haematologica
Journal of the Ferrata Storti Foundation

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Received: June 7, 2026.
Accepted: October 29, 2026.

Citation: Ayaka Nishihara, Shokichi Tsukamoto, Fumiaki Sakura, Takaaki Tanami, Sanshiro Nakao, Koji Takaishi, Chihiro Ri, Shintaro Izumi, Shinichiro Matsui, Arata Ishii, Nagisa Oshima-Hasegawa, Tomoya Muto, Yusuke Takeda, Tomoki Suichi, Wakaba Furuie, Toshihisa Semba, Sonoko Misawa, Satoshi Kuwabara, Koutaro Yokote, Yusuke Kawashima, Osamu Ohara, Jun-Ichiro Ikeda, Naoya Mimura and Emiko Sakaida. Platelet and megakaryocyte function in the pathogenesis of POEMS syndrome. Haematologica. 2026 Nov 5. doi: 10.3324/haematol.2026.301366 [Epub ahead of print]

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Original Article

Platelet and megakaryocyte function in the pathogenesis of POEMS syndrome

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Running title:

Platelet and megakaryocyte function in POEMS

Keywords:

POEMS syndrome, platelet, megakaryocyte, multi-omics analysis, VEGF

Authorship Contributions

A.N., S.T., N.M., and E.S. designed the study. A.N., S.T., T.T., S.N., K.T., S.I., S.M., A.I., N.O-H., T.M., and Y.T. collected patient samples. A.N., S.T., and O.O. performed experiments and analyzed and interpreted data. W.F., T.S., and Y.K. performed experiments and analyzed data. F.S. analyzed and interpreted data. A.N. and S.T. actively wrote the manuscript. F.S., T.S., Y.K., and O.O. helped write the manuscript. S.T., T.S., S.M., S.K., K.Y., J.I., N.M., and E.S. supervised the study. All authors discussed the manuscript and approved the final report.

Acknowledgements

We would like to thank Satoshi Takagi and Shinichiro Funayama for providing technical advice in this study; Yuka Imai, Satomi Hasegawa, and Tsubasa Matsusaka for technical support with immunohistochemistry; and Ayumi Koga for technical support with patient sample preparation. We also thank Sho Okamoto for technical support with RNA sequencing and cytokine assay. This work was supported partly by Grants-in-Aid for Scientific Research (#24K11510) from the Japan Society for the Promotion of Science (JSPS), Takeda Science Foundation, and Kanae Foundation for the Promotion of Medical Science.

Disclosure of Conflicts of Interest

The authors have no competing financial interests to declare

Data Deposition

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Data and Code Availability

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Artificial intelligence (AI)

AI tools were used solely for language editing, grammar checking, and spelling correction. No AI tools were used to generate, analyze, or interpret data in this manuscript.

Abstract

Polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes (POEMS) syndrome is a rare plasma cell dyscrasia characterized by multisystem manifestations and markedly elevated vascular endothelial growth factor (VEGF) levels, identified as a sensitive biomarker for disease progression. Serum VEGF exceeds plasma levels, implicating platelets as a potential reservoir of VEGF in POEMS syndrome. However, the precise cellular origin of VEGF and the underlying pathological mechanisms remain elusive and challenging to elucidate. We conducted the first comprehensive multi-omics analysis of platelets from patients with POEMS syndrome, integrating RNA sequencing and quantitative mass spectrometry–based proteomics. RNA sequencing revealed a distinct platelet transcriptome enriched for platelet activation-related genes. Flow cytometry and electron microscopy confirmed a highly activated platelet status. Mass spectrometry revealed an altered platelet proteome with upregulated protein synthesis and abundant ribosomes, indicating enhanced protein synthesis pathway in POEMS syndrome. Importantly, we demonstrated that the megakaryocyte–platelet axis, rather than plasma cells, is the primary source of excess VEGF. Platelet VEGF levels were significantly higher in patients than in healthy donors ($P < 0.0001$). *VEGFA* mRNA in situ hybridization confirmed significantly increased *VEGFA* expression in bone marrow megakaryocytes from patients ($P < 0.01$). These findings redefine the cellular origin of VEGF in POEMS syndrome and highlight the role of the megakaryocyte–platelet axis in the disease biology.

Our study provides critical mechanistic insights into a long-standing mystery of this disorder, underscoring the need to further elucidate its functional interplay with clonal plasma cells.

Introduction

Polyneuropathy, organomegaly, endocrinopathy, monoclonal protein (M-protein), and skin changes (POEMS) syndrome is a rare plasma cell disorder characterized by elevated serum vascular endothelial growth factor (VEGF) levels correlating with the disease activity and unique symptoms, including Castleman disease, sclerotic bone lesions, extravascular volume overload, papilledema, and thrombocytosis[1,2]. Abnormal plasma cells and M-proteins are detected at low numbers in POEMS syndrome than in multiple myeloma (MM), a representative plasma cell neoplasm[3,4]. However, MM treatments, including lenalidomide, bortezomib, and autologous peripheral blood stem cell transplantation after high-dose melphalan, are also effective in POEMS syndrome[5-7]. Hyperproduction of several cytokines, including interleukin (IL)-1β, IL-6, IL-12, and VEGF, is involved in causing the diverse symptoms of POEMS syndrome. In particular, VEGF may play a crucial role in the pathogenesis of this syndrome through increased angiogenesis and vascular permeability[1,8]. In our previous studies, bulk and single-cell RNA sequencing of bone marrow plasma cells revealed that *VEGFA* and *VEGFB* gene expression levels were not significantly different between patients with POEMS syndrome, MM, monoclonal gammopathy of undetermined significance, and normal controls[9,10]. Further, serum VEGF levels are higher than plasma levels in POEMS syndrome[11]. Therefore, we hypothesized that platelets, rather than plasma cells, are the source of VEGF specific to POEMS syndrome. However, this has yet to be conclusively demonstrated, and no targeted research has been conducted on platelets or megakaryocytes in POEMS syndrome. Moreover, the underlying

pathological mechanisms of POEMS syndrome, particularly those associated with VEGF-producing cells, remain poorly understood.

Until recently, the nucleic acid content of platelets was solely identified based on their progenitor cells, megakaryocytes. However, it is now understood that megakaryocytes specifically sort RNA transcripts for incorporation into platelets. Hence, the platelet transcriptome does not entirely reflect its parental megakaryocyte profile[12]. Platelet proteome analysis revealed that only 70%–90% of the platelet transcriptome is represented[13]. Accordingly, in this study, we investigated the function of platelet and megakaryocyte and their role in the overproduction of VEGF and various other cytokines in POEMS syndrome by conducting the first integrated multi-omics analysis of platelets, combining RNA sequencing (RNA-seq) and mass spectrometry (MS).

Methods

The details of the experiments are described in Online Supplementary Methods.

Study participants

This study enrolled patients with newly diagnosed or relapsed POEMS syndrome who visited Chiba University Hospital from May 2019 to February 2025 (Online Supplementary Table S1). POEMS syndrome was diagnosed following the international diagnostic criteria[1]. Peripheral blood samples were collected from patients with POEMS and healthy donors (HDs) as controls. Bone marrow examinations were performed as a part of clinical practice, and bone marrow biopsy specimens from

patients with POEMS, malignant lymphoma without bone marrow involvement, and MM, a representative plasma cell disorder, were analyzed. This study was conducted adhering to the Declaration of Helsinki and approved by the ethics committee of the Graduate School of Medicine, Chiba University (Approval Nos. 1058 and 1233). Written informed consent was obtained from all participants.

RNA sequencing

RNA-seq was conducted on total RNA extracted from platelets isolated from patients with POEMS and HDs using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and miRNeasy Micro Kit (Qiagen, Hilden, Germany), as described previously[14]. The Quanti Fluor® RNA system (Promega Co., Madison, Wisconsin, USA) and the Agilent 4150 TapeStation system (Agilent Technologies, Santa Clara, CA, USA) were used to quantify the total RNA concentration and quality, respectively. The QuantSeq-Pool 3' Library Prep Kit (Lexogen, Wien, Austria) was used to prepare RNA-seq libraries, sequenced on the NextSeq 2000 platform (Illumina, Sandiego, CA, USA).

Mass spectrometry

Liquid chromatography–MS (LC–MS) was performed on total proteins recovered from an organic layer of phase-separated TRIzol lysates of platelets isolated from patients with POEMS and HDs. All protein samples were fragmented into peptides with Trypsin/Lys-C Mix (Promega Co.) and then

analyzed with nano LC–MS/MS employing the DIA method with the UltiMate 3000 RSLCnano system and Orbitrap Exploris 480 MS (Thermo Fisher Scientific).

mRNA in situ hybridization (ISH) and immunohistochemistry (IHC)

VEGFA mRNA ISH followed by CD42b IHC were performed on the bone marrow biopsy specimens from patients with POEMS with reference to previous studies on double staining for ISH and IHC[15,16] to assess *VEGFA* gene expression in megakaryocytes. Patients with malignant lymphoma without bone marrow involvement, such as normal controls and MM—a representative plasma cell disorder—were also investigated (Online Supplementary Table S2). We used RNAscope with Probe-Hs-VEGFA (Advanced Cell Diagnostics, Inc., Newark, CA, USA) for ISH staining, detecting a single mRNA molecule as a dot signal. CD42b IHC was performed using an anti-CD42b antibody (Clone: SP202, Abcam Limited, Cambridge, UK) and a secondary antibody labeled with horseradish peroxidase (HRP). Gene expression levels were semi-quantified from a score of 0 to 4 by counting the average number of dots per cell based on the scoring criteria proposed by Advanced Cell Diagnostics, Inc. (Online Supplementary Table S3).

Statistical analysis

All continuous variables were tested for normality using the Shapiro–Wilk test and analyzed with Welch's t-test or Mann–Whitney U test. Brown–Forsythe and Welch analysis of variance with Dunnett

post hoc test or Kruskal–Wallis with Dunn post hoc test was employed to analyze three or more groups. Pearson or Spearman correlation was used for correlation analyses. GraphPad Prism version 10.3.1 (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analyses. Significant *P*-values are presented as *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ with a two-tailed method.

Results

Platelet transcriptome is altered in POEMS syndrome

We performed RNA-seq on platelets isolated from patients with POEMS (n = 12) and HDs (n = 6) to investigate the features of platelets and megakaryocytes in POEMS syndrome. First, we confirmed that the samples were highly purified platelet preparations. In both patients with POEMS and HDs, the levels of *PTPRC*—gene encoding CD45, a leukocyte common antigen—were significantly lower than *ITGA2B*—encoding the αIIb subunit of integrin αIIbβ3, a transmembrane receptor expressed explicitly on platelets and megakaryocytes[17-19]—and *PF4*—gene encoding platelet factor 4, a platelet-specific protein stored in α-granules[20]—in platelets from all samples (Online Supplementary Figure S1A, B). *ITGA2B* was one of the most enriched transcripts in platelets from patients with POEMS (Figure 1A). Further, *ITGA2B* mRNA expression levels were significantly higher in patients with POEMS (Figure 1B), indicating upregulated *ITGA2B* gene transcription in megakaryocytes of patients with POEMS. Hence, thrombopoiesis in megakaryocytes of patients with POEMS is accelerated compared with that in HDs. We then focused on altered platelet transcripts between patients with POEMS and HDs (Figure

1C). In analyzing DEGs between patients with POEMS and HDs, 235 genes were upregulated and 224 genes were downregulated in patients with POEMS (Figure 1D, E).

