Exosomes Derived From Heat Stroke Cases Carry miRNAs Associated With Inflammation and Coagulation Cascade

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Research

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Abstract

Background: The pathological mechanism of HS is associated with the dysbalanced inflammation and coagulation cascade. The cells-derived circulating extracellular vesicles (EVs) as a novel pathway mediating intercellular communication were evidenced to be associated with immune response and inflammation in critical inflammatory syndromes such as sepsis. Despite previous studies demonstrating that these vesicles contain genetic material related to their biological function, their molecular cargo during heat stroke is relatively unknown. In this study, we evaluated the presence of microRNAs (miRNAs) and messenger RNAs (mRNAs) related to inflammatory response and coagulation cascade in exosomes of patients with heat stroke.

Methods: Blood samples were collected from 3 patients with heat stroke at ICU admission. Three healthy volunteers were used as control subjects. Exosomes were isolated by ultracentrifugation, their miRNA content was profiled by next generation sequencing and mRNA content was evaluated by qRT-PCR array.

Results: As compared with healthy volunteers, exosomes from patients with heat stroke had significant changes in 202 exosomal miRNAs (154 miRNAs upregulated and 48 downregulated). The most upregulated miRNAs included miR-511-3p, miR-122-5p, miR-155-3p, miR-1290 and let7-5p whileas the most downregulated ones included miR-150-3p, 146a-5p and 151a-3p. The GO enrichment by the miRNAs of patients with heat stroke compared with control subjects were related mostly to inflammatory response including T cell activation, B cell receptor signaling, DC chemotaxis and leukocyte migration, and platelet activation and blood coagulation. KEGG pathway analysis determined those identified miRNAs were mainly enriched to the signal transduction pathways namely, T cell receptor signaling pathway, Ras signaling pathway, Chemokine signaling pathway, Platelet activation, and Leukocyte transendothelial migration. These pathways were mainly related to inflammation and hemostasis. Multiple targeted mRNAs associated with the inflammatory response, blood coagulation and platelet activation were further verified in serum exosomes.

Conclusions: Exosomes from patients with heat stroke convey miRNAs and mRNAs related to pathogenic pathways, including inflammatory response and coagulation cascade. Exosomes may represent a novel mechanism for intercellular communication during heat stroke.

Background

Heat stroke (HS) is one of the warranted causes of death during intensified exercise and military training in summer. It appears to be a major challenge to the intensivist due to the lack of definitive evidence-based therapy. Recently, HS shows an increasing trend because of the growing frequency of heat wave attack, and the resultant mortality rates are quite high, particularly the HS-induced multiple organ failure (MOF) with the mortality rate of 40%-60% [1, 2].

The molecular mechanism of HS pathogenesis is complicated and remains poorly documented [3]. Evidences have verified that mechanism of HS is associated with the dysbalanced inflammation and

coagulation cascade [4]. In recent years, the cells-derived circulating extracellular vesicles (EVs) shed from different cell types in a critical inflammatory syndrome such as sepsis, together with their roles in disease pathophysiology, have been extensively investigated, especially those associated with immune response and inflammation [5, 6]. In addition, it has been revealed in our previous study that the EVs derived from hepatocytes of HS patients are correlated with liver dysfunction, which is mediated by a route associated with activation of EV-induced inflammatory NOD-like receptor pathway and necrosis (an inflammatory death form) in hepatocytes [7, 8].

EVs may mediate the communication between cells under normal physical conditions or in some disorders via carrying the functional microRNAs (miRNAs) and messenger RNA (mRNA). By adopting pathway enrichment analysis, a pilot study suggests that the miRNA profiling in serum EVs from septic patients might exert potentially vital biological roles in sepsis [9]. The authors revealed a differential expression pattern of EV-miRNA in sepsis which was involved in the modulation of immune system and cell cycle. However, no existing study has examined the genetic content of exosomes in the context of HS.

This article aimed to analyze the changes of miRNA profiles in serum EVs and their biological functions in HS patients. Findings in this work can possibly shed more lights on the pathophysiology of HS and offer the research windows for developing new treatments.

Materials And Methods

Patient recruitment and clinical data collection

From June 2019 to June 2020, 6 patients admitted within the first 24 h of severe HS onset into the intensive care unit (ICU) of the General Hospital of South Theatre of People's Liberation Army (PLA) of China, a tertiary hospital, were retrospectively enrolled in this study. HS was diagnosed according to the Expert Consensus on Diagnosis and Treatment of Heat Stroke in China (2019) (10) released by the Expert Group on Prevention and Treatment of Heat Stroke and Critical Care Committee of PLA of China. The patients with active malignant tumors, chronic liver and kidney diseases, chronic cardiac insufficiency (New York grade 3-4), chronic pulmonary insufficiency, underlying central nervous system (CNS) disease, metabolic disorders or those using heparin or any other medications, were eliminated from this study. Additionally, three healthy people from the Physical Examination Center were enrolled as the control group. Baseline demographic characteristics were recorded upon admission into ICU (Day 1). Blood samples and organ function data were collected on Day 1; besides, the sequential organ failure assessment (SOFA) and the age and chronic health evaluation (APACHE II) scores were determined to evaluate the disease severity. Patients or the corresponding family members provided the informed consent. Our study protocol gained approval from the Medical Ethics Review Committee of General Hospital of Southern Theater of PLA of China.

Blood sample collection and exosome isolation

Altogether 30 ml peripheral venous blood sample was obtained from each patient using the ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Then, the blood collection tubes were left standing vertically for 30 min at 22–27 ℃. Afterwards, plasma was obtained from whole blood through 10 min of centrifugation under 4 °C and 2,500 × *g*. After the addition of protease inhibitors (supplemented with 3 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin, as well as 1 μg/ml aprotinin), the plasma was stored at -80 °C. When processing, plasma was diluted with equivalent volume of phosphate-buffered saline (PBS) and centrifuged at 4°C (30 min at 2,500 × *g*, 45 min at 12,000 × *g*, along with 2 h at 110,000 × *g*, SW 28 Ti Rotor, Optima L-90K Ultracentrifugue, Beckman Coulter, Fullerton, USA). At last, 50–200 μl PBS was used to resuspend the precipitate (exosomes), followed by storage under -80 °C.

Exosome morphology observed via transmission electron microscopy (TEM)

Exosome samples were collected from a healthy individual, a patient with mild HS, and a patient with severe HS (randomly selected), respectively, and prepared for TEM evaluation. Firstly, exosomes were dehydrated in 2% formalin. After adding 5 ml exosome suspension into the copper grid coated with formvar (Mecalab, QC, Canada), the sample was subjected to 30 min of incubation, washing with 100 μl PBS, 10 min of 2% paraformaldehyde fixation, and 15 min of 2% uranyl acetate staining (dissolved into 50% ethanol). Thereafter, the Philips CM10 transmission electron microscope (JEM-2100F, Netherlands) was utilized for sample visualization.

Nanoparticle tracking analysis (NTA)

For detecting exosome content and size distribution, NTA was performed using the NanoSight (NS3000 (Malvern Instruments, Worcestershire, UK) in accordance with specific protocols. In brief, exosome sample was diluted with sterile PBS at 1:5,000, and the NanoSight LM10 and NTA software (NanoSight Ltd, Amesbury, UK) was employed for sample analysis for thrice (60 s each).

Western blotting

Protein was extracted from 1 ml ultracentrifuged plasma (120,000 g for 2.5 h) using the DE buffer (consisting of 20 mM Tris-HCL, 12 mM 2-mercaptoethanol, 1 mM EGTA, 1 mM EDTA, 1% Triton-X 100, together with 10% glycerol) (5) that contained the protease inhibitor mix (GE Healthcare, Uppsala, Sweden). Total protein was quantified by Bradford, then, 12% SDS-PAGE was applied in separating 5 μg protein from each sample. Later, the proteins were transferred onto the nitrocellulose membranes and blotted using polyclonal antibodies against CD9 (ABcam ab10895, Cambridge, UK), CD63 (ABcam ab92726, Cambridge, UK) and Tsg-101 (ABcam ab30871, Cambridge, UK). GADPH was used as a loading control for protein extracts. Target protein expression level was quantified by densitometry analysis using the ImageJ software.

Exosomal RNA sequencing (next-generation sequencing, NGS)

Exosomal RNA extraction

The Trizol reagent was employed to extract the RNA from exosomes.

Library construction and sequencing

The NEB SmallRNA Library kit was adopted for library construction, while illumina HiSeq was applied for sequencing, with a data volume of 20M reads per sample.

Filtering and miRNA mapping

Clean data were filtered from raw reads after sequencing in accordance with criteria below: a) 30% base quality <20, b) read length < 17bp, and c) adaptor sequence. Afterwards, clean data were mapped to the Danio Rerio miRNA database (miRBase v21.0) together with the Danio Rerio genome (Zv10, NCBI) using BWA software. Later, those unmapped reads were used to map the Rat, Mouse and Human miRNA database (miRBase v21.0) to achieve the expression profiles of miRNAs.

New miRNA prediction

The novel miRNA prediction was applied using mirdeep based on the reads mapped to Zv10 genome but unmapped against the Danio Rerio miRNA database (miRBase v21.0), so as to identify new miRNA. The BWA software mapping was applied in measuring the expression of new miRNA. (miRDeep*: an integrated application tool for miRNA identification from RNA sequencing data. An J, et al. Nucleic Acids Res. 2013 Jan;41(2):727-37. doi: 10.1093/nar/gks1187.)

Mapping of RNA sequencing

Pair-end read mapping. Prior to read mapping, adaptor sequences, low quality reads with over 20% bases that had <20 quality, and those having over 5% ambiguous bases (N) were eliminated from raw reads to obtain clean reads. The zebrafish genome (version: Zv10) was used to align clean reads by Tophat program. [Trapnell C, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks.Nat Protoc. 2012 Mar 1;7(3):562-78. doi: 10.1038/nprot.2012.016.]

Differential-gene-finder

The DESeq2 package was adopted to identify miRNA and mRNA with differential expression according to the numbers of miRNA and mRNA, FDR and P-value[Benjamini Y, et al. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001 Nov 1;125(1-2):279-84.], with the selection criteria of i) Fold Change (FC)>2 or <0.5; ii) P-value<0.05 and FDR<0.05.

Target Analysis

Miranda target analysis [John B, Enright A J, Aravin A, et al. Human MicroRNA targets.[J]. Plos Biology, 2004, 2(11):: e363.] was adopted for predicting the target of miRNA in the mRNA of zebra fish. For discovering the possible target of miRNA in the present work, the target of miRNA with no negative association of mRNA with miRNA was filtered.

Gene ontology (GO) functional annotations

GO analysis was carried out for elucidating the biological roles of specific genes in typical or significant differentially expressed gene (DEG) profiles [Ashburner M, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000 May;25(1):25-9.]. First of all, those GO annotations were downloaded from Gene Ontology (http://www.geneontology.org/), UniProt (http://www.uniprot.org/), along with NCBI (http://www.ncbi.nlm.nih.gov/) databases. Then, those significant GO categories were identified by Fisher's exact test, while p-values were corrected by FDR.

Pathway enrichment analysis

Pathway enrichment analysis was also applied for discovering DEGs based on the KEGG database. Those significant pathways were selected by Fisher's exact test, with FDR and P-value being the significance thresholds. [Draghici S, et al. A systems biology approach for pathway level analysis. Genome Res. 2007 Oct;17(10):1537-45. Epub 2007 Sep 4.]

Analysis of miRNA and inflammation/coagulation-related gene expression

30 ng total RNA was collected for the reverse transcription of miRNAs using specific stem-loop primers. The TaqMan Low Density Array (TLDA, TaqMan MicroRNA Array v3.0, Life Technologies, Grand Island, EUA), a micro fluidic card simultaneously detecting 754 mature miRNAs, was used to analyze the pre-amplification reaction products by qPCR. Immune study was performed in 21 septic patients and 12 controls. The High Capacity RNA-to-cDNA Kit (Life Technologies) was used for the reverse transcription of 100 ng RNA into cDNA following manufacturer's instructions. cDNA was distributed in TLDA cards (TaqMan Low Density Array Immune Profiling, Life Technologies) designed to evaluate 90 genes related to immune response. The 7900HT thermocycler (Applied Biosystems) was used for real-time PCR using the SDS 2.4 software. All data collected were analyzed in the ExpressionSuite 1.0.3 software (Life Technologies), where both baseline and threshold were automatically set. Each gene was checked individually and the threshold was adjusted to the exponential scale as needed. In the analysis of gene expression associated with OS, the PCR array platform used in these experiments comprised 84 genes related to OS as well as the antioxidant defense (PAHS065-A, SABiosciences/Qiagen, Valencia, USA). A total of 21 patients and 10 healthy donors were evaluated. In line with specific protocols, 100 ng RNA was used in the reverse transcription into cDNA, and the specific primers were used in pre-amplification of cDNA. To avoid variation, the PCR plates were prepared in high precision robotic pipetting (QI Agility, Qiagen). Quantitative real time PCR (qRT-PCR) was completed in the 7300HT thermocycler (Life Technologies). Data were processed using the SDS 1.3 software, and the same baseline (3-15) and threshold (0.2) were considered for each gene.

qPCR data analysis

The Statminer v5 software (Integromics, Granada, Spain) was used to analyze the cycle threshold (Ct) data. According to specific manufacturer instructions, Ct values ≥32 were excluded from immune and

miRNAs studies. For OS studies, the cutoff value was set at 35 in line with manufacturer's protocols. With regard to miRNA analysis, the optimal reference was identified by geNorm approach, and the median miR17, miR20a and miR106a was applied in normalizing data. The 18S rRNA (7) was used to normalize the immune gene expression, whereas beta-actin was utilized to normalize OS gene expression. Besides, the 2^DDCt method was adopted to calculate relative expression of genes.

Statistical analysis

The normality of data was assesed by Kurtosis test. Data with normal distribution were expressed in the manner of mean ± standard deviation (SD), whereas those with abnormal distribution were expressed in the manner of median ± interquartile range. The two-tailed Student's t-test was applied in the statistical analysis between two groups, while the one-way analysis of variance (ANOVA) followed by post hoc test was adopted for comparison among multiple groups. Spearman's rank correlation test was adopted to analyze the correlations. A difference of *p*-value <0.05 (two tailed) indicated statistical significance. The GraphPad version 7.0 (La Jolla, CA, USA) was employed for all test.

Results

Baseline characteristics and outcomes of patients

All patients and healthy controls were male with a relatively young age and few comorbidities. Age was comparable between healthy controls and HS patients (25 ± 6 vs. 21 ± 4 years, respectively). No participant previously used any medication. Clinical and laboratory data are summarized in Table 1. Core body temperatures on admission were higher in HS patients (39.00 ± 1.94 vs. 36.42 ± 0.46°C, p<0.001). In addition, 3 of the 6 HS cases died during the ICU hospitalization. The patients stayed at the ICU for 5-10 (median, 7) days.

