A cell and transcriptome atlas of the human arterial vasculature

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SUMMARY:

Vascular beds show different propensities for different vascular pathologies, yet mechanisms explaining these fundamental differences remain unknown. We sought to build a transcriptomic, cellular, and spatial atlas of human arterial cells across multiple different arterial segments to understand this phenomenon. We found significant cell type-specific segmental heterogeneity. Determinants of arterial identity are predominantly encoded in fibroblasts and smooth muscle cells, and their differentially expressed genes are particularly enriched for vascular disease-associated loci and genes. Adventitial fibroblast-specific heterogeneity in gene expression coincides with numerous vascular disease risk genes, suggesting a previously unrecognized role for this cell type in disease risk. Adult arterial cells from different segments cluster not by anatomical proximity but by embryonic origin, with differentially regulated genes heavily influenced by developmental master regulators. Non-coding transcriptomes across arterial cells contain extensive variation in lnc-RNAs expressed in cell type- and segment-specific patterns, rivaling heterogeneity in protein coding transcriptomes, and show enrichment for non-coding genetic signals for vascular diseases.

Keywords
single-cell, vascular development, vascular cell populations, arterial segments, non-coding RNA, vascular diseases

INTRODUCTION:

Human vascular diseases are the leading cause of morbidity and mortality worldwide[1]. Different segments of the human aorta and its branches have seemingly identical morphology and structures, yet have dramatically distinct propensity for different vascular diseases[2-4]. Atherosclerotic disease frequently influences coronaries and carotids, while relatively sparing the descending thoracic aorta[5,6]. Hereditary aneurysmal diseases frequently affect the aortic root and ascending aorta but not other aortic segments[7,8]. Environmental triggers such as tobacco smoking and methamphetamine abuse disproportionally bring on pathologies in the infra-renal segments of the aorta and pulmonary arteries respectively[9,10]. Even inflammatory or infectious aortopathies, such as Takayasu's vasculitis, Kawasaki's diseases, or syphilis aortitis, have well documented segmental preferences[11-13]. While the differences in disease propensity are well documented, the underlying causal mechanisms are less clear. Prevalent hypotheses include differential exposure to life-long hemodynamic forces, along with distinct cellular lineages from different embryonic origins[14,15]. Understanding the cellular and molecular basis for the differences between these arteries may open the door to therapies for a variety of vascular diseases.

Lineage tracing data primarily from several animal models suggest a distinct pattern of embryonic origins for the different arteries that appears conserved through vertebrates[15-18]. The coronary artery is thought to derive from several sources[19,20]. The endothelium from coronary arteries can be traced to two distinct origins, the sinus venosus and the endocardium. Coronary endothelium is thought to instruct the surrounding mesenchyme, which consists of cells originating from the proepicardial organ, to turn into smooth muscle cells. [21,22]. The ascending aorta is thought to be made primarily of at least two populations of embryonic progenitor cells, arising from both the second heart field as well as the neural crest[21,23]. The two populations of cells mix in a distinct pattern, ending before the arch, where most smooth muscle cells are thought to come from neural crest cells of the 4th pharyngeal arch. The carotid artery arises from the 3rd pharyngeal arch. The pulmonary artery (PA) arises from the 6th pharyngeal arch, the thoracic descending aorta is thought to arise from the somitic mesoderm, and splenic mesoderm thought to be responsible for the iliac and femoral arteries. These distinct developmental origins have long been postulated to be causal for risk related to various diseases, as different segments have been shown to have differential expression of selected genes as studied with bulk RNA isolation[24,25]. Transplantation experiments in mammals have suggested arterial segments retain the disease propensities of their original site, suggesting intrinsic behavior of segments accounts for their disease propensity[26,27]. However, whether the same embryological developmental pattern exists in humans is unknown. In addition, the extent to which developmental origin influences cellular gene expression and behavior of adult vessels remains elusive.

To gain insight into these fundamental observations on differential arterial disease risk, we generated a human arterial cellular atlas spanning multiple segments of the arterial circulation. Single cell transcriptomic analysis revealed that different arterial segments exhibit distinct gene expression patterns in the adult. The distinct transcriptomic signatures were most discrepant between fibroblast and smooth muscle cells (SMC) from different vascular beds, while gene expression was relatively similar between endothelial cells and residential

inflammatory cells between the vascular beds. Taken together, these data suggest that vascular identity is encoded mostly in the vascular fibroblast and smooth muscle cell populations.

RESULTS:

To generate a baseline human vascular cellular atlas simultaneously from multiple arterial sites from the same person, we collaborated with the organ donor network to collect arterial tissues from organ donors. Up to 9 distinct arterial sites (ascending thoracic aorta (ASC), aortic arch (ARCH), descending thoracic aorta (DESC), infra-renal abdominal aorta (INFRARENAL), iliac artery (ILIAC), coronary artery (RCA), carotid artery (CAROTID), pulmonary artery (PA), and aortic root (ROOT)) were chosen to be evaluated due to their different embryonic origins and disease propensities. Available segments were collected using identical surgical techniques by a dedicated team of cardiothoracic surgeons to minimalize surgical variability (Figure 1A) and processed in an identical manner from 6 different patients. Tissues were dissociated into single cells using an enzymatic digestion approach we developed to overcome the dense vascular connective tissue [28-30]. This method provided for vascular smooth muscle cells to be the predominant population captured, matching histological cellular composition ratios, while preserving single-cell RNA (scRNA) quality and library complexity. Not all sites were collected from each patient as certain segments were retained along with organs that were utilized for patient care. The digested cells were then sorted for viability and then loaded onto the 10x Genomics platform to perform single-cell transcriptomic capture. Libraries that were made were then sequenced at a targeted depth of at least 50,000 reads per cell, and the resultant data processed using the Seurat platform. The dataset was then filtered to remove poor quality cells and doublets. The overall dataset contained 187,958 cells. Individual cell gene expression was then normalized based on read depth and mitochondrial/ribosomal fraction of reads, to allow for comparison between datasets. The resultant data was then dimensionally reduced onto a 2D representation using uniform manifold approximation and projection (UMAP) for ease of visualization.

The cell type identities were then identified first via unsupervised low dimensional clustering of the transcriptomic data. To avoid over-clustering, we initiated the analysis using very low-resolution clustering, which enables the identification of known distinct cell types, such as endothelial cells and smooth muscle cells. Visualization of the combined scRNA data revealed the presence of all the expected major cell types in the human arteries, namely endothelial cells (EC), smooth muscle cells (SMC), fibroblasts, microvascular cells and immune cells. These cell types are represented using existing canonical markers of each population (Figures 1B, S1A). The largest component of the variance in the dataset came from markers of different cell types, though the data contained a small level of expected inter-person variation such as gender-specific differences (Figure S1B). For ease of visualization and to focus on biology that exists between vascular beds instead of inter-person variations, we performed batch correction between different donors using Single-cell-transform (SCT)-based integration. These batch corrections allow us to better visualize the biology in question[31]. The resultant data are presented here (Figures 1C, S1C), and will be used as the main dimensional-reduction framework for data visualization.

Cells of the Human Arteries

We proceeded to identify each of these specific populations of cells found in our scRNAseq data. Currently, the cellular populations in the vessels are often defined by positive staining of one or two marker genes (i.e. *CDH5* for endothelial cells), which relies on the intrinsic assumption of "marker genes" being specific, and can fail to capture unexpected heterogeneity[17,32-35]. This had led to significant debate regarding the presence and identity of certain cell types, such as vascular mesenchymal stem cells, endothelial progenitor cells, adventitial progenitor cells, pericytes, fibrocytes, fibromyocytes, modulated smooth muscle cells, fibroblasts etc[17,28,32-36]. Discrete populations of cell types should have distinct transcriptomic signatures at rest. The single cell approach allows for identification of different populations of cells that exist in the human arteries a priori utilizing only the data encoded in the global transcriptomes instead of individual marker genes. Thus, we first sought to identify the location and specific markers for different subpopulations of cells that seem readily apparent based on clustering of the cells' global transcriptome using a very conservative clustering resolution. We subsequently applied the canonical "cell type markers" that have been classically utilized to identify SMC, EC, immune cells, fibroblast, pericytes, and various progenitor cells to identify each of these arterial cell populations, to test both the specificity of the markers as well as identify cellular heterogeneity within marker populations. Each of the sub-populations were then located to arterial sections via complementary approaches using RNAscope of specific markers identified from the dataset or mapped spatially with Slideseq (Figures 1D, S1D). Each one of these populations of cells was then compared across the vessel beds to understand what encodes arterial identity. The differentially expressed genes were then assessed for their relevance to human disease by enrichment analysis against human vascular genetics data. In addition, this dataset was then compared with existing murine datasets to identify differences between the human and murine arterial vasculature. Lastly, utilizing the rich information captured with this technique, we created an additional non-coding RNA atlas, to understand the variability and diversity of non-coding transcripts across different vessel beds and cell types.

Smooth Muscle Cells of Great Arteries

Histologically, the largest population of cells in the great arteries are medial SMC and their intimal derivatives that arise from phenotypic modulation of SMC. These cells make up the majority (47.2% in RCA and 53.7% in AORTA) of the arterial wall[28,36,37]. While existing scRNA datasets of arteries in the past have demonstrated inflammatory cells being the most numerous populations of isolated cells[28,38,39], our more vigorous tissue digestion protocol allowed for identification and isolation of SMC as by far the most numerous cell type, matching histological observations. SMC were classically identified using expression of mature smooth muscle contractile apparatus genes such as SMC heavy chain (*MYH11*) or calponin 1 (*CNN1*) (Figures 2A, 2B). Visualizing the population using several canonical SMC marker genes revealed a continuous gradient of SMC maturity in healthy vessels as marked by a gradient of expression of each of these "SMC-specific" markers. The medial and intimal SMC (as evidenced by RNAscope and spatial transcriptome), exist in a distinct gradient of hierarchical maturity, $MYOCD^{+}>CNN1^{+}>MYH11^{+}>ACTA2^{+}>TAGLN^{+}$ population. (Figure 2A). It is important to note that commonly applied smooth muscle markers (smooth muscle actin/*ACTA2*, and transgelin/*TAGLN*/SM22α) are expressed quite broadly, including robust expression of *ACTA2* and *TAGLN* in fibroblast and endothelial populations. This has significant implications regarding

lineage tracing models using *Tagln* or *Acta2* loci as the driver for Cre-recombinase lineage labeling, and perhaps explains the distinct results obtained using different Cre-recombinase in the existing literature.

The high depth of sequencing has allowed for the identification of two large transcriptomically distinct populations of SMC based on very conservative clustering of cells in all vessel beds. Both populations of SMC contain a contractile population, expressing mature SMC markers, and a more "phenotypically modulated" population. We first investigated the smaller population of cells expressing mature SMC markers.

Beyond medial arterial SMC, the other cell population that has been described previously to express mature SMC markers in the vessel wall are SMC -like cells associated with arterioles and pericytes, which are thought to represent mural cells of the microvasculature. Indeed, staining for specific contractile SMC marker *CNN1* revealed positive cells in both the media and small vessels in adventitia (Figure 2B). Pericytes are a heterogenous population of cells classically identified using expression of markers *RGS5* and *NG2/CSPG4*[34,40]. Interestingly, these "canonical pericyte markers" are not confined to one transcriptomically distinct population, and are frequently non-overlapping (Figures 2C, S2A), suggesting that cells previously thought to be pericytes in different studies may represent different heterogeneous populations of cells, including contractile SMC. Differentially expressed gene analysis across these two large SMC populations revealed 587 significantly differentially expressed genes between these two clusters of SMC populations (Figure 2D, Table S1), with pathway analysis demonstrating distinct biological functions (Figures 2E, S2B). For example, these data suggest very distinct pattern of expression of adrenergic receptors, with alpha adrenergic receptor 2A (*ADRA2A*) (Figure 2F) found only in the microvascular population whereas *ADRA2C* is found more in the medial SMC) (Figure S2C). Leveraging RNA in situ hybridization and spatial transcriptome with Slideseq, for markers unique for this microvascular populations identified with scRNAseq, revealed that this population of cells resides exclusively in the adventitia (Figures 2F, S2D). While these cells do express some of the classical "pericyte" markers, due to the potential imprecise nature of these markers as described above, we will call this population of cells "adventitial microvasculature" cells.

The larger SMC population, which is the most abundant cell type in the arteries, the markers map to the cells in the media and intima of the vessel. There was one subcluster of the population where the mature SMC markers were lowly expressed, and another sublcuster where the expression of contractile markers was lost altogether. To identify where these cells were located, we performed spatial transcriptomic profiling using Slideseq in conjunction with RNAscope for specific markers for this population. Consistent with previous work, one of the most specifically expressed genes in these SMC subclusters included *TNFRSF11B* and *FN1*. The less "mature SMC" cells localized to the intima (Figures 2G, 2H, S2E). In conjunction with *Myh11*-cre SMC lineage tracing in murine models[28,41], this lends further evidence to the notion that arterial intima, which is present in human but not mice, are likely smooth muscle derived, arising from media SMC in a process analogous to neointimal formation in murine atherosclerosis and intimal hyperplasia models.

Adventitial Fibroblast in Human Arteries

The second most abundant population of cells isolated in our dataset are adventitial fibroblasts, which form a continuum in the transcriptomic space with smooth muscle cells. This population of cells did not express mature SMC markers (Figures 1C, 2A), but did express high levels of extracellular matrix components and modifiers (Figure 3A). Marker gene RNAscope studies for this population, along with spatial transcriptomics, localized this population exclusively to the adventitia (Figures 3B, 1E). Based on marker gene expression, we assigned this population to be vascular adventitial fibroblast. Surprisingly, while it has been described that the adventitial fibroblast is a heterogeneous population, they appeared to be relatively homogenous in transcriptomic space within the same vascular segments. There were additional endothelial cells and other microvascular cells that are in the adventitia, which makes it more heterogenous than other layers, but the fibroblast themselves within each segment appeared to be one large population. However, there did appear to be significant heterogeneity across the adventitial fibroblasts among the different vascular beds (Figure S3A). Commonly used canonical fibroblast marker genes[32,42], including fibroblast specific protein (FSP1/*S100A4*), *PDGFRA*, *COL1A1*, *GLI1,* were expressed relatively diffusely in several different populations of cells (Figure S3B). This likely explains part of the heterogeneity of results that comes from different lineage tracing / manipulation Cre models in murine studies[43-45]. Using the scRNAseq data, we additionally identified *PI16*, *SFRP2*, and *MFAP5* as specific marker genes for this adventitial fibroblast population (Figure 3C, Table S2). Of note, genes highly expressed specifically in the adventitial fibroblast population were enriched for genes known to cause genetic aortopathies[46-48], including *FBN1*, *TGFBR2*, *LOX*, *FBLN1*, and *MFAP5* (Figure 3D). This suggests a potentially prominent role for this population of cells in the etiology of aortopathies and other vasculopathies.

Endothelial Cells

Endothelial cells heterogeneity across different types of vessels has been well described, including at the single cell level in the organ specific endothelial atlases[35,49,50]. Even at a specific arterial site, there is evidence of different populations of endothelial cells as determined by single cell RNA sequencing of murine aorta[35]. Well established markers for endothelial cells (*CDH5*, *VWF, CD31*) fall within two large distinct clusters (Figures 4A, S4A). A single layer of endothelial cells is found in the intimal surface of the arteries, but frequently many more are found in the adventitia associated with the adventitial micro-vasculature. In our data, the microvascular endothelial cells appear to be the most abundant population of endothelial cells (Figure 4B). The biggest determinant of the transcriptome of the endothelial cells appears to be which region of the vascular wall (intima/adventitia) these cells occupy (Figure S4B). The larger of the two clusters constitutes the majority of the endothelial cells (“Endo1”), and can be further subdivided into additional subclusters (Figure 4C). One subcluster expresses markers previously found to be associated with venous and capillary endothelium, (for example, *ACKR1*), whereas another subcluster expresses *HEY1*, a Notch signaling responsive gene previously associated with arterial endothelium (Figure 4D). Consistent with existing literature, the cluster that expresses *ACKR1* is found exclusively in the microvasculature in the adventitia, whereas the cluster that expresses *HEY1*, a key gene associated with arterial endothelial specification[51], is found both in the adventitia as well as on the luminal surfaces. These data suggest that these EC reside in arterioles in the adventitia as well as on the vascular surfaces (Figures 4E, S4C).

Arterial endothelial cells have been implicated in endothelial to mesenchymal transition (EndoMT), whereby these cells start to express mesenchymal genes[52,53]. This is observed even in our relatively healthy vessels, with expression of SMC marker genes *TAGLN* and *ACTA2* readily found in small groups of endothelial cells at a low level (Figures 2A, 4F). Of note, while it has been suggested that EndoMT may induce endothelial cell transition to smooth muscle cells, we do not detect significant levels of any of the more selective, mature SMC genes (i.e. *CNN1* or *MYH11*) being expressed in the endothelial subset. Lastly, using existing markers for lymphatic endothelial cells (*PROX1*, *LYVE1*)[54,55], we can observe a small population in the adventitial EC as well, though they consist of a very small minority (Figure 4F).

The second cluster of endothelial cells ("Endo2") are notably transcriptomically distinct from the remaining endothelial cells. Interestingly, even within the same segment, this population of cells appears to express different *HOX* genes compared to the other larger endothelial population, suggesting they likely come from distinct origins (Figure 4G). This endothelial cell population expresses a large number of distinct markers, many with known biological function and vascular disease relevance, including *ITLN1*, *NRG1*[56-58] (Figure 4H). The Endo2 population is found in all the different vascular territories, and accounts for a ~10% of the total endothelial population. Interestingly, one of the most specific markers, *ITLN1*, is known to be upregulated after large vascular ischemic events[59-61]. RNAscope using *ITLN1* revealed that this population exists exclusively on the luminal surface, and are not found in the adventitia similar to the other endothelial (Endo1) population (Figures 4I, 4J). Compared to previously reported data, the marker gene for this population appears distinct from the different endothelial populations reported in murine aortic endothelial cells[35,50]. However, the same distinct population of endothelial cells appears to be present in other human large artery single cell datasets[38,62]. Differentially expressed (DE) gene comparison between the two endothelial cell populations (Table S3) revealed that they likely have distinct functions. Compared to the numerically more abundant Endo1 population, the Endo2 population enriches for genes associated with TGFb/BMP signaling, angiogenesis and SMC crosstalk, as well as a strong disease association with atherosclerosis (Figures 4K, S4D). These findings suggest this Endo2 population may be uniquely involved in human vascular diseases.

Macrophages And Lymphocytes:

While classically not considered part of the vessel wall, emerging evidence suggests that inflammatory cells play an important part in vascular homeostasis[11,33,62-64]. Our data further supports this notion, as even though these donor samples were obtained from relatively healthy vessels without clinically overt diseases, there is a significant number of macrophages and lymphocytes present in all the vascular beds. There is a trend towards a slight increase in the total fraction of immune cells in the atherosclerosis prone arterial beds (coronary, infra-renal aorta) (Figure S5A). This may reflect early atherosclerosis, but there are no inflammatory cells or overt plaque seen in the existing intima. The majority of the macrophages and lymphocytes are found in the adventitia. Clusters of B and T cells can also occasionally be found in organized centers of what appears to be tertiary lymphoid organs that abut the adventitia (Figure 1D). Putative computational analysis of cell-to-cell crosstalk using CellChat[65] demonstrates significant cross talk between the adventitial fibroblast, smooth muscle, and the immune cell

populations (Figure S5B). These findings suggest a potential role for resident vascular mural cells in coordinating the vascular immune response.

Determinants of Arterial Identity

To understand differences between arterial segments, we first tried to determine whether there was a consistent relative composition of different cell types within different arteries. Using the low clustering resolution which identified the canonical populations of vascular cells, we discovered that with identical digestion protocols, the relative proportion of cell populations were mostly similar across different arterial beds (Figure S6A). There is a slight increase in inflammatory cells in the vascular beds that are more prone to atherosclerosis, such as the coronary artery, and infra-renal segment of the aorta (Figure S5A). However, there were no large differences in cellular composition across arterial segments, similar to histological studies.

Visualization of a large dataset using UMAP accentuates the drivers of the variance within the dataset[66,67]. Thus, visualization of the data in this manner also enables us to get a sense of relative heterogeneity of each population of cells across the different vascular beds. Simply visualizing this dataset coded by segmental origins, it became readily apparent that there is significant heterogeneity between adventitial fibroblasts and smooth muscle cells among the different vascular beds. However, there were relatively fewer differences between the microvascular, inflammatory and endothelial populations across different vessel beds, as demonstrated on the UMAP (Figure 5A). Because dimensional reduction such as UMAP can distort true distances between populations[66,68], we repeated the analysis by looking at the number of differentially expressed genes between the ascending and descending arteries, and between the coronary and descending pairs (Figures S6B, S6C), as well as the mean Euclidean distance[69] between the average global gene expression of each cell population across the vascular beds (Figures S6D, S6E). Consistent with the UMAP visualization, both of these analyses revealed fewer differences between endothelial cells and macrophages across different vascular beds compared to fibroblast and smooth muscle cells. Interestingly, the same analysis comparing Endo1 and Endo2 population showed much smaller difference between Endo1 population but significantly more difference in the Endo2 population across vascular beds (Figure S6E). These data suggest that the main determinant of arterial identities, at least transcriptomically, appears to be held mostly by fibroblast, smooth muscle cells, and possibly the smaller subset of intimal endothelial cells, Endo2 population, and much less so in the remaining endothelial cells, microvasculature, and inflammatory cells.

