nature portfolio

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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 (*n*) 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ 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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ 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 *d*, Pearson's *r*), 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	no software was used for data colection
Data analysis	R(4.3.2); Limma(3.64);DESeq2(1.48);GraphPad Prism 9.0.0; IBM SPSS (version 28.0); Fiji-ImageJ software (1.53c); Imaris 10 (Oxford Instruments)

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 microarray datasets described in the paper have been deposited in the Gene Expression Omnibus database with accession numbers GSE274954, GDS5083, and GSE21545. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org) via the iProX partner repository with the dataset identifier PXD053686 (https://www.iprox.cn/page/PSV023.html;?url=1726666205444yVEs, password: 14NC). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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	Participants self-reported their sex. This variable was included as covariate in all models. Descriptive results are reported in Table S1.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity or other socially relevant groupings were not reported.
Population characteristics	Table S1 of the manuscript provides descriptive statistics on the participants.
Recruitment	The patient cohort was recruited during hospitalization, and inclusion was determined based on relevant medical record information.
Ethics oversight	This study was approved by the Xiangya Hospital of Central South University, Hunan, China (202005379)

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

Life sciences study design

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

Sample size	During the study period, a total of 128 study participants was included in this study.
Data exclusions	Patients with any of the following conditions were excluded: Current infection. Autoimmune diseases. Active or recurrent cancer. Severe renal failure requiring dialysis. Active liver dysfunction, or physical examination findings of alanine aminotransferase (ALT) or aspartate transaminase (AST) levels greater than threefold the upper limit of normal. Anemia. Peripheral artery occlusive disease. Acute or chronic inflammatory diseases and chronic metabolic disorders, including rheumatic arthritis, rheumatoid arthritis, etc. Long-term use of anti-atherosclerotic medications.
Replication	We confirm that all attempts to replicate the key experimental findings presented in this manuscript were successful. The data shown in the figures are representative of the consistent results obtained across all independent replicates. In all cases, the sample size (n) for each experiment is explicitly defined in the figure legends as the number of independent biological replicates (e.g., individual animals, primary culture preparations). We believe that the approaches described above have ensured that our study is sufficiently powered to support the conclusions drawn.
Randomization	Not applicable, as the study is observational.
Blinding	The investigators were blinded to group allocation during data collection and analysis

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

Antibodies used

The following antibodies were used for human tissue staining and labeling: Anti-CD68 antibody (ab213363, Abcam), Anti-CD31 antibody (ab9498, Abcam), Anti-αSMA antibody (ab7817, Abcam), and Anti-GPNMB antibody (ab222109, Abcam).

The GPNMB Monoclonal Antibody (CTSREVL, eFluor™ 660, 50-5708-82) was purchased from eBioscience. Monoclonal antibodies against mouse GPNMB (ab188222), GFP (ab183734), TFEC (ab185226), SEC22B (ab181076), ATP1A1+ATP1A2+ATP1A3+ATP1A4 (ab300507), Rab5 (ab218624), LAMP1 (ab289548), and F4/80 (ab300421, Abcam) were purchased from Abcam. Monoclonal antibodies against ERGIC-53 (sc-365158), EEA1 (sc-137130), COPB (sc-393615), MITF (sc-515925), and TFEB (sc-166736) were purchased from Santa Cruz. Monoclonal antibody against Histone-H3 (68345-1) was purchased from Proteintech. Anti-β-Actin antibody (#4970) was purchased from Cell Signaling. Anti-GAPDH antibody (AC033,) was purchased from ABclonal. Polyclonal antibody against ATP6V0E2 (NBP1-55100) was purchased from Novus.
The dilution ratios for the antibodies described above strictly followed the guidelines specified by the manufacturer.

Validation

https://www.abcam.cn/products/primary-antibodies/cd68-antibody-epr20545-ab213363.html
https://www.abcam.cn/products/primary-antibodies/cd31-antibody-jc70a-ab9498.html
https://www.abcam.cn/products/primary-antibodies/alpha-smooth-muscle-actin-antibody-1a4-ab7817.html
https://www.abcam.cn/products/primary-antibodies/gpnmb-antibody-epr22011-11-ab222109.html
https://www.abcam.cn/products/primary-antibodies/gpnmb-antibody-epr18226-147-ab188222.html
https://www.abcam.cn/products/primary-antibodies/gfp-antibody-epr14104-ab183734.html
https://www.abcam.cn/products/primary-antibodies/sec22b-antibody-epr12335-ab181076.html
https://www.abcam.cn/products/primary-antibodies/sodiumpotassium-transporting-atpase-pan-alpha-subunit-antibody-epr25414-249-ab300507.html
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https://www.scbt.com/zh/p/eea1-antibody-g-4
https://www.scbt.com/zh/p/copb-antibody-d-10
https://www.scbt.com/zh/p/mitf-antibody-d-9
https://www.scbt.com/zh/p/tfeb-antibody-c-6
https://www.ptgcn.com/products/Histone-H3-Antibody-68345-1-Ig.htm
https://www.cellsignal.cn/products/primary-antibodies/b-actin-13e5-rabbit-mab/4970
https://abclonal.com.cn/catalog/AC033
https://www.novusbio.com/products/atp6v0e2-antibody_nbp1-55100