All severe HS patients were complicated with multiple organ dysfunction. There was plenty of organ support when patients were enrolled, because 67% patients received mechanical ventilation while 50% received norepinephrine. The overall disease severity, as assessed by the APACHE II and SOFA scores, were 17.05 ± 4.35 and 10.95 ± 5.64, respectively (Table 2). None of the enrolled patients received prophylactic heparin, or transfusion of red blood cells/platelet/fresh frozen plasma, or antibiotics on admission before the collection of study samples.

Characterization of plasma exosomes

TEM showed that the separated preparation from the plasma of healthy controls and HS patients exhibited a double-membrane vesicle-like structure about 100 nm in diameter (Fig. 1A). Besides, the NTA results indicated that the plasma exosome concentration of HS group increased compared with that of control group ($5.67 \pm 2.34\times10^9$ particles/ml vs. $0.47 \pm 0.15\times10^9$ particles/ml, respectively) (Fig. 1B). Finally, Western blotting analysis showed the positive expression of the membrane-associated proteins

related to endocytosis, as well as the exosomal markers (CD9, CD63 and Tsg-101) in both control and HS groups (Fig. 1C). Taken together, the extracted vesicles were mostly consistent with exosomes.

HS remarkably changed the expression patterns of circulating exosomal miRNAs

For testing whether HS changed exosomal miRNAs expression, the miRNA profiling analysis was conducted firstly among HS cases admitted at the ICU; then, the results were compared against the healthy controls. As a result, HS made no difference to 90.72% miRNAs accumulation (Fig. 2A). It was also observed from Fig. 2B that, differences in most identified miRNAs were not significant, due to the close abundance ratio of HS versus control EVs. Nonetheless, in HS patients, 202 miRNAs (9.28%) were identified as differentially expressed compared with controls [154 up-regulated (7.08%) and 48 down-regulated (2.21%)] (Fig.2A). Among them, the miRNAs expression FC in HS versus control EVs (ratio of HS to control group <0.6 or >1.5), along with statistically significant P-values are summarized in Table 1 and displayed in a volcano map (Fig. 2B).

Additionally, hierarchical cluster analysis was carried out on 202 differentially expressed miRNAs based on samples or stimulants, so as to examine the creditability of those selected target miRNAs (Fig. 3). There was significantly differential expression in HS patients versus controls, with low SD among 3 samples, which suggested that our results were highly repeatable. Some specific miRNAs with significant elevation within HS EVs relative to controls were identified, including miR-511-3p, miR-122-5p, miR-155-3p, miR-1290 and let7-5p. The most downregulated miRNAs included miR-150-3p, 146a-5p and 151a-3p. The ACC IDs of all the disregulated miRNAs and the corresponding FCs are presented in Table 3. The results suggested that, HS changed certain miRNAs levels within plasma EVs, and this was associated with the biological effect.

The up-regulated miRNAs in HS EVs were related to the inflammation and coagulation signal transduction pathways

Thereafter, each EV miRNAs discovered from NGS was classified for exploring the specific biological role by the use of GO functional annotation clustering approach. As suggested by the results of molecular functional analysis, most identified EV miRNAs participated in regulating the signaling and molecular functions. As a result, the targeted miRNAs were speculated to exert an important part in the EVs biological activity.

The 20 most significant biological terms related to the up-regulated or down-regulated miRNAs in HS EVs, as well as the miRNA enrichment significance determined for all clusters, are presented in Fig. 4. Particularly, inflammatory response including T cell activation, B cell receptor signaling, DC chemotaxis and leukocyte migration, and platelet activation and blood coagulation, as well as cell responses to various stimuli including cytokines, regulation of cell death and cell adhesion/migration and cell cycle were the most significantly up-regulated clusters and were all involved in the mechnism of HS.

KEGG enrichment helps to determine the miRNA enrichment significance of every pathway. In this study, KEGG analysis discovered that those identified miRNAs were mainly enriched to the signal transduction pathways below, namely, T cell receptor signaling pathway, Ras signaling pathway, Chemokine signaling pathway, Platelet activation, and Leukocyte transendothelial migration (Fig. 5). These pathways were mainly related to inflammation and hemostasis. Thus, it was speculated that the ciculating EVs might be related to HS-induced injury via the activation of these signal transduction pathways within target cells.

Targeted mRNAs associated with the inflammatory response, blood coagulation and platelet activation

Table 4, 5 and 6 list the targeted mRNAs related to the top three up-regulated clusters, inflammatory response, blood coagulation and platelet activation.

For assessing the presence or absence of immune response-related mRNAs within exosomes, qPCR was performed to compare the inflammation/immune-related gene expression levels based on RNA extracted from HS and healthy individuals. It was observed that 18 genes, such as PRKD1, SP100, IL25, CRLF2, NLRP2, AOAN, and CXCR1. were significantly up-regulated in HS patients ($p<0.05$) (Fig. 6).

As illustrated from Fig. 7 that, the platelet activation-related mRNAs expression in exosomes like VWF, TBXA2R, P2RY12, CD40LG, and THBS1 in HS patients were also up-regulated comparing to those in healthy controls.

We also investigated the expression of mRNAs related to blood coagulation and demonstrated an increased expression of exosomal mRNAs of PDE9A, LRRC16A, ITGAV, FCR, EGF, ITGA1, CD59, SERPING1, IRF2, etc (Fig. 8).

Putative pathways focusing the most enriched pathways, including chemokine signaling pathway, platelet activation pathway, T cell receptor signaling pathway and leukocyte transendothelial migration pathway were constructed based on KEGG mapping (Fig. 9).

Discussion

In the present work, the different circulating exosome-miRNAs expression profiles with corresponding targets involved in the immune and coagulation system, were firstly identified in HS patients.

The organ injury mechanism in HS remains unknown at present. The current opinion tends to believe that an inflammatory and coagulation response initiated by the hyperthermal injury may play a more important role than the direct physical damage from heat exposure [3]. The pathological findings of HS (core temperature attained 42.5~43°C) baboon models include massive transmural migration of leukocytes, widespread microthrombosis, microvascular endothelium injury, endothelial leukocyte-platelet interaction, and extensive apoptosis in multiple organs including spleen, gut, liver, kidney and lung [4].

As demonstrated in the present study, the enriched miRNAs conveyed in circulating exosomes might participate in the inflammatory response and coagulation cascade, suggesting that exosomal miRNAs

might potentially mediate the HS-associated damage. Little existing research demonstrates the presence of miRNAs in exosomes during HS. Our previous first relevant study is conducted in exosomes derived from human vascular endothelial cells exposed to 41℃ hyperthermal stress [10]. The gene expression profiles in lung of a HS rat model reveals that the miRNAs related to inflammatory/immune responses, including leukocyte migration, response to lipopolysaccharide, NIK/NF-kappaB signaling, and response to reactive oxygen species, are up-regulated, which is consistent with our results [11]. Whole blood mRNA and microRNA was identified as biomarkers of tissue damage and immune function resulting from heat stroke exposure [12].

In other acute critical inflammatory disease, miRNAs exert vital parts in the immune/inflammatory responses in the context of trauma and sepsis [13]. In one work, investigators carry out miRNA profiling analysis on 10 subjects with polytrauma, sepsis and septic shock, and some differentially expressed miRNAs are identified [14]. In Real et al's study that enrolled 24 septic shock patients, the circulating exosomal miRNAs significantly altered and the same expression kinetics was maintained during the course of disease [9]. Such results possibly suggested that the body responded in a similar way to that of severe inflammatory injury.

MiR-150, one of the earliest miRNAs detected in critically ill patients or those with sepsis. Through microarray gene expression profiling, numerous reviewers have reported that miR-150 is a part of the miRNA panels with abnormal expression within leukocytes/PBMC obtained from septic patients in comparison with normal subjects [15-17]. Such alterations are reflected by the consistent changes in the miR-150 expression in serum. For instance, Vasilescu et al. discovered that miR-150 expression was down-regulated in 16 cases with abdominal sepsis [15]. The reduced miR-150 expression in serum was related to the increased SOFA score and sepsis severity. Furthermore, Ma et al. [18] discovered that miR-150 expression was down-regulated in septic patients in comparison with those having non-infectious SIRS or normal subjects.

MiRNA-122 was one of the most significantly enriched miRNAs in our profilling. Changes in its serum concentration have also been confirmed to significantly increase in systemic inflammatory diseases such as sepsis, which is related to the inflammatory response level and considered to be a reliable biomarker for early disease stratification and prognostic evaluation [19]. The level of miRNA-122 can predict the degrees of inflammation and organ injury such as ARDS [20] and coagulation disorders [21]. According to the longitudinal samples obtained from septic patients, the miR-122 level markedly increased on day 14 upon admission to ICU, which was strongly related to antithrombin III ($R = 0.913$, $p < 0.001$) [21]. For determining the direct or indirect impacts of miRNAs on coagulation, the crosstalk between cytokines, miRNAs dysregulation, and thrombocyte synthesis/apoptosis should be identified in more studies.

MiRNA-155 was also among the most upregulated miRNAs in our study. In a heatstress cell model, microRNA-155 promotes heat stress-induced inflammatory responses in microglia by facilitating inflammatory factors expression by increasing NF-κB pathway activation via targeting liver X receptor α [22].

Little is known about the interactions between exosomal miRNAs and the corresponding targets for exerting the specific activities. A majority of investigators propose that exosomes can serve as the effective carriers to carry genetic materials and proteins to the surrounding or the distant cells. Nonetheless, it is still largely unknown about the exosome–recipient cell interaction mechanism. Such interaction generally begins with the internalization of exosomes via a variety of proposed pathways, such as the phagocytosis, clathrin-mediated endocytosis, as well as macropinocytosis [23]. The exosomal miRNAs and mRNAs in recipient cells have certain functions and may have interactions with related targets for synthesizing novel proteins or modulating the gene level [24]. It is usually suggested in the laboratory cells; as a result, the interaction of exosomal genetic contents with the target cells in the case of HS is still speculative.

Certain limitations should be noted in this work. Firstly, the present exploratory study enrolled few patients, and the results obtained were interpreted as hypothesis-generating. Additionally, only cases at the the first HS episode were enrolled in this work, for the sake of preventing subjects who had immunosuppression or chronic inflammation possibly affecting our results. It might thus restrict the generalizability of results in this study. The plasma exosome counts were not determined in either HS patients or normal controls, as a result, it was impossible to estimate the impacts of HS on exosome number, and the contents of exosomal miRNAs and mRNAs were not normalized relative to the plasma exosome amount. Moreover, only mRNAs associated with coagulation activation and inflammatory response, since they have been suggested in previous studies to regulate exosomes in HS. Using such method, it was feasible to assess additional pathophysiological targets for exosomal mRNAs. For comparing the effects between infectious and noninfectious injuries, the control group constituted by infected ICU cases was not enrolled in this work.

Conclusions

Exosomes from patients with heat stroke convey genetic material that may be related to key pathways in the pathogenesis of heat stroke, including inflammatory response, blood coagulation, and platelet activation. Further functional studies are required to clarify the exact contribution of these vesicles to the exchange of genetic material and intercellular communication during heat stroke.

Declarations

Ethics approval and consent to participate

All animal experiments were conducted in compliance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, 1985 revision) and were approved by the Animal Care and Use Committee of the General Hospital of Guangzhou Military Command. Blood sample collection from HS patients was approved by the Ethics Committee of the General Hospital of Guangzhou Military Command. Informed consent was obtained from the patients or their representatives.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Huasheng Tong supervised the complete study. Yue Li designed the complete study and performed the NTA, western-blotting, statistical analysis as well as manuscript writing. Qiang Wen performed the isolation of exsomes and data collection. Xinghui Wu and Huaisheng Chen performed the TEM. Lei Su performed the clinical data and blood sample collection.

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Tables

Table 1. Basic clinical characteristics and disease severity scores of the participants

Characteristics	Healthy controls (N=3)	Severe HS (N=6)	P value
ICU length of stay (days), median (interquartile range)	0 (0)	10 (3–17.45)	<0.001
T (℃)	36.42 ± 0.46	39.00 ± 1.94	<0.001
APACHE II score	0.47 ± 0.83	17.05 ± 4.35	<0.001
SOFA score	0.6 ± 0.63	10.95 ± 5.64	<0.001

APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; SOFA, Sequential Organ Failure Assessment; T, body temperature

Table 2. Comparison of clinical and laboratory indexs of patients with heat stroke and healthy controls according to day of admission and outcome status

Characteristics	Healthy controls (N=3)	Severe HS (N=6)	P value
Hemodynamic data			
HR (beats/min)	74.3 ± 7.74	96.85 ± 32.43	<0.001
MAP (mmHg)	77.6 ± 6.99	72.15 ± 20.95	0.782
Vasoactive drug, n (%)	0 (0)	3 (50)	<0.001
Lactate (μmol/L)	1.07 ± 0.47	3.11 ± 2.55	<0.001
Ventilatory data			
PaO_2/FiO_2	378.7 ± 72.25	312.3 ± 67.56	0.007
MV, n (%)	0 (0)	6 (67)	<0.001
Inflammatory data			
WBC ($\times 10^9$ cells/L)	9.42 ± 3.14	10.81 ± 4.47	<0.001
PCT (ng/ml)	0.34 ± 0.34	4.06 ± 4.14	<0.001
Hepatic data			
ALT (U/L)	25.51 ± 13.7	1448 ± 2360	<0.001
AST (U/L)	22.4 ± 13.8	2144 ± 3861	0.001
TBil (μmol/L)	9.23 ± 4.45	62.83 ± 93.67	<0.001
ALB (g/L)	40.79 ± 5.36	37.56 ± 3.51	0.302
Renal data			
Cr (μmol/L)	95.4 ± 27.28	161.1 ± 84.24	<0.001
BUN (mmol/L)	5.51 ± 2.19	8.03 ± 6.73	<0..001
Urine output (ml/d)	2680 ± 727.2	2095 ± 1369	<0.001
Coagulation data			
PT (s)	13.39 ± 0.93	25.67 ± 15.61	<0.001
INR	13.39 ± 0.93	2.50 ± 2.21	<0.001
Fib (g/L)	3.53 ± 0.66	2.05 ± 0.76	<0.001
PLT ($\times 10^9$/L)	219.6 ± 65.05	101.2±61.35	<0.001
D-dimer	1.46 ± 1.32	15.77 ± 5.96	<0.001
FDP	6.83 ± 2.88	100.8 ± 184.3	0.001
Rhabdomyo data			

