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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	Untargeted metabolomics data was collected by an Ultimate 3000 HPLC system coupled to a LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Mass Spectrometer. Targeted metabolomics data was collected for ImP quatification for APOEKO mice and for the PESA cohort by an HPLC system 1290 Infinity series coupled to a triple quadrupole 6460 MS, whereas for the IGT cohort by Acquity UPLC I-class system coupled to a Xevo TQ-XS triple quadrupole mass spectrometer. Fluorescence-Activated Cell Sorting data was collected by LSRFortessa SORP (Becton Dickinson) or a FACSymphony (Becton Dickinson). 16S rRNA amplicon sequencing data was collected by MiSeq Illumina platform. Single-cell and bulk RNA data was collected by Illumina NextSeq 2000 sequencer. Proteomics and phosphoproteomics data was collected by liquid chromatography with tandem mass spectrometry (LC–MS/MS) using Q Exactive HF Orbitrap MS. Other data were collected with Microsoft Excel 2019.
Data analysis	Heatmap and all charts were generated using GraphPad Prism software v.9.0 (GraphPad Software, San Diego, CA). SPSS version 23.0 and Stata version 18 (StataCorp LP, College Station, TX) were used for statistical analysis. Partial least squares discriminant analysis (PLS-DA) and receiver operating characteristic (ROC) analyses were performed in MetaboAnalyst V5 (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml) and Stata. The Kolmogorov-Smirnov test and Shapiro–Wilk normality test were used to determine the normal distribution of samples. Nominal variables were presented as percentages and frequencies. Comparisons between two groups were performed using two-tailed unpaired Student's t-tests (normal distribution) or Mann–Whitney U-tests (non-normal distribution) for continous variables or Fisher exact test for categorical variables. Unless otherwise stated, comparisons across multiple groups were assessed using one-way ANOVA (normal distribution) followed by Tukey's post hoc test, or Kruskal–Wallis test (non-normal distribution) followed by Dunn's test as indicated in each figure legend. Continuous variables were compared across tertiles of ImP by ANOVA or non-parametric Kruskall Wallis tests as appropriate, whereas nominal variables by applying the Cohran-Armitage test (dichotomous variables) and the Jonckheere-Terpstra test (ordinal variable) for trend. Restricted cubic splines (natural splines, RCS) with 3 knots across ImP distribution were applied to produce a dose-response curves and to flexibly model the potential nonlinear association of continuous ImP with the main endpoints, as previously described (PMID: 29799975; PMID: 36049808). In both cohorts, the 3 knots for RCS were fixed at the 10th, 50th and 90th percentile of the ImP

distribution. Number of knots was selected on the basis of improvement in log likelihood among regression models with 3- or 4-knot RCS. To enhance visual clarity, plots with RCS were truncated at values exceeding the 95th perecentile of the ImP distribution. We evaluated the non-linear relationships of ImP with log odd ratio (OR) of the main atherosclerosis outcomes by examining (Wald test) the non-linear terms (RCS). Dose–response curves in both cohorts were controlled for a set of confounders, constructed by combining LASSO regression and variables with biological plausibility. LASSO for logistic regression was used for multivariable model selection with respect to the outcome presence of atherosclerosis. Adaptive LASSO with cross validation was employed to select the value of the lasso penalty parameter lambda. Adjusted differences in levels of ImP across groups of interest were estimated by multivariable linear regression analysis. To evaluate the association between ImP levels and the presence and activity of atherosclerosis within the PESA cohort multinomial logistic regression was adjusted on a set of confounders (i.e. age, sex, smoking status, fasting glucose, high-sensitivity C Reactive Protein (hs-CRP), and hemoglobin concentration) based on previous evidence (PMID: 35567559)). The proportional odds assumption was tested with the Brant Wald test. The discriminatory capability of ImP for prevalence of subclinical or active atherosclerosis on top of traditional risk factors, including LDL-cholesterol and high-sensitive C Reactive Protein, was assessed by comparing Area(s) Under the Curve (AUC) from corresponding Receiver Operating Characteristic (ROC) analysis. AUCs were compared using the DeLong test (PMID: 3203132). Bootstrapping with 1,000 replicates was used to derive 95% bias-corrected confidence interval (CI) around AUCs.All tests were two tailed and the level of statistical significance was pre-specified at P<0.05.