Platelets are activated in patients with POEMS syndrome

Enrichment analysis of the RNA-seq data revealed that several gene sets involved in platelet activation, including response to increased platelet cytosolic Ca_2^+, platelet degranulation, platelet activation signaling and aggregation, and hemostasis, were enriched in platelets from patients with POEMS compared with HDs (Figure 2A, B). We then conducted a flow cytometry analysis of platelets to investigate the platelet activation status in POEMS syndrome. P-selectin expression levels on the platelet surface were significantly higher in patients with POEMS (n = 16) compared with HDs (n = 9), indicating that platelets in patients with POEMS are highly activated compared with HDs at both transcripts and protein levels (Figure 2C). Further, this finding was explored based on morphological observation using a TEM on fixed platelets isolated from patients with POEMS and HD. Platelets from HD were disc-shaped, whereas platelets from patients with POEMS were spherical with protruding filopodia, centralized α-granules and dense granules, and extended open canalicular system on the membrane, indicating that the platelets were in the activated state (Figure 2D). TEM observation and P-selectin flow cytometry testing revealed that platelets are activated in POEMS syndrome, supporting the results from RNA-Seq analysis. Furthermore, to assess whether platelet activation was associated with disease activity, we also investigated platelets from patients who had achieved remission, defined

as improvement in clinical symptoms and normalization or a marked decrease in serum VEGF levels, following plasma cell-directed therapy. P-selectin expression levels were significantly lower in patients in remission (n = 9) than in those with newly diagnosed or relapsed disease (n = 16, the same cohort shown in Figure 2C), suggesting a potential association between platelet activation and disease activity in POEMS syndrome (Online Supplementary Figure S2).

Platelet proteome is altered in POEMS syndrome

We then conducted MS analysis on platelets isolated from patients with POEMS (n = 3) and HDs (n = 3) to further investigate the features of platelets and megakaryocytes in POEMS syndrome. *PTPRC* levels were significantly lower than *ITGA2B* and *PF4* in platelets from all samples (Online Supplementary Figure S1C, D). MS analysis indicated that our method of isolating platelets was sufficient to purify platelets from whole blood samples, similar to RNA-seq. Patients with POEMS demonstrated distinct platelet proteome profiles compared with HDs, as evidenced by the identification of 137 upregulated proteins and 88 downregulated proteins in patients with POEMS (Figure 3A-C).

Upregulation of protein synthesis and abundant ribosomes in platelets of patients with POEMS syndrome

Enrichment analysis of MS data revealed that proteins involved in translation were enriched in platelets from patients with POEMS compared with HDs (Figure 4A, B). MS analysis revealed that proteins

that form ribosomes, including both large ribosomal subunits (RPLs), a component of the large 60S subunit of eukaryotic ribosomes, and small ribosomal subunits (RPSs), a component of the small 40S subunit[21], were enriched in platelets from patients with POEMS (Figure 4C-E). RNA-seq analysis revealed that the total RNA recovered from platelets isolated from the same volume of whole blood between all samples was significantly higher in patients with POEMS (Online Supplementary Figure S3A). This indicated that the increased total RNA content per platelet in POEMS as the median platelet count in the peripheral blood samples of 12 patients with POEMS included in the RNA-seq analysis was within the institution's standard values (Online Supplementary Table S1). Further, the number of mapped reads from total RNA was significantly lower in platelets from patients with POEMS compared with HDs, despite preparing the samples to the same extent (Online Supplementary Figure S3B). These observations indicate that rRNA levels may be relatively increased in platelets from patients with POEMS, which is further supported by MS analysis revealing an increase in ribosomal proteins in platelets from these patients. TEM morphological observation confirmed this finding. Platelets from patients with POEMS had abundant ribosomes attached to the endoplasmic reticulum (Figure 4F), indicating upregulated protein synthesis in the megakaryocyte–platelet axis compared with HDs.

We then focused on the discordance between RNA and protein expression levels in our RNA-seq analysis and MS data, as the continuous secretion of proteins to other tissues may cause the discordance[22]. Interestingly, enrichment analysis of MS data revealed that α-granules, platelet-specific

secretory granules containing chemokines and adhesion molecules, were significantly decreased in patients with POEMS compared with HDs (Online Supplementary Figure S4A). The analysis revealed that proteins implicated in immune responses, including cytokine production involved in immune response and leukocyte-mediated immunity, were significantly downregulated in patients with POEMS (Online Supplementary Figure S4B). In contrast, several gene sets related to them, such as the immune system and neutrophil degranulation, were enriched in the RNA-seq analysis (Figure 2A). This indicates that secreted proteins, such as chemokines and adhesion molecules, may be continuously released from platelets because of platelet activation and increased degranulation involved in the pathogenesis of POEMS syndrome.

VEGF and IL-8 accumulation is higher in platelets from patients with POEMS syndrome than in HDs

We next performed ELISA for VEGF on platelet protein lysates from patients (n = 18) and HDs (n = 6) to investigate the accumulation of VEGF as well as several other cytokines in platelets in POEMS syndrome. In each sample, the VEGF concentration identified using ELISA was corrected for the total protein concentration, considering differences in the number of isolated platelets between samples. A significant positive correlation was found between the total protein content extracted from isolated platelets and the platelet count (Online Supplementary Figure S5A). Platelet VEGF levels were significantly higher in patients with POEMS than in HDs (Figure 5A). Further, we observed that

platelet VEGF levels were correlated with serum VEGF levels (Figure 5B), but not with peripheral blood platelet count (Online Supplementary Figure S5B), indicating that VEGF accumulates more in platelets in POEMS syndrome. To assess whether platelet VEGF levels were associated with disease activity, we also investigated platelets from patients who had achieved remission. Platelet VEGF levels were significantly lower in patients in remission (n = 12) than in those with newly diagnosed or relapsed disease (n = 18, the same cohort shown in Figure 5A), suggesting a potential association between platelet VEGF accumulation and disease activity in POEMS syndrome (Online Supplementary Figure S5C). We then conducted a multiplex cytokine assay on platelet protein lysates from patients with POEMS (n = 17) and HDs (n = 7). Cytokines to be measured included IL-1β, IL-6, IL-8, IL-12p70, macrophage inflammatory protein-1α (MIP-1α), tumor necrosis factor alpha (TNF-α), and VEGF-A, which have been reported to be higher in the serum of patients with POEMS[1,8]. VEGF-A levels were significantly higher in platelets from patients than in HDs (Figure 5C). Further, significantly higher IL-8 levels were observed in patients' platelets. However, no significant differences in the platelet levels of IL-6 and TNF-α were observed between the two groups, and IL-1β, IL-12p70, and MIP-1α were challenging to assess, as their concentrations were below the lower detection limit. These results indicate that the hyperproduction of several cytokines in POEMS syndrome cannot be solely attributed to platelets.

VEGFA gene expression levels in megakaryocytes are higher in patients with POEMS syndrome than in controls

VEGF is abundant in platelets in POEMS syndrome; thus, we assessed VEGF production in bone marrow megakaryocytes by double staining for *VEGFA* mRNA ISH and CD42b IHC on the bone marrow biopsy specimens from patients with POEMS. Megakaryocytes positive for ISH, which means the stainability of a score of 1–4 in the scoring criteria, were observed in samples from patients with POEMS but not in normal controls (Figure 6A). *VEGFA* gene expression levels in megakaryocytes were significantly higher in patients with POEMS than in normal controls and those with MM, a representative plasma cell neoplasm (Figure 6B). Further, the number of megakaryocytes in POEMS observed in the bone marrow biopsy specimen was increased than that in normal controls but was not significantly different from patients with MM (Figure 6C), indicating that both *VEGFA* gene expression per megakaryocyte and the number of megakaryocytes are increased in POEMS syndrome compared with normal controls. Furthermore, the comparison of patients with POEMS and essential thrombocythemia, a myeloproliferative disorder with increased megakaryocytes, revealed increased *VEGFA* gene expression per megakaryocyte in POEMS syndrome (Online Supplementary Figure S6A, B). These results indicate that the megakaryocyte–platelet axis, rather than plasma cells, is one of the sources of VEGF specific to POEMS syndrome.

Discussion

This study identifies several key features and functional abnormalities of platelets and megakaryocytes in the pathogenesis of POEMS syndrome. Furthermore, this work represents the first integrated multi-omics analysis of platelets in patients with POEMS syndrome, combining RNA-seq and MS analysis.

Our RNA-seq data indicated that platelet transcriptome profiles are altered in POEMS syndrome. In particular, *ITGA2B*, which encodes a platelet- and megakaryocyte-specific integrin subunit[17-19], is expressed at significantly higher levels in platelets from patients with POEMS than in HDs. This alteration in the platelet transcriptome profiles is significant as it indicates upregulated *ITGA2B* gene transcription in megakaryocytes and accelerated thrombopoiesis in patients with POEMS compared with HDs, which is consistent with the fact that thrombocytosis is one of the clinical features of POEMS syndrome[1,23].

Furthermore, we observed that transcripts involved in platelet activation are enriched in platelets from patients with POEMS. Platelets are well-known to play the most crucial role in primary hemostasis. Platelets bind to von Willebrand factors and fibrinogens in the plasma via integrins on the cell membrane during vascular injury, leading to adhesion to collagen fibers beneath the endothelial cells and platelet aggregation[24,25]. It is well recognized that patients with POEMS syndrome have a high incidence of both venous and arterial thrombotic complications[23,26]. The platelet activation identified in our study, in combination with thrombocytosis, may contribute to a prothrombotic state, potentially increasing the risk of thrombosis in these patients. Recent studies reveal that platelets play a crucial role in immune responses by promoting the secretion of inflammatory cytokines in monocytes

and other leukocytes and neutrophil migration[27-32]. Platelet activation is mediated by the intracellular calcium signaling in response to external stimuli, such as intravascular wall shear stress and the binding of mediators from leukocytes and vascular endothelial cells to platelets[33,34]. Several molecules stored in α-granules and dense granules inside the cells degranulate or are expressed on the membrane, of which P-selectin (CD62P) from α-granules can be detected by ELISA or flow cytometry, and morphological changes occur in platelets when activated[35,36]. Our study revealed that platelet transcripts involved in platelet activation are enriched and that P-selectin expression levels on the platelet surface are significantly higher in patients with POEMS than in HDs. Further, TEM observation revealed that α-granules were clustered at the center of platelets from patients with POEMS. RNA-seq analysis revealed enriched genes involved in platelet degranulation, whereas the number of α-granules in platelets was significantly lower in patients than in HDs. These results indicate that platelets are highly activated, thereby improving degranulation, and that transcription of genes associated with platelet activation is increased in megakaryocytes in POEMS syndrome.

Our MS analysis revealed altered platelet proteome profiles and upregulation of proteins involved in translation and forming ribosomes in platelets from patients with POEMS. TEM morphological observation confirmed that platelets from patients with POEMS had abundant ribosomes attached to the endoplasmic reticulum, and RNA-seq analysis indicated an increase in platelet rRNA levels, both supporting the results of MS analysis. Notably, previous studies have demonstrated that platelets synthesize proteins using mRNA and ribosomes derived from

megakaryocytes and platelet activation improves their function[37,38]. The present study supports these previous findings by presenting compelling evidence from patients with POEMS syndrome: (i) platelets are activated, (ii) protein synthesis is markedly upregulated, and (iii) ribosomes within the platelets are notably increased, highlighting a robust improvement in their protein production machinery.

