

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

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	No software was used to collect data.
Data analysis	Statistical analyses was performed using Graphpad Prism 9 (v9.5.0), Qjagen's Ingenuity Pathway Analysis platform (v01-22-01), and the Seurat (v4.3.0), CellChat (v1.1.3), propeller (1.1.0), and ggpubr (v0.6.0) packages as well as base functions in R (v4.0.3). Single-cell RNAseq data were analyzed with the Seurat (v4.3.0) and CellChat (v1.1.3) packages in R (v4.0.3). Cytometry by time-of-flight was analyzed on the Cytobank Premium online platform (Beckman Coulter Life Sciences) and later using the cytoqc (v0.99.2), flowWorkspace (v4.14.3), flowCore (v2.14.2), and cytofCore (v0.4) packages in R (v4.0.3). Pathway analysis was performed with Qjagen's Ingenuity Pathway Analysis software (v01-22-01) Code used for data analysis will be made available on GitHub at https://github.com/giannarelli-lab

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](#)

Single-cell RNA sequencing (scRNA-seq) data from human carotid plaque specimens that were previously published are available via the Gene Expression Omnibus (GEO) hosted by NCBI (GSE224273 and GSE235437). Similarly, scRNA-seq from previous mouse studies can be found on the same platform (GSE141038, GSE161494, GSE168389, and GSE252243). Previously published human coronary artery scRNA-seq data can be accessed from GSE252243. Additional high-throughput sequencing data from previously unpublished human carotid plaque specimens, coronary artery tissue, and blood, as well as murine plaque scRNA-seq, can be accessed from GSE246318. Data from scRNA-seq of human carotid plaque, coronary tissue, and PBMC were aligned against GRCh38 Ensembl 93, 107, and 98, respectively. Human PBMC CITE-seq was aligned against GRCh38 Ensembl 93 and mouse aortic arch scRNA-seq was aligned against GRCm38 Ensembl 84. Coronary tissue scRNA-seq was also aligned against the SARS-CoV-2 genome (GCA_009937905.1). Human CyTOF raw data are available at <https://zenodo.org/records/12734792>.

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	We refer to biological sex in the manuscript. We did not actively select the sex or gender of the enrolled patients.
Reporting on race, ethnicity, or other socially relevant groupings	We do not refer to race, ethnicity or any other socially relevant grouping in the manuscript. We did not actively select the race, ethnicity or other socially relevant grouping of the enrolled patients.
Population characteristics	Supplementary Tables 1, 2, 3, 4, and 6 describe the demographics and clinical history of patients enrolled in the ATHERO-IN (Atherosclerosis-Inflammation) study. Supplementary Tables 7 and 8 describes the demographics and clinical history of patients enrolled in the CHORD (Cholesterol lowering and residual risk in type 2 diabetes) study.
Recruitment	ATHERO-IN Twenty-two participants undergoing carotid endarterectomy (CEA) and five patients presenting end-stage heart failure and undergoing orthotopic heart transplantation were enrolled in this study, which was approved by the Institutional Review Board (IRB) of New York University (NYU) Langone Health (IRB no. 21-00429) and the Icahn School of Medicine at Mount Sinai (IRB no. 11-01427). All patients were over 18 years old and gave informed written consent. Exclusion criteria: current infection, autoimmune disease, active or recurrent cancer, severe renal failure requiring dialysis or peripheral arterial occlusive disease-causing pain at rest. CHORD De-identified PBMC specimens from 12 subjects with or without type 2 diabetes (T2D) enrolled in this study (NCT04369664) at NYU Langone Health (IRB no. 19-01964) were obtained for scRNAseq analysis. All subjects were between 18 and 90 years old, had elevated plasma cholesterol levels (LDL-c > 100 mg/dl or Lp(a) > 50 mg/dl) with or without T2D, and gave informed written consent. Exclusion criteria for subjects with T2D: established cardiovascular disease on antithrombotic therapy, triglycerides > 250 mg/dl, use of a PCSK9 inhibitor, HbA1c > 10%, recent infection in the past 30 days, any hospitalization in the past 30 days, use of immunosuppressive therapy, use of any antithrombotic therapy, use of aspirin, use of NSAID within the past 72 h, pregnancy, anemia (hemoglobin < 9 g/dl), thrombocytopenia (platelet count < 75 × 1000 per µl), thrombocytosis (platelet count > 600 × 1000 per µl), a history of severe bleeding or bleeding disorders and chronic kidney disease (CrCl < 30 ml/min). Exclusion criteria for subjects without known diabetes included: diabetes (type 1 or type 2) and all exclusions for subjects with T2D.
Ethics oversight	Patients undergoing CEA or orthotopic heart transplantation were enrolled in the ATHERO-IN study, an observational clinical study approved by the IRB of the Icahn School of Medicine at Mount Sinai (IRB no. 11-01427) and NYU Langone Health (IRB no. 21-00429). Patients with elevated plasma cholesterol levels with or without T2D were enrolled in the CHORD study at NYU Langone Health (IRB no. 19-01964).