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Human monocyte line THP1 cells (American Type Culture Collection, ATCC,TIB-202)
HEK-293T cells (American Type Culture Collection, ATCC,CRL-11268)

Authentication

Not authenticated

Mycoplasma contamination

Mycoplasma testing quarterly always negative

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

C57BL/6J mice, GpnmbR150X (DBA/2) mice, ApoE−/− mice (on C57BL/6 background), Gpnmb-flox mice, Lyz2cre/cre mice

Wild animals	no wild animals were used
Reporting on sex	Animals of both sexes were used in all studies. The gender of the animals used in different experiments has been described in the Methods section.
Field-collected samples	no field-collected samples were used
Ethics oversight	All experimental procedures in mice were approved by the Institutional Animal Care and Use Committee at the Beijing Institute of Lifeomics (IACUC-20220707-47MB).

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	This study was approved by the Xiangya Hospital of Central South University, Hunan, China (202005379)
Study protocol	Research Objective: This study aims to collect blood samples from healthy individuals and patients with varying degrees of asymptomatic arteriosclerosis to measure plasma levels of soluble GPNMB (sGPNMB). The primary objective is to explore the correlation between plasma sGPNMB levels and the progression of arteriosclerosis. Additionally, arterial plaque tissues from patients undergoing carotid endarterectomy will be collected to investigate the potential mechanisms through which GPNMB influences the development and progression of arteriosclerosis. Patient Inclusion Criteria: 1. Clinically diagnosed asymptomatic arteriosclerosis. 2. Age range: 15–80 years. 3. Presence of clinical symptoms, including asymptomatic myocardial ischemia (latent coronary heart disease), angina pectoris, myocardial infarction, and ischemic heart failure. Exclusion Criteria: 1. Concurrent infections. 2. Autoimmune diseases. 3. Active or recurrent cancers. 4. Severe renal failure requiring dialysis. 5. Active liver dysfunction or elevated alanine transaminase (ALT) or aspartate transaminase (AST) levels exceeding three times the upper limit of normal. 6. Anemia. 7. Peripheral arterial occlusive disease. 8. Acute or chronic inflammatory diseases and chronic metabolic diseases, including rheumatoid arthritis. 9. Long-term use of medications for arteriosclerosis treatment. Research Methods: 1. Blood samples will be collected from each participant, and plasma will be isolated for further analysis. Soluble GPNMB levels in the plasma will be quantified using enzyme-linked immunosorbent assay (ELISA) kits. 2. Plaque tissues from patients undergoing carotid endarterectomy will be collected, immediately snap-frozen in liquid nitrogen after surgical removal, and subsequently used for histological analysis.
Data collection	Participants were recruited from the patient registry of Xiangya Hospital between March 2024 to September 2024. We acknowledge that our recruitment method may introduce the sampling Bias: Our sample was drawn primarily from a single clinical center, which may not be fully representative of the broader target population. However, we believe these biases are less likely to threaten the internal validity of the study. The relationships and correlations observed within our sample are likely still valid and reproducible for this specific participant profile.
Outcomes	The outcomes include the levels of sGPNMB measured in the plasma of cohort members, as well as the severity of atherosclerosis assessed using distinct scoring systems, which integrate other clinical indicators related to the progression of atherosclerosis from the medical records.

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	Mice were perfused with at least 10 mL of PBS containing sodium heparin to eliminate blood contamination before isolating the aorta. The whole aortas were carefully dissected and cut into 2–5 mm pieces and incubated at 37 °C for 75 min with gentle shaking in a PBS solution with calcium and magnesium containing DNase I (90 U/mL), collagenase I (675 U/mL), collagenase XI (187.5 U/mL), hyaluronidase (90 U/mL) 3. After enzyme digestion, the samples were disaggregated by passing through a 70μm nylon sieve (BD Bioscience) and then were centrifuge at 500g for 5min at 4 °C to collect cell pellet. Cells were first blocked by anti-CD16/32 antibody and then stained with other antibodies against surface markers at 4°C for 30 min. After centrifugation and cleaning, cells were stained with Ghost DyesTM (TONBO) and then were permeabilized with the FoxP3 transcription factor-staining buffer set (eBioscience). After permeabilization and cleaning, foam cells were labeled with 40 nmol/L BODIPY493/503 (D3922, Invitrogen) in PBS at 4 °C for 30 min.
Instrument	Flow cytometric analyses were performed using a fluorescence-associated cell sorter (FACS) Fortessa or LSRII (BD Biosciences) instrument.
Software	FlowJo software (version 10.3; Tree Star Inc, Ashland, OR)
Cell population abundance	>10,000 cell events were gated for analysis.
Gating strategy	FSC and SSC parameters were used toto gate cell samples and exclude cell debris and large aggregates. Positive and negative populations were defined using isotype control antibodies or negative staining samples.

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