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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

A power analysis with 95% power and α=0.05 indicated that a sample size of at least 6 mice per group was required for morphometry and immunofluorescence analysis and a minimum of 3 mice per group for the bead assay. We routinely exceeded the minimal number of mice required for each experiment as indicated in the figure legends.

2. Data exclusions

Describe any data exclusions.

All data were included and outliers, if any, were not excluded. Only mice with a plasma cholesterol level <500 g/dL and thus not considered hypercholesterolemic were excluded from analyses.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Replications were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

We randomly distributed litter mates in either the IL-1b antibody treated group or the IgG control treated group.

For IL1R1 KO studies, we utilized Il1r1wt/wt and Il1r1fl/fl littermates to ensure that the IL-1R1 deficient and control animals were genetically identical with exception to the Il1r1 locus. All mice were on a C57Bl/6 background and had been backcrossed for at least 8 generations.

Although it would be ideal to study IL-1β neutralization in both males and females, the location of the Myh11 Cre ERT2 transgene on the Y chromosome prevents us from using females in this study.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The investigators were blinded regarding treatment or genotype during experimental procedures or data acquisition.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Digitized images were analyzed with Image Pro Plus Software 7.0 (Media Cybernetics). Immunofluorescent staining was imaged using a Zeiss LSM700 confocal microscope. Zen 2009 Light Edition Software (Zeiss) was used to acquire a series of eight z-stack images at 1um intervals. Zen 2009 Light Edition Software (Zeiss) was used for analysis of each z-stack image and single-cell counting was performed for phenotyping and quantifying the cell population comprised within the 30um thick layer proximal to the lumen (i.e., fibrous cap area).
FlowJo Version 10 software was used for flow cytometry analyses.
For RNAseq analysis, base calls was performed using CASAVA; reads were aligned to the mm9 genome using STAR version 3.0; aligned reads were counted against genes from the UCSC mm9 annotation using the featureCounts from the SubRead package; DESeq2 was used to normalize (normalized for library size using the DESeq2 median ration normalization procedure), and to perform differential gene expression; pathway analysis was performed using GAGE.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

There is restriction in availability of the IL-1b neutralizing antibody provided by Novartis. Other reagents are available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Brand/Cat#/Clone# (if applicable)/Lot#/concentration):
DAB staining:
- rabbit IRAK phospho-T209 (Abcam; ab218130; GR3199915-1; 4 ug/mL)
- rabbit IL-6 (Bioss antibodies; bs-0379R; 9K14V2; 4 ug/mL)
- rabbit IL1R1 (Bioss Antibodies, bs-2594R, 980890W, 4 ug/mL)
- rabbit MMP3 (Abcam, ab53015, GR248745-6, 7.7 ug/mL)
- rabbit MMP9 (Abcam, ab38898, 573149; 2 ug/mL)
- rat TER-119 (Santa-Cruz Biotechnology; sc-19592; lot unknown; 1 ug/mL)
Antibody specificity was assessed by using a matched control IgG as primary antibody. Optimization testing a large range of dilutions is initially performed using each individual primary antibody or IgG control at the same dilution.

For immunofluorescent staining, the following antibodies were used:
- mouse monoclonal SM α-actin-FITC (Sigma, F3777, clone 1A4, 086M4820V; 4.4 µg/mL)
- goat polyclonal GFP (Abcam, ab6673, GR287379-17, 4 µg/mL)
- rat LGALS3 (Cedarlane, CL8942AP, 1542219B, 2 µg/mL)
- rabbit Ki67 (Abcam, ab15580, GR3196372-1, 4 µg/mL)
- rabbit iNOS (Abcam, ab15323, GR295447-2, 0.52 µg/mL)
- Rabbit Arg1 (GeneTex, GTX109242, 42263, 9.2 µg/mL)
- rabbit RUNX2 (Abcam; ab192256; clone: EPR14334; GR204559; 1.374 µg/mL)
- goat PECAM-1 (1 µg/mL, Santa Cruz)
- Rabbit cleaved caspase 3 (Cell Signaling Technology, 9661S, D175, 42, 0.84 ug/mL)
- rabbit IRAK phospho-T209 (Abcam; ab218130; GR3199915-1; 4 ug/mL)
Antibody specificity was assessed by using a matched control IgG as primary antibody. Optimization testing a large range of dilutions is initially performed using each individual primary antibody or IgG control at the same dilution.