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	No a-priori sample size calculation was performed for the study. We have included all possible samples that were available at the time of the experiments.
Data exclusions	No samples were excluded from the analysis.
Replication	A minimum of two-three biological replicates per group or condition were used in all comparisons, with the exception of the in vitro ICI experiment followed by CITE-seq, for which we were able to validate transcriptomic phenotypes at the surface protein level. Statistical analyses showed replicable results across experiments.
Randomization	Since the tissues were processed from prospectively enrolled patients, we were unable to employ randomization.
Blinding	We used unbiased computational approaches to analyze the data. Investigators were blinded to the study conditions and experimental designs for the analysis, so blind group allocation during data collection was not relevant to this study. In addition, blinding was not possible in many cases human study participants were recruited prospectively and specimens used in experiments were subjected to both treatments and controls whenever possible.

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 in multiplex staining experiments: anti-CD3e (Akoya Biosciences, 4550119; EP449E; 1:200), anti-CD11c (Akoya Biosciences, 4550114; 118/A5; 1:200), anti-PD-L1 (Akoya Biosciences, 4550072; 73-10; 1:100), and anti-PD-1 (Akoya Biosciences, 4550038; D4W2J; 1:200). The following antibodies were used in single-plex immunofluorescent staining experiments: anti-CD86 (abcam, ab220188; C86/1146; 1:100), anti-CD4 (abcam, ab133616; EPR6855; 1:100), anti-CTLA4 (Invitrogen, PA5-47547; polyclonal; 1:100), anti-Galectin 3 (Invitrogen, 14-5301-82; eBioM3/38; 1:100), anti-CD8a (abcam, ab17147; C8/144B; 1:100), anti-LAG3 (abcam ab209236; EPR20261; 1:100), anti-CD3e (abcam, ab11089; CD3-12; 1:100), anti-CD11c (Invitrogen, MA1-46052; 3.9; 1:200), and anti-Fascin 1 (Invitrogen, MA5-32728; JM12-53; 1:100). The following antibodies were used in immune checkpoint inhibition experiments in vitro followed by CITE-seq: anti-human PD-1 (Bio X Cell, SIM0003), and anti-human CTLA4 (Bio X Cell, SIM0004). The following antibodies were used in the purification of murine plaque immune cells: TruStain FcX (anti-mouse CD16/32; Biolegend, 101319; 93; 1:100) and PerCP/Cyanine5.5 anti-mouse CD45 (Biolegend, 103132; 30-F11; 1:100). The following antibodies were used for intracellular staining in the PD-1 inhibition experiment in vitro (CyTOF): Anti-Human IFNγ-165Ho (Standard BioTools, 3165002B, B27; 1:50), Anti-Human IL-2-112Cd (Standard BioTools, 3112002B; MQ1-17H12; 1:50), Anti-Human Perforin1-175Lu (Standard BioTools, 3175004B, B-D48; 1:50), and Anti-Human Granzyme B-198Pt (Standard BioTools, 3198002B; GB11; 1:50). Corresponding information about the antibodies used for surface staining in CyTOF experiments appears in Supplementary Tables 11 and 12. Usage is described in the Methods section of the manuscript.
Validation	All primary antibodies were validated by the corresponding manufacturer for use in human or mouse in their respective applications (single- or multiplex immunofluorescence, FACS, CyTOF) and using control samples to confirm specificity. Antibodies used in inhibition experiments were validated by Western blot by Bio X Cell.

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	We used eight-week-old Ldlr knockout mice (The Jackson Laboratory, 002207) housed in 12-hour light/dark cycles, at 20–40 °C, and 30–70% humidity.
Wild animals	We did not use wild animals in this study.
Reporting on sex	We refer to biological sex in the manuscript but did not actively select the sex or gender of the enrolled patients.
Field-collected samples	No samples in this study were field-collected.
Ethics oversight	All experimental procedures were done in accordance with the US Department of Agriculture Animal Welfare Act and the US Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the NYU Grossman School of Medicine.

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	NCT04369664
Study protocol	All participants (with and without T2D) in the CHORD study were given evolocumab (a cholesterol-lowering medication) plus atorvastatin or ezetimibe for one month with the same risk factors being measured following cholesterol reduction. They were asked to undergo blood draw, receive study medication, and undergo additional optional vascular health testing including endothelial cell harvesting and glycocalyx (tongue probe). A detailed protocol can be found at: https://clinicaltrials.gov/study/NCT04369664?cond=NCT04369664&rank=1
Data collection	For every study participant, blood draws from which peripheral blood mononuclear cells were isolated were collected before and after evolocumab treatment at NYU Langone Health between 2020 and 2023.
Outcomes	Primary outcomes were defined as changes in platelet activity before and after evolocumab treatment, as measured by monocyte-platelet aggregate quantification and light transmission aggregometry tests.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA