**Figure S1**. **Overview of animal models**. （**A**） and (**B**), workflow of generation of CD45.1 fl/fl , CD45.2 $^{-/-}$ (EC-iCD45KO) /ApoE$^{-/-}$mouse , ApoE $^{-/-}$ as control group.

**Figure S2**. **Loss of endothelial CD45 reduces atherosclerosis in ApoE$^{-/-}$ atherosclerotic mice.**

Aortic root (**A**), and aortic aneurysm sections(**C**) from WT and EC-iCD45KO mice (16 week-WD) stained with Oil Red O. Statistics for *En face* ORO staining in aortic root (**B**) and aortic aneurysm sections (**D**). **E**, Brachiocephalic artery (BCA) sections from ApoE-/- or EC-iCD45KO

ApoE-/- mice were stained with the macrophage marker CD68 and α-SMA. **F**, Van Gieson's stain gives collagen a pink color (arrows) and other tissue elements a yellow color in mouse aortic BCA (Scale bars = 20 μM).

**S3**. **Loss of endothelial CD45 reduces inflammation in ApoE−/− atherosclerotic mice. A,** VCAM-1 (Vascular cell adhesion molecule 1 red), CD31(green), and DAPI (blue) on the luminal surface of aortic root sections (12-week WD). **B**, ICAM-1 (intercellular adhesion molecule-1, red) CD31 (green), and DAPI (blue) on the luminal surface of aortic root sections (16-week WD).

**Figure S4. Steps for preparation of aortic cells scRNA-seq data.** WT and EC-iCD45KO male mice were fed a western diet for 16 weeks, followed by the isolation of aortas, enzyme digestion, CD31 beads enrichment endothelial cells, and single cell suspension were prepared by manufacturer's instructions. 10X Genomics- Single-cell cDNA library preparation: barcoding cells for 10 x genomics, cDNA library construction and sc-RNA sequencing. cDNA library sequencing and computational analysis: Then the sequencing reads were preprocessed by cell ranger, and downstream analysis was done.

**S5. Data quality control (QC) for sc-RNA sequencing.** Data quality control (QC) for sc-RNA sequencing: ApoE$^{-/-}$:21745 genes from 11497 cells; EC-iCD45KO/ApoE$^{-/-}$: 21436 genes from 16273 cells.

**S6. Cell type clusters of ApoE$^{-/-}$ and EC-iCD45KO/ApoE$^{-/-}$ mice.**

**A,** Cell type clusters of ApoE$^{-/-}$ and EC-iCD45KO/ApoE$^{-/-}$ mice, including endothelial cells (ECs), endothelial-to-mesenchymal transition (EndoMT) cells, vascular smooth muscle cells (VSMCs), fibroblasts, VSMC-like cells, proliferation cells, mesenchymal-like cells, MEndoT cells, B cells, T cells, Schwann cells, Trem2 macrophages, inflammatory macrophages, resident – like

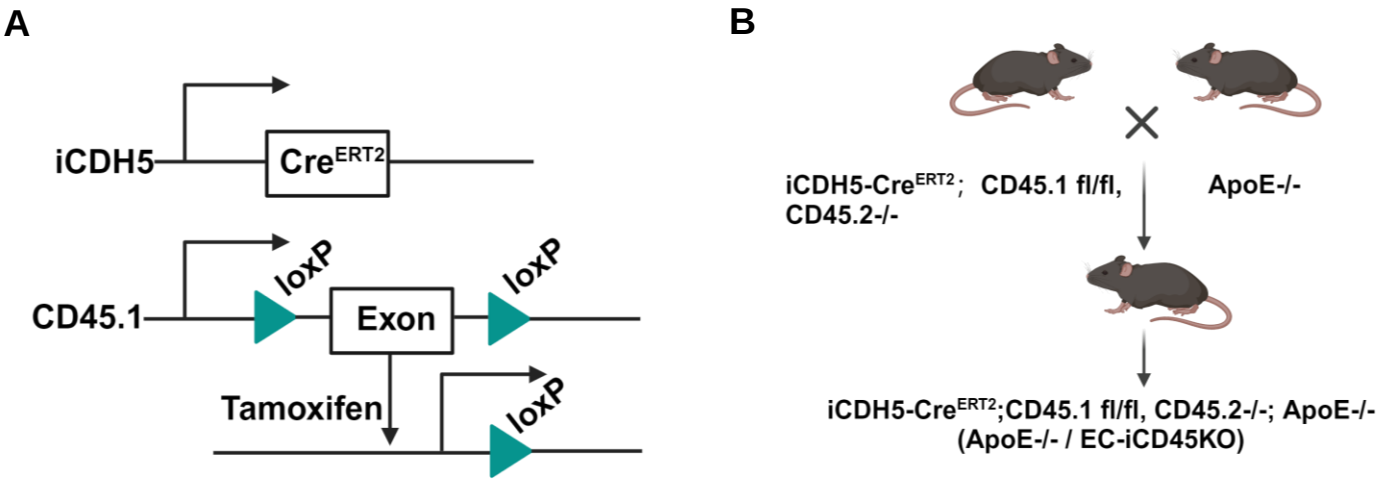
macrophages, monocytes and monocytes derived dendritic cells. **B**, Proportion of endothelial cells (ECs), endothelial-to-mesenchymal transition (EndoMT) cells, vascular smooth muscle cells (VSMCs), fibroblasts, VSMC-like cells, proliferation cells, mesenchymal-like cells, MEndoT cells, B cells, T cells, Schwann cells, Trem2 macrophages, inflammatory macrophages, resident – like macrophages, monocytes and monocytes derived dendritic cells.

**S7. scRNA-seq analysis for ECs and SMCs markers in ApoE$^{-/-}$ and EC-iCD45KO/ApoE$^{-/-}$ mouse**. **A**, RNA velocity analysis showed EndoMT transition down-regulated in EC-iCD45KO/ApoE$^{-/-}$ mouse. **B**, CDH5 is up-regulated in MEndoT cells of EC-iCD45KO/ApoE$^{-/-}$ that indicates Mesenchymal to EC transition in endothelial specific knock-out mice. **C**, Gene expression of representative markers specific to ECs (Cldn5, Egfl7, Cdh5) and SMCs (Myh11, Tagln, Fbln5) through the EC→EndoMT→SMC process is shown as normalized expression vs UMAP_1.

**S8. scRNA-seq analysis for ECs and SMCs markers on EndMT1 and EndMT2 subcluster between ApoE$^{-/-}$ and EC-iCD45KO/ApoE$^{-/-}$ mouse**. **A**, Trajectory analysis for ApoE$^{-/-}$ and EC-iCD45KO/ApoE$^{-/-}$ in mouse aortic cells (ECs as root). **B**, Proportion of ECs, EndoMT1, EndoMT2, VSMCs, fibroblasts, VSMC-like cells, and proliferation cells. **C-D**, Gene expression of representative markers specific to ECs (Cdh5) and SMCs (*Acta2, Col1a2, Sparc, Ly6a, and Pde4d*) through the EC→EndoMT→SMC process is shown as normalized expression vs UMAP_1. Violin plots of EC (**E**), SMC (**F**), and EndoMT (**G**) markers differentially expressed in ApoE-/- and EC-iCD45KO/ApoE-/- mice. In the EndoMT1 subgroups of EC marker–positive cells (Ly6a, Emcn, Sem6a), SMC marker–positive cells (Foxp1, Col8a1, Itga4) and EndoMT marker-positive cells (Ebf1, Bgn) were analyzed. In the EndoMT2 subgroups of EC marker–positive cells (Calr, Nf1,

Nr2f2), SMC marker–positive cells (Silk) and EndoMT marker-positive cells (Ets1, Btg1) were analyzed.

# Figure S1


A
iCDH5
Cre^ERT2
CD45.1
loxP
Exon
loxP
Tamoxifen
loxP
B
iCDH5-Cre^ERT2; CD45.1 fl/fl, CD45.2-/-
ApoE-/-
iCDH5-Cre^ERT2;CD45.1 fl/fl, CD45.2-/-; ApoE-/-
(ApoE-/- / EC-iCD45KO)

# Figure S2

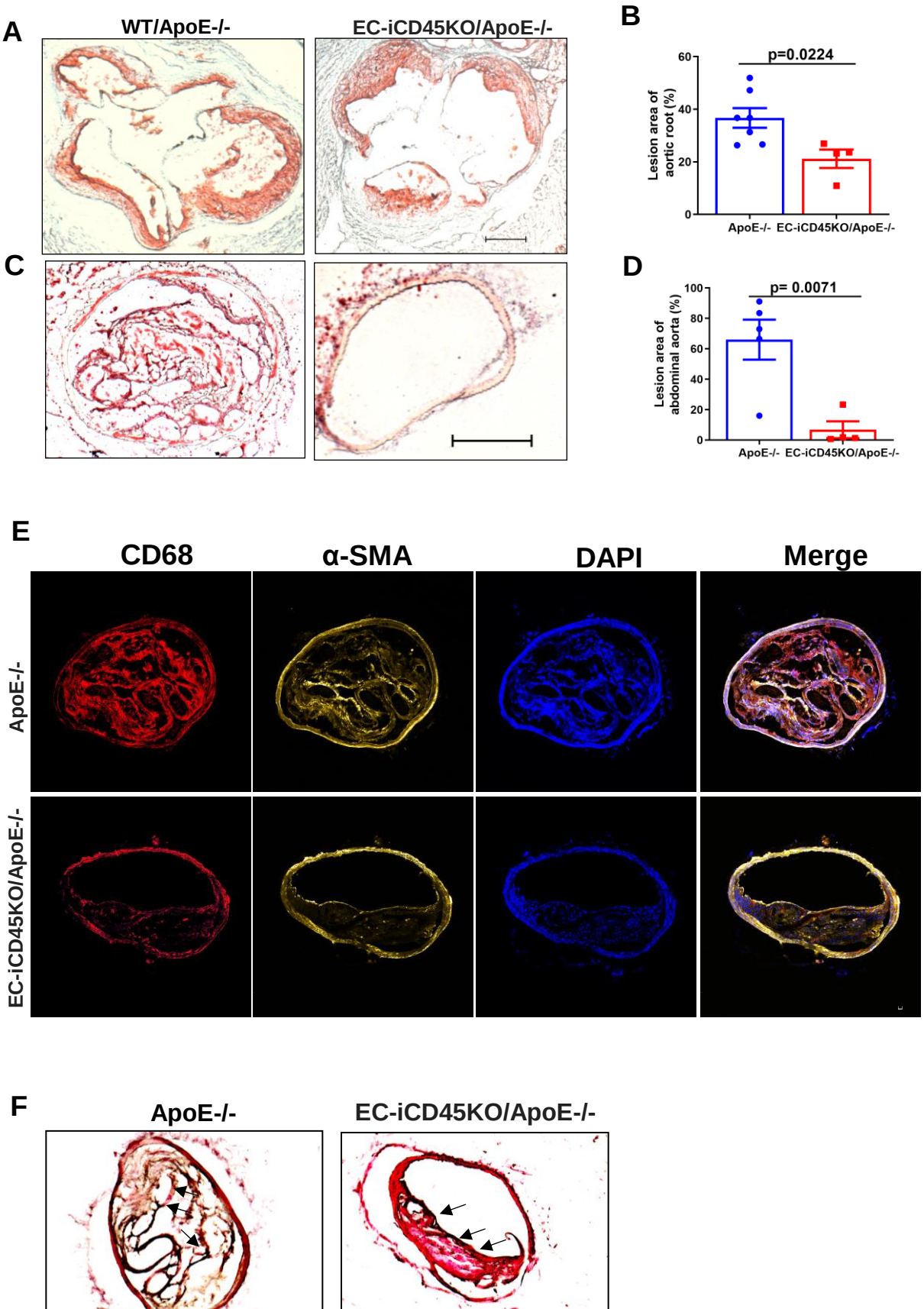

A
WT/ApoE-/-
EC-iCD45KO/ApoE-/-
B
p=0.0224
Lesion area of aortic root (%)
60
40
20
0
ApoE-/-
EC-iCD45KO/ApoE-/-
C
D
p= 0.0071
Lesion area of abdominal aorta (%)
100
80
60
40
20
0
ApoE-/-
EC-iCD45KO/ApoE-/-
E
CD68
α-SMA
DAPI
Merge
ApoE-/-
EC-iCD45KO/ApoE-/-
F
ApoE-/-
EC-iCD45KO/ApoE-/-

Figure S3

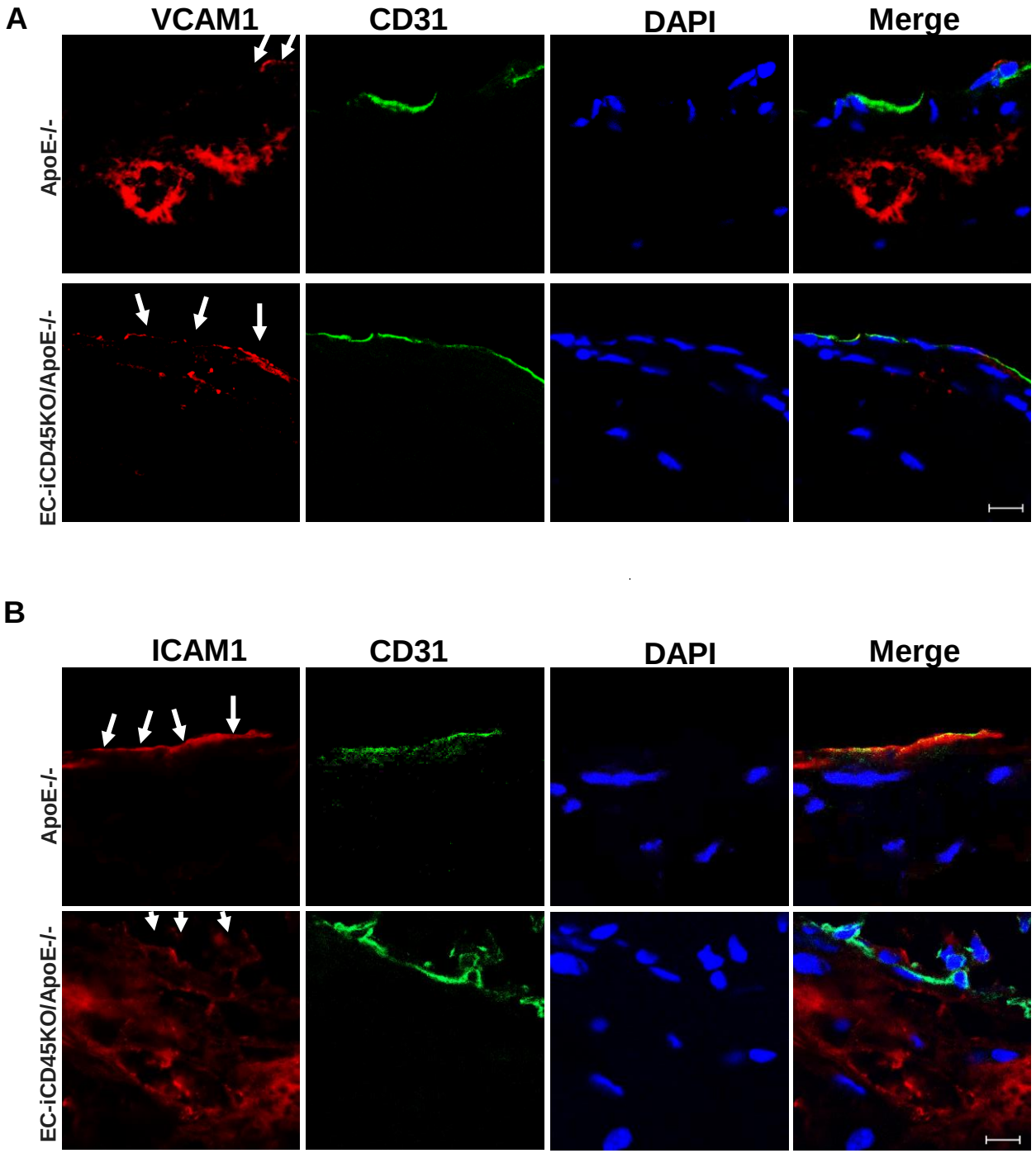

A
VCAM1
CD31
DAPI
Merge
ApoE-/-
EC-iCD45KO/ApoE-/-
B
ICAM1
CD31
DAPI
Merge
ApoE-/-
EC-iCD45KO/ApoE-/-

**Figure S4**

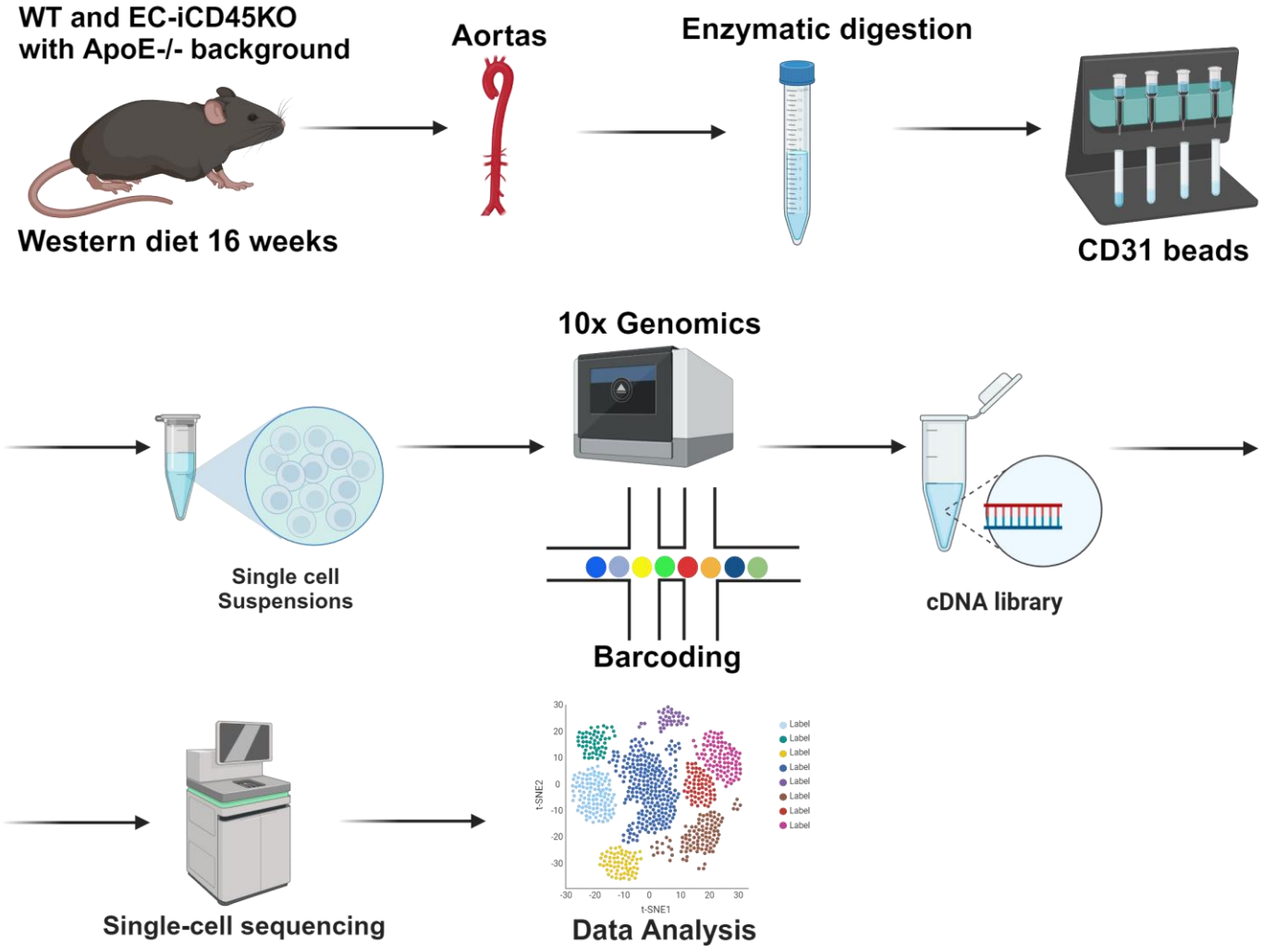
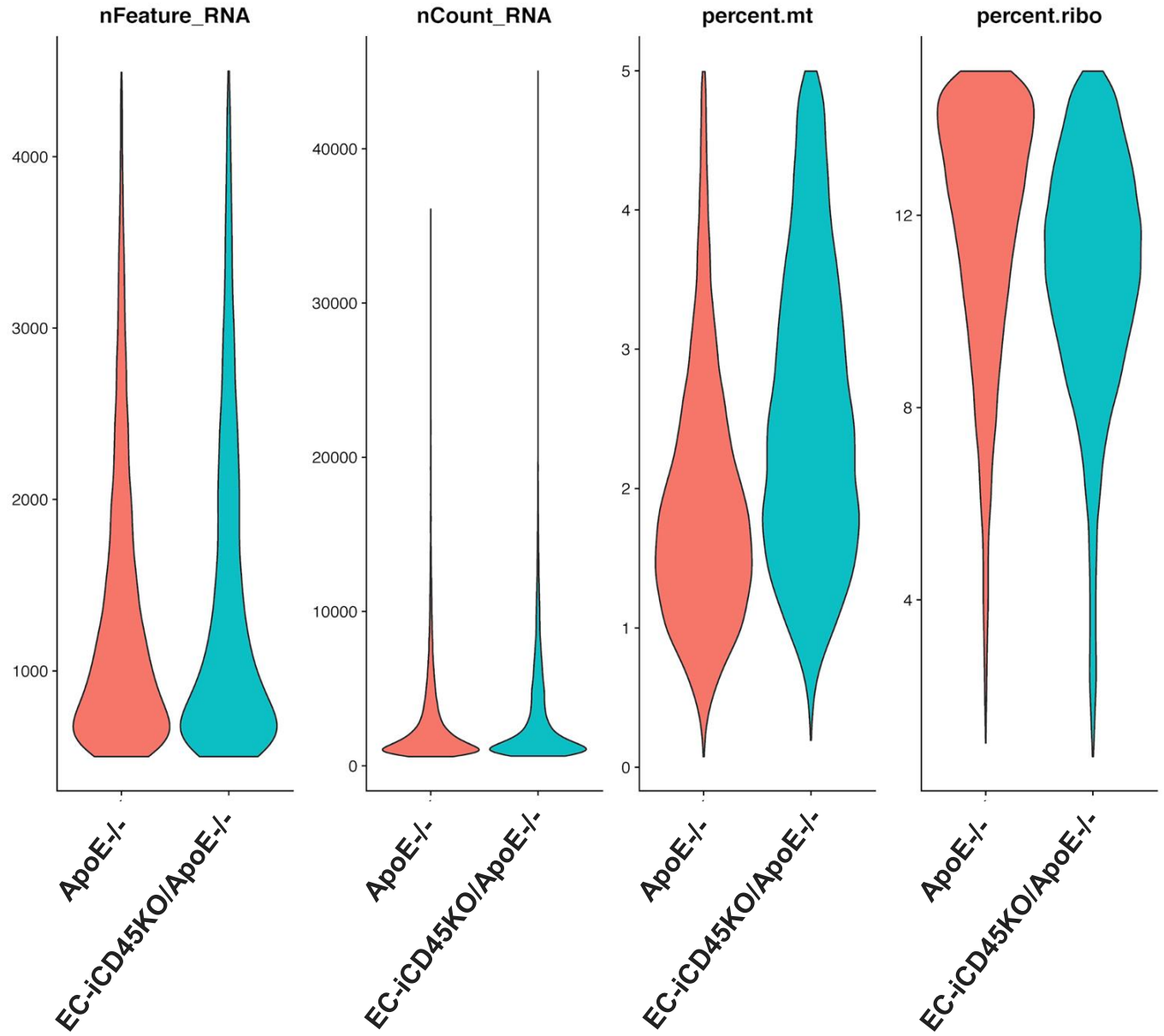
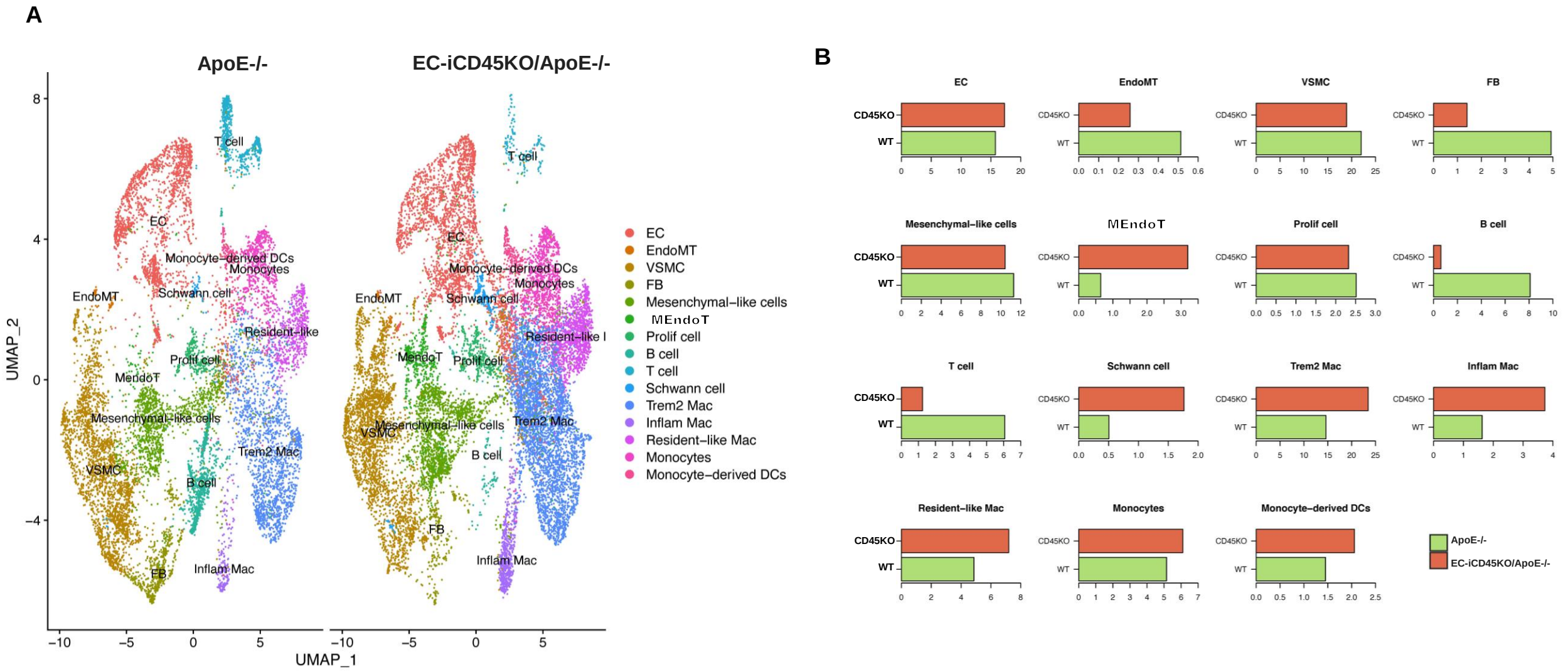

