

## **Supplemental information**

### **Single-nucleus profiling of Ossabaw pig atherosclerosis model**

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Supplemental material

Supplementary Figures S1-S8

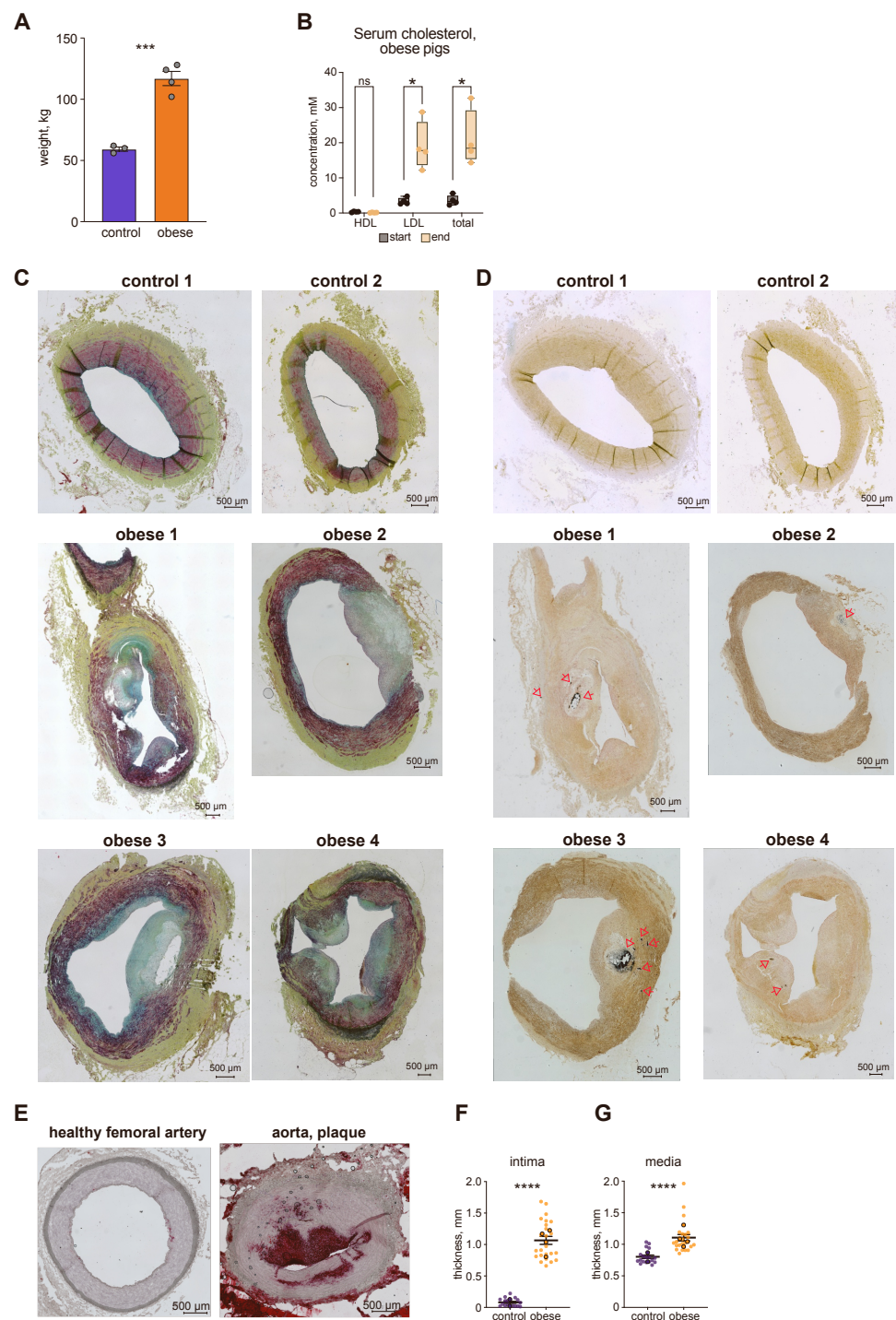
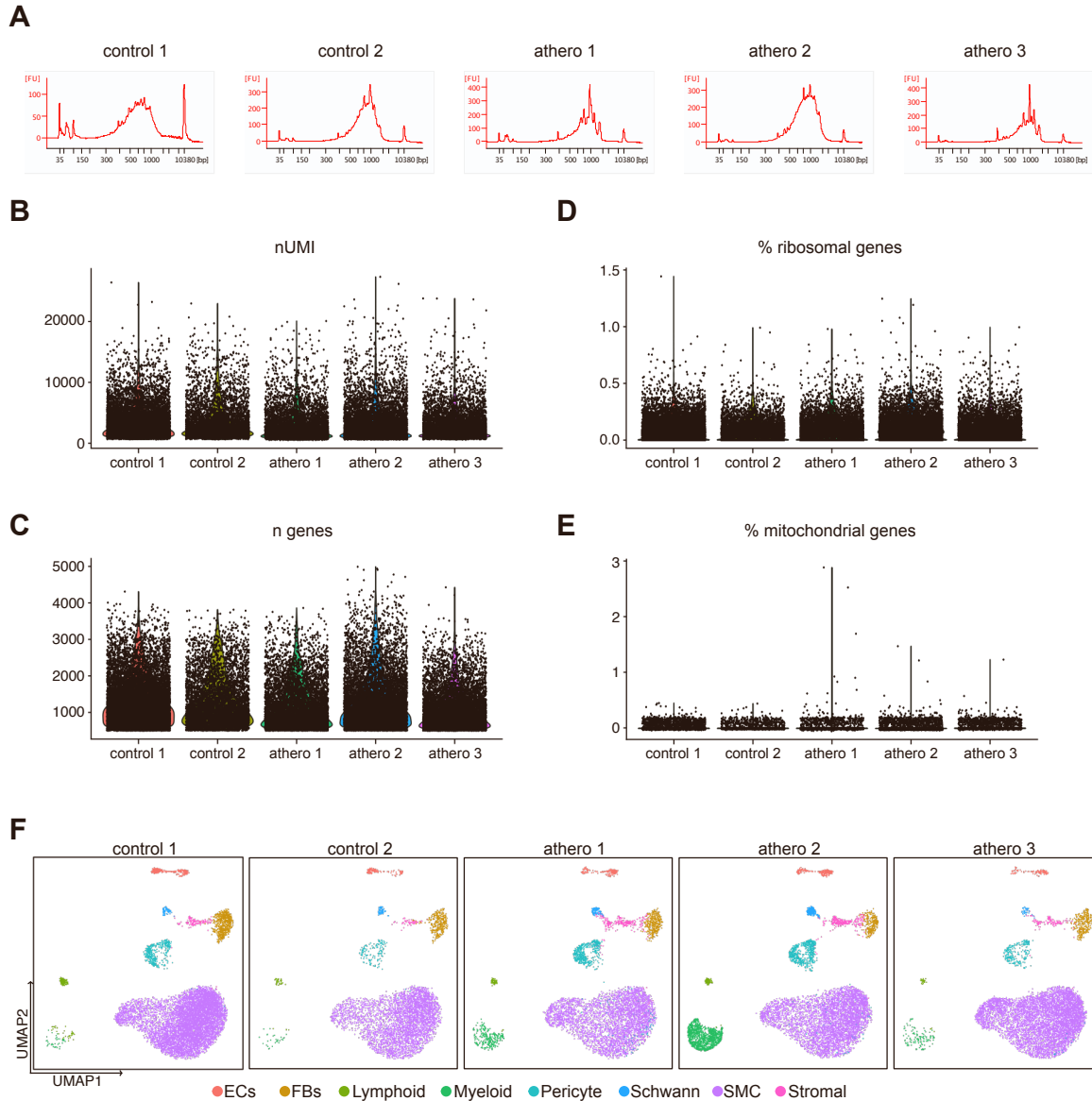