CK (μg/L)	54.33 ± 23.52	5155 ± 5888	<0·001
MYO (μg/L)	48.15 ± 24.91	1374 ± 964.4	<0·001
Cardiac data			
CK-MB	2.54 ± 1.45	39.83 ± 50.51	<0·001
cTnI	12.76 ± 9.94	874.2 ± 1134	0.02
CNS data			
GCS score	15 ± 0	8.3 ± 4.28	<0.001

ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, CK-myocardial band; CNS, central nervous system; Cr, creatinine; cTnI, cardiac troponin I; FDP, fibrin degradation product; Fib, fibrin; FiO_2, percentage of inspired oxygen; GCS, Glasgow Coma Scale; HR, heart rate; INR, international normalized ratio; MAP, mean arterial pressure; MV, mechanical ventilation; MYO, myoglobin; PaO_2, partial pressure of arterial oxygen; PCT, procalcitonin; PLT, platelet; PT, prothrombin time; TBil, total bilirubin; WBC, white blood cell

Table 3 miRNAs dysregulated in HS-hepatocyte EVs identified by NGS analysis

AccID	log2FC	Pvalue	FDR	Style
hsa-miR-511-3p	10.44555535	0.001051524	0.014788254	up
hsa-miR-122-5p	8.276723708	0.005868702	0.046320826	up
hsa-miR-155-3p	7.658481446	5.18604E-05	0.002694095	up
hsa-miR-1290	7.535022672	1.23381E-07	3.42525E-05	up
hsa-let-7b-5p	7.081014395	0.000227207	0.004955503	up
hsa-miR-1298-5p	6.74576804	2.58948E-05	0.001820878	up
hsa-miR-193a-5p	6.602101886	7.49325E-05	0.002851128	up
hsa-miR-855-5p	6.418510241	0.000272081	0.005628786	up
hsa-miR-let-7c-5p	6.297028039	2.95983E-09	1.52628E-06	up
hsa-miR-363-3p	6.269409954	1.54789E-13	1.19729E-10	up
hsa-miR-618	6.231316715	0.000276527	0.005628786	up
hsa-miR-29b-3p	6.164685475	0.000733793	0.011519171	up
hsa-miR-99b-3p	6.147758159	4.93893E-14	7.64053E-11	up
hsa-miR-485-3p	6.083130973	6.25082E-07	0.000123803	up
hsa-miR-1243	6.080205186	6.40223E-07	0.000123803	up
hsa-miR-193a-5p	6.069448799	1.32848E-07	3.42525E-05	up
hsa-miR-137-5p	6.047009206	0.000763775	0.011815601	up
hsa-miR-590-5p	6.037288962	0.000383296	0.007144091	up
hsa-miR-511-5p	5.905742669	1.7233E-06	0.000242359	up
hsa-miR-1267	5.896199662	3.28785E-05	0.002088438	up
hsa-miR-148a-3p	5.865441423	0.001261164	0.016395129	up
hsa-miR-30d-5p	5.831116516	0.002248497	0.024495949	up
hsa-miR-486-5p	5.564447631	1.41289E-05	0.001264144	up
hsa-miR-548a-3p	5.552914827	7.19604E-05	0.002851128	up
hsa-miR-99a-5p	5.539603918	5.92159E-06	0.000654335	up
hsa-miR-520d-5p	5.53697579	0.000148742	0.003900067	up
hsa-miR-518b-3p	5.51551199	0.001133096	0.015360113	up
hsa-miR-140-3p	5.481357457	0.000181949	0.004264777	up

hsa-miR-508-5p	5.458111243	0.006282607	0.048114816	up
hsa-miR-592	5.447297769	0.000167126	0.004147149	up
hsa-miR-324-3p	5.445343033	0.002847146	0.029760376	up
hsa-miR-296-5p	5.440772306	0.003753563	0.036066845	up
hsa-miR-1285-3p	5.426482304	0.001343155	0.017066652	up
hsa-miR-598-3p	5.413461255	0.004338065	0.03791518	up
hsa-miR-766-3p	5.384247124	0.000518532	0.008680444	up
hsa-miR-614	5.346661476	0.002538486	0.027271096	up
hsa-miR-1183	5.313071929	0.000110185	0.003626728	up
hsa-miR-339-3p	5.306714553	0.000124834	0.003696458	up
hsa-miR-1233-3p	5.264430569	0.000274062	0.005628786	up
hsa-miR-188-3p	5.226803605	0.003360793	0.033327859	up
hsa-miR-625-3p	5.192911687	0.000778011	0.011916657	up
hsa-miR-875-3p	5.174336191	0.00086226	0.012950644	up
hsa-miR-378b	5.170813667	8.04617E-06	0.000829828	up
hsa-miR-758-3p	5.167747909	2.39966E-06	0.000309356	up
hsa-miR-54a-5p	5.152096449	1.20951E-07	3.42525E-05	up
hsa-miR-27a	5.141395196	0.002041626	0.023395526	up
hsa-miR-34a	5.138801488	0.00125247	0.016395129	up
hsa-miR-125b-5p	5.122676194	1.33884E-05	0.001264144	up
hsa-miR-15a	5.110036409	0.000359735	0.006786705	up
hsa-miR-558	5.051441121	0.005306746	0.043667749	up
hsa-miR-483-5p	4.985278141	6.82155E-05	0.002851128	up
hsa-miR-1277-3p	4.96612105	0.001033352	0.014666017	up
hsa-miR-1260a	4.931265335	0.000137917	0.003743124	up
hsa-miR-122-3p	4.882978843	6.46948E-05	0.002851128	up
hsa-miR-16-5p	4.870571508	4.4788E-05	0.002474535	up
hsa-miR-378c	4.865473522	3.50998E-05	0.002088438	up
hsa-miR-192-5p	4.819906165	0.000130442	0.003696458	up

hsa-miR-122b-5p	4.813043234	7.55632E-05	0.002851128	up
hsa-miR-548b-3p	4.787050472	0.005772356	0.046320826	up
hsa-miR-194-5p	4.781402495	0.00015155	0.003907475	up
hsa-miR-658	4.766242612	0.004135298	0.036983224	up
hsa-miR-195-5p	4.723631785	0.003657473	0.035363195	up
hsa-miR-19a-3p	4.701557418	0.003604788	0.035294977	up
hsa-miR-885-5p	4.670729991	6.79172E-05	0.002851128	up
hsa-miR-25-3p	4.668210424	0.002787529	0.029335427	up
hsa-miR-676-3p	4.635212949	0.000147017	0.003900067	up
hsa-miR-30a-5p	4.633464441	0.000104981	0.003530551	up
hsa-miR-378e	4.573833884	6.24059E-05	0.002851128	up
hsa-miR-320a	4.545202766	7.5698E-07	0.000130116	up
hsa-miR-518d-3p	4.523946854	1.83062E-05	0.001415983	up
hsa-miR-532-3p	4.438273684	0.005937185	0.046435358	up
hsa-miR-152-3p	4.434831855	1.47089E-05	0.001264144	up
hsa-miR-885-3p	4.343597305	0.000104869	0.003530551	up
hsa-miR-548c-3p	4.298413102	0.001422001	0.017598688	up
hsa-miR-597-5p	4.291099833	0.00423662	0.037238931	up
hsa-miR-636	4.286856265	0.00041197	0.00741067	up
hsa-miR-645	4.261328603	0.000119754	0.003696458	up
hsa-miR-720	4.245115	0.004098041	0.036983224	up
hsa-miR-378g	4.242244907	7.1321E-05	0.002851128	up
hsa-miR-449b-3p	4.190458798	0.005779387	0.046320826	up
hsa-miR-886-5p	4.181509179	0.000737167	0.011519171	up
hsa-miR-133a-5p	4.163298014	5.89909E-05	0.002851128	up
hsa-miR-92a-3p	4.117957927	0.001603042	0.019526823	up
hsa-miR-549a	4.093083013	0.002382692	0.025776395	up
hsa-miR-455-3p	4.085035465	0.000201408	0.004650421	up
hsa-miR-875-5p	4.084769606	0.004764889	0.04028024	up

hsa-miR-378j	4.067935221	0.000172496	0.004147149	up
hsa-miR-100-5p	4.064359746	0.000528185	0.008692578	up
hsa-miR-378f	4.058976779	0.000214544	0.004880873	up
hsa-miR-323b-5p	4.049295395	0.002076178	0.023569945	up
hsa-miR-378d	3.967150304	0.000303754	0.006024464	up
hsa-miR-378a-3p	3.887692985	0.000172964	0.004147149	up
hsa-miR-99b-5p	3.87989185	0.000860374	0.012950644	up
hsa-miR-675-3p	3.863440724	0.000984691	0.014370908	up
hsa-miR-125b-1-3p	3.858649521	0.000355423	0.006786705	up
hsa-miR-133a-3p	3.856130758	0.000397748	0.007316155	up
hsa-miR-156-3p	3.828665539	0.001070782	0.014909039	up
hsa-miR-422a	3.821697972	0.000227434	0.004955503	up
hsa-miR-508-5p	3.773055535	0.004143019	0.036983224	up
hsa-miR-455-5p	3.74855908	3.54643E-06	0.000422025	up
hsa-miR-30a-5p	3.722860052	1.74445E-05	0.001415983	up
hsa-miR-362-3p	3.708667691	0.002089088	0.023569945	up
hsa-miR-210-3p	3.706738617	0.001018893	0.014594692	up
hsa-miR-80	3.690830266	0.004398526	0.038227641	up
hsa-miR-428	3.690701315	0.00017425	0.004147149	up
hsa-miR-686-5p	3.563674461	0.004159716	0.036983224	up
hsa-miR-557-3p	3.54859907	0.005170255	0.042854747	up
hsa-miR-127-5p	3.534359038	0.005180244	0.042854747	up
hsa-miR-574-5p	3.460709408	0.001686106	0.020064661	up
hsa-miR-95-5p	3.450983644	0.004151843	0.036983224	up
hsa-miR-672-5p	3.426905205	0.000131419	0.003696458	up
hsa-miR-378h	3.399669168	0.001305573	0.016831014	up
hsa-miR-499b-3p	3.367588817	0.000512354	0.008680444	up
hsa-miR-499a-5p	3.321524527	0.000401987	0.007316155	up
hsa-miR-873-5p	3.303504919	0.002108672	0.023569945	up

hsa-miR-468	3.242848143	0.000313963	0.006071253	up
hsa-miR-188-5p	3.202653388	0.001920073	0.022166816	up
hsa-miR-501-5p	3.201586587	0.001662516	0.019937302	up
hsa-miR-375-3p	3.198625055	0.004051092	0.036983224	up
hsa-miR-601	3.183032738	0.001142479	0.015360113	up
hsa-miR-313-3p	3.181596542	0.003044582	0.031191848	up
hsa-miR-125b-5p	3.177573994	0.001729595	0.020425067	up
hsa-miR-480-3p	3.139771854	0.000225445	0.004955503	up
hsa-miR-141-5p	3.135945816	0.004088257	0.036983224	up
hsa-miR-475-5p	3.123371628	0.004584576	0.039401888	up
hsa-miR-660-3p	3.107484568	0.001847427	0.021651279	up
hsa-miR-208b-3p	3.076327192	0.003236933	0.032306675	up
hsa-miR-619-5p	3.068163387	0.000131118	0.003696458	up
hsa-miR-36a-5p	3.0546317	0.000115998	0.003666773	up
hsa-miR-650-3p	3.050923963	0.003925388	0.036983224	up
hsa-miR-497-5p	3.01762823	2.81112E-05	0.001890783	up
hsa-miR-480-5p	3.014405265	0.001079387	0.014909039	up
hsa-miR-148a-3p	2.988162684	0.00594996	0.046435358	up
hsa-miR-124-5p	2.951062151	0.00395291	0.036983224	up
hsa-miR-21-3p	2.934507665	0.004686581	0.039835941	up
hsa-miR-391	2.929568628	0.000559096	0.009009607	up
hsa-miR-193b-3p	2.918888063	0.005446315	0.044344472	up
hsa-miR-95-3p	2.908344044	0.000872945	0.01298506	up
hsa-miR-365b-3p	2.888195604	0.001576713	0.019358531	up
hsa-miR-675-5p	2.883829012	0.001414023	0.017598688	up
hsa-miR-500a-3p	2.828996605	0.005840891	0.046320826	up
hsa-miR-463	2.820851921	8.29863E-05	0.002989247	up
hsa-miR-770	2.80885692	0.000460923	0.008011765	up
hsa-miR-133b	2.733475223	0.002157145	0.023667401	up

hsa-miR-467	2.727764422	0.006033295	0.046435358	up
hsa-miR-130-5p	2.583022076	0.004642189	0.039676606	up
hsa-miR-125a-5p	2.581138745	0.003159096	0.031941968	up
hsa-miR-99	2.579135083	0.003921596	0.036983224	up
hsa-miR-365a-3p	2.507688657	0.005501005	0.044555261	up
hsa-miR-448	2.39972093	0.004084038	0.036983224	up
hsa-miR-394-5p	2.283892989	0.006021612	0.046435358	up
hsa-miR-29a-3p	2.268261556	0.00115176	0.015360113	up
hsa-miR-512-3p	2.126801502	0.005430677	0.044344472	up
hsa-miR-475-3p	1.990553632	0.005843288	0.046320826	up
hsa-miR-30e-3p	-1.835550942	0.004945721	0.0415597	down
hsa-miR-222-3p	-1.856156913	0.00164635	0.019897684	down
hsa-miR-584-5p	-2.009084984	0.004226844	0.037238931	down
hsa-miR-671-3p	-2.014744985	0.002636901	0.02813301	down
hsa-miR-340-5p	-2.030456926	0.003124884	0.031803918	down
hsa-miR-654-5p	-2.110003521	0.003565424	0.035131917	down
hsa-miR-30b-3p	-2.192120837	0.004969971	0.0415597	down
hsa-miR-766-5p	-2.212703259	0.000521837	0.008680444	down
hsa-miR-30c-1-3p	-2.31408293	0.003994987	0.036983224	down
hsa-miR-374b-5p	-2.343572476	0.003917189	0.036983224	down
hsa-miR-432-5p	-2.358199166	0.002717244	0.028791614	down
hsa-miR-374c-3p	-2.358739972	0.002871652	0.029815068	down
hsa-miR-331-3p	-2.435047906	0.001003414	0.014507302	down
hsa-miR-130b-5p	-2.469418509	0.002127476	0.023569945	down
hsa-miR-224-3p	-2.477360429	0.006010819	0.046435358	down
hsa-miR-135a-5p	-2.506163407	0.001895315	0.022045503	down
hsa-miR-199a-5p	-2.513372321	0.003633528	0.035352625	down
hsa-miR-98-5p	-2.557471672	0.000449668	0.007904952	down
hsa-miR-186-5p	-2.72167449	0.001115982	0.015278093	down

