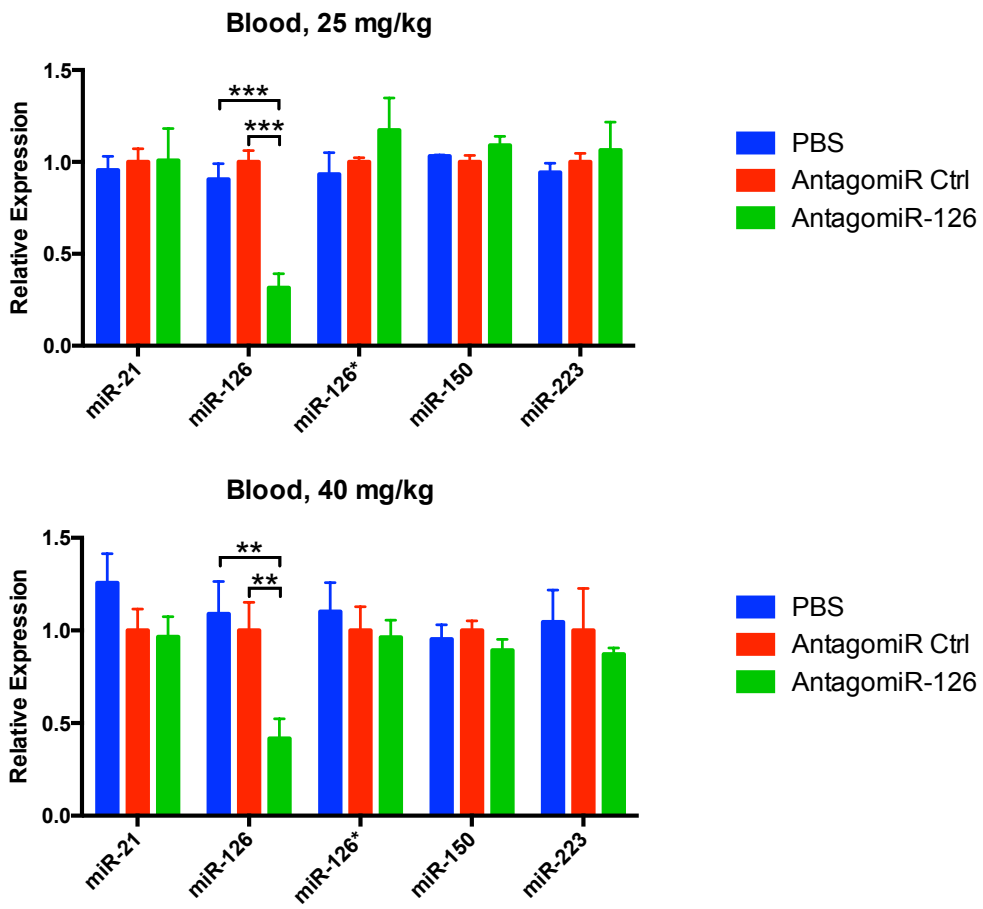
Online Figure VIII. Dependency of plasma proteins on the rs4636297 SNP. Dependencies of plasma levels of 219 proteins on the AA genotype of the rs4636297 SNP were assessed by Wilcoxon test in the Bruneck cohort. Protein IDs are shown on the x-axis and P-values on the logarithmic y-axis. Black color indicates annotation with the GO term "platelet activation", gray color, no such annotation. Kolmogorov-Smirnov test yielded an enrichment P value of 0.019, indicating that annotated proteins and proteins not annotated differed in their distributions of P values for association with the AA genotype. PF4, PPBP and SELP are highlighted in red. For independent validation of the ELISA data, mass-spectrometry-based measurements of PF4 were used for this analysis. For PPBP and SELP, only ELISA data were available **(Fig. 6B, Online Table V)**. PF4, platelet factor 4; PPBP, pro-platelet basic protein; SELP, P-selectin.



Online Fig. IX. Effect of miRNA inhibitors on platelet aggregation. Human PRP was incubated with PBS, collagen, ADP, fully phosphorothioate (PS)-modified LNAs with (19, 21, 23 nt) or without (15 nt) cholesterol modification and with cholesterol-conjugated antagomiR (22 nt, PS modifications of 2 and 4 nt at 5' and 3' end, respectively). None of the PS-modified oligonucleotides tested induced platelet aggregation.

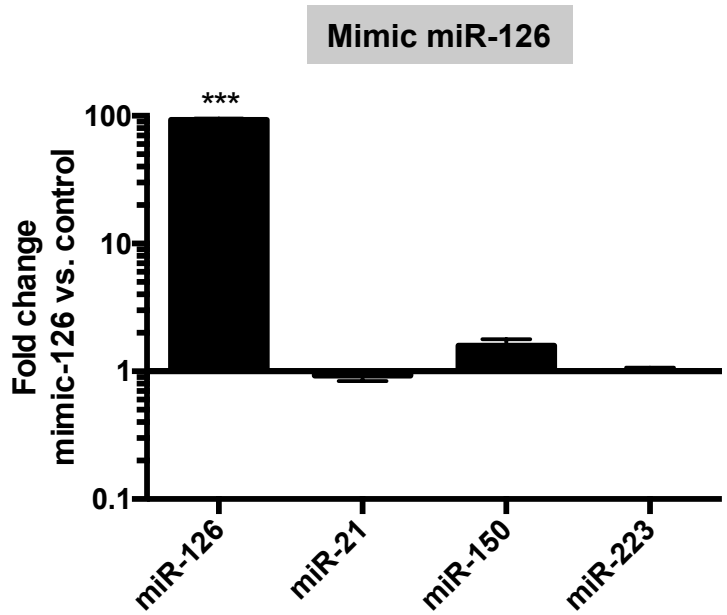


Online Fig. X. MiRNA expression in antagomiR-treated mice. MiRNAs were analyzed by qPCR using RNA isolated from whole blood. Graphs represent mean+SEM (n=4 per group), asterisks denote statistical significance in a two-way ANOVA with Bonferroni post-test (**, p<0.01; ***, p<0.001).



Online Fig. XI. Effects of miR-126 in MEG-01 cells. MiRNA expression was analysed in MEG-01 cells transfected with mimic-126 (**A**) or LNA-126 (**B**) or the respective controls. MiRNA expression was normalised to endogenous U6. Graphs represent mean+SEM (n=4 per condition), asterisks denote statistical significance in a two-way ANOVA with Bonferroni post-test (***, p<0.001).

A



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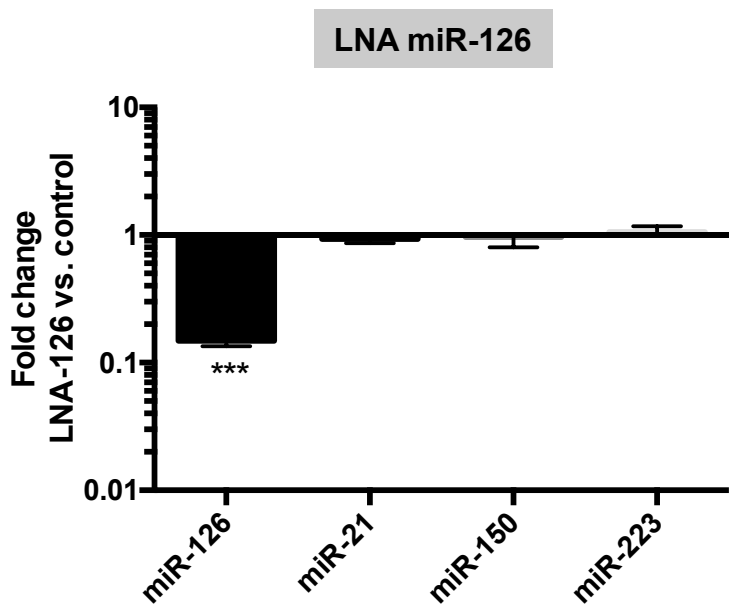

Online Table I. miRNAs identified by NGS. MiRNAs identified by NGS and their raw read count as well as their normalised read count (per million reads) are shown for each sample (Samples 1 & 2: PPP, Samples 3 & 4: PRP). MiRNAs that were not consistently identified in all four samples were omitted.

