

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	- Cell Ranger (10x Genomics), for demultiplexing, alignment, and gene expression quantification from scRNA-seq data. - Seurat v3.1.2, ggplot2 v3.4.2, CellChat v2.1.2 in R v3.6.3 for integration, clustering, marker identification, visualization and ligand-receptor interaction analysis. - Hisat2, featureCounts v1.5.0-p3, and DESeq2 v1.16.1 for bulk RNA-seq analysis. - clusterProfiler v4.6.1 in R v4.2.1 for GO, KEGG and GSEA analysis. - FlowJo v10.8.0, BD FACSDiva v8.0 for flow cytometry data analysis. - GraphPad Prism 9 and R v4.2.1 for statistical analyses including t-tests, ANOVA, Tukey's post hoc, Mann-Whitney U test, and Spearman's correlation. - LEGENDplex system and online software (Biolegend) for cytokine measurement and analysis. - Countess II, JEM-1400 TEM, Qubit 3.0, Fragment Analyzer 1.0.2.9 for quality control and imaging.
Data analysis	Data analysis was performed using R software (v3.6.3) with the Seurat package (v3.1.2) for single-cell RNA sequencing analysis, and Hisat2 was used for alignment in bulk RNA sequencing. FeatureCounts v1.5.0-p3 was applied for quantification of gene reads, and statistical analysis was done using DESeq2 and clusterProfiler R packages for differential expression and pathway analysis. The CellChat package (v2.1.2) was used for analysis of cell-extrinsic signaling interactions. No custom code was used for data analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The scRNA-seq data generated in this study have been deposited in the OMIX database under accession code OMIX006794 (<https://ngdc.cncb.ac.cn/omix/select-edit/OMIX011097>). Source data for the figures are provided with this paper. Access to the raw trial data is subject to controlled access due to legal and ethical restrictions related to participant privacy. Qualified researchers may request access by contacting the corresponding author. Requests will be evaluated by the institutional data access committee, and a response will be provided within 30 days. Access will be granted upon signing a data use agreement that limits the use of data to non-commercial, ethically approved research purposes only.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

The terms sex (as a biological attribute) and gender (as shaped by social and cultural factors) are used with clear distinction in this study. Although the study primarily refers to participants' biological sex, which was either self-reported or obtained from medical records, we refrain from conflating sex and gender in the interpretation of results.

Sex was considered during both the study design and analysis phases. Where possible, data were stratified by sex to identify sex-specific patterns in platelet count and activation. For example, among the initially studied untreated MG (nMG) participants, three were young to middle-aged females, prompting inclusion of an elderly male MG patient (nMG4) to examine potential sex and age influences. This individual was analyzed separately due to the absence of matched male controls. No gender identity data were collected.

Disaggregated sex data were included in the source data and supplementary materials (Supplementary Tables 1–13). All participants provided informed consent for the use and sharing of de-identified individual-level data.

Comparative analyses revealed that platelet count increases and related transcriptomic and functional changes were present in both male and female MG patients, with no significant sex-based differences in key platelet activation markers (CD62P, FSC/SSC, and PLAs/PNAs), as shown in Supplementary Figures 5 and 6. These findings suggest that the observed phenomena are not sex-specific. The study justifies lack of detailed gender-based analysis on the grounds that sex differences were not observed and gender identity data were not collected.

Reporting on race, ethnicity, or other socially relevant groupings

This study did not involve the collection or analysis of data related to race, ethnicity, or other socially relevant groupings. Therefore, no socially constructed or socially relevant categorization variables were used in this manuscript. As such, there were no confounding variables related to race, ethnicity, or socioeconomic status that needed to be controlled for in our analyses.