hsa-miR-487b-3p	-2.729215601	0.000241089	0.005180058	down
hsa-miR-454-3p	-2.748583439	0.002930923	0.030227591	down
hsa-miR-495-3p	-2.758027548	0.000420334	0.00747421	down
hsa-miR-26a-1-3p	-2.763384301	0.003216004	0.03230622	down
hsa-miR-409-3p	-2.820796229	0.001345916	0.017066652	down
hsa-miR-543	-2.850172237	0.000680847	0.010858462	down
hsa-miR-296-5p	-2.931642939	8.30883E-05	0.002989247	down
hsa-miR-1185-1-3p	-2.947501718	0.000538761	0.008773296	down
hsa-miR-329-3p	-3.010672012	0.000102978	0.003530551	down
hsa-miR-548j-5p	-3.066580896	0.001162522	0.015371118	down
hsa-miR-556-5p	-3.086513534	0.00095938	0.014134865	down
hsa-miR-654-3p	-3.09343416	2.53667E-05	0.001820878	down
hsa-miR-340-3p	-3.09443468	0.000302301	0.006024464	down
hsa-miR-200a-5p	-3.107804542	6.20481E-05	0.002851128	down
hsa-miR-221-3p	-3.134305694	0.001390292	0.017486025	down
hsa-miR-26a-5p	-3.171505823	4.01985E-05	0.002303226	down
hsa-miR-758-3p	-3.216623778	0.000256334	0.005432171	down
hsa-miR-323b-3p	-3.255988987	0.000508585	0.008680444	down
hsa-miR-224-5p	-3.363071212	0.002133027	0.023569945	down
hsa-miR-485-3p	-3.422140644	0.004446777	0.038431083	down
hsa-miR-487a-5p	-3.426603802	0.000123266	0.003696458	down
hsa-miR-194-5p	-3.902033656	0.000116142	0.003666773	down
hsa-miR-411-3p	-3.936102911	5.22449E-05	0.002694095	down
hsa-miR-196a-5p	-3.960486658	7.3528E-05	0.002851128	down
hsa-miR-379-3p	-4.028025819	3.48353E-05	0.002088438	down
hsa-miR-16-5p	-4.037789955	0.000311387	0.006071253	down
hsa-miR-151a-3p	-4.170054878	1.18001E-06	0.000182547	down
hsa-miR-146a-5p	-4.181358731	0.000158479	0.004019119	down
hsa-miR-150-3p	-4.335973238	0.000134263	0.003709015	down

Table 4 Differentially expressed targeted mRNAs associated with inflammatory response

Gene symbol	Protein Name
NFAM1	NFAT activating protein with ITAM motif 1
LYN	LYN proto-oncogene, Src family tyrosine kinase
NRROS	negative regulator of reactive oxygen species
DAB2IP	DAB2 interacting protein
IGFBP4	insulin-like growth factor binding protein 4
PRKD1	protein kinase D1
SP100	SP100 nuclear antigen
IL25	interleukin 25
CRLF2	cytokine receptor-like factor 2
PSTPIP1	proline-serine-threonine phosphatase interacting protein 1
NLRP2	NLR family, pyrin domain containing 2
NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
AOAH	acyloxyacyl hydrolase (neutrophil)
PIK3C2A	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha
CXCR1	chemokine (C-X-C motif) receptor 1
THEMIS2	thymocyte selection associated family member 2
RELA	v-rel avian reticuloendotheliosis viral oncogene homolog A
IL17C	interleukin 17C
CCR4	chemokine (C-C motif) receptor 4
NDST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1
FOLR2	folate receptor 2 (fetal)
CHST4	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4
TBXA2R	thromboxane A2 receptor
CCL5	chemokine (C-C motif) ligand 5
APOL3	apolipoprotein L, 3
IL13	interleukin 13
TNIP2	TNFAIP3 interacting protein 2
TGFB1	transforming growth factor, beta 1

HRH1	histamine receptor H1
CCL21	chemokine (C-C motif) ligand 21
PROK2	prokineticin 2
CXCR6	chemokine (C-X-C motif) receptor 6
BMPR1B	bone morphogenetic protein receptor, type IB
PTGDR	prostaglandin D2 receptor (DP)
PRDX5	peroxiredoxin 5
CXCL6	chemokine (C-X-C motif) ligand 6
CXCL10	chemokine (C-X-C motif) ligand 10
ADORA2A	adenosine A2a receptor
NLRP4	NLR family, pyrin domain containing 4
CCL2	chemokine (C-C motif) ligand 2
CYBA	cytochrome b-245, alpha polypeptide
JAK3	Janus kinase 3
PIK3CD	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit delta
CXCR4	chemokine (C-X-C motif) receptor 4
CCR5	chemokine (C-C motif) receptor 5 (gene/pseudogene)
TNFAIP6	tumor necrosis factor, alpha-induced protein 6
APOC3	apolipoprotein C-III
IKBKG	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma
CD40LG	CD40 ligand
CHST2	carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2
AIMP1	aminoacyl tRNA synthetase complex-interacting multifunctional protein 1
ADORA1	adenosine A1 receptor
TSPAN2	tetraspanin 2
TLR7	toll-like receptor 7
CXCL11	chemokine (C-X-C motif) ligand 11
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
KDM6B	lysine (K)-specific demethylase 6B

LGALS9	lectin, galactoside-binding, soluble, 9
AFAP1L2	actin filament associated protein 1-like 2
VNN1	vanin 1
S100A9	S100 calcium binding protein A9
PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta
NOD1	nucleotide-binding oligomerization domain containing 1
TNIP3	TNFAIP3 interacting protein 3
CCL26	chemokine (C-C motif) ligand 26
NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
FFAR2	free fatty acid receptor 2
IL1RAP	interleukin 1 receptor accessory protein
IL2RA	interleukin 2 receptor, alpha
CCL22	chemokine (C-C motif) ligand 22
PIK3C2G	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 gamma
TLR5	toll-like receptor 5
TPST1	tyrosylprotein sulfotransferase 1
BMP6	bone morphogenetic protein 6
CCR3	chemokine (C-C motif) receptor 3
HDAC9	histone deacetylase 9
CCL7	chemokine (C-C motif) ligand 7
OLR1	oxidized low density lipoprotein (lectin-like) receptor 1
IDO1	indoleamine 2,3-dioxygenase 1
IL23R	interleukin 23 receptor
TRIL	TLR4 interactor with leucine-rich repeats
TUSC2	tumor suppressor candidate 2
C5AR1	complement component 5a receptor 1
S1PR3	sphingosine-1-phosphate receptor 3
IL10RB	interleukin 10 receptor, beta

ELF3	E74-like factor 3 (ets domain transcription factor, epithelial-specific)
JMJD7-PLA2G4B	JMJD7-PLA2G4B readthrough
CD40	CD40 molecule, TNF receptor superfamily member 5
SCN9A	sodium channel, voltage-gated, type IX, alpha subunit
ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
CAMK1D	calcium/calmodulin-dependent protein kinase ID
LY86	lymphocyte antigen 86
PLA2G4C	phospholipase A2, group IVC (cytosolic, calcium-independent)
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5
FPR2	formyl peptide receptor 2
TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B
GPR68	G protein-coupled receptor 68
ATRN	attractin
SYK	spleen tyrosine kinase
XCL2	chemokine (C motif) ligand 2
MS4A2	membrane-spanning 4-domains, subfamily A, member 2
F2RL1	coagulation factor II (thrombin) receptor-like 1
IL36A	interleukin 36, alpha
PXK	PX domain containing serine/threonine kinase
HDAC5	histone deacetylase 5
PRKCQ	protein kinase C, theta
SPHK1	sphingosine kinase 1
ADAM8	ADAM metallopeptidase domain 8
CXCL9	chemokine (C-X-C motif) ligand 9
TICAM2	toll-like receptor adaptor molecule 2
PLA2G2D	phospholipase A2, group IID

CCL25	chemokine (C-C motif) ligand 25
IL22	interleukin 22
MIF	macrophage migration inhibitory factor (glycosylation-inhibiting factor)
LYZ	lysozyme
NGF	nerve growth factor (beta polypeptide)
PDPN	podoplanin
PRKCZ	protein kinase C, zeta
PIK3C2B	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 beta
KNG1	kininogen 1
CCL3L3	chemokine (C-C motif) ligand 3-like 3
NFATC4	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
AZU1	azurocidin 1
IL5	interleukin 5
ADGRE2	egf-like module containing, mucin-like, hormone receptor-like 2
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
HRH4	histamine receptor H4
CCR1	chemokine (C-C motif) receptor 1
CSF1	colony stimulating factor 1 (macrophage)
IL10	interleukin 10
THBS1	thrombospondin 1
NAIP	NLR family, apoptosis inhibitory protein
HYAL3	hyaluronoglucosaminidase 3
CALCA	calcitonin-related polypeptide alpha
MMP17	matrix metallopeptidase 17 (membrane-inserted)
ALOX15	arachidonate 15-lipoxygenase
IL24	interleukin 24
CNR2	cannabinoid receptor 2 (macrophage)
CYP26B1	cytochrome P450, family 26, subfamily B, polypeptide 1
CCL24	chemokine (C-C motif) ligand 24

IL18	interleukin 18
TNFRSF4	tumor necrosis factor receptor superfamily, member 4
BCL6	B-cell CLL/lymphoma 6
CCL4L2	chemokine (C-C motif) ligand 4-like 2
ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
IL20	interleukin 20
HCK	HCK proto-oncogene, Src family tyrosine kinase
IL23A	interleukin 23, alpha subunit p19
GGT5	gamma-glutamyltransferase 5
BDKRB2	bradykinin receptor B2
TLR9	toll-like receptor 9
GPER1	G protein-coupled estrogen receptor 1
CCL8	chemokine (C-C motif) ligand 8
TLR8	toll-like receptor 8
TLR1	toll-like receptor 1
NFKBID	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta
MEFV	Mediterranean fever
HDAC4	histone deacetylase 4
IL18RAP	interleukin 18 receptor accessory protein
CSF1R	colony stimulating factor 1 receptor
KLRG1	killer cell lectin-like receptor subfamily G, member 1
NOX4	NADPH oxidase 4
C3AR1	complement component 3a receptor 1
ADGRE5	CD97 molecule
TLR10	toll-like receptor 10
KCNJ10	potassium inwardly-rectifying channel, subfamily J, member 10
ITGB6	integrin, beta 6
C4A	complement component 4A (Rodgers blood group)
CXCR2	chemokine (C-X-C motif) receptor 2

P2RX7	purinergic receptor P2X, ligand-gated ion channel, 7
MMP25	matrix metallopeptidase 25
CXCL5	chemokine (C-X-C motif) ligand 5
SELE	selectin E
NCF1	neutrophil cytosolic factor 1
SDC1	syndecan 1
ALOX5	arachidonate 5-lipoxygenase
BLNK	B-cell linker
IL17D	interleukin 17D
RPS6KA4	ribosomal protein S6 kinase, 90kDa, polypeptide 4
IL1RL2	interleukin 1 receptor-like 2
F2R	coagulation factor II (thrombin) receptor
CXCR3	chemokine (C-X-C motif) receptor 3
FFAR3	free fatty acid receptor 3
SCG2	secretogranin II
PTAFR	platelet-activating factor receptor
SCUBE1	signal peptide, CUB domain, EGF-like 1
TICAM1	toll-like receptor adaptor molecule 1
IL17A	interleukin 17A
NLRP3	NLR family, pyrin domain containing 3
AKT1	v-akt murine thymoma viral oncogene homolog 1
NFE2L1	nuclear factor, erythroid 2-like 1
TLR6	toll-like receptor 6
SERPINA3	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
TNFAIP3	tumor necrosis factor, alpha-induced protein 3
PLA2G4B	phospholipase A2, group IVB (cytosolic)
PTGS1	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
TLR2	toll-like receptor 2
MECOM	MDS1 and EVI1 complex locus

REL	v-rel avian reticuloendotheliosis viral oncogene homolog
CCL16	chemokine (C-C motif) ligand 16
MGLL	monoglyceride lipase
CYBB	cytochrome b-245, beta polypeptide
XCR1	chemokine (C motif) receptor 1
CAMK4	calcium/calmodulin-dependent protein kinase IV
CHST1	carbohydrate (keratan sulfate Gal-6) sulfotransferase 1
IL6	interleukin 6
IFI16	interferon, gamma-inducible protein 16
NCR3	natural cytotoxicity triggering receptor 3
TP73	tumor protein p73
TACR1	tachykinin receptor 1
CCL13	chemokine (C-C motif) ligand 13
C4B	complement component 4B (Chido blood group)
NFRKB	nuclear factor related to kappaB binding protein
CHIA	chitinase, acidic
LAT	linker for activation of T cells
CCR2	chemokine (C-C motif) receptor 2
F11R	F11 receptor
LTB4R	leukotriene B4 receptor
MEP1B	meprin A, beta
CCL19	chemokine (C-C motif) ligand 19
TNFSF4	tumor necrosis factor (ligand) superfamily, member 4
CRP	C-reactive protein, pentraxin-related
RARRES2	retinoic acid receptor responder (tazarotene induced) 2
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma
LY75	lymphocyte antigen 75
SEMA7A	semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)
HNRNPA0	heterogeneous nuclear ribonucleoprotein A0

RAC1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
SIGLEC1	sialic acid binding Ig-like lectin 1, sialoadhesin
TNIP1	TNFAIP3 interacting protein 1
NLRP1	NLR family, pyrin domain containing 1
SELP	selectin P (granule membrane protein 140kDa, antigen CD62)
TLR3	toll-like receptor 3
CCR7	chemokine (C-C motif) receptor 7
SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
ACKR2	atypical chemokine receptor 2
ITCH	itchy E3 ubiquitin protein ligase
AOX1	aldehyde oxidase 1
IL1B	interleukin 1, beta
RXRA	retinoid X receptor, alpha
MYD88	myeloid differentiation primary response 88
CCL17	chemokine (C-C motif) ligand 17
SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transporter), member 1
NTRK2	neurotrophic tyrosine kinase, receptor, type 2
LIPA	lipase A, lysosomal acid, cholesterol esterase
CYP4F11	cytochrome P450, family 4, subfamily F, polypeptide 11
CD180	CD180 molecule
HYAL1	hyaluronoglucosaminidase 1
IL34	interleukin 34
KLKB1	kallikrein B, plasma (Fletcher factor) 1
ADORA3	adenosine A3 receptor
IL27	interleukin 27
IL1A	interleukin 1, alpha
PLP1	proteolipid protein 1