The secondary antibodies:
- donkey anti-rat conjugated to Dylight 550 (Abcam; ab102261; GR231498-2; 5ug/mL)
- donkey anti-rat conjugated to Dylight 650 (Abcam; ab102263; GR3176376374-2; 5 ug/mL)
- donkey anti-goat conjugated to Alexa 555 (Invitrogen; A21432; Ab_2535853; 1818686; 5 ug/mL)
- donkey anti-goat conjugated to Alexa 647 (Invitrogen; A21447; Ab_2535864; 1841382; 4 ug/mL)
- donkey anti-rabbit alexa 488 (Invitrogen; A21206; AB_2535792; 1874771; 5ug/ml)
- donkey anti-rabbit alexa 555 (Invitrogen; A31572; AB_162543; 1891766; 5ug/ml)
- donkey anti-goat alexa 488 (Invitrogen; A11055; AB_2534102; 1869589; 5ug/ml)
DAPI (0.05 mg/mL, D3571, ThermoFisher Scientific) was used as a nuclear counterstain and slides were mounted using Prolong Gold Antifade mountant (Invitrogen).

For flow cytometry:
- CD45 (eBiosciences; 103106, clone: 30-F11, B218387, 5 ug/mL)
- CD11b (eBiosciences; 101230; clone: M1/70; B204233; 2.5 ug/mL),
- Ly6C (Biolegend; 17-5932-82; clone: HK1.4; E10761-1637; 2.5 ug/mL)
- Ly6G (Biolegend; 127616; clone: 1A8; B196548; 2.5 ug/uL)
- CD115 (eBiosciences; 17-1152-82; clone: AFS98; E107201-1632; 0.6 ug/mL).
OneComp ebeads (50uL/sample; eBiosciences; 01-1111-42) were used for single stain controls and all experiments used fluorescent minus one (FMO) controls for each marker.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used. — No eukaryotic cell lines were used

b. Describe the method of cell line authentication used. — No eukaryotic cell lines were used

c. Report whether the cell lines were tested for mycoplasma contamination. — No eukaryotic cell lines were used

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use. — No eukaryotic cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Animal protocols were approved by the University of Virginia Animal Care and Use Committee. The Apoe-/- Myh11 Cre ERT2 R26R-YFP mice used in the intervention study have been described in previous studies. For the intervention studies, mice are injected with tamoxifen between 6 and 8 weeks of age and fed a Western Diet between 8 and 29 or 34 weeks of age. Mice are euthanized at 29 or 34 weeks of age. These mice were crossed with Il1r1fl/fl mice that were generated, characterized, and provided by Dr. Emmanuel Pinteaux. Male Apoe-/- Myh11 Cre ERT2 Il1r1fl/wt R26R-YFP and Apoe-/- Il1r1fl/wt R26R-YFP female mice were bred to generate Apoe-/- Myh11Cre ERT2 Il1r1fl/fl or wt/wt R26R-YFP littermate experimental mice. Apoe-/- LysM Cre Il1r1fl/fl or wt/wt R26R-YFP littermates were generated in a similar fashion. All mice were carefully genotyped by PCR as previously described. Apoe-/- Myh11Cre ERT2 Il1r1fl/fl and Apoe-/- LysM Cre Il1r1fl/fl mice are fed a Western Diet for 18 weeks and euthanized at 26 weeks of age.

All mice were on a C57Bl/6 background and had been backcrossed for at least 8 generations. Only males were used in these studies due to the location of the Myh11 Cre ERT2 transgene on the Y chromosome preventing using females.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.