

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 <i>P</i> 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	Leica MZFLIII Stereo Microscope (Leica Microsystems, Germany) was used to obtain aorta brightfield images. Leica Aperio AT2 slide scanner (Leica Microsystems, Germany) was used to obtain images of H&E and Movat-stained cross sections. Leica TCS SP8 confocal microscope (Leica Microsystems, Germany) was used to obtain immunofluorescence images. Beckman Coulter AU480 Chemistry Analyzer (Beckman Coulter, USA) was used to quantify plasma cholesterol, triglycerides, and glucose. LSR II Flow Cytometer (BD Biosciences, USA) with BD FACSDiva (v6.1.3) was used obtain flow cytometry data. 10x Genomics Chromium platform (10x Genomics, USA) was used to obtain scRNA-seq data. Illumina NovaSeq 6000 (Illumina, USA) was used to obtain bulk ATAC-seq data for human tissue and bulk RNA-seq data for teloHAECs Illumina HiSeq 2500 (Illumina, USA) was used to obtain bulk ATAC-seq data for teloHAECs
Data analysis	ImageJ (v1.54k) was used to quantify images. BD FlowJo (v10.8.1) was used to analyze flow cytometry data. Cell Ranger (v7.2.0) was used to align scRNA-seq data and analysis was conducted using Seurat (v5.1.0) in R (v4.4.1); the following R packages were loaded for data analysis: AnnotationDbi (1.66.0); Augur (1.0.3); biomaRt (2.60.1); CellChat (2.1.2); circlize (0.4.16); cluster (2.1.6); clusterProfiler (4.12.6); ComplexHeatmap (2.20.0); cowplot (1.1.3); data.table (1.17.2); DESeq2 (1.44.0); dplyr (1.1.4); edgeR (4.2.2); enrichplot (1.24.4); enrichR (3.4); ggalluvial (0.12.5); ggradar (0.2); ggh4x (0.2.8); ggplot2 (3.5.2); ggpubr (0.6.0); ggrastr (1.0.2); ggrepel (0.9.6); gplots (3.2.0); harmony (1.2.3); MASS (7.3.61); Matrix (1.7.1); monocle3 (1.3.7); NMF (0.28); org.Hs.eg.db (3.19.1); org.Mm.eg.db (3.19.1); patchwork (1.3.0); PCAtools (2.16.0); pbapply (1.7.2); pheatmap (1.0.12); readr (2.1.5); reshape2 (1.4.4); rstatix (0.7.2); scales (1.4.0);

scraper (1.32.0); Seurat (5.1.0); SeuratWrappers (0.3.5); tidy (1.3.1); tidyverse (2.0.0); tsne (0.1.3.1); UCell (2.8.0); viridis (0.6.5); vsn (3.72.0); WriteXLS (6.7.0); xlsx (0.6.5).
 The PEPATAC pipeline was used to process bulk ATAC-seq data and differential chromatin accessibility analysis was performed with DESeq2 (v1.45.1); ATAC-seq tracks were visualized on the UCSC genome browser; ATAC-seq peaks were annotated to nearest protein coding genes using GenomicRanges (v1.48.0) and Ensembl (v86); candidate transcription factor binding sites were identified using the Cistrome Database (Cistrome DB; 2015); motif deviations were generated using chromVAR (v1.12.0).
 TeloHAEC RNA-seq data was processed with Trimmomatic (v0.32) and aligned with STAR (v2.5.1b) and feature quantification was performed using Salmon (v1.09). DE analysis was performed using DESeq2 (v1.44.0).
 TeloHAEC ATAC-seq data was processed with Trimmomatic (v0.32) and aligned with BWA (v0.7.8) and peaks were called with Genrich.
 Differential peaks analysis was performed using DESeq2 (v1.44.0) and peak annotation was performed with ChIPseeker. HOMER and TOBIAS (v0.14.0) were used for motif enrichment analysis.
 Statistical analysis for non-omics data was performed using GraphPad Prism version 10.1.1 (270) (Dotmatics, USA).