		Raw read count				Normalised read count			
		PPP		PRP		PPP		PRP	
miRNA	**Arm**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
hsa-mir-486	hsa-miR-486-5p	73344	190238	863651	822870	20832.33	15392.46	22977.07	22038.35
hsa-mir-191	hsa-miR-191-5p	5811	11081	528126	634227	1650.53	896.58	14050.57	16986.06
hsa-mir-26a-2	hsa-miR-26a-5p	2846	8515	580866	550146	808.37	688.96	15453.70	14734.17
hsa-mir-26a-1	hsa-miR-26a-5p	2845	8515	580844	550121	808.08	688.96	15453.11	14733.50
hsa-mir-92a-1	hsa-miR-92a-3p	10086	29876	380405	354038	2864.79	2417.31	10120.52	9481.95
hsa-mir-92a-2	hsa-miR-92a-3p	9165	26966	318271	299437	2603.19	2181.86	8467.47	8019.61
hsa-mir-22	hsa-miR-22-3p	4192	14439	309062	310113	1190.68	1168.28	8222.47	8305.54
hsa-let-7f-2	hsa-let-7f-5p	2863	5718	310026	335991	813.19	462.65	8248.11	8998.61
hsa-let-7f-1	hsa-let-7f-5p	2707	5499	286771	305939	768.89	444.93	7629.42	8193.75
hsa-let-7a-1	hsa-let-7a-5p	1437	3176	225007	230722	408.16	256.98	5986.22	6179.27
hsa-let-7a-2	hsa-let-7a-5p	1439	3179	224920	230649	408.73	257.22	5983.90	6177.31
hsa-let-7a-3	hsa-let-7a-5p	1440	3179	224873	230392	409.01	257.22	5982.65	6170.43
hsa-mir-423	hsa-miR-423-5p	5026	11050	164157	216687	1427.56	894.07	4367.33	5803.38
hsa-mir-181a-1	hsa-miR-181a-5p	2557	6371	146992	148051	726.28	515.49	3910.66	3965.15
hsa-mir-181a-2	hsa-miR-181a-5p	2557	6371	146992	148051	726.28	515.49	3910.66	3965.15
hsa-mir-126	hsa-miR-126-5p	1397	4042	100014	182946	396.80	327.04	2660.83	4899.71
hsa-mir-16-1	hsa-miR-16-5p	1860	9541	105725	114609	528.31	771.98	2812.77	3069.49
hsa-mir-16-2	hsa-miR-16-5p	1860	9538	105713	114593	528.31	771.73	2812.45	3069.06
hsa-mir-30d	hsa-miR-30d-5p	1031	2900	110610	131657	292.84	234.64	2942.73	3526.08
hsa-mir-142	hsa-miR-142-5p	2853	7244	86187	99281	810.35	586.12	2292.97	2658.97
hsa-mir-21	hsa-miR-21-5p	878	2130	83479	136576	249.38	172.34	2220.92	3657.82
hsa-mir-151a	hsa-miR-151a-5p	591	1641	106964	90541	167.87	132.78	2845.73	2424.90
hsa-mir-423	hsa-miR-423-3p	841	2047	99312	73779	238.87	165.63	2642.15	1975.97
hsa-mir-30e	hsa-miR-30e-5p	774	2948	69342	77971	219.84	238.53	1844.81	2088.24
hsa-mir-222	hsa-miR-222-3p	468	860	88295	68599	132.93	69.58	2349.05	1837.24
hsa-mir-151a	hsa-miR-151a-3p	723	1151	64798	77515	205.36	93.13	1723.92	2076.03
hsa-mir-27b	hsa-miR-27b-3p	1166	2979	47042	71992	331.19	241.04	1251.53	1928.11
hsa-mir-28	hsa-miR-28-3p	820	999	51598	62225	232.91	80.83	1372.74	1666.53
hsa-mir-10b	hsa-miR-10b-5p	4704	8255	16717	22218	1336.11	667.93	444.75	595.05
hsa-mir-25	hsa-miR-25-3p	1372	4209	46231	37665	389.70	340.56	1229.96	1008.76
hsa-let-7i	hsa-let-7i-5p	396	1066	42313	53215	112.48	86.25	1125.72	1425.22
hsa-mir-10a	hsa-miR-10a-5p	1831	4027	34598	28720	520.07	325.83	920.47	769.19
hsa-mir-148a	hsa-miR-148a-3p	845	1641	35587	42867	240.01	132.78	946.78	1148.08
hsa-mir-409	hsa-miR-409-3p	245	371	54060	34531	69.59	30.02	1438.24	924.82
hsa-mir-186	hsa-miR-186-5p	639	2124	37684	40550	181.50	171.86	1002.57	1086.02
hsa-mir-451a	hsa-miR-451a	745	6840	35986	20742	211.61	553.44	957.39	555.52
hsa-mir-146a	hsa-miR-146a-5p	362	606	33606	42475	102.82	49.03	894.07	1137.58
hsa-let-7d	hsa-let-7d-5p	153	476	37413	35916	43.46	38.51	995.36	961.91
hsa-mir-103a-2	hsa-miR-103a-3p	283	1162	30138	33231	80.38	94.02	801.81	890.00
hsa-mir-103a-1	hsa-miR-103a-3p	283	1162	30087	33195	80.38	94.02	800.45	889.04
hsa-mir-30c-2	hsa-miR-30c-5p	203	804	34571	27972	57.66	65.05	919.75	749.15
hsa-mir-30c-1	hsa-miR-30c-5p	203	803	34542	27948	57.66	64.97	918.98	748.51
hsa-mir-199a-2	hsa-miR-199a-3p	166	495	31928	31043	47.15	40.05	849.43	831.40
hsa-mir-199a-1	hsa-miR-199a-3p	166	495	31907	31017	47.15	40.05	848.87	830.71
hsa-mir-199b	hsa-miR-199b-3p	166	495	31907	31017	47.15	40.05	848.87	830.71
hsa-mir-26b	hsa-miR-26b-5p	130	490	27158	35250	36.92	39.65	722.53	944.08
hsa-let-7b	hsa-let-7b-5p	169	666	30104	30350	48.00	53.89	800.90	812.84
hsa-let-7g	hsa-let-7g-5p	177	587	25299	27025	50.27	47.50	673.07	723.79
hsa-mir-744	hsa-miR-744-5p	152	292	25010	27407	43.17	23.63	665.38	734.02
hsa-mir-93	hsa-miR-93-5p	262	1411	20321	17837	74.42	114.17	540.63	477.72
hsa-mir-143	hsa-miR-143-3p	393	1051	19648	18191	111.63	85.04	522.73	487.20
hsa-mir-127	hsa-miR-127-3p	165	278	20069	22026	46.87	22.49	533.93	589.91
hsa-mir-223	hsa-miR-223-3p	153	1087	20944	15471	43.46	87.95	557.21	414.35
hsa-mir-126	hsa-miR-126-3p	143	610	15758	21322	40.62	49.36	419.24	571.05
hsa-mir-182	hsa-miR-182-5p	605	1886	11429	14560	171.84	152.60	304.06	389.95
hsa-mir-192	hsa-miR-192-5p	1256	2594	8025	8867	356.75	209.88	213.50	237.48

hsa-mir-320a	hsa-miR-320a	385	767	13930	11803	109.35	62.06	370.60	316.11
hsa-mir-146b	hsa-miR-146b-5p	213	292	11688	15446	60.50	23.63	310.95	413.68
hsa-let-7d	hsa-let-7d-3p	255	540	14588	10135	72.43	43.69	388.11	271.44
hsa-mir-584	hsa-miR-584-5p	49	98	11961	15095	13.92	7.93	318.22	404.28
hsa-mir-654	hsa-miR-654-3p	51	124	13633	13265	14.49	10.03	362.70	355.27
hsa-mir-425	hsa-miR-425-5p	154	528	11703	12477	43.74	42.72	311.35	334.16
hsa-mir-221	hsa-miR-221-3p	102	309	13335	10058	28.97	25.00	354.77	269.38
hsa-mir-98	hsa-miR-98-5p	27	47	11275	13574	7.67	3.80	299.97	363.54
hsa-mir-125a	hsa-miR-125a-5p	217	459	10054	7915	61.64	37.14	267.48	211.98
hsa-mir-484	hsa-miR-484	211	728	11711	4543	59.93	58.90	311.57	121.67
hsa-mir-30a	hsa-miR-30a-5p	493	1332	5138	5891	140.03	107.77	136.69	157.77
hsa-mir-4433b	hsa-miR-4433b-5p	19	24	10824	8378	5.40	1.94	287.97	224.38
hsa-let-7e	hsa-let-7e-5p	75	138	7967	9786	21.30	11.17	211.96	262.09
hsa-mir-23a	hsa-miR-23a-3p	112	425	8126	7164	31.81	34.39	216.19	191.87
hsa-mir-150	hsa-miR-150-5p	614	1242	4162	3231	174.40	100.49	110.73	86.53
hsa-mir-27a	hsa-miR-27a-3p	138	814	5837	5737	39.20	65.86	155.29	153.65
hsa-mir-130a	hsa-miR-130a-3p	61	209	8507	5643	17.33	16.91	226.33	151.13
hsa-mir-101-2	hsa-miR-101-3p	135	516	5152	7032	38.34	41.75	137.07	188.33
hsa-mir-92b	hsa-miR-92b-3p	290	677	4494	5458	82.37	54.78	119.56	146.18
hsa-mir-128-1	hsa-miR-128-3p	88	288	7523	5749	25.00	23.30	200.15	153.97
hsa-mir-107	hsa-miR-107	131	714	6372	4912	37.21	57.77	169.52	131.55
hsa-mir-101-1	hsa-miR-101-3p	132	504	5029	6844	37.49	40.78	133.79	183.30
hsa-mir-21	hsa-miR-21-3p	78	241	4807	7296	22.15	19.50	127.89	195.40
hsa-mir-411	hsa-miR-411-5p	16	48	4585	8654	4.54	3.88	121.98	231.77
hsa-mir-99b	hsa-miR-99b-5p	176	305	5237	4408	49.99	24.68	139.33	118.06
hsa-mir-17	hsa-miR-17-5p	28	106	5911	5669	7.95	8.58	157.26	151.83
hsa-mir-410	hsa-miR-410-3p	23	37	5690	5543	6.53	2.99	151.38	148.45
hsa-mir-128-2	hsa-miR-128-3p	77	255	5639	4326	21.87	20.63	150.02	115.86
hsa-mir-1307	hsa-miR-1307-5p	61	161	4423	5935	17.33	13.03	117.67	158.95
hsa-mir-30b	hsa-miR-30b-5p	27	138	5563	4862	7.67	11.17	148.00	130.22
hsa-mir-15a	hsa-miR-15a-5p	108	671	4045	3532	30.68	54.29	107.62	94.60
hsa-mir-181b-2	hsa-miR-181b-5p	140	274	3214	4659	39.77	22.17	85.51	124.78
hsa-mir-181b-1	hsa-miR-181b-5p	138	273	3175	4568	39.20	22.09	84.47	122.34
hsa-mir-122	hsa-miR-122-5p	105	86	4262	3480	29.82	6.96	113.39	93.20
hsa-mir-340	hsa-miR-340-5p	37	94	3265	4291	10.51	7.61	86.86	114.92
hsa-mir-140	hsa-miR-140-3p	75	183	2806	4039	21.30	14.81	74.65	108.17
hsa-mir-375	hsa-miR-375	226	589	2300	1695	64.19	47.66	61.19	45.40
hsa-mir-30e	hsa-miR-30e-3p	53	65	3329	4065	15.05	5.26	88.57	108.87
hsa-mir-941-1	hsa-miR-941	50	161	3577	3527	14.20	13.03	95.16	94.46
hsa-mir-941-2	hsa-miR-941	50	161	3577	3527	14.20	13.03	95.16	94.46
hsa-mir-941-3	hsa-miR-941	50	161	3577	3527	14.20	13.03	95.16	94.46
hsa-mir-941-4	hsa-miR-941	50	161	3577	3527	14.20	13.03	95.16	94.46
hsa-mir-144	hsa-miR-144-5p	88	597	2227	2233	25.00	48.30	59.25	59.80
hsa-mir-301a	hsa-miR-301a-3p	22	53	3212	3586	6.25	4.29	85.45	96.04
hsa-mir-15b	hsa-miR-15b-5p	21	119	3225	3099	5.96	9.63	85.80	83.00
hsa-mir-197	hsa-miR-197-3p	59	171	3333	2218	16.76	13.84	88.67	59.40
hsa-mir-199a-1	hsa-miR-199a-5p	20	34	3415	2874	5.68	2.75	90.85	76.97
hsa-mir-199a-2	hsa-miR-199a-5p	20	34	3414	2873	5.68	2.75	90.83	76.95
hsa-mir-320b-2	hsa-miR-320b	207	368	1404	1830	58.80	29.78	37.35	49.01
hsa-mir-320b-1	hsa-miR-320b	207	368	1402	1830	58.80	29.78	37.30	49.01
hsa-mir-28	hsa-miR-28-5p	8	39	2822	3415	2.27	3.16	75.08	91.46
hsa-mir-378a	hsa-miR-378a-3p	120	320	1985	2144	34.08	25.89	52.81	57.42
hsa-mir-148b	hsa-miR-148b-3p	22	41	2822	2932	6.25	3.32	75.08	78.53
hsa-mir-106b	hsa-miR-106b-3p	68	277	2381	2043	19.31	22.41	63.35	54.72
hsa-mir-323b	hsa-miR-323b-3p	8	32	3674	1880	2.27	2.59	97.75	50.35
hsa-mir-1307	hsa-miR-1307-3p	21	85	2290	2765	5.96	6.88	60.92	74.05
hsa-mir-335	hsa-miR-335-5p	26	74	2923	2041	7.38	5.99	77.77	54.66
hsa-mir-142	hsa-miR-142-3p	18	74	2099	2878	5.11	5.99	55.84	77.08
hsa-mir-181c	hsa-miR-181c-5p	21	85	2180	2527	5.96	6.88	58.00	67.68
hsa-mir-19b-2	hsa-miR-19b-3p	30	160	2308	2077	8.52	12.95	61.40	55.63
hsa-mir-19b-1	hsa-miR-19b-3p	30	160	2292	2060	8.52	12.95	60.98	55.17
hsa-mir-181a-2	hsa-miR-181a-2-3p	23	57	2174	2507	6.53	4.61	57.84	67.14
hsa-mir-136	hsa-miR-136-3p	16	25	1731	3054	4.54	2.02	46.05	81.79
hsa-mir-421	hsa-miR-421	22	56	2255	2328	6.25	4.53	59.99	62.35
hsa-mir-363	hsa-miR-363-3p	56	330	1888	1389	15.91	26.70	50.23	37.20