Prominent Roles of Embryonic Origin in Adult Vascular Identity

We next investigated the main differences between SMC and fibroblasts from different vascular beds. Examination of the differentially expressed genes across SMC and Fibroblasts of different vascular beds revealed a large number of developmentally important transcription factors[28,70-75], including *HOX* family genes, *ISL1*, *MEF2C*, *TCF21, HAND2, GATA4* etc. (Figure 5C). We hypothesize, therefore, that embryonic origins are the dominant factor in vascular identity. We regenerated a UMAP visualization focusing on the top transcriptomic variances that exist in each individual population. This UMAP readily revealed distinct transcriptomic sub-clusters of Fibroblast and SMC (Figures S7A, S7B). Consistent with our hypothesis, the proximity of different populations of fibroblast by either Euclidean distance or visualization in UMAP space

appears to correspond to each segments presumed embryonic origin reported in animal models (Figures 5D, 5E, S7C). Cells from arteries of different caliber and flow with similar embryonic origins (i.e.aortic arch, and ascending aorta/carotid arteries) cluster closer together than their connected counterparts (such as descending thoracic artery to aortic arch). This suggests that embryonic origins of each of these arterial segments play a dominant role in adult gene expression. Curiously, this developmental pattern is less preserved in Identical analysis of macrophage, microvasculature, and Endo1, suggesting that in these cells the resting gene expression may be less influenced by their embryonic origin (Figures S7D, S7E, S7F).

Many of the differentially regulated genes found on the list has been known to be regulated by these transcription factors in different processes. To unbiasedly determine if these lowly expressed developmental master-regulators continue to play an important role in adult gene expression globally, we investigated whether the binding sites for these transcription factors are enriched in differentially expressed genes between segments compared to other variable genes in the dataset, focusing on the ascending and descending thoracic aorta as the main test case. Transcription factors *ISL1*, *HAND2*, *MEF2C* are expressed in the second heart field and neural crest cells which give rise to the ascending aorta, whereas *HOX* genes are expressed in the somitic mesoderm which gives rise to the descending thoracic aorta (Figures 5F, S7G). Consistent with these transcription factors playing an important role, we saw a very strong enrichment of ISL1, HAND2, MEF2C, and HOX binding sites in the regulatory regions of differentially expressed genes in fibroblast and SMC across these vessel beds of distinct embryonic origin (Figures 5G, S7H). These data suggest the embryonic origin and the persistent expression of these critical transcription factors into adulthood plays a critical role in arterial identity and their global gene expression in adult.

Adult Vascular Hox Gene Expression Patterns

While *HOX* genes' roles are best elucidated in development, as one of the most potent cell fate and patterning signals[70,76], their role in adult tissue identity and disease is less well understood. It has been shown that different arterial segments express different *HOX* genes by bulk RNAseq[25]. Given the impressive enrichment of *HOX* gene binding motifs in regulatory regions of most differentially expressed genes, we leveraged our dataset to better understand *HOX* gene expression across the different cell types within each segment of the aorta.

It appears that adult *HOX* gene expression patterns from the same vessel segment are conserved in adulthood and thus mirror their embryonic origin (Figures 6A, S8A). Descending thoracic aorta, abdominal aorta, and iliac arteries each expresses distinct subsets of *HOX* genes that are conserved from one person to the next (Figure S8B). However, we observed that within the different cell types of a segment, each cell type expresses a different subset of *HOX* genes. For example, in descending thoracic aorta, *HOXD8* is most highly expressed only by Endo1, *HOX A4/A5* predominantly by Endo2, and *HOXA3* in SMC, and *HOX A5/B7* in fibroblast. While smooth muscle and microvasculature are transcriptionally and phenotypically similar, and AdvFib and microvasculature are geographically close, they express very distinct *HOX* genes patterns (Figures 6A, S8C). On the other hand, adventitial fibroblast *HOX* gene expression is quite similar to those of the medial SMC. This potentially reflects these two populations of cells (medial SMC and AdvFib) have more closely shared embryonic origins compared to those of microvasculature in the adventitia. As further evidence that the *HOX* genes likely play an

important role in adult arterial identity, we clustered the cells and segments based on *HOX* genes alone. Hierarchical clustering of SMC and fibroblast based on *HOX* genes alone mirrors hierarchical clustering of whole transcriptomes, and readily reflects each segment's developmental origin (Figures 6B, 6C). Given that most immune cells are likely formed uniformly in the marrow and migrate into each segment, unsurprisingly there is less distinction between them. While the endothelial cells are some of the earliest to form during embryonic development of the great vessels, with each segments' endothelial cells having distinct origins, they are significantly less heterogeneous compared to neighboring segments of different embryonic origin, with the potential exception of the Endo2 population (Figure S8D). Those populations that differ from one vascular bed to another tend to have a very distinct pattern of *HOX* gene expression, whereas cell types with similar *HOX* gene expressions tend to have globally more similar transcriptomes. The persistence of this very distinct pattern of *HOX* gene expression across individuals suggests that conserved transcriptomic regulation of these genes in adulthood, along with enrichment in HOX binding motifs in differentially expressed genes noted above suggests critical roles for *HOX* genes in cell type specific adult segmental specific gene expression.

Enrichment of Vascular Disease Rare and Common Variant Genetic Signals in Segmental Transcriptomic Differences

Our original hypothesis was that the transcriptomic differences between these vascular beds are important for differences in disease propensities, and that the single cell atlas will help identify novel pathologic populations and genetic programs. To determine if these identified transcriptomic differences play a role in adult diseases, we then asked whether the differentially regulated genes were enriched in human signals of genetic diseases. We again focused our attention on the difference between ascending and descending thoracic aortic segments, which have a known strong propensity for aortic aneurysms[77,78]. While these disease segments are subject to changes in flow, there was relatively few differences between the global endothelial cell expression of cells from these two vessel beds (Table S4). However, there were significantly more transcriptomic differences between fibroblast and SMC from these different connected arterial segments. Most notably, there were a large number of differentially regulated genes in the fibroblast population that have known roles in inherited aortopathies[79]. A number of mendelian aortopathies involve haploinsufficiency of critical ECM components, including fibrilin 1 (*FBN1*), lysyl oxidase (*LOX*), microfibril associated protein 4 (*MFAP4*), microfibril associated protein 5 (*MFAP5*), and collagen 3A1 (*COL3A1*). Pathological decreased expression of these genes is known to be causal for thoracic aortic aneurysms, with a strong preference for affecting the root and ascending aorta. Interestingly, the expression of each of these genes is significantly lower in the ascending than the descending aorta (Figure 7A). Closer examination revealed that differences in expression were seen nearly exclusively in the adventitial fibroblasts, and not in the medial SMC (Figure 7B). Conversely, *PRKG1* is a gene whose gain of function is known to increase the risk of aneurysm[80-82], also has higher expression in the aortic root and ascending compared to descending (Figure 7C).

To determine if other arterial segment specific vascular traits showed segregation of genetic signals, we subsequently performed the same analysis on common genetic variants associated with human aortic diameter. Most GWAS lead variants are located in non-coding

regions of the genome, likely exerting its effect on gene dosages. Thus, GWAS “hits” enrich for genes whose dose are likely to contribute to pathology, with the added benefit that GWAS “hits” do not hold bias towards known pathways. We leveraged recently published GWAS on aortic diameter[83], and asked how often GWAS genes for aortic diameter are differentially expressed in different populations of cells in aneurysm prone (i.e. ascending) vs resistant (i.e. descending) segments. For those within the non-coding region of the genome, we used the putative target gene suggested in the original manuscript, which mostly reflected the closest gene. From the list of GWAS genes, we then determined how often these dose sensitive genes were differentially expressed at the individual cell level in ascending and descending aorta. After Bonferroni correction for multiple hypothesis testing, our analysis revealed 31 diameter sensitive genes are differentially expressed in ascending vs descending fibroblasts, and 18 diameter sensitive genes are differentially expressed in smooth muscle cells, and 5, 3, and 4 genes are differentially expressed in microvasculature, macrophage, and endothelial cells, respectively (Table S5). These analyses suggest a large proportion of segment-specific modifiers of aortic propensity for aneurysm act through fibroblast and SMC, with surprisingly little transcriptomic differences in the endothelial cells. These findings confirm the known role of SMC in aneurysms, while suggesting a prominent unrecognized role of AdvFib in the same disease process.

We subsequently performed the same analysis in atherosclerotic disease and observed a similar pattern. Coronary artery and carotid arteries are known to be more prone to atherosclerosis whereas descending thoracic aorta is less so[84]. Thus, these vessel beds are used for the atherosclerosis disease propensity comparisons to identify causal cells and causal genes for the reasons for the differences in disease propensity. A significant subset of common variant associated genes for atherosclerotic disease (in this case, coronary artery disease) are differentially expressed in the different population of cells in the vessel wall (Figures 7D, 7E, S9A). Our analysis revealed 45 CAD GWAS genes differentially expressed in fibroblast, 30 in SMC, 11 in microvasculature, 15 in Endo1, 7 in Endo2, and 27 in macrophages (Table S6). Functional analysis of enriched genes in each population of cells revealed angiogenesis related biological processes are significantly enriched in SMC, and PDGF and VEGF signaling enriched in the fibroblast population (Figure 7F). These analyses suggest that segment specific differences in vascular disease propensities appears to be mostly encoded in fibroblast and SMC, and much less so in endothelial cells.

Sex-specific differences

At a population level, there is a distinct difference in the susceptibility for vascular diseases between patients of different biological sex[85-88]. Coronary artery disease and Marfan syndrome, for example, are known to have worse vascular manifestation in men compared to women[89,90], whereas Takayasu arteritis or spontaneous coronary artery dissections are much more common in women than men[91,92]. The underlying cause of these sex specific differences is poorly understood. While many of the causes of the sex specific differences may be related to numerous systemic and other confounding factors that are unrelated to the cell biology at the vessel wall, this dataset containing an equal number of men versus women, offers an opportunity to explore potential differences between male and female arteries. We performed differential expression analysis comparing different cellular populations in our dataset. This analysis revealed different cell-type specific transcriptomes between male and female sex.

Unsurprisingly, many sex chromosome specific genes, such as *XIST*, were differentially regulated in all cells. The analysis revealed 119, 176, 201, 79, 196, 281, 172 sex-specific differentially expressed (DE) genes between SMC, fibroblast, macrophage, Endo1, microvasculature, Endo2, and lymphocytes respectively (Table S7). Unlike cross-segmental identities that appears to lie more in fibroblast and SMC, sex-specific differences were seen across all cell types and are more profound in endothelial and immune cells. This parallels the observations of sex specific differences observed in incidence of vasculitis and vascular endothelial dysfunction. Sex differences in different populations appears to govern different aspects of vascular biology and different diseases. Pathway analysis with the GO biological process performed on each of these DE genes revealed distinct biological process enrichments (Table S8). Different cell type DE gene overlap with OMIM data revealed distinct vascular disease associations with known sex preferences (i.e. SMC association with Ehler-Danlos, and EC association with macular degeneration) (Table S9).