REG3A	regenerating islet-derived 3 alpha
RELB	v-rel avian reticuloendotheliosis viral oncogene homolog B
MAP2K3	mitogen-activated protein kinase kinase 3
CHUK	conserved helix-loop-helix ubiquitous kinase
CRH	corticotropin releasing hormone
HMGB1	high mobility group box 1
BMP2	bone morphogenetic protein 2
IL19	interleukin 19
IRAK2	interleukin-1 receptor-associated kinase 2
CCL18	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
NOS2	nitric oxide synthase 2, inducible
CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)
LY75-CD302	LY75-CD302 readthrough
AXL	AXL receptor tyrosine kinase
MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2

Table 5 Differentially expressed targeted mRNAs associated with platelet activation

LYN	LYN proto-oncogene, Src family tyrosine kinase
MMRN1	multimerin 1
VWF	von Willebrand factor
RAF1	Raf-1 proto-oncogene, serine/threonine kinase
ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1
RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)
PFN1	profilin 1
EGF	epidermal growth factor
PIK3C2A	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha
DGKH	diacylglycerol kinase, eta
MAPK14	mitogen-activated protein kinase 14
GNAI3	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3
SERPING1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
TBXA2R	thromboxane A2 receptor
IGF2	insulin-like growth factor 2
PRKCD	protein kinase C, delta
RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4
TGFB1	transforming growth factor, beta 1
GP1BB	glycoprotein Ib (platelet), beta polypeptide
SPARC	secreted protein, acidic, cysteine-rich (osteonectin)
F5	coagulation factor V (proaccelerin, labile factor)
P2RY12	purinergic receptor P2Y, G-protein coupled, 12
MAPK1	mitogen-activated protein kinase 1
RAP2B	RAP2B, member of RAS oncogene family
APBB1IP	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
PIK3CD	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit delta
PPIA	peptidylprolyl isomerase A (cyclophilin A)
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta

ENTPD2	ectonucleoside triphosphate diphosphohydrolase 2
CD9	CD9 molecule
GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)
CSK	c-src tyrosine kinase
CD40LG	CD40 ligand
CFL1	cofilin 1 (non-muscle)
ARRB2	arrestin, beta 2
PTK2	protein tyrosine kinase 2
BCAR1	breast cancer anti-estrogen resistance 1
RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)
PIK3R5	phosphoinositide-3-kinase, regulatory subunit 5
PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta
LAMP2	lysosomal-associated membrane protein 2
DAGLA	diacylglycerol lipase, alpha
HGF	hepatocyte growth factor (hepapoietin A; scatter factor)
ACTN2	actinin, alpha 2
CRK	v-crk avian sarcoma virus CT10 oncogene homolog
PIK3C2G	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 gamma
DGKI	diacylglycerol kinase, iota
TLN1	talin 1
F2RL2	coagulation factor II (thrombin) receptor-like 2
PLSCR1	phospholipid scramblase 1
TGFB3	transforming growth factor, beta 3
RHOG	ras homolog family member G
TGFB2	transforming growth factor, beta 2
PIK3R6	phosphoinositide-3-kinase, regulatory subunit 6
TRPC3	transient receptor potential cation channel, subfamily C, member 3
DGKA	diacylglycerol kinase, alpha 80kDa
GNG2	guanine nucleotide binding protein (G protein), gamma 2

CD40	CD40 molecule, TNF receptor superfamily member 5
ACTN1	actinin, alpha 1
DGKK	diacylglycerol kinase, kappa
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
ITPR3	inositol 1,4,5-trisphosphate receptor, type 3
SYK	spleen tyrosine kinase
PTPN1	protein tyrosine phosphatase, non-receptor type 1
SOS1	son of sevenless homolog 1 (Drosophila)
PDPK1	3-phosphoinositide dependent protein kinase 1
CALM2	calmodulin 2 (phosphorylase kinase, delta)
PRKCQ	protein kinase C, theta
RHOB	ras homolog family member B
SERPINF2	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2
COL1A1	collagen, type I, alpha 1
PRKCZ	protein kinase C, zeta
PIK3C2B	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 beta
LEFTY2	left-right determination factor 2
VAV3	vav 3 guanine nucleotide exchange factor
KNG1	kininogen 1
DGKG	diacylglycerol kinase, gamma 90kDa
RHOA	ras homolog family member A
FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
ADRA2A	adrenoceptor alpha 2A
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1
THBS1	thrombospondin 1
GNAQ	guanine nucleotide binding protein (G protein), q polypeptide
TIMP1	TIMP metallopeptidase inhibitor 1
VAV1	vav 1 guanine nucleotide exchange factor
GNA14	guanine nucleotide binding protein (G protein), alpha 14

GNAT3	guanine nucleotide binding protein, alpha transducing 3
SRC	SRC proto-oncogene, non-receptor tyrosine kinase
GRB2	growth factor receptor-bound protein 2
P2RY1	purinergic receptor P2Y, G-protein coupled, 1
GNA13	guanine nucleotide binding protein (G protein), alpha 13
FN1	fibronectin 1
PLEK	pleckstrin
PRKCA	protein kinase C, alpha
GP9	glycoprotein IX (platelet)
PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)
TREML1	triggering receptor expressed on myeloid cells-like 1
CAP1	CAP, adenylate cyclase-associated protein 1 (yeast)
COL3A1	collagen, type III, alpha 1
F8A1	coagulation factor VIII-associated 1
PRKCH	protein kinase C, eta
SHC1	SHC (Src homology 2 domain containing) transforming protein 1
GNB1	guanine nucleotide binding protein (G protein), beta polypeptide 1
FLNA	filamin A, alpha
LCK	LCK proto-oncogene, Src family tyrosine kinase
CLU	clusterin
APOA1	apolipoprotein A-I
TMSB4X	thymosin beta 4, X-linked
FYN	FYN proto-oncogene, Src family tyrosine kinase
TYRO3	TYRO3 protein tyrosine kinase
ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
VEGFA	vascular endothelial growth factor A
LCP2	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)
VCL	vinculin
F2R	coagulation factor II (thrombin) receptor

DGKB	diacylglycerol kinase, beta 90kDa
ADRA2B	adrenoceptor alpha 2B
PSAP	prosaposin
AKT1	v-akt murine thymoma viral oncogene homolog 1
BRPF3	bromodomain and PHD finger containing, 3
MPL	MPL proto-oncogene, thrombopoietin receptor
ITPR2	inositol 1,4,5-trisphosphate receptor, type 2
PDGFB	platelet-derived growth factor beta polypeptide
ITGA2B	integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41)
ARRB1	arrestin, beta 1
ACTN4	actinin, alpha 4
POTEM	POTE ankyrin domain family, member M
GNAI2	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2
CALM1	calmodulin 1 (phosphorylase kinase, delta)
PRKCG	protein kinase C, gamma
DAGLB	diacylglycerol lipase, beta
MGLL	monoglyceride lipase
F2RL3	coagulation factor II (thrombin) receptor-like 3
VEGFB	vascular endothelial growth factor B
IGF1	insulin-like growth factor 1 (somatomedin C)
IL6	interleukin 6
GAS6	growth arrest-specific 6
CALM3	calmodulin 3 (phosphorylase kinase, delta)
VAV2	vav 2 guanine nucleotide exchange factor
ADRA2C	adrenoceptor alpha 2C
CD63	CD63 molecule
LAT	linker for activation of T cells
F8	coagulation factor VIII, procoagulant component
ADAMTS13	ADAM metallopeptidase with thrombospondin type 1 motif, 13

SCG3	secretogranin III
SRF	serum response factor (c-fos serum response element-binding transcription factor)
WNT3A	wingless-type MMTV integration site family, member 3A
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma
PLCG2	phospholipase C, gamma 2 (phosphatidylinositol-specific)
RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)
GNA12	guanine nucleotide binding protein (G protein) alpha 12
RAC1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
CALU	calumenin
GNA15	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)
WDR1	WD repeat domain 1
DGKQ	diacylglycerol kinase, theta 110kDa
SELP	selectin P (granule membrane protein 140kDa, antigen CD62)
VEGFC	vascular endothelial growth factor C
SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
THPO	thrombopoietin
HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
TRPC7	transient receptor potential cation channel, subfamily C, member 7
PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)
FGB	fibrinogen beta chain
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
BLOC1S3	biogenesis of lysosomal organelles complex-1, subunit 3
RAPGEF3	Rap guanine nucleotide exchange factor (GEF) 3
RAP1A	RAP1A, member of RAS oncogene family
GP5	glycoprotein V (platelet)
CFD	complement factor D (adipsin)
PRKCB	protein kinase C, beta
PECAM1	platelet/endothelial cell adhesion molecule 1

PLG	plasminogen
AXL	AXL receptor tyrosine kinase
SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
CX3CL1	chemokine (C-X3-C motif) ligand 1
TUBA4A	tubulin, alpha 4a

Table 6 Differentially expressed targeted mRNAs associated with blood coagulation

Gene symbol	Protein Name
LYN	LYN proto-oncogene, Src family tyrosine kinase
GGCX	gamma-glutamyl carboxylase
PDE9A	phosphodiesterase 9A
SIRPA	signal-regulatory protein alpha
MMRN1	multimerin 1
EFEMP2	EGF containing fibulin-like extracellular matrix protein 2
HPS1	Hermansky-Pudlak syndrome 1
VWF	von Willebrand factor
RAF1	Raf-1 proto-oncogene, serine/threonine kinase
KIF9	kinesin family member 9
KDM1A	lysine (K)-specific demethylase 1A
ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1
LRRC16A	leucine rich repeat containing 16A
ANO6	anoctamin 6
ITGAV	integrin, alpha V
CDC42	cell division cycle 42
RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)
PFN1	profilin 1
SELPLG	selectin P ligand
FGR	FGR proto-oncogene, Src family tyrosine kinase
HDAC1	histone deacetylase 1
EGF	epidermal growth factor
KIF23	kinesin family member 23
DGKH	diacylglycerol kinase, eta
AKAP1	A kinase (PRKA) anchor protein 1
ITGA1	integrin, alpha 1
EHD2	EH-domain containing 2
MAPK14	mitogen-activated protein kinase 14

ITPK1	inositol-tetrakisphosphate 1-kinase
CD59	CD59 molecule, complement regulatory protein
GNAI3	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3
KRAS	Kirsten rat sarcoma viral oncogene homolog
EHD1	EH-domain containing 1
SERPING1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
TBXA2R	thromboxane A2 receptor
IRF2	interferon regulatory factor 2
CDK2	cyclin-dependent kinase 2
IGF2	insulin-like growth factor 2
F13B	coagulation factor XIII, B polypeptide
CAV1	caveolin 1, caveolae protein, 22kDa
MAG	myelin associated glycoprotein
PRKCD	protein kinase C, delta
RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4
TGFB1	transforming growth factor, beta 1
PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta
INPP5D	inositol polyphosphate-5-phosphatase, 145kDa
GP1BB	glycoprotein Ib (platelet), beta polypeptide
AKAP10	A kinase (PRKA) anchor protein 10
IFNB1	interferon, beta 1, fibroblast
SPARC	secreted protein, acidic, cysteine-rich (osteonectin)
F5	coagulation factor V (proaccelerin, labile factor)
P2RY12	purinergic receptor P2Y, G-protein coupled, 12
MAPK1	mitogen-activated protein kinase 1
DOK2	docking protein 2, 56kDa
HMCN1	hemicentin 1
NFE2	nuclear factor, erythroid 2
H3F3B	H3 histone, family 3B (H3.3B)

PRKACB	protein kinase, cAMP-dependent, catalytic, beta
ADORA2A	adenosine A2a receptor
PRKG1	protein kinase, cGMP-dependent, type I
CAPZB	capping protein (actin filament) muscle Z-line, beta
JAM2	junctional adhesion molecule 2
LMAN1	lectin, mannose-binding, 1
PDE3A	phosphodiesterase 3A, cGMP-inhibited
APBB1IP	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
PPIA	peptidylprolyl isomerase A (cyclophilin A)
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta
KIF3A	kinesin family member 3A
CD9	CD9 molecule
STIM1	stromal interaction molecule 1
SERPINA10	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10
PRTN3	proteinase 3
GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)
KIF11	kinesin family member 11
TEK	TEK tyrosine kinase, endothelial
CSK	c-src tyrosine kinase
ITGAM	integrin, alpha M (complement component 3 receptor 3 subunit)
SHH	sonic hedgehog
LYST	lysosomal trafficking regulator
ACTB	actin, beta
CFL1	cofilin 1 (non-muscle)
CD2	CD2 molecule
IRF1	interferon regulatory factor 1
ARRB2	arrestin, beta 2
NOS3	nitric oxide synthase 3 (endothelial cell)
ATP2B2	ATPase, Ca++ transporting, plasma membrane 2

PRKG2	protein kinase, cGMP-dependent, type II
PTK2	protein tyrosine kinase 2
BCAR1	breast cancer anti-estrogen resistance 1
SLC16A8	solute carrier family 16 (monocarboxylate transporter), member 8
GUCY1A2	guanylate cyclase 1, soluble, alpha 2
EHD3	EH-domain containing 3
RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)
PIK3R5	phosphoinositide-3-kinase, regulatory subunit 5
F3	coagulation factor III (thromboplastin, tissue factor)
RCOR1	REST corepressor 1
SLC8A1	solute carrier family 8 (sodium/calcium exchanger), member 1
PDE2A	phosphodiesterase 2A, cGMP-stimulated
RAB27A	RAB27A, member RAS oncogene family
ITGA10	integrin, alpha 10
PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta
LRP8	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor
ESAM	endothelial cell adhesion molecule
LAMP2	lysosomal-associated membrane protein 2
DAGLA	diacylglycerol lipase, alpha
GATA2	GATA binding protein 2
HGF	hepatocyte growth factor (hepapoietin A; scatter factor)
ACTN2	actinin, alpha 2
CRK	v-crk avian sarcoma virus CT10 oncogene homolog
DGKI	diacylglycerol kinase, iota
TLN1	talin 1
F2RL2	coagulation factor II (thrombin) receptor-like 2
OLR1	oxidized low density lipoprotein (lectin-like) receptor 1
CBX5	chromobox homolog 5
TGFB3	transforming growth factor, beta 3

PLAT	plasminogen activator, tissue
CD47	CD47 molecule
APOB	apolipoprotein B
SIRPG	signal-regulatory protein gamma
SLC7A5	solute carrier family 7 (amino acid transporter light chain, L system), member 5
GRB7	growth factor receptor-bound protein 7
RHOG	ras homolog family member G
TGFB2	transforming growth factor, beta 2
PIK3R6	phosphoinositide-3-kinase, regulatory subunit 6
TRPC3	transient receptor potential cation channel, subfamily C, member 3
GATA6	GATA binding protein 6
DGKA	diacylglycerol kinase, alpha 80kDa
CD44	CD44 molecule (Indian blood group)
HNF4A	hepatocyte nuclear factor 4, alpha
GNG2	guanine nucleotide binding protein (G protein), gamma 2
MFN2	mitofusin 2
ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
TREM1	triggering receptor expressed on myeloid cells 1
GATA5	GATA binding protein 5
KCNMB2	potassium large conductance calcium-activated channel, subfamily M, beta member 2
ACTN1	actinin, alpha 1
ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide
KLC1	kinesin light chain 1
DGKK	diacylglycerol kinase, kappa
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2
AP3B1	adaptor-related protein complex 3, beta 1 subunit
ITPR3	inositol 1,4,5-trisphosphate receptor, type 3
GATA3	GATA binding protein 3

CD84	CD84 molecule
KLC2	kinesin light chain 2
PDE1B	phosphodiesterase 1B, calmodulin-dependent
SYK	spleen tyrosine kinase
ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
PTPN1	protein tyrosine phosphatase, non-receptor type 1
SOS1	son of sevenless homolog 1 (Drosophila)
PDPK1	3-phosphoinositide dependent protein kinase 1
F2RL1	coagulation factor II (thrombin) receptor-like 1
CALM2	calmodulin 2 (phosphorylase kinase, delta)
CDK5	cyclin-dependent kinase 5
PRKCQ	protein kinase C, theta
CYP4F2	cytochrome P450, family 4, subfamily F, polypeptide 2
ATP2B4	ATPase, Ca++ transporting, plasma membrane 4
RHOB	ras homolog family member B
HMG20B	high mobility group 20B
SERPINF2	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2
SLC7A6	solute carrier family 7 (amino acid transporter light chain, y+L system), member 6
VKORC1	vitamin K epoxide reductase complex, subunit 1
JAM3	junctional adhesion molecule 3
KCNMB4	potassium large conductance calcium-activated channel, subfamily M, beta member 4
ANXA8	annexin A8
COL1A1	collagen, type I, alpha 1
MAFK	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog K
PRKCZ	protein kinase C, zeta
DOCK9	dedicator of cytokinesis 9
SLC8A3	solute carrier family 8 (sodium/calcium exchanger), member 3
SERPINA5	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5
C6orf25	chromosome 6 open reading frame 25

KIF2C	kinesin family member 2C
LEFTY2	left-right determination factor 2
VAV3	vav 3 guanine nucleotide exchange factor
PLAUR	plasminogen activator, urokinase receptor
GATA4	GATA binding protein 4
KNG1	kininogen 1
PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha
RAD51B	RAD51 paralog B
KIAA1715	KIAA1715
DGKG	diacylglycerol kinase, gamma 90kDa
RHOA	ras homolog family member A
FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
ADRA2A	adrenoceptor alpha 2A
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1
THBS1	thrombospondin 1
KCNMA1	potassium large conductance calcium-activated channel, subfamily M, alpha member 1
DOCK1	dedicator of cytokinesis 1
GNAQ	guanine nucleotide binding protein (G protein), q polypeptide
TIMP1	TIMP metallopeptidase inhibitor 1
VAV1	vav 1 guanine nucleotide exchange factor
PDE5A	phosphodiesterase 5A, cGMP-specific
PLAU	plasminogen activator, urokinase
GNA14	guanine nucleotide binding protein (G protein), alpha 14
GNAT3	guanine nucleotide binding protein, alpha transducing 3
ATP2A3	ATPase, Ca++ transporting, ubiquitous
SRC	SRC proto-oncogene, non-receptor tyrosine kinase
GRB2	growth factor receptor-bound protein 2
P2RY1	purinergic receptor P2Y, G-protein coupled, 1
CXADR	coxsackie virus and adenovirus receptor

PDE1A	phosphodiesterase 1A, calmodulin-dependent
GNA13	guanine nucleotide binding protein (G protein), alpha 13
FN1	fibronectin 1
PLEK	pleckstrin
PRKCA	protein kinase C, alpha
ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
GP9	glycoprotein IX (platelet)
PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)
ABL1	ABL proto-oncogene 1, non-receptor tyrosine kinase
CAP1	CAP, adenylate cyclase-associated protein 1 (yeast)
PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta
CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1
AK3	adenylate kinase 3
PRKCH	protein kinase C, eta
TSPAN32	tetraspanin 32
SHC1	SHC (Src homology 2 domain containing) transforming protein 1
GNB1	guanine nucleotide binding protein (G protein), beta polypeptide 1
ANGPT2	angiopoietin 2
FLNA	filamin A, alpha
LCK	LCK proto-oncogene, Src family tyrosine kinase
SLC7A8	solute carrier family 7 (amino acid transporter light chain, L system), member 8
CLU	clusterin
KCNMB1	potassium large conductance calcium-activated channel, subfamily M, beta member 1
PTPN11	protein tyrosine phosphatase, non-receptor type 11
APOA1	apolipoprotein A-I
KIF5A	kinesin family member 5A
DOCK6	dedicator of cytokinesis 6
TMSB4X	thymosin beta 4, X-linked
C4BPB	complement component 4 binding protein, beta

YES1	YES proto-oncogene 1, Src family tyrosine kinase
ZFPM1	zinc finger protein, FOG family member 1
CD177	CD177 molecule
FYN	FYN proto-oncogene, Src family tyrosine kinase
PLCG1	phospholipase C, gamma 1
SELE	selectin E
ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
WAS	Wiskott-Aldrich syndrome
KIF15	kinesin family member 15
SLC16A1	solute carrier family 16 (monocarboxylate transporter), member 1
CABLES1	Cdk5 and Abl enzyme substrate 1
THBD	thrombomodulin
ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
KIF2A	kinesin heavy chain member 2A
MMP1	matrix metallopeptidase 1 (interstitial collagenase)
VEGFA	vascular endothelial growth factor A
LCP2	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)
ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide
MAFG	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog G
VCL	vinculin
RACGAP1	Rac GTPase activating protein 1
ATP1B2	ATPase, Na+/K+ transporting, beta 2 polypeptide
F2R	coagulation factor II (thrombin) receptor
ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
DGKB	diacylglycerol kinase, beta 90kDa
ADRA2B	adrenoceptor alpha 2B
SCUBE1	signal peptide, CUB domain, EGF-like 1
KIF3C	kinesin family member 3C
PTPRJ	protein tyrosine phosphatase, receptor type, J

PSAP	prosaposin
AKT1	v-akt murine thymoma viral oncogene homolog 1
BRPF3	bromodomain and PHD finger containing, 3
RBSN	zinc finger, FYVE domain containing 20
MPL	MPL proto-oncogene, thrombopoietin receptor
HPS5	Hermansky-Pudlak syndrome 5
ITPR2	inositol 1,4,5-trisphosphate receptor, type 2
CD48	CD48 molecule
ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
PDGFB	platelet-derived growth factor beta polypeptide
ORAI1	ORAI calcium release-activated calcium modulator 1
GATA1	GATA binding protein 1 (globin transcription factor 1)
PRKAR2A	protein kinase, cAMP-dependent, regulatory, type II, alpha
ITGA2B	integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41)
ARRB1	arrestin, beta 1
ACTN4	actinin, alpha 4
GUCY1A3	guanylate cyclase 1, soluble, alpha 3
SELL	selectin L
VPS45	vacuolar protein sorting 45 homolog (S. cerevisiae)
POTEM	POTE ankyrin domain family, member M
GNAI2	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2
CALM1	calmodulin 1 (phosphorylase kinase, delta)
PRKCG	protein kinase C, gamma
KIF4B	kinesin family member 4B
F11	coagulation factor XI
DAGLB	diacylglycerol lipase, beta
MGLL	monoglyceride lipase
ITGAX	integrin, alpha X (complement component 3 receptor 4 subunit)
SH2B1	SH2B adaptor protein 1

ITGA6	integrin, alpha 6
F2RL3	coagulation factor II (thrombin) receptor-like 3
PRCP	prolylcarboxypeptidase (angiotensinase C)
VEGFB	vascular endothelial growth factor B
IGF1	insulin-like growth factor 1 (somatomedin C)
GAS6	growth arrest-specific 6
CALM3	calmodulin 3 (phosphorylase kinase, delta)
VAV2	vav 2 guanine nucleotide exchange factor
CEACAM8	carcinoembryonic antigen-related cell adhesion molecule 8
PTGIR	prostaglandin I2 (prostacyclin) receptor (IP)
ADRA2C	adrenoceptor alpha 2C
CD63	CD63 molecule
LAT	linker for activation of T cells
SLC7A10	solute carrier family 7 (neutral amino acid transporter light chain, asc system), member 10
ATP2B1	ATPase, Ca++ transporting, plasma membrane 1
F11R	F11 receptor
PTPN6	protein tyrosine phosphatase, non-receptor type 6
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
F8	coagulation factor VIII, procoagulant component
ADAMTS13	ADAM metallopeptidase with thrombospondin type 1 motif, 13
SLC8A2	solute carrier family 8 (sodium/calcium exchanger), member 2
NOS1	nitric oxide synthase 1 (neuronal)
SCG3	secretogranin III
PPIL2	peptidylprolyl isomerase (cyclophilin)-like 2
MRVI1	murine retrovirus integration site 1 homolog
SH2B2	SH2B adaptor protein 2
NBEAL2	neurobeachin-like 2
ATP2A1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1
HRAS	Harvey rat sarcoma viral oncogene homolog

PDE11A	phosphodiesterase 11A
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma
SIN3A	SIN3 transcription regulator family member A
PRKACA	protein kinase, cAMP-dependent, catalytic, alpha
PLCG2	phospholipase C, gamma 2 (phosphatidylinositol-specific)
ENPP4	ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative)
RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)
GNA12	guanine nucleotide binding protein (G protein) alpha 12
MAFF	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F
RAC1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
CALU	calumenin
GNA15	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)
WDR1	WD repeat domain 1
CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
SLC7A11	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11
DGKQ	diacylglycerol kinase, theta 110kDa
SELP	selectin P (granule membrane protein 140kDa, antigen CD62)
KIF26A	kinesin family member 26A
VEGFC	vascular endothelial growth factor C
MYB	v-myb avian myeloblastosis viral oncogene homolog
ANGPT4	angiopoietin 4
SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
CEACAM6	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)
THPO	thrombopoietin
HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
TRPC7	transient receptor potential cation channel, subfamily C, member 7
GUCY1B3	guanylate cyclase 1, soluble, beta 3

PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)
IFNA8	interferon, alpha 8
TP53	tumor protein p53
KIFAP3	kinesin-associated protein 3
FBLN5	fibulin 5
L1CAM	L1 cell adhesion molecule
CYP4F11	cytochrome P450, family 4, subfamily F, polypeptide 11
BSG	basigin (Ok blood group)
FGB	fibrinogen beta chain
KLKB1	kallikrein B, plasma (Fletcher factor) 1
SLC16A3	solute carrier family 16 (monocarboxylate transporter), member 3
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
BLOC1S3	biogenesis of lysosomal organelles complex-1, subunit 3
RAPGEF3	Rap guanine nucleotide exchange factor (GEF) 3
RAP1A	RAP1A, member of RAS oncogene family
TFPI	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)
GP5	glycoprotein V (platelet)
CFD	complement factor D (adipsin)
PRKCB	protein kinase C, beta
PECAM1	platelet/endothelial cell adhesion molecule 1
F7	coagulation factor VII (serum prothrombin conversion accelerator)
NOS2	nitric oxide synthase 2, inducible
PLG	plasminogen
CD58	CD58 molecule
KIF4A	kinesin family member 4A
SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
HPS6	Hermansky-Pudlak syndrome 6
SPN	sialophorin
SH2B3	SH2B adaptor protein 3

TUBA4A	tubulin, alpha 4a

Figures

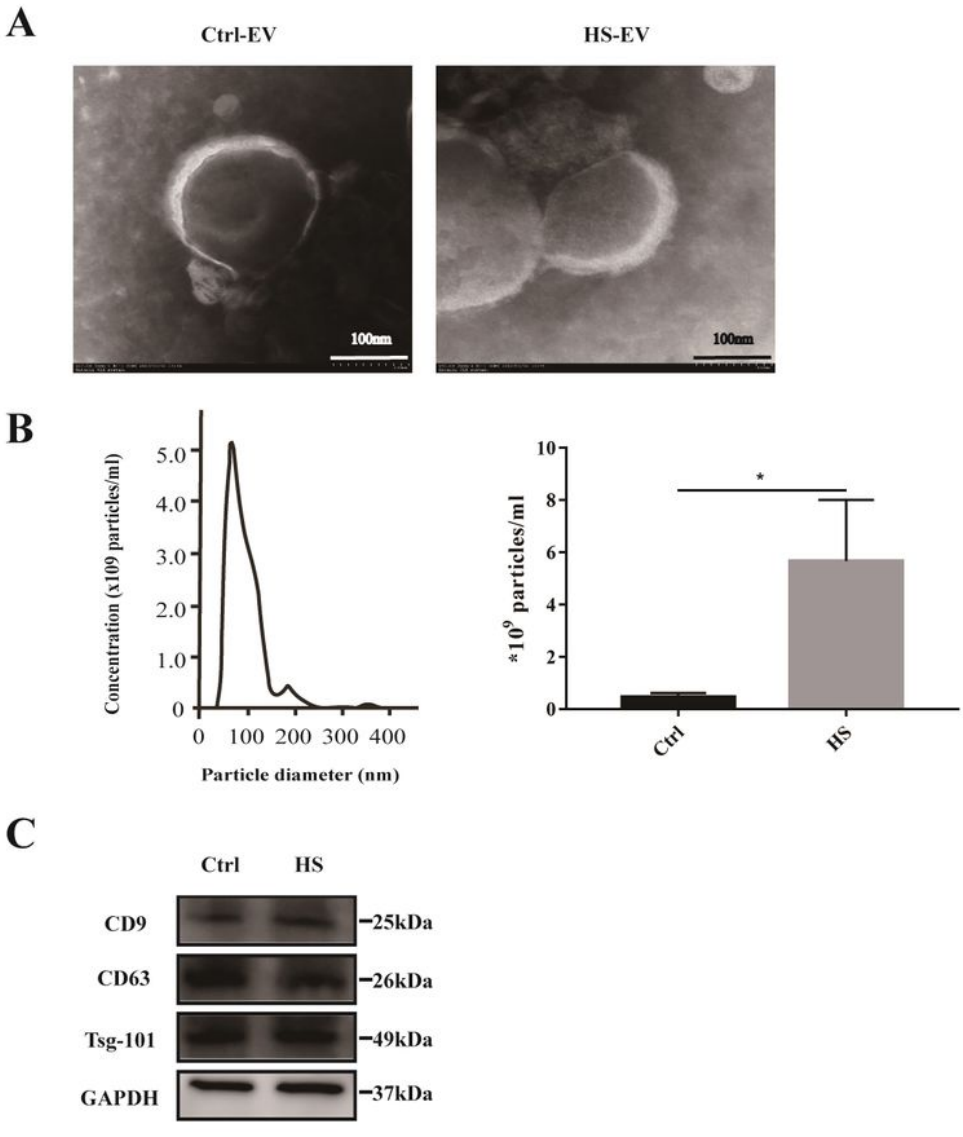

Figure 1

Characterization of plasma exosomes in both healthy controls and HS patients. (A) Morphology of plasma exosomes visualized under TEM. Bar,100 nm. (B) Exosome size distribution examined through NTA. (C) Levels of representative exosomal surface markers (CD9, CD63 along with Tsg-101) by Western blot analysis. All experiments were repeated thrice.