hsa-mir-23b	hsa-miR-23b-3p	32	117	1991	2044	9.09	9.47	52.97	54.74
hsa-mir-381	hsa-miR-381-3p	7	16	1479	3118	1.99	1.29	39.35	83.51
hsa-mir-16-2	hsa-miR-16-2-3p	48	332	1883	1223	13.63	26.86	50.10	32.75
hsa-mir-24-1	hsa-miR-24-3p	43	146	2317	1405	12.21	11.81	61.64	37.63
hsa-mir-24-2	hsa-miR-24-3p	43	146	2317	1405	12.21	11.81	61.64	37.63
hsa-mir-20a	hsa-miR-20a-5p	4	46	2212	2171	1.14	3.72	58.85	58.14
hsa-mir-328	hsa-miR-328-3p	25	48	2361	1620	7.10	3.88	62.81	43.39
hsa-mir-345	hsa-miR-345-5p	67	229	1464	1455	19.03	18.53	38.95	38.97
hsa-mir-130b	hsa-miR-130b-5p	9	29	2148	1958	2.56	2.35	57.15	52.44
hsa-mir-144	hsa-miR-144-3p	48	477	1121	1205	13.63	38.59	29.82	32.27
hsa-mir-486	hsa-miR-486-3p	59	195	1816	1121	16.76	15.78	48.31	30.02
hsa-let-7c	hsa-let-7c-5p	18	61	1566	1933	5.11	4.94	41.66	51.77
hsa-mir-432	hsa-miR-432-5p	5	9	1726	2053	1.42	0.73	45.92	54.98
hsa-mir-130b	hsa-miR-130b-3p	28	91	1488	1680	7.95	7.36	39.59	44.99
hsa-mir-151b	hsa-miR-151b	21	52	1621	1649	5.96	4.21	43.13	44.16
hsa-mir-769	hsa-miR-769-5p	27	57	1507	1630	7.67	4.61	40.09	43.66
hsa-mir-431	hsa-miR-431-5p	11	17	1470	1922	3.12	1.38	39.11	51.48
hsa-mir-342	hsa-miR-342-3p	76	181	1159	913	21.59	14.64	30.83	24.45
hsa-mir-3615	hsa-miR-3615	44	134	1169	1344	12.50	10.84	31.10	36.00
hsa-mir-134	hsa-miR-134-5p	3	6	2270	936	0.85	0.49	60.39	25.07
hsa-mir-671	hsa-miR-671-3p	16	29	1310	1475	4.54	2.35	34.85	39.50
hsa-mir-106b	hsa-miR-106b-5p	9	98	1443	1186	2.56	7.93	38.39	31.76
hsa-mir-574	hsa-miR-574-3p	30	90	1654	745	8.52	7.28	44.00	19.95
hsa-mir-29a	hsa-miR-29a-3p	48	203	984	863	13.63	16.43	26.18	23.11
hsa-mir-485	hsa-miR-485-5p	5	21	1673	1089	1.42	1.70	44.51	29.17
hsa-mir-589	hsa-miR-589-5p	20	31	1236	1312	5.68	2.51	32.88	35.14
hsa-mir-487b	hsa-miR-487b-3p	4	11	1331	1347	1.14	0.89	35.41	36.08
hsa-mir-625	hsa-miR-625-3p	6	26	2106	489	1.70	2.10	56.03	13.10
hsa-mir-4446	hsa-miR-4446-3p	17	16	1213	1103	4.83	1.29	32.27	29.54
hsa-mir-1273h	hsa-miR-1273h-3p	17	7	1049	1217	4.83	0.57	27.91	32.59
hsa-mir-181d	hsa-miR-181d-5p	10	15	1104	1210	2.84	1.21	29.37	32.41
hsa-mir-6852	hsa-miR-6852-5p	1	10	1043	1343	0.28	0.81	27.75	35.97
hsa-mir-335	hsa-miR-335-3p	7	17	1198	949	1.99	1.38	31.87	25.42
hsa-mir-652	hsa-miR-652-3p	6	79	1083	838	1.70	6.39	28.81	22.44
hsa-mir-181c	hsa-miR-181c-3p	8	17	1002	1052	2.27	1.38	26.66	28.17
hsa-mir-889	hsa-miR-889-3p	8	13	793	1250	2.27	1.05	21.10	33.48
hsa-mir-361	hsa-miR-361-5p	10	25	1020	958	2.84	2.02	27.14	25.66
hsa-mir-532	hsa-miR-532-5p	37	107	821	569	10.51	8.66	21.84	15.24
hsa-mir-340	hsa-miR-340-3p	6	23	1104	853	1.70	1.86	29.37	22.85
hsa-mir-223	hsa-miR-223-5p	14	42	867	860	3.98	3.40	23.07	23.03
hsa-mir-485	hsa-miR-485-3p	1	7	1378	581	0.28	0.57	36.66	15.56
hsa-mir-155	hsa-miR-155-5p	12	30	722	981	3.41	2.43	19.21	26.27
hsa-mir-148b	hsa-miR-148b-5p	3	6	744	1122	0.85	0.49	19.79	30.05
hsa-mir-4732	hsa-miR-4732-3p	56	142	440	362	15.91	11.49	11.71	9.70
hsa-mir-339	hsa-miR-339-3p	7	47	1158	436	1.99	3.80	30.81	11.68
hsa-mir-181a-1	hsa-miR-181a-3p	5	22	695	930	1.42	1.78	18.49	24.91
hsa-mir-454	hsa-miR-454-3p	5	19	875	707	1.42	1.54	23.28	18.94
hsa-mir-301b	hsa-miR-301b	2	4	712	893	0.57	0.32	18.94	23.92
hsa-mir-19a	hsa-miR-19a-3p	9	33	746	638	2.56	2.67	19.85	17.09
hsa-mir-500a	hsa-miR-500a-3p	25	53	453	633	7.10	4.29	12.05	16.95
hsa-mir-374b	hsa-miR-374b-5p	6	11	718	688	1.70	0.89	19.10	18.43
hsa-mir-548k	hsa-miR-548k	1	19	644	758	0.28	1.54	17.13	20.30
hsa-mir-493	hsa-miR-493-5p	3	5	536	786	0.85	0.40	14.26	21.05
hsa-mir-185	hsa-miR-185-3p	6	10	719	546	1.70	0.81	19.13	14.62
hsa-mir-221	hsa-miR-221-5p	5	11	617	575	1.42	0.89	16.42	15.40
hsa-mir-361	hsa-miR-361-3p	4	32	479	594	1.14	2.59	12.74	15.91
hsa-mir-26b	hsa-miR-26b-3p	2	23	542	567	0.57	1.86	14.42	15.19
hsa-mir-543	hsa-miR-543	3	3	766	374	0.85	0.24	20.38	10.02
hsa-mir-224	hsa-miR-224-5p	7	20	500	485	1.99	1.62	13.30	12.99
hsa-mir-664a	hsa-miR-664a-3p	7	5	636	357	1.99	0.40	16.92	9.56
hsa-mir-424	hsa-miR-424-3p	18	94	433	170	5.11	7.61	11.52	4.55
hsa-mir-342	hsa-miR-342-5p	25	51	338	295	7.10	4.13	8.99	7.90
hsa-mir-191	hsa-miR-191-3p	4	14	557	411	1.14	1.13	14.82	11.01
hsa-mir-370	hsa-miR-370-3p	6	7	449	505	1.70	0.57	11.95	13.53
hsa-mir-374a	hsa-miR-374a-3p	5	11	343	605	1.42	0.89	9.13	16.20