WT and EC-iCD45KO
with ApoE-/- background
Western diet 16 weeks
Aortas
Enzymatic digestion
CD31 beads
10x Genomics
Single cell
Suspensions
Barcoding
cDNA library
Single-cell sequencing
Data Analysis
t-SNE1
t-SNE2
Label

**Figure S5**

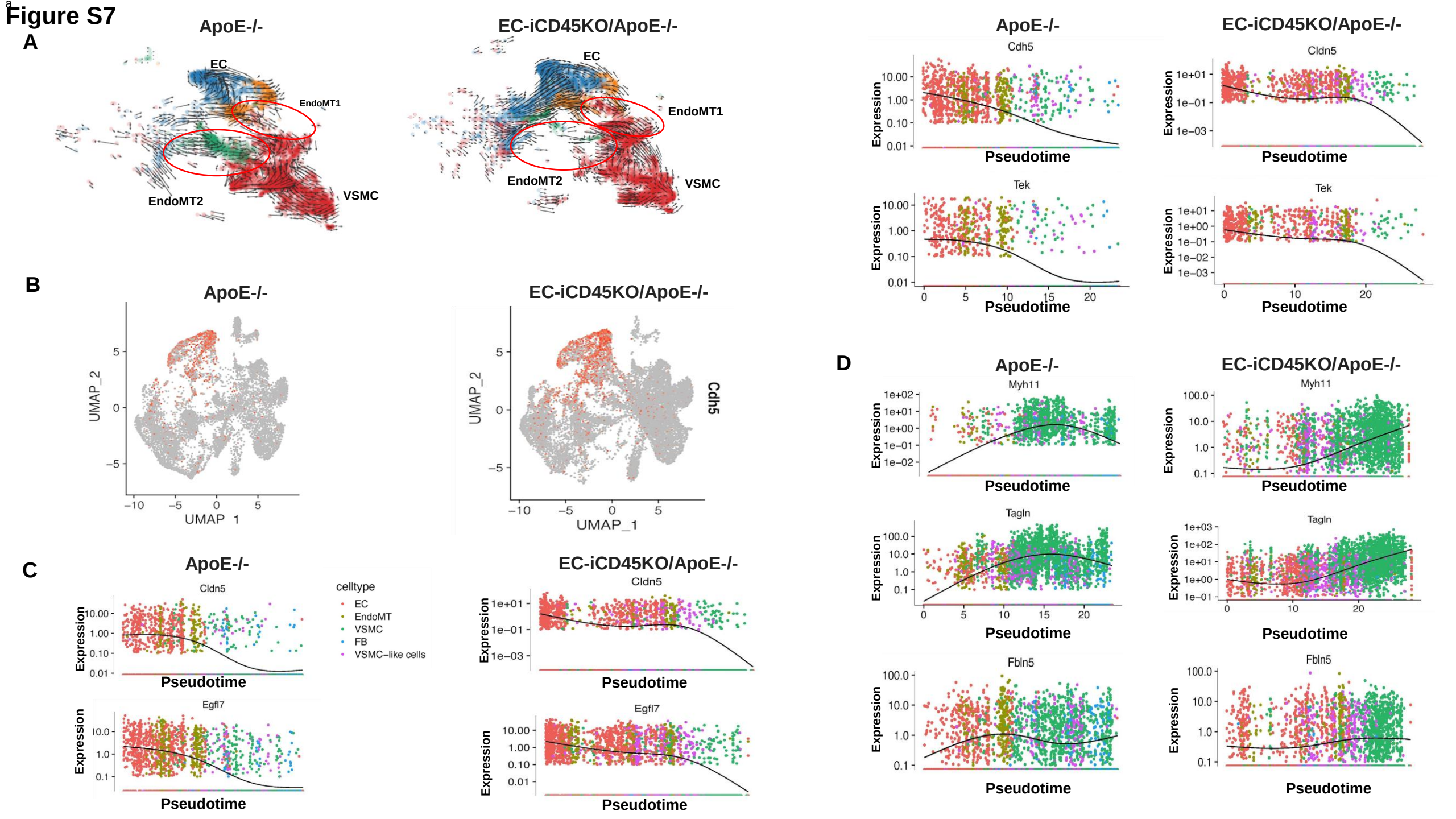



*nFeature_RNA > 500 & nFeature_RNA < 4500 & percent.mt < 5 & percent.ribo < 15*

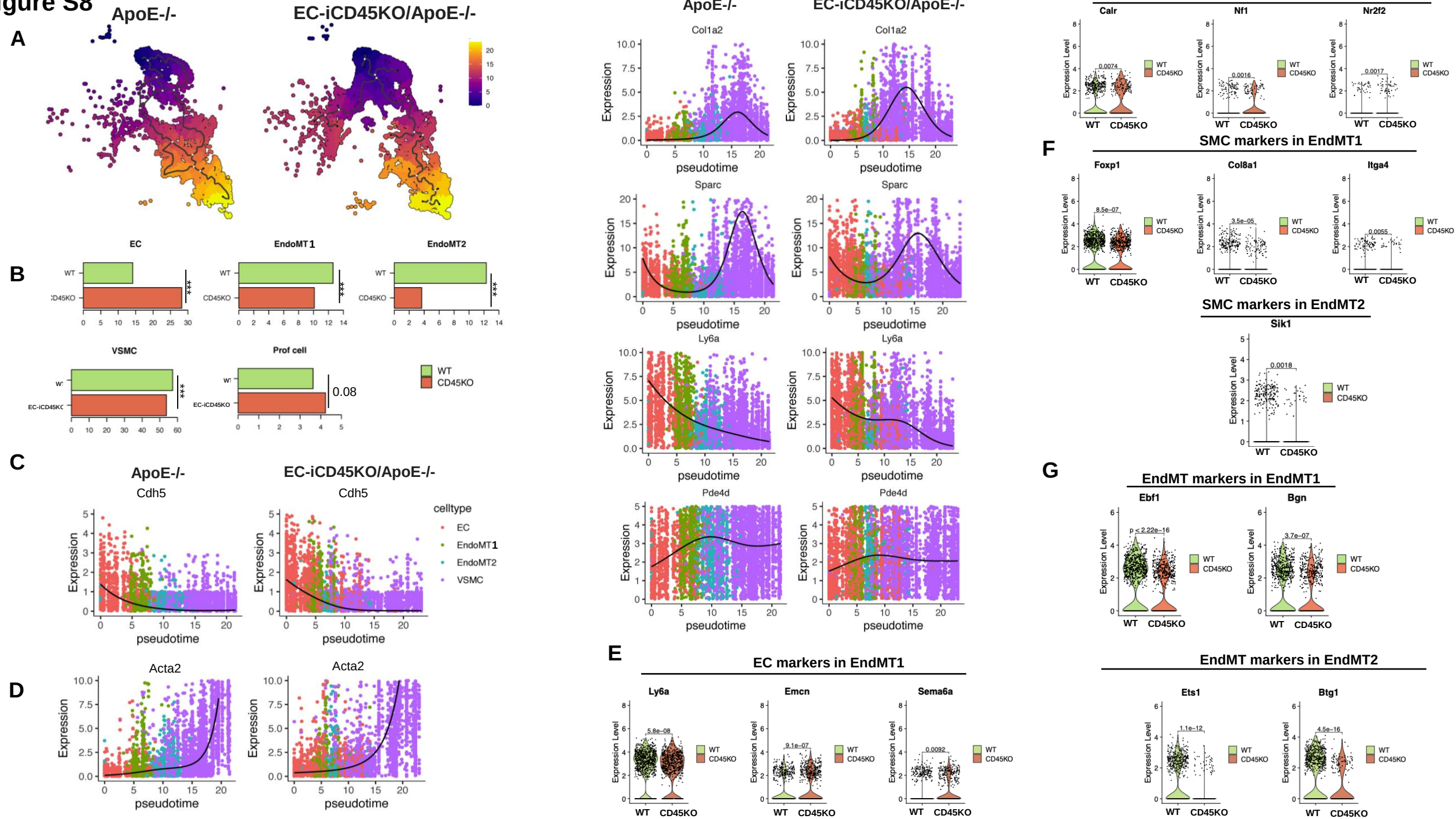
# Figure S6


A
ApoE-/-
EC-iCD45KO/ApoE-/-
UMAP_2
UMAP_1
EC
EndoMT
VSMC
FB
Mesenchymal–like cells
MEndoT
Prolif cell
B cell
T cell
Schwann cell
Trem2 Mac
Inflam Mac
Resident–like Mac
Monocytes
Monocyte–derived DCs
B
CD45KO
WT
Mesenchymal–like cells
MEndoT
Prolif cell
B cell
T cell
Schwann cell
Trem2 Mac
Inflam Mac
Resident–like Mac
Monocytes
Monocyte–derived DCs
ApoE-/-
EC-iCD45KO/ApoE-/-

# Figure S7


A
ApoE-/-
EC-iCD45KO/ApoE-/-
EC
EndoMT1
EndoMT2
VSMC
B
ApoE-/-
EC-iCD45KO/ApoE-/-
UMAP_1
UMAP_2
Cdh5
C
ApoE-/-
EC-iCD45KO/ApoE-/-
Cldn5
Egfl7
Expression
Pseudotime
celltype
EC
EndoMT
VSMC
FB
VSMC–like cells
D
ApoE-/-
EC-iCD45KO/ApoE-/-
Cdh5
Cldn5
Tek
Myh11
Tagln
Fbln5

# Figure S8


A
ApoE-/-
EC-iCD45KO/ApoE-/-
B
EC
EndoMT1
EndoMT2
VSMC
Prof cell
WT
CD45KO
0.08
C
ApoE-/-
EC-iCD45KO/ApoE-/-
Cdh5
celltype
EC
EndoMT1
EndoMT2
VSMC
Expression
pseudotime
D
Acta2
Col1a2
Sparc
Ly6a
Pde4d
E
EC markers in EndMT1
Ly6a
Emcn
Sema6a
F
EC markers in EndMT2
Calr
Nf1
Nr2f2
SMC markers in EndMT1
Foxp1
Col8a1
Itga4
SMC markers in EndMT2
Sik1
G
EndMT markers in EndMT1
Ebf1
Bgn
EndMT markers in EndMT2
Ets1
Btg1
Expression Level

## Supplemental Materials and Methods

### Animal models

In this study, all animal procedures were performed in compliance with institutional guidelines and mouse protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Boston Children's Hospital, MA, USA. Both male and female mice were used. C57BL/6 mice (stock #00664), ApoE-/- mice (stock #002052), EC-specific Cre deleter mice (iCDH5CreERT2). We crossed CD45 floxed mice with tamoxifen-inducible EC-specific Cre deleter mice (iCDH5 CreER$^{T2}$) to create an inducible EC-specific CD45 deficient mouse strain (EC-iCD45KO). We then bred EC-iCD45KO mice to the ApoE$^{-/-}$ (C57BL/6) background to obtain ApoE$^{-/-}$/EC-iCD45KO mice. For controls, we used wild-type C57BL/6 mice bearing iCDH5 CreERT2 (denoted as WT). These mice crossed to the ApoE$^{-/-}$ (C57BL/6) background to obtain control ApoE$^{-/-}$/WT (ApoE$^{-/-}$) mice. Mice were fed a Western diet (WD, D12079B, Research Diet) starting at the age of around 8 months old for 8-24 weeks. Mice were sacrificed at different time points based on the experiment.

### Mouse Genotyping for CD45.1 by Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from mouse tail biopsy using the DNeasy Kit (Qiagen). A triple-primer method was used for genotyping ApoE, CD45.1 and their mutants with neomycin-resistant gene inserts, respectively, using the following primer sequences: Mouse CD45 wild-type primer: 5'-'; Mouse CD45.1 mutant: 5'- -3'; Mouse CD45 common primer: 5'- -3'; ApoE forward-1: 5'- -3', ApoE forward-2: 5'- -3', and ApoE reverse: 5'- -3'. The PCR condition was as follows: jump start for 2 min at 95°C, denaturation for 1 min at 95°C, annealing for 1 min at 56 °C, and extension at 72°C for 1 min, for 40 cycles. The resulting PCR products were resolved on a 1.5 % agarose

ethidium bromide gel and the amplified bands were visualized with ultraviolet light, after which the PCR products were purified and sequence identity was confirmed by sequencing

**Mouse Genotyping for CD45.2 by flow cytometry**

Mouse blood was collected from the tail nick into an Eppendorf tube that contains 200µL PBS/ 10mM EDTA (anticoagulant) and stored on ice. Then, red blood cells were lysis by using red blood cell lysis buffer (Millipore Sigma Cat# 11814389001), and cell pellets were spun down. We then added 500µL buffer (2%HBSS without phenol red + 0.1% BSA + 0.02% Na azide) to wash RBC lysis buffer from cells and spun down. Next, remove the supernatant from the pellets. Cell pellets were resuspended in FACS buffer with Trustain (anti-mouse CD16/32) from BioLegend, anti-mouse CD45.1-PE (BioLegend) and CD45.2-FITC (BioLegend) at 4°C, 30 min before flow cytometry analysis using BD instrument.

**MAEC isolation**

To isolate mouse aortic endothelial cells (MAECs), aortas were collected and washed twice with PBS at 4 °C and then carefully stripped of fat and connective tissue. The aortas were cut into 3 mm long sections, and segments were put on Matrigel-coated (Corning) plate with EC medium. After 4 days, vascular networks were visible under the light microscope and tissue segments were removed. ECs were detached, spun down and cultured in fresh EC medium. The identity of isolated ECs was confirmed by immunofluorescent staining using EC markers CD31. A full list of reagents including antibodies and primers is included in the supplementary information (Supplementary Data file 4). Cultured MAECs were treated with 5 µM tamoxifen for 3 days to induce the deletion of CD45 gene from EC-iCD45KO/ApoE$^{-/-}$ mice, MAECs isolated from ApoE$^{-/-}$ mice as controls

(without tamoxifen treatment). Cells were treated with 100 µg/mL oxLDL or 10ng/mL TGFβ1 at different times as indicated while maintaining 2 µM tamoxifen in the culture medium.

**Flow cytometry analysis**

We applied the triple-label flow cytometric method we developed for mitral valve analyses to total aortic ECs from age and gender-matched wild-type (WT) and ApoE$^{-/-}$ mice fed a WD for 16-20 weeks. As ApoE$^{-/-}$ mice fed a normal diet also develop lesions, albeit at a slower rate compared to WD-fed ApoE$^{-/-}$ mice, we chose to use WT mice that were fed a normal chow as our true negative control for EndoMT occurrence to rule out the potential effect of high cholesterol content in WD-fed mice and lesion development in normal chow-fed ApoE$^{-/-}$ mice. Blood was thoroughly flushed from aortas to enrich vessel wall-localized cells. Antibodies were rat anti-mouse CD45 (BD Biosciences #564590), anti-VE-cadherin-FITC(), anti-CD45-CF647(), anti-FGFR1-PE(), and rabbit anti-human VE-cadherin (ABD Serotec #AHP628Z) coupled to APC using the LYNX Rapid APC Antibody Conjugation Kit (ABD Serotec #LNK031APC) as well as rabbit anti-human α-SMA-PE (Abcam #ab209478) with isotype-matched control IgGs and matching fluorescent tags. Antibodies were authenticated by showing binding to murine CD45, VE-cadherin, and α-SMA, respectively, and using appropriate positive and negative controls.

# Supplement Table 1 : Major Resources Table

Antibodies

| Target antigen | Vendor or Source | Catalog # | Working concentration |
|---|---|---|---|
| Primary Antibodies | | | |
| Mouse VE-Cadherin Antibody | R & D systems | AF1002 | 1:50(IF) |
| Anti-Klf2 Antibody (Rabbit Polyclonal Antibody | EMD Millipore | 09-820 | 1:200(IF) |
| Mouse monoclonal anti-CD68 | Santa Cruz Biotechnology | Sc20060 | 1:200(IF) |
| Anti-Actin, α-Smooth Muscle - Cy3™ antibody, Mouse monoclonal α-SMA | Sigma-Aldrich | C6198 | 1:200(IF) |
| Rat-anti Mouse CD31 | BD Pharmingen | 550274 | 1:50(IF) |
| Mose Anti-CD45 | Santa Cruz Biotechnology | Sc-28369 | 1:200 |
| Mouse monoclonal ICAM-1 | Santa Cruz Biotechnology | Sc8439 | 1:200(IF) |
| Mouse monoclonal VCAM-1 | Santa Cruz Biotechnology | sc13160 | 1:70(IF) |
| Alexa Fluor Conjugated Second Antibodies | | REF | 2mg/ML |
| Goat anti-rabbit 647 | Invitrogen | A-21244 | 1:200(IF) |
| Goat anti-rat 647 | Invitrogen | A-21247 | 1:200(IF) |
| Donkey anti-mouse 594 | Invitrogen | A-21203 | 1:200(IF) |
| Donkey anti-rabbit 594 | Invitrogen | A-21207 | 1:150(IF) |
| Donkey anti-rat 594 | Invitrogen | A-21209 | 1:150(IF, Flow) |
| Donkey anti-rat 488 | Invitrogen | A-21208 | 1:200(IF) |
| Donkey anti-mouse 488 | Invitrogen | A-21202 | 1:150(IF, Flow) |
| Donkey anti-rabbit 488 | Invitrogen | A-21206 | 1:150(IF, Flow) |
| IF | | | |
| Donkey anti-rabbit 647 | Invitrogen | A31573 | 1:200(IF) Lot:2674379 |

| | | | |
|---|---|---|---|
| Donkey anti-goat 594 | Invitrogen | A11058 | 1:200(IF) |
| Donkey anti-Rat 647 | Invitrogen | A21247 | 1:200(IF) |
| Donkey anti-mouse 647 | Invitrogen | A31571 | 1:200(IF) |

Reagents

| Description | Source / Repository | Catalog # |
|---|---|---|
| Oil Red O (ORO) | Thermo Scientific | Cat# A12989 |
| Hematoxylin and Eosin stain kit | Vector Laboratories | Cat# H-3502 |
| Propylene glycol | VWR | 0575 |
| Oxidized LDL (ox-LDL) | Athens Research and Technology | Cat#12-16-120412-ox |
| Low Density Lipoprotein from Human Plasma, oxidized, DiI conjugate (DiI-OxLDL) | ThermoFisher Scientific | Cat# L34358 |
| TopFluor Cholesterol | Avanti Polar Lipids,Inc | Cat# 810255p |
| SlowFade mount with DAPI | Invitrogen | Cat# 1896320 |
| Protein inhibitor cocktail | **cOmplete ™** | REF:11836170001 |
| Isopropanol, molecular biology grade | ThermoFisher Scientific | Cat# T036181000CS |

| | | |
|---|---|---|
| Qiagen RNeasy Mini Kit | Qiagen | Cat# 74104 |
| RNase-free DNAse Set | Qiagen | Cat# 79254 |

| | | |
|---|---|---|
| HiScript II One Step qRT-PCR SYBR Green Kit | Vazyme | Q221-01 |
| SYPR Green qPCR Master Mix reagent | Vazyme | Cat# Q712 |
| Fluoroshield | R & D Systems | F6812 |
| Ciprofloxacin Hydrochloride | TCI AMERICA | C2227 |
| Triton™ X-100 Surfactant | MilliporeSigma | TX15681 |
| Matrigel Matrix | Corning | REF#356237 |
| CD31 MicroBeads (Mouse) | Miltenyi Biotec | Cat# 130-097-418 |
| Collagenase type I | Gibco by Life technologies | Ref 17100-017 |
| Collagenase type IV | Gibco by Life technologies | Ref 17104-019 |
| Liberase | Roche | Cat# 05401127001 |
| GEM Single Cell 3'GEM Kit v3.1 | 10X Genomics | PN-1000123 |
| GEM Single Cell 3'Library Kit v3.1 | 10X Genomics | PN-1000157 |
| GEM Single Cell 3'Gel Bead Kit v3.1 | 10X Genomics | PN-1000122 |
| Dynabeads™ MyOne™ SILANE | 10X Genomics | PN-2000048 |
| GEM Chip G Single Cell Kit, 48rxns | 10X Genomics | PN-1000120 |

| | | |
|---|---|---|
| Single Index Kit T Set A, 96 rxns | 10X Genomics | PN-1000213 |

Oligonucleotides

| Description | Source / Repository | Catalog # |
|---|---|---|
| TGFβ2 | This study | N/A |
| Forward: 5'- GGACCCTACTCTGTCTGTGG -3'<br>Reverse: 5'- AGCCATGGAGTAGACATCCG -3' | | |
| TGFβ3 | This study | N/A |
| Forward: 5'- GACATCCCTTCCACCCAAGA-3'<br>Reverse: 5'- CAGGAGGAATGGTGTGGACT -3' | | |
| Beta-actin | This study | N/A |
| Forward: 5'- TTACTGCTCTGGCTCCTAGCA-3'<br>Reverse: 5'-CCACCGATCCACACAGAGTAC-3' | | |

Software and Algorithms

| Description | Persistent ID / URL |
|---|---|
| Image J | NIH website: https://imagej.nih.gov/ij/ |
| GraphPad Prism | GraphPad software company: https://www.graphpad.com/scientificsoftware/prism/ |
| Zeiss Zen lite | https://www.zeiss.com/microscopy/us/products/microscope-software/zen-lite.html |
| Image J | NIH website: https://imagej.nih.gov/ij/ |

| | |
|---|---|
| GraphPad Prism | GraphPad software company: https://www.graphpad.com/scientificsoftware/prism/ |