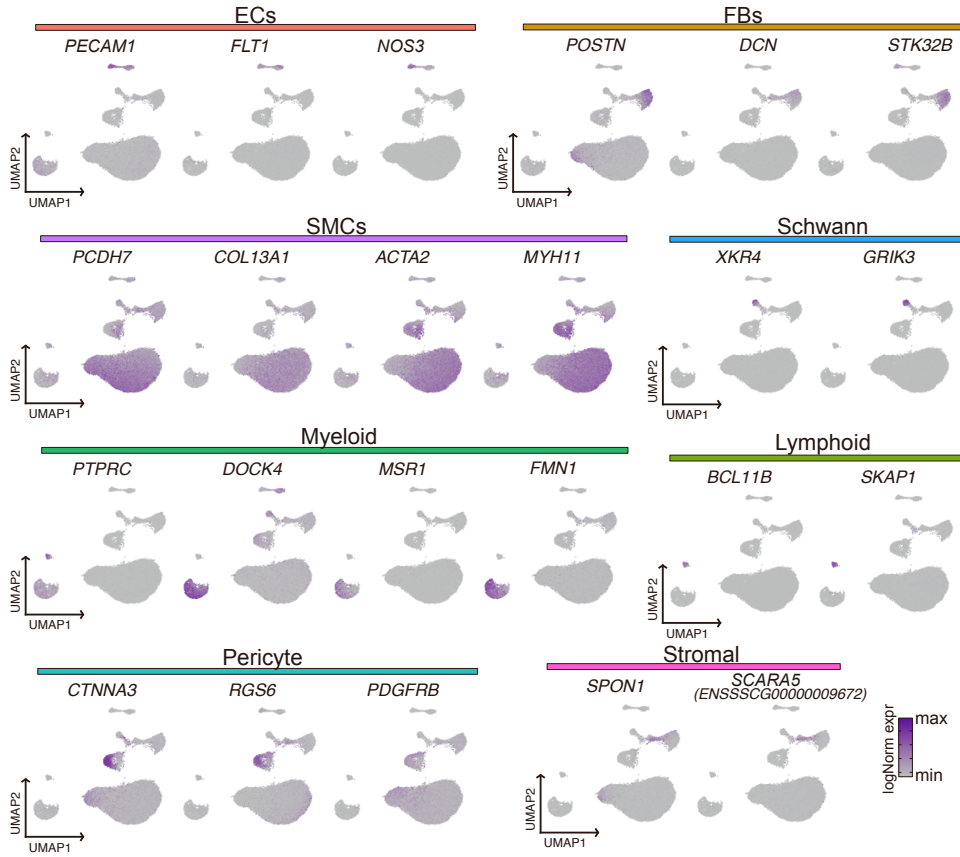
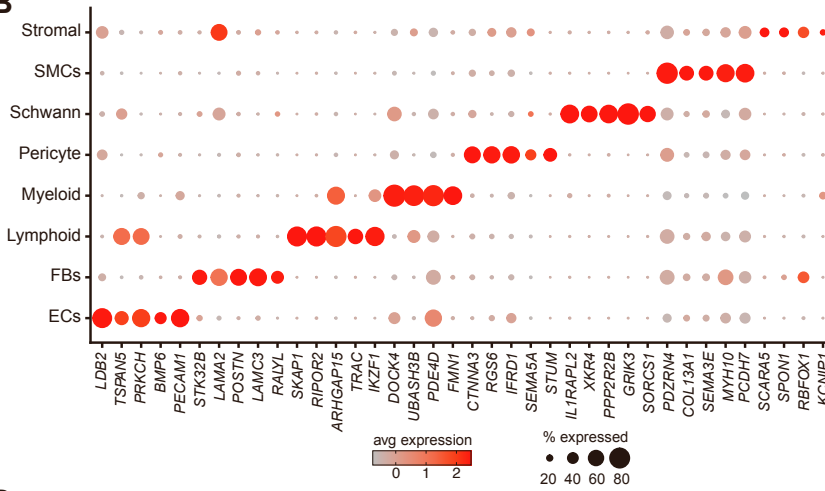
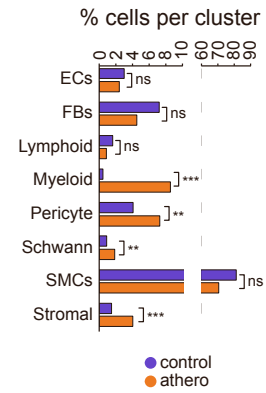
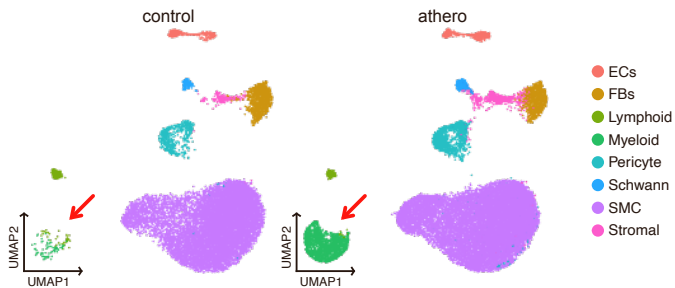