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](#)

All raw and processed mouse aortic intimal scRNA-seq data are available in the Gene Expression Omnibus database (GEO: GSE300155). The raw and processed human carotid artery scRNA-seq dataset is available through GEO: GSE159677.35 The raw and processed human coronary artery bulk ATAC-seq datasets are available through GEO: GSE273054 and GEO: GSE188422. The raw and processed human coronary artery single-nucleus ATAC-seq dataset is available through GEO: GSE175621.39 The raw and processed teloHAEC RNA-seq data are part of a larger dataset that is available through GEO: GSE292727. The raw and processed teloHAEC ATAC-seq data are part of a larger dataset that is available through GEO: GSE292724. Published ChIP-seq data from Ctrl and ERG siRNA-treated HUVECs are available through GEO: GSE124892.12 Published ATAC-seq data from human MRC-5 fibroblasts are available through GEO: GSE121053.82 Sample metadata for human studies are available in the cited publications or GEO datasets. Remaining data generated in this study are provided in the Supplementary Material and Source Data files.

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	Sex and gender were not considered for the study design as the study was not powered to assess these subgroups.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially related groupings data were not available for the human tissue analyzed in this study.
Population characteristics	Human carotid artery biospecimens were obtained at the Peter Munk Cardiac Centre (ON, Canada) from patients undergoing carotid endarterectomy (CEA) that provided written informed consent for research studies as previously described, and according to established clinical guidelines (n = 2 unique samples; median age 81; 50% female). Ischemic and non-ischemic explanted human coronary artery tissue biospecimens from diseased heart transplant donors and normal coronary artery biospecimens from non-diseased donor hearts rejected for orthotopic heart transplantation were obtained with written informed research consent at Stanford University (CA, USA) as previously described (n = 119 unique samples; median age 54; 35% female).
Recruitment	Deidentified individuals were recruited with research consent to REB-approved studies at the University Health Network (ON, Canada) and IRB-approved studies at Stanford University (CA, USA).
Ethics oversight	All human carotid artery research complies with ethical guidelines for human subjects research under approved REB protocols at the University Health Network (no. 18-5282), for the procurement and use of human tissues and information. All human coronary artery research herein complies with ethical guidelines for human subjects research under approved IRB protocols at Stanford University (no. 4237 and no. 11925) and the University of Virginia (no. 20008 and no. 302407), for the procurement and use of human tissues and information, respectively.

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

Life sciences study design

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

Sample size	No sample size calculation was performed. Sample sizes were determined empirically based on experience in our research group or previously published studies in similar areas of research.
Data exclusions	No data was excluded from analyses.
Replication	Each group included at least three replicates for all experiments, except for scRNA-seq analysis (where 6 mice were pooled per single sample). AAV8-PCSK9 atherosclerosis experiments were conducted across three cohorts with successful replication. The effects of Erg knockout were replicated in two models of atherosclerosis (AAV8-PCSK9 induction and Apoe knockout).
Randomization	Sex- and age-matched mice were randomly allocated for experiments.
Blinding	Investigators were not blinded to group allocation, but the analysis of results was performed blinded when possible (e.g., image quantification).

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

Immunofluorescence staining primary antibodies (1:100 unless otherwise stated):
 anti-human/mouse ACTA2-Cy3 (1:1000) (Sigma-Aldrich # C6198): <https://www.sigmaaldrich.com/CA/en/product/sigma/c6198>
 anti-mouse CD68-AF647 (BioLegend # 137003): <https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-mouse-cd68-antibody-6422>
 anti-mouse ERG (Abcam # ab133264): <https://www.abcam.com/en-us/products/primary-antibodies/erg-antibody-epr38642-ab133264>
 anti-human/mouse FN1 (Abcam # ab2413): <https://www.abcam.com/en-us/products/primary-antibodies/fibronectin-antibody-ab2413>
 anti-GFP (Rockland Immunochemicals # 600-101-215): <https://www.rockland.com/categories/primary-antibodies/gfp-antibody-600-101-215>
 anti-human CD31 (Synaptic Systems # HS-351 004): <https://sysy.com/product/HS-351004>
 anti-human/mouse ERG (Abcam # ab110639): <https://www.abcam.com/en-us/products/primary-antibodies/erg-antibody-epr3863-ab110639>
 anti-mouse CD45-AF647 (BioLegend # 103124): <https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-mouse-cd45-antibody-3101>