Serum VEGF levels are higher than plasma levels in POEMS syndrome[11], and we hypothesized that that platelets are the source of VEGF specific to POEMS syndrome. However, this has yet to be conclusively demonstrated. This study revealed that VEGF accumulates abundantly in platelets and that megakaryocytes are one of the VEGF-producing cells in POEMS syndrome. In patients with POEMS, platelets are activated, indicating that stored VEGF in platelets is released into the blood circulation by platelet activation. In addition, serum VEGF levels were significantly correlated with platelet VEGF levels, supporting the evidence that platelets are the primary source of VEGF specific to POEMS. Conversely, we observed other cells with *VEGFA* mRNA expression in addition to megakaryocytes on the bone marrow specimens from patients with POEMS in the double staining study for ISH and IHC (data not shown). Moreover, we could not detect the accumulation of cytokines other than VEGF, except for IL-8, in platelets from patients with POEMS. As previously mentioned, platelets play a crucial role in immune responses by stimulating cytokine production in monocytes and other leukocytes by releasing chemokines and adhesion molecules stored in intracellular granules when activated[27,31]. Interestingly, our study revealed the downregulated immune

response-related proteome in patients with POEMS, whereas the corresponding transcripts were upregulated. This indicates that secreted proteins, including chemokines and adhesion molecules, may be continuously released and stimulate leukocytes to produce the cytokines implicated in the pathogenesis of POEMS syndrome, which is consistent with a previous report stating that discordance between RNA and protein expression levels could be caused by the continuous secretion of proteins to other tissues[22]. Further research is warranted to identify the involvement of cells other than megakaryocytes in producing disease-specific VEGF and several other cytokines and whether and how platelets and megakaryocytes interact with them in POEMS syndrome.

There have been conflicting reports regarding the primary source of VEGF in POEMS syndrome. Wang et al. demonstrated marked expression of VEGF in bone marrow plasma cells of patients with POEMS syndrome[39]. In contrast, in our bulk and single-cell RNA sequencing analyses, we consistently observed no significant elevation or selective upregulation of *VEGFA* transcript levels in bone marrow plasma cells from patients with POEMS syndrome compared to disease or controls[9,10]. Based on these robust findings, we maintain that bone marrow plasma cells are unlikely to be the primary or specific source of elevated VEGF in POEMS syndrome. Nevertheless, clonal plasma cells could still act as an initial trigger or minor source that secondarily activates megakaryocytes, and further research is warranted to clarify the precise mechanisms by which abnormal plasma cells influence the megakaryocyte–platelet axis.

This study has several limitations. First, due to the rarity of POEMS syndrome, the sample size was relatively small, and all samples were obtained from a single institution. Second, functional validation evaluating VEGF secretion following platelet activation or inhibition was not performed in this study due to the limited availability of clinical samples; further functional studies are warranted to directly confirm this mechanism. Finally, although our findings demonstrated altered platelet and megakaryocyte function in POEMS syndrome, POEMS syndrome remains fundamentally a plasma cell disorder in which plasma cell-targeted therapies are clinically effective[5-7]. Therefore, the functional crosstalk between clonal plasma cells and the megakaryocyte–platelet axis needs to be fully elucidated in future studies.

In conclusion, this study demonstrates that platelets and megakaryocytes play a pivotal role in the pathogenesis of POEMS syndrome. Our findings provide novel insights that resolve a long-standing mystery of this paraneoplastic disorder, underscoring the need to further elucidate its functional interplay with clonal plasma cells.

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Figure Legends

Figure 1. Patients with POEMS syndrome have different transcriptome profiles from healthy donors.

RNA-seq was performed on platelets isolated from patients with POEMS (n = 12) and healthy donors (HDs, n = 6). (A) The MA plot was created using the ggpubr package (https://rpkgs.datanovia.com/ggpubr/), with red indicating upregulated and blue indicating downregulated transcripts in patients with POEMS, respectively. *ITGA2B* was one of the most enriched transcripts in patients with POEMS. (B) *ITGA2B* mRNA expression levels in platelets, corrected for variance stabilizing normalization, were significantly higher in patients with POEMS than in HDs. (C-E) Principal component analysis of normalized gene expression levels (C), Heatmap (D), and volcano plot (E) of DEGs (adjusted-$P < 0.05$, log2 fold-change > 1). Red and blue in the heatmap indicate relatively increased and decreased gene expression levels, respectively. The volcano plot illustrates increased (red), decreased (blue), and not significantly changed (black) genes in platelets from patients with POEMS. ****, $P < 0.0001$.

Figure 2. Platelets are activated in POEMS syndrome.

(A, B) Gene enrichment analysis using pathway analysis (A) and gene set enrichment analysis (GSEA) (B) based on the Reactome database of transcripts from RNA-seq (POEMS, n = 12; healthy donors (HDs), n = 6). Top-ranked pathways with adjusted *P*-values of <0.05 are listed in the pathway analysis. (C) Flow cytometry for P-selectin on the platelet surface using peripheral blood samples from patients

with POEMS (n = 16) and HDs (n = 9). P-selectin expression levels were assessed as the percentage of platelets expressing P-selectin out of all platelets analyzed and were compared between patients with POEMS and HDs. (D) Representative transmission electron microscope (TEM) images of platelets isolated from patients with POEMS (n = 3) and an HD (n = 1); scale bars indicate 2 μm (left) and 0.5 μm (right). **, $P < 0.01$.

Figure 3. Patients with POEMS syndrome have different proteome profiles from healthy donors.

Mass spectrometry (MS) analysis of platelets isolated from patients with POEMS (n = 3) and healthy donors (HDs) (n = 3). (A-C) Principal component analysis of normalized protein expression levels (A), Heatmap (B), and volcano plot (C) of DEGs, meaning protein-coding genes (adjusted-$P < 0.05$, log2 fold-change > 1). Red and blue in the heatmap indicate relatively increased and decreased gene expression levels. The volcano plot illustrates increased (red), decreased (blue), and not significantly changed (black) genes in platelets from patients with POEMS.

Figure 4. Upregulation of protein synthesis and abundant ribosomes in the platelets of patients with POEMS syndrome.

(A) Gene Ontology analysis of protein-coding genes from MS data focused on the biological process. Top-ranked cellular characteristics with adjusted *P*-values of <0.05 are listed in the analysis. (B) GSEA based on Gene Ontology database of proteome from MS. **(**C) Gene Ontology analysis of protein-

coding genes from MS data focused on cellular component. Top-ranked cellular characteristics with adjusted *P*-values of <0.05 are listed in the analysis. (D) Gene set enrichment analysis (GSEA) based on the Gene Ontology database of the proteome. (E) Enhanced volcano highlighting an increase in ribosomal proteins in patients with POEMS from MS data. Red in the volcano plot indicates significantly increased genes in patients with POEMS (right) and HDs (left) with adjusted *P*-values of <0.05 and log2 fold-change of >1, and gray indicates genes not significantly changed between the two groups. (F) Representative transmission electron microscope (TEM) images of ribosomes attached to the endoplasmic reticulum in platelets isolated from patients with POEMS (n = 3); scale bars indicate 0.5 μm (left), 0.2 μm (middle), and 0.05 μm (right); RPS: ribosomal protein S (small); RPL: ribosomal protein L (large).

Figure 5. VEGF and IL-8 accumulate at high levels in the platelets of patients with POEMS syndrome.

(A) VEGF levels in platelets isolated from patients with POEMS (n = 18) and HDs (n = 6). Platelet VEGF levels were defined as the VEGF concentration in the platelet protein lysate measured by ELISA, normalized to the platelet total protein concentration determined using the BCA assay. (B) Correlation between platelet VEGF levels measured by ELISA and serum VEGF levels in patients with POEMS (n = 18). (C) IL-6, IL-8, TNF-α, and VEGF-A levels in platelets isolated from patients with POEMS (n = 17) and HDs (n = 7), measured using a cytokine assay. The concentration of each cytokine in the

platelet protein lysate determined by the cytokine assay was normalized to the platelet total protein concentration, as described for ELISA. ****, $P < 0.0001$.

Figure 6. *VEGFA* gene expression levels in megakaryocytes are increased in POEMS syndrome.

Double staining for *VEGFA* mRNA in situ hybridization (ISH) and CD42b immunohistochemistry (IHC) on the bone marrow biopsy specimens from patients with POEMS (n = 16), malignant lymphoma without bone marrow involvement as normal controls (n = 7), and multiple myeloma (MM) (n = 5). (A) Representative optical microscopic images of megakaryocytes at 100× magnification. Megakaryocytes have brown cytoplasm stained with HRP/DAB and blue nuclei stained with hematoxylin by IHC. A red dot indicates one molecule of mRNA stained by ISH. *VEGFA* gene expression level per megakaryocyte was semi-quantified to a score of 0 (left, control) and 3 (right, patient with POEMS), respectively, by the scoring system described in the Methods section. (B) *VEGFA* gene expression levels in megakaryocytes in patients with POEMS, MM, and normal controls. (C) Counts of megakaryocytes observed in each specimen in the three groups. HRP, horseradish peroxidase; DAB, diaminobenzidine.