Figure S1

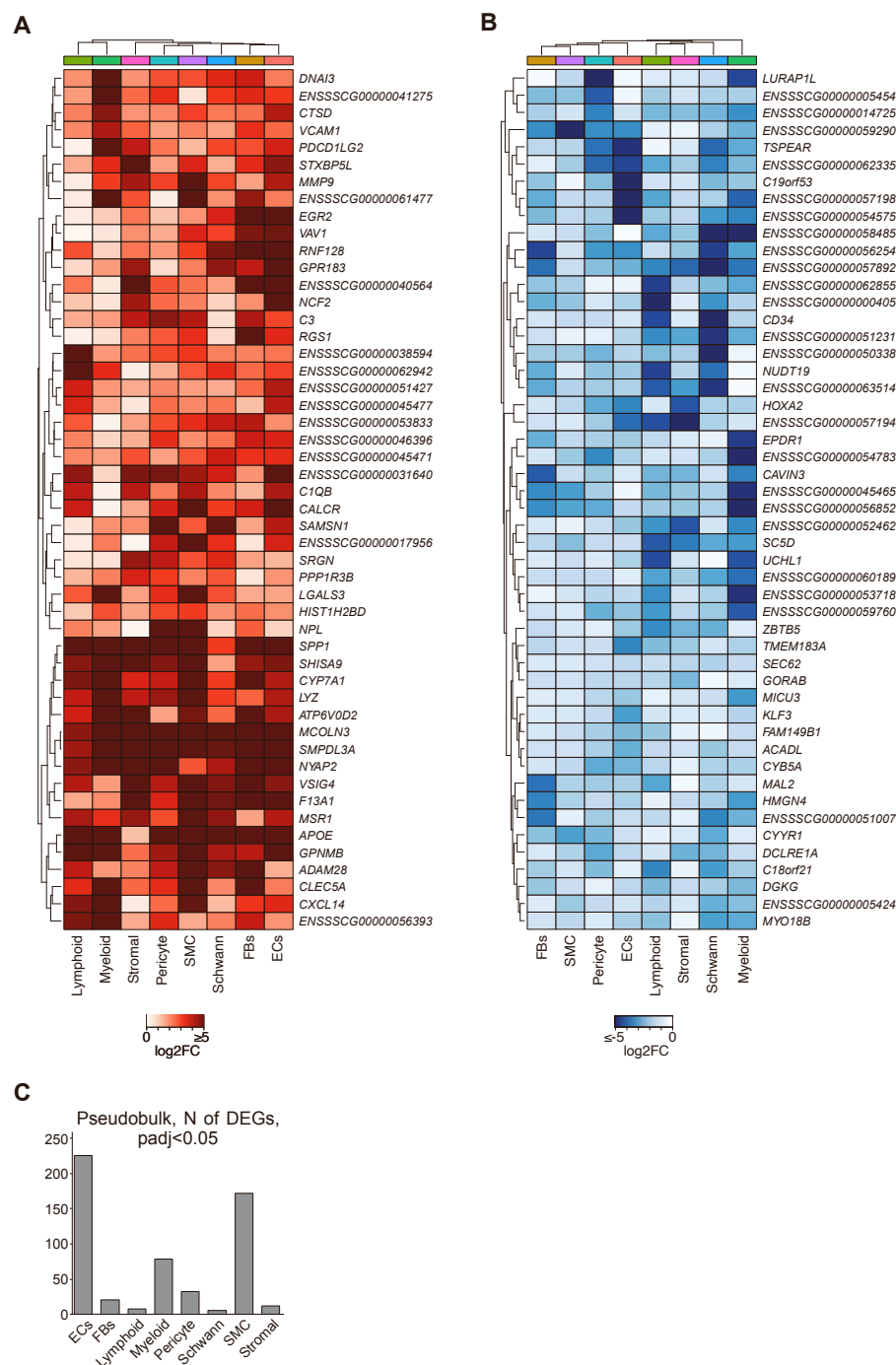
**Figure S1.** Animal metrics and histological stainings of aortas, related to Figure 1. **A.** Animal weights at the end of the experiment, N=3 and 4 per group, p-value = 0.0004, unpaired Student's t-test. **B.** Serum cholesterol levels in obese pigs measured by ELISA assay before and after HF/HFru diet, two-tailed Mann-Whitney test, p = 0.04. **C.** Movat pentachrome modified Russell-Movat staining. Scale bar 500  $\mu$ m. **D.** Von Kossa silver staining. Red arrows indicate calcification areas. Scale bar 500  $\mu$ m. **E.** Representative Oil Red O staining of healthy femoral artery and atherosclerotic aorta. Red – lipid deposits. Scale bar 500  $\mu$ m. **F-G.** Quantification of **(F)** intima and **(G)** media thickness. Pale dots represent technical replicates, outlined dots represent biological replicates, i.e. mean of technical replicates per each animal, p-values < 0.0001, Mann-Whitney test.



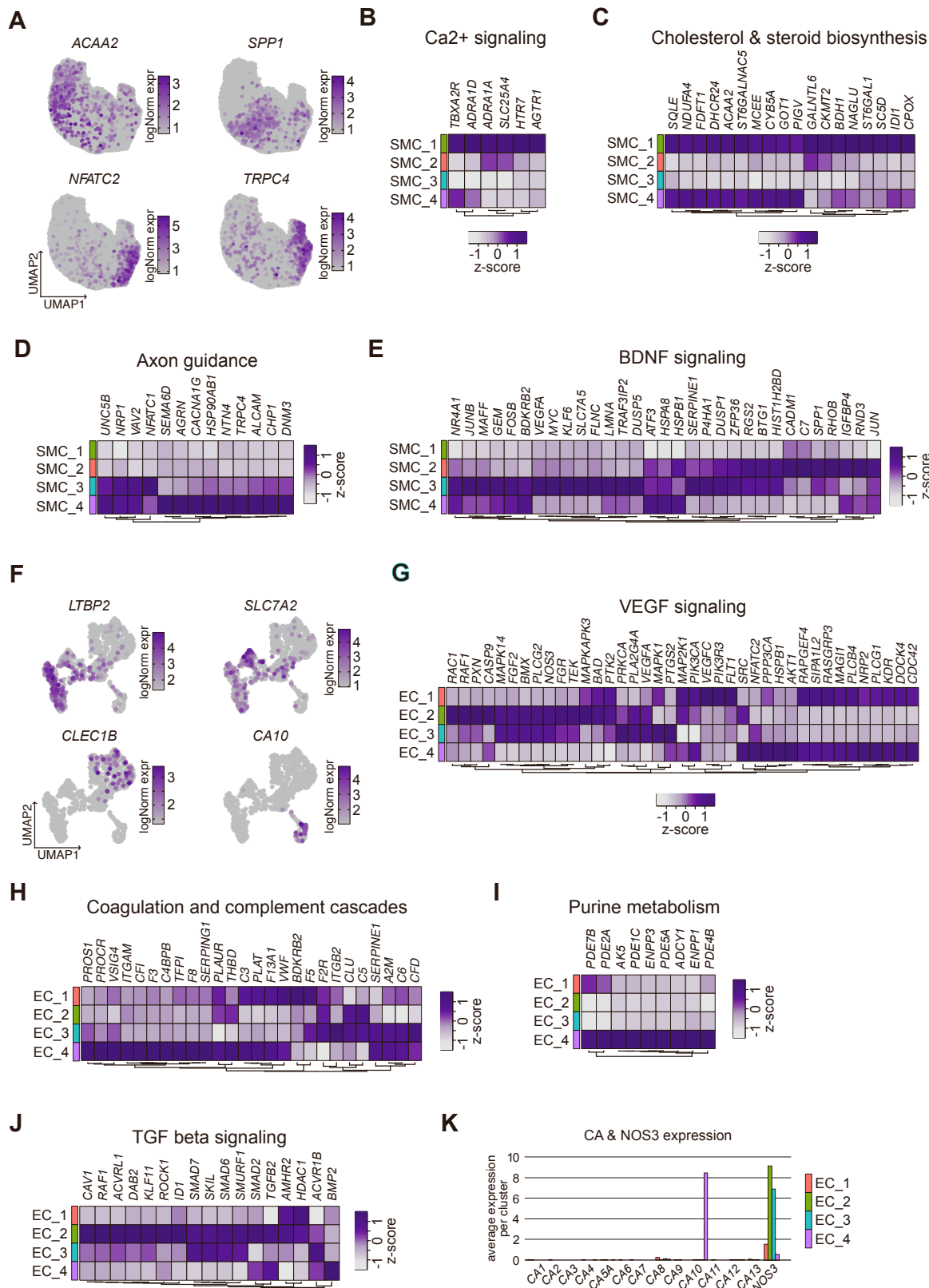
**Figure S2.** Quality controls of snRNA-seq per replicate, related to Figure 1. **A.** Bioanalyser traces of cDNA from each replicate produced with 10x Next GEM Single Cell 3 GEM kit v3.1. **B.** Violin plot showing the number of unique molecular identifiers (UMI) per nucleus detected in each replicate after quality filtering. **C.** Violin plot showing the number of genes per nucleus detected in each replicate after quality filtering. **D.** Violin plot showing percentage of ribosomal genes per nucleus detected in each replicate after quality filtering. **E.** Violin plot showing percentage of mitochondrial genes per nucleus detected in each replicate after quality filtering. **F.** UMAPs showing cell types clustering of snRNA-seq data across all replicates.

**A****B****C****D**

**Figure S3.** Cell type identification based on marker gene expression, related to Figure 1. **A.** UMAPs showing expression of cell type-specific marker genes across all nuclei. **B.** Dot plot depicting enrichment of top 5 expressed genes per cluster. Dot size indicates the percentage of cells expressing gene of interest, color indicates the average expression level. **C.** Bar plot showing percentage of nuclei in control and obese condition per cell type cluster.  $\chi^2$  test, \*\* indicate p-values < 0.01, \*\*\* < 0.001, \*\*\*\* < 0.0001. **D.** UMAP of integrated snRNA-seq from control and atherosclerotic aortae, split by condition.

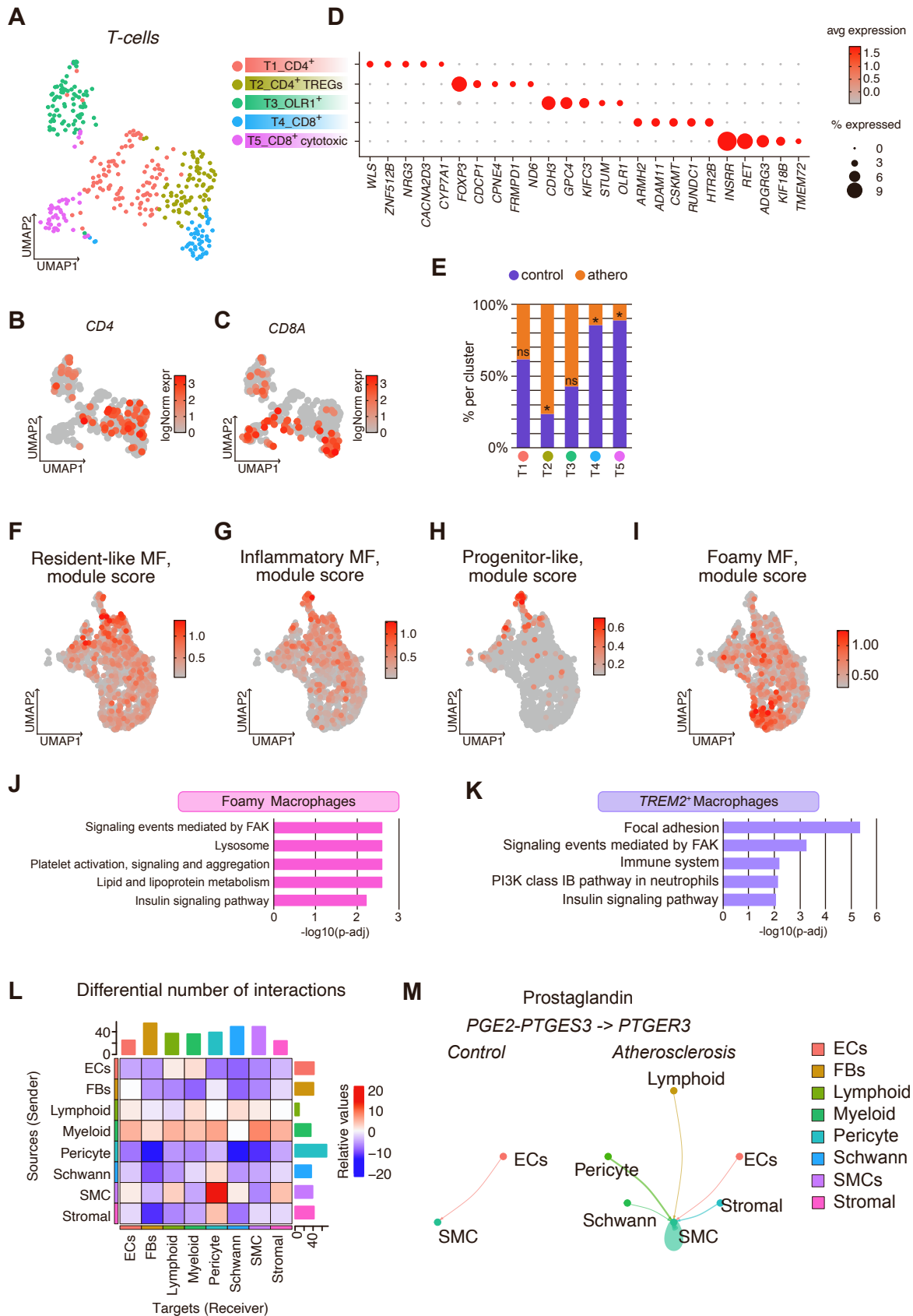


**Figure S4.** Ranked top 50 most deregulated genes across all cell types, related to Figure 2. **A-B.** Heatmaps showing log<sub>2</sub>FC of top 50 **(A)** upregulated and bottom 50 **(B)** downregulated genes across all cell types. **C.** Nuber of DEGs per cell type defined by pseudobulk analysis, p-adj < 0.05.





**Figure S5.** Subcluster analysis of enriched genes in SMCs and ECs, related to Figure 3. **A.** UMAPs showing expression of subcluster marker genes in SMCs. **B.** Heatmap of  $\text{Ca}^{2+}$  signaling genes in SMC subclusters, z-scores. **C.** Heatmap of cholesterol and steroid biosynthesis genes in SMC subclusters, z-scores. **D.** Heatmap of axon guidance signaling genes in SMC subclusters, z-scores. **E.** Heatmap of BDNF signaling genes in SMC subclusters, z-scores. **F.** UMAPs showing expression of subcluster marker genes in ECs. **G.** Heatmap of VEGF signaling genes, z-scores. **H.** Heatmap of coagulation and complement cascade-related genes in EC subclusters, z-scores. **I.** Heatmap of purine metabolism genes in EC subclusters, z-scores. **J.** Heatmap of  $\text{TGF}\beta$  pathway genes in EC subclusters, z-scores. **K.** Average expression of carbonic anhydrases and nitric oxide synthase (*NOS3*) across EC subclusters, log normalized counts.



**Figure S6.** Subcluster analysis of enriched genes in immune cells, and cell interaction analysis, related to Figure 4 and 5. **A.** UMAP showing subclustering of T-cells. **B-C.** UMAPs showing expression of **(B)** *CD4* and **(C)** *CD8A* in T-cells. **D.** Dot plot of top 5 genes enriched in each T-cell subcluster. Dot size indicates the percentage of cells expressing gene of interest, color indicates the average expression level. **E.** Bar plot showing percentage of nuclei in control and obese condition per T-cell subcluster.  $\chi^2$  test, \* indicate p-values < 0.01. **F.** UMAP showing resident-like MF module score enrichment in MF clusters. **G.** UMAP showing inflammatory MF module score enrichment in MF clusters. **H.** UMAP showing progenitor-like module score enrichment in MF clusters. **I.** UMAP showing foamy MF module score enrichment in MF clusters. **J.** Selected top Bioplanet GO terms upregulated in Foamy MF vs. *TREM2*<sup>+</sup> MF, the Benjamini-Hochberg adjusted p-value of enrichment. **K.** Selected top Bioplanet GO terms downregulated in Foamy MF vs. *TREM2*<sup>+</sup> MF, the Benjamini-Hochberg adjusted p-value of enrichment. **L.** Interaction heatmap between cell types, blue – reduced interaction numbers, red – increased interaction numbers. **M.** Prostaglandin signaling in control and atherosclerotic aortas.