For untargeted metabolomics data: generated data was firstly aligned using Compound Discoverer (ThermoFisher Scientific); signals were extracted and grouped into features (isotopic traces from a single analyte at a particular charge state) using the Metaboprofiler node in Compound discoverer (the open source plug-in freely available from OpenMS, http://www.openms.de/downloads/). For targeted metabolomics data: Raw signals for multiple reaction monitoring (MRM) transitions were checked and peaks corresponding to all the targeted compounds were integrated by MassHunter Quantitative B.10.00 (Agilent Technologies).
For 16S rRNA gene sequencing analysis from both mice and human samples, the paired-end sequences were curated, binned into operational taxonomic units at >97% identity level, and annotated with SILVA release v.132 using DADA2 for mice data and v.138 and RDP version 18 databases using Mothur (v.1.40.5) for human data.
For dietary pattern assessment a factor analysis was performed in SPSS V.23.0 (SPSS Inc., Armonk, NY, USA) to identify common underlying dimensions (factors or patterns) of food consumption by deriving factor loadings for each predefined food group. Factors were subsequently rotated using a Varimax procedure to maintain uncorrelated factors. Analysis of eigenvalues, scree plot, and the interpretability of the factor solution were used to support a final decision on retaining a 5-factor solution, where each factor had an eigenvalue > 0.3.
For single-cell RNA seq data raw sequencing data processing was performed from FASTQ file from each port using Cell Ranger (v6.1.2) with default parameters and the mm10 (GRCm38.p6) mouse genome reference provided by 10x Genomics. Obtained raw unique molecular identifier (UMI) count matrices of valid barcoded cells for each port were loaded into R (v4.1.2) for further analyses using Bioconductor packages and Seurat (v4.0.6). Cells were clustered using the Louvain algorithm for modularity optimization using KNN graph as input and visualized using the Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) algorithm with the first 25 principal components as input. Main clusters were identified by using known markers and calculating differentially expressed genes with Wilcoxon-rank sum test using presto R package (v1.0.0)). Cell proportions were calculated for each condition and time point in every cell population, and a two-proportions Z-test using the prop.test() R function was chosen to determine significance. Differential expression analyses between conditions were performed using the MAST R package (v1.20.0). Genes were ranked according to the calculated logFCs, and resulting ranks were used as input for the FGSEA algorithm along with the Hallmark gene sets from the Molecular Signature Data Base (MSigDB; https://www.gsea-msigdb.org/gsea/msigdb). Finally, overrepresentation analysis was performed along with the GO database (www.geneontology.org/).
For RNA-seq resulted reads were mapped to the reference transcriptome GRCm38.102 using STAR25 and gene expression levels were estimated using RSEM26. Further analyses were performed in R (v 4.3.2). In brief, counts per million (CPM) were calculated, and size factors for inter-sample normalization were determined using the trimmed mean of M values (TMM) method. Then, log-normalized data considering only genes with ≥ 20 counts in three samples were used for differential expression analysis, making all possible comparisons within the same cell type using the DESeq2 R package. Gene-set enrichment analysis was performed using the fgsea R package comparing 1h and 2h after ImP stimulation with 0h (unstimulated) within each cell type. Genes were ranked in each comparison according to logFC, and fgsea was used along with the Hallmark gene sets from the Molecular Signature Data Base (MSigDB; https://www.gsea-msigdb.org/gsea/msigdb). Finally, normalized enrichment scores of significant selected pathways were represented as a heatmap using the ComplexHeatmap R package.
Proteome and phosphoproteome analyses were accomplished using 18-plex isobaric TMT labeling as described previously. The statistical analysis of quantitative data was made using iSanXoT, and the comparative analysis between conditions was performed using the normalized values X_p^m and X_q^m, which represent the binary logarithm of the ratio between the abundance in sample m and its control for peptide p and protein q, respectively. Gene Set Enrichment Analysis (GSEA) using X_p values of peptides was employed to evaluate the phosphorylation levels in proteins of the mTOR signalling pathway among conditions (i.e. cells stimulated with ImP vs unstimulated). In addition, a Wilcoxon Signed Rank Test was performed using paired phosphopeptide abundance X_p values to determine the effects of AGN192403 in the phosphorylation levels of the mTOR pathway among cells treated with ImP and co-incubated or not with AGN192403.

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

Metabolomics data are deposited in Metabolomics workbench (ST003928, ST003929, ST003932 and ST003938). The bulk RNA-seq and scRNA-seq data generated in this study are available in the NCBI GEO database under BioProject IDs GSE297831 and GSE298392, respectively. The proteomics and phosphoproteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD063955. Source data are provided with this paper.

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 current study is focused on subclinical atherosclerosis and explore the blood concentration of imidazole propionate, urocanic acid and histidine in middle-aged asymptomatic participants (56±4 years). Considering the delayed onset of subclinical atherosclerosis that is approximately 10 years later in women (PMID: 34543072 and PMID: 25882487), our cohort included 400 participants of which 310 (78%, by sex) were male participants. The information regarding the sex of each participant was collected by self-reporting via a questionnaire. These data are indicated in Extended Data Table 1a and 2b and Table 3. The IGT cohort that was used to validate the results in an independent cohort comprises men and women aged 50-64 years from the Gothenburg area (Sweden). In this case the cohort included 1844 participants of which 841 (46%, by sex), were male participants thus including an higher the percentage of women. The participants were recruited on a random basis from the Swedish population register and information regarding the sex of each participant was collected by self-reporting via a questionnaire. These data are indicated in Extended Data Table 2a and 2b.
Reporting on race, ethnicity, or other socially relevant groupings	This study was conducted in a subset of participants from the PESA study (Progression of Early Subclinical Atherosclerosis), an ongoing observational prospective cohort study of caucasian asymptomatic employees of the Santander Bank in Madrid (PMID: 34238438). The information regarding race and other socially relevant groupings of each participant was collected by self-reporting via a questionnaire and was not used as exclusion factor. In the IGT study, the population includes men and women aged 50-64 years from the Gothenburg area (Sweden), recruited on a random basis from the Swedish population register. Among the exclusion criteria, cognitive dysfunction and inability to understand written and spoken Swedish as well as subjects born outside Sweden were included.
Population characteristics	This study was conducted in a subset of participants (n=400) from the PESA-CNIC-Santander. Based on multiterritorial/ multimodal imaging (i.e. 2D/3D vascular ultrasonography and non-contrast computed tomography), we classified 295 participants with subclinical atherosclerosis and 105 controls without atherosclerosis. Extent of atherosclerosis was defined according to the number of affected vascular sites and the coronary artery calcium (CAC) score. Subjects with subclinical atherosclerosis were further stratified by Fluorodeoxyglucose (18F-FDG) uptake and bone marrow activation in: inactive atherosclerosis (FDGneg, n=74), arterial 18 F-FDG uptake (FDG+_A, n=57), bone marrow activation (FDG+_BM, n=40), and both arterial uptake and bone marrow activation (FDG+_SYS, n=124) as indicatives of metabolically active atherosclerosis (FDG+, n=221). Detailed population characteristics and group allocation are presented in Extended Data Table 1a,b and Table 3 and Extended Data Fig. 2c. The IGT study population is composed of men and women aged 50-64 years from the Gothenburg area (Sweden), recruited on a random basis from the Swedish population register and included based on their glucose status. The applied exclusion criteria included: known diabetes, inflammatory diseases (e.g. Crohn's disease, ulcerative colitis, rheumatic diseases), treatment with steroids or immunomodulatory drugs, cancer (unless relapse free for the preceding 5 years), cognitive dysfunction, treatment for infectious diseases and with antibiotics in the past three months, inability to understand written and spoken Swedish as well as subjects born outside Sweden. Atherosclerosis in the carotid arteries was assessed by ultrasound imaging using a standardized protocol including a Siemens Acuson S2000 ultrasound scanner and by presence of coronary artery calcium assessed by CT scan. Systolic blood pressure (SBP) was measured twice with an automatic device (Omron M10-IT, Omron Health care Co, Kyoto, Japan) and the mean of the measurements was used. A questionnaire was used to collect detailed information on medication and family history. A venous blood sample (100 mL) was collected from participants after an overnight fast and was used for immediate biochemical analysis of glucose, glycated haemoglobin and total cholesterol. SCORE2 (Systematic Coronary Risk Evaluation) was calculated as previously. Body weight, height waist and hip circumferences were measured on calibrated equipment with subjects dressed in light clothing without shoes and according to current WHO recommendations. Characteristics of the participants are listed in Extended Data Table 2,b.
Recruitment	Participants were recruited following the inclusion criteria of the main PESA-CNIC-Santander study (NCT01410318, https://clinicaltrials.gov/ct2/show/NCT01410318). Exclusion criteria included those previously used for the main study and those that could affect imidazole propionate production or associations previously described (e.g. type 2 diabetes): 1. Active treatment for inflammation or cardiovascular disease. 2. Participants with family history inflammatory illness 3. Active treatment for cancer, history of transplant with active immunosuppressive or immunomodulator treatment. 4. Employees who are pregnant or breast-feeding. 5. Clinically relevant renal or hepatic insufficiency. 6. Unable to consent 7. Any other clinically significant condition that may jeopardize the study or be dangerous for the participant. 8. Active drug or alcohol abuse. 9. Participants taking antibiotics in the three months prior to the collection of the faecal samples. 10. Participants taking proton pump inhibitors, laxatives, and non-steroidal anti-inflammatory drugs 3 days prior to the collection of the faecal sample. 11.Subjects with known Type 2 diabetes or treated for diabetes. 12. Subjects with intestinal disorders. IGT cohort (Ethics Dnr 560-13) was recruited n a random basis from the Swedish population register and included based on their glucose status. The applied exclusion criteria included: known diabetes, inflammatory diseases (e.g. Crohn's disease, ulcerative colitis, rheumatic diseases), treatment with steroids or immunomodulatory drugs, cancer (unless relapse free for the preceding 5 years), cognitive dysfunction, treatment for infectious diseases and with antibiotics in the past three months, inability to understand written and spoken Swedish as well as subjects born outside Sweden. The participants were invited by letter and gave all informed consent. Despite the application of rigorous recruitment and exclusion criteria, several potential sources of bias should be acknowledged. First, self-selection bias may be present, as participation was voluntary; individuals who chose to enroll may differ systematically from those who declined, potentially leading to a study population with higher health literacy or distinct risk profiles compared to the general population. In the PESA-CNIC-Santander subset, participants were all employees of Santander Bank in Madrid, which may introduce a "healthy worker effect" and limit the cohort's socioeconomic and

occupational diversity. Moreover, the exclusive inclusion of Spanish individuals in the PESA cohort and Swedish individuals in the IGT cohort may introduce geographic and population-related bias. As a result, the findings may not fully represent the spectrum of cardiovascular risk or disease presentation seen in more ethnically and socioeconomically diverse or non-European populations. In the IGT cohort, although recruitment was population-based, the exclusion of non-Swedish-born individuals and those unable to understand Swedish may have introduced additional cultural or socioeconomic bias.

Ethics oversight

The Instituto de Salud Carlos III research ethics committee approved the study protocol (CEI PI 32_2018) and all participants were provided with a written informed consent.
All participants gave informed consent, and IGT study was approved by the Ethics Review Board in Gothenburg (560-13)

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

To calculate the sample size needed for a study in mice with the given parameters (two-tailed test, unpaired samples, significance level of 0.05, power of 0.8, and detecting at least a 1 standard deviation difference), we use the formula for sample size estimation in a two-sample t-test. These numbers were refined based on our previous knowledge of the reproducibility, balancing statistical robustness and animal welfare, following the reduction criterium. The formula for the sample size (per group) in a two-sample t-test is given by:

n≈2 · (Z1−α/2+Z1−β)2 · SD2/d2

Where:

n: Sample size per group

Z1−α/2: Critical value for significance level (e.g., 1.96 for α=0.05)

Z1−β: Critical value for power (e.g., 0.84 for 80% power)

SD: Standard deviation (assumed equal between groups)

d: Effect size (minimum detectable difference between group means)

For our parameters, we would need approximately 15.7 mice per group to achieve a significance level of 0.05, a power of 0.8, and detect at least a 1 standard deviation difference. We increase the sample group in 1 mouse to be able to discard a mouse where the experimental technique may fail. Total sample size is usually about 17 mice to be tested in (at least) two independent experiments.

For the human cohorts (PESA and IGT), no sample size calculation was conducted, as samples were retrospectively collected from observational studies. Instead, sample sizes were determined based on the availability of well-characterized patients with complete clinical, imaging, and biosamples from ongoing prospective observational studies. The cohorts were assembled with the goal of maximizing biological and phenotypic depth rather than targeting a predefined effect size. The inclusion of two independent cohorts with complementary phenotypes enabled replication and validation across datasets. Post hoc power considerations indicated that the sample size of the derivation cohort (PESA cohort, n=400) was adequately powered at the 0.85 level to detect a minimum 5nMol difference in ImP levels between the group of subjects without atherosclerosis and patients without atherosclerosis. A ratio of 1:3 between patients without and with atherosclerosis was selected to reflect the distribution of AT in the PESA cohort, and measures of dispersion (i.e., standard deviation) were derived from the group of subjects without atherosclerosis in the PESA cohort. Respectively, the IGT cohort was adequately sized to detect with 0.85 power a difference of 2 nMoL in ImP levels between subjects without and with AT. Power considerations were based on the Mann-Whitney test for 2 independent groups. Type I error was prespecified at 0.05 for power calculations. Power analysis was performed with G*Power 3.1.9.6 (University of Kiel., Germany).

Data exclusions

No data were excluded from the analyses shown in the manuscript.

Replication

For mice and primary cell culture experiments, multiple independent biological replicates and independent experiments (at least twice) were performed, as indicated in figure legends. The results from the paper are consistent across multiple experimental approaches performed by different researchers. All attempts at replication obtained consistent data.

Randomization

Mice were homogeneous in terms of age (8 weeks) and randomly assigned to different cages for experimental treatments (antibiotics, imidazole propionate and AGN192403 administration). Human and animal samples were analysed in a randomized fashion on the mass spectrometer.

Blinding

For animal studies and cell-based in vitro studies, numbers were assigned to each experimental sample and analysis was blinded to group allocation during data collection.
For human study, samples were collected by blinded clinical staff and all data were generated by investigators that were blinded to the

metadata. Once data were generated, computational analysis was performed with all of the necessary clinical information to test between groups.

Behavioural & social sciences study design

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

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

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

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

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

Brilliant Violet 650m anti-mouse CDllc Antibody ;Biolegend;117339; Clone N418 PE anti CDllc;BD bioscience;553802; Clone HL3
PE anti-mouse NK-1.l Antibody ;Biolegend;l56504; Clone S17016D
FITC Anti-Mouse CD3e;Tonbo;35-0031-U500; clone 145-2C11
Anti-mouse B220-FITC;eBioscience;ll-0452-82; clone RA3-6B2
PerCP anti-mouse/human CD45R/B220 Antibody;Biolegend;l03234; Clone RA3-6B2
Anti-Human/Mouse CD45R (B220) PerCP-Cy5.5;eBioscience;45-0452-82; clone RA3-6B2
Brilliant Violet 605m anti-mouse/human CDllb Antibody;Biolegend;l01237; Clone M1/70
anti-mouse CDllb eFluor 660;eBioscience;50-0112-80; Clone M1/70
Anti-Mouse CDllb PerCP-Cyanine5.5;eBioscience;45-0112-82; Clone M1/70
Anti-mouse CD206-eF450 ;eBioscience;48-2069-42; Clone 19.2
PE anti-mouse CD31 Antibody;Biolegend;l02407; Clone 390
Anti-mouse CD4-BV570;Biolegend;l00542; Clone RM4-5
PE/Cy7 anti-mouse CD4 Antibody;Biolegend;l00528 ; Clone RM4-5
Anti-Mouse CD45 APC-eFluor" 780;eBioscience;47-0451-82 ; Clone 30-F11
PE anti-mouse CD49b (pan-NK cells) Antibody;Biolegend;l08908 ; Clone DX5
Anti-mouse CD8-PE;eBioscience;12-0081-83; Clone 53-6.7
CD86 (B7-2) Monoclonal Antibody (Gll), PE-Cyanine7;eBioscience;25-0862-80 ; Clone GL1
CD90.2 Rat anti-Mouse, BUV805, Clone: 53-2.l;BD Biosciences;BDB741908; Clone 53-2.1
BV786 Rat Anti-Mouse CD90.2 Clone 30-H12 (RUO);BD Biosciences; 740841; Clone 53-21
PerCP/CyS.5 anti-mouse F4/80 Antibody;Biolegend;123128; Clone BM8
anti-mouse/rat Foxp3 FITC FJK-16s;eBioscience;ll-5773-82; Clone FJK-16s
Anti-mouse Ly6C-BV711;Biolegend;128037 ; Clone HK1.4
ANTI-MOUSE LY6G PE ;BD PHARMINGEN;551461; Clone 1A8
APC rat anti-Mouse Ly6G ;BD PHARMINGEN;560599; Clone 1A8
anti-Ly6G v450;BD PHARMINGEN;560603; Clone 1A8
Alexa Fluor" 700 anti-mouse I-A/1-E [M5/114.15.2];Biolegend; 107622 ; Clone M5/114.15.2
Anti-mouse MHCII-UV737;BD Biosciences;367-5321-82; Clone M5/114.15.2
Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb;Cell signaling;4858S; Clone D57.2.2.E Anti-Mouse ROR gamma (t) APC;eBioscience;l 7-6981-80 ; Clone B2D
PE Rat Anti-Mouse Siglec-F Clone ES0-2440 (RUO);BD PHARMINGEN;552126; Clone E50-2440
Anti-mouse SiglecH FITC;eBioscience;ll-0333-81; eBIO440c
Anti-mouse T-bet-PECF594;BD Biosciences;562467; Clone O4-46
Mouse VCAM-1/CD106 Alexa Fluor® 594-conjugated Antibody;biotechne;FAB6432T; Clone 112734
Brilliant Violet 78S'M anti-mouse/rat XCRl Antibody;Biolegend;148225 ; Clone ZET