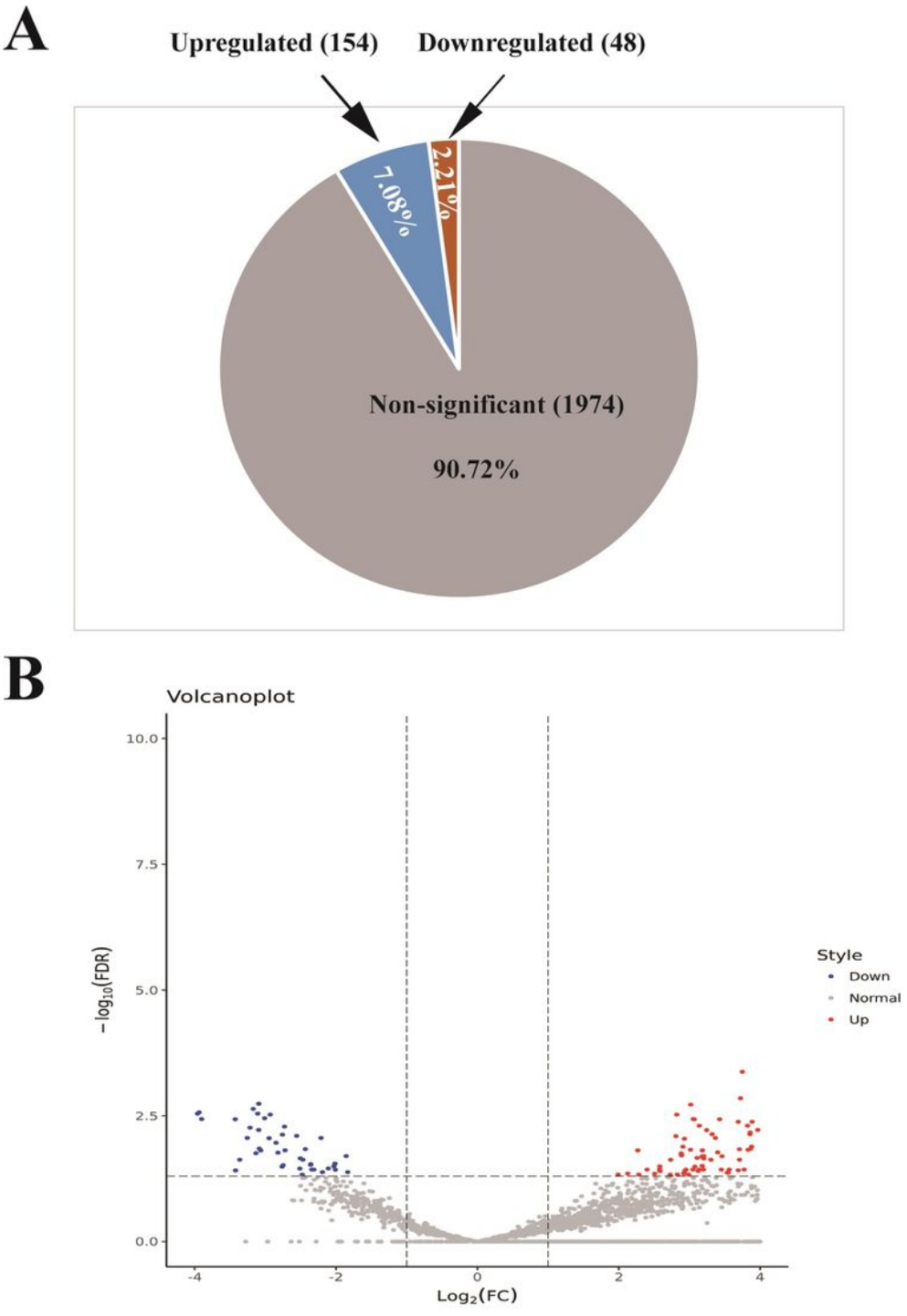

Figure 2

(A) HS altered the miRNA expression patterns of hepatocyte-derived EVs as analyzed by NGS. The number and proportion of differentially regulated (up-regulated, down-regulated, and non-significant) EV miRNAs isolated from HS compared with the control (n = 3 samples/group). Numbers indicate proportions. Data base: HomoSapiens. (B) Volcano plot representing the miRNA fold change and RNA intesity in the HS and control hepatocyte EVs. X-axis shows miRNA fold-change (HS/Control, log2), while Y-axis depicted the summary of RNA intensity (LFQ intensity, lg).

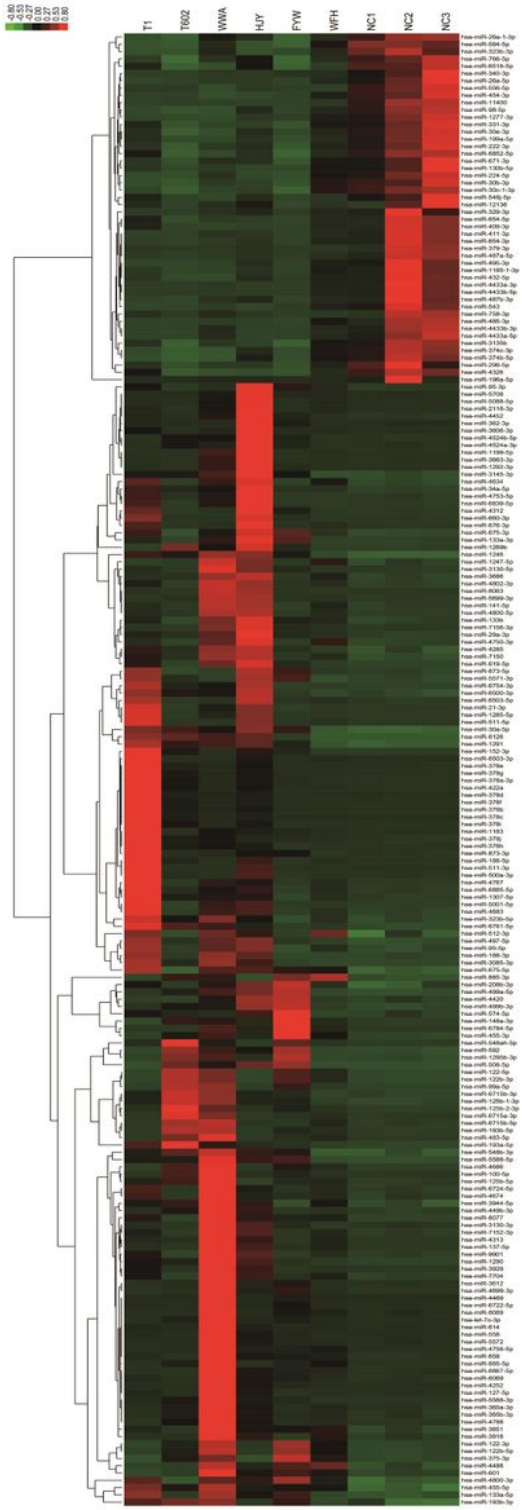

Figure 3

Cluster analysis of the disreguralted miRNA components from HS EVs. The list of all of the 202 significantly differentially expressed (HS-EV group/control EV group) has been shown in heat map.

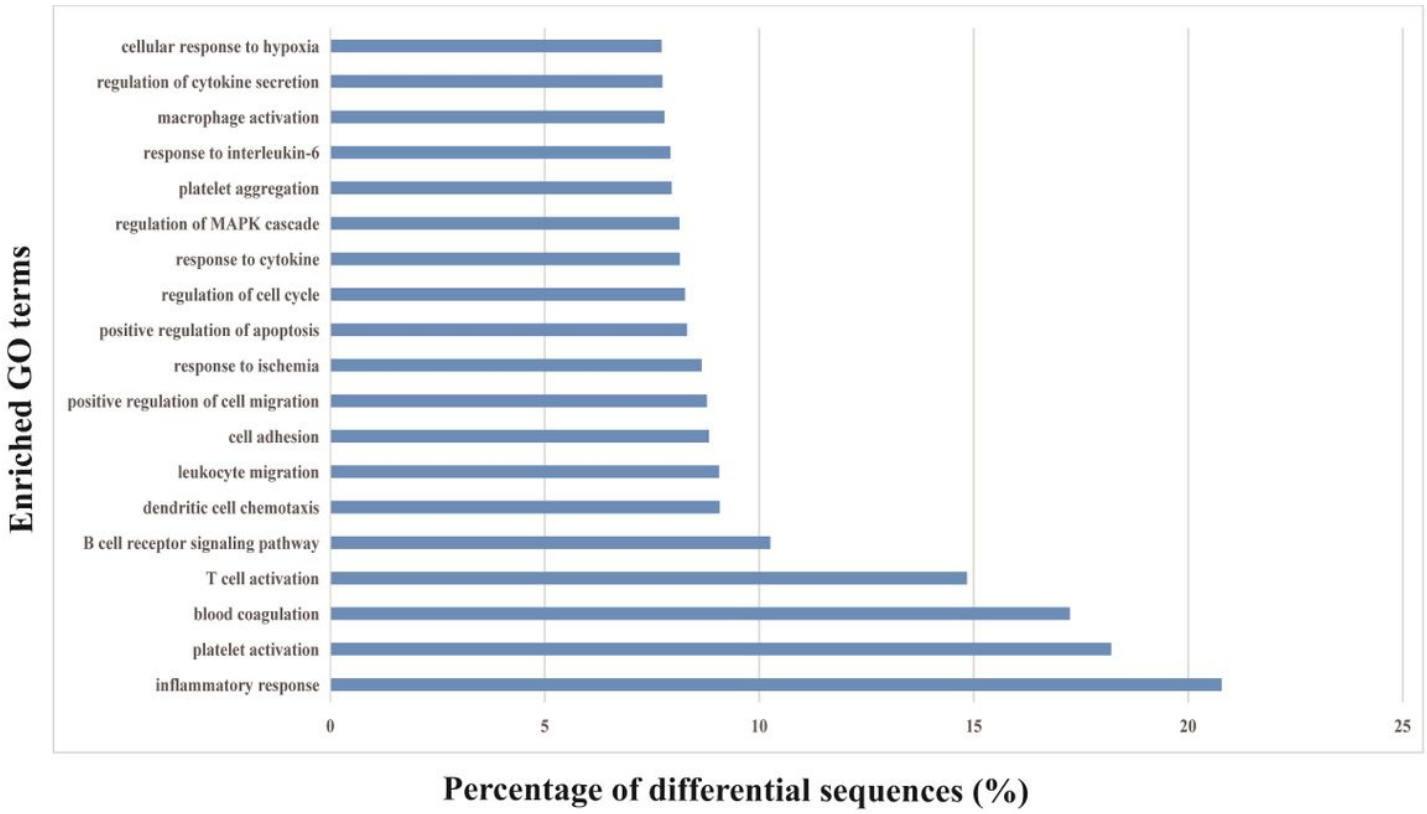

Figure 4

Gene Ontology (GO) enrichment analysis of the differentially regulated proteins. The top 20 enriched terms according to the GO functional annotation clustering of the 202 differentially expressed miRNAs in the heat-stroked hepatocyte-derived EVs. Percentages of the sequences involved are shown.

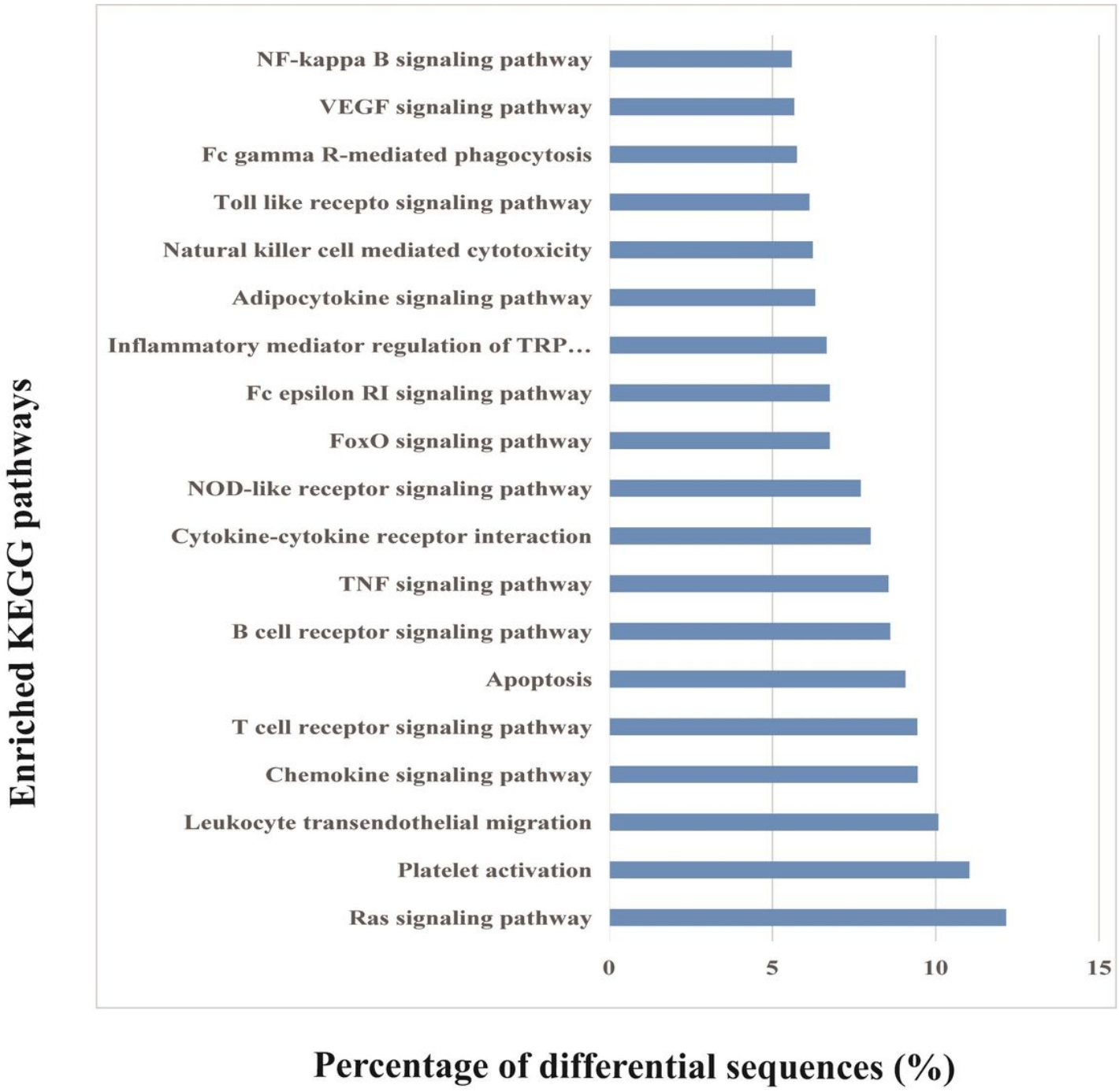

Figure 5

Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis of the differentially expressed EV miRNAs. The top 20 enriched KEGG pathways in the heat-stroked hepatocyte-derived EV miRNA and the percentages of the sequences involved in each pathway are shown.