hsa-mir-3605	hsa-miR-3605-3p	26	40	298	329	7.38	3.24	7.93	8.81
hsa-mir-133a-1	hsa-miR-133a-3p	8	48	658	138	2.27	3.88	17.51	3.70
hsa-mir-133a-2	hsa-miR-133a-3p	8	48	658	138	2.27	3.88	17.51	3.70
hsa-mir-433	hsa-miR-433-3p	5	1	436	525	1.42	0.08	11.60	14.06
hsa-mir-215	hsa-miR-215-5p	27	81	156	328	7.67	6.55	4.15	8.78
hsa-mir-1260b	hsa-miR-1260b	1	2	633	365	0.28	0.16	16.84	9.78
hsa-mir-24-2	hsa-miR-24-2-5p	3	7	527	427	0.85	0.57	14.02	11.44
hsa-mir-148a	hsa-miR-148a-5p	1	11	394	537	0.28	0.89	10.48	14.38
hsa-mir-210	hsa-miR-210-3p	23	66	309	212	6.53	5.34	8.22	5.68
hsa-mir-1249	hsa-miR-1249	9	31	571	193	2.56	2.51	15.19	5.17
hsa-mir-493	hsa-miR-493-3p	1	8	327	546	0.28	0.65	8.70	14.62
hsa-mir-625	hsa-miR-625-5p	1	5	526	345	0.28	0.40	13.99	9.24
hsa-mir-374a	hsa-miR-374a-5p	4	16	364	436	1.14	1.29	9.68	11.68
hsa-mir-323a	hsa-miR-323a-3p	2	6	482	337	0.57	0.49	12.82	9.03
hsa-mir-454	hsa-miR-454-5p	1	6	399	370	0.28	0.49	10.62	9.91
hsa-mir-1468	hsa-miR-1468-5p	9	14	304	344	2.56	1.13	8.09	9.21
hsa-mir-125b-2	hsa-miR-125b-5p	17	70	235	156	4.83	5.66	6.25	4.18
hsa-mir-125b-1	hsa-miR-125b-5p	17	70	233	155	4.83	5.66	6.20	4.15
hsa-mir-877	hsa-miR-877-5p	3	9	327	367	0.85	0.73	8.70	9.83
hsa-mir-758	hsa-miR-758-3p	2	2	343	363	0.57	0.16	9.13	9.72
hsa-mir-766	hsa-miR-766-3p	6	9	355	283	1.70	0.73	9.44	7.58
hsa-mir-194-2	hsa-miR-194-5p	7	33	299	249	1.99	2.67	7.95	6.67
hsa-mir-139	hsa-miR-139-5p	4	25	217	382	1.14	2.02	5.77	10.23
hsa-mir-18a	hsa-miR-18a-5p	2	9	387	278	0.57	0.73	10.30	7.45
hsa-mir-194-1	hsa-miR-194-5p	5	33	304	239	1.42	2.67	8.09	6.40
hsa-mir-29c	hsa-miR-29c-3p	5	41	259	257	1.42	3.32	6.89	6.88
hsa-mir-204	hsa-miR-204-5p	11	26	224	271	3.12	2.10	5.96	7.26
hsa-mir-339	hsa-miR-339-5p	4	4	390	229	1.14	0.32	10.38	6.13
hsa-mir-100	hsa-miR-100-5p	18	52	119	161	5.11	4.21	3.17	4.31
hsa-mir-1301	hsa-miR-1301-3p	6	5	327	220	1.70	0.40	8.70	5.89
hsa-mir-628	hsa-miR-628-3p	4	13	282	248	1.14	1.05	7.50	6.64
hsa-mir-338	hsa-miR-338-3p	3	21	263	252	0.85	1.70	7.00	6.75
hsa-mir-146b	hsa-miR-146b-3p	10	19	219	227	2.84	1.54	5.83	6.08
hsa-mir-93	hsa-miR-93-3p	5	14	330	172	1.42	1.13	8.78	4.61
hsa-mir-6741	hsa-miR-6741-3p	1	2	317	258	0.28	0.16	8.43	6.91
hsa-mir-30a	hsa-miR-30a-3p	13	20	215	165	3.69	1.62	5.72	4.42
hsa-mir-183	hsa-miR-183-5p	14	53	125	136	3.98	4.29	3.33	3.64
hsa-mir-27b	hsa-miR-27b-5p	5	3	199	269	1.42	0.24	5.29	7.20
hsa-mir-141	hsa-miR-141-3p	12	46	134	126	3.41	3.72	3.57	3.37
hsa-mir-660	hsa-miR-660-5p	5	32	173	200	1.42	2.59	4.60	5.36
hsa-mir-6511a-1	hsa-miR-6511a-3p	7	15	212	186	1.99	1.21	5.64	4.98
hsa-mir-6511a-2	hsa-miR-6511a-3p	7	15	212	186	1.99	1.21	5.64	4.98
hsa-mir-6511a-3	hsa-miR-6511a-3p	7	15	212	186	1.99	1.21	5.64	4.98
hsa-mir-6511a-4	hsa-miR-6511a-3p	7	15	212	186	1.99	1.21	5.64	4.98
hsa-mir-196b	hsa-miR-196b-5p	2	4	282	197	0.57	0.32	7.50	5.28
hsa-mir-668	hsa-miR-668-3p	1	2	304	174	0.28	0.16	8.09	4.66
hsa-mir-539	hsa-miR-539-3p	1	2	196	280	0.28	0.16	5.21	7.50
hsa-mir-483	hsa-miR-483-3p	17	67	91	8	4.83	5.42	2.42	0.21
hsa-mir-548o	hsa-miR-548o-3p	2	12	181	230	0.57	0.97	4.82	6.16
hsa-mir-548o-2	hsa-miR-548o-3p	2	12	180	230	0.57	0.97	4.79	6.16
hsa-mir-1306	hsa-miR-1306-5p	2	21	239	137	0.57	1.70	6.36	3.67
hsa-mir-25	hsa-miR-25-5p	4	12	220	160	1.14	0.97	5.85	4.29
hsa-mir-99a	hsa-miR-99a-5p	13	34	110	97	3.69	2.75	2.93	2.60
hsa-mir-5010	hsa-miR-5010-5p	4	7	135	249	1.14	0.57	3.59	6.67
hsa-mir-874	hsa-miR-874-3p	10	17	171	119	2.84	1.38	4.55	3.19
hsa-mir-576	hsa-miR-576-5p	4	13	155	208	1.14	1.05	4.12	5.57
hsa-mir-4662a	hsa-miR-4662a-5p	3	4	134	251	0.85	0.32	3.57	6.72
hsa-mir-7706	hsa-miR-7706	10	21	109	150	2.84	1.70	2.90	4.02
hsa-mir-369	hsa-miR-369-3p	2	2	176	218	0.57	0.16	4.68	5.84
hsa-mir-505	hsa-miR-505-3p	2	6	234	136	0.57	0.49	6.23	3.64
hsa-mir-4286	hsa-miR-4286	2	13	187	143	0.57	1.05	4.98	3.83
hsa-mir-6087	hsa-miR-6087	6	51	96	76	1.70	4.13	2.55	2.04
hsa-mir-330	hsa-miR-330-5p	1	1	193	168	0.28	0.08	5.13	4.50
hsa-mir-548e	hsa-miR-548e-3p	1	7	156	184	0.28	0.57	4.15	4.93
hsa-mir-501	hsa-miR-501-3p	9	15	115	91	2.56	1.21	3.06	2.44

hsa-mir-6511b-1	hsa-miR-6511b-3p	2	22	137	111	0.57	1.78	3.64	2.97
hsa-mir-629	hsa-miR-629-5p	1	11	153	131	0.28	0.89	4.07	3.51
hsa-mir-7849	hsa-miR-7849-3p	2	5	128	163	0.57	0.40	3.41	4.37
hsa-mir-641	hsa-miR-641	1	1	135	177	0.28	0.08	3.59	4.74
hsa-mir-664a	hsa-miR-664a-5p	3	1	123	141	0.85	0.08	3.27	3.78
hsa-mir-15b	hsa-miR-15b-3p	4	17	99	101	1.14	1.38	2.63	2.71
hsa-mir-199b	hsa-miR-199b-5p	5	12	98	103	1.42	0.97	2.61	2.76
hsa-mir-20b	hsa-miR-20b-5p	1	11	141	99	0.28	0.89	3.75	2.65
hsa-mir-5189	hsa-miR-5189-5p	2	7	60	165	0.57	0.57	1.60	4.42
hsa-mir-320c-1	hsa-miR-320c	3	13	95	96	0.85	1.05	2.53	2.57
hsa-mir-532	hsa-miR-532-3p	4	18	85	76	1.14	1.46	2.26	2.04
hsa-mir-132	hsa-miR-132-3p	1	17	76	118	0.28	1.38	2.02	3.16
hsa-mir-1296	hsa-miR-1296-5p	2	6	116	95	0.57	0.49	3.09	2.54
hsa-mir-150	hsa-miR-150-3p	8	17	52	59	2.27	1.38	1.38	1.58
hsa-mir-320c-2	hsa-miR-320c	3	13	89	86	0.85	1.05	2.37	2.30
hsa-mir-4508	hsa-miR-4508	3	30	53	68	0.85	2.43	1.41	1.82
hsa-mir-152	hsa-miR-152-3p	2	7	109	88	0.57	0.57	2.90	2.36
hsa-mir-598	hsa-miR-598-3p	2	1	94	116	0.57	0.08	2.50	3.11
hsa-mir-125b-2	hsa-miR-125b-2-3p	9	15	50	39	2.56	1.21	1.33	1.04
hsa-mir-128-1	hsa-miR-128-1-5p	6	1	72	89	1.70	0.08	1.92	2.38
hsa-mir-3173	hsa-miR-3173-5p	5	9	75	69	1.42	0.73	2.00	1.85
hsa-mir-32	hsa-miR-32-5p	1	15	77	87	0.28	1.21	2.05	2.33
hsa-mir-18a	hsa-miR-18a-3p	2	7	103	74	0.57	0.57	2.74	1.98
hsa-mir-1255a	hsa-miR-1255a	1	1	47	157	0.28	0.08	1.25	4.20
hsa-mir-550a-1	hsa-miR-550a-3p	3	19	66	62	0.85	1.54	1.76	1.66
hsa-mir-550a-2	hsa-miR-550a-3p	3	19	66	62	0.85	1.54	1.76	1.66
hsa-mir-550a-3	hsa-miR-550a-3p	3	19	66	62	0.85	1.54	1.76	1.66
hsa-mir-885	hsa-miR-885-5p	9	25	36	10	2.56	2.02	0.96	0.27
hsa-mir-326	hsa-miR-326	1	2	108	90	0.28	0.16	2.87	2.41
hsa-mir-324	hsa-miR-324-5p	1	6	95	89	0.28	0.49	2.53	2.38
hsa-mir-338	hsa-miR-338-5p	1	6	81	83	0.28	0.49	2.15	2.22
hsa-let-7b	hsa-let-7b-3p	3	24	44	44	0.85	1.94	1.17	1.18
hsa-mir-7848	hsa-miR-7848-3p	1	2	93	82	0.28	0.16	2.47	2.20
hsa-mir-548e	hsa-miR-548e-5p	1	3	76	95	0.28	0.24	2.02	2.54
hsa-mir-205	hsa-miR-205-5p	6	24	19	34	1.70	1.94	0.51	0.91
hsa-mir-502	hsa-miR-502-3p	3	14	51	61	0.85	1.13	1.36	1.63
hsa-mir-324	hsa-miR-324-3p	1	2	105	63	0.28	0.16	2.79	1.69
hsa-mir-3158-1	hsa-miR-3158-3p	5	6	70	41	1.42	0.49	1.86	1.10
hsa-mir-3158-2	hsa-miR-3158-3p	5	6	70	41	1.42	0.49	1.86	1.10
hsa-mir-33b	hsa-miR-33b-5p	1	9	74	70	0.28	0.73	1.97	1.87
hsa-mir-656	hsa-miR-656-3p	1	2	79	71	0.28	0.16	2.10	1.90
hsa-mir-7-1	hsa-miR-7-1-3p	3	3	52	73	0.85	0.24	1.38	1.96
hsa-mir-6772	hsa-miR-6772-3p	1	1	61	77	0.28	0.08	1.62	2.06
hsa-mir-106a	hsa-miR-106a-5p	3	2	56	56	0.85	0.16	1.49	1.50
hsa-mir-29b-2	hsa-miR-29b-3p	2	6	47	54	0.57	0.49	1.25	1.45
hsa-mir-2110	hsa-miR-2110	1	3	61	59	0.28	0.24	1.62	1.58
hsa-mir-5010	hsa-miR-5010-3p	7	5	36	14	1.99	0.40	0.96	0.37
hsa-mir-29b-1	hsa-miR-29b-3p	2	6	46	54	0.57	0.49	1.22	1.45
hsa-mir-378c	hsa-miR-378c	1	12	51	39	0.28	0.97	1.36	1.04
hsa-mir-550a-1	hsa-miR-550a-5p	1	8	44	57	0.28	0.65	1.17	1.53
hsa-mir-550a-2	hsa-miR-550a-5p	1	8	44	57	0.28	0.65	1.17	1.53
hsa-mir-329-1	hsa-miR-329-3p	1	1	73	34	0.28	0.08	1.94	0.91
hsa-mir-329-2	hsa-miR-329-3p	1	1	73	34	0.28	0.08	1.94	0.91
hsa-mir-1277	hsa-miR-1277-5p	1	2	28	70	0.28	0.16	0.74	1.87
hsa-mir-365a	hsa-miR-365a-3p	5	11	11	7	1.42	0.89	0.29	0.19
hsa-mir-365b	hsa-miR-365b-3p	5	11	11	7	1.42	0.89	0.29	0.19
hsa-mir-6859-1	hsa-miR-6859-5p	2	1	33	46	0.57	0.08	0.88	1.23
hsa-mir-212	hsa-miR-212-3p	3	8	25	21	0.85	0.65	0.67	0.56
hsa-mir-873	hsa-miR-873-5p	4	8	28	6	1.14	0.65	0.74	0.16
hsa-let-7a-1	hsa-let-7a-3p	1	1	47	40	0.28	0.08	1.25	1.07
hsa-let-7a-3	hsa-let-7a-3p	1	1	47	40	0.28	0.08	1.25	1.07
hsa-mir-636	hsa-miR-636	4	5	23	17	1.14	0.40	0.61	0.46
hsa-mir-4326	hsa-miR-4326	1	6	30	30	0.28	0.49	0.80	0.80
hsa-mir-331	hsa-miR-331-5p	1	2	23	47	0.28	0.16	0.61	1.26
hsa-mir-937	hsa-miR-937-3p	2	8	16	24	0.57	0.65	0.43	0.64