Validation

All antibodies are validated by the manufacturer

https://www.biolegend.com/en-gb/products/brilliant-violet-650-anti-mouse-cd11c-antibody-8840
https://www.fishersci.com/shop/products/anti-cd11c-r-pe-clone-hl3bd/BDB553802
https://www.biolegend.com/en-us/products/pe-anti-mouse-nk-1-1-antibody-16926?GroupID=GROUP20
https://www.citeab.com/antibodies/2204479-35-0031-u500-anti-mouse-cd3e
https://www.thermofisher.com/antibody/product/CD45R-B220-Antibody-clone-RA3-6B2-Monoclonal/11-0452-82
https://www.biolegend.com/fr-ch/search-results/percp-anti-mouse-human-cd45r-b220-antibody-4266?Clone=RA3-6B2
https://www.thermofisher.com/antibody/product/CD45R-B220-Antibody-clone-RA3-6B2-Monoclonal/45-0452-82
https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-human-cd11b-antibody-7637?GroupID=BLG10530
https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/50-0112-82
https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/45-0112-82
https://www.thermofisher.com/antibody/product/CD206-MMR-Antibody-clone-19-2-Monoclonal/48-2069-42
https://www.biolegend.com/nl-nl/products/pe-anti-mouse-cd31-antibody-122
https://www.biolegend.com/nl-be/products/brilliant-violet-570-anti-mouse-cd4-antibody-7379
https://www.biolegend.com/en-us/cell-separation/pe-cyanine7-anti-mouse-cd4-antibody-1932
https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/47-0451-82
https://www.biolegend.com/en-gb/products/pe-anti-mouse-cd49b-pan-nk-cells-antibody-234?GroupID=BLG4768
https://www.citeab.com/antibodies/2037994-12-0081-82-cd8a-monoclonal-antibody-53-6-7-pe-eb
https://www.thermofisher.com/antibody/product/CD86-B7-2-Antibody-clone-GL1-Monoclonal/25-0862-82
https://www.fishersci.com/shop/products/cd90-2-rat-anti-mouse-buv805-clone-53-2-1-bd-biosciences/BDB741908
https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-f480-antibody-4303?GroupID=BLG5319
https://www.citeab.com/antibodies/2041744-11-5773-82-foxp3-monoclonal-antibody-fjk-16s-fitc
https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-ly-6c-antibody-8935?GroupID=BLG7242
https://www.citeab.com/antibodies/2410368-551461-bd-pharmingen-pe-rat-anti-mouse-ly-6g
https://www.citeab.com/antibodies/2410938-560599-bd-pharmingen-apc-rat-anti-mouse-ly-6g
https://www.citeab.com/antibodies/2408945-560603-bd-horizon-v450-rat-anti-mouse-ly-6g
https://www.biolegend.com/fr-ch/products/alexa-fluor-700-anti-mouse-i-a-i-e-antibody-3413
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-rat-anti-mouse-i-a-i-e.742894
https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser235-236-d57-2-2e-xp-rabbit-mab/4858
https://www.labome.com/product/Invitrogen/17-6981-80.html
https://www.citeab.com/antibodies/2411876-552126-bd-pharmingen-pe-rat-anti-mouse-siglec-f
https://www.labome.com/product/Invitrogen/11-0333-82.html
https://www.bdbiosciences.com/en-de/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cf594-mouse-anti-t-bet.562467
https://www.bio-techne.com/p/antibodies/mouse-vcam-1-cd106-alexa-fluor-594-conjugated-antibody-112734_fab6432t
https://www.biolegend.com/fr-ch/products/brilliant-violet-785-anti-mouse-rat-xcr1-antibody-16711

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Mouse aortic endothelial cells (MAECs) were isolated from mouse thoracic aortas as described previously23. Cells were cultured in Medium 199 (Gibco, Invitrogen Life Technologies) + 20% fetal bovine serum + Penicillin/Streptomycin 2 mM + Glutamine 2 mM + HEPES 10 mM + endothelial cell growth supplement 30μg/mL + Heparin 100 mg/mL, all from Sigma–Aldrich, under 5% CO2 at 37ºC.

Mouse embryo fibroblast (MEF) cell lines were isolated from 13.5 dpc from embryos using standard protocol. Each embryo was dissected into 10 ml of sterile PBS, voided of its internal organs, head, and legs. After 30 min incubation with gentle shaking at 37°C with 5ml 0,1 % trypsin, cells were plated in two 100 mm dishes and incubated for 24-48 h.To establish the immortalized MEF lines, early passage MEFs were seeded in 60 mm plates and infected with 10E5 IU/ml packaged retrovirus carrying the human papillomavirus (HPV) 16 E6/E7 genes. Selection was performed with 400 μg/ml of G418 during 10 days.

L929 cell line (ATCC® CCL-1TM) used for production of the M-CSF supernatant, was grown on 175 cm2 cell culture flasks (Stemcell) and resuspended in RPMI 1640 (Sigma) supplemented with 10 % heat-inactivated fetal calf serum (FCS, Sigma), 1 mM pyruvate (Lonza, Bassel, Switzerland), 100 μM non-essential aminoacids (Thermo Fisher Scientific, Walthman, MA), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all three from Lonza) and 50 μM 2-mercaptoethanol (Merck, Darmstad,Germany), herein called R10. Supernatants were obtained by filtering 15-days long cultures through 0.22μm Stericup Filter units (Merck Millipore) and were used to subsequently supplement the medium for the generation of bone marrow derived macrophages.

Bone marrow derived macrophages (BMDMs) were generated as previously described with some modifications. BM cells from WT C57BL/6J mice were cultured in RPMI 1640 supplemented with 30 % M-CSF obtained from L929 cell line and 10 % FBS, 100 μg/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 1nM sodium pyruvate (all from Gibco) during 5 days in sterile, but not tissue-culture treated, 10-cm Petri dishes.

Authentication

L929 were directly purchased from ATCC (ATCC® CCL-1TM). The rest of cells used were primary lines. Primary cell lines were identified by morphology and phenotype characterization by flow cytometry.