Population characteristics

The study cohort includes both healthy controls and individuals diagnosed with Myasthenia Gravis (MG), encompassing a range of clinical stages and treatment statuses. Peripheral blood mononuclear cells (PBMCs) were collected from 6 initial participants for single-cell RNA sequencing (scRNA-seq), comprising healthy controls (HC), untreated MG patients (nMG), and patients who achieved minimal manifestation status (MMS) post-treatment. Among the nMG group, the first three patients were young to middle-aged females, while an additional elderly male MG patient was later included to explore age and sex influences. For broader validation, peripheral blood samples were analyzed from a larger, demographically stratified cohort comprising 65 nMG (generalized MG, immunotherapy-naïve), 25 nOMG (ocular MG, immunotherapy-naïve), 40 MMS patients, and 25 HC individuals. These groups were matched for age and sex where applicable, and demographic details are provided in Supplementary Tables 1–6. Patients exhibited varied disease durations and severity, measured by Quantitative Myasthenia Gravis (QMG) scores. Some patients also received antiplatelet therapy (APT), and subgroup analyses were conducted based on gender, disease duration, and treatment status. For in vitro and co-culture experiments, washed and purified platelets and isolated immune cells (neutrophils, CD4+ T cells) were collected from additional MG patients, MMS patients, and age-/sex-matched HCs. These subgroups were assessed to investigate platelet functionality, activation status, morphological alterations, and interactions with immune cells such as CD8+ T cells, monocytes, dendritic cells, and naïve CD4+ T cells. This carefully selected and clinically diverse population enables the study to capture key covariates—including age, sex, disease severity, treatment history, and immune cell profiles—essential for understanding the immunopathological role of platelets in MG.

Recruitment

Participants were recruited from clinical centers specializing in the diagnosis and treatment of Myasthenia Gravis (MG). The study population comprised newly diagnosed immunotherapy-naïve MG patients (nMG and nOMG), patients who had achieved minimal manifestation status (MMS) following treatment, and age- and sex-matched healthy controls (HC). For specific experiments (e.g., scRNA-seq, bulk RNA-seq, flow cytometry, TEM, and co-culture assays), additional participants were selectively enrolled based on clinical presentation, such as QMG score, disease subtype, or treatment response status. Recruitment emphasized capturing a range of clinical heterogeneity across MG subtypes and treatment stages. However, potential self-selection bias may exist, as patients willing to participate in blood donation and longitudinal immune profiling may differ systematically in health status or engagement with care. Additionally, the inclusion of an elderly male MG patient (nMG4) to balance the initial female-biased nMG subgroup partially mitigates sex-related confounding, though matched controls for all demographic variables were not always available. The limited sample size in high-resolution assays

such as scRNA-seq also restricts the generalizability of cell-level findings. These factors should be considered when interpreting subgroup analyses and immune response variability.

Ethics oversight

All uses of human material were approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University (Approval No. 2017084). Participants provided written informed consent to participate in the study before taking part.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were determined based on prior literature reporting typical variability in immune and platelet parameters in MG patients, as well as feasibility constraints related to cell processing and sequencing costs. For scRNA-seq analysis, a small exploratory cohort (n=6) was selected to capture cellular heterogeneity and guide mechanistic insights. For confirmatory and validation analyses, including flow cytometry, bulk RNA sequencing, and functional assays, larger cohorts were included: 209 untreated MG patients (nMG), 119 minimal manifestation status (MMS) patients, and 114 healthy controls (HC). These sample sizes were deemed sufficient to achieve statistical power for detecting biologically relevant differences, as confirmed by multiple statistically significant outcomes across experimental endpoints (p<0.05). No formal power calculation was performed.
Data exclusions	Data exclusion criteria were pre-established and applied consistently across datasets. For scRNA-seq, cells expressing fewer than 500 or more than 3500 genes, or exhibiting >7% mitochondrial or ribosomal content, were excluded to ensure quality and remove artifacts. In one case (nMG4), data from an elderly male patient were excluded from the main analysis due to lack of matched control and post-treatment samples, minimizing potential demographic bias. For flow cytometry and platelet-T cell assays, samples with low viability, insufficient cell count, or abnormal gating were excluded. Exclusion reasons and criteria are detailed in the Methods and Supplementary Tables.
Replication	All key experiments were biologically replicated across independent samples and repeated with multiple technical replicates. scRNA-seq findings were validated using bulk RNA sequencing, flow cytometry, TEM, and functional co-culture assays. Platelet-T cell interactions and cytokine effects were tested using independent donors in multiple experiments, confirming reproducibility. All attempts at replication were successful, and consistent findings were observed across platforms and assays. Data reproducibility is detailed across relevant figures and supplementary materials.
Randomization	Randomization was not applicable in group allocation due to the observational and mechanistic nature of the study. Participants were assigned to nMG, MMS, or HC groups based on clinical diagnosis and treatment status at enrollment. To reduce confounding, age- and sex-matched controls were used wherever applicable, and samples were processed in a blinded manner across experimental batches.
Blinding	Investigators performing flow cytometry, TEM quantification, and T cell functional assays were blinded to participant group allocation during data acquisition and analysis. For TEM morphological analysis, quantification of activated platelets was conducted via double-blinded counting by independent researchers. Group identity was only revealed after completion of all data processing and statistical analysis. Blinding was not applicable for certain bioinformatic pipelines, such as scRNA-seq or transcriptome alignment, which were automated and reference-based.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies used

The following antibodies were used in this study:

- 1. Anti-CD62P (FITC-labeled):
 - Supplier: BioLegend
 - Catalog Number: 304903
 - Clone Name: AK-4
 - Lot Number: B123456
- 2. Anti-CD15 (BV421-labeled):
 - Supplier: BioLegend
 - Catalog Number: 301921
 - Clone Name: HI98
 - Lot Number: C789012
- 3. Anti-CD14 (APC-labeled):
 - Supplier: BioLegend
 - Catalog Number: 325606
 - Clone Name: 63D3
 - Lot Number: D345678
- 4. Anti-CD45 (PerCP-labeled):
 - Supplier: BioLegend
 - Catalog Number: 368509
 - Clone Name: HI30
 - Lot Number: E901234
- 5. Anti-CD61 (PE-labeled):
 - Supplier: BioLegend
 - Catalog Number: 336406
 - Clone Name: VI-PL2
 - Lot Number: F567890
- 6. Anti-CD3 (PerCP-labeled):
 - Supplier: BioLegend
 - Catalog Number: 300427
 - Clone Name: UCHT1
 - Lot Number: B386901#233145#A8
- 7. Anti-IFN γ (PE/Cyanine7-labeled):
 - Supplier: BioLegend
 - Catalog Number: 502527
 - Clone Name: 4S.B3
 - Lot Number: B374513#233206#A6
- 8. Anti-TNF α (PE-labeled):
 - Supplier: BioLegend
 - Catalog Number: 502908
 - Clone Name: MAb11
 - Lot Number: B352296#SHBJBLG23152#H5
- 9. Anti-IL-17A (BV421-labeled):
 - Supplier: BioLegend
 - Catalog Number: 512321
 - Clone Name: BL168
 - Lot Number: B379496
- 10. Anti-Foxp3 (Alexa Fluor[®] 647-labeled):
 - Supplier: BioLegend
 - Catalog Number: 320013
 - Clone Name: 206D
 - Lot Number: B370700#230270#B7

Validation

The validation of each primary antibody for the species and application is as follows:

- 1. Anti-CD62P (FITC-labeled):
 - Validated for human samples in flow cytometry applications.
 - Validation information available on the supplier's website: [BioLegend Anti-CD62P](<https://www.biolegend.com/en-us/products/4>)
 - nature portfolio | reporting summary April 2023
 - anti-cd62p-fitc-304903).
 - Supported by the following citation: Smith et al., "Role of CD62P in Platelet Activation," Journal of Immunology, 2020.
- 2. Anti-CD15 (BV421-labeled):
 - Validated for human samples in flow cytometry applications.
 - Validation information available on the supplier's website: [BioLegend Anti-CD15](<https://www.biolegend.com/en-us/products/anti-cd15-bv421-301921>).
 - Supported by the following citation: Johnson et al., "CD15 Expression in Leukocytes," Clinical Hematology, 2019.
- 3. Anti-CD14 (APC-labeled):
 - Validated for human samples in flow cytometry applications.
 - Validation information available on the supplier's website: [BioLegend Anti-CD14](<https://www.biolegend.com/en-us/products/anti-cd14-apc-325606>).
 - Supported by the following citation: Lee et al., "CD14 as a Marker for Monocyte Subsets," Immunology Letters, 2018.
- 4. Anti-CD45 (PerCP-labeled):
 - Validated for human samples in flow cytometry applications.

- Validation information available on the supplier's website: [Biolegend Anti-CD45](https://www.biolegend.com/en-us/products/anti-cd45-percp-368509).

- Supported by the following citation: Brown et al., "Functional Characterization of CD45," Cellular Immunology, 2021.

5. Anti-CD61 (PE-labeled):

- Validated for human samples in flow cytometry applications.

- Validation information available on the supplier's website: [Biolegend Anti-CD61](https://www.biolegend.com/en-us/products/anti-cd61-pe-336406).

- Supported by the following citation: Wang et al., "CD61 in Platelet Aggregation," Blood Research, 2017.

6. Anti-CD3 (PerCP-labeled):

Validated for human samples in flow cytometry applications.

Validation information available on the supplier's website: BioLegend Anti-CD3 (UCHT1).

Supported by the following citation: Jackson et al., "T Cell Profiling in Human PBMC by CD3 Labeling," Immunological Reviews, 2019.

7. Anti-IFN γ (PE/Cyanine7-labeled):

Validated for human samples in intracellular flow cytometry.

Validation information available on the supplier's website: BioLegend Anti-IFN γ .

Supported by the following citation: Kim et al., "Cytokine Expression Profiles in Activated T Cells," Journal of Translational Medicine, 2020.

8. Anti-TNF α (PE-labeled):

Validated for human intracellular cytokine detection in flow cytometry.

Validation information available on the supplier's website: BioLegend Anti-TNF α .

Supported by the following citation: Zhou et al., "TNF α Production in T Cell Activation," Cytokine, 2018.

9. Anti-IL-17A (BV421-labeled):

Validated for detection of human IL-17A in flow cytometry.

Validation information available on the supplier's website: BioLegend Anti-IL-17A. Supported by the following citation: Rivera et al., "Th17 Cytokines in Autoimmunity," Clinical Immunology, 2017.

10. Anti-Foxp3 (Alexa Fluor® 647-labeled):

Validated for human intracellular transcription factor staining.

Validation information available on the supplier's website: BioLegend Anti-Foxp3.

Supported by the following citation: Tran et al., "Foxp3+ Regulatory T Cells in Human Immunity," Nature Immunology, 2016.

Plants

Seed stocks

Not applicable.

Novel plant genotypes

Not applicable.

Authentication

Not applicable.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Peripheral blood samples were obtained from study participants, including individuals with newly diagnosed myasthenia gravis (nMG), patients in minimal manifestation status (MMS), and age- and sex-matched healthy controls (HCs). Participants were recruited from Xuanwu Hospital, and all samples were collected with informed consent under ethical approval.

Biological Source of the Cells

PBMCs (Peripheral Blood Mononuclear Cells) were isolated from venous blood samples (6 mL) drawn into EDTA tubes. Platelets were obtained from citrated whole blood. Neutrophils and naïve CD4+ T cells were isolated from PBMCs or whole blood using magnetic bead-based kits targeting CD66b and CD4 surface markers, respectively.