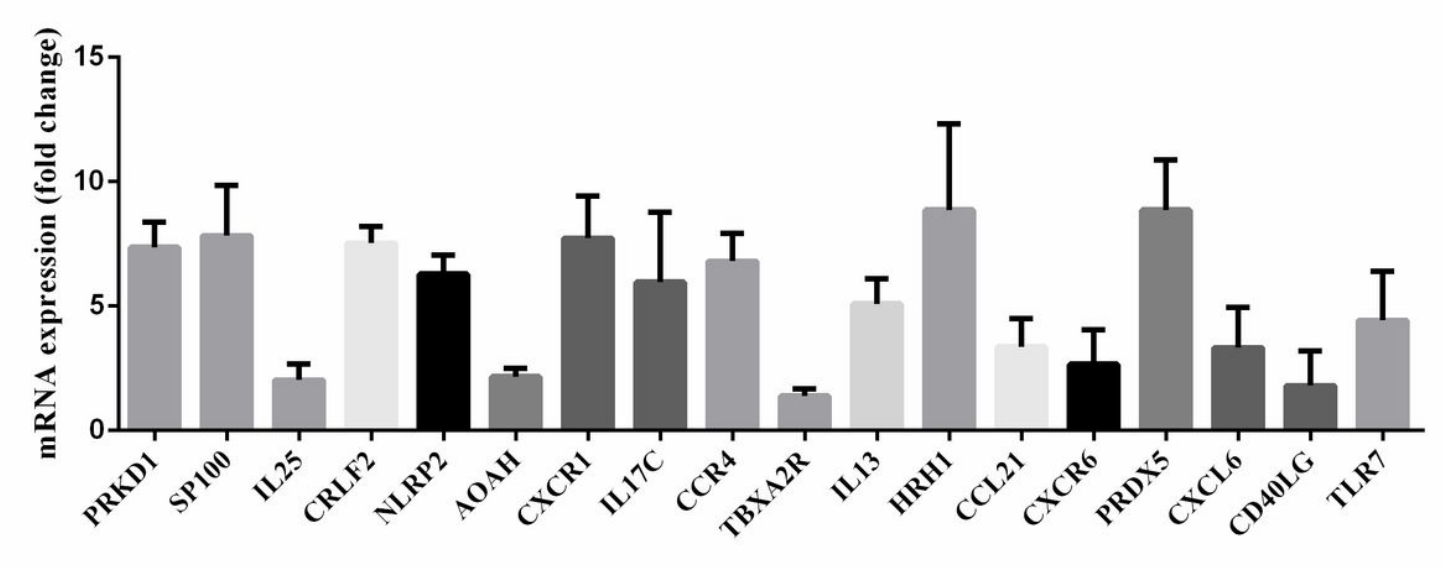

Figure 6

Gene expression of messenger RNA (mRNA) related to inflammatory response in the comparison between patients with heat stroke versus healthy control subjects.

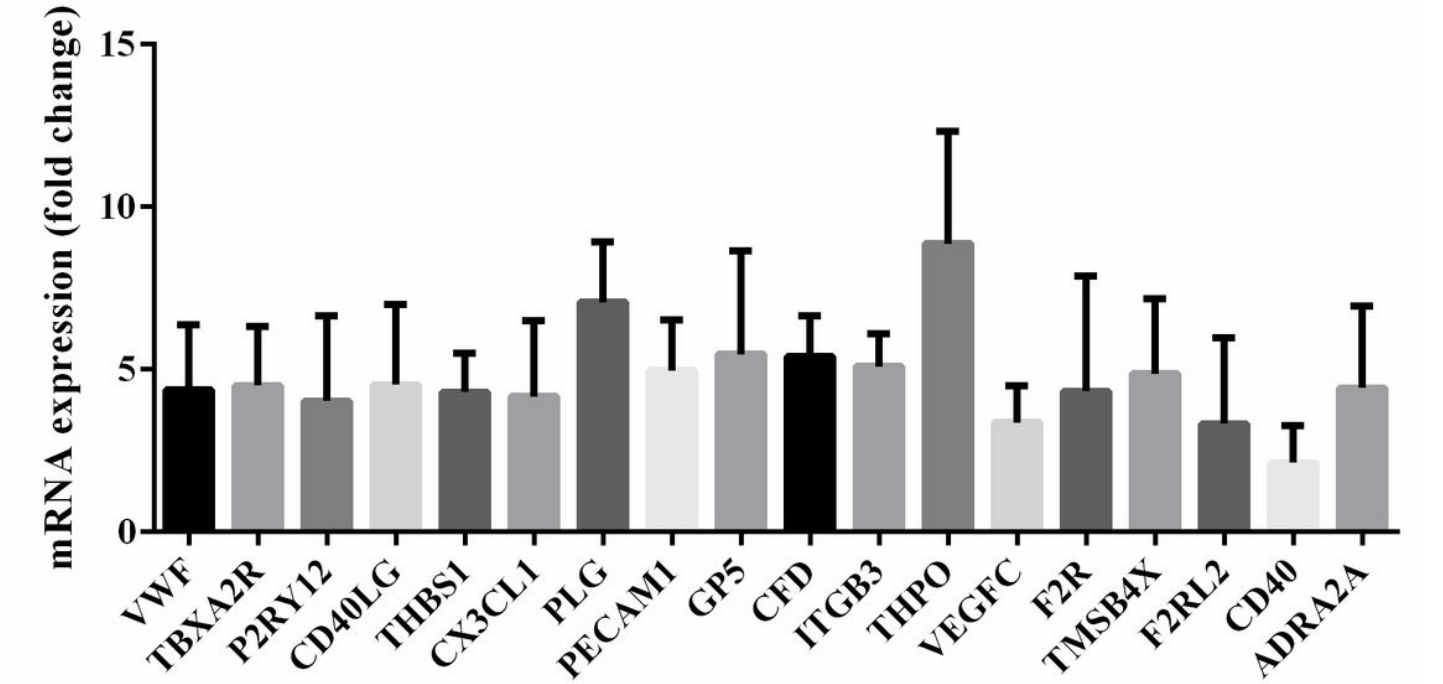

Figure 7

Gene expression of messenger RNA (mRNA) related to platelet activation in the comparison between patients with heat stroke versus healthy control subjects.

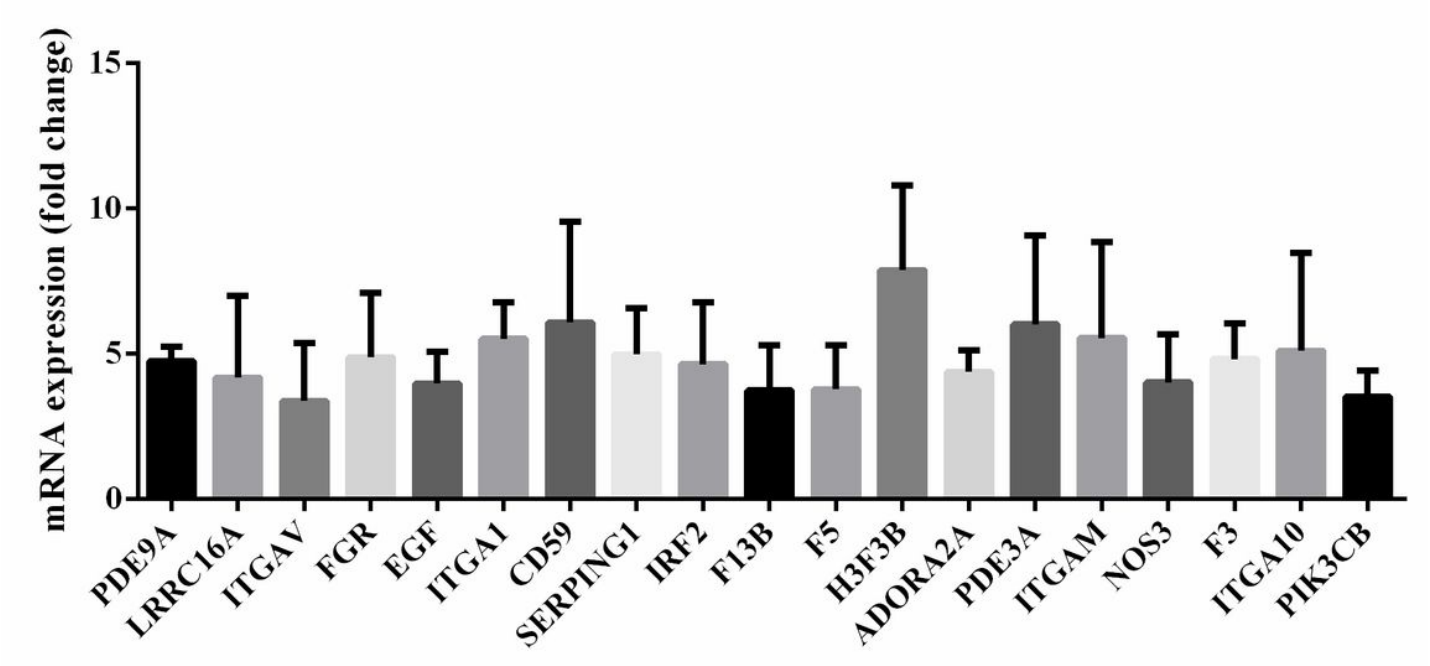

Figure 8

Gene expression of messenger RNA (mRNA) related to blood coagulation in the comparison between patients with heat stroke versus healthy control subjects.

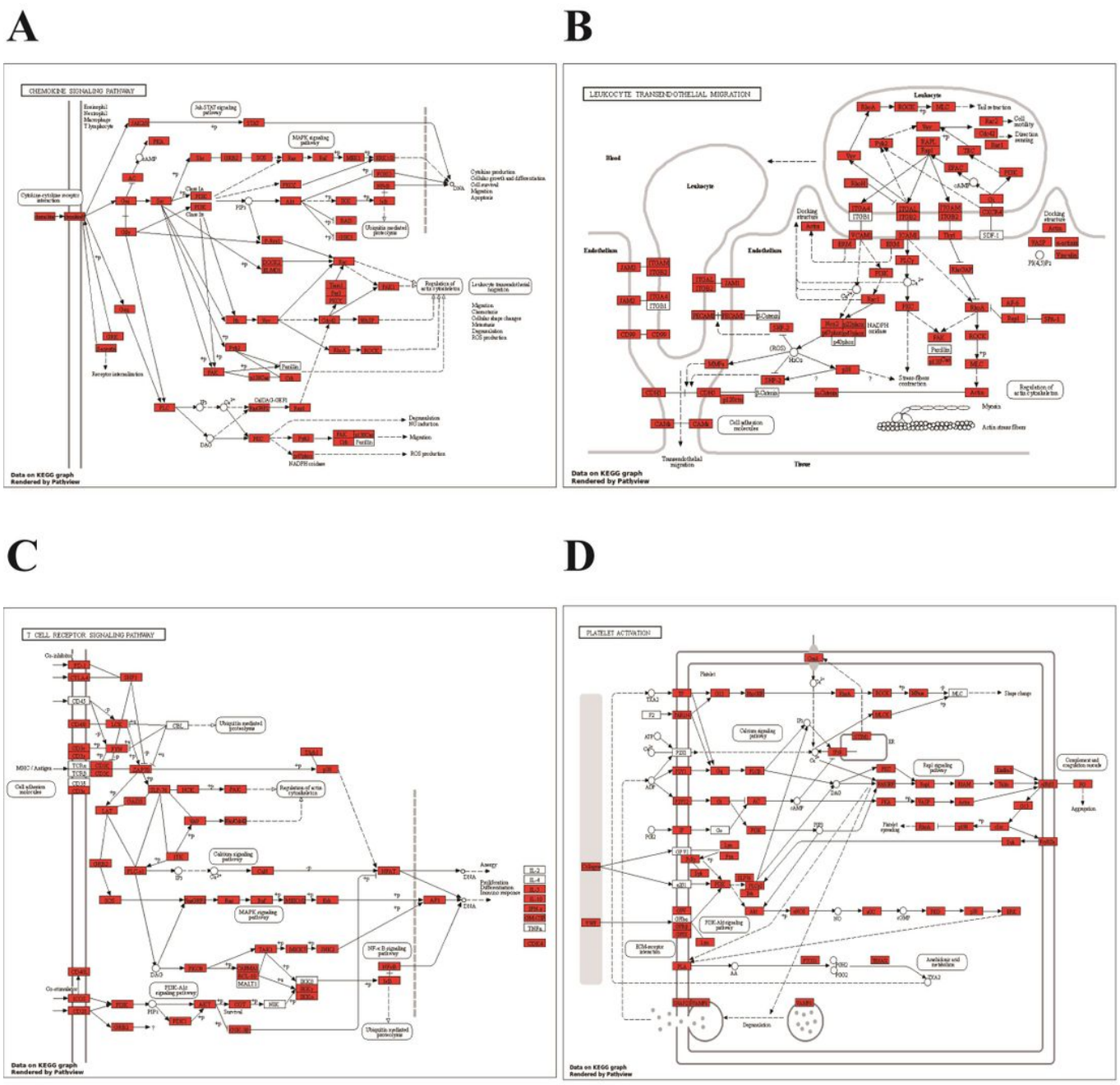

Figure 9

Putative chemokine signaling pathway, platelet activation pathway, T cell receptor signaling pathway and leukocyte transendothelial migration pathway were constructed based on KEGG mapping. Red squares indicate proteins identified as differentially expressed miRNAs; white circles indicate miRNAs not identified as differentially expressed in our study.