Mycoplasma contamination

All cells lines were continuously surveyed and tested negative for mycoplasma contamination by PCR.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in the study

Palaeontology and Archaeology

Specimen provenance | *Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*

Specimen deposition | *Indicate where the specimens have been deposited to permit free access by other researchers.*

Dating methods | *If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight | *Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

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 | All mice (Mus musculus) were housed at an ambient temperature around 21-23 C°, humidity of 40-60% and a light dark cycle of 12 hours. Mice were bred and maintained in groups of 5 animals per cage at the CNIC under specific pathogen-free conditions.
We used proatherogenic background B6.129P2-Apoetm1Unc/J mice (The Jackson Laboratory,strain 002052, ApoE−/−); and B6.129S7-Ldlrtm1Her/J mice (The Jackson Laboratory, strain 002207, Ldlr−/−) backcrossed with B6.SJL-Ptprca Pepcb/BoyJ mice expressing the CD45.1 congenic marker (The Jackson Laboratory, strain 002014, B6 CD45.1). For initiation of experiments, 6-8 week-old male mice were used.
Rag1tm1Mom/J mice (The Jackson Laboratory, strain 002216, Rag1–/–); B6.Cg-Rptortm1.1Dmsa/J mice (The Jackson Laboratory, strain 013188, RaploxP) were mated with B6.129P2-Lyz2tm1(cre)Ifo/J mice (The Jackson Laboratory, strain 004781, Lyz2-Cre) to generate Lyz2ΔRaptor (Cre+) and control Raptorf/f (Cre−) littermates used as controls. Sperm from B6.Nischtm1a(EUCOMM)Hmgu>/H mice (Mary Lyon Centre at MRC Harwell, United, EM:08808) was used for in vitro fertilization of ROSA-Flpe mice (Gt(ROSA)26Sortm1(FLP1)Dym) to generate Nischf/f mice (tm1c). Nischf/f mice were mated with Lyz2-Cre (B6.129P2-Lyz2tm1(cre)Ifo/J) mice to generate Lyz2ΔNisch (Cre+) and control Nischf/f (Cre−) littermates used as controls.

Wild animals | No wild animals were used in the study

Reporting on sex | Our findings in mouse models include both males and females. The human cohorts also include female participants that account for the 22% and the 54% of the whole PESA and IGT cohorts, respectively.

Field-collected samples | No field collected samples were used in the study.

Ethics oversight | Animal studies were approved by the local ethics committee. All animal procedures conformed to EU Directive 2010/63EU and Recommendation 2007/526/EC regarding the protection of animals used for experimental and other scientific purposes, enforced by Spanish law under Real Decreto 1201/2005.

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 | The discovery cohort was conducted in a subset of participants from the PESA-CNIC-Santander study (Progression of Early Subclinical Atherosclerosis): NCT01410318 (https://clinicaltrials.gov/ct2/show/NCT01410318).
The IGT cohort used for the validation was approved by Ethics Review Board in Gothenburg (IGTM [560-13])

Study protocol | Volunteers were recruited following the inclusion and exclusion criteria of the main PESA-CNIC-Santander study https://clinicaltrials.gov/ct2/show/NCT01410318.
Exclusion criteria for the IGT study were: known diabetes, inflammatory diseases (e.g. Crohn's disease, ulcerative colitis, rheumatic diseases), treatment with steroids or immunomodulatory drugs, cancer (unless relapse free for the preceding 5 years), cognitive dysfunction, treatment for infectious diseases and with antibiotics in the past three months, inability to understand written and spoken Swedish as well as subjects born outside Sweden.

Data collection | For the PESA study, plasma and faecal samples were collected from December 2018 to May 2021. Metabolomics data were collected and analysed in 2021. 16S gene sequencing data was collected and analysed in 2022.
In the IGT cohort, men and women aged 50 to 64 years and born in the region of Gothenburg, Sweden, were screened between 2013

and 2018. Metabolomics data were collected and analysed in 2023.

Outcomes

For the PESA ohort, blood and stool samples were collected from the participants followed by targeted mass spectrometry and 16S rRNA gene sequencing, respectively. Subclinical atherosclerosis was assessed by assessed by imaging studies including 2D and 3D vascular ultrasonography of carotid and iliofemoral arteries and presence of coronary artery calcium assessed by CT scan (PMID:24268213). Subjects with subclinical AT underwent a whole body 18F-FDG PET/MRI study to characterize arterial 18F-FDG uptake and bone marrow metabolic activity (PMID:30922468 and PMID: 35567559, respectively). Fasting blood test included biochemistry and determination of high-sensitivity C-reactive protein (hs-CRP). In this study, hypertension (HTN) was as systolic > 130 mmHg and/or diastolic > 85 mmHg and/or subjects taking antihypertensive drugs. Dyslipidaemia was defined as total cholesterol ≥ 240 mg/dL, LDL-cholesterol ≥160 mg/dL, HDL-cholesterol < 40 mg/dL, or use of lipid-lowering drugs. Anthropometry and bioelectrical impedance analysis assessments were performed during the same appointment for each participant. Height and weight were measured using calibrated equipment (Tanita BC-545N,) with participants wearing underwear and barefoot. BMI was calculated as weight (kg) divided by height squared (metre).
For the IGT cohort blood samples were collected from the participants followed by targeted mass spectrometry. Atherosclerosis in the carotid arteries was assessed by ultrasound imaging using a standardized protocol including a Siemens Acuson S2000 ultrasound scanner and by presence of coronary artery calcium assessed by CT scan. Systolic blood pressure (SBP) was measured twice with an automatic device (Omron M10-IT, Omron Health care Co, Kyoto, Japan) and the mean of the measurements was used. A questionnaire was used to collect detailed information on medication and family history. SCORE2 (Systematic Coronary Risk Evaluation) was calculated as previously. Body weight, height waist and hip circumferences were measured on calibrated equipment with subjects dressed in light clothing without shoes and according to current WHO recommendations.