Of mice and men

Our dataset enables us to compare cellular transcriptomes and cell populations of healthy human arteries with murine ones. One of the biggest differences observed between human and murine arteries is the presence of an intima at baseline. It is well-established that even a healthy human has a significant layer of intimal cells as early as the second or third decade of life[93-95]. This is well reflected in this dataset. While healthy murine vessels do not contain a population contiguous with the fibroblast population[28-30], we see a population of cells in healthy human vessels that appears to be correspond to “phenotypically modulated smooth muscle cells” in mice (Figure S10A). These modulated SMC are found almost exclusively in the human arterial intima, and appear to comprise the majority of healthy intimal cells (Figure 2H). Beyond the differences in the intimal cells that exist in the resting vessel, a comparison of the human and mouse single cell RNA datasets processed in a similar manner reveals notable differences in a few cellular populations. There is a significantly larger population of microvascular cells, associated with the vaso-vasorum in human arteries compared to murine tissue. This finding is expected given the increased thickness of human arterial tissues. As a result, the majority of endothelial cells isolated from human arteries actually lines the microvasculature that supplies the vessels instead of those on the luminal surface. In addition, there appears to be two transcriptionally distinct subsets of endothelial cells in the human data, whereas the endothelial populations appear less distinct in mice. While heterogeneity in endothelial cells in murine arteries has also been described, no previously described population of endothelial cells in mice matches the “Endo2” population found in humans[28,29,35].

A larger number of immune cells (both monocyte/macrophages and lymphocytes) are present in donor human vasculature without overt disease compared to young/healthy mice. This may reflect subclinical disease in these donors after decades of hyperlipidemia and hypertension or rather fundamental differences between murine and human vasculature[29,30]. Lastly, while pro-calcific progenies of the SMC often accompany intimal formation or other vascular damage in murine models, the same population/markers (*COL2A1*, *IBSP*) are not found in healthy human arteries with extensive intima (Figure S10B), which may suggest a different process causing calcification in mice compared to humans.

Cell type- and Segment- Specific Non-coding Transcriptome

While existing analysis of scRNAseq data has mostly focused on protein coding genes, there is an increasing recognition that non-coding transcripts such as long non-coding RNAs (lncRNAs) play an important role in health and disease[96-98]. Taking advantage of the fact that a significant portion of lncRNAs is poly-adenylated and thus captured by the 10x platform, we asked whether lncRNAs also follow segmental and cell-type specific expression by aligning these sequencing data to a library of known lncRNAs. Given the library preparation of 10x does not allow for strand specific capture, one cannot readily determine if a read is from the coding or antisense strand of a transcript. To remove any potential confounding reads from protein coding genes, we leveraged a reference lncRNA transcriptome that eliminated any segments of overlap with any protein coding mRNAs. We will refer to this as the sc-lncRNA transcriptome.

This dataset contains 53,247 unique non-coding transcripts that are found in the vascular cells (compared to 18,088 protein coding genes). Despite our stringent filtering, on average, ~1601 (median 1284) non-coding transcripts were detected in each cell, encoding an average of ~397 (median 323) lncRNAs per cell. This represents ~10% in number of genes or reads compared to the protein coding genome. Differential gene expression analysis of these data found that a significant portion of the sc-lncRNA transcriptome also exhibited cell-type and segmental specific expression (Figures 8A, 8B).

Employing these data, we investigated how much of the cell type specific identities are encoded in this limited subset of the non-coding transcriptome. To accomplish this, we performed unbiased clustering of the sc-lncRNA transcriptome using standard methods, along with UMAP based visualization. Utilizing no protein coding gene data, analyzing sc-lncRNA transcriptome alone, standard unbiased expression clustering approach could readily segregate the dataset into cell type specific clusters that recapitulate those identified using the entire transcriptome (Figure 8C). Comparison of cell type identities assigned via unsupervised low-resolution clustering to those identified by protein coding transcriptome demonstrated that ~92% of the cells were clustered in identical ways using sc-lncRNA data alone without any protein coding transcripts. The cells exhibiting discordance were primarily situated at the interfaces between distinct cell types as visualized on the UMAP (Figure S11A). Hierarchical clustering based on sc-lncRNA data itself in SMC and fibroblast could recapitulate the developmental origin (Figure 8D). This suggests that a significant portion of the identities of each cell types, behavior, and embryological origin are embedded within the non-coding transcriptome.

The majority of common variants that are identified in humans that carry heritable risk of vascular diseases lie within the non-coding region of the genome[99-101]. One hypothesis has been that some of these non-coding regions of the genome may overlap with functional lncRNAs and alter their function. To test this hypothesis, we asked whether these vascular cell specific lncRNAs enriched for genome-wide significant GWAS SNPs for different vascular conditions. Interestingly, there appears to be a significant enrichment of GWAS SNPs for coronary artery diseases that lie within the exons of lncRNAs compared to expected (18 lncRNAs with 158 MVP SNPs) (Figure 8E, Table S10) [(cite MVP)]. We subsequently intersected the differentially expressed lncRNAs with the loci from the GWAS catalog, revealing a significant intersection of these lncRNAs with loci associated with coronary artery disease (CAD) and heart disease (Figure S11B). This finding is consistent with results obtained with analysis of the Million Veterans Program CAD GWAS [100].

DISCUSSION

By performing cellular transcriptomic and spatial localization of donor arteries across a variety of arterial segments, we created a resource for the vascular research community to better understand the cellular composition and distribution of gene expression programs across human arteries. We identified major cellular populations characterized not by individual markers, but by entire transcriptomic profiles and spatial localizations. We contribute to the existing literature of single cell characterization of human arteries by improving optimal digestion of highly fibrous arterial tissue to obtain high quality single cell RNAseq data superior to the cellular populations and ratios observed by histology[28,30]. These techniques also enabled us to achieve deeper characterization of vascular cell transcriptomes, including lowly expressed transcription factors, to better elucidate their biology. We hope this dataset can serve as a useful reference to aid the cardiovascular research community in future single cell characterization of vascular conditions.

Utilizing entire cellular transcriptomes instead of specific marker genes to define cellular heterogeneity, this dataset both validates and challenges existing approaches to study vascular diseases in murine and cellular models. One immediate benefit of this approach is the ability to identify specific markers empirically in an unbiased manner for populations of cells that are transcriptomically distinct. This large number of cell-type specific markers can then be utilized to enable other vascular discoveries, including generating more specific murine Cre-lox models to understand individual cell populations. Furthermore, our data can help reconcile seemingly paradoxical findings of existing cellular and lineage tracing models. For example, our analyses have revealed surprising heterogeneity and limitations of many commonly used vascular population markers (*RGS5*, *TAGLN*, *GLI1*, *ACTA2* etc), which may explain some of the contradictory findings in animal models that arise from modification using different “marker” genes[102]. By combining single cell RNAseq data with complementary spatial transcriptomic techniques, we were able to localize transcriptomically unique populations that reside in different parts of the vessel wall. This includes identification of multiple “smooth muscle marker” positive populations, and multiple subsets of endothelial cells with distinct transcriptomes and localizations. This extensive heterogeneity that exists in healthy vessels may help explain conflicting findings that sometimes occur from in vitro studies of primary human arterial cells, as in vitro “arterial SMC” or “arterial endothelial” cells using canonical markers may be isolated from one or multiple populations of cells. Our dataset may also be used to deconvolute the population(s) of vascular endothelial cells or SMC[103,104].

One of our main goals with this dataset was to identify underlying differences in the biology and disease propensities across different human arterial segments. Current understanding of such differences mainly attributes them to flow characteristics and hemodynamics[105-107]. Indeed, turbulent flow, laminar flow, and static flow are known to create significant alterations in transcriptomic profiles of endothelial cells that are relevant to diseases[108,109]. On the other hand, numerous pieces of evidence also exist suggesting that developmental influences may alter vascular disease propensity[110-112], such as the frequently observed changes in atherosclerotic disease lesion quality and severity near junctions of vessels segments deriving from distinct developmental origins[113]. This nature vs nurture debate

of vascular disease remains unsettled. Our analysis of healthy vessels prior to initiation of disease, yet still subjected to decades of differential flow patterns, reveals relatively few differences between the transcriptomes of endothelial cells across different vascular segments. However, adventitial fibroblasts and medial smooth muscle cells are clearly responsible for the main transcriptomic differences across different vascular beds. Differentially expressed genes in these populations of cells enrich for both common and rare variants of vascular diseases. Furthermore, the regulatory regions of these differentially regulated genes significantly enrich for transcription factor binding sites of developmentally critical master regulators. These findings suggest that developmental origins of these vessels play a large role in different disease propensities at least equally and maybe more so than decades of differences in vascular flow and pressure. Developmental master regulators may therefore possess key pathogenic roles. Given the clinical observation of strong associations of different arterial segments for diseases within the same person[5,78,93,114], modulation of these developmental master regulators' function may be sufficient to alter disease development or progression. Lessons learned from developmental biology and additional discoveries aimed to better understand these factors' exact functions are attractive targets for future therapies.

One of the largest populations of cells identified from this dataset are adventitial fibroblasts. This population of cells carries remarkable heterogeneity across vascular segments with different disease predilections, including a large number of genetic signals for aneurysmal diseases and atherosclerosis. Yet, this population of cells has traditionally not been the focus of pathogenesis of vascular diseases. Further evaluation of this population of cells and their roles in vascular pathologies are warranted.

Analysis of the human endothelial population reveals significant heterogeneity as previously demonstrated in murine vascular single cell analysis. However, unlike murine models[35,50], the majority of human endothelial cells in the vessel walls reside in the microvasculature of the adventitia. Consistent with distinct developmental origins and early arterial/venous/lymphatic endothelial fate commitment demonstrated in animal models[14,15,18,35,50,54,55], the most significant determinant of the endothelial transcriptome is the type of vessel it covers. A distinct population of relatively rare endothelial cells is located at the luminal surface of the great arteries. The lack of an equivalent endothelial cell population in murine single cell vascular data suggests the function of this endothelial population may not be accurately measured in murine models. Given the enrichment of this population of cells for distinct disease-related transcriptomic programs, further dedicated studies of this population of cells may yield distinct insight into their pathophysiological function.

Murine disease models with SMC lineage tracing have identified "de-differentiated" and "phenotypically-modulated" SMC as key pathogenic cells in a variety of vascular conditions[17,28,36,37]. The importance of this population of cells is well-established through a variety of lineage tracing and manipulation models of different diseases[28,41,115,116]. However, in macroscopically healthy vessels as obtained in these studies, an apparent de-differentiated SMC subset is universally present across all vascular segments and populates the intima. This population of cells expresses a similar global transcriptomic program as those SMC-lineage traced cells that have lost their contractile genes in murine models. This potentially reflects the presence of subclinical diseases in all these donors given years of relative hyperlipidemia due to exposure to a western diet[117]. Alternatively, another explanation for this observation is that SMC

phenotypic modulation itself is part of the normal physiological spectrum and not necessarily pathologic. Instead, the specific ways which seemingly similar SMC populations phenotypically modulate in different ways may govern the quality and burden of disease. This notion is further supported by the fact that there appears to be particularly large global transcriptomic differences in the “modulated” /intimal SMC population observed across the vascular beds, more so than “contractile” smooth muscle cells. This same population of cells also carries more genetic signals of vascular diseases than other populations. Existing data are unfortunately inadequate to discern these two possibilities. Additional characterization of a larger number of healthy and diseased vascular tissues at different ages may help elucidate the answer.

Lastly, while the focus of pathogenic factors for vascular disease is often on protein coding genes[83,99-101], analysis of non-protein-coding transcriptomes of the vascular beds reveals surprisingly rich information and heterogeneity. Global analysis of lncRNAs alone can readily identify the main vascular wall cell types and differentially expressed lncRNA across vascular beds disproportionally enrich for non-coding variants that influence vascular disease risk. While the analysis is preliminary, the enrichment of genetic signals suggests these differentially enriched lncRNAs may contain critical regulators of disease risk.

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AUTHOR CONTRIBUTIONS

P.C., T.Q., and A.K conceived and supervised the study. Q.Z., D.S., and P.C performed bioinformatic analyses. P.C., A.P., W.G., A.D., C.W., W.J., A.B. and R.W. performed single cell captures, spatial transcriptomics, histology, and IHC. D.Y.L., H.S., and R.W. helped analyses. A.P., A.D., R.S. L.L. M.F. performed human tissue collection, D.Y.L., Y.R., T.N., R.S., B.T.P., J.P.M., M.W., A.B., M.I., R.K., L.L., helped conducting experiments. P.C., T.Q., and Q.Z. interpreted data and wrote the manuscript. R.W., M.F., and J.B.K contributed to experiments and manuscript. The authors read and approved the final manuscript.

DECLARATION OF INTERESTS

A.K. is on the scientific advisory board of SerImmune, AINovo, TensorBio, Arcadia, Inari and OpenTargets; and has financial stake in Illumina, DeepGenomics, Immunai and Freenome.

SUPPLEMENTAL INFORMATION

Figures S1–S11, Word file containing additional figure.
Figure S1. Single cell RNA and spatial Slide-Seq of human arteries, related to Figure 1.
Figure S2. Smooth muscle cells lineage, related to Figure 2.
Figure S3. Fibroblast in different embryonic origins, related to Figure 3.
Figure S4. Endothelial cell populations across vessels, related to Figure 4.
Figure S5. Macrophages and lymphocytes.
Figure S6. Determinants of arterial identity.
Figure S7. The roles of embryonic origin in different cell types, related to Figure 5.
Figure S8. Vascular *HOX* gene expression patterns, related to Figure 6.
Figure S9. GWAS genes enrichment in different cell types, related to Figure 7.
Figure S10. Smooth muscle cell modulation in human arteries.
Figure S11. Concordant of lncRNA cell type annotation and GWAS catalog enrichment, related to Figure 8.
Table S1-S11. Excel files containing additional data too large to fit in a PDF.
Table S1. Differentially expressed genes between these two clusters of SMC populations, related to Figure 2.
Table S2. Specific markers defined adventitial fibroblast population, related to Figure 3.

Table S3. Differentially expressed genes between the two endothelial cell populations, , related to Figure 3.
Table S4. Differentially expressed genes between ascending and descending thoracic aorta, related to Figure 7.
Table S5. Diameter sensitive genes, related to Figure 7.
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Table S7. Sex-specific differentially expressed genes.
Table S8. GO biological processes of sex-specific differentially expressed genes.
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Table S10. lncRNAs overlapped with MVP GWAS loci for coronary artery diseases, related to Figure 8.
Table S11. The metadata for donors includes details about the collected tissues and the assays performed.

FIGURE LEGENDS

Figure 1. Single cell RNA and spatial landscape of human arteries.
(A) Schematic diagram of the 8 distinct human arterial sites collection strategy.
(B) Workflow of the experimental design and the sample processing for scRNA-seq and Slide-seq.
(C) RNA expression of existing canonical markers of each cell type population.
(D) UMAP visualization and clustering of cells labeled by cell type.
(E) Spatial dim plot visualization of the right coronary artery (RCA), annotated by cell type (left). The right panel presents a split view, illustrating the positions of individual cell types on the same slide.
See also Figure S1.

Figure 2. Smooth muscle cells of great arteries.
(A) RNA expression of mature smooth muscle contractile genes *MYOCD*, *CNN1*, *MYH11*, *ACTA2* and *TAGLN*, shown on the UMAPs.
(B) Immuno-fluorescence staining of CNN1 (green) and DAPI (blue) in ascending aorta.
(C) UMAP showing the *RGS5* RNA expression level.
(D) The differentially expressed genes between the two main clusters of SMC population, ranked by the average log2 fold change level.
(E) Dot plot showing the z scores of enriched biological processes in the differentially expressed gene between the two clusters of SMC population.
(F) The UMAPs showing the RNA expression of the unique makers in adventitial microvasculature cell cluster (left panels). RNAscope validated the expression of these markers with specific probes (right panels).
(G) RNA expression of medial and intimal smooth muscle genes *MYH11*, *FN1* and *TNFRSF11B*, shown on the UMAPs.
(H) Spatial localization of the cells expressed *MYH11* and *FN1* in RCA (left, middle). And the RNAscope staining of *TNSRSF11B* expression (right).

See also Figure S2 and Table S1.

Figure 3. Adventitial fibroblast in human arteries.
(A) The scores derived from the expression levels of collagen (middle) and extracellular matrix genes (right) depicted on UMAP plots were analyzed alongside cell type annotations (left).
(B) *PI16* RNA expression levels on RNAseq UMAP plot (left), Slide-seq dim plot alongside the fibroblast localization in RCA (middle), and the RNAscope staining with probes (right).
(C) The differentially expressed genes between fibroblast cells and all other cell types, plotted by their percent of expressed cells and the average log2 fold changes.
(D) The violin plots illustrate the specific expression of the genes *FBN1*, *TGFBR2*, *LOX*, *FBLN1*, and *MFAP5* within the adventitial fibroblast population.
See also Figure S3 and Table S2.

Figure 4. Distinct endothelial cell populations across vessels.
(A) Two large distinct clusters of endothelial cells shown on the UMAP.
(B) Endothelial cells identified by the canonical marker *CDH5* RNAscope staining (left), and the endothelial cells localization on the Slide-seq dim plot of aorta (right).
(C) The sub-clustering of endothelial 1 population.
(D) The UMAP (top panels) and violin (bottom panels) plots showing the expression of cluster-specific genes *ACKR1* (left panels) and *HEY1* (right panels) in endothelial1 cells.
(E) The expression of cluster-specific genes *ACKR1* (left) and *HEY1* (right) are stained by RNAscope probes in ascending aorta.
(F) The UMAP (left panels) and violin (right panels) plots showing the expression of SMC genes *TAGLN* and *ACTA2* (left panels), or lymphatic genes *PROX1* and *LYVE1* (right panels) in endothelial1 cells.
(G) Heatmap showing the z scores of *HOX* genes expression in each of the vessel segments in both endothelial 1 and 2.
(H) Endothelial 2 specific genes *ITLN1* and *NRG1* expression levels on RNAseq UMAP plot.
(I) RNAscope staining of *ITLN1* gene expression ascending aorta.
(K) The co-staining of *ITLN1* (blue) and *CDH5* (red) expression in ascending aorta.
(L) Dot plot showing the z scores of enriched biological processes in the differentially expressed gene between endothelial 1 and 2.
See also Figure S4 and Table S3.

Figure 5. Prominent roles of embryonic origin in adult vascular identity.
(A) The heterogeneity of each population of cells across the different vascular beds on UMAP.
(B) A split view UMAP of the segmental origins presents the heterogeneity of each cell types.
(C) Heatmap showing the z scores of *HOX* genes expression in each of the vessel segments in both SMC and fibroblast.
(D) Dendrogram Clustering using Euclidean distance calculated by all expressed genes showing the correlation of each segment with its anticipated embryonic lineage within fibroblasts.
(E) Heatmap showing the percentage of each segment depicted within the distinct UMAP clusters.

(F) Violin plots showing the differential expression patterns of transcription factors *ISL1*, *HAND2*, *MEDF2C*, and *HOX* genes in fibroblast of ascending and descending sites.
(G) Enrichment of ISLET1, HAND2, MEF2C, and HOX binding motifs on the promoter regions of differentially expressed genes between ascending and descending fibroblasts, with macrophages serving as controls.
See also Figure S7.

Figure 6. Adult vascular *HOX* gene expression patterns.
(A) Heatmap showing the log10 normalized expression values of *HOX* genes across the different vascular segment in fibroblast, SMC, endothetial1, endothelial2, microvasculature, or macrophage.
(B) Dendrogram clustering using Euclidean distance calculated by all *HOX* genes showing the correlation of each segment with its anticipated embryonic lineage in SMC (bottom) or fibroblasts (top).
(C) The heatmap illustrates the Pearson correlation coefficients among all arterial segments, derived from the expression values of all *HOX* genes. These segments are clustered according to Euclidean distance in SMC (left) or fibroblast (right).
See also Figure S8.

Figure 7. Enrichment of vascular disease variants in segmental transcriptomic differences.
(A) Dot plot showing the differential expression patterns of mendelian aortopathies genes *FBN1*, *FBNL1*, *LOX*, *MFAP4*, *MFAP5*, and *COL3A1* genes in fibroblast of ascending and descending sites.
(B) Bar plot showing the differential expression significances of these mendelian aortopathies genes *FBN1*, *FBNL1*, *LOX*, *MFAP4*, *MFAP5*, and *COL3A1* between ascending and descending sites in fibroblast.
(C) Violin plots showing the differential expression patterns of aneurysm risk in fibroblast (left) or SMC (right) of ascending and descending sites.
(D) The bar graph displays the count of Million Veteran Program (MVP) GWAS genes that are differentially expressed in each cell type when comparing the coronary artery and carotid with descending segments.
(E) Genetic association between the MVP GWAS genes and the differentially expressed genes across the coronary artery, carotid, and descending segments within each cell type, ranked by the number of overlapping genes.
(F) Heatmap showing the z scores of fold enrichment of biological processes in the differentially expressed genes across the coronary artery, carotid, and descending segments within each cell type.
See also Figure S9 and Table S4, S5, S6.

Figure 8. Cell type and segment specific non-coding transcriptome.
(A) Heatmap showing the z scores of non-coding RNAs expression in each of the cell types.
(B) Heatmap showing the z scores of non-coding RNAs expression in each of the vascular segments.

(C) UMAP visualization and clustering of cells using the sc-lncRNA transcriptome, annotated based on either whole transcriptome-derived cell types (left) or lncRNA-derived cell type identification (right).
(D) Dendrogram clustering using Euclidean distance calculated by all expressed lncRNAs showing the correlation of each segment with its anticipated embryonic lineage in SMC (top) or fibroblasts (bottom).
(E) The dot plot (top) illustrating the MVP SNPs positioned directly within the loci of differentially expressed lncRNAs. The violin plot displays the expression levels of these intersected lncRNAs. See also Figure S11 and Table S10.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- **QUANTIFICATION AND STATISTICAL ANALYSIS**

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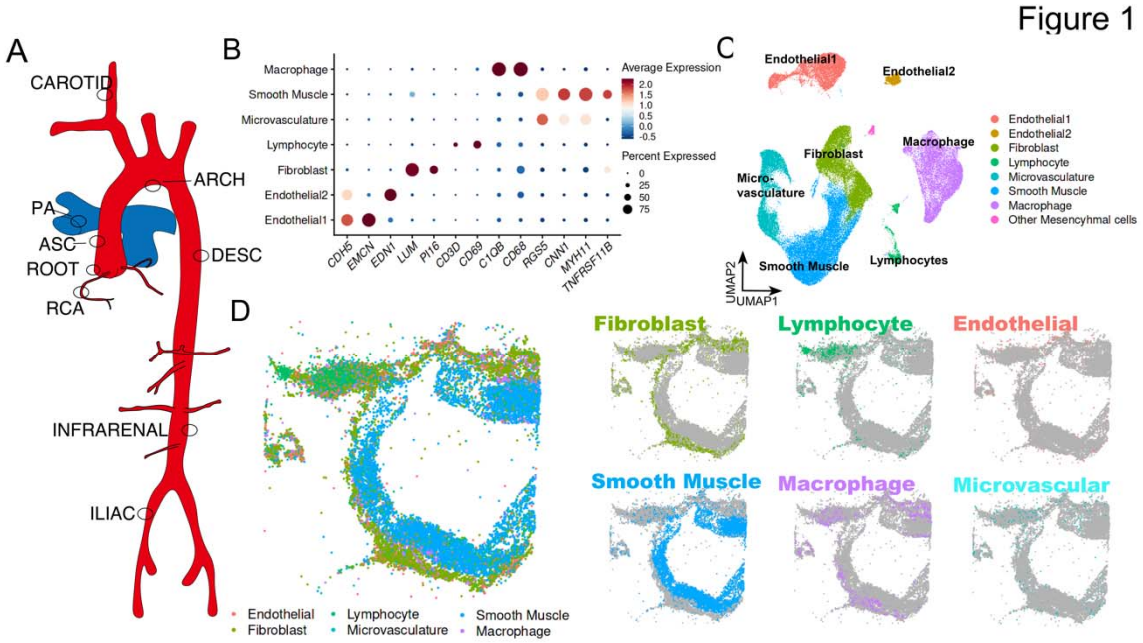

Figure 1. Single cell RNA and spatial landscape of human arteries.

(A) Schematic diagram of the 8 distinct human arterial sites collection strategy.

(B) Workflow of the experimental design and the sample processing for scRNA-seq and Slide-seq.

(C) RNA expression of existing canonical markers of each cell type population.

(D) UMAP visualization and clustering of cells labeled by cell type.

(E) Spatial dim plot visualization of the right coronary artery (RCA), annotated by cell type (left). The right panel presents a split view, illustrating the positions of individual cell types on the same slide.

See also Figure S1.

Figure 2

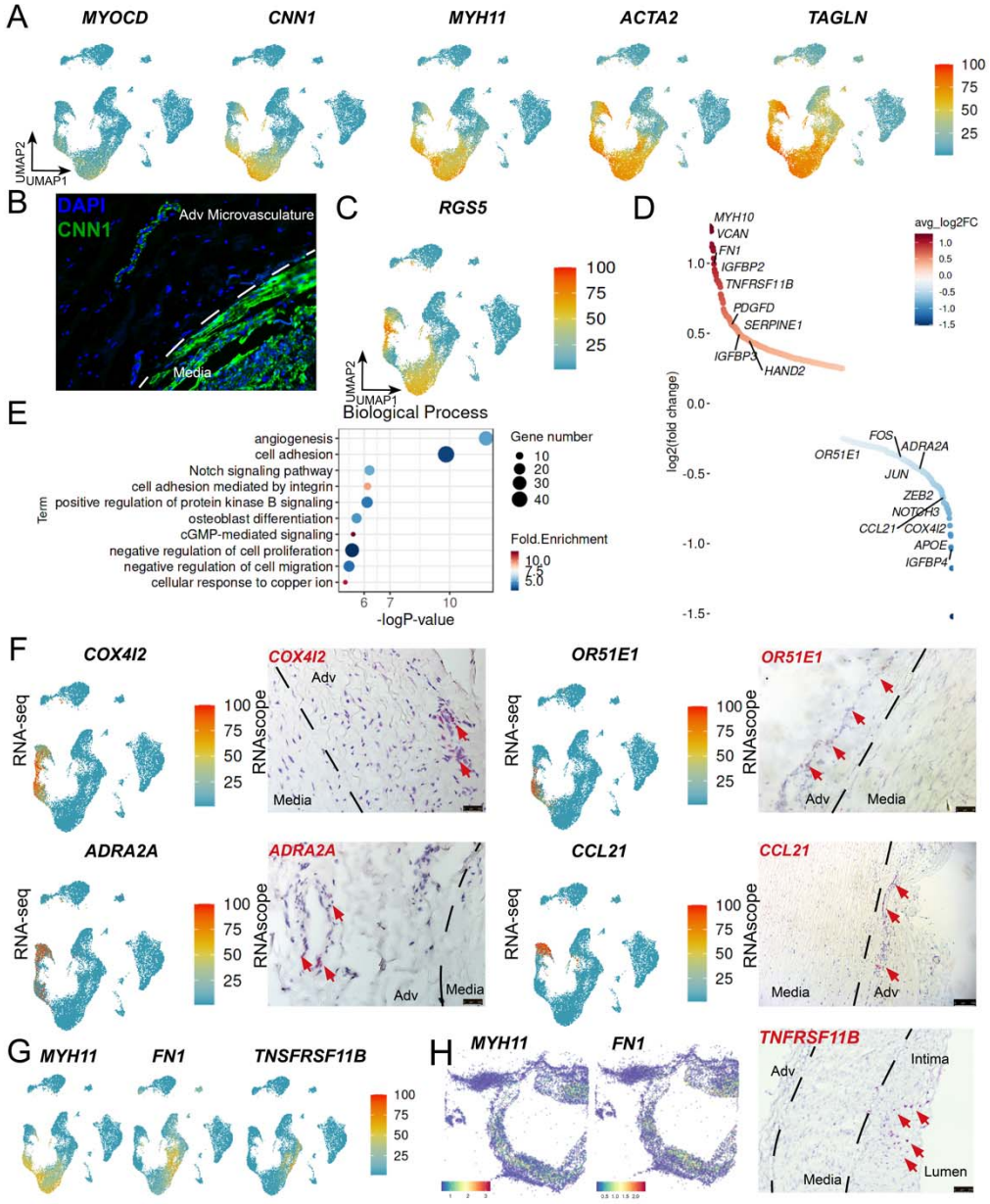

Figure 2. Smooth muscle cells of great arteries.

(A) RNA expression of mature smooth muscle contractile genes *MYOCD*, *CNN1*, *MYH11*, *ACTA2* and *TAGLN*, shown on the UMAPs.

(B) Immuno-fluorescence staining of CNN1 (green) and DAPI (blue) in ascending aorta.

(C) UMAP showing the *RGS5* RNA expression level.

(D) The differentially expressed genes between the two main clusters of SMC population, ranked by the average log2 fold change level.

(E) Dot plot showing the z scores of enriched biological processes in the differentially expressed gene between the two clusters of SMC population.

(F) The UMAPs showing the RNA expression of the unique makers in adventitial microvasculature cell cluster (left panels). RNAscope validated the expression of these markers with specific probes (right panels).

(G) RNA expression of medial and intimal smooth muscle genes *MYH11*, *FN1* and *TNFRSF11B*, shown on the UMAPs.

(H) Spatial localization of the cells expressed *MYH11* and *FN1* in RCA (left, middle). And the RNAscope staining of *TNSRSF11B* expression (right).

See also Figure S2 and Table S1.

Figure 3

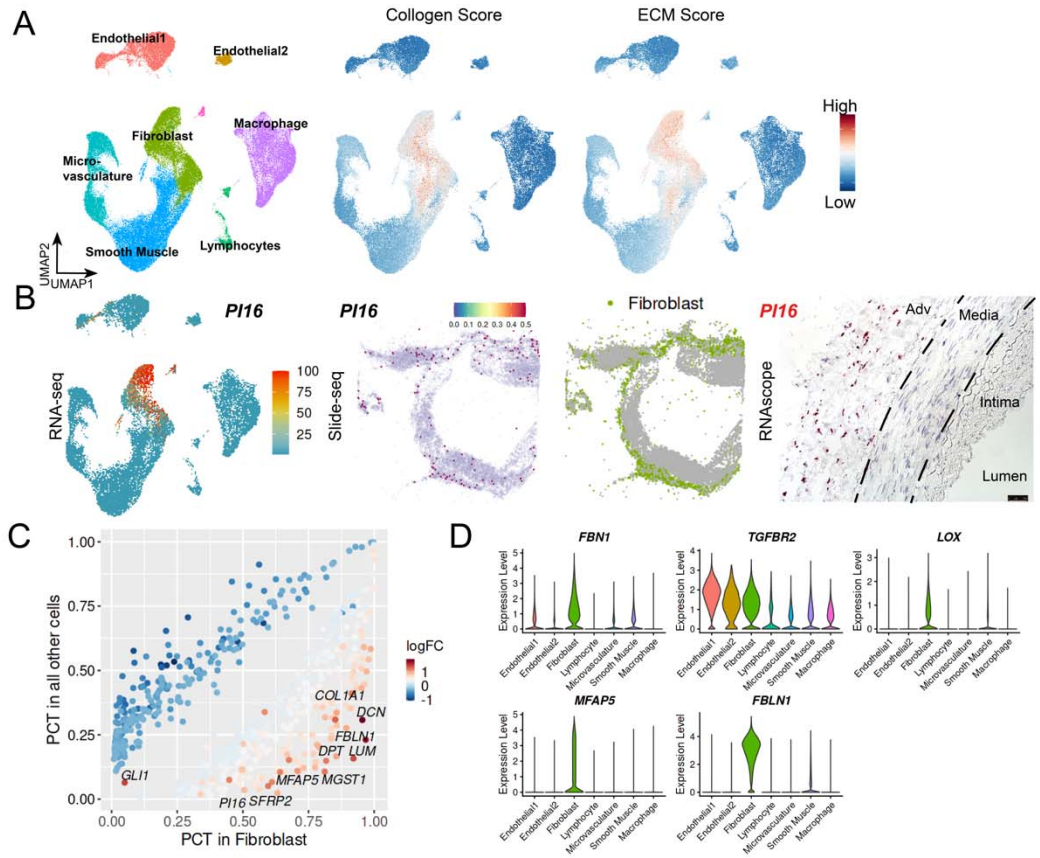

Figure 3. Adventitial fibroblast in human arteries.

(A) The scores derived from the expression levels of collagen (middle) and extracellular matrix genes (right) depicted on UMAP plots were analyzed alongside cell type (left).

(B) *PI16* RNA expression levels on RNAseq UMAP plot (left), Slide-seq dim plot alongside the fibroblast localization in RCA (middle), and the RNAscope staining with probes (right).

(C) The differentially expressed genes between fibroblast cells and all other cell types, plotted by their percent of expressed cells and the average log2 fold changes.

(D) The violin plots illustrate the specific expression of the genes *FBN1*, *TGFBR2*, *LOX*, *FBLN1*, and MFAP5 within the adventitial fibroblast population.

See also Figure S3 and Table S2.

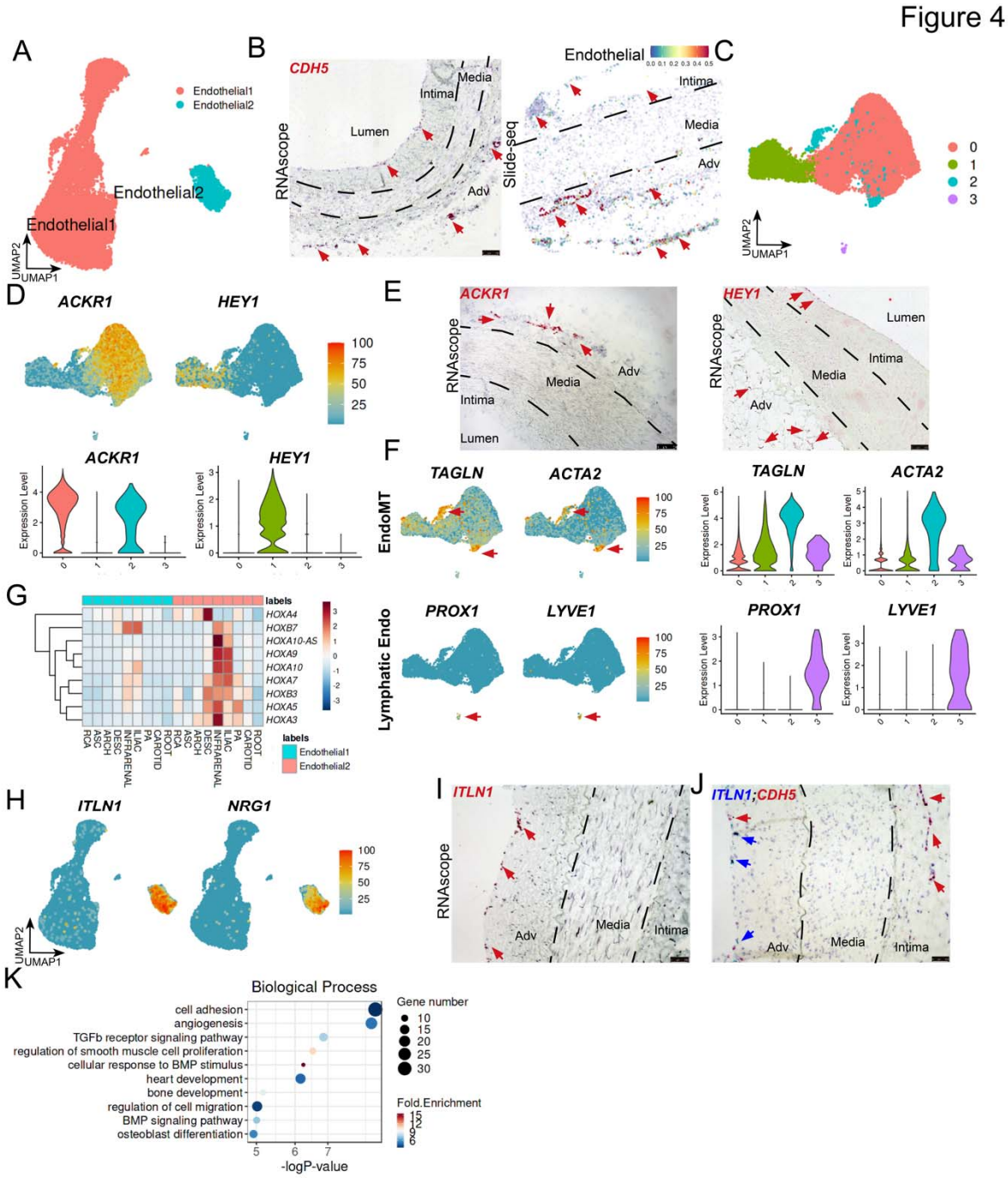

Figure 4. Distinct endothelial cell populations across arteries.

(A) Two large distinct clusters of endothelial cells shown on the UMAP.

(B) Endothelial cells identified by the canonical marker CDH5 RNAscope staining (left), and the endothelial cells localization on the Slide-seq dim plot of aorta (right).

(C) The sub-clustering of endothelial 1 population.

(D) The UMAP (top panels) and violin (bottom panels) plots showing the expression of cluster-specific genes *ACKR1* (left panels) and *HEY1* (right panels) in endothelial1 cells.

(E) The expression of cluster-specific genes *ACKR1* (left) and *HEY1* (right) are stained by RNAscope probes in ascending aorta.
(F) The UMAP (left panels) and violin (right panels) plots showing the expression of SMC genes *TAGLN* and *ACTA2* (left panels), or lymphatic genes *PROX1* and *LYVE1* (right panels) in endothelial1 cells.
(G) Heatmap showing the z scores of *HOX* genes expression in each of the vessel segments in both endothelial 1 and 2.
(H) Endothelial 2 specific genes *ITLN1* and *NRG1* expression levels on RNAseq UMAP plot.
(I) RNAscope staining of *ITLN1* gene expression in ascending aorta
(K) The co-staining of *ITLN1* (blue) and *CDH5* (red) expression in ascending aorta
(L) Dot plot showing the z scores of enriched biological processes in the differentially expressed gene between endothelial 1 and 2.
See also Figure S4 and Table S3.

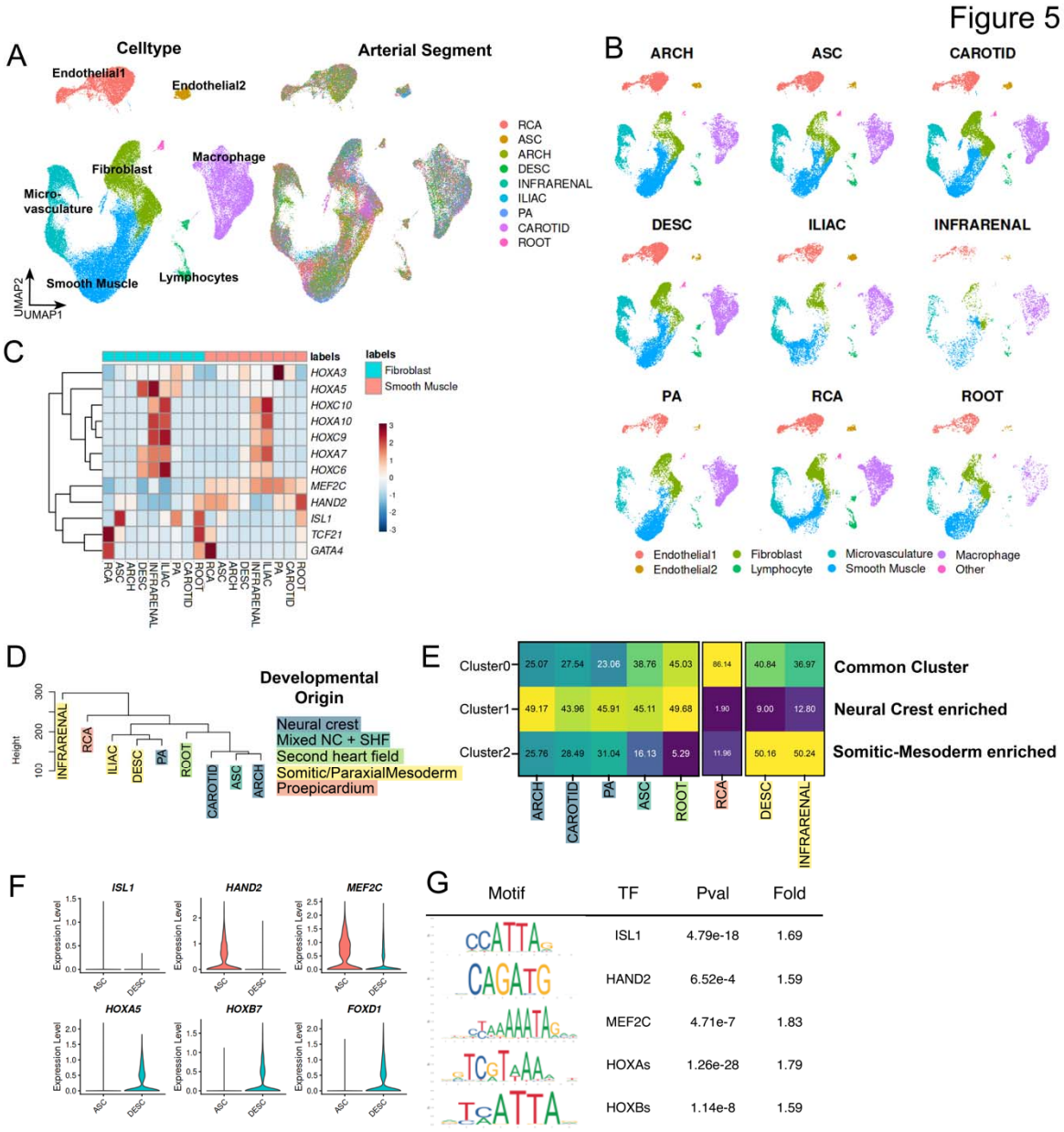

Figure 5. Prominent roles of embryonic origin in adult vascular identity.
(A) The heterogeneity of each population of cells across the different vascular beds on UMAP.
(B) A split view UMAP of the segmental origins presents the heterogeneity of each cell types.
(C) Heatmap showing the z scores of *HOX* genes expression in each of the vessel segments in both SMC and fibroblast.
(D) Dendrogram Clustering using Euclidean distance calculated by all expressed genes showing the correlation of each segment with its anticipated embryonic lineage within fibroblasts.
(E) Heatmap showing the percentage of each segment depicted within the distinct UMAP clusters.
(F) Violin plots showing the differential expression patterns of transcription factors *ISL1*, *HAND2*, *MEDF2C*, and *HOX* genes in fibroblast of ascending and descending sites.

(G) Enrichment of ISLET1, HAND2, MEF2C, and HOX binding motifs on the promoter regions of differentially expressed genes between ascending and descending fibroblasts, with macrophages serving as controls.

See also Figure S7.

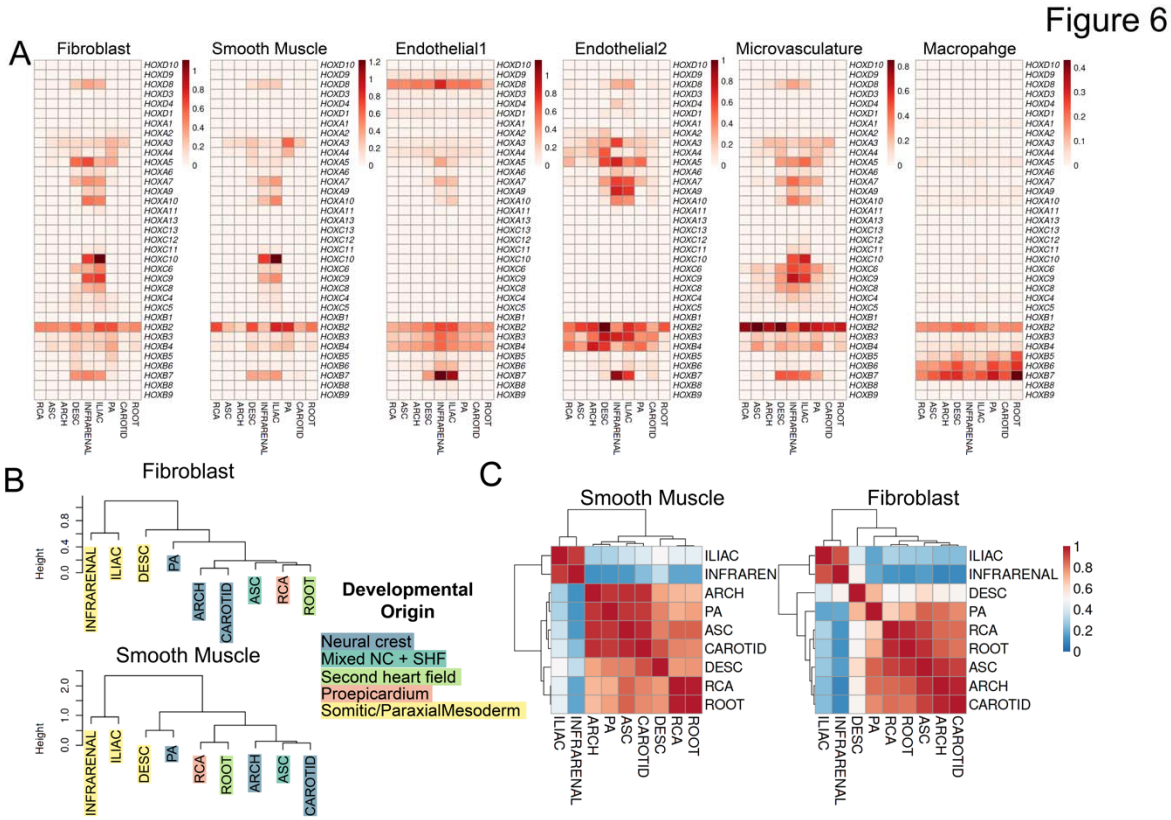

Figure 6. Adult vascular *HOX* gene expression patterns.

(A) Heatmap showing the log10 normalized expression values of *HOX* genes across the different vascular segment in fibroblast, SMC, endothetial1, endothelial2, microvasculature, or macrophage.

(B) Dendrogram clustering using Euclidean distance calculated by all *HOX* genes showing the correlation of each segment with its anticipated embryonic lineage in SMC (bottom) or fibroblasts (top).

(C) The heatmap illustrates the Pearson correlation coefficients among all arterial segments, derived from the expression values of all *HOX* genes. These segments are clustered according to Euclidean distance in SMC (left) or fibroblast (right).

See also Figure S8.

Figure 7

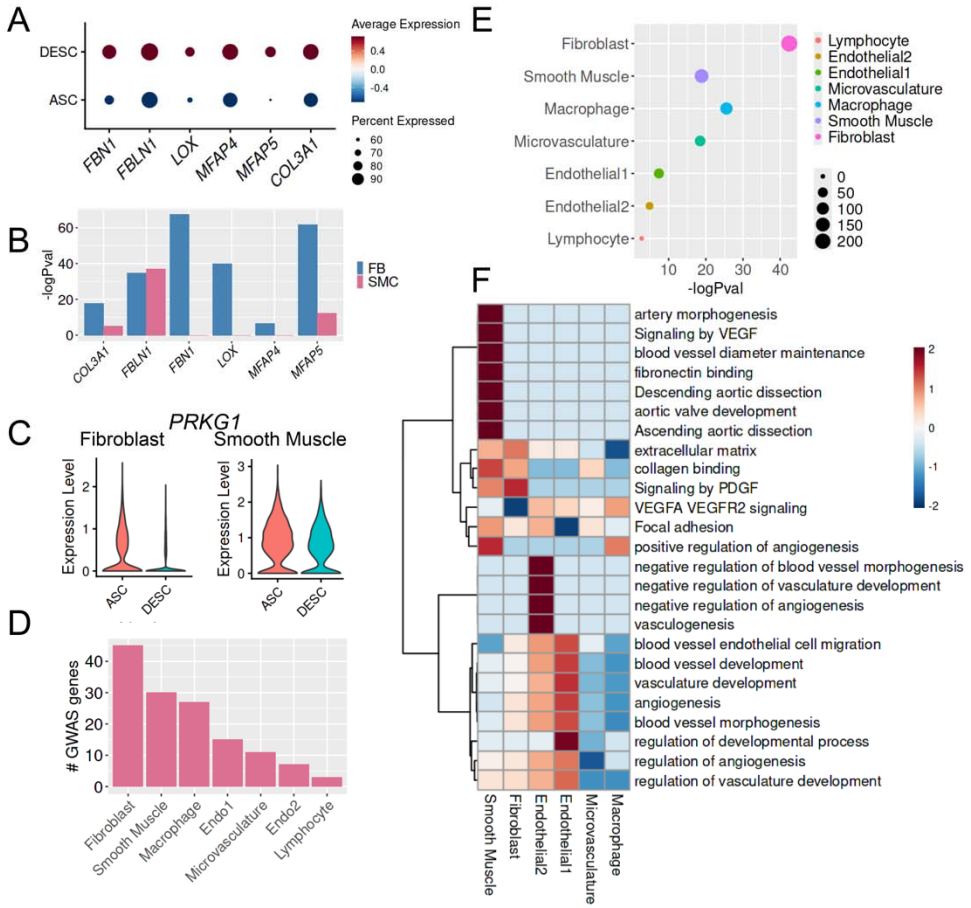

Figure 7. Enrichment of vascular disease variants in segmental transcriptomic differences.

(A) Dot plot showing the differential expression patterns of mendelian aortopathies genes *FBN1*, *FBNL1*, *LOX*, *MFAP4*, *MFAP5*, and *COL3A1* genes in fibroblast of ascending and descending sites.

(B) Bar plot showing the differential expression significances of these mendelian aortopathies genes *FBN1*, *FBNL1*, *LOX*, *MFAP4*, *MFAP5*, and *COL3A1* between ascending and descending sites in fibroblast.

(C) Violin plots showing the differential expression patterns of aneurysm risk in fibroblast (left) or SMC (right) of ascending and descending sites.

(D) The bar graph displays the count of Million Veteran Program (MVP) GWAS genes that are differentially expressed in each cell type when comparing atherosclerosis prone (coronary / carotid) with relatively atherosclerosis resistant descending aorta.

(E) Genetic association between the MVP GWAS genes and the differentially expressed genes across the coronary artery, carotid, and descending segments within each cell type, ranked by the number of overlapping genes.

(F) Heatmap showing the z scores of fold enrichment of biological processes in the differentially expressed genes across the coronary artery, carotid, and descending segments within each cell type.

See also Figure S9 and Table S4, S5, S6.

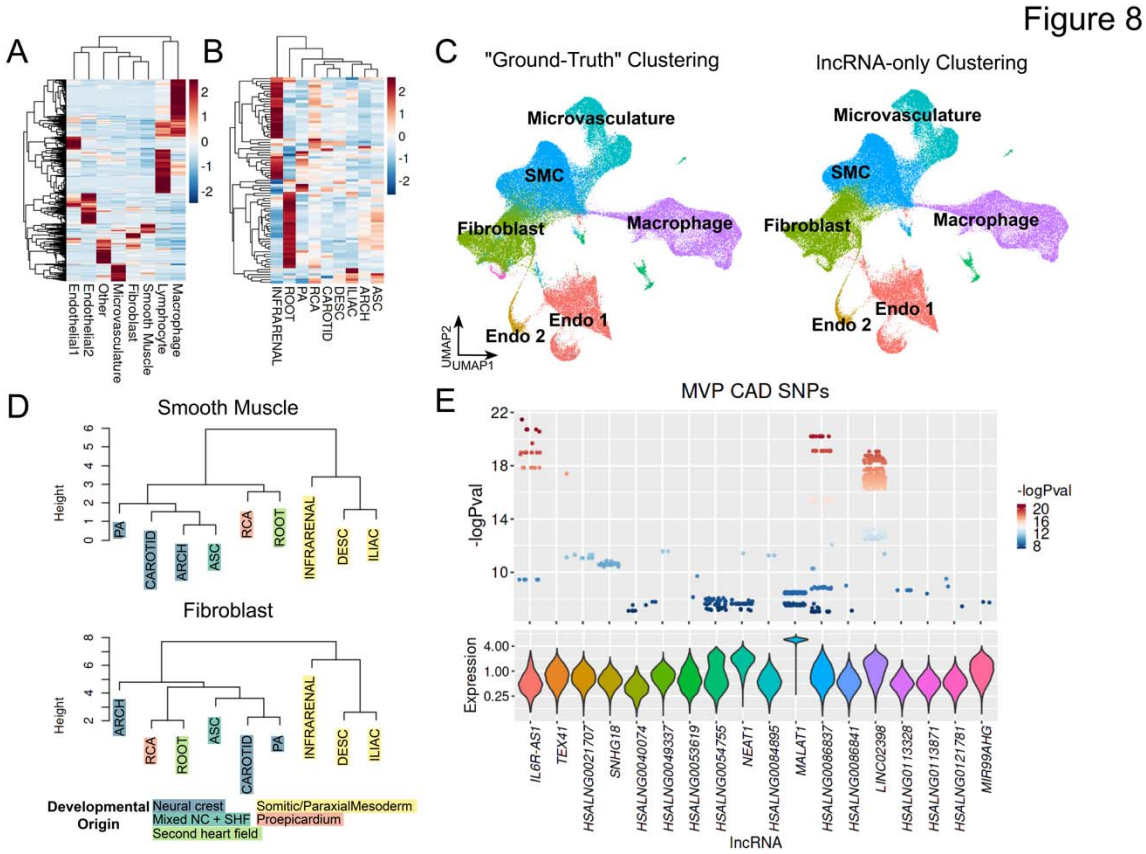

Figure 8. Cell type and segment specific non-coding transcriptome.
(A) Heatmap showing the z scores of non-coding RNAs expression in each of the cell types.
(B) Heatmap showing the z scores of non-coding RNAs expression in each of the vascular segments.
(C) UMAP visualization and clustering of cells using the sc-lncRNA transcriptome, annotated based on either whole transcriptome-derived cell types (left) or lncRNA-derived cell type identification (right).
(D) Dendrogram clustering using Euclidean distance calculated by all expressed lncRNAs showing the correlation of each segment with its anticipated embryonic lineage in SMC (top) or fibroblasts (bottom).
(E) The dot plot (top) illustrating the MVP SNPs positioned directly within the loci of differentially expressed lncRNAs. The violin plot displays the expression levels of these intersected lncRNAs. See also Figure S11 and Table S10.