hsa-mir-371b	hsa-miR-371b-5p	5	4	11	6	1.42	0.32	0.29	0.16
hsa-mir-497	hsa-miR-497-5p	1	4	30	26	0.28	0.32	0.80	0.70
hsa-mir-483	hsa-miR-483-5p	3	4	17	14	0.85	0.32	0.45	0.37
hsa-let-7i	hsa-let-7i-3p	1	3	34	20	0.28	0.24	0.90	0.54
hsa-mir-96	hsa-miR-96-5p	3	6	13	5	0.85	0.49	0.35	0.13
hsa-mir-30c-2	hsa-miR-30c-2-3p	3	2	14	15	0.85	0.16	0.37	0.40
hsa-mir-200a	hsa-miR-200a-3p	1	6	17	21	0.28	0.49	0.45	0.56
hsa-mir-3200	hsa-miR-3200-5p	1	9	17	10	0.28	0.73	0.45	0.27
hsa-mir-548l	hsa-miR-548l	1	1	16	35	0.28	0.08	0.43	0.94
hsa-mir-424	hsa-miR-424-5p	1	3	29	16	0.28	0.24	0.77	0.43
hsa-mir-122	hsa-miR-122-3p	3	7	7	4	0.85	0.57	0.19	0.11
hsa-mir-6735	hsa-miR-6735-5p	1	1	25	25	0.28	0.08	0.67	0.67
hsa-mir-2467	hsa-miR-2467-5p	1	1	29	20	0.28	0.08	0.77	0.54
hsa-mir-101-1	hsa-miR-101-5p	1	5	15	19	0.28	0.40	0.40	0.51
hsa-mir-195	hsa-miR-195-3p	2	4	9	17	0.57	0.32	0.24	0.46
hsa-mir-5193	hsa-miR-5193	1	3	14	25	0.28	0.24	0.37	0.67
hsa-mir-193b	hsa-miR-193b-5p	1	11	9	4	0.28	0.89	0.24	0.11
hsa-mir-2355	hsa-miR-2355-3p	1	2	18	21	0.28	0.16	0.48	0.56
hsa-mir-1323	hsa-miR-1323	2	7	9	1	0.57	0.57	0.24	0.03
hsa-mir-1304	hsa-miR-1304-3p	1	1	19	16	0.28	0.08	0.51	0.43
hsa-mir-887	hsa-miR-887-3p	2	3	7	7	0.57	0.24	0.19	0.19
hsa-mir-2116	hsa-miR-2116-3p	1	1	8	22	0.28	0.08	0.21	0.59
hsa-mir-1247	hsa-miR-1247-5p	2	3	8	5	0.57	0.24	0.21	0.13
hsa-mir-4762	hsa-miR-4762-5p	1	1	12	17	0.28	0.08	0.32	0.46
hsa-mir-203a	hsa-miR-203a	1	1	9	18	0.28	0.08	0.24	0.48
hsa-mir-1910	hsa-miR-1910-5p	1	7	3	2	0.28	0.57	0.08	0.05
hsa-mir-378d-1	hsa-miR-378d	2	2	4	5	0.57	0.16	0.11	0.13
hsa-mir-378d-2	hsa-miR-378d	2	2	4	5	0.57	0.16	0.11	0.13
hsa-mir-7854	hsa-miR-7854-3p	1	1	13	6	0.28	0.08	0.35	0.16
hsa-mir-3157	hsa-miR-3157-3p	1	1	10	6	0.28	0.08	0.27	0.16
hsa-mir-4772	hsa-miR-4772-3p	1	3	4	5	0.28	0.24	0.11	0.13
hsa-mir-188	hsa-miR-188-5p	1	1	8	5	0.28	0.08	0.21	0.13
hsa-mir-1255b-1	hsa-miR-1255b-5p	1	1	6	4	0.28	0.08	0.16	0.11
hsa-mir-1255b-2	hsa-miR-1255b-5p	1	1	6	4	0.28	0.08	0.16	0.11
hsa-mir-577	hsa-miR-577	1	1	3	1	0.28	0.08	0.08	0.03

Online Table II. Layout for custom-made Exiqon miRNA qPCR assays. 92 miRNAs (plus U6 snRNA and exogenous controls *Cel*-miR-39-3p, UniSp6 CP and UniSp3 IPC) are listed according to their positions on the 384-well plate. Every sequence was present in four wells per plate, e.g. hsa-let-7e-5p was in wells A01, A02, B01 and B02.

microRNA Name	Well	Target sequence
hsa-let-7e-5p	A01	UGAGGUAGGAGGUUGUAUAGUU
hsa-miR-16-5p	A03	UAGCAGCACGUAAAUAUUGGCG
hsa-miR-17-5p	A05	CAAAGUGCUUACAGUGCAGGUAG
hsa-miR-19b-3p	A07	UGUGCAAAUCCAUGCAAAACUGA
hsa-miR-20a-5p	A09	UAAAGUGCUUAUAGUGCAGGUAG
hsa-miR-20b-5p	A11	CAAAGUGCUCAUAGUGCAGGUAG
hsa-miR-24-3p	A13	UGGCUCAGUUCAGCAGGAACAG
hsa-miR-28-3p	A15	CACUAGAUUGUGAGCUCCUGGA
hsa-miR-30b-5p	A17	UGUAAACAUCCUACACUCAGCU
hsa-miR-30c-5p	A19	UGUAAACAUCCUACACUCUCAGC
hsa-miR-92a-3p	A21	UAUUGCACUUGUCCCGGCCUGU
hsa-miR-93-5p	A23	CAAAGUGCUGUUCGUGCAGGUAG
hsa-miR-106a-5p	C01	AAAAGUGCUUACAGUGCAGGUAG
hsa-miR-125a-5p	C03	UCCCUGAGACCCUUUAACCUGUGA
hsa-miR-126-3p	C05	UCGUACCGUGAGUAAUAAUGCG
hsa-miR-139-5p	C07	UCUACAGUGCACGUGUCUCCAGU
hsa-miR-146a-5p	C09	UGAGAACUGAAUUCCAUGGGUU
hsa-miR-150-5p	C11	UCUCCCAACCCUUGUACCAGUG
hsa-miR-146b-5p	C13	UGAGAACUGAAUUCCAUAGGCU
hsa-miR-186-5p	C15	CAAAGAAUUCUCCUUUUGGGCU
hsa-miR-191-5p	C17	CAACGGAAUCCCAAAAGCAGCUG
hsa-miR-197-3p	C19	UUCACCACCUUCUCCACCCAGC
hsa-miR-222-3p	C21	AGCUACAUCUGGCUACUGGGU
hsa-miR-223-3p	C23	UGUCAGUUUGUCAAAUACCCCA
hsa-miR-320a	E01	AAAAGCUGGGUUGAGAGGGCGA
hsa-miR-331-3p	E03	GCCCCUGGGCCUAUCCUAGAA
hsa-miR-342-3p	E05	UCUCACACAGAAAUCGCACCCGU
hsa-let-7b-5p	E07	UGAGGUAGUAGGUUGUGUGGUU
hsa-miR-451a	E09	AAACCGUUACCAUUACUGAGUU
hsa-miR-454-3p	E11	UAGUGCAAUAUUGCUUAUAGGGU
hsa-miR-484	E13	UCAGGCUCAGUCCCCUCCCGAU
hsa-miR-486-5p	E15	UCCUGUACUGAGCUGCCCCGAG
U6 snRNA	E17	
hsa-let-7g-5p	E19	UGAGGUAGUAGUUUGUACAGUU
UniSp6 CP	E21	
Cel-miR-39-3p CP	E23	
hsa-miR-19a-3p	G02	UGUGCAAAUCUAUGCAAAACUGA
hsa-miR-21-5p	G04	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-25-3p	G06	CAUUGCACUUGUCUCGGUCUGA
hsa-miR-26a-5p	G08	UUCAAGUAAUCCAGGAUAGGCU
hsa-miR-26b-5p	G10	UUCAAGUAAUUCAGGAUAGGU
hsa-miR-27a-3p	G12	UUCACAGUGGCUAAGUUCCGC
hsa-miR-27b-3p	G14	UUCACAGUGGCUAAGUUCUGC
hsa-miR-28-5p	G16	AAGGAGCUCACAGUCUAUUGAG
hsa-miR-29a-3p	G18	UAGCACCAUCUGAAAUCGGUUA
hsa-miR-29b-3p	G20	UAGCACCAUUUGAAAUCAGUGUU
hsa-miR-103a-3p	G22	AGCAGCAUUGUACAGGGCUAUGA
hsa-miR-107	G24	AGCAGCAUUGUACAGGGCUAUCA