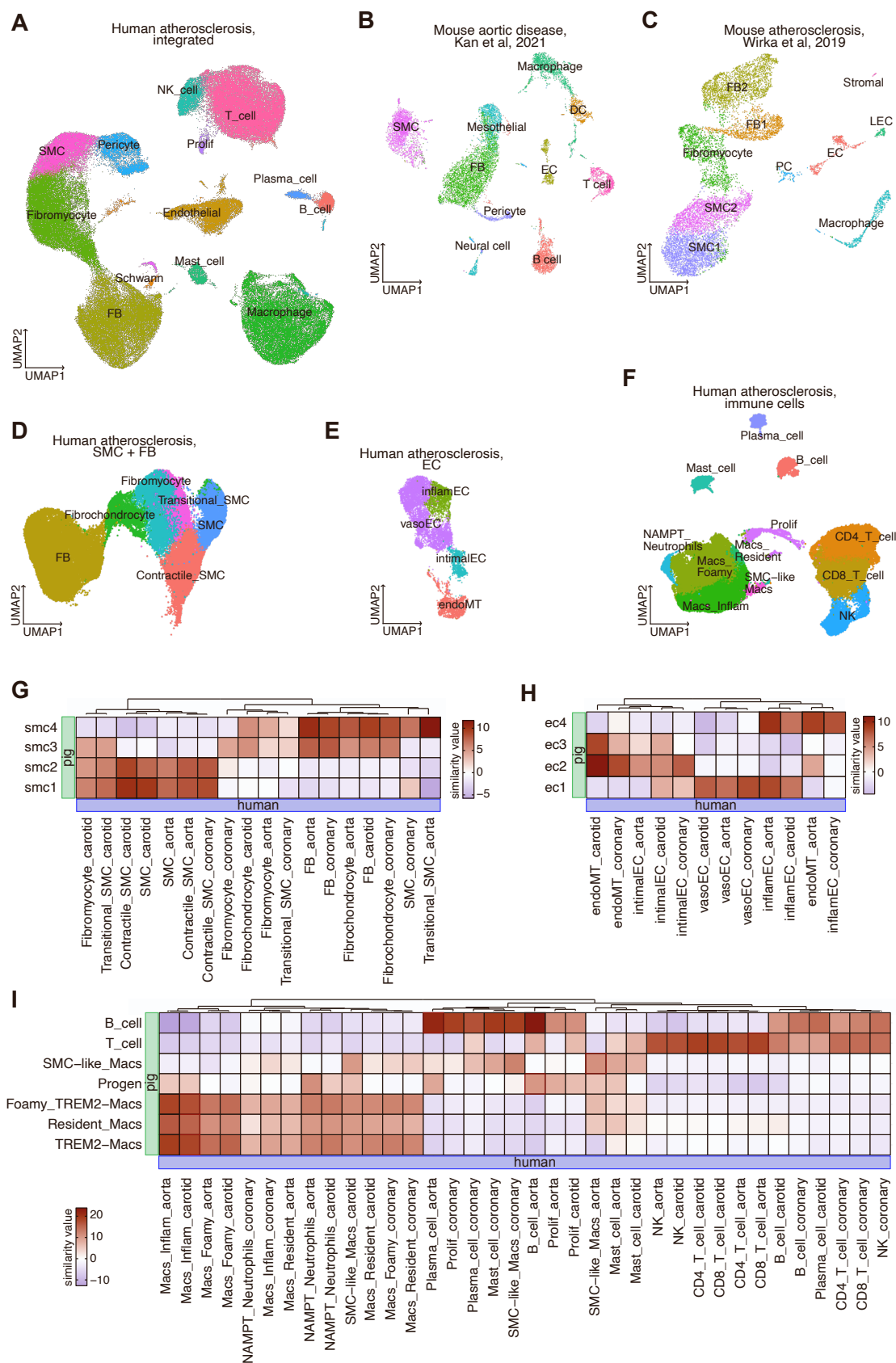
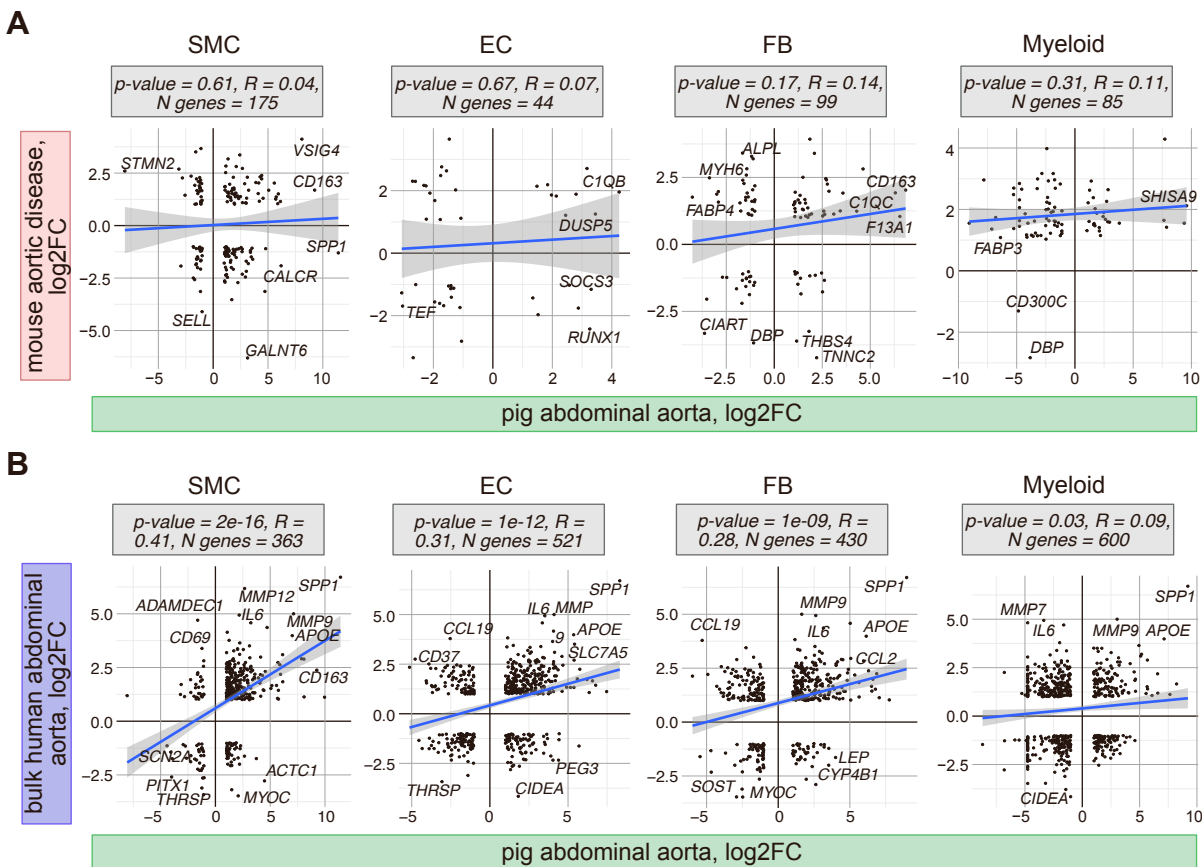