Immunofluorescence staining secondary antibodies (1:200):
 anti-goat AF488 (Jackson ImmunoResearch # 705-546-147): <https://www.jacksonimmuno.com/catalog/products/705-546-147>
 anti-rabbit AF647 (Jackson ImmunoResearch # 711-606-152): <https://www.jacksonimmuno.com/catalog/products/711-606-152>
 and anti-rabbit Cy3 (Jackson ImmunoResearch # 711-166-152): <https://www.jacksonimmuno.com/catalog/products/711-166-152>
 anti-guinea pig AF488 (Jackson ImmunoResearch # 706-545-148): <https://www.jacksonimmuno.com/catalog/products/706-545-148>
 anti-rabbit AF647 (Jackson ImmunoResearch # 711-606-152): <https://www.jacksonimmuno.com/catalog/products/711-606-152>

Flow cytometry primary antibodies:
 anti-mouse B220-PE-Cy7 (BD Biosciences # 552772): <https://www.bdbiosciences.com/en-fi/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-rat-anti-mouse-cd45r-b220.552772>
 anti-mouse CD115-BV711 (BioLegend # 135515): <https://www.biolegend.com/en-ie/products/brilliant-violet-711-anti-mouse-cd115-csf-1r-antibody-9030>
 anti-mouse CD11b-BV605 (BD Biosciences # 563015): <https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-rat-anti-mouse-cd45r-b220.552772>

reagents/research-reagents/single-color-antibodies-ruo/bv605-rat-anti-cd11b.563015
 anti-mouse CD3-FITC (BD Biosciences # 555274): <https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fits-rat-anti-mouse-cd3-molecular-complex.555274>
 anti-mouse CD45-BV650 (BD Biosciences # 563410): <https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv650-rat-anti-mouse-cd45.563410>
 anti-mouse Ly6C-AF700 (BioLegend # 128024): <https://www.biolegend.com/ja-jp/products/alexa-fluor-700-anti-mouse-ly-6c-antibody-6757>
 anti-mouse Ly6G-APC-Cy7 (BD Biosciences # 560600): <https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-rat-anti-mouse-ly-6g.560600>
 anti-mouse SiglecF-PerCP-Cy5.5 (BD Biosciences # 565526): <https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/percp-cy-5-5-rat-anti-mouse-siglec-f.565526>

Validation

All antibodies used in this study were validated for antigen and application specificity by the manufacturer. Validation methods and associated data are available at the manufacturer URL's provided above.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

ATCC TeloHAEC; Aortic Endothelial Cells; Human (Homo sapiens), Normal (Thermo Fisher Scientific 50-238-1690): <https://www.fishersci.com/shop/products/telohaec-aortic-endothelial-ce/502381690>

Authentication

The teloHAEC cell line used in this study was authenticated by the RNA-seq and ATAC-seq experiments reported in this manuscript.