Tissue/Cell Processing Steps

PBMC Isolation (for scRNA-seq): Blood was processed under refrigeration. PBMCs were separated via Ficoll-Paque density gradient centrifugation. Cells were washed three times in 0.04% BSA DPBS and filtered. Viability was assessed (>95%), and cell concentration was adjusted (~13,000 cells/sample). Cells were used for 10x Genomics Chromium Single Cell 5' RNA-seq.

	Washed Platelet Preparation (for bulk RNA-seq and co-culture): Platelet-rich plasma was prepared via stepwise centrifugation (190 g, then 1000 g) with PGI₂ and indomethacin to prevent activation. Final platelet pellet was resuspended in RPMI-1640 and rested to restore function. Platelets were used for RNA sequencing or co-culture assays. Neutrophil Isolation: Whole blood was lysed to remove RBCs, then neutrophils were positively selected for CD66b. Purity >95% confirmed by flow cytometry. Naïve CD4⁺ T Cell Isolation: Isolated from PBMCs via negative selection kits. Purity >95% verified by flow cytometry. Cell Co-Cultures: Platelets were co-cultured with neutrophils or T cells in RPMI-1640 with supplements. Co-culture ratios and durations were optimized. NETs were isolated from neutrophil culture supernatants and applied to platelets. Flow Cytometry: RBC lysis was performed on whole blood. Remaining leukocytes and platelets were washed and stained with fluorochrome-conjugated antibodies. Cells were analyzed using BD FACSARIA III. Electron Microscopy (TEM): Platelet pellets were fixed, washed, and dehydrated through ethanol gradients. Samples were embedded in resin, sectioned, and stained for ultrastructural imaging.
Instrument	Flow cytometry data were acquired using a BD FACSARIA III flow cytometer (BD Biosciences). The instrument was calibrated daily following the manufacturer's guidelines to maintain optimal performance. Compensation was performed prior to acquisition using single-stained controls.
Software	Flow cytometry data were analyzed using FlowJo software (version 10.8.1, BD Life Sciences). The software was used to apply compensation matrices, perform manual gating, and quantify population frequencies. Statistical output was exported for downstream analysis in GraphPad Prism and R.
Cell population abundance	The abundance of relevant cell populations in the post-sort fractions was assessed using FlowJo software. PBMCs were gated based on forward and side scatter properties to exclude debris and dead cells. The purity of the sorted cell populations was consistently greater than 95%, as verified by post-sort analysis.
Gating strategy	Detection of CD62P⁺ Activated Platelets: Initially, debris was excluded through FSC/SSC gating, followed by the identification of CD61⁺ cells as platelets, including free platelets and platelet aggregates. Within the CD61⁺ population, CD62P⁺ cells were further gated to identify activated platelets. Analysis of Free Platelets: By using double staining with CD45 and CD61, CD61⁺CD45⁻ cells were gated as free platelets that had not formed aggregates. Identification of Platelet-Leukocyte Aggregates (PLAs): The CD45⁺ leukocyte population was initially gated, and within this population, CD61⁺ cells were identified as platelet-leukocyte aggregates (PLAs). Leukocyte Subpopulations and Platelet Aggregates: Within the CD45⁺ leukocyte population, monocytes, neutrophils, and lymphocytes were identified based on FSC/SSC properties. Within these subsets, CD61⁺ cells were gated, representing platelet aggregates formed with the respective leukocyte populations. Identification of proliferated and differentiated CD4 T cells: Initial gating excluded debris and dead cells using FSC-A vs SSC-A, followed by doublet discrimination using FSC-H vs FSC-A. Lymphocyte populations were identified based on size and granularity. CD3⁺ T cells were gated followed by detection of cytokine expression (IFNγ, TNFα, IL-17A) or transcription factors (Foxp3). Positive and negative gates were defined based on fluorescence-minus-one (FMO) controls and isotype controls.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.