The targeted metabolomics analyses showed that imidazole propionate (ImP), but not histidine or urocanic acid, plasma concentrations were selectively increased in subjects with subclinical atherosclerosis when compared to controls. Linear and nonlinear associations were also observed between ImP concentrations and atherosclerosis and extent of atherosclerosis, respectively. The prevalence of atherosclerosis endpoints and the CAC score also increased across ascending ImP tertiles. These findings were validated in an independent cohort, the IGT cohort. ImP was also found significantly increased in subjects with metabolically active atherosclerosis (FDG+) when compared to inactive atherosclerosis, particularly those with bone marrow activation (FDG+_BM) and systemic inflammation (FDG+_SYS). After adjusting for traditional risk factors in both cohorts, higher ImP levels were independently associated with main atherosclerosis outcomes, suggesting high ImP levels as indicator of heightened atherosclerosis risk. Notably, ImP also showed additive value when added to established blood-derived atherosclerosis biomarkers such as LDL-cholesterol and high sensitive C reactive protein (hs-CRP) in discriminating atherosclerosis prevalence in both cohorts and active atherosclerosis in the PESA cohort. These findings reveal a strong association between ImP and atherosclerosis, particularly active atherosclerosis, suggesting its potential use as an indicator of early active atherosclerosis before other co-morbidities become confounding factors.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks: Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes: Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication: Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.: For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission: Provide a list of all files available in the database submission.

Genome browser session (e.g. UCSC): Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates: Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth: Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies: Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters: Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality: Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software: Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

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: For blood samples, after erythrocytes lysis buffer, single cell suspensions were stained for 30 min at 4°C with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Life Technologies). After washing with PBS, cells were stained in FACS buffer containing anti-CD16/32 (BioXcell), 3 % FBS and 0.05 % EDTA with the corresponding antibody cocktail for 30 min on ice. Cells were stained with a cocktail of antibodies.

For cells infiltrating the in aorta, mice were perfused with 10 ml of Phosphate Buffered Saline (PBS) via cardiac puncture to remove blood contamination from vascular tissue.

The aortas were kept in cold Dulbecco's Modified Eagle Medium (DMEM) to be digested. Perivascular fat was removed and the thoracic aorta with arch was opened longitudinally and cut into smaller pieces that were incubated for 30 min at 37oC in water bath in digestion buffer (Collagenase A (25 mg/ml), (Roche/Sigma #10103586001). Dispase II (25 mg/ml), (Roche/Sigma #04942078001), DNase I (250µg/ml) (Roche/Sigma #10104159001) elastase (25 µg/ml) and Libersase TL (0.2 Wünsch units/mL) Cat#5401119001. After incubation time the digested tissue was mixed by pipetting, filtered through a 70 µm strainer and spinned at 400g 5 min 4C. Single cells were stained with a cocktail of antibodies.

Instrument

LSRFortessa SORP (BD Biosciences)
FACSymphony (BD Biosciences)
FACSAria Cell Sorter (BD Biosciences)

Software

FlowJo software version 10 (TreeStar)

Cell population abundance

The number of cells is indicated in the respective graph corresponding to each population.

Gating strategy

Gating strategy on blood and aorta tissues:

1. FSC and SSC
2. FSC-H and FSC-A to gate on single cell population
3. Viability to gate on live cells, this is defined as negative cells to staining
4. CD45+ cells
5. CD90 cells for T cells, B220 cell for B cells and non t or b cells as double negative.
6. In CD90-;B220-; was further gated on Ly6C high Monocytes
7. In CD90-;B220-; was further gated on F4/80+CD86+ and F4/80+CD11c+ (Macrophages)
8. CD90 cells was further gated CD4+Rorg-t+ (Th17), CD4+t-Bet+ (Th1), CD4+Foxp3+ (Treg)

Gating strategy on blood to isolated monocytes:

1. FSC and SSC
2. FSC-H and FSC-A to gate on single cell population
3. Viability to gate on live cells, this is defined as negative cells to staining
4. CD45+ cells
5. CD3-, B220-, NK1.1-, LY6G-, CD49-, SiglecF- cells
6. Monocytes was gated as CD11B+ and Ly6C+/-

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

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template | *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal | *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring | *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings | *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested | *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference | *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

(See Eklund et al. 2016)

Correction | *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity | *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis | *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis | *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*