microRNA Name	Well	Target sequence
hsa-miR-122-5p	I02	UGGAGUGUGACAAUGGUGUUUG
hsa-miR-125b-5p	I04	UCCCUGAGACCCUAACUUGUGA
hsa-miR-127-3p	I06	UCGGAUCCGUCUGAGCUUGGCU
hsa-miR-130a-3p	I08	CAGUGCAAUGUUAAAAGGGCAU
hsa-miR-140-5p	I10	CAGUGGUUUUACCCUAUGGUAG
hsa-miR-142-3p	I12	UGUAGUGUUUCCUACUUUAUGGA
hsa-miR-143-3p	I14	UGAGAUGAAGCACUGUAGCUC
hsa-miR-152	I16	UCAGUGCAUGACAGAACUUGG
hsa-miR-185-5p	I18	UGGAGAGAAAGGCAGUUCCUGA
hsa-miR-192-5p	I20	CUGACCUAUGAAUUGACAGCC
hsa-miR-193b-3p	I22	AACUGGCCCUCAAAGUCCCGCU
hsa-miR-194-5p	I24	UGUAACAGCAACUCCAUGUGGA
hsa-miR-195-5p	K02	UAGCAGCACAGAAAUAUUGGC
hsa-miR-199a-3p	K04	ACAGUAGUCUGCACAUUGGUUA
hsa-miR-200b-3p	K06	UAAUACUGCCUGGUAAUGAUGA
hsa-miR-221-3p	K08	AGCUACAUUGUCUGCUGGGUUUC
hsa-miR-18a-5p	K10	UAAGGUGCAUCUAGUGCAGAUAG
hsa-miR-503-5p	K12	UAGCAGCGGGAACAGUUCUGCAG
hsa-miR-328	K14	CUGGCCCUCUCUGCCCUUCCGU
hsa-miR-335-5p	K16	UCAAGAGCAAUAACGAAAAAUGU
hsa-miR-342-5p	K18	AGGGGUGCUAUCUGUGAUUGA
hsa-miR-155-5p	K20	UUAAUGCUAAUCGUGAUAGGGGU
UniSp3 IPC	K22	
hsa-miR-340-5p	K24	UUAUAAAGCAAUGAGACUGAUU
hsa-miR-363-3p	M02	AAUUGCACGGUAUCCAUCUGUA
hsa-miR-374a-5p	M04	UUAUAAUACAACCUGAUAAGUG
hsa-miR-381-3p	M06	UAUACAAGGGCAAGCUCUCUGU
hsa-miR-423-5p	M08	UGAGGGGCAGAGAGCGAGACUUU
hsa-miR-518b	M10	CAAAGCGCUCCCCUUUAGAGGU
hsa-miR-518f-3p	M12	GAAAGCGCUUCUCUUUAGAGG
hsa-miR-532-3p	M14	CCUCCCACACCCAAGGCUUGCA
hsa-miR-574-3p	M16	CACGCUCAUGCACACACCCACA
hsa-miR-590-5p	M18	GAGCUUAUUCAUAAAAGUGCAG
hsa-miR-628-5p	M20	AUGCUGACAUAUUUACUAGAGG
hsa-miR-660-5p	M22	UACCCAUUGCAUAUCGGAGUUG
hsa-miR-744-5p	M24	UGCGGGGCUAGGGCUAACAGCA
hsa-miR-758-3p	O02	UUUGUGACCUGGUCCACUAACC
hsa-miR-210	O04	CUGUGCGUGUGACAGCGGCUGA
hsa-miR-214-3p	O06	ACAGCAGGCACAGACAGGCAGU
hsa-miR-885-5p	O08	UCCAUUACACUACCCUGCCUCU
hsa-miR-10a-5p	O10	UACCCUGUAGAUCCGAAUUUGUG
hsa-miR-15a-5p	O12	UAGCAGCACAUAAUGGUUUGUG
hsa-miR-130b-3p	O14	CAGUGCAAUGAUGAAAGGGCAU
hsa-miR-375	O16	UUUGUUCGUUCGGCUCGCGUGA
hsa-miR-202-3p	O18	AGAGGUAUAGGGCAUGGGAA
hsa-miR-126-5p	O20	CAUUAUUACUUUUGGUACGCG
hsa-miR-376c-3p	O22	AACAUAGAGGAAAUUCCACGU
hsa-miR-195-3p	O24	CCAAUAUUGGCUGUGCUGCUCC

Online Table III. Antiplatelet medication in the ACS cohort (n=125). Plasma was taken 30 days after the acute event when the inflammation associated with the acute injury has receded and anti-platelet drugs had been administered for a month. Importantly, none of the ACS patients had received heparin at the time of sampling.

	Number of patients				
Assay	**ASA only**	**ASA +Clopidogrel**	**ASA +Prasugrel**	**ASA +others**	**Total**
Platelet count on day 30 (10^9/L)	13	78	30	2	123
OPTICAL AGGREGOMETRY (FINAL) WITH ADP	13	79	31	2	125
OPTICAL AGGREGOMETRY (MAX) WITH ADP	13	79	31	2	125
OPTICAL AGGREGOMETRY (MAX) WITH AA	13	79	31	2	125
Verify Now PRU, P2Y12 AGGREGATION ASSAYS	7	28	4	1	40
VASP P2Y12 AGGREGATION ASSAYS	12	78	29	2	121

Online Table IV. Allele and genotype frequencies for SNP rs4636297. The counts and frequencies for the different alleles and genotypes of SNP rs4636297 in the Bruneck cohort (n=628) and the European population analyzed in the 1000 genomes project (n=503, release 16 - Oct 2014).[6]

	Bruneck (n=628)		**European Population (n=503)**	
	Count	**Frequency**	**Count**	**Frequency**
Allele				
A	490	0.390	366	0.364
G	766	0.610	640	0.636
Genotype				
AA	105	0.167	70	0.139
GA	280	0.446	226	0.449
GG	243	0.387	207	0.412

Online Table V. Protein associations with the rs4636297 AA genotype. In the Bruneck cohort, 219 plasma proteins were measured and for each protein levels were compared between subjects with the AA genotype of the SNP rs4636297 and subjects with other genotypes. P values were derived from Wilcoxon-Mann-Whitney tests and proteins are ranked ascending by P value. Three measures of effect size are shown: Median ratio refers to the ratio of the median concentration in AA subjects over that in other subjects; mean ratio to an analogous ratio of means; and median difference to the difference in medians scaled by the overall interquartile range. The last column indicates whether a protein has the GO annotation "platelet activation". Proteins tested by ELISA (Fig. 6B) are shown in bold. ELISA, enzyme-linked immunosorbent assay; FDR, false discovery rate; IQR, interquartile range; MS, mass spectrometry; PEA, proximity extension assay; PF4, platelet factor 4; PPBP, pro-platelet basic protein; SELP, P-selectin.

Rank	Gene Name	Protein	P-value	FDR q-value	Median Ratio	Mean Ratio	Median Difference over IQR	n (GG/AG)	n (AA)	Detection Method	Annotation Platelet Activation
1	CFI	CFAI	2.76E-05	0.01	1.12	1.10	0.420	515	100	MS	-
2	HPX	HEMO	9.47E-05	0.01	1.07	1.07	0.284	515	100	MS	-
3	KNG1	KNG1	0.00013	0.01	1.09	1.07	0.371	515	100	MS	+
4	KLKB1	KLKB1	0.00013	0.01	1.10	1.09	0.346	515	100	MS	-
5	C4BPA	C4BPA	0.00032	0.01	1.12	1.11	0.356	515	100	MS	-
6	VTN	VTNC	0.00045	0.02	1.07	1.07	0.292	515	100	MS	-
7	SERPIND1	HEP2	0.00054	0.02	1.09	1.08	0.308	515	100	MS	-
8	C3	CO3	0.00094	0.03	1.13	1.09	0.373	515	100	MS	-
9	F2	THRB	0.00110	0.03	1.06	1.07	0.266	515	100	MS	+
10	C1S	C1S	0.00124	0.03	1.05	1.07	0.268	515	100	MS	-
11	**PF4**	**PF4**	**0.00163**	**0.03**	**1.18**	**1.47**	**0.212**	**515**	**100**	**MS**	**+**
12	SERPINF2	A2AP	0.00171	0.03	1.06	1.05	0.293	515	100	MS	+
13	CFH	CFAH	0.00183	0.03	1.07	1.06	0.286	515	100	MS	-
14	**PPBP**	**PPBP**	**0.00207**	**0.03**	**1.16**	**1.49**	**0.196**	**513**	**100**	**ELISA**	**+**
15	AGT	ANGT	0.00269	0.04	1.06	1.07	0.215	515	100	MS	-
16	SERPINA3	AACT	0.00273	0.04	1.09	1.05	0.350	515	100	MS	-
17	HP	HPT	0.00315	0.04	1.15	1.12	0.260	515	100	MS	-
18	AFM	AFAM	0.00381	0.05	1.07	1.08	0.235	515	100	MS	-
19	EIF4EBP1	4E-BP1	0.00448	0.05	1.17	1.47	0.252	512	98	PEA	-
20	ITIH1	ITIH1	0.00476	0.05	1.03	1.06	0.146	515	100	MS	-
21	APOH	APOH	0.00503	0.05	1.08	1.07	0.297	515	100	MS	-
22	ORM2	A1AG2	0.00510	0.05	1.10	1.08	0.238	515	100	MS	-
23	PDGFB	PDGFB	0.00617	0.06	1.17	1.26	0.185	515	102	PEA	+
24	AHSG	FETUA	0.00622	0.06	1.06	1.05	0.261	515	100	MS	-
25	SERPINA7	THBG	0.00654	0.06	1.08	1.08	0.225	515	100	MS	-
26	ITIH4	ITIH4	0.00668	0.06	1.05	1.10	0.193	515	100	MS	-
27	SERPING1	IC1	0.00723	0.06	1.03	1.06	0.133	515	100	MS	+
28	CLU	CLUS	0.01173	0.09	1.08	1.05	0.324	515	100	MS	+
29	APOB	APOB	0.01248	0.09	1.12	1.09	0.260	515	100	MS	-
30	HBD	HBD	0.01265	0.09	1.16	1.16	0.238	515	100	MS	-
31	C1R	C1R	0.01269	0.09	1.05	1.05	0.228	515	100	MS	-
32	IL1RN	IL-1ra	0.01602	0.11	1.13	1.10	0.212	515	102	PEA	-
33	A1BG	A1BG	0.01651	0.11	1.07	1.05	0.256	515	100	MS	-
34	SERPINF1	PEDF	0.01704	0.11	1.07	1.05	0.238	515	100	MS	-
35	DKK1	Dkk-1	0.01763	0.11	1.07	1.14	0.144	515	102	PEA	-
36	CXCL16	CXCL16	0.01942	0.12	1.11	1.06	0.336	515	102	PEA	-
37	C2	CO2	0.01954	0.12	1.03	1.05	0.142	515	100	MS	-
38	APOA2	APOA2	0.02026	0.12	1.08	1.06	0.278	515	100	MS	-
39	CCL25	CCL25	0.02158	0.12	1.12	1.11	0.197	512	98	PEA	-
40	APOA4	APOA4	0.02318	0.13	1.06	1.06	0.179	515	100	MS	-
41	HBEGF	HB-EGF	0.02321	0.13	1.05	1.08	0.212	515	102	PEA	-
42	FAS	FAS	0.02404	0.13	1.05	1.04	0.173	515	102	PEA	-
43	C1QB	C1QB	0.02460	0.13	1.03	1.06	0.125	515	100	MS	-
44	FIGF	VEGF-D	0.02683	0.14	0.89	0.94	-0.290	515	102	PEA	+