Mycoplasma contamination

Not tested for mycoplasma contamination

Commonly misidentified lines
(See [ICLAC](#) register)

NA

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

The Cdh5CreERT2; Rosa26lox-STOP-lox-Sun1-sfGFP; Ergfl/fl mice used in this study were generated at the University of Virginia School of Medicine (VA, USA). This strain was derived from the following mice: Tg(Cdh5-cre/ERT2)1Rha mice (MGI # 3848982) obtained from Dr. Ralf Adams (Max Planck Institute for Molecular Biomedicine, Germany); Rosa26-Sun1-2xsfGFP-6xMyc mice (B6;129-Gt(ROSA)26Sortm5(CAG-Sun1/sfGFP)Nat/J; Jackson Laboratory # 021039); and floxed Erg mice that were derived from Ergtm1a(KOMP)Wtsi mice (MGI # 4455490) as previously described. Unfloxed and floxed Erg mice on ApoE+/+ and Apoetm1Unc/tm1Unc backgrounds were generated by breeding Cdh5CreERT2; Rosa26lox-STOP-lox-Sun1-sfGFP; Ergfl/fl mice with ApoE-deficient mice (B6.129P2-Apoetm1Unc/J; Jackson Laboratory # 002052) and selecting for desired genotypes. Mice were bred and housed under pathogen-free conditions in cages containing up to 5 animals on vented racks with 12/12 h light/dark cycles at 21–22°C and 35–40% relative humidity at the University Health Network Animal Resources Centre (ARC). Erg knockout and hypercholesterolemia were induced in male and female 6–8 week-old mice.

Wild animals

No wild animals were used in this study.

Reporting on sex

Sex- and age-matched mice were randomly allocated for experiments. Sex-specific effects were not observed for initial experiments in this study and were not evaluated separately thereafter.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

Experiments were conducted in accordance with the current Canadian Council on Animal Care guidelines and with institutional research ethics board approval at the University Health Network (Animal Resources Centre / ARC; Animal Use Protocol # 2291).

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

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, mosaicism, off-target gene editing) were examined.

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

Mouse peripheral blood was aliquoted for leukocyte counting and assessment with flow cytometry. For leukocyte counting, 10 µL of each blood sample was diluted 1:20 with 1x RBC lysis buffer (BioLegend # 420301), triturated, incubated for seven minutes on ice, and enumerated to obtain the average of two independent counts. The remaining blood volume was added to 1 mL 1x RBC lysis buffer, triturated, incubated for 12 minutes on ice, and neutralized with 10 mL PBS-/-. Samples were centrifuged at 300 g for 10 minutes at 4°C and supernatant was discarded. Cells were resuspended in 100 µL ice-cold FACS buffer (1x PBS-/-, 0.5% BSA), vortexed, and 100 µL of cells were added to flow tubes. Samples were incubated with primary antibodies for 30 minutes on ice and fixed with Cytofix fixation buffer (BD Biosciences # 554655) prior to flow cytometry analysis. Primary antibodies were used with a 1:300 dilution.

Instrument

LSR II Flow Cytometer (BD Biosciences, USA)

Software

BD FACSDiva (v6.1.3) and BD FlowJo (v10.8.1)

Cell population abundance

The proportion of B cells varied from 34.0% to 47.7%.
 The proportion of T cells varied from 15.1% to 32.2%.
 The proportion of neutrophils varied from 8.60% to 22.3%.
 The proportion of ly6Chi monocytes varied from 1.20% to 7.76%.
 The proportion of ly6Cint monocytes varied from 0.0650% to 0.980%.
 The proportion of ly6Cneg monocytes varied from 0.0740% to 6.00%.
 The proportion of eosinophils varied from 0.320% to 4.56%.

Gating strategy

All cells were gated by forward scatter, side scatter, and doublets discrimination.
 B cells were further gated by CD45+, CD11b-, Ly6G-, CD3-, B220+.
 T cells were further gated by CD45+, CD11b-, Ly6G-, B220-, CD3+.
 Neutrophils were further gated by CD45+, CD11b+, Ly6G+
 Ly6Chi monocytes were further gated by CD45+, CD11b+, Ly6G-, CD115+, Ly6Chi
 Ly6Cint monocytes were further gated by CD45+, CD11b+, Ly6G-, CD115+, Ly6Cint
 Ly6Cneg monocytes were further gated by CD45+, CD11b+, Ly6G-, CD115+, Ly6C-
 Eosinophils were further gated by CD45+, CD11b+, CD115-, Ly6G-, SiglecF+

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