**, $P < 0.01$; *, $P < 0.05$.

Figure 1

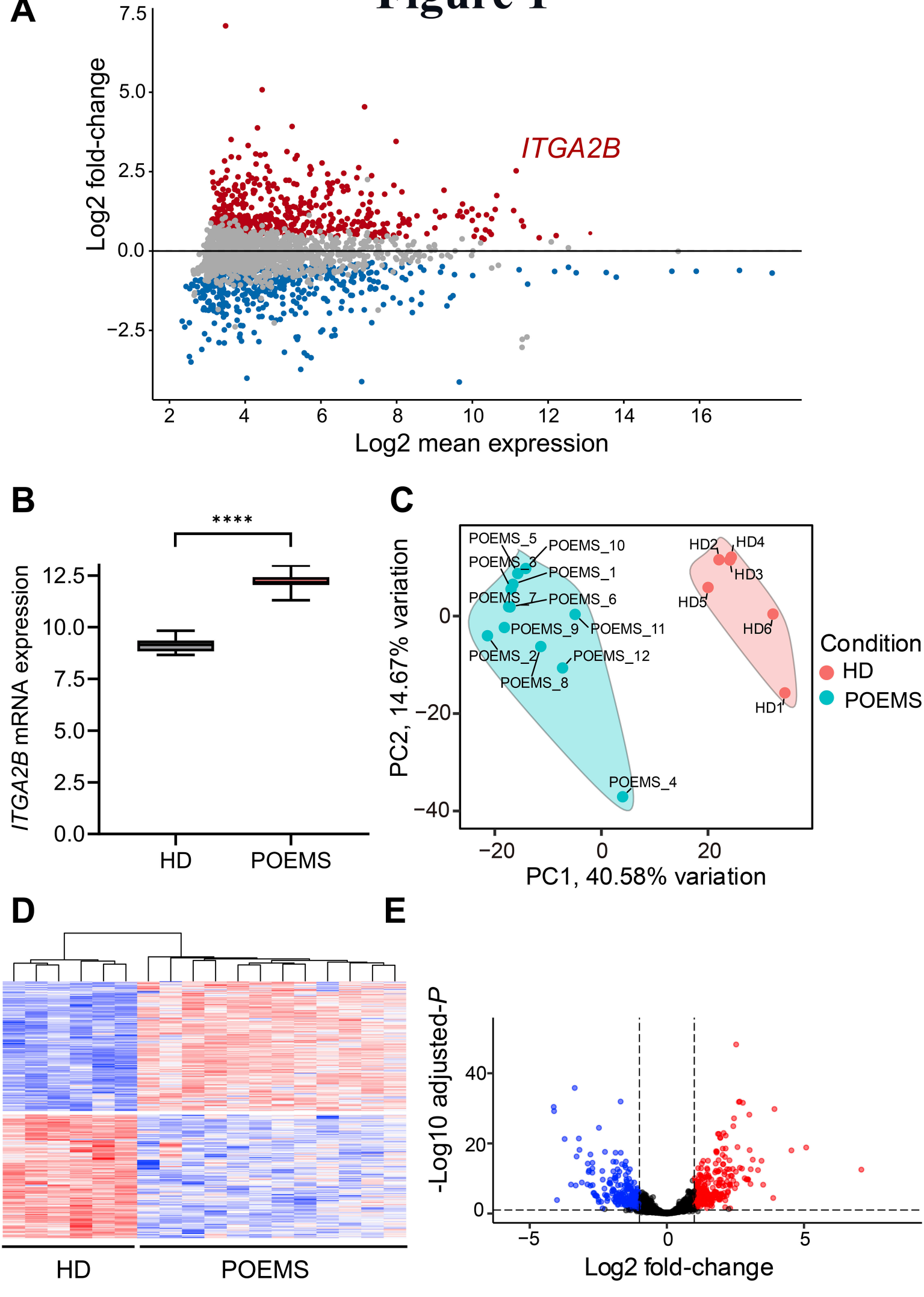

A
Log2 fold-change
Log2 mean expression
ITGA2B
B
ITGA2B mRNA expression
****
HD
POEMS
C
PC2, 14.67% variation
PC1, 40.58% variation
POEMS_5
POEMS_10
POEMS_3
POEMS_1
POEMS_7
POEMS_6
POEMS_9
POEMS_11
POEMS_2
POEMS_12
POEMS_8
POEMS_4
HD2
HD4
HD3
HD5
HD6
HD1
Condition
HD
POEMS
D
HD
POEMS
E
-Log10 adjusted-P
Log2 fold-change

Figure 2

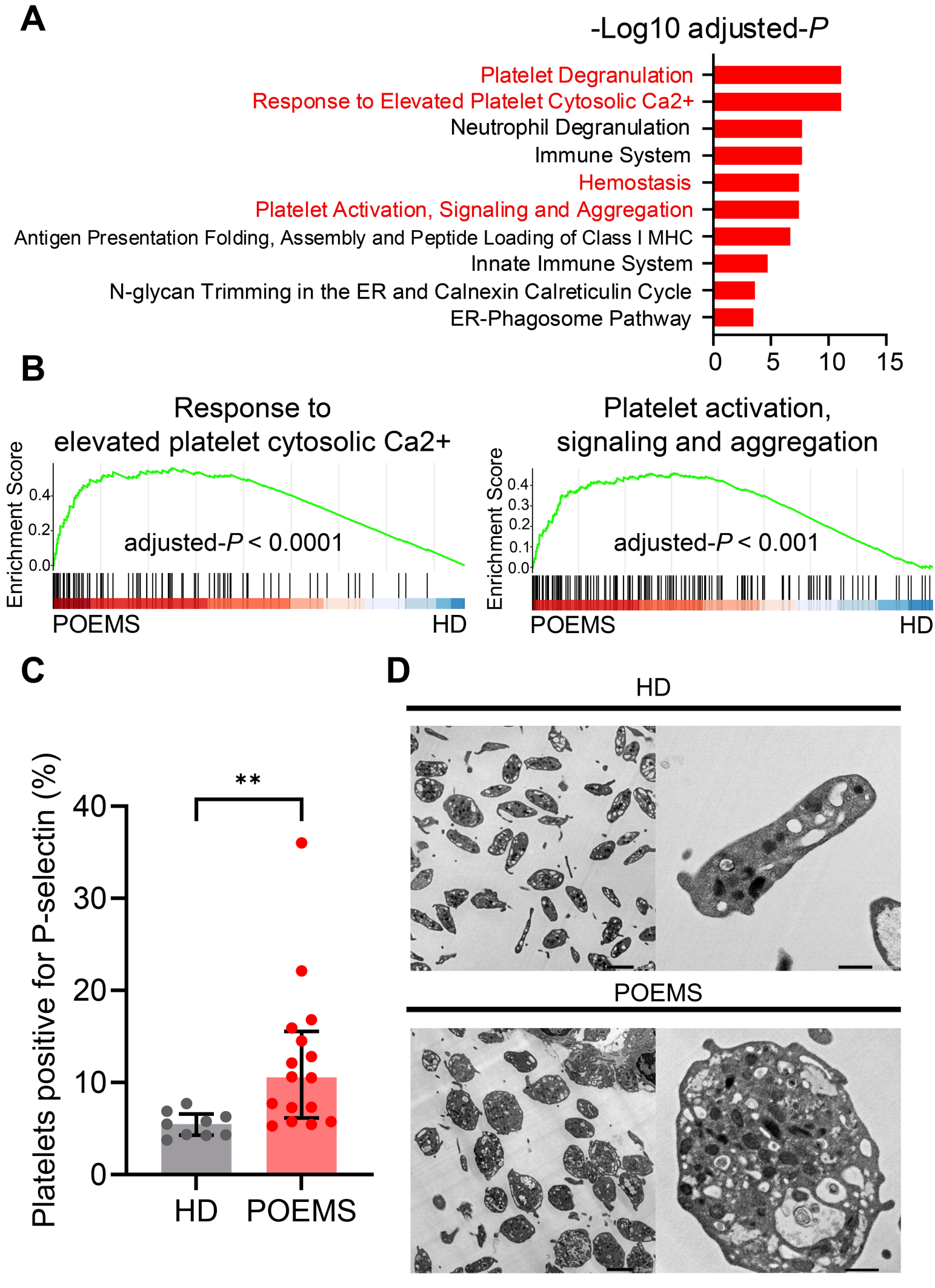

A
-Log10 adjusted-P
Platelet Degranulation
Response to Elevated Platelet Cytosolic Ca2+
Neutrophil Degranulation
Immune System
Hemostasis
Platelet Activation, Signaling and Aggregation
Antigen Presentation Folding, Assembly and Peptide Loading of Class I MHC
Innate Immune System
N-glycan Trimming in the ER and Calnexin Calreticulin Cycle
ER-Phagosome Pathway
0
5
10
15
B
Response to elevated platelet cytosolic Ca2+
Enrichment Score
0.4
0.2
0.0
adjusted-P < 0.0001
POEMS
HD
Platelet activation, signaling and aggregation
Enrichment Score
0.4
0.3
0.2
0.1
0.0
adjusted-P < 0.001
POEMS
HD
C
Platelets positive for P-selectin (%)
**
40
30
20
10
0
HD
POEMS
D
HD
POEMS

Figure 3

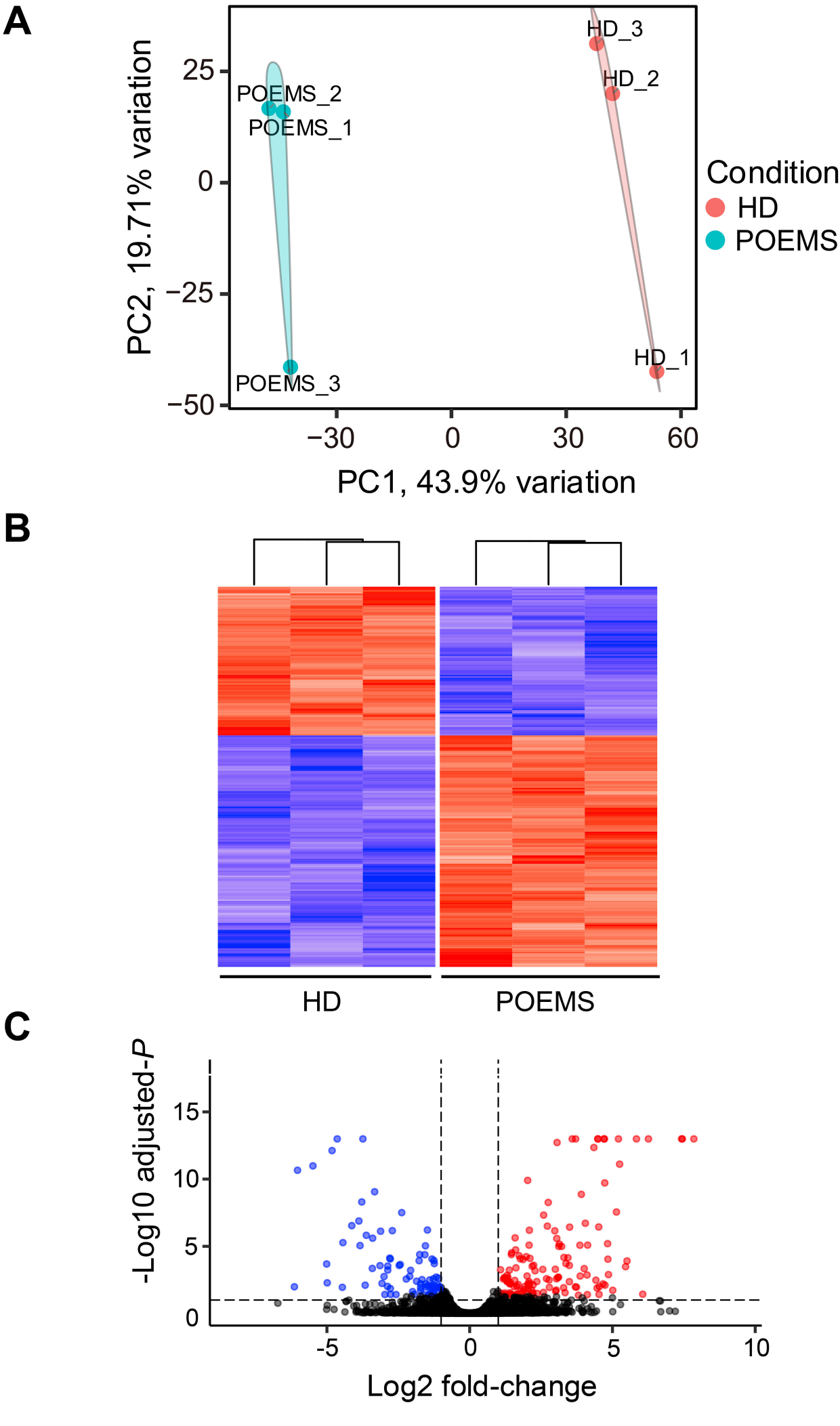

A
HD_3
HD_2
POEMS_2
POEMS_1
HD_1
POEMS_3
25
0
−25
−50
−30
0
30
60
PC2, 19.71% variation
PC1, 43.9% variation
Condition
HD
POEMS
B
HD
POEMS
C
-Log10 adjusted-P
15
10
5
0
-5
0
5
10
Log2 fold-change

Figure 4

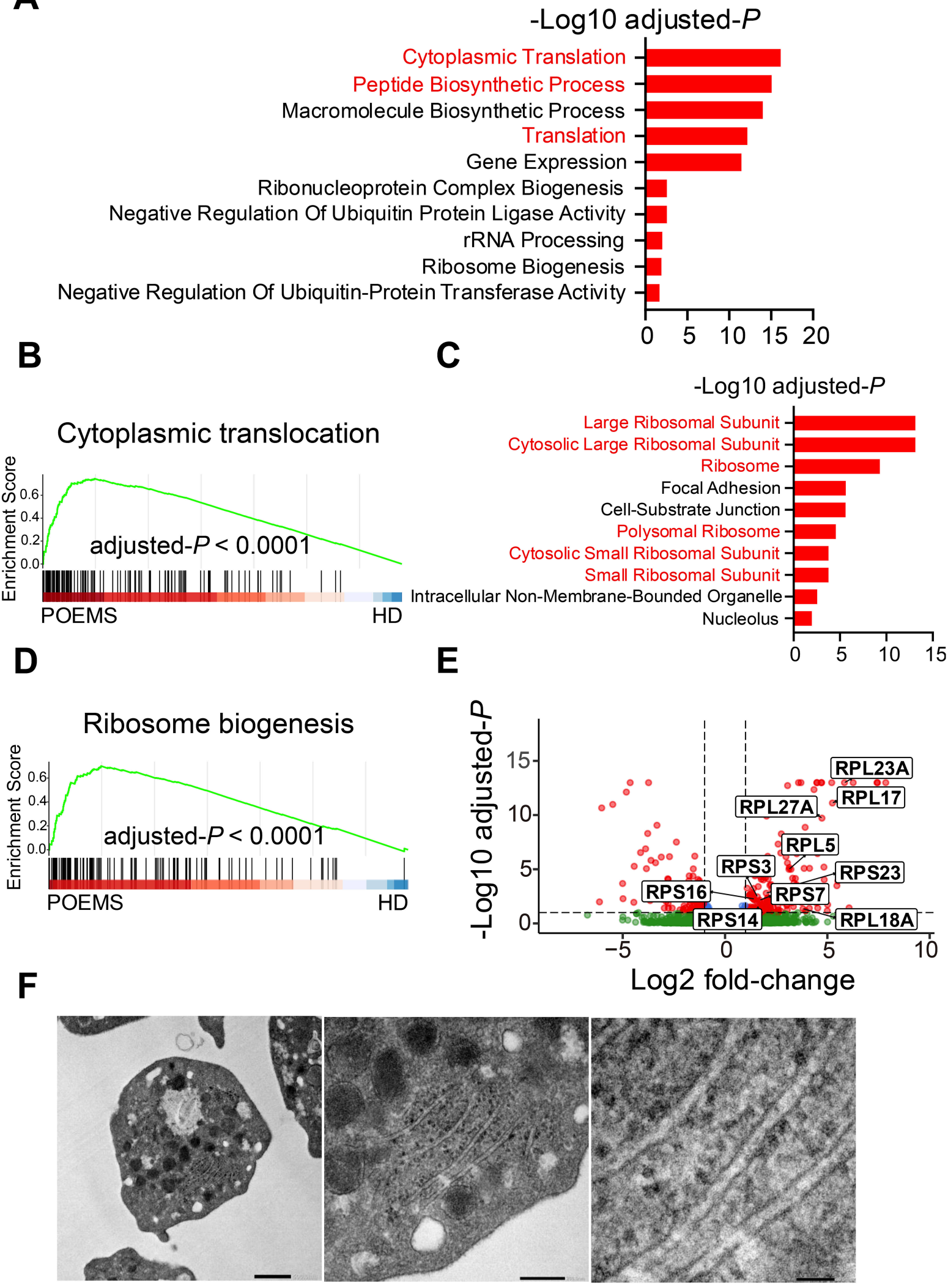

A
-Log10 adjusted-P
Cytoplasmic Translation
Peptide Biosynthetic Process
Macromolecule Biosynthetic Process
Translation
Gene Expression
Ribonucleoprotein Complex Biogenesis
Negative Regulation Of Ubiquitin Protein Ligase Activity
rRNA Processing
Ribosome Biogenesis
Negative Regulation Of Ubiquitin-Protein Transferase Activity
0 5 10 15 20
B
Cytoplasmic translocation
Enrichment Score
0.6
0.4
0.2
0.0
adjusted-P < 0.0001
POEMS
HD
C
-Log10 adjusted-P
Large Ribosomal Subunit
Cytosolic Large Ribosomal Subunit
Ribosome
Focal Adhesion
Cell-Substrate Junction
Polysomal Ribosome
Cytosolic Small Ribosomal Subunit
Small Ribosomal Subunit
Intracellular Non-Membrane-Bounded Organelle
Nucleolus
0 5 10 15
D
Ribosome biogenesis
Enrichment Score
0.6
0.4
0.2
0.0
adjusted-P < 0.0001
POEMS
HD
E
-Log10 adjusted-P
15
10
5
0
RPL23A
RPL17
RPL27A
RPL5
RPS3
RPS23
RPS16
RPS7
RPS14
RPL18A
−5 0 5 10
Log2 fold-change
F

Figure 5

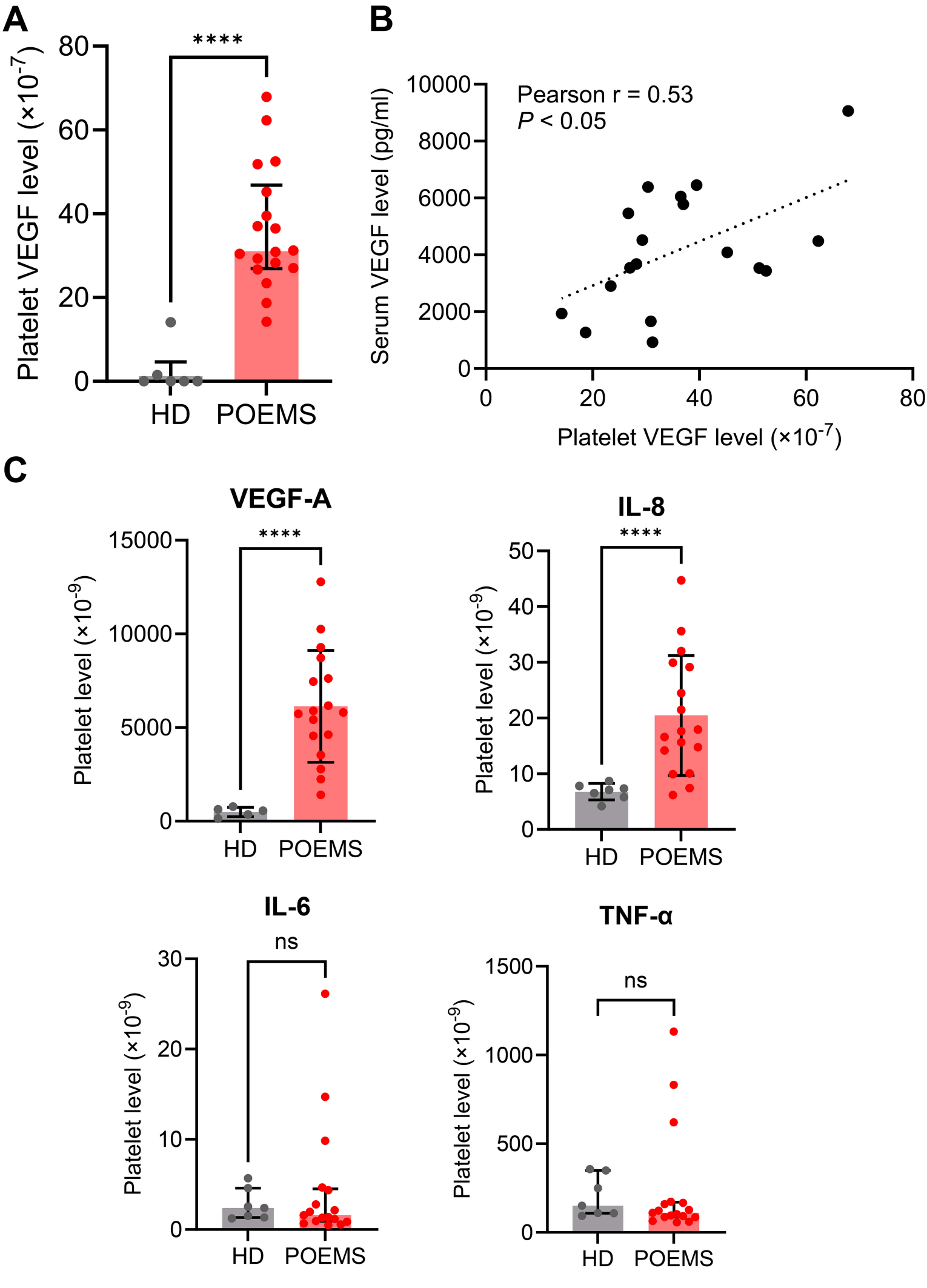
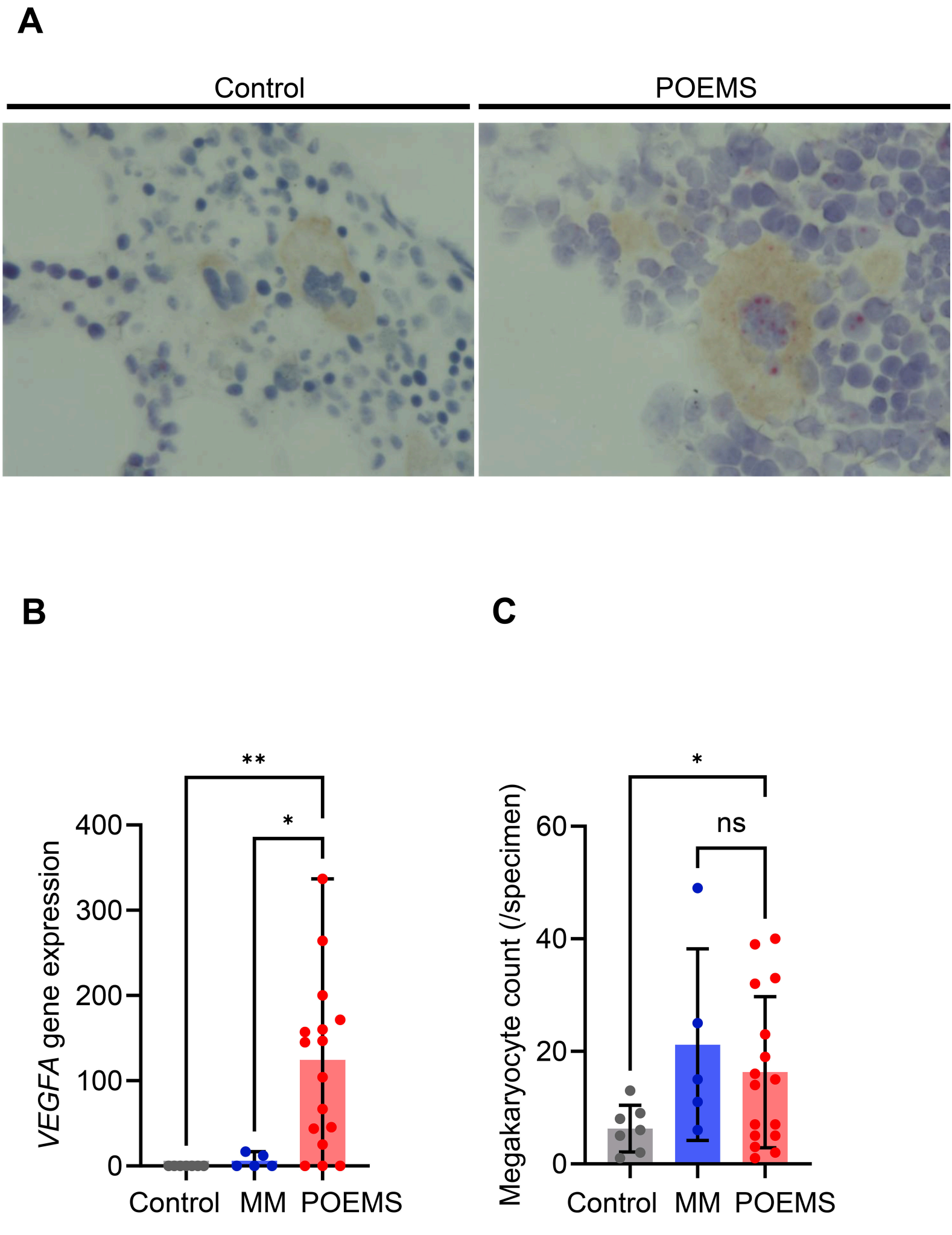

A
Platelet VEGF level (×10-7)
80
60
40
20
0
****
HD
POEMS
B
Serum VEGF level (pg/ml)
10000
8000
6000
4000
2000
0
Pearson r = 0.53
P < 0.05
0
20
40
60
80
Platelet VEGF level (×10-7)
C
VEGF-A
Platelet level (×10-9)
15000
10000
5000
0
****
HD
POEMS
IL-8
Platelet level (×10-9)
50
40
30
20
10
0
****
HD
POEMS
IL-6
Platelet level (×10-9)
30
20
10
0
ns
HD
POEMS
TNF-α
Platelet level (×10-9)
1500
1000
500
0
ns
HD
POEMS

Figure 6


A
Control
POEMS
B
**
*
400
300
200
100
0
VEGFA gene expression
Control
MM
POEMS
C
*
ns
60
40
20
0
Megakaryocyte count (/specimen)
Control
MM
POEMS

Supplementary Methods

Isolation of platelets

Platelets were isolated and purified as previously described[1-3]. Platelet-rich plasma (PRP) was obtained from 10 mL of EDTA-2Na-anticoagulated whole blood of patients with POEMS and HDs by centrifugation at 200 ×g for 20 min, followed by spinning at 100 ×g for 5 min to pellet contaminating red and white blood cells. Platelets were isolated as pellets from PRP by centrifugation at 1000 ×g for 10 min and washed with Tyrode's Salts ($CaCl_2 \cdot H_2O$ 0.265 g/L; $MgCl_2 \cdot 6H_2O$ 0.214 g/L; KCl 0.2 g/L; $NaHCO_3$ 1.0 g/L; NaCl 8.0 g/L; NaH_2PO_4 [anhydrous] 0.05 g/L; D-Glucose 1.0 g/L). All centrifuge processes were performed with the addition of prostaglandin I2 (0.5 μM) to prevent platelet activation.

RNA sequencing data analysis

RNA-seq data were aligned to the human reference genome sequence (hg38), analyzed utilizing Lexogen tools (https://github.com/Lexogen-Tools/quantseqpool_analysis), and converted into gene expression values according to the number of reads per million mapped reads. Principal component analysis (PCA) was conducted using PCAtools (DOI: 10.18129/B9.bioc.PCAtools) based on normalized expression levels. A log2 fold-change threshold of 1 and an FDR-adjusted P-value threshold of 0.05 were used to identify differentially expressed genes (DEGs). To identify significantly enriched pathways in platelets in POEMS syndrome, Reactome (https://reactome.org/) and the clusterProfiler R package (DOI: 10.18129/B9.bioc.clusterProfiler) were used for pathway analysis of

upregulated DEGs and gene set enrichment analysis (GSEA), respectively. In these analyses, pathways with an FDR threshold of 0.05 indicated significance. DEGs in the heatmap clustering analysis were analyzed using the DESeq2 R package (DOI: 10.18129/B9.bioc.DESeq2) and visualized utilizing the pheatmap R package (https://github.com/raivokolde/pheatmap). The EnhancedVolcano R package (https://github.com/kevinblighe/EnhancedVolcano) with the same threshold as the DEGs was used to produce a volcano plot.

Flow cytometry analysis

Whole blood flow cytometry was performed to assess the platelet activation status. Peripheral blood samples were collected in ACD-anticoagulated tubes from patients with POEMS and HDs, and flow cytometry testing for P-selectin (CD62P) expression on the surface of platelets was conducted within 30 min of blood collection. APC anti-CD61 antibody (Clone: VI-PL2, BioLegend, San Diego, CA, USA), PE anti-CD62P antibody (Clone: AK4, BD Biosciences, Franklin Lakes, New Jersey, USA), and isotype controls combined with each fluorescent dye were incubated with blood samples, and flow cytometric analysis was conducted using Navios EX flow cytometer (Beckman Coulter, Indianapolis, IN, USA). FlowJo software (BD Biosciences) was used to analyze the resulting data. P-selectin expression levels were evaluated as the percentage of platelets expressing P-selectin from all platelets analyzed.

Transmission electron microscopy

Platelets isolated from patients with POEMS and HDs were respectively fixed with 2% glutaraldehyde in 0.1 μmol/L of phosphate buffer (pH 7.4) overnight, and post-fixed in 2% osmium tetroxide for 2 h at 4℃. Samples were dehydrated in a graded ethanol and embedded in the epoxy resin. Ultrathin sections were obtained employing the ultramicrotome technique and stained with uranyl acetate for 15 min and lead staining solution for 2 min. Preparation of thin-section specimens and sample observation using a transmission electron microscope (TEM) with the JEOL JEM-1400Flash was performed by Hanaichi UltraStructure Research Institute, Co., Ltd. (Aichi, Japan).

Mass spectrometry data analysis

The resulting MS data were reanalyzed using DIA-NN software (https://github.com/vdemichev/DiaNN), and proteins with a threshold of 0.01 for Precursor FDR and Protein FDR were quantified. The DEP R package (10.18129/B9.bioc.DEP) with a log2 fold-change threshold of 1 and an FDR-adjusted P-value threshold of 0.05 was used for differential expression analysis. The ComplexHeatmap R package (https://github.com/jokergoo/ComplexHeatmap) was used for hierarchical clustering analysis based on the result of differential expression analysis, and the EnhancedVolcano R package (10.18129/B9.bioc.EnhancedVolcano) was utilized to produce the volcano plot. The clusterProfiler R package with the same methodology described for RNA-seq was used for Gene Ontology analysis and GSEA.

ELISA for VEGF

Protein lysates were prepared from isolated platelets from patients with POEMS and HDs using RIPA buffer (50 mmol/L Tris-HCl, pH 8.0, 150 mmol/L sodium chloride, 0.5 wt/vol% sodium deoxycholate, 0.1 wt/vol% sodium dodecyl sulfate, 1.0 wt/vol% NP-40 substitute). The total protein concentration of each sample was identified using the BCA Protein Assay Kit (Thermo Fisher Scientific). VEGF levels in the platelet protein lysates were measured utilizing ELISA using DuoSet ELISA (R&D Systems, Inc., Minneapolis, MN, USA) and then corrected for the total protein concentration of each sample, considering differences in the number of isolated platelets between samples.

Cytokine assay

A MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A kit with Luminex 200 Instrument System (Merck KGaA, Darmstadt, Germany) was used to identify the multiple cytokine levels in the platelet protein lysate from patients with POEMS and HDs. Each cytokine level was corrected for the total protein concentration as in ELISA.

Evaluation of mRNA in situ hybridization (ISH) and immunohistochemistry (IHC) results

VEGFA gene expression levels in megakaryocytes from patients with POEMS, those with MM, and normal controls were assessed using this scoring criterion and the IHC staining assessment method using the H-score[4]. The score was calculated based on the following formula as *VEGFA* gene

expression level for each megakaryocyte observed in the specimen of each sample: [1 × (% of score 1 cells) + 2 × (% of score 2 cells) + 3 × (% of score 3 cells) + 4 × (% of score 4 cells)]. Observations were conducted using the BZ-X700 microscope (Keyence, Osaka, Japan) at 100× magnification.

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3. Campbell RA, Franks Z, Bhatnagar A, et al. Granzyme A in Human Platelets Regulates the Synthesis of Proinflammatory Cytokines by Monocytes in Aging. *J Immunol.* 2018;200(1):295-304.
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Figure S1

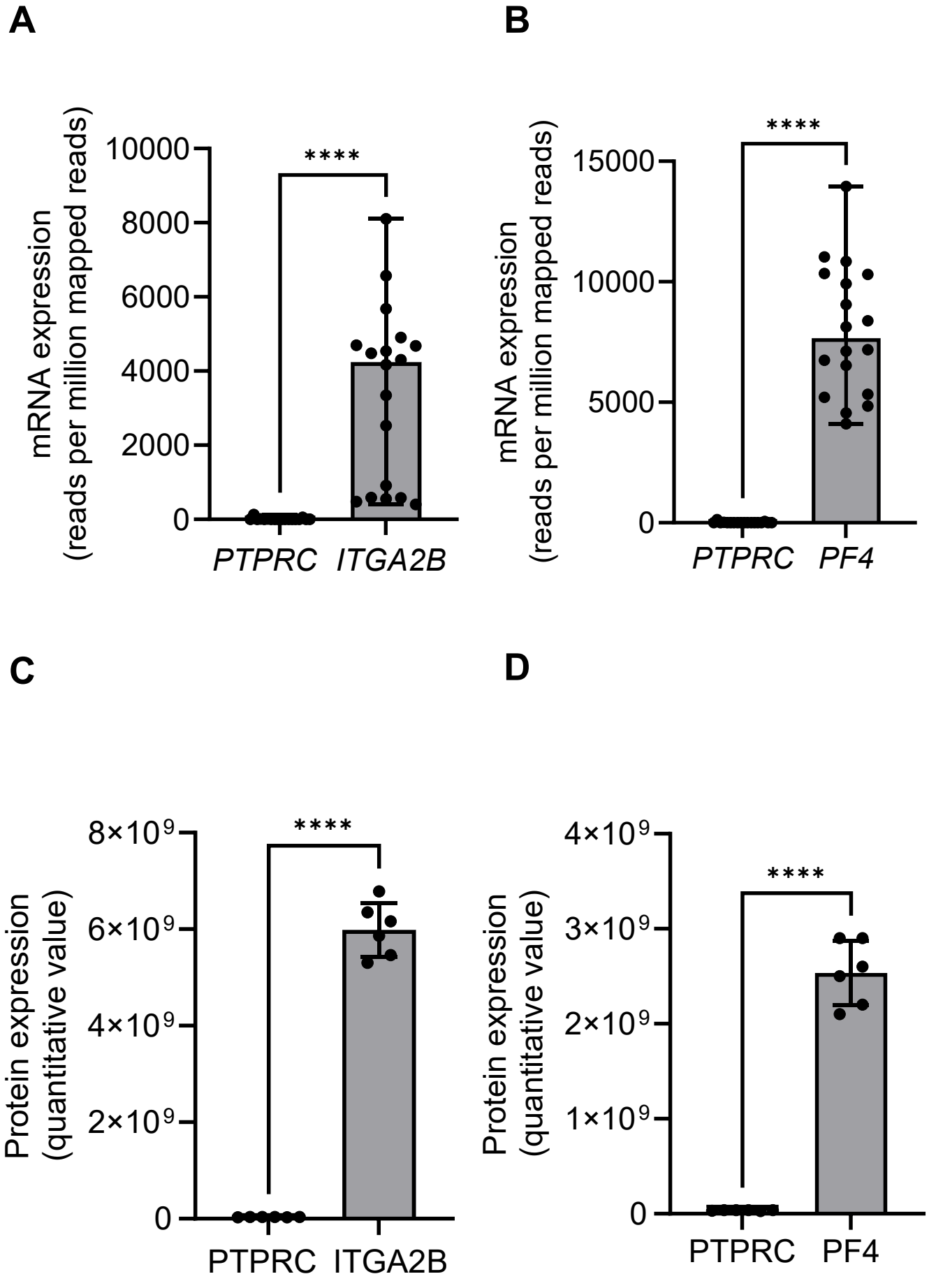

A
B
C
D
mRNA expression
(reads per million mapped reads)
10000
8000
6000
4000
2000
0
****
PTPRC
ITGA2B
15000
10000
5000
PF4
Protein expression
(quantitative value)
8×10⁹
6×10⁹
4×10⁹
2×10⁹
4×10⁹
3×10⁹
2×10⁹
1×10⁹

Figure S1. Platelets in all samples are highly purified from whole blood.

(A, B) *PTPRC* and *ITGA2B* (A) or *PF4* (B) mRNA expression levels in platelets from all samples (n = 18) corrected for reads per million mapped reads in RNA-seq analysis. (C, D) PTPRC and ITGA2B (C) or PF4 (D) protein expression levels in platelets from all samples (n = 6) in MS analysis. Protein expression levels refer to the quantitative values of each protein. ****, $P < 0.0001$

Figure S2

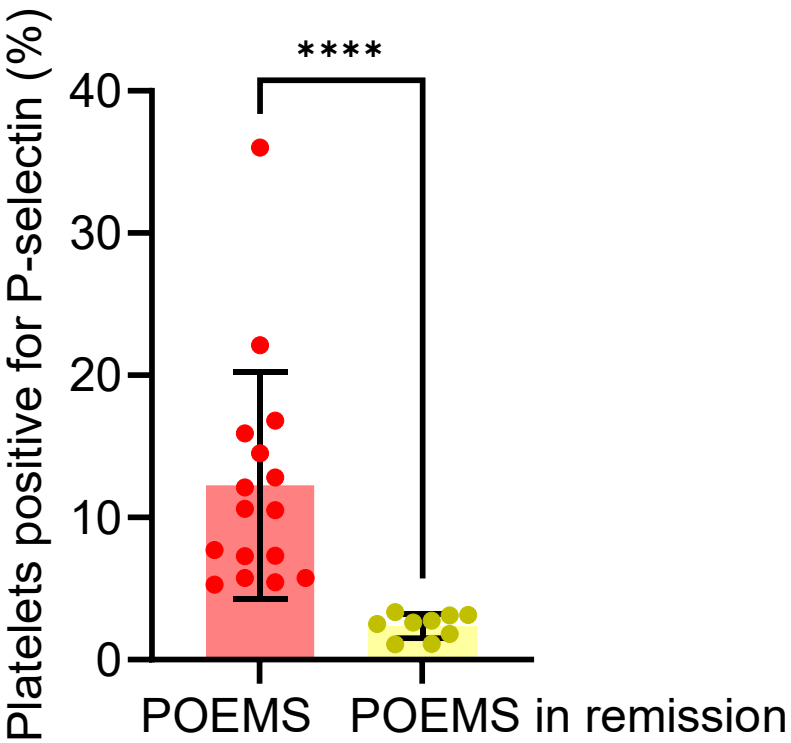
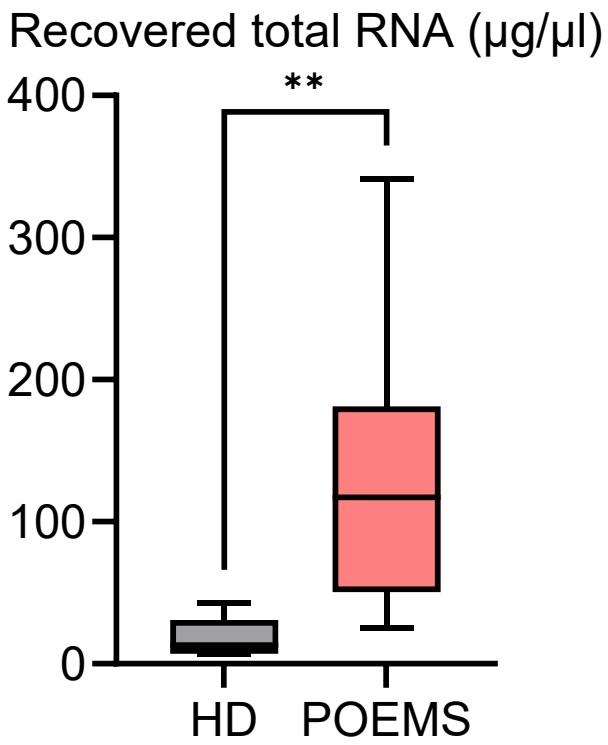

****
Platelets positive for P-selectin (%)
40
30
20
10
0
POEMS
POEMS in remission

Figure S2. Platelet P-selectin expression is significantly lower in patients with POEMS syndrome in remission than in those with newly diagnosed or relapsed disease.

Flow cytometry for P-selectin on the platelet surface using peripheral blood samples from patients with POEMS syndrome in remission (n = 9) and those with newly diagnosed or relapsed disease (n = 16; the same cohort shown in Figure 2C). Among patients in remission, five were male and four were female, with a median age of 65 years (range, 51−75) and a median serum VEGF level of 368.4 pg/mL (range, 280.3−1230.6). P-selectin expression levels were assessed as the percentage of P-selectin-positive platelets and compared between the two groups. ****, $P < 0.0001$

Figure S3

A


Recovered total RNA (μg/μl)
400
300
200
100
0
**
HD
POEMS

B

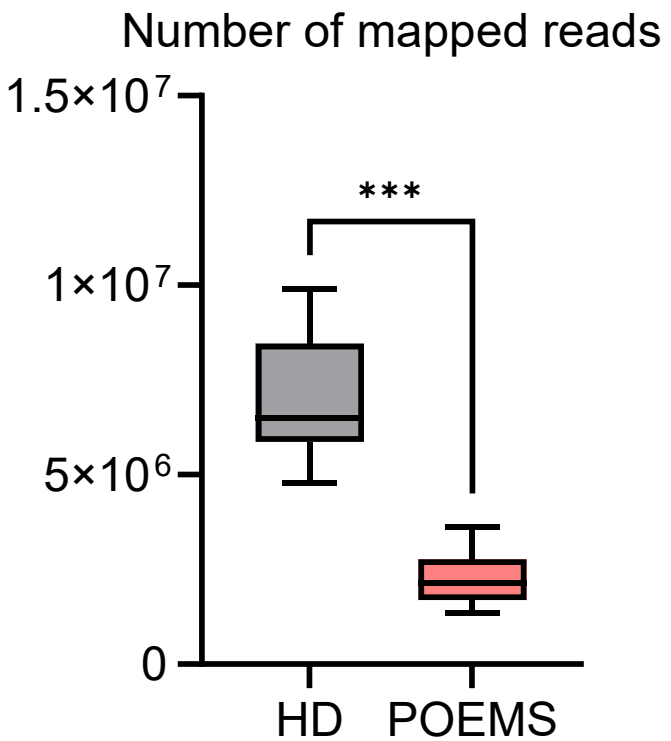

Number of mapped reads
1.5×10^7
1×10^7
5×10^6
0
***
HD
POEMS

Figure S3. Platelet mRNA in total RNA is significantly lower in patients with POEMS than in healthy donors.

(A) The total RNA recovered from platelets isolated from 5 mL of whole blood between all samples (n = 18). (B) The number of mapped reads obtained from the platelet RNA-seq libraries from all samples (n = 18). It corresponds to the amount of mRNA in the total RNA prepared in the same amount. **, $P < 0.01$; ***, $P < 0.001$

Figure S4

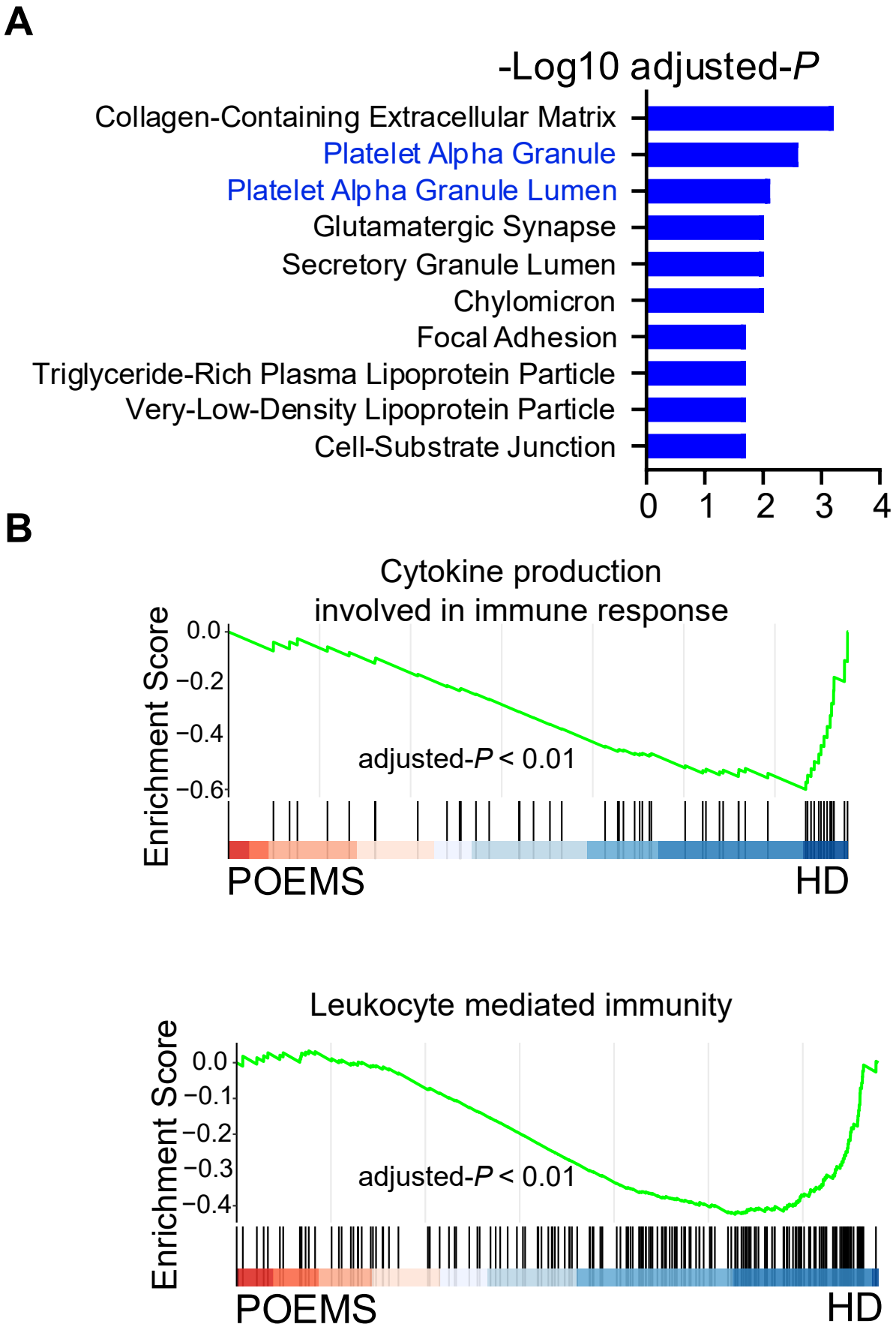
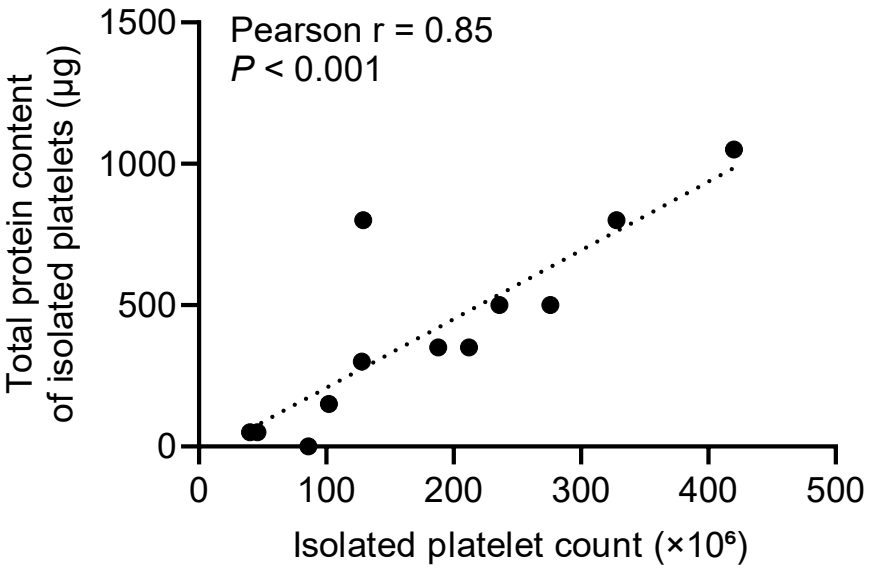

A
-Log10 adjusted-P
Collagen-Containing Extracellular Matrix
Platelet Alpha Granule
Platelet Alpha Granule Lumen
Glutamatergic Synapse
Secretory Granule Lumen
Chylomicron
Focal Adhesion
Triglyceride-Rich Plasma Lipoprotein Particle
Very-Low-Density Lipoprotein Particle
Cell-Substrate Junction
0
1
2
3
4
B
Cytokine production
involved in immune response
Enrichment Score
0.0
−0.2
−0.4
−0.6
adjusted-P < 0.01
POEMS
HD
Leukocyte mediated immunity
Enrichment Score
0.0
−0.1
−0.2
−0.3
−0.4
adjusted-P < 0.01
POEMS
HD

Figure S4. Platelet proteome involved in immune responses is downregulated in patients with POEMS.

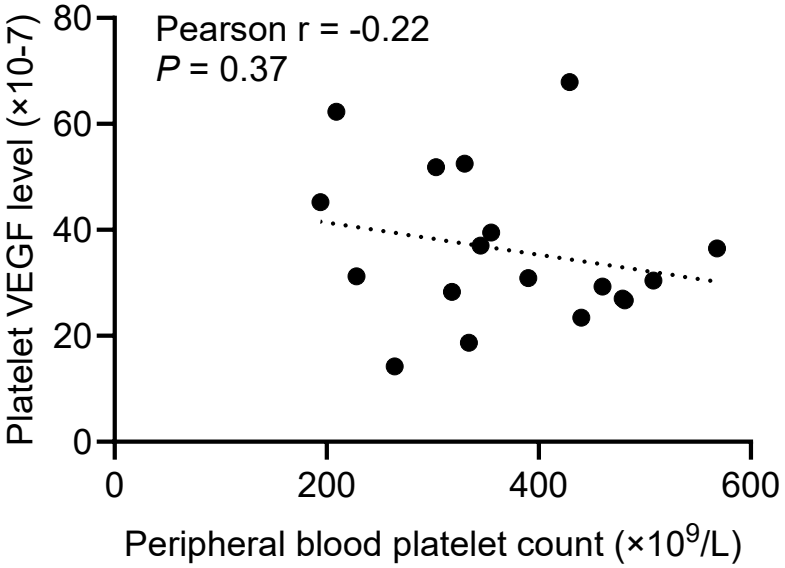
(A) Gene Ontology analysis of protein-coding genes from MS data focused on cellular component [POEMS, n = 3; healthy donors (HDs), n = 3]. In the analysis, top-ranked cellular characteristics with adjusted-$P < 0.05$ that are downregulated in patients are listed. (B) Gene sets exhibiting decreased expression in POEMS patients in gene set enrichment analysis (GSEA) based on Gene Ontology database of proteome from MS.

Figure S5

A



B



C

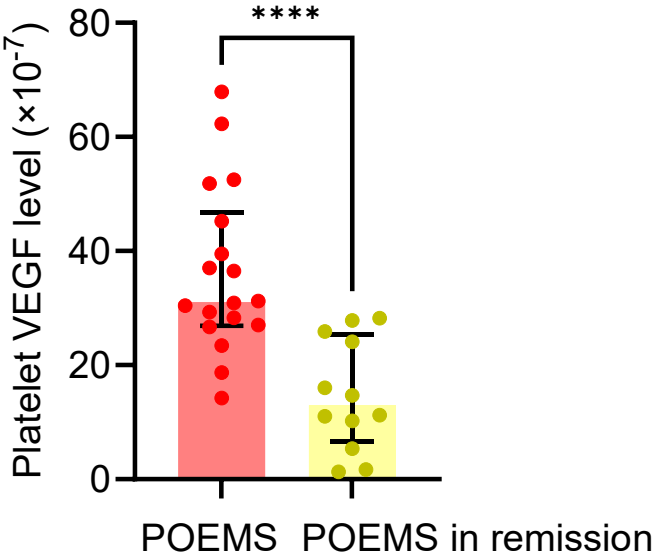
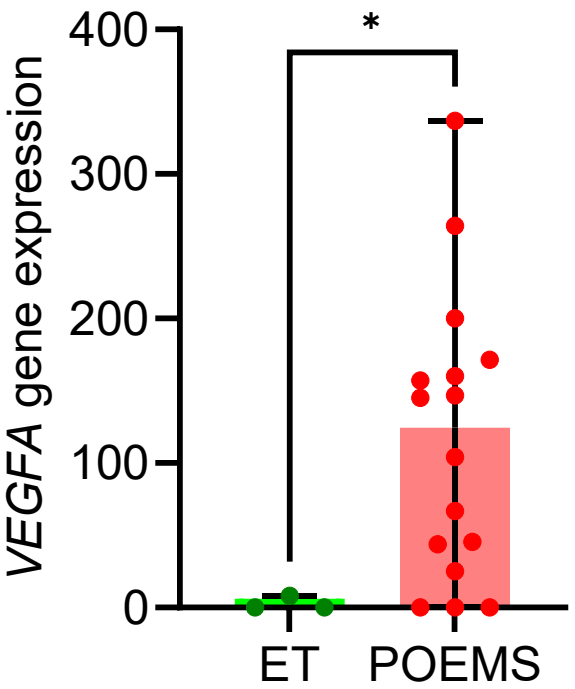


Figure S5. Platelet VEGF levels in relation to platelet count and disease status in POEMS syndrome

(A) Correlation between platelet count and total protein content of isolated platelets, measured by the BCA assay, in patients with POEMS syndrome (n = 4) and healthy donors (HDs) (n = 8). (B) Correlation between peripheral blood platelet count and platelet VEGF levels, measured by ELISA. (C) VEGF levels in platelets isolated from patients with POEMS syndrome in remission (n = 12) and those with newly diagnosed or relapsed disease (n = 18; the same cohort shown in Figure 5A). Platelet VEGF levels were defined as the VEGF concentration in the platelet protein lysate measured by ELISA, normalized to the platelet total protein concentration determined using the BCA assay. Among patients in remission, seven were male and five were female, with a median age of 62 years (range, 22−75) and a median serum VEGF level of 384.5 pg/mL (range, 280.3−1230.6). ****, $P < 0.0001$

Figure S6

A


400
300
200
100
0
*
VEGFA gene expression
ET
POEMS

B

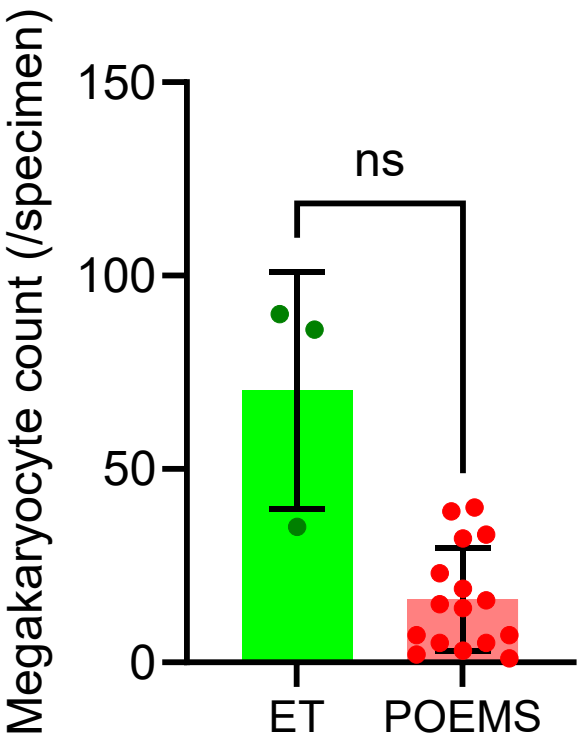

150
100
50
0
ns
Megakaryocyte count (/specimen)
ET
POEMS

Figure S6. *VEGFA* gene expression levels in megakaryocytes are higher in patients with POEMS than in those with ET.

(A) *VEGFA* gene expression levels in megakaryocytes in patients with POEMS (n = 16) and essential thrombocythemia (ET) (n = 3), a myeloproliferative disorder with increased megakaryocytes. (B) Counts of megakaryocytes observed in each specimen in the two groups. *, $P < 0.05$; **, $P < 0.01$

Table S1. Characteristics of patients with POEMS syndrome

VEGF, vascular endothelial growth factor

Table S2. Patients with malignant lymphoma without bone marrow involvement

F, female; M, male; MALT, mucosa-associated lymphoid tissue; DLBCL, diffuse large B-cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; AITL, angioimmunoblastic T-cell lymphoma.

Table S3. Scoring criteria for mRNA in situ hybridization using RNAscope

Table S1

Patient characteristics	Overall (n = 31)
Median age, y (range)	57 (25-71)
Male, n (%)	14 (45.16)
Median serum VEGF level, pg/mL (range)	3679 (927-18231)
Median platelet count, $\times 10^9$/L (range)	334 (147-568)
Relapsed, n (%)	7 (22.58)
Type of M-protein, n (%)	
IgG-λ	11 (35.48)
IgA-λ	11 (35.48)
IgA-κ	3 (9.68)
IgG-κ	2 (6.45)
BJP-λ	1 (3.23)
not detected	3 (9.68)

Table S2

Age, y	Sex	Disease
51	F	MALT lymphoma
86	M	MALT lymphoma
30	F	DLBCL
23	F	PMBCL
66	F	MALT lymphoma
63	M	DLBCL
37	M	AITL

Table S3

Score	Criteria
0	No staining or less than 1 dot/10 cells
1	1-3 dots/cell
2	4-9 dots/cell with no or very few clusters of dots
3	10-15 dots/cell with less than 10% of dots in clusters
4	More than 15 dots/cell with more than 10% of dots in clusters