45	CFB	CFAB	0.02757	0.14	1.04	1.06	0.149	515	100	MS	-
46	CXCL5	CXCL5	0.02825	0.14	1.35	1.41	0.274	512	98	PEA	-
47	SIRT2	SIRT2	0.02921	0.14	1.17	1.72	0.132	512	98	PEA	-
48	IL27	IL27-A	0.03102	0.14	0.95	0.94	-0.156	515	102	PEA	-
49	ITIH2	ITIH2	0.03248	0.15	1.05	1.05	0.167	515	100	MS	-
50	ORM1	A1AG1	0.03516	0.15	1.14	1.07	0.294	515	100	MS	-
51	CCL4	CCL4	0.03935	0.16	1.05	1.28	0.113	512	98	PEA	-
52	GH1	GH	0.04003	0.16	0.40	0.86	-0.207	515	102	PEA	-
53	LEP	LEP	0.04070	0.16	1.24	1.20	0.221	515	102	PEA	-
54	GC	VTDB	0.04480	0.17	1.03	1.05	0.110	515	100	MS	-
55	PLG	PLMN	0.04523	0.17	1.03	1.04	0.156	515	100	MS	+
56	CXCL1	CXCL1	0.04689	0.18	1.11	1.23	0.122	512	98	PEA	-
57	AZGP1	ZA2G	0.04738	0.18	1.04	1.04	0.156	515	100	MS	-
58	TNFSF10	TRAIL	0.04864	0.18	1.05	1.07	0.117	512	98	PEA	-
59	CASP8	CASP-8	0.05002	0.18	1.11	1.38	0.187	512	98	PEA	-
60	GSN	GELS	0.05002	0.18	1.01	1.05	0.038	515	100	MS	-
61	BDNF	BDNF	0.05024	0.18	0.46	0.61	-0.013	512	98	PEA	-
62	AMBP	AMBP	0.05192	0.18	1.05	1.04	0.236	515	100	MS	-
63	CP	CERU	0.05422	0.18	1.06	1.06	0.165	515	100	MS	-
64	CXCL11	CXCL11	0.05608	0.19	1.05	1.13	0.074	512	98	PEA	-
65	IL18R1	IL-18R1	0.05755	0.19	1.08	1.07	0.173	512	98	PEA	-
66	STAMBP	STAMPB	0.05998	0.19	1.19	1.91	0.165	512	98	PEA	-
67	AGER	RAGE	0.06025	0.19	0.94	0.95	-0.145	515	102	PEA	-
68	SERPINA6	CBG	0.06037	0.19	1.03	1.08	0.086	515	100	MS	-
69	APOL1	APOL1	0.06308	0.20	1.07	1.05	0.201	515	100	MS	-
70	SERPINA1	A1AT	0.07053	0.22	1.03	1.06	0.087	515	100	MS	+
71	SELE	SELE	0.07216	0.22	1.06	1.05	0.110	515	102	PEA	-
72	EGF	EGF	0.07510	0.23	1.03	1.35	0.027	515	102	PEA	+
73	TTR	TTHY	0.08263	0.24	1.05	1.07	0.110	515	100	MS	-
74	SERPINC1	ANT3	0.08345	0.24	1.06	1.04	0.230	515	100	MS	-
75	TF	TRFE	0.08351	0.24	1.02	1.04	0.098	515	100	MS	+
76	**SELP**	**SELP**	**0.08472**	**0.24**	**1.13**	**1.16**	**0.237**	**513**	**100**	**ELISA**	**+**
77	CD40LG	CD40-L	0.08638	0.24	1.10	1.38	0.072	515	102	PEA	+
78	F13A1	F13A	0.08856	0.25	1.06	1.07	0.156	515	100	MS	+
79	PGLYRP2	PGRP2	0.09320	0.26	1.04	1.03	0.165	515	100	MS	-
80	HRG	HRG	0.10340	0.28	1.03	1.04	0.075	515	100	MS	+
81	ALB	ALBU	0.10557	0.28	1.03	1.02	0.165	515	100	MS	+
82	CLEC3B	TETN	0.10569	0.28	1.03	1.03	0.133	515	100	MS	-
83	SEPP1	SEPP1	0.10733	0.28	1.03	1.04	0.100	515	100	MS	-
84	APOC3	APOC3	0.10892	0.28	1.04	1.07	0.094	515	100	MS	-
85	FABP4	FABP4	0.10935	0.28	1.06	1.09	0.091	515	102	PEA	-
86	HPR	HPTR	0.11460	0.29	1.08	1.06	0.128	515	100	MS	-
87	RBP4	RET4	0.11595	0.29	1.02	1.05	0.052	515	100	MS	-
88	PLAT	t-PA	0.11681	0.29	1.06	1.04	0.117	515	102	PEA	-
89	PECAM1	PECAM-1	0.11867	0.29	1.02	1.11	0.034	515	102	PEA	+
90	CCL8	MCP-2	0.11935	0.29	1.06	1.11	0.125	512	98	PEA	-
91	CCL7	MCP-3	0.12533	0.30	1.09	1.33	0.184	512	98	PEA	-
92	CSF1	CSF-1	0.12726	0.30	1.03	1.05	0.076	512	98	PEA	-
93	ADA	ADA	0.12836	0.30	1.04	1.09	0.077	512	98	PEA	-
94	HSPB1	HSP-27	0.13642	0.32	1.09	1.23	0.070	515	102	PEA	+
95	VEGFA	VEGF-A	0.13872	0.32	1.06	1.12	0.176	512	98	PEA	+
96	CCL13	MCP-4	0.14107	0.32	1.07	1.10	0.154	512	98	PEA	-
97	IL7	IL-7	0.14318	0.32	1.06	1.16	0.129	512	98	PEA	-
98	THBD	TM	0.15107	0.33	1.03	1.04	0.121	515	102	PEA	+
99	C9	CO9	0.15159	0.33	1.05	1.05	0.104	515	100	MS	-
100	AXIN1	AXIN1	0.15221	0.33	1.17	2.12	0.133	512	98	PEA	-
101	RETN	RETN	0.15351	0.33	1.06	1.04	0.133	515	102	PEA	-
102	FGA	FIBA	0.15983	0.34	1.05	1.01	0.150	515	100	MS	+
103	SAA4	SAA4	0.16286	0.34	1.05	1.05	0.120	515	100	MS	-
104	CSTB	CSTB	0.18173	0.38	1.05	1.01	0.107	515	102	PEA	-
105	MMP12	MMP-12	0.18333	0.38	0.90	0.86	-0.144	515	102	PEA	-

106	HGF	HGF	0.19736	0.40	1.06	1.02	0.135	512	98	PEA	+
107	C8A	CO8A	0.19949	0.40	1.01	1.03	0.045	515	100	MS	-
108	GDNF	hGDNF	0.20771	0.42	0.97	0.95	-0.088	512	98	PEA	-
109	CST5	CST5	0.21324	0.42	0.97	0.94	-0.054	512	98	PEA	-
110	CHI3L1	CHI3L1	0.21453	0.42	1.15	0.91	0.134	515	102	PEA	-
111	IGHG2	IGHG2	0.22133	0.43	0.98	0.93	-0.027	515	100	MS	-
112	IGHM	IGHM	0.22743	0.44	0.87	0.91	-0.163	515	100	MS	-
113	CD5L	CD5L	0.23839	0.45	0.88	0.93	-0.167	515	100	MS	-
114	C5	CO5	0.24022	0.45	1.01	1.02	0.034	515	100	MS	-
115	IKBKG	NEMO	0.24345	0.45	1.02	1.19	0.020	515	102	PEA	-
116	PAPPA	PAPPA	0.24505	0.45	0.98	0.92	-0.046	515	102	PEA	-
117	ADM	AM	0.24542	0.45	1.02	1.04	0.038	515	102	PEA	-
118	APOA1	APOA1	0.24670	0.45	1.03	1.02	0.103	515	100	MS	+
119	FN1	FINC	0.25035	0.46	1.08	1.00	0.121	515	100	MS	+
120	AGRP	AGRP	0.25417	0.46	1.05	1.01	0.149	515	102	PEA	-
121	C1QC	C1QC	0.25881	0.46	1.02	1.03	0.101	515	100	MS	-
122	OSM	OSM	0.26280	0.47	1.11	1.05	0.231	512	98	PEA	-
123	CD244	CD244	0.26530	0.47	1.03	1.22	0.069	512	98	PEA	-
124	SULT1A1	ST1A1	0.27158	0.48	1.13	1.23	0.155	512	98	PEA	-
125	LGALS3	Gal-3	0.28665	0.50	1.05	1.05	0.131	515	102	PEA	-
126	NPPB	NT-pro-BNP	0.28688	0.50	0.97	0.75	-0.023	515	102	PEA	-
127	PGF	PlGF	0.29024	0.50	0.98	0.97	-0.076	515	102	PEA	-
128	GPX3	GPX3	0.30204	0.51	1.02	1.02	0.086	515	100	MS	-
129	TEK	TIE2	0.31271	0.53	1.03	1.01	0.118	515	102	PEA	-
130	FST	FS	0.31446	0.53	1.01	1.05	0.035	515	102	PEA	-
131	CCL3	MIP-1-alpha	0.32153	0.53	1.03	1.03	0.065	512	98	PEA	-
132	IL10RB	IL-10RB	0.32336	0.53	1.02	1.03	0.039	512	98	PEA	-
133	TNFSF11	TRANCE	0.32984	0.54	1.01	1.04	0.010	512	98	PEA	-
134	TNFSF12	TWEAK	0.33265	0.54	1.04	1.02	0.125	512	98	PEA	-
135	CD5	CD5	0.34210	0.55	1.04	1.09	0.093	512	98	PEA	-
136	SRC	SRC	0.34678	0.55	1.03	1.05	0.049	515	102	PEA	+
137	IGHG3	IGHG3	0.34734	0.55	1.02	1.06	0.044	515	100	MS	-
138	IGHG1	IGHG1	0.35098	0.55	0.97	0.96	-0.082	515	100	MS	-
139	CCL2	MCP-1	0.35204	0.55	1.06	1.02	0.143	512	98	PEA	-
140	CXCL10	CXCL10	0.35269	0.55	1.05	1.06	0.064	512	98	PEA	-
141	TNFRSF1B	TNF-R2	0.35336	0.55	1.05	0.99	0.130	515	102	PEA	-
142	MMP7	MMP-7	0.35462	0.55	0.98	0.93	-0.053	515	102	PEA	-
143	TNFSF14	TNFSF14	0.36995	0.56	1.06	1.46	0.117	512	98	PEA	-
144	SHBG	SHBG	0.37134	0.56	0.96	0.94	-0.060	515	100	MS	-
145	RNASE3	ECP	0.37489	0.57	0.94	0.96	-0.085	515	102	PEA	-
146	CCL19	CCL19	0.39155	0.59	1.06	0.93	0.077	512	98	PEA	-
147	CX3CL1	CX3CL1	0.39571	0.59	1.02	1.07	0.035	512	98	PEA	-
148	IL17C	IL-17C	0.40793	0.60	1.04	1.13	0.072	512	98	PEA	-
149	KITLG	SCF	0.41085	0.60	1.01	1.04	0.024	512	98	PEA	-
150	CD40	CD40	0.42306	0.61	1.03	1.21	0.037	512	98	PEA	+
151	FGF21	FGF-21	0.42451	0.61	1.05	1.83	0.051	512	98	PEA	-
152	LRG1	A2GL	0.43132	0.62	1.05	1.01	0.120	515	100	MS	-
153	MMP1	MMP-1	0.43662	0.62	1.08	1.00	0.110	515	102	PEA	-
154	CXCL6	CXCL6	0.45525	0.64	1.00	1.37	0.004	512	98	PEA	-
155	CCL3	CCL3	0.45769	0.64	1.02	0.98	0.063	515	102	PEA	-
156	MMP3	MMP-3	0.46397	0.65	1.01	1.00	0.018	515	102	PEA	-
157	TNFRSF11B	OPG	0.47706	0.66	0.95	0.97	-0.126	512	98	PEA	-
158	PLAUR	U-PAR	0.48958	0.67	1.00	0.98	-0.009	515	102	PEA	-
159	APOE	APOE	0.49539	0.67	1.01	1.03	0.021	515	100	MS	+
160	CCL11	CCL11	0.50063	0.67	1.03	1.08	0.085	512	98	PEA	-
161	TGFA	TGFA	0.50183	0.67	0.99	0.98	-0.029	512	98	PEA	-
162	SPON1	SPON1	0.50228	0.67	0.99	0.97	-0.025	515	102	PEA	-
163	APOC2	APOC2	0.50300	0.67	1.09	1.02	0.146	515	100	MS	-
164	CTSD	CTSD	0.50499	0.67	1.04	1.01	0.073	515	102	PEA	-
165	SLAMF1	SLAMF1	0.50575	0.67	1.05	1.01	0.124	512	98	PEA	-
166	PRL	PRL	0.51554	0.68	1.03	1.11	0.040	515	102	PEA	-

167	ESM1	ESM-1	0.52303	0.68	0.98	0.95	-0.040	515	102	PEA	-
168	APOM	APOM	0.52561	0.68	1.01	0.98	0.019	515	100	MS	-
169	F2R	PAR-1	0.52659	0.68	0.98	1.05	-0.042	515	102	PEA	+
170	IL18	IL-18	0.53258	0.69	1.03	1.07	0.069	512	98	PEA	-
171	IL6R	IL-6RA	0.54179	0.69	1.02	1.02	0.049	515	102	PEA	-
172	CD6	CD6	0.55290	0.70	1.06	1.06	0.117	512	98	PEA	-
173	APOC1	APOC1	0.58391	0.74	0.99	0.99	-0.046	515	100	MS	-
174	DNER	DNER	0.58860	0.74	1.04	1.01	0.103	512	98	PEA	-
175	CCL20	CCL20	0.58860	0.74	1.10	1.26	0.099	512	98	PEA	-
176	MUC16	CA-125	0.59410	0.74	1.07	0.99	0.089	515	102	PEA	-
177	OLR1	LOX-1	0.61617	0.76	0.99	0.99	-0.024	515	102	PEA	-
178	TGFB1	TGF-beta-1	0.62179	0.76	1.00	1.36	0.001	512	98	PEA	+
179	TNFRSF9	TNFRSF9	0.62267	0.76	1.05	0.98	0.106	512	98	PEA	-
180	KLK11	hK11	0.62605	0.76	0.98	0.98	-0.048	515	102	PEA	-
181	CCL28	CCL28	0.63195	0.77	0.98	1.01	-0.059	512	98	PEA	-
182	FBLN1	FBLN1	0.63557	0.77	0.96	0.99	-0.099	515	100	MS	-
183	LIFR	LIF-R	0.63913	0.77	1.04	1.01	0.113	512	98	PEA	-
184	IL12B	IL-12B	0.64451	0.77	1.02	1.00	0.029	512	98	PEA	-
185	PLAU	uPA	0.64496	0.77	1.02	0.98	0.051	512	98	PEA	-
186	SELPLG	PSGL-1	0.68063	0.80	1.01	1.00	0.050	515	102	PEA	-
187	NGF	Beta-NGF	0.69879	0.82	1.00	1.16	0.011	515	102	PEA	-
188	MPO	MPO	0.70553	0.82	0.99	1.00	-0.024	515	102	PEA	-
189	MMP10	MMP-10	0.70854	0.82	0.96	0.93	-0.074	512	98	PEA	-
190	IGHA1	IGHA1	0.71074	0.82	1.03	0.98	0.046	515	100	MS	-
191	TNFRSF1A	TNF-R1	0.71141	0.82	1.02	1.00	0.054	515	102	PEA	-
192	IL16	IL-16	0.72596	0.83	0.98	0.99	-0.053	515	102	PEA	-
193	F3	TF	0.74843	0.85	1.03	1.00	0.094	515	102	PEA	-
194	LCN2	LCN2	0.76820	0.87	1.00	0.98	0.008	513	100	ELISA	-
195	CCL23	CCL23	0.78526	0.89	0.99	1.00	-0.017	512	98	PEA	-
196	LTA	TNFB	0.81329	0.91	1.01	0.99	0.025	512	98	PEA	-
197	CXCL9	CXCL9	0.81475	0.91	1.06	0.94	0.067	512	98	PEA	-
198	MB	MB	0.81847	0.91	1.05	0.99	0.113	515	102	PEA	-
199	IGHA2	IGHA2	0.82741	0.91	1.02	1.00	0.018	515	100	MS	-
200	FLT3LG	Flt3L	0.83179	0.91	1.02	1.02	0.041	512	98	PEA	-
201	IL1RL1	ST2	0.83220	0.91	1.02	0.96	0.039	515	102	PEA	-
202	FGF19	FGF-19	0.85038	0.92	0.90	1.05	-0.107	512	98	PEA	-
203	FGF23	FGF-23	0.85873	0.92	1.01	0.94	0.017	512	98	PEA	-
204	S100A12	EN-RAGE	0.85946	0.92	0.93	0.94	-0.108	512	98	PEA	-
205	NTF3	NT-3	0.86709	0.92	0.99	1.00	-0.032	512	98	PEA	-
206	GDF15	GDF-15	0.87272	0.92	0.98	0.94	-0.041	515	102	PEA	-
207	LPA	APOA	0.87368	0.92	1.03	0.89	0.013	515	100	MS	-
208	REN	REN	0.87895	0.92	1.06	0.97	0.083	515	102	PEA	-
209	CTSL	CTSL1	0.88615	0.93	0.98	1.00	-0.069	515	102	PEA	-
210	CXCL8	IL-8	0.89275	0.93	1.03	1.20	0.053	512	98	PEA	-
211	IL10	IL-10	0.89374	0.93	1.00	0.91	-0.003	512	98	PEA	-
212	APOD	APOD	0.89967	0.93	1.04	1.01	0.101	515	100	MS	-
213	GAL	GAL	0.90394	0.93	0.96	1.00	-0.062	515	102	PEA	-
214	KLK6	KLK6	0.94886	0.97	1.00	0.99	0.003	515	102	PEA	-
215	TNFRSF10B	TRAIL-R2	0.94983	0.97	1.02	0.52	0.057	515	102	PEA	-
216	CDCP1	CDCP1	0.96033	0.97	1.03	0.98	0.044	512	98	PEA	-
217	HAVCR1	TIM	0.96969	0.98	0.98	0.97	-0.032	515	102	PEA	-
218	A2M	A2MG	0.97571	0.98	0.96	1.00	-0.074	515	100	MS	+
219	IL6	IL-6	0.99476	0.99	1.00	0.43	0.003	512	98	PEA	+

Online Table VI. Selection of miR-126 targets and platelet-related genes for gene expression analysis. Validated miR-126 targets (green) and targets of miR-126 predicted by at least 5 out of 9 algorithms available in miRWalk[7] (blue) were selected if they also had a reported role in platelet function. References are given for the experimental confirmation as miR-126 targets and for the involvement of the target protein in platelet function. The column "Platelet web" lists whether a target was found in the proteome, serial analysis of gene expression (SAGE) analysis or both according to the Platelet web database.[8] "PaxDb" indicates whether a protein was identified in human platelets in the studies listed in the PaxDb website.[9]

	Gene Name	Predicted Target	Validated Target	References for Validation	Platelet web	PaxDb	References for Platelet Function
Validated miR-126 Targets	ADAM9	6/9	+	10, 11, 12	Proteome	-	10
	CXCL12	0/9	+	13	-	+	14
	IRS1	7/9	+	15	Proteome	-	16
	PIK3R2	4/9	+	17, 18	Proteome	+	19
	RGS16	0/9	+	20	-	-	21
	SPRED1	7/9	+	22	-	-	-
	VEGFA	1/9	+	23	Proteome	-	24
Predicted mR-126 Targets	IRS2	5/9	-		Both	+	16
	ITGA6	5/9	-		Proteome	+	25
	PIK3CD	5/9	-		Proteome	+	19
	PTPN9	6/9	-		SAGE	+	26
	TSC1	5/9	-		Proteome	-	27
Platelet Proteins	ITGA2B	0/9	-		Both	+	
	P2RY12	0/9	-		Proteome	+	
	PPBP	0/9	-		Both	+	
	PF4	0/9	-		Both	+	
	SELP	0/9	-		Proteome	+	

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