Figure S7

**Figure S7.** Integration of published human and mouse scRNA-seq data and cluster similarity based on vessel types, related to Figure 6. **A.** UMAP of integrated human atherosclerosis scRNA-seq datasets [1-4]. **B.** UMAP of mouse atherosclerosis scRNA-seq dataset [5]. **C.** UMAP of mouse atherosclerosis scRNA-seq dataset [4]. **D-F.** UMAP showing subsets of **(D)** SMC and FB cells; **(E)** ECs; **(F)** immune cells of integrated human atherosclerosis datasets, re-integrated and subclustered. **G-I.** Similarity heatmaps between pig abdominal aorta atherosclerosis scRNA-seq (green bar) and integrated human atherosclerosis scRNA-seq datasets (blue bar) split by type of the vessel origin [1-4]: **(G)** SMC and FB subpopulations; **(H)** EC subpopulations; **(I)** immune subpopulations.



**Figure S8.** Correlation of published mouse and human DEGs to Ossabaw pig per cell type, related to Figure 6. **A.** Correlation of DEGs found in mouse aortic disease [5] with DEGs identified in pig atherosclerosis cell type by cell type: SMCs; ECs; FBs; myeloid cells. **B.** Correlation of DEGs found in human abdominal aorta atherosclerosis (bulk RNA-

seq [6]) with: pig SMCs; pig ECs; pig FBs; pig myeloid cells. Used significant DEGs ( $p\text{-adj} \leq 0.001$ ,  $|\log_2\text{FC}| \geq 1$ ), R – Pearson's correlation coefficient.

1. Alsaigh, T., et al., *Decoding the transcriptome of calcified atherosclerotic plaque at single-cell resolution*. Commun Biol, 2022. **5**(1): p. 1084.
2. Hu, Z., et al., *Single-Cell Transcriptomic Atlas of Different Human Cardiac Arteries Identifies Cell Types Associated With Vascular Physiology*. Arterioscler Thromb Vasc Biol, 2021. **41**(4): p. 1408-1427.
3. Pan, H., et al., *Single-Cell Genomics Reveals a Novel Cell State During Smooth Muscle Cell Phenotypic Switching and Potential Therapeutic Targets for Atherosclerosis in Mouse and Human*. Circulation, 2020. **142**(21): p. 2060-2075.
4. Wirka, R.C., et al., *Atheroprotective roles of smooth muscle cell phenotypic modulation and the TCF21 disease gene as revealed by single-cell analysis*. Nat Med, 2019. **25**(8): p. 1280-1289.
5. Kan, H., et al., *Single-cell transcriptome analysis reveals cellular heterogeneity in the ascending aortas of normal and high-fat diet-fed mice*. Exp Mol Med, 2021. **53**(9): p. 1379-1389.
6. Sulkava, M., et al., *Differentially expressed genes and canonical pathway expression in human atherosclerotic plaques - Tampere Vascular Study*. Sci Rep, 2017. **7**: p. 41483.

## Tables S1–S5

Table S1. Post-sequencing quality statistics, related to STAR methods.							
Sample	Est number of cells	Mean reads per cell	Seq saturation	Reads mapped to genome	Fraction reads in cells	Median genes/cell	Median UMI/cell
control 1	12319	41071	80.2%	95.2%	78.2%	1127	2694
control 2	5689	78154	87.5%	94.7%	71.6%	1074	2490
obese 1	7615	51469	85.4%	92.2%	68.0%	926	1827
obese 2	10108	46139	83.3%	94.5%	74.8%	1078	2240
obese 3	9840	40883	80.2%	94.7%	76.5%	840	1847

Table S2. Updated pig to human gene orthologs.	
<i>Sus Scrofa Ensemble gene ID</i>	<i>Homo Sapiens gene name</i>
ENSSSCG00000009633	TNFRSF10A
ENSSSCG00000022925	SLC2A3
ENSSSCG00000040100	ITPKB
ENSSSCG00000035622	UBE2D3
ENSSSCG00000029160	HSPA1A/B
ENSSSCG00000010212	ANK3
ENSSSCG00000031706	MICAL2
ENSSSCG00000010943	AOPEP
ENSSSCG00000009672	SCARA5
ENSSSCG00000033894	LAMA2
ENSSSCG00000061997	MYH10
ENSSSCG00000033721	TRAC
ENSSSCG00000039222	BASP1
ENSSSCG00000004082	SYNE1
ENSSSCG00000048856	MALAT1
ENSSSCG00000007520	GNAS
ENSSSCG00000056754	lncRNA56754
ENSSSCG00000038144	ENSSSCG00000038144*
ENSSSCG00000008193	ZAP70
ENSSSCG00000013785	EMR2
ENSSSCG00000038606	CUPIN1
ENSSSCG00000040820	KIAA1210

\* Myosin motor domain-containing protein

**Table S3. Excel file with the combined list of significant DEGs for all cell types, related to Figure 2.**

**Table S4. Excel file with the list of upregulated ligand-receptor interactions in atherosclerosis, CellChat, related to Figure 5.**

**Table S5. Excel file with the list of downregulated ligand-receptor interactions in atherosclerosis, CellChat, related to Figure 5.**