

Transcription factors regulating vasculogenesis and angiogenesis

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Abstract

Transcription factors (TFs) play a crucial role in regulating the dynamic and precise patterns of gene expression required for the initial specification of endothelial cells (ECs), and during endothelial growth and differentiation. While sharing many core features, ECs can be highly heterogeneous. Differential gene expression between ECs is essential to pattern the hierarchical vascular network into arteries, veins and capillaries, to drive angiogenic growth of new vessels, and to direct specialization in response to local signals. Unlike many other cell types, ECs have no single master regulator, instead relying on differing combinations of a necessarily limited repertoire of TFs to achieve tight spatial and temporal activation and repression of gene expression. Here, we will discuss the cohort of TFs known to be involved in directing gene expression during different stages of mammalian vasculogenesis and angiogenesis, with a primary focus on development.

KEYWORDS

endothelial cell, angioblast, transcriptional regulation, vasculature, endothelium, ETS

1 | INTRODUCTION

In the early mammalian embryo, endothelial cells (ECs) are formed de novo through a process known as vasculogenesis, canonically defined as the differentiation of specific mesodermal precursors into endothelial progenitor cells known as angioblasts. This may occur via multipotential blood islands, although the existence of a shared progenitor population for hematopoietic and endothelial lineages remains subject to debate. Hemato-endothelial progenitor cells can be detected in the mouse from embryonic days (E) 6.75–7.0, with angioblasts identified within the lateral plate mesoderm by E7.5.^{1–6} By E8.0 angioblasts have begun to form the first vascular structures, organizing into two parallel tracts to form the primitive paired dorsal aorta, and along the cardiac

crescent as a precursor to the endocardium.⁵ Vein primordia form soon after at the yolk sac/embryo interface, presaging the future sinus venosus, and by E8.5 the first intra-embryonic veins coalesce and a primitive vascular plexus can be found throughout the embryo.⁵

Once the vascular plexus has been established, subsequent growth of blood vessels in the embryo and in the adult primarily occurs via angiogenesis. While angiogenesis by definition refers to the formation of blood vessels from existing ones via any mechanism, the best described and apparently most abundant form of angiogenesis is sprouting angiogenesis. In this dynamic process, a subset of ECs within the pre-existing vasculature responds to growth factors such as VEGF-A (via VEGFR2) and CXCL12 (via CXCR4) to become filopodia-rich migratory tip cells and invade the interstitial space.^{7–9} In turn, tip

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cells use DLL4/JAG-Notch signaling to suppress migratory behavior in neighboring ECs, which instead become highly proliferative stalk cells, enabling vessel elongation and lumen formation.¹⁰ Further, tip cells with high Notch activity can also migrate toward, and differentiate into, arterial ECs.⁹ In addition to VEGF-VEGFR2, CXCL12-CXCR4, and NOTCH signaling, multiple additional growth factors and signaling pathways provide both positive and negative regulatory inputs to control angiogenesis, as do cell adhesion interactions with the extracellular matrix. Thus sprouting angiogenesis allows a vascular bed to expand in response to hypoxia and insufficient nutrients, both during development and during physiological processes in the adult such as wound healing and changes in the lining of the endometrium. Pathological angiogenesis can also contribute to and aid the development of conditions such as cancer, atherosclerosis, and age-related macular degeneration.

The transition from mesodermal progenitors into a differentiated, functional endothelium depends on the activity of lineage-defining genes. Similarly, the coordinated response of ECs to the complex signals directing sprouting angiogenesis is dependent on the tight spatio-temporal expression and repression of specific cohorts of genes. Transcription factors (TFs) play a key role in this dynamic gene regulation. These DNA-binding proteins influence transcription by binding to specific motifs within cis-regulatory elements (enhancers and promoters), in combinations that often include both lineage-specifying factors (e.g. ETS factors for ECs) and transcriptional effectors of different signaling pathways (e.g., RBPJ for Notch, SMADs for TGF β /BMP). In this way, multiple different environmental cues can collectively influence enhancer/promoter activity and subsequent gene expression.^{11,12} While most TFs have a defined “consensus” DNA binding motif, usually established using either ChIP-seq (chromatin immunoprecipitation combined with sequencing) or HT-SELEX (high-throughput systematic evolution of ligands by exponential enrichment), TFs are also often able to bind slightly alternative (non-consensus) sequences, a process that can be influenced by other bound or accessory proteins and that likely also contributes to the differing patterns of gene activation downstream of different enhancers. In this review, we aim to summarize what is currently understood about the complex network of TFs that orchestrate the formation of a functional circulatory network in the mammalian embryo through the processes of vasculogenesis and sprouting angiogenesis. In addition, we provide three tables covering specific aspects of this regulation, including the known binding motifs for each endothelial TF (Table 1), the expression patterns of each TF during mammalian embryonic development and available ChIP-

seq data sets (Table 2), and a detailed assessment of all in vivo-characterized mammalian endothelial enhancers and their cognate binding TFs (Table 3).

This review will focus on data from mouse models of vasculogenesis and angiogenesis, which predominantly involves analysis of vascular development during embryonic and early postnatal growth. Vasculogenesis research focuses primarily on the E7.5–E9.5 embryo, whereas a variety of later embryonic timepoints have been used to study angiogenesis (with the E10–E12 hindbrain providing a useful angiogenic-only vascular bed^{7,96}). Additionally, the post-natal retina (from post-natal days (P)4–7) has been a favored model for many angiogenic studies,^{96,97} while angiogenesis can also be studied in the adult after insertion of Matrigel plug or tumor cells. These models are often used in combination with different Cre-driver transgenes to delete floxed genes-of-interest, permitting endothelial specific (e.g., Tie2-Cre), induced (e.g., RosaCreER) or induced endothelial specific (e.g., CDH5-CreERT2 and PDGFB-iCreERT2) patterns of gene deletion (reviewed by 98), although constitutive gene deletion has also been a useful approach for those genes largely specific to ECs. Because the efficacy of Cre and CreERT2 transgenes can vary considerably (both between different types of drivers, and between different versions of the same Cre transgene⁹⁸), and tamoxifen administration alongside Cre can itself result in endothelial phenotypes,⁹⁹ analysis of results should always consider the Cre driver utilized, the evidence of gene knockdown provided and the quality of controls. In vitro models can also provide powerful information (e.g., analysis of embryonic stem cell differentiation to study vasculogenesis¹⁰⁰; angiogenesis assays using cultured ECs to investigate sprouting from EC spheroids¹⁰¹) and are included alongside animal studies where relevant.

2 | ETS

ETS proteins comprise a large family of TFs characterized by a common DNA-binding domain (the ETS domain) which mediates binding to a core GGA^A/T motif (reviewed by¹⁰² and summarised in Table 1). While ETS factors are not specific to ECs and contribute to the differentiation of other cell types (e.g., PU.1 in hematopoiesis,¹⁰³ ELK1 in neurogenesis,¹⁰⁴ see Table 2), it is increasingly appreciated that ETS factors play a key role in EC identity. In particular, ETS binding motifs are a defining and essential feature of all known endothelial-expressed promoters and enhancers (e.g., 45,48,57,58,76 and Table 3). However, analysis of the direct role(s) of ETS TFs within the vasculature has been complicated by

TABLE 1 Consensus binding motifs for common EC transcription factors (TFs)

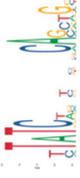
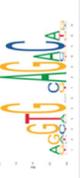
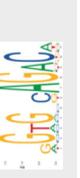
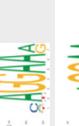
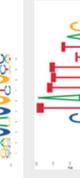
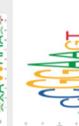
TF	Sequence logo	Type	Species/cell type	Source	TF	Sequence logo	Type	Species/cell type	Source
ETV2		HT-SELEX	Human Transfected LoVo/ Hek293	JASPAR ^{13,14}	GATA/ TAL1		ChIP-seq	Mouse Erythroid progenitors	Primary publication ¹⁵
ETV2		ChIP-seq	Mouse Differentiated ES cell	Primary publication ¹	TAL1		ChIP-seq	Mouse Erythroid progenitors	Primary publication ¹⁵
FLI1		ChIP-seq	Human Not specified	JASPAR/ PAZAR ^{14,16}	SMAD4		ChIP-seq	Human Unspecified	JASPAR2016/ PAZAR ^{16,17}
FLI1		HT-SELEX	Human Transfected LoVo/ Hek293	JASPAR ^{13,14}	SMAD4		ChIP-seq	Human Embryonic stem cells	Primary publication ¹⁸
FLI1		ChIP-seq	Human HUVEC	Primary publication ¹⁹	SMAD1 SMAD5		ChIP-seq	Human HUVEC	Primary publication ²⁰
ERG		ChIP-seq	Human HUVEC	Primary publication ¹⁹	HIF1A		ChIP-seq	Human Not specified	JASPAR/ ReMap2018 ^{14,21}
ERG		ChIP-seq	Mouse Not specified	JASPAR/ PAZAR ^{14,16}	HIF1A		ChIP-seq	Human HUVEC	Primary publication ²²
ETS1		ChIP-seq	Human HUVEC	Primary publication ²³	RBPI		ChIP-seq	Human Not specified	JASPAR/ ReMap2018 ^{14,21}
ETS1		ChIP-seq	Mouse Not specified	JASPAR/ PAZAR ^{14,16}	HEY1		ChIP-seq	Human Hek293, Mouse hearts	JASPAR ^{13,14}
ELK3		ChIP-seq	Human HUVEC	Primary publication ²⁴	MEF2A		ChIP-seq	Human Not specified	JASPAR/ ReMap2018 ^{14,21}
ELK3		HT-SELEX	Human Transfected LoVo/ Hek293	JASPAR ^{13,14}	MEF2C		ChIP-seq	Human Not specified	JASPAR/ENCODE ¹⁴
FOXC1		HT-SELEX	Human Transfected LoVo/ Hek293	JASPAR ^{13,14}	JUNB		ChIP-seq	Human Not specified	JASPAR/ENCODE ¹⁴
FOXC2		HT-SELEX	Human Transfected LoVo/ Hek293	JASPAR ^{13,14}	MAFB		ChIP-seq	Mouse Not specified	JASPAR ¹⁴

TABLE 1 (Continued)

TF	Sequence logo	Type	Species/cell type	Source	TF	Sequence logo	Type	Species/cell type	Source
FOXO2		ChIP-chip	Human Lymphatic ECs	Primary publication ²⁵	MAFF		ChIP-seq	Human Not specified	JASPAR/ENCODE ¹⁴
FOXO1		ChIP-seq	Mouse Not specified	JASPAR/ ^{14,16} PAZAR	TEAD1		ChIP-seq	Human Not specified	JASPAR/ ReMap2022 ^{14,26}
FOXP1		ChIP-seq	Human Not specified	JASPAR/ PAZAR 228,230	SOX7		ChIP-seq	Mouse ESC-embryoid body	Primary publication ²⁷
GATA2		ChIP-seq	Human Not specified	JASPAR/ ENCODE ¹⁴	SOX17		ChIP-seq	Mouse Not specified	JASPAR/ ReMap2022 ^{14,26}
GATA2		ChIP-seq	Human HUVEC	Primary publication ²⁸	SOX18		ChIP-seq	Human HUVEC	Primary publication ²⁹

Note: JASPAR refers to JASPAR 2022 unless specified, logos from primary publications are adapted by authors from the original paper. ChIP-seq refers to chromatin immunoprecipitation combined with sequencing; HT-SELEX refers to high-throughput systematic evolution of ligands by exponential enrichment, bold denotes that ECs were experimental model used in motif designation. Note that consensus motifs should be viewed as only a guide to potential TF binding locations, as TFs can also bind non-consensus motifs (often similar to consensus) and conversely do not always bind sequences containing the exact consensus motif.

the sheer number of ETS family members expressed in ECs. Adult ECs express at least 13 different ETS factor genes (*Ets1*, *Ets2*, *Erg*, *Fli1*, *Etv3*, *Etv6/Tel*, *Elk1*, *Elk3/Net*, *Elk4/Sap1*, *Elf1*, *Elf2/Nerf*, *Elf4*, and *Gabpa*), although consistent high expression across adult aorta, lung and brain ECs is principally restricted to *Ets1*, *Ets2*, *Erg*, *Fli1*, *Elk3*, *Elf1*, *Elf2*, and *Gabpa*, the latter three of which are also highly expressed in many other cell types.^{102,105,106} Of these adult ETS, all but *Elk1* are also expressed in angioblasts in E8.0 embryos.² Additionally, *Etv2* (*Etsrp71*) is strongly expressed in hemato-endothelial progenitors in the early mouse embryo,¹⁰⁷ although little expression is reported after mid-gestation. Similarly, *Fev*, *Etv4*, *Etv5*, and *Erf* are expressed just after *Etv2* in the same progenitor population,^{2,108} although none are strongly expressed in adult ECs. Consequently, analysis of phenotypes after deletion of a single ETS factor must always consider the potential for functional redundancy and compensation. Nonetheless, gene deletional studies in both mouse and zebrafish have repeatedly found significant endothelial defects downstream of ETS factor deletion, strongly supporting a key role for ETS factors in the regulation of vasculogenesis and angiogenesis.^{102,109} It is, however, unlikely that ETS factors regulate their gene targets alone: gene enhancers with entirely different patterns of EC expression (e.g., arterial-specific, angiogenic-specific, and vein-specific) can all be robustly bound and activated by ETS factors (e.g., 32,48-51,57,69,85; see also Table 3) indicating that ETS-driven regulation of many aspects of endothelial behavior is likely to involve combinatorial interaction with other families of TFs to achieve the required spatio-temporal specificity.

2.1 | ETV2

Of all the ETS TFs investigated in endothelial knock-out mouse models, only loss of ETV2 (ETSRP71/ER71) ablates vasculogenesis, resulting in a complete absence of both endothelial and blood cell lineages and subsequent lethality by E9.0.^{61,107} Conversely, prolonged activation of *Etv2* leads to an overly dense capillary bed, hemorrhage, and an absence of hematopoietic cell differentiation.¹¹⁰ Predating these discoveries, knockdown of the zebrafish orthologue, *etsrp/etv2*, was also shown to result in cessation of embryonic circulation.^{109,111}

Etv2 is expressed within a narrow time window during mouse development between E6.75 and E9.5 (Table 2), a transience that is crucial for endothelial maturation. It is first observed in a subset of cells in the posterolateral mesoderm at E6.75–7.0,^{2,108,112} with the strongest *Etv2* expression occurring in hemato-endothelial progenitor populations rather than

TABLE 2 Expression dynamics of common endothelial TFs and information on publicly deposited ChIP-seq data sets

Transcription factor gene	Earliest time-point expressed in hemato-endothelial progenitors (from ²) * = expression in most cells	Cell type with highest expression of TF in E9–E13 embryos (from ³⁰)	Other tissues expressing TF List of next five highest cell types expressing as indicated and defined by Mouse Atlas data set, ³⁰ italics indicate expression is <50% of EC level, cut off is 10% of EC expression	Chip-seq data from ECs, progenitor cells or whole tissues Cell type, GEO accession number, reference
<i>Etv2</i>	E6.75	Not expressed	Not expressed at detectable levels	In-vitro differentiated ES cells expressing inducible ETV2-V5 GSE5940 ¹
<i>Fli1</i>	E6.75–E7.0	Endothelial cells	Megakaryocytes, white blood cells, jaw/tooth progenitors, <i>early mesenchyme, limb mesenchyme</i>	HUVECs with and without VEGFA stimulation, GSE109625 ³¹ ; HUVECs with and without ERG and FLI siRNA, GSE109695 ¹⁹
<i>Erg</i>	E7.75	Endothelial cells	<i>Jaw/tooth progenitors, chondrocytes/osteoblasts, limb mesenchyme, osteoblasts, neutrophils</i>	HUVECs with and without VEGFA stimulation, GSE109625 ³¹ ; HUVECs and HUAEC, GSE128382 ³² ; HUVECs GSE124893 ³³ ; HAECs under control or inflammatory stimuli, GSE89970 ³⁴
<i>Ets1</i>	E7.25	Endothelial cells	<i>Melanocytes, chondrocyte progenitors, Schwann cell precursors, intermediate mesoderm, connective tissue progenitors</i>	HUVECs with and without VEGFA stimulation, GSE109625 ³¹ ; HUVECs with and without VEGFA stimulation GSE93030 ²³
<i>Ets2</i>	E7.25*	Endothelial cells	Limb mesenchyme, <i>stromal cells, early mesenchyme, osteoblast, cardiac muscle lineages</i>	
<i>Elk3</i>	E7.0	Endothelial cells	<i>Limb mesenchyme, white blood cells, jaw/tooth progenitors, early mesenchyme, chondrocytes/osteoblasts</i>	HUVECs in normoxia, GSE60156 ²⁴
<i>Foxc1</i>	E8.25	Chondrocyte progenitors	Jaw/tooth progenitors, stromal cells, osteoblasts, early mesenchyme, endothelial cells	
<i>Foxc2</i>	E7.75	Chondrocyte progenitors	Jaw/tooth progenitors, stromal cells, osteoblasts, early mesenchyme, endothelial cells	
<i>Foxo1</i>	E7.75	Endothelial cells	Myocytes, <i>Schwann cell precursor, hepatocytes, chondrocyte progenitors, epithelial cells</i>	HUVECs with or without constitutively active FOXO1, GSE128635 ³⁵ ; mouse heart after isoproterenol, transverse aortic constriction or vehicle/sham, GSE144011 ³⁶

TABLE 2 (Continued)

Transcription factor gene	Earliest time-point expressed in hemato-endothelial progenitors (from ²) * = expression in most cells	Cell type with highest expression of TF in E9–E13 embryos (from ³⁰)	Other tissues expressing TF List of next five highest cell types expressing as indicated and defined by Mouse Atlas data set, ³⁰ italics indicate expression is <50% of EC level, cut off is 10% of EC expression	Chip-seq data from ECs, progenitor cells or whole tissues Cell type, GEO accession number, reference
<i>Foxo3</i>	N/A	Primary erythroid lineage	Definitive erythroid lineage, white blood cells, megakaryocytes, hepatocytes, intermediate mesoderm	
<i>Foxo4</i>	N/A	Primary erythroid lineage	Myocytes, cardiac muscle lineages, definitive erythroid lineage, lens, osteoblasts	
<i>Foxm1</i>	E7.0*	Premature oligodendrocyte,	Oligodendrocyte progenitors, neural tube, radial glia, isthmic organizer cells, primitive erythroid lineage.	
<i>Gata2</i>	E7.0	Megakaryocytes	White blood cells, inhibitory neuron progenitors, endothelial cells , neural progenitor cells, intermediate mesoderm	HUVECs, GSE29531 ²⁸ ; HUVECs GSM935347, as part of 37
<i>Gata3</i>	E7.0	Inhibitory neuron progenitors,	Inhibitory neurons, epithelial cells, lens, neutrophils, excitatory neurons	
<i>Gata6</i>	E7.0	Intermediate mesoderm	Cardiac muscle lineages, hepatocytes, endothelial cells , stromal cells, connective tissue progenitors	
<i>Tal1</i>	E7.0	Primitive erythroid lineage	Definitive erythroid lineage, megakaryocytes, endothelial cells , inhibitory neuron progenitors, white blood cells	
<i>Smad1</i>	E7.0*	Sensory neurons	Endothelial cells , neural progenitor cells, stromal cells, postmitotic premature neurons	HUVECs with BMP6/9 stimulation, GSE27661 ²⁰
<i>Smad2</i>	E7.0*	Stromal cells	<i>Limb mesenchyme, early mesenchyme, Schwann cell precursor, hepatocytes, neural tube</i>	
<i>Smad3</i>	E8.25*	Epithelial cells	Isthmic organizer cells, chondrocyte progenitors, stromal cells, limb mesenchyme	

(Continues)

TABLE 2 (Continued)

Transcription factor gene	Earliest time-point expressed in hemato-endothelial progenitors (from ²) * = expression in most cells	Cell type with highest expression of TF in E9–E13 embryos (from ³⁰)	Other tissues expressing TF List of next five highest cell types expressing as indicated and defined by Mouse Atlas data set, ³⁰ italics indicate expression is <50% of EC level, cut off is 10% of EC expression	Chip-seq data from ECs, progenitor cells or whole tissues Cell type, GEO accession number, reference
<i>Smad4</i>	E6.75*	Stromal cells	Early mesenchyme, limb mesenchyme, neural tube, neural progenitor cells, cardiac muscle lineages	
<i>Smad5</i>	E7.0*	Limb mesenchyme	Stromal cells, endothelial cells , melanocytes, chondrocyte/osteoblasts, osteoblasts	HUVECs with BMP6/9 stimulation, GSE27661 ²⁰
<i>Smad6</i>	E7.0	Endothelial cells	Intermediate mesoderm, cardiac muscle lineages, stromal cells, epithelial cells, limb mesenchyme	
<i>Smad7</i>	E6.75*	Stromal cells	Limb mesenchyme, endothelial cells , intermediate mesoderm, chondrocytes/osteoblast, cardiac muscle lineage	
<i>Smad9</i>	N/A	Intermediate mesoderm	Jaw and tooth progenitors, neutrophils, cardiac muscle lineages, ependymal cell, osteoblasts	
<i>Hif1a</i>	E7.0*	Cardiac muscle lineages	Stromal cells, melanocytes, early mesenchyme, chondrocytes/osteoblasts, jaw and tooth progenitors	HUVEC under normoxia and hypoxia, GSE39089, ²² HUVECs grown in hypoxia, GSE89836 ³⁸
<i>Hif1b</i>	E7.5*	Connective tissue progenitors	Chondrocyte progenitors, primitive erythroid lineage, stromal cells, definitive erythroid lineage, hepatocytes	HUVECs grown in hypoxia, GSE89836 ³⁸
<i>Hif2a</i>	E8.5	Endothelial cells	Hepatocytes, chondrocyte progenitors, intermediate mesoderm, osteoblasts, megakaryocytes	HUVECs grown in hypoxia, GSE89836 ³⁸
<i>Rbpj</i>	E7.0*	White blood cells	Neural progenitor cells, myocytes, stromal cells, granule neurons, neural tube	HUVECs with and without VEGFA stimulation, GSE109625 ³¹ ; quiescent and confluent HUVECs, GSE85987 ³⁹
<i>Notch1</i>	E7.0	Radial glia	Neural tube, oligodendrocyte progenitors, endothelial cells , neural progenitor cells, Schwann cell precursor	Quiescent and confluent HUVECs, GSE85987 ³⁹

TABLE 2 (Continued)

Transcription factor gene	Earliest time-point expressed in hemato-endothelial progenitors (from ²) * = expression in most cells	Cell type with highest expression of TF in E9–E13 embryos (from ³⁰)	Other tissues expressing TF List of next five highest cell types expressing as indicated and defined by Mouse Atlas data set, ³⁰ italics indicate expression is <50% of EC level, cut off is 10% of EC expression	Chip-seq data from ECs, progenitor cells or whole tissues Cell type, GEO accession number, reference
<i>Notch4</i>	E8.25	Endothelial cells	Chondrocyte progenitors, osteoblasts, lens, cardiac muscle lineages, stromal cells	
<i>Hey1</i>	E7.75	Notocord cells	Endothelial cells, <i>premature oligodendrocyte, myocytes, Schwann cell precursor, limb mesenchyme</i>	
<i>Hey2</i>	E8.0	Schwann cell precursor	Cardiac muscle lineages, chondrocyte progenitors, chondrocytes/osteoblasts, endothelial cells, <i>premature oligodendrocyte</i>	
<i>Hlx</i>	E7.75	Endothelial cells	Intermediate mesoderm, neutrophils, white blood cells, <i>lens, connective tissue progenitors</i>	
<i>Mef2a</i>	E7.5*	Endothelial cells	Myocytes, white blood cells, cardiac muscle lineages, chondrocyte progenitors, connective tissue progenitors	Mouse hearts, GSE124008 ⁴⁰
<i>Mef2c</i>	E7.75	White blood cells	Endothelial cells, <i>myocytes, Schwann cell precursor, megakaryocytes, cardiac muscle lineages</i>	HUVECs with and without statins, GSE32644 ⁴¹ ; Mouse hearts, GSE124008 ⁴⁰
<i>Mef2d</i>	E7.75	Endothelial cells	Myocytes, hepatocytes, <i>white blood cells, primitive erythroid lineage, definitive erythroid lineage</i>	
<i>Junb</i>	E7.0	Primitive erythroid lineage	Endothelial cells, <i>definitive erythroid lineage, white blood cells, hepatocytes, megakaryocytes</i>	HAECs under control or inflammatory stimuli, GSE89970 ³⁴
<i>Jun</i>	E6.75*	Osteoblasts	Cholinergic neurons, cardiac muscle lineages, endothelial cells, <i>neutrophils, stromal cells</i>	HAECs under control or inflammatory stimuli, GSE89970 ³⁴
<i>Ma1b</i>	E8.5	White blood cells	Cholinergic neurons, ependymal cells, osteoblasts, neutrophils, inhibitory neurons	

(Continues)

TABLE 2 (Continued)

Transcription factor gene	Earliest time-point expressed in hemato-endothelial progenitors (from ²) * = expression in most cells	Cell type with highest expression of TF in E9–E13 embryos (from ³⁰)	Other tissues expressing TF List of next five highest cell types expressing as indicated and defined by Mouse Atlas data set, ³⁰ italics indicate expression is <50% of EC level, cut off is 10% of EC expression	Chip-seq data from ECs, progenitor cells or whole tissues Cell type, GEO accession number, reference
<i>Maff</i>	E7.75	Hepatocytes	Endothelial cells , lens, <i>cardiac muscle lineages</i> , <i>stromal cells</i> , <i>epithelial cells</i>	
<i>Mafg</i>	E7.0*	Primitive erythroid lineage	White blood cells, definitive erythroid lineage, megakaryocytes, hepatocytes, lens	
<i>Mafk</i>	E8.5	Primitive erythroid lineage	Definitive erythroid lineage, megakaryocytes, white blood cells, endothelial cells , <i>cardiac muscle lineages</i>	
<i>Tead1</i>	E7.5*	Ependymal cells	Epithelial cells, notochord cells, neural tube, radial glia, melanocytes	Mouse hearts, GSE124008 ⁴⁰
<i>Tead2</i>	E6.75*	Stromal cells	Limb mesenchyme, early mesenchyme, premature oligodendrocyte, chondrocyte/osteoblasts, oligodendrocyte progenitors	
<i>Tead4</i>	E7.75	Myocytes	Endothelial cells , epithelial cells, intermediate mesoderm, melanocytes, chondrocyte progenitors	
<i>Sox7</i>	E7.0	Endothelial cells		HUVECs overexpressing SOX7-mCherry, ChIP of mCherry E-MTAB-4480 (ArrayExpress database) ²⁹
<i>Sox17</i>	E7.75	Endothelial cells		
<i>Sox18</i>	E7.75	Endothelial cells	<i>Osteoblasts</i> , <i>cardiac muscle lineages</i>	HUVECs overexpressing SOX18-mCherry, ChIP of mCherry E-MTAB-4481 (ArrayExpress database) ²⁹

differentiated ECs by E8.5.^{2,19,113} While it is possible that the uniquely early expression pattern of *Etv2* contributes to the severe consequences of deletion, overexpression of *Fli1*, *Erg* and *Ets1* in ETV2-null embryoid body culture systems could not fully rescue the formation of the endothelial lineage.¹ Alternatively, the importance of ETV2 in

the specification of ECs may, at least in part, be attributed to its crucial role in regulating the expression of *Flk1* (also known as *Kdr* and encoding the VEGFR2 receptor). In the mouse embryo, loss of ETV2 markedly reduces the number of VEGFR2+ cells,¹¹⁴ likely due to direct regulation of *Flk1* through ETV2 binding at *Flk1*

enhancer elements^{69,115,116} (Table 3). While not all *Flk1* expression is lost, those VEGFR2+ cells that persist have a cardiac rather than EC lineage.¹¹⁷ This is supported by studies in embryonic stem cell cultures, which also found that cells differentiate into cardiac lineages in the absence of ETV2.^{112,114,118} ETV2 is therefore not essential for the expression of *Flk1* per se, but instead up-regulates expression levels to create a subpopulation of high VEGFR2+ cells driven towards an endothelial fate.¹ VEGFA signaling through VEGFR2 is a potent activator of MAPK/ERK signaling (reviewed in 119), which in turn phosphorylates ETS factors and increases their binding affinity (reviewed in 120,121). Therefore, VEGFA-VEGFR2 signaling enhances *Flk1* gene transcription through an ETV2-dependent positive feedback loop.

In addition to *Flk1*, ETV2 is implicated in the direct activation of many other key endothelial lineage specifying genes, including *Cdh5*, *Tek*, *Tal1(SCL)*, *Notch4*, *Nfatc1*, and *Sox7*,^{45,114,122,123} often but not always in combination with Forkhead TFs (see below and Table 3). Additionally, ETV2 can play a role in the activation of other endothelial ETS factors, including *Fli1*, *Ets1*, *Ets2*, *Erg*, and *Fev*.^{1,108} It has been hypothesized that ETV2 may act as a pioneer factor, creating an endothelial lineage-specific epigenetic landscape.¹²⁴ Supporting this, ETV2 binding can directly result in demethylation of its binding sites, and this hypomethylation can be maintained in blood and vascular systems as an epigenetic memory.^{1,125} Further, this role may be facilitated by complexing with TET1/TET2 enzymes and thereby directly promoting locus-specific reversal of methylation marks.¹²⁶ ChIP-seq data show that binding sites at regulatory elements previously occupied by ETV2 become occupied by other ETS factors at later stages of development.¹ Cultured amniotic cells can also transition into immature ECs through transient *Etv2* expression, while co-expression with *Fli1* and *Erg1* is required for maturation.¹¹⁸ It is therefore clear that ETV2 plays a unique role in establishing EC fate during vasculogenesis, both specifying the endothelial epigenetic landscape and directly establishing the expression of lineage-specifying genes that will maintain EC fate once ETV2 itself is gone.

2.2 | FLI1

FLI1 is expressed in hemato-endothelial progenitors soon after ETV2 and remains highly expressed in ECs throughout development (see Table 2). However, unlike *Etv2*, constitutive deletion of *Fli1* in mice does not affect vasculogenesis, although *Fli1* null mice die at E11 due to cerebral hemorrhage and loss of vessel integrity. Instead, FLI1 may play a role in the differentiation of angioblasts

into a functional vascular network,^{127,128} and has been implicated in the direct regulation of many genes involved in maintaining vascular homeostasis (including *Cdh5*, *Cd31*, *Col4a1*, *Mmp9*, *Pdgfb*, and *Slpr1*).¹²⁹ However, endothelial-specific deletion of *Fli1* mediated by Tie2-Cre results in a milder phenotype and mice are born at expected mendelian ratios.¹²⁹ While this could indicate an EC-independent role for FLI1 in vascular assembly, it is also possible that critical EC functions of FLI1 occur before the onset of Tie2-Cre activity. Further, the limited phenotype may be explained by functional redundancy between FLI1 and the homologous ETS factor ERG. ERG shares over 70% amino acid sequence similarity and a near-identical ETS domain with FLI1,¹³⁰ but is expressed slightly later during development.^{2,19,131} Of note, it has recently been shown that induced endothelial-specific compound knockdown of *Fli1* and *Erg* together in adult mice results in rapid lethality alongside transcriptional silencing of core EC genes, strongly indicating both significant functional redundancy and a shared yet crucial role for FLI1/ERG in EC identity.¹³²

2.3 | ERG

ERG is the most highly expressed ETS factor in the mature mouse vasculature, with robust EC expression beginning at E7.75–8.0 and maintained throughout the lifespan across all EC subtypes^{2,133–135} (Table 2). Although highly expressed in ECs, ERG is not endothelial-specific, with expression also observed in some blood cell lineages, in osteoblasts and in chondrocytes (reviewed by 136). Endothelial-specific deletion of *Erg* leads to growth defects, cardiovascular abnormalities, hemorrhage, and embryonic lethality at E10–E12.5, indicating a crucial and independent role for ERG in vascular development.^{137,138} Analysis of ERG binding patterns in cultured ECs suggests that it promotes endothelial homeostasis via directly binding a majority of active endothelial enhancers and super-enhancers.³³ Such genome-wide enhancer occupancy underscores the critical role ERG plays in the regulation of endothelial function, although unlike ETV2, ERG enhancer occupancy requires a pre-specified chromatin landscape.³³

ERG is also known to directly regulate many pro-angiogenic genes, including those involved in EC migration, apoptosis, and vascular stability (reviewed in 136). VEGF-VEGFR2 signaling during angiogenesis can induce phosphorylation and activation of ERG via the MAPK/ERK pathway, resulting in increased ERG binding and co-factor recruitment at regulatory elements driving angiogenic gene expression (e.g., *Dll4* and *Hlx*⁴⁹), although this VEGF-enriched ERG binding is non-

TABLE 3 A list of in vivo-validated EC enhancers alongside approximate location and associated regulatory TFs

Gene (species)	Enhancer name in original paper	Enhancer location	Validation method	EC specificity (age investigated)	Transcription factors with experimental validation method	References
<i>Acvr11/Alk1 (ms)</i>	Alk1 pXh4.5-in2-SIB	Promoter/ intronic −3 to +6 kb (9 kb piece)	Tg mouse	Arterial EC (E11.5-adult)	SP1: mo AP2: mo NFKB: mo GATA: mo CREB: mo ETS: mo	42,43
<i>Apln (ms)</i>	Apln	Downstream +28 kb	Tg mouse	Pan-EC (E11.5)		44
<i>CDH5/VE-cadherin (hm/ms)</i>	hVEcad promoter	Upstream to promoter −1 kb to TSS	Tg mouse	Pan-EC (E7.5–E12.5/ adult)	FOXC/O: mo, em, ch, oe/OE, MU ETS: mo, em (ETS1), oe/OE (ETV2), MU GATA: mo, oe, mu TAL1: mo, oe, mu	45-47
<i>Dab2 (ms)</i>	Dab2	Upstream −240 kb	Tg mouse	Pan-EC (E11.5)		44
<i>Dll4 (ms)</i>	Dll4in3/Dll4-F2	Intronic +2 kb	Tg mouse Tg zebrafish	Arterial EC Angiogenic EC (E8–P8, 24– 72 hpf)	ETS: mo, em (ETS1/ERG), ch (ERG), mu/MU, KD (ERG) SOXF: mo, ch (SOX7/18), em (SOX7/18), MU, KD (<i>sox7/sox18</i>) RBPJ: mo, ch, em, MU, KD NOTCH: ch MEF2: mo, chs (MEF2C), em (MEF2A/C/D), MU, KD/KO KLF4: mo, ch, oe B-catenin: ch, oe	48-55
<i>Dll4 (ms)</i>	Dll4-12	Upstream −12 kb	Tg mouse	Arterial EC (E8–P8)	SOXF: mo, ch (SOX7/18), em (SOX7/18), MU, KD (<i>sox7/sox18</i>) RBPJ: mo, ch, em, MU, KD ETS (all): mo	50,54
<i>ECE1 (hm)</i>	ECE1 enhancer	Promoter or intronic +10 kb ^a	Tg mouse	Arterial EC (E7.75–E12.5)	FOXC/O: mo, em (FOXC2), oe, MU ETS: mo, em (ETV2), oe, MU SOXF: mo, em (SOX17), oe, mu, MU	45,56
<i>Egfl7 (ms)</i>	Egfl7_E1	Upstream −9 kb	Tg mouse	Pan-EC (E11.5)		44
<i>Egfl7 (ms)</i>	Egfl7_E3	Upstream −2 kb	Tg mouse	Pan-EC (E11.5)		44
<i>EMCN (hm)</i>	EMCN-22	Upstream −22 kb	Tg mouse	Venous EC (E11.5)	ETS: mo SMAD: mo, chs (SMAD1/5)	57,58
<i>Eng (ms)</i>	Eng-8	Upstream −8 kb	Tg mouse	Pan-EC (E11.5)	ETS: mo, ch (FLI1/ERG/ ELF1), mu/MU	59
<i>Eng (ms)</i>	End+9	Intronic +9 kb	Tg mouse	EC ^a (E11.5)	ETS: mo, ch (FLI1) MU	60

TABLE 3 (Continued)

Gene (species)	Enhancer name in original paper	Enhancer location	Validation method	EC specificity (age investigated)	Transcription factors with experimental validation method	References
<i>Ephb4 (ms)</i>	Ephb4-2	Upstream –2 kb	Tg mouse Tg zebrafish	Venous EC (E9–E15.5, 24–72 hpf)	ETS: mo, em (ETS1/ERG), ch (ERG), chs (ETS1/ERG), MU, KD SMAD: mo, ch (SMAD1/5), chs (SMAD1/5), MU, KO	44,57,58
<i>Etv2 (ms)</i>	Etv2-enhancer	Upstream to promoter –3 kb to TSS	Tg mouse	Early ECs (E7.75–E8.75)	ETS: mo, mu, oe NFAT: mo, oe SMAD: mo GATA: mo, oe MESP/CREB: mo, mu, oe NKX2-5: mo, em, ch	61-65
<i>Fli1 (ms)</i>	Fli1+12	Intronic +12 kb	Tg mouse	Pan-EC (E9.5–E12.5)	GATA: mo, em, ch (GATA2) ETS: mo, mu/MU, ch (FLI1/ELF) TAL1: mo (Ebox), ch	66,67
<i>Flk1 (ms)</i>	Flk1 minimal enhancer/ Flklin1	Intronic +3.5 kb	Tg mouse	Pan-EC (E11.5)	ETS: mo, em (ETS1), oe/OE (ETV2) GATA: mo, em (GATA2), MU TAL1: mo, em, MU FOXC/O: mo, em, ch, oe/OE	45,68
<i>Flk1 (ms)</i>	Flklin10	Intronic +16 kb	Tg mouse Tg zebrafish	Pan-EC (E9.5), Arterial EC (E10–E16)	ETS: mo, em (ETV2), MU GATA: mo, em (GATA2), MU, KD SOXF: mo, em (SOX7), MU RBPJ: mo, em, MU, KD, MU FOXC: mo, em (FOXC2)	69
<i>FLT4/VEGFR3 (hm)</i>	FLT4 enhancer/ In11-12TBE	Intronic +26 kb	Tg mouse	Pan-EC (E9.5) Lymphatic EC (E15.5) Arterial EC (E15.5)	FOXC/O: mo ETS: mo TBX1: mo, mu, ch, oe	45,70
<i>FOXP1 (hm)</i>	FOXP1 enhancer	Intronic +138 kb ^a	Tg mouse	Pan-EC tail region (E9.5)	FOXC/O: mo ETS: mo	45
<i>Gata2 (ms)</i>	G2-EHRD G2-5H G2-D3.1	Upstream –3 kb	Tg mouse	EC ^a (E9.5) Hemogenic EC (E10.5–E11.5)	GATA: mo, em, ch (GATA2) ETS: mo, mu/MU, ch (FLI1) TAL1: mo (Ebox), mu, ch	66,71
<i>Gata2 (ms/ zf)</i>	Gata2intron4 Gata2-TKVE Gata2+9.5	Intronic +9 kb	Tg mouse Tg zebrafish	Pan-EC (E10.5, 27 hpf)	GATA: mo ETS: mo, MU ^b TAL1: mo, em, MU MEF2: mo SMAD: mo	72-74
<i>Hey1 (ms)</i>	Hey1-18k	Upstream –18 kb	Tg mouse	Arterial EC (E9.5–10.5)	RBPJ: mo, em, MU, oe	75

(Continues)

TABLE 3 (Continued)

Gene (species)	Enhancer name in original paper	Enhancer location	Validation method	EC specificity (age investigated)	Transcription factors with experimental validation method	References
<i>HLX (hm)</i>	HLX-3/HLX-3b	Upstream −3 kb	Tg mouse Tg zebrafish	Angiogenic ECs (E11.5, 27–42 hpf)	ETS: mo, chs (ERG), mu, MU MEF2: mo, MU	49,51
<i>Mef2 (ms)</i>	Mef2F10	Intronic +79 kb ^a	Tg mouse Tg zebrafish	Pan-EC (E9.5, 48 hpf); Venous EC (E11.5)	ETS: mo, em (ETS1/ETV2) mu/MU, oe/OE (ETV2) FOXC/O: mo, em (FOXC1/2, FOXO1) ch (FOXC2), mu/MU, oe/OE (FOXC2)	44,45
<i>Mef2 (ms)</i>	Mef2F7	Intronic +68 kb ^a	Tg mouse	Pan-EC (E7.5-adult)	ETS: mo, em (ETS1), MU	76
<i>NOS3/eNOS (hm/ms)</i>	eNOSprom	Upstream to promoter −5 kb to TSS		Arterial EC (E11.5–E15.5)	KLF2: mo, oe, em GATA: mo AP2: mo, em MZF-like: mo, em ETS1: mo, em (ERG), mu	77–81
<i>NOTCH1 (hm)</i>	NOTCH1+16	Intronic +16 kb	Tg mouse	Arterial EC (E9.5–E12.5)	ETS: mo, em (ETV2) SOXF: mo, em (SOX7/18), MU	82
<i>notch1b (zf)</i>	notch1b-15	Upstream −15 kb	Tg zebrafish	Arterial EC (28–48 hpf)	ETS: mo, em (ETV2) SOXF: mo, em (SOX7/18), MU, KD	82
<i>NOTCH1 (hm and ms)</i>	NOTCH1+33/ Notch1_enh1	Intronic +33 kb	Tg mouse	Pan-EC (E11–E13)		44,82
<i>NOTCH4 (hm)</i>	NOTCH4proIN1	Promoter/ intronic +0.7 kb ^a	Tg mouse	EC ^a (E10.5)	API: em (FRA1), ch (all-Fos/FRA1), mu/MU FOXC/O: mo, em, ch, oe/OE ETS: mo, em (ETS1), oe/OE (ETV2)	45,83
<i>Nr2f2/CouptfII (ms)</i>	CouptfII-965	Upstream −965 kb	Tg mouse Tg zebrafish	Venous EC Lymphatic EC (E9–E15.5, 24–72 hpf)	ETS: mo, em (ETS1/ERG), ch (ERG), chs (ETS1/ERG), MU, KD SMAD: mo, ch (SMAD1/5), chs (SMAD1/5), MU, KO	57,58
<i>NRP1 (hm)</i>	NRP1 enhancer	Intronic +32 kb	Tg mouse	Pan-EC (E9.5)	FOXC/O: mo ETS: mo	45
<i>Nrp2 (ms)</i>	Nrp2+26	Intronic +26 kb	Tg mouse	ECs around neural tube ^a (E11.5)	ETS: mo SMAD: mo, chs (SMAD1/5)	57,58
<i>PDGFRB (hm)</i>	PDGFRB enhancer	Intronic +18 kb	Tg mouse	Pan-EC (E9.5)	FOXC/O: mo ETS: mo	45
<i>Procr/Epcr (ms)</i>	-5.5HS	Upstream −5 kb		Pan-EC (E12.5)	ETS: mo, mu, ch (ETS1/ELF1/FLI1/ERG) GATA: mo, mu, ch (GATA2) TAL1: mo, mu, ch	84

TABLE 3 (Continued)

Gene (species)	Enhancer name in original paper	Enhancer location	Validation method	EC specificity (age investigated)	Transcription factors with experimental validation method	References
<i>PROX1</i> (hm/ <i>ms</i>)	PROX1-11	Upstream –11 kb	Tg mouse	Lymphatic ECs, particularly in valves (E12–P4)	ETS: mo GATA: mo, ch and chs (GATA2) FOXC: mo, ch (FOXC2) NFAT: mo, ch (NFATc1) API: mo	85,86
<i>Sema6d</i> (<i>ms</i>)	Sema6d	Upstream –55 kb	Tg mouse	Arterial ECs (E11.5)		44
<i>SOX7</i> (hm and <i>ms</i>)	Sox7/CRE3	Upstream –14 kb (<i>ms</i>) –10 kb (hm)	Tg mouse Tg zebrafish	Arterial ECs (E11.5, 48 hpf)		44,87
<i>Tal1/Scl</i> (<i>ms</i>)	Scl+19	Downstream +19 kb	Tg mouse	EC ^a (E11.5)	MYB: mo ETS: mo, em (FLI1/ELF1) GATA: mo, em (GATA2)	88
<i>Tal1/Scl</i> (<i>ms</i>)	TAL1-3.8	Upstream –4 kb	Tg mouse	Pan-EC (E11.5)	ETS: mo, em (FLI1/ELF1), oe (FLI1/ELF1)	89
<i>Tie1</i> (<i>ms</i>)	Tie promoter	Upstream/ promoter –1 kb	Tg mouse	Pan-EC (E8.5–E10.5) Organ specific (E15–E17) Large vessels (adult)	ETS: mo, mu, oe API1/2/4: mo	90-92
<i>Tie2</i> (<i>ms</i>)	Tie2 HHXK and Tie2 HHNS	Promoter/ intronic +1 kb	Tg mouse	Pan-EC (E9.5-adult)	FOXC/O: mo, em, ch, oe/OE ETS: mo, em (ETS1), oe/OE (ETV2), mu	45,93-95

Note: Enhancer location is given as approximate kb from TSS of assigned gene and species. Categories of experimental validation methods linking TFs to particular enhancers include mo (motif for TF identified within enhancer); ch (ChIP confirms TF binding); chs (ChIP-seq confirms TF binding), em (electromobility shift assay confirms TF binding), mu/MU (mutation tested in vitro/in vivo confirms role of motif); KO/KD (knock-out/knock-down analysis confirms role of TF in enhancer regulation) and oe/OE (overexpression of TF in vitro/in vivo confirms role of TF in enhancer regulation).

^aIndicates multiple TSS, value given to first.

^bIndicates mutation did not include all available motifs for that TF.

specific and is equally found at arterial and venous-specific enhancers.⁵⁷ Additionally, siRNA-mediated knockdown of ERG reduces vascularization of Matrigel plugs, a phenotype linked to reduced *Hdac6* expression.^{139,140} ERG-deficient ECs also have reduced WNT signaling, with ERG influencing angiogenesis by promoting β -catenin stability via the Wnt receptor FZD4 and CDH5 (VE-Cadherin).¹³⁷

2.4 | ETS1/2

Ets1, the founding member of the ETS family, is first seen in hemato-endothelial progenitors from E7.0 to E7.25 and is strongly expressed in mature endothelium as well as in other tissues^{2,141,142} (Table 2). Like several other

non-EC ETS factors, ETS1 exhibits autoinhibition (meaning the full-length protein has less affinity for DNA than the binding domain alone), which is reinforced by phosphorylation and counteracted by protein partnerships.¹³⁰ ETS1 is expressed in ECs at the sites of both developmental and tumor angiogenesis,¹⁴³⁻¹⁴⁵ and is up-regulated by both pro-angiogenic VEGF-VEGFR2 and hypoxia signaling pathways.¹⁴⁶ Although constitutive deletion of *Ets1* results in few vascular defects, ETS1 shows significant functional redundancy with its close homolog ETS2, with which it shares a PNT domain targeted by Raf/Mek/Erk-mediated phosphorylation.¹³⁰ While single knockout of *Ets2* also does not result in significant vascular phenotypes, compound *Ets1*; *Ets2* knockout mice are embryonic lethal by E12 with dilated vessels, failed blood vessel branching, edema, and hemorrhage, suggesting a key but

redundant role for ETS1/2 in angiogenesis.¹¹³ Dominant negative ETS1 also inhibits retinal angiogenesis during proliferative retinopathy,¹⁴⁵ while antisense oligonucleotides directed against ETS1 inhibit EC migration and VEGF-induced EC proliferation.¹⁴⁴ The many characterized EC direct target genes include *Flt1*, *Tie2 (Tek)*, *Angpt2*, *Nrp1*, *Vwf*, *Cd31*, *Cdh5*, and crucially *Flk1*.^{90,142,147-150} ETS1 binding is enriched at gene promoters, and strongly correlates with transcription²³: in cultured ECs, VEGFA stimulation results in increased ETS1 occupation at both P300-bound enhancers and promoters of activated angiogenic genes.^{23,151} High expression of ETS1 in EC lines can also drive the switch from a quiescent to angiogenic state, which is attributed to increased expression of matrix metalloproteinases.^{113,152}

2.5 | ELK3

Among the ETS factors expressed strongly and early in ECs, the function of ELK3 (NET) is probably the least understood. ELK3 is part of the ternary complex factor (TCF) subfamily of ETS alongside ELK1 and ELK4 (SAP1), all of which contain an additional B-box domain to mediate interaction with serum response factor (SRF). *Elk3* expression is first seen in hemato-endothelial progenitors from E7.0 (concurrent with *Fli1* but after *Etv2*) and persists in ECs throughout development and in the adult.^{2,153,154} (Table 2). In the absence of MAPK activation (particularly isoforms ERK2, JNK, or p38), ELK3 strongly inhibits transcription,¹³⁰ and the switch from inhibitor to activator has been implicated in the role of ELK3 in angiogenesis.¹⁵⁵ However, while tumor cells with reduced ELK3 form fewer vessels, and *Elk3* downregulation inhibits VEGFA expression¹⁵⁵ and modulates HIF1 stability,¹⁵⁶ constitutive *Elk3* deletion results in viable mice with only mild vascular defects.¹⁵⁷ Compound deletion of orthologues *Elk1* and *Elk4* also fail to show angiogenic defects, leading to the conclusion that the crucial role of SRF in angiogenesis occurs via alternative MRTF co-factors.¹⁵⁸⁻¹⁶⁰ While no true *Elk1;Elk3;Elk4* triple knockout mice have been studied, neither *Elk1* or *Elk4* are strongly expressed during early endothelial development, and consequently the precise role of ELK3, and of the TCF ETS subfamily, in vasculogenesis and angiogenesis is still not fully defined.

3 | FORKHEAD

Forkhead (FOX) TFs are characterized by a winged-helix Forkhead box DNA binding domain which binds a core $C_{/T}AAA_{/T}A$ motif (Table 1). The 44 different FOX

proteins found in mice and humans can be divided into 22 subclasses (denoted as FOXA through to FOXS) according to sequence similarities,¹⁶¹ with members of the FOXC and FOXO subfamilies the principal FOX factors implicated in the regulation of vasculogenesis and angiogenesis.

3.1 | FOXC1/2

Both *Foxc1 (Mf1)* and *Foxc2 (Mfh1)* are expressed in ECs from early in embryonic development, detected by scRNA-seq from around E8.0 and by in situ hybridization from E9.5^{2,162} (Table 2). Compound *Foxc1;Foxc2* deletion results in a failure of blood vessel development and lethality by E9.5. Although ECs are specified and an initial vascular plexus forms in these mutant mice, the plexus does not remodel into a functional vascular network,^{162,163} indicating important functions for FOXC factors in endothelial differentiation and angiogenesis. In particular, FOXC factors have been implicated in angiogenesis via direct induction of *Itgb3*-mediated EC adhesion and migration,¹⁶⁴ and of *Dll4*-mediated Notch signaling downstream of VEGF.^{165,166} Analysis of endothelial enhancers also identified a shared role for FOXC and ETS factors in endothelial specification. FOXC1/2 and the ETS factor ETV2 combinatorially bind compound FOX:ETS motifs within gene enhancers¹⁶⁷ and promoters to synergistically activate the transcription of crucial endothelial lineage-identity genes, including those for *Flk1*, *Flt4*, *Tal1*, *Cdh5*, *Tie2*, *Notch4*, and *Pdgfr β* ⁴⁵ (see Table 3). Further, FOXC factors are also important regulators of endothelial patterning in the maturing vasculature. Embryos in which only a single FOXC allele remains show severe arterio-venous malformations and lack expression of arterial/angiogenic-associated genes including *Notch1*, *Dll4*, and *Jag1*,¹⁶³ while lymphatic-specific compound deletion of *Foxc1;Foxc2* results in increased lymphatic EC proliferation and abnormal lymphatic vessel morphogenesis.¹⁶⁸

3.2 | FOXO1/3/4

Mammalian *Foxo1 (Fkhr)*, *Foxo3 (Fkhr1)*, and *Foxo4 (Afx)* encode the evolutionarily conserved FOXO subfamily, which act as key nuclear effectors of the PI3K/AKT pathway. In the absence of active PI3K/AKT signaling, FOXOs localize to the nucleus, while PI3K activation results in FOXO phosphorylation and subsequent nuclear exclusion and proteasomal degradation.¹⁶⁹⁻¹⁷¹ FOXO1, the most robustly expressed *Foxo* gene in ECs (Table 2), is expressed from E7.75 and plays a crucial

role in vascular development in both the embryo and the adult: constitutive and EC-specific deletion of *Foxo1* results in a failure of angiogenesis-dependant vascular remodeling and lethality by E10.5.¹⁷²⁻¹⁷⁴ It is also able to bind the compound FOX:ETS motifs found in many early EC enhancers.⁴⁵ *Foxo3a* null and *Foxo4* null mice do not die during embryogenesis; however, post-natal angiogenic capacity is increased in *Foxo3a* knockout mice.¹⁷⁵ FOXO1 is also required to direct angiogenesis in the post-natal retina, with deletion resulting in uncoordinated vascular growth, increased endothelial number, density, and vessel diameter.¹⁷⁴ Mechanistically, FOXO factors function by coupling changes in metabolism with changes in gene transcription and cell activity. In ECs, the transcriptional targets of FOXOs include antioxidants, cell cycle inhibitors, and metabolic regulators, and they act as potent negative regulators of MYC activity.¹⁷⁴ FOXO activity itself is also regulated by energy-sensing post-transcriptional modifiers.¹⁷⁶ Therefore, in ECs, FOXO1 activity reduces metabolic activity, reduces MYC-induced glycolysis and mitochondrial respiration, thus mediating angiogenesis in response to changes in metabolism.¹⁷⁴

3.3 | Other FOX factors

Foxm1 is ubiquitously expressed in the early embryo but may play a role in vascular growth. Although EC-specific deletion of *Foxm1* results in no overt phenotype, pulmonary vascular injury in mice lacking or overexpressing *Foxm1* revealed a role for EC FOXM1 in the restoration of endothelial barrier function.^{177,178} Additionally, both *Foxp1* and *Foxp4* are expressed during early endothelial development,² with *Foxp1* most highly expressed in ECs in culture, and up-regulated in ECs during injury-induced neovascular growth. Knockdown of *Foxp1* in ECs in culture inhibits EC proliferation, migration, and tube formation,¹⁷⁹ while constitutive *Foxp1* knockout mice die at E14.5 with complex cardiovascular defects including vascular hemorrhage.¹⁸⁰ However, the vessel defects are thought to be secondary to severe cardiac and valve defects and were not seen after EC-specific *Foxp1* deletion.¹⁸¹

4 | GATA

The GATA family consists of six TFs characterized by a highly conserved zinc finger domain binding a core GATA motif (reviewed by 182; Table 1). GATA1, GATA2, and GATA3 have well-established roles in the specification of hematopoietic cells from the hemogenic endothelium (a specialized subset of ECs that give rise to the

hematopoietic stem and progenitor cells, reviewed by 183), but are also implicated in EC specification and development.

4.1 | GATA2

Gata2 is expressed in hemato-endothelial progenitors from E7.0 (Table 2) and has been implicated in the regulation of EC lineage specification via interaction with ETV2.¹⁸⁴ Motif analysis of endothelial enhancers also supports a role for GATA2 in the expression of angiogenic genes, with all characterized *Flk1* EC enhancers containing essential GATA-binding motifs^{69,185} (Table 3). GATA2 binding at the *Flk1* promoter also increases expression in response to changes in matrix stiffness,¹⁸⁶ while expression of *Emcn* (Endomucin), a type I integral membrane glycoprotein important in EC signaling and angiogenesis, requires GATA2-mediated chromatin remodeling.¹⁸⁷ Furthermore, GATA2 binds a unique set of gene loci in ECs compared to other cell lines,²⁸ dynamic GATA2 binding has been observed at key EC enhancers in response to VEGFA stimulation,³¹ and siRNA-mediated GATA2 knockdown in the post-natal retina inhibits angiogenic sprouting.¹⁸⁶ However, constitutive deletion of *Gata2* had no clear effect on early vascular formation before embryonic lethality at E10.5 due to hematopoietic defects,¹⁸⁸ although it is possible that the deletion strategy used to generate these mice (which removes only the C-terminal zinc finger of GATA2) did not ablate all GATA2 DNA binding.¹⁸⁹ Alternatively, the early hematopoietic lethality may predate overt EC phenotypes. Supporting this, both constitutive deletion of the GATA2+9.5 enhancer (strongly active in developing embryonic ECs)⁷² and endothelial-specific deletions of *Gata2* result in lethality at E13–16.5 with vascular abnormalities including hemorrhage, edema, and anemia. However, important roles for GATA2 in lymphangiogenesis underpins many of these defects.^{85,190}

GATA3 and GATA6 are also expressed in ECs in culture and have been implicated in the regulation of *Tie2*.¹⁹¹ Although it is possible that endothelial GATA2 may be functionally redundant to some degree with these other GATA factors, there is little evidence that GATA3 or GATA6 are robustly expressed in the developing endothelium (Table 2). Consequently, the precise roles of GATA factors in vasculogenesis and angiogenesis remain unclear.

4.2 | GATA/TAL1

Combinatorial binding of GATA alongside the TAL1/SCL TF at a composite GATA-E-box binding motif plays

a key role in hematopoietic development.^{192,193} In particular, GATA/TAL1 binding motifs are essential for the maintenance of hematopoietic stem cells and for the terminal differentiation of select blood cell lineages.¹⁹²⁻¹⁹⁴ GATA/TAL1 binding may also play a role in maintaining endothelial identity in the early embryo: constitutive *Tal1* knockout embryos display irregular yolk sac vasculature,^{195,196} while EC-specific deletion of *Tal1* results in edema, hemorrhage, and defective vascular remodeling in the yolk sac.¹⁹⁷ These defects have been attributed to dual roles for TAL1, in both activating hemogenic endothelium and repressing ectopic cardiomyogenesis in the yolk sac endothelium and endocardium.^{198,199} ChIP-seq experiments that assess chromatin states of enhancer elements suggest that TAL1 exploits a pre-established epigenetic landscape, likely generated by ETV2, to bind and repress enhancers of the cardiac lineage, while binding together with GATA to activate endothelial/hematopoietic lineage specification.²⁰⁰

5 | SMAD

SMAD proteins are the transcriptional effectors of the TGF β superfamily and can be subdivided into receptor-regulated SMADs (R-SMADs, consisting of SMAD1, 2, 3, 5, and 8), common SMAD (SMAD4), and inhibitory SMADs (I-SMADs, SMAD6 and 7).^{201,202} R-SMADs act as the primary signal transducers, forming complexes with SMAD4 after phosphorylation and subsequently translocating to the nucleus to directly regulate gene transcription.²⁰² R-SMADs can be further subdivided into those primarily downstream of canonical BMP signaling (SMAD1/5/8) and those primarily downstream of canonical TGF β signaling (SMAD2-3). Both BMP and TGF β signaling pathways play complex and sometimes contradictory roles in angiogenesis. TGF β signaling via ALK1 and endoglin can stimulate EC activation, proliferation, and migration, whereas TGF β signaling via ALK5 can inhibit proliferation and promote vascular maturation (reviewed by 203). Similarly, BMP signaling is implicated in both the promotion of angiogenesis and the maintenance of endothelial homeostasis via interactions of different ligands, receptors, and co-factors (reviewed by 202). Most SMADs are expressed early during EC development but also widely in non-ECs (Table 2). While endothelial-specific deletion of *Smad2/3* did not affect vasculogenesis and early angiogenesis (although defects in vascular maturation and mural cell assembly resulted in hemorrhage and lethality by E12.5), endothelial-specific deletion of either *Smad4* or *Smad1/5* led to defective angiogenic sprouting and embryonic lethality by E10.5.^{204,205} Analysis of SMAD1/5 binding in ECs

identified numerous direct targets, including *Id1*, Notch pathway genes *Hey1*, *Hey2*, *Hes1*, and *Jag1*, and venous identity genes *Ephb4* and *Coup-TFII* (*Nr2f2*)^{20,58,204} (Table 3). These targets have also been verified in knockout models, with endothelial-specific deletion of *Smad4* resulting in embryos with reduced *Ephb4* and *Coup-TFII* expression and defective venous differentiation, while endothelial-specific deletion of *Smad1/5* resulted in embryos with impaired DLL4-Notch signaling. The inhibitory SMAD6 may also play a role in this process downstream of Notch and upstream of target gene activation.²⁰⁶ Additionally, analysis of retinal angiogenesis after postnatal deletion of *Smad4* found increased EC proliferation and distorted arteriovenous gene expression, implicating SMADs in the pathogenic formation of arteriovenous malformations downstream of BMP9/10-ALK1 and upstream of angiopoietin-Tek signaling.²⁰⁷⁻²⁰⁹

6 | HYPOXIA-INDUCIBLE FACTOR

Hypoxia-inducible factor (HIF) TFs are master regulators of oxygen homeostasis²¹⁰ and therefore have significant direct and indirect influences on vascular growth. HIF1 α and HIF2 α (EPAS) subunits are stabilized in low oxygen conditions, permitting them to translocate to the nucleus and bind DNA at consensus ^A/_GCGTG motifs (Table 1) as a heterodimer with HIF1 β (ARNT), inducing gene programs that respond to the hypoxic environment (reviewed by 211). This adaptive response includes the activation of physiological and pathological angiogenesis through both cell autonomous and non-autonomous mechanisms.^{212,213} While *Hif1a* is ubiquitously expressed, *Hif1b* is predominantly EC-specific from E8.5 (see Table 2) where it is up-regulated by hypoxia^{214,215} (Table 2). Fewer than 20% of HIF target genes are regulated by both isoforms in ECs indicating predominantly non-overlapping functions, with HIF2 α inducing a larger and more diverse transcriptional response.²¹⁶ Overexpression of HIF isoforms in cultured ECs increases expression of a range of pro-angiogenic genes, including *Vegfa*, *Angpt2*, and *Pdgfb* (by HIF1 α)²¹⁷⁻²¹⁹ and *Flt1* (by HIF2 α).²²⁰ However, studies into the role of HIFs in ECs during developmental angiogenesis in mice have produced conflicting results. EC-specific expression (driven by a *Flk1* enhancer/promoter) of a dominant negative HIF mutant (which inhibits transcriptional responses by both HIF1 α and EPAS1/HIF2 α) results in embryonic lethality by E11.5 alongside severe cardiovascular defects and loss of vascular sprout formation attributed to reduced *Tie2* (*Tek*) expression.²²¹ Embryonic lethality and severe vascular defects (with reduced *Tie2*) were also seen after constitutive deletion of *Hif2a*.²²²

However, *Cdh5-Cre*-driven EC-specific deletion of *Hif2a* alone or in combination with *Hif1a* resulted in no major developmental vascular abnormalities,^{223,224} although increased vascular permeability and pulmonary hypertension were seen in the adult.²²⁴ This discrepancy in phenotype may be due to an early role for HIFs before robust *Cdh5-Cre* activity or incomplete Cre-mediated deletion (in part because the *Cdh5-Cre* driver used here is known to be only weakly and sporadically expressed before E14.5,^{98,225} whereas the *Flk1* driver of dominant negative HIF is active more robustly and much earlier), or may instead indicate a key function for HIF proteins in the activation of *Vegfa* in non-EC lineages.^{226,227} Alternatively, off-target effects of the dominant negative form of HIF may have contributed to the severity of phenotype seen in the dominant negative mutant mouse.

7 | RBPJ

The RBPJ (CSL) TF acts as the nuclear effector of the Notch signaling pathway, forming a complex with Notch intracellular domain (NICD) and the MAML1 co-activator to promote transcription via direct binding to Notch target genes at a consensus TGGGAA motif (reviewed by 228 and Table 1). Analysis of many deletion models strongly indicates that Notch signaling via RBPJ plays an essential role in vasculogenesis, angiogenesis, and arteriovenous differentiation.^{8,9,229,230} Loss of multiple different Notch pathway components results in early lethality in mice associated with severe vascular defects including aberrant arteriovenous specification (e.g., compound *Notch1/Notch4* deletion,²³¹ deletion of ligand *Dll4*^{232,233}), while ablation of Notch signaling in the post-natal retina results in significant defects in sprouting angiogenesis (reviewed by 8,10). Loss of RBPJ largely recapitulates defects downstream of Notch receptor/ligand deletion,^{9,232,234} and the Notch signaling components *Hey1* and *Dll4* are both direct targets of RBPJ via arterial/angiogenic-specific enhancers.^{50,75} Further, multiple different signaling pathways can converge upon, and influence, RBPJ binding. For example, Ang1/Tie2 signaling induces *Dll4* via AKT-mediated activation of β -catenin, which complexes with RBPJ to bind and activate an intronic enhancer,^{52,235} whereas KLF4 can inhibit *Dll4* expression during angiogenesis by interfering with RBPJ binding to the same element⁵³ (Table 3). In the absence of NICD, RBPJ can also act as a transcriptional repressor, and may repress EC gene expression in some circumstances. For example, RBPJ binding to an *Flk1* arterial enhancer represses its activity in veins,⁶⁹ while RBPJ binding to a *Vegfa* promoter negatively regulates its

expression.⁹ However, although it was long assumed that Notch-RBPJ influenced vascular patterning by directly regulating key arteriovenous genes, postnatal deletion of RBPJ does not ablate arterial gene expression,^{234,236} and ECs lacking MYC (a key driver of metabolism and proliferation) require neither RBPJ nor Notch for correct arteriovenous gene expression.²³⁷ Consequently, it is now thought that Notch-RBPJ regulates arteriovenous specification by reducing metabolism and cell cycle rather than via direct activation or repression of arteriovenous identity genes.²³⁷ Given that the signaling processes involved in angiogenic sprouting are often coupled to arterial formation,⁹ it is still unclear to what degree RBPJ directly versus indirectly regulates gene expression in arterial and angiogenic ECs.

8 | HEY FACTORS

The Hey family of transcriptional repressors are basic helix-loop-helix proteins that act downstream of Notch signaling and are directly activated by RBPJ^{75,238} (Table 3). *Hey1* and *Hey2* are expressed in ECs from early during mammalian development (see Table 1) and directly bind DNA at E-box motifs.²³⁸ While the orthologue of *Hey2* in zebrafish (*gridlock*) is essential for arterial morphogenesis,²³⁹ deletion of either *Hey1* or *Hey2* separately does not result in gross embryonic vascular phenotypes, although *Hey2* null mice die soon after birth and *Hey1* null mice show anomalies of the thoracic great vessels.^{240,241} However, compound deletion of both *Hey1* and *Hey2* results in embryonic lethality and significant endothelial defects including in arteriovenous differentiation.^{242,243} Loss of HEY1/HEY2 resulted in both reduced *Ephb2* and *Jag1* expression and increased *Robo4* and *Flk1* expression.²⁴²⁻²⁴⁴ However, there are currently few well-characterized direct endothelial targets of HEY1/2 described in the literature, and it is unclear to what extent the phenotype in the compound *Hey1/2* null mice can be attributed to vascular versus cardiac defects.²⁴²⁻²⁴⁴

9 | HLX

HLX1 is a homeobox TF with a currently undefined DNA binding motif expressed in hemato-endothelial progenitors from E7.75 (Table 2). HLX1 was first implicated as a key regulator of sprouting angiogenesis in zebrafish (as the orthologue *hlx*), where it maintains endothelial stalk-cell fate in a cell-autonomous manner.²⁴⁵ In mammalian EC culture, HLX1 regulates expression of cell guidance molecules including *Unc5b*, *Plxna1*, and

Sema3g downstream of VEGF-VEGFR2 signaling,²⁴⁶ and is a direct transcriptional target of both ERG and MEF2 TFs downstream of VEGFA-VEGFR2 signaling.^{49,51} However, although both overexpression and knockdown of *Hlx1* in cultured ECs disrupt sprouting angiogenesis,^{246,247} constitutive *Hlx1* deletion in mice causes only a mild vascular remodeling phenotype.²⁴⁷ Consequently, the precise role and direct transcriptional targets of HLX1 in angiogenesis are yet to be clearly elucidated.

10 | MEF2

The MEF2 family of TFs is characterized by their highly conserved MADS-box and MEF2 domains which mediate dimerization, co-factor interactions, and DNA binding to a consensus $C_{/T}TA^A_{/T}A_{/T}A_{/T}A_{/T}TA^A_G$ motif.²⁴⁸ They are found in multiple cell lineages and play key roles in a diverse range of developmental processes.²⁴⁸ While constitutive *Mef2c* deletion results in both cardiac and vascular defects,^{249,250} endothelial-specific *Mef2c* deletion has no effect on embryonic vascular development regardless of Cre driver,^{251,252} although subtle defects are detected in pathological angiogenesis.²⁵¹ However, *Mef2a*, *Mef2c*, and *Mef2d* are all expressed in the early endothelium^{2,51} (Table 3), and studies from other cell types show strong functional redundancy between the three proteins.²⁵³

Analysis of direct MEF2 targets in ECs alongside combinatorial gene deletion has strongly implicated MEF2 factors in angiogenesis downstream of VEGFA-VEGFR2 signaling, and in endothelial homeostasis downstream of blood flow.^{51,254} Supporting this, induced EC-specific deletion of *Mef2a* alongside *Mef2c* reduces sprouting angiogenesis and *Dll4* expression in the postnatal retina, while MEF2 proteins directly bind and activate many angiogenic-specific genes including *Dll4* and *Hlx1*.⁵¹ Further, compound endothelial-specific deletion of *Mef2a*, *Mef2c*, and *Mef2d* in adult mice results in systemic inflammation, hemorrhage, and rapid lethality,²⁵⁴ closely phenocopying that of *Klf2/4* deletion and supporting a key role for MEF2 factors directly upstream of *Klf2/4* transcription in response to fluid shear stress.²⁵⁴⁻²⁵⁶ MEF2 factors also directly regulate the expression of *Mmp10* to regulate vascular integrity.²⁵⁷ It is, however, unclear how the widely expressed MEF2 factors are able to regulate such disparate vascular behavior. Although MEF2 factor activity can itself be regulated by both ERK5 and by complexing with class IIa HDACs, it is likely that additional transcriptional cofactors are required to enable MEF2 factors to achieve their diverse gene expression patterns in the endothelium.

11 | AP1

AP1 proteins are made up of a ubiquitously expressed family of transcription complexes most commonly defined as a collection of dimers from the Jun and Fos families, although it can also be considered to include members of the ATF and MAF subfamilies.²⁵⁸ AP1 TFs bind a consensus TGA^C/GTC^A motif, although the ATF and MAF subfamilies have slightly differing preferences (see Table 1).

11.1 | JUNB

Induced EC-specific deletion of *Junb* in the retina leads to reduced angiogenic vascular growth and diminished expression of neurovascular guidance genes including *Sema3a*, likely via direct binding at AP1 motifs within enhancer elements.²⁵⁹ Although often considered ubiquitous, *Junb* expression is enriched in hemato-endothelial progenitors and angioblasts in the E8.5 embryo.² Further, JUNB is spatially restricted at the angiogenic front by a combination of VEGFA-VEGFR2 and S1P-S1PR signaling: induction of *Junb* by VEGFA-VEGFR2 is countered by the circulating vascular maturation factor sphingosine 1-phosphate (S1P), which restricts *Junb* expression in the perfused vasculature via S1PR-dependent VE-cadherin assembly.²⁵⁹ The related factor JUN/c-JUN may also play a role in angiogenesis in some contexts and is implicated in the regulation of expansion of the vasa vasorum downstream of extracellular ATP signaling.²⁶⁰

11.2 | MAFB

MAFB, a member of the large MAF TF subfamily, has been implicated in the regulation of both lymphangiogenesis and sprouting angiogenesis.^{261,262} Analysis of the actively translated transcriptome at different stages of postnatal retinal angiogenesis combined with promoter analysis identified MAFB as a key regulator of postnatal angiogenesis.²⁶¹ *Mafb* is enriched at the angiogenic front in postnatal retinas, while induced EC-specific deletion results in defective angiogenic expansion. MAFB expression up-regulates *Git1* and down-regulates *Arhgdib* expression, two Rho GTPase modulators that activate and inhibit Rac1/Cdc42 signaling, respectively, leading to cytoskeletal changes and EC migration during sprouting angiogenesis.²⁶¹ However, constitutive deletion of *Mafb* in mice results in no vascular-related lethality and embryonic angiogenesis is unaffected,²⁶³ suggesting organotypic specificity of its angiogenic role or potential

redundancy. In lymphatic ECs, *Mafb* expression is strongly up-regulated by VEGFC, while *Mafb* overexpression results in increased levels of many lymphatic EC genes including *Prox1*.²⁶⁴ Additionally, although viable both constitutive and lymphatic EC-specific *Mafb* deletion results in impaired lymphatic patterning, further supporting a role for MAFB in lymphatic ECs.^{264,265}

11.3 | Small MAFs

Analysis of the epigenetic and transcriptional changes in cultured ECs following VEGFA stimulation identified small MAF proteins MAFF, MAFG, and MAFK as key regulators of the angiogenic transcriptional response alongside ETS1, ERG, MEF2C, and FOXO1.³¹ This is supported by in vitro sprouting angiogenesis assays, which indicate partially redundant but collectively critical functions for small MAFs in EC migration, proliferation, and tube formation.³¹ However, these observations have yet to be validated in animal models.

12 | TEAD

The TEAD/TEF TF family consists of four highly homologous proteins (TEAD1–4), all containing the conserved TEA DNA-binding domain recognizing a consensus GGAATG motif²⁶⁶ (Table 1). TEAD proteins are the mediators of YAP/TAZ-dependent gene regulation downstream of the highly conserved Hippo signaling pathway. In their active state, YAP and TAZ translocate to the nucleus and form YAP/TAZ-TEAD complexes, which are associated with the expression of genes controlling cell proliferation, migration, and apoptosis.²⁶⁷ YAP/TAZ activity is limited through phosphorylation by Hippo pathway components, leading to cytoplasmic retention and destabilization.²⁶⁸

EC-specific deletion of *Yap/Taz* in mice leads to embryonic lethality associated with severe vascular defects throughout the embryo and yolk sac.^{269,270} Similarly, induced EC deletion of *Yap/Taz* in the postnatal retina results in reduced vessel growth, blunted-ended tip cells with fewer filipodia and defective lumen formation.²⁶⁹ Lowered levels of tip cell-associated ANGPT2 and ESM1 are observed, the number of ERG-positive ECs is reduced, and fewer actively proliferating ECs are detected.²⁶⁹ Conversely, overexpression of a stabilized TAZ protein results in a dense and hyperplastic vascular network with increased EC proliferation.²⁷¹ Notably, induced EC-specific compound deletion of *Tead1*, *Tead2*, and *Tead4* together results in a similar phenotype to that seen after *Yap/Taz* deletion, validating TEADs as crucial

transcriptional effectors of endothelial YAP/TAZ signaling.²⁷¹ Potential mechanisms for YAP/TAZ/TEAD involvement in angiogenesis include activation of actin cytoskeleton remodeling downstream of VEGF-VEGFR2,^{269,270} modulation of MYC signaling,²⁶⁹ activation of the small GTPase CDC42,²⁷² and fuelling nutrient-dependent mTORC1 signaling via transcriptional activation of cell-surface transporters.²⁷¹

13 | SOXF

The SOXF proteins, comprising SOX7, SOX17, and SOX18, are the only members of the SOX TF family strongly expressed in the developing endothelium, and recognize a consensus ^A/_TCAA^A/_T DNA motif.²⁷³ *Sox7* and *Sox17* are expressed concurrently with *Etv2* in angioblasts from the onset of vasculogenesis at E7.5, with *Sox18* also expressed in endothelial progenitors from E7.75.^{2,123,274–276} While SOX17 has been specifically implicated in arterial differentiation²⁷⁷ and SOX18 plays a key role in the initiation of lymphangiogenesis,²⁷⁸ SOXF factors also play crucial, although redundant, roles in vasculogenesis and angiogenesis. Although *Sox7* deficiency does not impact the emergence of ECs, vasculogenic defects to dorsal aorta formation are visible by E8.5, impaired angiogenesis is seen from E9.5, and both constitutive and EC-specific deletion of *Sox7* results in significant growth retardation, severe vascular defects, and lethality by E10.5–E11.5.^{273,274} EC-specific deletion of *Sox17* results in a similar loss of angiogenic sprouting in some mouse backgrounds,²⁷⁹ as does compound heterozygous deletion of *Sox7* alongside *Sox17*.²⁷³ Complicating analysis, genetic levels of compensation between SOXF factors can vary depending on mouse background.²⁸⁰ This is evident in analysis of the role of SOXF factors in postnatal retinal angiogenesis, with the severity of single or compound deletion of *Sox7*, *Sox17*, and *Sox18* varying between mouse models. Notably, however, the resultant phenotypes are similar, resulting in ablation of tip and stalk cell identity, reduced vascular outgrowth and branching, and compromised tube formation and perfusion.^{273,281} Overexpression of *Sox17* also promotes tumor angiogenesis and vascular abnormalities, while *Sox17* deletion in ECs reduces tumor angiogenesis and normalized tumor vessels, inhibiting tumor growth.²⁸²

The known direct SOXF target genes align with the range of vascular functions associated with these TFs. SOX17 directly binds arterial enhancers regulating *Dll4*, *Notch1*, and *Ece1*, while SOX18 directly targets a *Prox1* enhancer/promoter.^{50,56,277,278,283} SOX7 and/or SOX17 can also directly up-regulate angiogenic *Flk1*,^{69,273} *Lef1*, and β -catenin²⁸⁴ expression, while enforced expression of

Sox17 in EC-like cells increases expression of *Col18a1* and *Cd31* as well as *Flk1*.²⁸⁵ However, given the wide and overlapping expression patterns of each SOXF factor both within and beyond the endothelium, it is apparent that additional co-factors must be involved in the regulation of genes downstream of SOXF, while expression of the *SoxF* genes is themselves likely to be controlled by multiple upstream inputs.

14 | FUTURE DIRECTIONS

Our understanding of the array of TFs involved in vasculogenesis and angiogenesis has greatly increased in the last ten years, as has our ability to link these factors both to specific aspects of EC biology and to their direct target genes. Progress in these areas will continue as new technologies increasingly provide information of gene expression patterns, enhancer marks, and protein binding patterns at a single-cell resolution, and as computational pathways are developed to process and analyze such complex information simply and efficiently. Alongside this progress sit innovations in our ability to experimentally examine the role of novel EC transcriptional regulators, including the increasing ease of genetic manipulation, the generation of more diverse and specific methods to alter gene expression selectively in certain ECs, and higher-throughput methods to validate and interrogate enhancer elements directly in animal models. However, while such approaches will improve our knowledge of the roles of known vascular TFs and identify new factors, a better understanding of gene regulation within the vasculature also requires a greater appreciation of the manner in which the limited cohort of TFs work together to achieve different outputs. As is made clear in this review, ECs contain no single lineage-defining TF. Instead, the vast majority of endothelial transcriptional regulators are both expressed outside of ECs and involved in activating genes with more than one type of endothelial expression pattern and/or in response to more than one stimulus. Consequently, while improved genetic information and animal models will provide a more complete picture of the TF repertoire coordinating vasculogenesis and angiogenesis, this must be coupled with a greater understanding of the different combinatorial, synergistic, and antagonistic ways in which these factors work together to enable this limited number of proteins to achieve such complex and responsive patterns of gene expression.

AUTHOR CONTRIBUTIONS

Sophie Payne: Writing – original draft (equal). **Alice Neal:** Writing – original draft (equal). **Sarah De Val:** Writing – review and editing (lead).

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REFERENCES

- Liu F, Li D, Yu YYL, et al. Induction of hematopoietic and endothelial cell program orchestrated by ETS transcription factor ER71/ETV2. *EMBO Rep.* 2015;16(5):654-669. doi:10.15252/embr.201439939
- Pijuan-Sala B, Griffiths JA, Guibentif C, et al. A single-cell molecular map of mouse gastrulation and early organogenesis. *Nature.* 2019;566(7745):490-495. doi:10.1038/s41586-019-0933-9
- Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G. A common precursor for hematopoietic and endothelial cells. *Development.* 1998;125(4):725-732. <http://dev.biologists.org/content/125/4/725.long>
- Nishikawa SI, Nishikawa S, Hirashima M, Matsuyoshi N, Kodama H. Progressive lineage analysis by cell sorting and culture identifies FLK1+VE-cadherin+ cells at a diverging point of endothelial and hemopoietic lineages. *Development.* 1998;125(9):1747-1757. doi:10.1242/dev.125.9.1747
- Chong DC, Koo Y, Xu K, Fu S, Cleaver O. Stepwise arteriovenous fate acquisition during mammalian vasculogenesis. *Dev Dyn.* 2011;240(9):2153-2165. doi:10.1002/dvdy.22706
- Gong W, Rasmussen TL, Singh BN, Koyano-Nakagawa N, Pan W, Garry DJ. Dpath software reveals hierarchical haemato-endothelial lineages of Etv2 progenitors based on single-cell transcriptome analysis. *Nat Commun.* 2017;8(1):14362. doi:10.1038/ncomms14362
- Gerhardt H, Golding M, Fruttiger M, et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol.* 2003;161(6):1163-1177. doi:10.1083/jcb.200302047
- Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. *Cell.* 2011;146(6):873-887. doi:10.1016/j.cell.2011.08.039
- Pitulescu ME, Schmidt I, Giaimo BD, et al. Dll4 and Notch signalling couples sprouting angiogenesis and artery formation. *Nat Cell Biol.* 2017;19(8):915-927. doi:10.1038/ncb3555
- Geudens I, Gerhardt H. Coordinating cell behaviour during blood vessel formation. *Development.* 2011;138(21):4569-4583. doi:10.1242/dev.062323
- Long HK, Prescott SL, Wysocka J. Ever-changing landscapes: transcriptional enhancers in development and evolution. *Cell.* 2016;167(5):1170-1187. doi:10.1016/j.cell.2016.09.018
- Panigrahi A, O'Malley BW. Mechanisms of enhancer action: the known and the unknown. *Genome Biol.* 2021;22(1):108. doi:10.1186/s13059-021-02322-1
- Jolma A, Yan J, Whittington T, et al. DNA-binding specificities of human transcription factors. *Cell.* 2013;152(1-2):327-339. doi:10.1016/j.cell.2012.12.009
- Castro-Mondragon JA, Riudavets-Puig R, Rauluseviciute I, et al. JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. *Nucleic Acids Res.* 2021;30:D165-D173. doi:10.1093/nar/gkab1113

15. Kassouf MT, Hughes JR, Taylor S, et al. Genome-wide identification of TAL1's functional targets: insights into its mechanisms of action in primary erythroid cells. *Genome Res.* 2010; 20(8):1064-1083. doi:10.1101/gr.104935.110
16. Portales-Casamar E, Kirov S, Lim J, et al. PAZAR: a framework for collection and dissemination of cis-regulatory sequence annotation. *Genome Biol.* 2007;8(10):R207. doi:10.1186/gb-2007-8-10-r207
17. Mathelier A, Fornes O, Arenillas DJ, et al. JASPAR 2016: a major expansion and update of the open-access database of transcription factor binding profiles. *Nucleic Acids Res.* 2016; 44(D1):D110-D115. doi:10.1093/nar/gkv1176
18. Yoon SJ, Wills AE, Chuong E, Gupta R, Baker JC. HEB and E2A function as SMAD/FOXH1 cofactors. *Gene Dev.* 2011; 25(15):1654-1661. doi:10.1101/gad.16800511
19. Nagai N, Ohguchi H, Nakaki R, et al. Downregulation of ERG and FLI1 expression in endothelial cells triggers endothelial-to-mesenchymal transition. *PLoS Genet.* 2018;14(11): e1007826. doi:10.1371/journal.pgen.1007826
20. Morikawa M, Koinuma D, Tsutsumi S, et al. CHIP-seq reveals cell type-specific binding patterns of BMP-specific Smads and a novel binding motif. *Nucleic Acids Res.* 2011;39(20):8712-8727. doi:10.1093/nar/gkr572
21. Chèneby J, Gheorghie M, Artufel M, Mathelier A, Ballester B. ReMap 2018: an updated atlas of regulatory regions from an integrative analysis of DNA-binding ChIP-seq experiments. *Nucleic Acids Res.* 2018;46(Database issue):D267-D275. doi:10.1093/nar/gkx1092
22. Mimura I, Nangaku M, Kanki Y, et al. Dynamic change of chromatin conformation in response to hypoxia enhances the expression of GLUT3 (SLC2A3) by cooperative interaction of hypoxia-inducible factor 1 and KDM3A. *Mol Cell Biol.* 2012; 32(15):3018-3032. doi:10.1128/mcb.06643-11
23. Chen J, Fu Y, Day DS, et al. VEGF amplifies transcription through ETS1 acetylation to enable angiogenesis. *Nat Commun.* 2017;8(1):383. doi:10.1038/s41467-017-00405-x
24. Robertson ED, Wasyluk C, Ye T, Jung AC, Wasyluk B. The oncogenic microRNA Hsa-miR-155-5p targets the transcription factor ELK3 and links it to the hypoxia response. *PLoS One.* 2014;9(11):e113050. doi:10.1371/journal.pone.0113050
25. Norrmén C, Ivanov KI, Cheng J, et al. FOXC2 controls formation and maturation of lymphatic collecting vessels through cooperation with NFATc1. *J Cell Biol.* 2009;185(3):439-457. doi:10.1083/jcb.200901104
26. Hammal F, de Langen P, Bergon A, Lopez F, Ballester B. ReMap 2022: a database of Human, Mouse, Drosophila and Arabidopsis regulatory regions from an integrative analysis of DNA-binding sequencing experiments. *Nucleic Acids Res.* 2021;50(D1):D316-D325. doi:10.1093/nar/gkab996
27. Doyle MJ, Magli A, Estharabadi N, Amundsen D, Mills LJ, Martin CM. Sox7 regulates lineage decisions in cardiovascular progenitor cells. *Stem Cells Dev.* 2019;28(16):1089-1103. doi:10.1089/scd.2019.0040
28. Linnemann AK, O'Geen H, Keles S, Farnham PJ, Bresnick EH. Genetic framework for GATA factor function in vascular biology. *Proc Natl Acad Sci U S A.* 2011;108(33): 13641-13646. doi:10.1073/pnas.1108440108
29. Overman J, Fontaine F, Moustaqil M, et al. Pharmacological targeting of the transcription factor SOX18 delays breast cancer in mice. *eLife Sci.* 2017;6:6. doi:10.7554/elife.21221
30. Cao J, Spielmann M, Qiu X, et al. The single-cell transcriptional landscape of mammalian organogenesis. *Nature.* 2019; 566(7745):496-502. doi:10.1038/s41586-019-0969-x
31. Wang S, Chen J, Garcia SP, et al. A dynamic and integrated epigenetic program at distal regions orchestrates transcriptional responses to VEGFA. *Genome Res.* 2019;29(2):193-207. doi:10.1101/gr.239053.118
32. Sissaoui S, Yu J, Yan A, et al. Genomic characterization of endothelial enhancers reveals a multifunctional role for NR2F2 in regulation of arteriovenous gene expression. *Circ Res.* 2020;126(7):875-888. doi:10.1161/circresaha.119.316075
33. Kalna V, Yang Y, Peghaire CR, et al. The transcription factor ERG regulates super-enhancers associated with an endothelial-specific gene expression program. *Circ Res.* 2019; 124(9):1337-1349. doi:10.1161/circresaha.118.313788
34. Hogan NT, Whalen MB, Stolze LK, et al. Transcriptional networks specifying homeostatic and inflammatory programs of gene expression in human aortic endothelial cells. *Elife.* 2017; 6:e22536. doi:10.7554/elife.22536
35. Andrade J, Shi C, Costa ASH, et al. Control of endothelial quiescence by FOXO-regulated metabolites. *Nat Cell Biol.* 2021;5: 1-33. doi:10.1038/s41556-021-00637-6
36. Pflieger J, Coleman RC, Ibeti J, et al. Genomic binding patterns of forkhead box protein O1 reveal its unique role in cardiac hypertrophy. *Circulation* 2020;142(9):882-898. doi:10.1161/circulationaha.120.046356
37. The ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature.* 2012; 489(7414):57-74. doi:10.1038/nature11247
38. Tiana M, Acosta-Iborra B, Puente-Santamaria L, et al. The SIN3A histone deacetylase complex is required for a complete transcriptional response to hypoxia. *Nucleic Acids Res.* 2018; 46(1):120-133. doi:10.1093/nar/gkx951
39. Poulsen LC, Edelmann RJ, Krüger S, et al. Inhibition of endothelial NOTCH1 signaling attenuates inflammation by reducing cytokine-mediated histone acetylation at inflammatory enhancers. *Arterioscler Thromb Vasc Biol.* 2018;38(4):854-869. doi:10.1161/atvbaha.117.310388
40. Akerberg BN, Gu F, Van Dusen NJ, et al. A reference map of murine cardiac transcription factor chromatin occupancy identifies dynamic and conserved enhancers. *Nat Commun.* 2019;22:1-16. doi:10.1038/s41467-019-12812-3
41. Maejima T, Inoue T, Kanki Y, et al. Direct evidence for pitavastatin induced chromatin structure change in the KLF4 gene in endothelial cells. *PLoS One.* 2014;9(5):e96005. doi:10.1371/journal.pone.0096005
42. Li X, Yonenaga Y, Seki T. Shortened ALK1 regulatory fragment maintains a specific activity in arteries feeding ischemic tissues. *Gene Ther.* 2019;11:1-8. doi:10.1038/gt.2009.53
43. Seki T, Hong KH, Yun J, Kim SJ, Oh SP. Isolation of a regulatory region of activin receptor-like kinase 1 gene sufficient for arterial endothelium-specific expression. *Circ Res.* 2004;94(8): e72-e77. doi:10.1161/01.res.0000127048.81744.31
44. Zhou P, Gu F, Zhang L, et al. Mapping cell type-specific transcriptional enhancers using high affinity, lineage-specific

- Ep300 bioChIP-seq. *eLife Sci.* 2017;6:e22039. doi:[10.7554/elife.22039](https://doi.org/10.7554/elife.22039)
45. De Val S, Chi NC, Meadows SM, et al. Combinatorial regulation of endothelial gene expression by Ets and forkhead transcription factors. *Cell.* 2008;135(6):1053-1064. doi:[10.1016/j.cell.2008.10.049](https://doi.org/10.1016/j.cell.2008.10.049)
 46. Prandini MH, Dreher I, Bouillot S, Benkerri S, Moll T, Huber P. The human VE-cadherin promoter is subjected to organ-specific regulation and is activated in tumour angiogenesis. *Oncogene.* 2005;24(18):2992-3001. doi:[10.1038/sj.onc.1208483](https://doi.org/10.1038/sj.onc.1208483)
 47. Deleuze V, Chalhoub E, El-Hajj R, et al. TAL-1/SCL and its partners E47 and LMO2 up-regulate VE-cadherin expression in endothelial cells. *Mol Cell Biol.* 2007;27(7):2687-2697. doi:[10.1128/mcb.00493-06](https://doi.org/10.1128/mcb.00493-06)
 48. Wythe JD, Dang LTH, Devine WP, et al. ETS factors regulate Vegf-dependent arterial specification. *Dev Cell.* 2013;26(1):45-58. doi:[10.1016/j.devcel.2013.06.007](https://doi.org/10.1016/j.devcel.2013.06.007)
 49. Fish JE, Gutierrez MC, Dang LT, et al. Dynamic regulation of VEGF-inducible genes by an ERK/ERG/p300 transcriptional network. *Development.* 2017;144(13):2428-2444. doi:[10.1242/dev.146050](https://doi.org/10.1242/dev.146050)
 50. Sacilotto N, Monteiro R, Fritzsche M, et al. Analysis of Dll4 regulation reveals a combinatorial role for Sox and Notch in arterial development. *Proc Natl Acad Sci U S A.* 2013;110(29):11893-11898. doi:[10.1073/pnas.1300805110](https://doi.org/10.1073/pnas.1300805110)
 51. Sacilotto N, Chouliaras KM, Nikitenko LL, et al. MEF2 transcription factors are key regulators of sprouting angiogenesis. *Genes Dev.* 2016;30(20):2297-2309. doi:[10.1101/gad.290619.116](https://doi.org/10.1101/gad.290619.116)
 52. Zhang J, Fukuhara S, Sako K, et al. Angiopoietin-1/Tie2 signal augments basal Notch signal controlling vascular quiescence by inducing delta-like 4 expression through AKT-mediated activation of β -Catenin*. *J Biol Chem.* 2011;286(10):8055-8066. doi:[10.1074/jbc.m110.192641](https://doi.org/10.1074/jbc.m110.192641)
 53. Boriushkin E, Zhang H, Becker M, et al. Kruppel-like factor 4 regulates developmental angiogenesis through disruption of the RBP-J-NICD-MAML complex in intron 3 of Dll4. *Angiogenesis.* 2019;22(2):295-309. doi:[10.1007/s10456-018-9657-y](https://doi.org/10.1007/s10456-018-9657-y)
 54. Payne S, Gunadasa-Rohling M, Neal A, et al. Regulatory pathways governing murine coronary vessel formation are dysregulated in the injured adult heart. *Nat Commun.* 2019;10(1):3276-3219. doi:[10.1038/s41467-019-10710-2](https://doi.org/10.1038/s41467-019-10710-2)
 55. Yamamizu K, Matsunaga T, Uosaki H, et al. Convergence of Notch and beta-catenin signaling induces arterial fate in vascular progenitors. *J Cell Biol.* 2010;189(2):325-338. doi:[10.1083/jcb.200904114](https://doi.org/10.1083/jcb.200904114)
 56. Robinson AS, Materna SC, Barnes RM, De Val S, Xu SM, Black BL. An arterial-specific enhancer of the human endothelin converting enzyme 1 (ECE1) gene is synergistically activated by Sox17, FoxC2, and Etv2. *Dev Biol.* 2014;395(2):379-389. doi:[10.1016/j.ydbio.2014.08.027](https://doi.org/10.1016/j.ydbio.2014.08.027)
 57. Neal A, Nornes S, Louphrasitthiphol P, et al. ETS factors are required but not sufficient for specific patterns of enhancer activity in different endothelial subtypes. *Dev Biol.* 2021;473:1-14. doi:[10.1016/j.ydbio.2021.01.002](https://doi.org/10.1016/j.ydbio.2021.01.002)
 58. Neal A, Nornes S, Payne S, et al. Venous identity requires BMP signalling through ALK3. *Nat Commun.* 2019;10(1):453. doi:[10.1038/s41467-019-08315-w](https://doi.org/10.1038/s41467-019-08315-w)
 59. Pimanda JE, Chan WYI, Donaldson IJ, Bowen M, Green AR, Göttgens B. Endoglin expression in the endothelium is regulated by Fli-1, Erg, and Elf-1 acting on the promoter and a -8-kb enhancer. *Blood.* 2006;107(12):4737-4745. doi:[10.1182/blood-2005-12-4929](https://doi.org/10.1182/blood-2005-12-4929)
 60. Pimanda JE, Chan WYI, Wilson NK, et al. Endoglin expression in blood and endothelium is differentially regulated by modular assembly of the Ets/Gata hemangioblast code. *Blood.* 2008;112(12):4512-4522. doi:[10.1182/blood-2008-05-157560](https://doi.org/10.1182/blood-2008-05-157560)
 61. Ferdous A, Caprioli A, Iacovino M, et al. Nkx2-5 transactivates the Ets-related protein 71 gene and specifies an endothelial/endocardial fate in the developing embryo. *Proc Natl Acad Sci U S A.* 2009;106(3):814-819. doi:[10.1073/pnas.0807583106](https://doi.org/10.1073/pnas.0807583106)
 62. Sinha T, van Bueren KL, Dickel DE, et al. Differential Etv2 threshold requirement for endothelial and erythropoietic development. *Cell Rep.* 2022;39(9):110881. doi:[10.1016/j.celrep.2022.110881](https://doi.org/10.1016/j.celrep.2022.110881)
 63. Koyano-Nakagawa N, Shi X, Rasmussen TL, Das S, Walter CA, Garry DJ. Feedback mechanisms regulate Ets variant 2 (Etv2) gene expression and hematoendothelial lineages. *J Biol Chem.* 2015;290(47):28107-28119. doi:[10.1074/jbc.m115.662197](https://doi.org/10.1074/jbc.m115.662197)
 64. Shi X, Zirbes KM, Rasmussen TL, et al. The transcription factor Mesp1 interacts with cAMP-responsive element binding protein 1 (Creb1) and coactivates Ets variant 2 (Etv2) gene expression. *J Biol Chem.* 2015;290(15):9614-9625. doi:[10.1074/jbc.m114.614628](https://doi.org/10.1074/jbc.m114.614628)
 65. Yamamizu K, Matsunaga T, Katayama S, et al. PKA/CREB signaling triggers initiation of endothelial and hematopoietic cell differentiation via Etv2 induction. *Stem Cells.* 2012;30(4):687-696. doi:[10.1002/stem.1041](https://doi.org/10.1002/stem.1041)
 66. Pimanda JE, Ottersbach K, Knezevic K, et al. Gata2, Fli1, and Scl form a recursively wired gene-regulatory circuit during early hematopoietic development. *Proc Natl Acad Sci U S A.* 2007;104(45):17692-17697. doi:[10.1073/pnas.0707045104](https://doi.org/10.1073/pnas.0707045104)
 67. Donaldson IJ, Chapman M, Kinston S, et al. Genome-wide identification of cis-regulatory sequences controlling blood and endothelial development. *Hum Mol Genet.* 2005;14(5):595-601. doi:[10.1093/hmg/ddi056](https://doi.org/10.1093/hmg/ddi056)
 68. Kappel A, Schlaeger TM, Flamme I, Orkin SH, Risau W, Breier G. Role of SCL/Tal-1, GATA, and ets transcription factor binding sites for the regulation of flk-1 expression during murine vascular development. *Blood.* 2000;96(9):3078-3085.
 69. Becker PW, Sacilotto N, Nornes S, et al. An intronic Flk1 enhancer directs arterial-specific expression via RBPJ-mediated venous repression. *Arterioscler Thromb Vasc Biol.* 2016;36(6):1209-1219. doi:[10.1161/atvbaha.116.307517](https://doi.org/10.1161/atvbaha.116.307517)
 70. Chen L, Mupo A, Huynh T, et al. Tbx1 regulates Vegfr3 and is required for lymphatic vessel development. *J Cell Biol.* 2010;189(3):417-424. doi:[10.1083/jcb.200912037](https://doi.org/10.1083/jcb.200912037)
 71. Kobayashi-Osaki M, Ohneda O, Suzuki N, et al. GATA motifs regulate early hematopoietic lineage-specific expression of the Gata2 gene. *Mol Cell Biol.* 2005;25(16):7005-7020. doi:[10.1128/mcb.25.16.7005-7020.2005](https://doi.org/10.1128/mcb.25.16.7005-7020.2005)
 72. Johnson KD, Hsu AP, Ryu MJ, et al. Cis-element mutated in GATA2-dependent immunodeficiency governs hematopoiesis and vascular integrity. *J Clin Invest.* 2012;122(10):3692-3704. doi:[10.1172/jci61623](https://doi.org/10.1172/jci61623)

73. Khandekar M, Brandt W, Zhou Y, et al. A Gata2 intronic enhancer confers its pan-endothelia-specific regulation. *Development*. 2007;134(9):1703-1712. doi:10.1242/dev.001297
74. Dobrzycki T, Mahony CB, Krecsmarik M, et al. Deletion of a conserved Gata2 enhancer impairs haemogenic endothelium programming and adult Zebrafish haematopoiesis. *Commun Biol*. 2020;7:1-14. doi:10.1038/s42003-020-0798-3
75. Watanabe Y, Seya D, Ihara D, et al. Importance of endothelial Hey1 expression for thoracic great vessel development and its distal enhancer for Notch-dependent endothelial transcription. *J Biol Chem*. 2020;295(51):17632-17645. doi:10.1074/jbc.ra120.015003
76. De Val S, Anderson JP, Heidt AB, Khiem D, Xu SM, Black BL. Mef2c is activated directly by Ets transcription factors through an evolutionarily conserved endothelial cell-specific enhancer. *Dev Biol*. 2004;275(2):424-434. doi:10.1016/j.ydbio.2004.08.016
77. Teichert AM, Miller TL, Tai SC, et al. In vivo expression profile of an endothelial nitric oxide synthase promoter-reporter transgene. *Am J Physiol Heart Circ Physiol*. 2000;278(4):H1352-H1361. doi:10.1152/ajpheart.2000.278.4.h1352
78. Teichert AM, Scott JA, Robb GB, et al. Endothelial nitric oxide synthase gene expression during murine embryogenesis. *Circ Res*. 2008;103(1):24-33. doi:10.1161/circresaha.107.168567
79. Guillot PV, Liu L, Kuivenhoven JA, Guan J, Rosenberg RD, Aird WC. Targeting of human eNOS promoter to the Hprt locus of mice leads to tissue-restricted transgene expression. *Physiol Genom*. 2000;2(2):77-83. doi:10.1152/physiolgenomics.2000.2.2.77
80. SenBanerjee S, Lin Z, Atkins GB, et al. KLF2 is a novel transcriptional regulator of endothelial proinflammatory activation. *J Exp Med*. 2004;199(10):1305-1315. doi:10.1084/jem.20031132
81. Laumonier Y, Nadaud S, Agrapart M, Soubrier F. Characterization of an upstream enhancer region in the promoter of the human endothelial nitric-oxide synthase gene. *J Biol Chem*. 2000;275(52):40732-40741. doi:10.1074/jbc.m004696200
82. Chiang IKN, Fritzsche M, Pichol-Thievend C, et al. Correction: SoxF factors induce Notch1 expression via direct transcriptional regulation during early arterial development. *Development*. 2017;144(20):3847-3848. doi:10.1242/dev.159715
83. Wu J, Iwata F, Grass JA, et al. Molecular determinants of NOTCH4 transcription in vascular endothelium. *Mol Cell Biol*. 2005;25(4):1458-1474. doi:10.1128/mcb.25.4.1458-1474.2005
84. Mollica LR, Crawley JTB, Liu K, et al. Role of a 5'-enhancer in the transcriptional regulation of the human endothelial cell protein C receptor gene. *Blood*. 2006;108(4):1251-1259. doi:10.1182/blood-2006-02-001461
85. Kazenwadel J, Betterman KL, Chong CE, et al. GATA2 is required for lymphatic vessel valve development and maintenance. *J Clin Invest*. 2015;125(8):2979-2994. doi:10.1172/jci78888
86. Kazenwadel J, Venugopal P, Oszmiana A, et al. A Prox1 enhancer represses haematopoiesis in the lymphatic vasculature. *Nature*. 2023;614:1-6. doi:10.1038/s41586-022-05650-9
87. Andersson R, Gebhard C, Miguel-Escalada I, et al. An atlas of active enhancers across human cell types and tissues. *Nature*. 2014;507(7493):455-461. doi:10.1038/nature12787
88. Göttgens B, Nastos A, Kinston S, et al. Establishing the transcriptional programme for blood: the SCL stem cell enhancer is regulated by a multiprotein complex containing Ets and GATA factors. *EMBO J*. 2002;21(12):3039-3050. doi:10.1093/emboj/cdf286
89. Göttgens B, Broccardo C, Sanchez MJ, et al. The scl +18/19 stem cell enhancer is not required for hematopoiesis: identification of a 5' bifunctional hematopoietic-endothelial enhancer bound by Fli-1 and Elf-1. *Mol Cell Biol*. 2004;24(5):1870-1883. <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=14966269&retmode=ref&cmd=prlinks>
90. Iljin K, Dube A, Kontusaari S, et al. Role of ets factors in the activity and endothelial cell specificity of the mouse Tie gene promoter. *FASEB J*. 1999;13(2):377-386.
91. Korhonen J, Lahtinen I, Halmekytö M, et al. Endothelial-specific gene expression directed by the tie gene promoter in vivo. *Blood*. 1995;86(5):1828-1835.
92. Chen-Konak L, Guetta-Shubin Y, Yahav H, et al. Transcriptional and post-translation regulation of the Tie1 receptor by fluid shear stress changes in vascular endothelial cells. *FASEB J*. 2003;17(14):2121-2123. doi:10.1096/fj.02-1151fj
93. Dube A, Akbarali Y, Sato TN, Libermann TA, Oettgen P. Role of the Ets transcription factors in the regulation of the vascular-specific Tie2 gene. *Circ Res*. 1999;84(10):1177-1185.
94. Minami T, Kuivenhoven JA, Evans V, Kodama T, Rosenberg RD, Aird WC. Ets motifs are necessary for endothelial cell-specific expression of a 723-bp Tie-2 promoter/enhancer in Hprt targeted transgenic mice. *Arterioscler Thromb Vasc Biol*. 2003;23(11):2041-2047. doi:10.1161/01.atv.0000089326.63053.9a
95. Schlaeger TM, Bartunkova S, Lawitts JA, et al. Uniform vascular-endothelial-cell-specific gene expression in both embryonic and adult transgenic mice. *Proc Natl Acad Sci U S A*. 1997;94(7):3058-3063. <http://www.pnas.org/content/94/7/3058.long>
96. Fantin A, Ruhrberg C. The embryonic mouse hindbrain and postnatal retina as in vivo models to study angiogenesis. *Methods Mol Biol*. 2022;2475:275-287. doi:10.1007/978-1-0716-2217-9_20
97. Pitulescu ME, Schmidt I, Benedito R, Adams RH. Inducible gene targeting in the neonatal vasculature and analysis of retinal angiogenesis in mice. *Nat Protoc*. 2010;5(9):1518-1534. doi:10.1038/nprot.2010.113
98. Payne S, De Val S, Neal A. Endothelial-specific cre mouse models. *Arterioscler Thromb Vasc Biol*. 2018;38(11):2550-2561. doi:10.1161/atvbaha.118.309669
99. Brash JT, Bolton RL, Rashbrook VS, Denti L, Kubota Y, Ruhrberg C. Tamoxifen-activated CreERT impairs retinal angiogenesis independently of gene deletion. *Circ Res*. 2020;127(6):849-850. doi:10.1161/circresaha.120.317025
100. McCracken IR, Taylor RS, Kok FO, et al. Transcriptional dynamics of pluripotent stem cell-derived endothelial cell differentiation revealed by single-cell RNA sequencing. *Eur Heart J*. 2020;41:1024-1036. doi:10.1093/eurheartj/ehz351
101. Heiss M, Hellström M, Kalén M, et al. Endothelial cell spheroids as a versatile tool to study angiogenesis in vitro. *FASEB J*. 2015;29(7):3076-3084. doi:10.1096/fj.14-267633

102. Randi AM, Sperone A, Dryden NH, Birdsey GM. Regulation of angiogenesis by ETS transcription factors. *Biochem Soc Trans.* 2009;37(Pt 6):1248-1253. doi:10.1042/bst0371248
103. Rothenberg EV, Hosokawa H, Ungerback J. Mechanisms of action of hematopoietic transcription factor PU.1 in initiation of T-Cell development. *Front Immunol.* 2019;10:228. doi:10.3389/fimmu.2019.00228
104. Besnard A, Galan-Rodriguez B, Vanhoutte P, Caboche J. Elk-1 a transcription factor with multiple facets in the brain. *Front Neurosci.* 2011;5:35. doi:10.3389/fnins.2011.00035
105. Engelbrecht E, Levesque MV, He L, et al. Sphingosine 1-phosphate-regulated transcriptomes in heterogenous arterial and lymphatic endothelium of the aorta. *eLife Sci.* 2020;9:3665. doi:10.7554/elife.52690
106. Vanlandewijck M, He L, Mäe MA, et al. A molecular atlas of cell types and zonation in the brain vasculature. *Nature.* 2018;554(7693):475-480. doi:10.1038/nature25739
107. Lee D, Park C, Lee H, et al. ER71 acts downstream of BMP, Notch, and Wnt signaling in blood and vessel progenitor specification. *Cell Stem Cell.* 2008;2(5):497-507. doi:10.1016/j.stem.2008.03.008
108. Pijuan-Sala B, Wilson NK, Xia J, et al. Single-cell chromatin accessibility maps reveal regulatory programs driving early mouse organogenesis. *Nat Cell Biol.* 2020;22(4):487-497. doi:10.1038/s41556-020-0489-9
109. Pham VN, Lawson ND, Mugford JW, et al. Combinatorial function of ETS transcription factors in the developing vasculature. *Dev Biol.* 2007;303(2):772-783. doi:10.1016/j.ydbio.2006.10.030
110. Hayashi M, Pluchinotta M, Momiyama A, Tanaka Y, Nishikawa SI, Kataoka H. Endothelialization and altered hematopoiesis by persistent Etv2 expression in mice. *Exp Hematol.* 2012;40(9):738-750.e11. doi:10.1016/j.exphem.2012.05.012
111. Sumanas S, Joraniak T, Lin S. Identification of novel vascular endothelial-specific genes by the microarray analysis of the zebrafish cloche mutants. *Blood.* 2005;106(2):534-541. doi:10.1182/blood-2004-12-4653
112. Kataoka H, Hayashi M, Nakagawa R, et al. Etv2/ER71 induces vascular mesoderm from Flk1+PDGFR α + primitive mesoderm. *Blood.* 2011;118(26):6975-6986. doi:10.1182/blood-2011-05-352658
113. Wei G, Srinivasan R, Cantemir-Stone CZ, et al. Ets1 and Ets2 are required for endothelial cell survival during embryonic angiogenesis. *Blood.* 2009;114(5):1123-1130. doi:10.1182/blood-2009-03-211391
114. Liu F, Kang I, Park C, et al. ER71 specifies Flk-1+ hemangiogenic mesoderm by inhibiting cardiac mesoderm and Wnt signaling. *Blood.* 2012;119(14):3295-3305. doi:10.1182/blood-2012-01-403766
115. Kappel A, Röncke V, Damert A, Flamme I, Risau W, Breier G. Identification of vascular endothelial growth factor (VEGF) receptor-2 (Flk-1) promoter/enhancer sequences sufficient for angioblast and endothelial cell-specific transcription in transgenic mice. *Blood.* 1999;93(12):4284-4292.
116. Murakami M, Nguyen LT, Hatanaka K, et al. FGF-dependent regulation of VEGF receptor 2 expression in mice. *J Clin Invest.* 2011;121(7):2668-2678. doi:10.1172/jci44762
117. Rasmussen TL, Shi X, Wallis A, et al. VEGF/Flk1 signaling cascade transactivates Etv2 gene expression. *PLoS One.* 2012;7(11):e50103. doi:10.1371/journal.pone.0050103
118. Ginsberg M, James D, Ding BS, et al. Efficient direct reprogramming of mature amniotic cells into endothelial cells by ETS factors and TGF β suppression. *Cell.* 2012;151(3):559-575. doi:10.1016/j.cell.2012.09.032
119. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell.* 2010;141(7):1117-1134. doi:10.1016/j.cell.2010.06.011
120. Yordy JS, Muise-Helmericks RC. Signal transduction and the Ets family of transcription factors. *Oncogene.* 2000;19(55):6503-6513. doi:10.1038/sj.onc.1204036
121. Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res.* 2002;12(1):9-18. doi:10.1038/sj.cr.7290105
122. Palencia-Desai S, Kohli V, Kang J, Chi NC, Black BL, Sumanas S. Vascular endothelial and endocardial progenitors differentiate as cardiomyocytes in the absence of Etsrp/Etv2 function. *Development.* 2011;138(21):4721-4732. doi:10.1242/dev.064998
123. Behrens AN, Zierold C, Shi X, et al. Sox7 is regulated by ETV2 during cardiovascular development. *Stem Cells Dev.* 2014;23(17):2004-2013. doi:10.1089/scd.2013.0525
124. Sabbagh MF, Heng JS, Luo C, et al. Transcriptional and epigenomic landscapes of CNS and non-CNS vascular endothelial cells. *eLife Sci.* 2018;7. doi:10.7554/elife.36187
125. Zhang B, Zhou Y, Lin N, et al. Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. *Genome Res.* 2013;23(9):1522-1540. doi:10.1101/gr.156539.113
126. Tanaka T, Izawa K, Maniwa Y, et al. ETV2-TET1/TET2 complexes induce endothelial cell-specific Robo4 expression via promoter demethylation. *Sci Rep.* 2018;8(1):5653. doi:10.1038/s41598-018-23937-8
127. Spyropoulos DD, Pharr PN, Lavenburg KR, et al. Hemorrhage, impaired hematopoiesis, and lethality in mouse embryos carrying a targeted disruption of the Flil1 transcription factor. *Mol Cell Biol.* 2000;20(15):5643-5652.
128. Hart A, Melet F, Grossfeld P, et al. Flil-1 is required for murine vascular and megakaryocytic development and is hemizygotously deleted in patients with thrombocytopenia. *Immunity.* 2000;13(2):167-177. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WSP-417DGV3-2&_user=126524&_coverDate=08%2F01%2F2000&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_acct=C000010360&_version=1&_urlVersion=0&_userid=126524&md5=70fd6be43539ece4bcb6e072fe5d9a1d&searchtype=a
129. Asano Y, Stawski L, Hant F, et al. Endothelial Flil1 deficiency impairs vascular homeostasis A role in scleroderma vasculopathy. *Am J Pathol.* 2010;176(4):1983-1998. doi:10.2353/ajpath.2010.090593
130. Hollenhorst PC, McIntosh LP, Graves BJ. Genomic and biochemical insights into the specificity of ETS transcription factors. *Annu Rev Biochem.* 2011;80:437-471. doi:10.1146/annurev.biochem.79.081507.103945
131. Ben-David Y, Giddens EB, Letwin K, Bernstein A. Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the ets gene family, Flil-1, closely linked to c-ets-1. *Gene Dev.* 1991;5(6):908-918. doi:10.1101/gad.5.6.908

132. Gomez-Salinerio JM, Itkin T, Houghton S, et al. Cooperative ETS transcription factors enforce adult endothelial cell fate and cardiovascular homeostasis. *Nat Cardiovasc Res.* 2022; 1(10):882-899. doi:10.1038/s44161-022-00128-3
133. Vlaeminck-Guillem V, Carrere S, Dewitte F, Stehelin D, Desbiens X, Duterque-Coquillaud M. The Ets family member Erg gene is expressed in mesodermal tissues and neural crests at fundamental steps during mouse embryogenesis. *Mech Dev.* 2000;91(1-2):331-335. doi:10.1016/s0925-4773(99)00272-5
134. Yuan L, Nikolova-Krstevski V, Zhan Y, et al. Antiinflammatory effects of the ETS factor ERG in endothelial cells are mediated through transcriptional repression of the interleukin-8 gene. *Circ Res.* 2009;104(9):1049-1057. doi:10.1161/circresaha.108.190751
135. Hewett PW, Nishi K, Daft EL, Murray JC. Selective expression of erg isoforms in human endothelial cells. *Int J Biochem Cell Biol.* 2001;33(4):347-355. doi:10.1016/s1357-2725(01)00022-x
136. Shah AV, Birdsey GM, Randi AM. Regulation of endothelial homeostasis, vascular development and angiogenesis by the transcription factor ERG. *Vasc Pharmacol.* 2016;86:3-13. doi:10.1016/j.vph.2016.05.003
137. Birdsey GM, Shah AV, Dufton N, et al. The endothelial transcription factor ERG promotes vascular stability and growth through Wnt/ β -catenin signaling. *Dev Cell.* 2015;32(1):82-96. doi:10.1016/j.devcel.2014.11.016
138. Han R, Pacifici M, Iwamoto M, Trojanowska M. Endothelial Erg expression is required for embryogenesis and vascular integrity. *Organogenesis.* 2015;11(2):75-86. doi:10.1080/15476278.2015.1031435
139. Birdsey GM, Dryden NH, Shah AV, et al. The transcription factor Erg regulates expression of histone deacetylase 6 and multiple pathways involved in endothelial cell migration and angiogenesis. *Blood.* 2012;119(3):894-903. doi:10.1182/blood-2011-04-350025
140. Birdsey GM, Dryden NH, Amsellem V, et al. Transcription factor Erg regulates angiogenesis and endothelial apoptosis through VE-cadherin. *Blood.* 2008;111(7):3498-3506. doi:10.1182/blood-2007-08-105346
141. Kola I, Brookes S, Green AR, et al. The Ets1 transcription factor is widely expressed during murine embryo development and is associated with mesodermal cells involved in morphogenetic processes such as organ formation. *Proc Natl Acad Sci U S A.* 1993;90(16):7588-7592. doi:10.1073/pnas.90.16.7588
142. Maroulakou IG, Papas TS, Green JE. Differential expression of ets-1 and ets-2 proto-oncogenes during murine embryogenesis. *Oncogene.* 1994;9(6):1551-1565.
143. Vandenbunder B, Wernert N, Stehelin D. Does oncogene c-ets 1 participate in the regulation of tumor angiogenesis? *Bull Cancer.* 1993;80(1):38-49.
144. Chen Z, Fisher RJ, Riggs CW, Rhim JS, Lautenberger JA. Inhibition of vascular endothelial growth factor-induced endothelial cell migration by ETS1 antisense oligonucleotides. *Cancer Res.* 1997;57(10):2013-2019.
145. Watanabe D, Takagi H, Suzuma K, et al. Transcription factor Ets-1 mediates ischemia- and vascular endothelial growth factor-dependent retinal neovascularization. *Am J Pathol.* 2004;164(5):1827-1835. doi:10.1016/s0002-9440(10)63741-8
146. Oikawa M, Abe M, Kurosawa H, Hida W, Shirato K, Sato Y. Hypoxia induces transcription factor ETS-1 via the activity of hypoxia-inducible factor-1. *Biochem Bioph Res Commun.* 2001; 289(1):39-43. doi:10.1006/bbrc.2001.5927
147. Schwachtgen JL, Janel N, Barek L, et al. Ets transcription factors bind and transactivate the core promoter of the von Willibrand factor gene. *Oncogene.* 1997;15(25):3091-3102. doi:10.1038/sj.onc.1201502
148. Lelièvre E, Mattot V, Huber P, Vandenbunder B, Soncin F. ETS1 lowers capillary endothelial cell density at confluence and induces the expression of VE-cadherin. *Oncogene.* 2000; 19(20):2438-2446. doi:10.1038/sj.onc.1203563
149. Hasegawa Y, Abe M, Yamazaki T, et al. Transcriptional regulation of human angiopoietin-2 by transcription factor Ets-1. *Biochem Bioph Res Commun.* 2004;316(1):52-58. doi:10.1016/j.bbrc.2004.02.019
150. Elvert G. Cooperative interaction of hypoxia-inducible factor-2alpha (HIF-2alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). *J Biol Chem.* 2002;278(9):7520-7530. doi:10.1074/jbc.m211298200
151. Zhang B, Day DS, Ho JW, et al. A dynamic H3K27ac signature identifies VEGFA-stimulated endothelial enhancers and requires EP300 activity. *Genome Res.* 2013;23(6):917-927. doi:10.1101/gr.149674.112
152. Oda N, Abe M, Sato Y. ETS-1 converts endothelial cells to the angiogenic phenotype by inducing the expression of matrix metalloproteinases and integrin β 3. *J Cell Physiol.* 1999;178(2): 121-132. doi:10.1002/(sici)1097-4652(199902)178:2<121::aid-jcp1>3.0.co;2-f
153. Ayadi A, Zheng H, Sobieszczuk P, et al. Net-targeted mutant mice develop a vascular phenotype and up-regulate egr-1. *EMBO J.* 2001;20(18):5139-5152. doi:10.1093/emboj/20.18.5139
154. Ayadi A, Suelves M, Dollé P, Wasylyk B. Net, an Ets ternary complex transcription factor, is expressed in sites of vasculogenesis, angiogenesis, and chondrogenesis during mouse development. *Mech Dev.* 2001;102(1-2):205-208. doi:10.1016/s0925-4773(01)00289-1
155. Zheng H, Wasylyk C, Ayadi A, et al. The transcription factor Net regulates the angiogenic switch. *Genes Dev.* 2003;17(18): 2283-2297. doi:10.1101/gad.272503
156. Gross C, Dubois-Pot H, Wasylyk B. The ternary complex factor Net/Elk-3 participates in the transcriptional response to hypoxia and regulates HIF-1 alpha. *Oncogene.* 2008;27(9): 1333-1341. doi:10.1038/sj.onc.1210736
157. Weigl C, Wasylyk C, Garrido MG, et al. Elk3 deficiency causes transient impairment in post-natal retinal vascular development and formation of tortuous arteries in adult murine retinae. *PLoS One.* 2014;9(9):e107048. doi:10.1371/journal.pone.0107048
158. Franco CA, Mericskay M, Parlakian A, et al. Serum response factor is required for sprouting angiogenesis and vascular integrity. *Dev Cell.* 2008;15(3):448-461. doi:10.1016/j.devcel.2008.07.019
159. Franco CA, Blanc J, Parlakian A, et al. SRF selectively controls tip cell invasive behavior in angiogenesis. *Development.* 2013;140(11):2321-2333. doi:10.1242/dev.091074
160. Weigl C, Riehle H, Park D, et al. Endothelial SRF/MRTF ablation causes vascular disease phenotypes in murine retinae. *J Clin Invest.* 2013;123(5):2193-2206. doi:10.1172/jci64201

161. Golson ML, Kaestner KH. Fox transcription factors: from development to disease. *Development*. 2016;143(24):4558-4570. doi:[10.1242/dev.112672](https://doi.org/10.1242/dev.112672)
162. Kume T, Jiang H, Topczewska JM, Hogan BL. The murine winged helix transcription factors, Foxc1 and Foxc2, are both required for cardiovascular development and somitogenesis. *Genes Dev*. 2001;15(18):2470-2482. doi:[10.1101/gad.907301](https://doi.org/10.1101/gad.907301)
163. Seo S, Fujita H, Nakano A, Kang M, Duarte A, Kume T. The forkhead transcription factors, Foxc1 and Foxc2, are required for arterial specification and lymphatic sprouting during vascular development. *Dev Biol*. 2006;294(2):458-470. doi:[10.1016/j.ydbio.2006.03.035](https://doi.org/10.1016/j.ydbio.2006.03.035)
164. Hayashi H, Kume T. Forkhead transcription factors regulate expression of the chemokine receptor CXCR4 in endothelial cells and CXCL12-induced cell migration. *Biochem Biophys Res Commun*. 2008;367(3):584-589. doi:[10.1016/j.bbrc.2007.12.183](https://doi.org/10.1016/j.bbrc.2007.12.183)
165. Hayashi H, Kume T. Foxc transcription factors directly regulate Dll4 and Hey2 expression by interacting with the VEGF-Notch signaling pathways in endothelial cells. *PLoS One*. 2008;3(6):e2401. doi:[10.1371/journal.pone.0002401](https://doi.org/10.1371/journal.pone.0002401)
166. Xia S, Menden HL, Korfhagen TR, Kume T, Sampath V. Endothelial immune activation programmes cell-fate decisions and angiogenesis by inducing angiogenesis regulator DLL4 through TLR4-ERK-FOXC2 signalling. *J Physiol*. 2018;596(8):1397-1417. doi:[10.1113/jp275453](https://doi.org/10.1113/jp275453)
167. Hayashi H, Kume T. Foxc2 transcription factor as a regulator of angiogenesis via induction of integrin β 3 expression. *Cell Adhes Migr*. 2009;3(1):24-26. doi:[10.4161/cam.3.1.7252](https://doi.org/10.4161/cam.3.1.7252)
168. Fatima A, Wang Y, Uchida Y, et al. Foxc1 and Foxc2 deletion causes abnormal lymphangiogenesis and correlates with ERK hyperactivation. *J Clin Invest*. 2016;126(7):2437-2451. doi:[10.1172/jci80465](https://doi.org/10.1172/jci80465)
169. Graupera M, Potente M. Regulation of angiogenesis by PI3K signaling networks. *Exp Cell Res*. 2013;319(9):1348-1355. doi:[10.1016/j.yexcr.2013.02.021](https://doi.org/10.1016/j.yexcr.2013.02.021)
170. Link W. Introduction to FOXO biology. *Methods Mol Biol*. 2018;1890:1-9. doi:[10.1007/978-1-4939-8900-3_1](https://doi.org/10.1007/978-1-4939-8900-3_1)
171. Oellerich MF, Potente M. FOXOs and sirtuins in vascular growth, maintenance, and aging. *Circ Res*. 2012;110(9):1238-1251. doi:[10.1161/circresaha.111.246488](https://doi.org/10.1161/circresaha.111.246488)
172. Hosaka T, Biggs WH, Tieu D, et al. Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. *Proc Natl Acad Sci U S A*. 2004;101(9):2975-2980. doi:[10.1073/pnas.0400093101](https://doi.org/10.1073/pnas.0400093101)
173. Furuyama T, Kitayama K, Shimoda Y, et al. Abnormal angiogenesis in Foxo1 (Fkhr)-deficient mice. *J Biol Chem*. 2004;279(33):34741-34749. doi:[10.1074/jbc.m314214200](https://doi.org/10.1074/jbc.m314214200)
174. Wilhelm K, Happel K, Eelen G, et al. FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature*. 2016;529(7585):216-220. doi:[10.1038/nature16498](https://doi.org/10.1038/nature16498)
175. Potente M, Urbich C, Sasaki K, et al. Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization. *J Clin Invest* 2005;115(9):2382-2392. doi:[10.1172/jci23126](https://doi.org/10.1172/jci23126)
176. Tzivion G, Dobson M, Ramakrishnan G. FoxO transcription factors; regulation by AKT and 14-3-3 proteins. *Biochim Biophys Acta Mol Cell Res*. 2011;1813(11):1938-1945. doi:[10.1016/j.bbamcr.2011.06.002](https://doi.org/10.1016/j.bbamcr.2011.06.002)
177. Zhao YY, Gao XP, Zhao YD, et al. Endothelial cell-restricted disruption of FoxM1 impairs endothelial repair following LPS-induced vascular injury. *J Clin Invest*. 2006;116(9):2333-2343. doi:[10.1172/jci27154](https://doi.org/10.1172/jci27154)
178. Huang X, Zhao YY. Transgenic expression of FoxM1 promotes endothelial repair following lung injury induced by polymicrobial sepsis in mice. *PLoS One*. 2012;7(11):e50094. doi:[10.1371/journal.pone.0050094](https://doi.org/10.1371/journal.pone.0050094)
179. Grundmann S, Lindmayer C, Hans FP, et al. FoxP1 stimulates angiogenesis by repressing the inhibitory guidance protein semaphorin 5B in endothelial cells. *PLoS One*. 2013;8(9):e70873. doi:[10.1371/journal.pone.0070873](https://doi.org/10.1371/journal.pone.0070873)
180. Wang B, Weidenfeld J, Lu MM, et al. Foxp1 regulates cardiac outflow tract, endocardial cushion morphogenesis and myocyte proliferation and maturation. *Development*. 2004;131(18):4477-4487. doi:[10.1242/dev.01287](https://doi.org/10.1242/dev.01287)
181. Liu J, Zhuang T, Pi J, et al. Endothelial forkhead box transcription factor P1 regulates pathological cardiac remodeling through transforming growth factor- β 1-endothelin-1 signal pathway. *Circulation*. 2019;140(8):665-680. doi:[10.1161/circulationaha.119.039767](https://doi.org/10.1161/circulationaha.119.039767)
182. Lentjes MH, Niessen HE, Akiyama Y, de Bruïne AP, Melotte V, van Engeland M. The emerging role of GATA transcription factors in development and disease. *Expert Rev Mol Med*. 2016;18:e3. doi:[10.1017/erm.2016.2](https://doi.org/10.1017/erm.2016.2)
183. Katsumura KR, Bresnick EH, Group the GFM. The GATA factor revolution in hematology. *Blood*. 2017;129(15):2092-2102. doi:[10.1182/blood-2016-09-687871](https://doi.org/10.1182/blood-2016-09-687871)
184. Shi X, Richard J, Zirbes KM, et al. Cooperative interaction of Etv2 and Gata2 regulates the development of endothelial and hematopoietic lineages. *Dev Biol*. 2014;389(2):208-218. doi:[10.1016/j.ydbio.2014.02.018](https://doi.org/10.1016/j.ydbio.2014.02.018)
185. Ishitobi H, Wakamatsu A, Liu F, et al. Molecular basis for Flk1 expression in hemato-cardiovascular progenitors in the mouse. *Development*. 2011;138(24):5357-5368. doi:[10.1242/dev.065565](https://doi.org/10.1242/dev.065565)
186. Mammoto A, Connor KM, Mammoto T, et al. A mechanosensitive transcriptional mechanism that controls angiogenesis. *Nature*. 2009;457(7233):1103-1108. doi:[10.1038/nature07765](https://doi.org/10.1038/nature07765)
187. Kanki Y, Kohro T, Jiang S, et al. Epigenetically coordinated GATA2 binding is necessary for endothelium-specific endomucin expression. *EMBO J*. 2011;30(13):2582-2595. doi:[10.1038/emboj.2011.173](https://doi.org/10.1038/emboj.2011.173)
188. Tsai FY, Keller G, Kuo FC, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature*. 1994;371(6494):221-226. doi:[10.1038/371221a0](https://doi.org/10.1038/371221a0)
189. Bates DL, Chen Y, Kim G, Guo L, Chen L. Crystal structures of multiple GATA zinc fingers bound to DNA reveal new insights into DNA recognition and self-association by GATA. *J Mol Biol*. 2008;381(5):1292-1306. doi:[10.1016/j.jmb.2008.06.072](https://doi.org/10.1016/j.jmb.2008.06.072)
190. Lim KC, Hosoya T, Brandt W, et al. Conditional *Gata2* inactivation results in HSC loss and lymphatic mispatterning. *J Clin Invest*. 2012;122(10):3705-3717. doi:[10.1172/jci61619](https://doi.org/10.1172/jci61619)
191. Song H, Suehiro J, Kanki Y, et al. A critical role for GATA3 in mediating Tie2 expression and function in large vessel endothelial cells. *J Biol Chem*. 2009;284(29):29109-29124. doi:[10.1074/jbc.m109.041145](https://doi.org/10.1074/jbc.m109.041145)

192. Porcher C, Chagraoui H, Kristiansen MS. SCL/TAL1: a multifaceted regulator from blood development to disease. *Blood*. 2017;129(15):2051-2060. doi:10.1182/blood-2016-12-754051
193. Wu W, Morrissey CS, Keller CA, et al. Dynamic shifts in occupancy by TAL1 are guided by GATA factors and drive large-scale reprogramming of gene expression during hematopoiesis. *Genome Res*. 2014;24(12):1945-1962. doi:10.1101/gr.164830.113
194. Fujiwara T, O'Geen H, Keles S, et al. Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy. *Mol Cell*. 2009;36(4):667-681. doi:10.1016/j.molcel.2009.11.001
195. Shivdasani RA, Mayer EL, Orkin SH. Absence of blood formation in mice lacking the T-cell leukaemia oncogene tal-1/SCL. *Nature*. 1995;373(6513):432-434. doi:10.1038/373432a0
196. Robb L, Lyons I, Li R, et al. Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. *Proc Natl Acad Sci U S A*. 1995;92(15):7075-7079. <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7624372&retmode=ref&cmd=prlinks>
197. Schlaeger TM, Mikkola HKA, Gekas C, Helgadottir HB, Orkin SH. Tie2Cre-mediated gene ablation defines the stem-cell leukemia gene (SCL/tal1)-dependent window during hematopoietic stem-cell development. *Blood*. 2005;105(10):3871-3874. doi:10.1182/blood-2004-11-4467
198. Handel BV, Montel-Hagen A, Sasidharan R, et al. Scl represses cardiomyogenesis in prospective hemogenic endothelium and endocardium. *Cell*. 2012;150(3):590-605. doi:10.1016/j.cell.2012.06.026
199. Wu Y, Hirschi KK. Regulation of hemogenic endothelial cell development and function. *Annu Rev Physiol*. 2020;83(1):1-21. doi:10.1146/annurev-physiol-021119-034352
200. Org T, Duan D, Ferrari R, et al. Scl binds to primed enhancers in mesoderm to regulate hematopoietic and cardiac fate divergence. *EMBO J*. 2015;34(6):759-777. doi:10.15252/embj.201490542
201. Hill CS. Transcriptional control by the SMADs. *Cold Spring Harb Perspect Biol*. 2016;8(10):a022079. doi:10.1101/cshperspect.a022079
202. Kulikauskas MR, X S, Bautch VL. The versatility and paradox of BMP signaling in endothelial cell behaviors and blood vessel function. *Cell Mol Life Sci*. 2022;79(2):77. doi:10.1007/s00018-021-04033-z
203. Goumans MJ, ten Dijke P. TGF- β signaling in control of cardiovascular function. *Cold Spring Harb Perspect Biol*. 2018;10(2):a022210. doi:10.1101/cshperspect.a022210
204. Moya IM, Umans L, Maas E, et al. Stalk cell phenotype depends on integration of Notch and Smad1/5 signaling cascades. *Dev Cell*. 2012;22(3):501-514. doi:10.1016/j.devcel.2012.01.007
205. Lan Y, Liu B, Yao H, et al. Essential role of endothelial Smad4 in vascular remodeling and integrity. *Mol Cell Biol*. 2007;27(21):7683-7692. doi:10.1128/mcb.00577-07
206. Mouillessaux KP, Wiley DS, Saunders LM, et al. Notch regulates BMP responsiveness and lateral branching in vessel networks via SMAD6. *Nat Commun*. 2016;11:13247. <http://www.nature.com/articles/ncomms13247>
207. Ola R, Künzel SH, Zhang F, et al. SMAD4 prevents flow induced arteriovenous malformations by inhibiting casein kinase 2. *Circulation*. 2018;138(21):2379-2394. doi:10.1161/circulationaha.118.033842
208. Crist AM, Zhou X, Garai J, et al. Angiotensin-2 inhibition rescues arteriovenous malformation in a Smad4 hereditary hemorrhagic telangiectasia mouse model. *Circulation*. 2019;139(17):2049-2063. doi:10.1161/circulationaha.118.036952
209. Crist AM, Lee AR, Patel NR, Westhoff DE, Meadows SM. Vascular deficiency of Smad4 causes arteriovenous malformations: a mouse model of hereditary hemorrhagic telangiectasia. *Angiogenesis*. 2018;21(2):363-380. doi:10.1007/s10456-018-9602-0
210. Semenza GL. Hypoxia-inducible factor 1 and cardiovascular disease. *Physiology*. 2014;76(1):39-56. doi:10.1146/annurev-physiol-021113-170322
211. Dengler VL, Galbraith MD, Espinosa JM. Transcriptional regulation by hypoxia inducible factors. *Crit Rev Biochem Mol*. 2013;49(1):1-15. doi:10.3109/10409238.2013.838205
212. Zimna A, Kurpisz M. Hypoxia-inducible factor-1 in physiological and pathophysiological angiogenesis: applications and therapies. *BioMed Res Int*. 2015;2015(4):1-13. doi:10.3390/cancers3033610
213. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med*. 2003;9(6):677-684. doi:10.1038/nm0603-677
214. Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev*. 1997;11(1):72-82.
215. Wiesener MS. Widespread, hypoxia-inducible expression of HIF-2 α in distinct cell populations of different organs. *FASEB J*. 2002;17:271-273. doi:10.1096/fj.02-0445fje
216. Downes NL, Laham-Karam N, Kaikkonen MU, Ylä-Herttua S. Differential but complementary HIF1 α and HIF2 α transcriptional regulation. *Mol Ther*. 2018;26(7):1735-1745. doi:10.1016/j.ymthe.2018.05.004
217. Kelly BD, Hackett SF, Hirota K, et al. Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. *Circ Res J Am Heart Assoc*. 2003;93(11):1074-1081. doi:10.1161/01.res.0000102937.50486.1b
218. Yamakawa M, Liu LX, Date T, et al. Hypoxia-inducible factor-1 mediates activation of cultured vascular endothelial cells by inducing multiple angiogenic factors. *Circ Res J Am Heart Assoc*. 2003;93(7):664-673. doi:10.1161/01.res.0000093984.48643.d7
219. Manalo DJ, Rowan A, Lavoie T, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood*. 2005;105(2):659-669. doi:10.1182/blood-2004-07-2958
220. Takeda N, Maemura K, Imai Y, et al. Endothelial PAS domain protein 1 gene promotes angiogenesis through the transactivation of both vascular endothelial growth factor and its receptor, Flt-1. *Circ Res*. 2004;95(2):146-153. doi:10.1161/01.res.0000134920.10128.b4
221. Licht AH. Inhibition of hypoxia-inducible factor activity in endothelial cells disrupts embryonic cardiovascular development. *Blood*. 2006;107(2):584-590. doi:10.1182/blood-2005-07-3033
222. Duan LJ, Zhang-Benoit Y, Fong GH. Endothelium-intrinsic requirement for Hif-2 α during vascular development.

- Circulation*. 2005;111(17):2227-2232. doi:10.1161/01.cir.0000163580.98098.a3
223. Kapitsinou PP, Sano H, Michael M, et al. Endothelial HIF-2 mediates protection and recovery from ischemic kidney injury. *J Clin Invest*. 2014;124(6):2396-2409. doi:10.1172/jci69073
 224. Skuli N, Liu L, Runge A, et al. Endothelial deletion of hypoxia-inducible factor-2alpha (HIF-2alpha) alters vascular function and tumor angiogenesis. *Blood*. 2009;114(2):469-477. doi:10.1182/blood-2008-12-193581
 225. Alva JA, Zovein AC, Monvoisin A, et al. VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. *Dev Dyn*. 2006;235(3):759-767. doi:10.1002/dvdy.20643
 226. Iyer NV, Kotch LE, Agani F, et al. Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1alpha. *Genes Dev*. 1998;12(2):149-162. doi:10.1101/gad.12.2.149
 227. Forsythe JA, Jiang BH, Iyer NV, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol*. 1996;16(9):4604-4613. doi:10.1128/mcb.16.9.4604
 228. Faló-Sanjuan J, Bray SJ. Decoding the Notch signal. *Dev Growth Differ*. 2020;62(1):4-14. doi:10.1111/dgd.12644
 229. Gaudio FD, Liu D, Lendahl U. Notch signalling in healthy and diseased vasculature. *Open Biol*. 2022;12(4):220004. doi:10.1098/rsob.220004
 230. Phng LK, Gerhardt H. Angiogenesis: a team effort coordinated by notch. *Dev Cell*. 2009;16(2):196-208. doi:10.1016/j.devcel.2009.01.015
 231. Krebs LT, Xue Y, Norton CR, et al. Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev*. 2000;14(11):1343-1352. <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10837027&retmode=ref&cmd=prlinks>
 232. Krebs LT, Shutter JR, Tanigaki K, Honjo T, Stark KL, Gridley T. Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. *Genes Dev*. 2004;18(20):2469-2473. doi:10.1101/gad.1239204
 233. Duarte A, Hirashima M, Benedito R, et al. Dosage-sensitive requirement for mouse Dll4 in artery development. *Genes Dev*. 2004;18(20):2474-2478. doi:10.1101/gad.1239004
 234. Nielsen CM, Cuervo H, Ding VW, Kong Y, Huang EJ, Wang RA. Deletion of Rbpj from postnatal endothelium leads to abnormal arteriovenous shunting in mice. *Development*. 2014;141(19):3782-3792. doi:10.1242/dev.108951
 235. Yamamizu K, Matsunaga T, Uosaki H, et al. Convergence of Notch and β -catenin signaling induces arterial fate in vascular progenitors. *J Cell Biol*. 2010;189(2):325-338. doi:10.1083/jcb.200904114
 236. Ehling M, Adams S, Benedito R, Adams RH. Notch controls retinal blood vessel maturation and quiescence. *Development*. 2013;140(14):3051-3061. doi:10.1242/dev.093351
 237. Luo W, Garcia-Gonzalez I, Fernández-Chacón M, et al. Arterialization requires the timely suppression of cell growth. *Nature*. 2021;589:1-29. doi:10.1038/s41586-020-3018-x
 238. Weber D, Wiese C, Gessler M. Hey bHLH transcription factors. *Curr Top Dev Biol*. 2014;110:285-315. doi:10.1016/b978-0-12-405943-6.00008-7
 239. Zhong TP, Rosenberg M, Mohideen MAPK, Weinstein B, Fishman MC. gridlock, an HLH gene required for assembly of the aorta in zebrafish. *Science*. 2000;287(5459):1820-1824. doi:10.1126/science.287.5459.1820
 240. Donovan J, Kordylewska A, Jan YN, Utset MF. Tetralogy of fallot and other congenital heart defects in Hey2 mutant mice. *Curr Biol*. 2002;12(18):1605-1610. doi:10.1016/s0960-9822(02)01149-1
 241. Fujita M, Sakabe M, Ioka T, et al. Pharyngeal arch artery defects and lethal malformations of the aortic arch and its branches in mice deficient for the Hrt1/Hey1 transcription factor. *Mech Dev*. 2016;139:65-73. doi:10.1016/j.mod.2015.11.002
 242. Fischer A, Schumacher N, Maier M, Sendtner M, Gessler M. The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. *Genes Dev*. 2004;18(8):901-911. doi:10.1101/gad.291004
 243. Kokubo H, Miyagawa-Tomita S, Nakazawa M, Saga Y, Johnson RL. Mouse hesr1 and hesr2 genes are redundantly required to mediate Notch signaling in the developing cardiovascular system. *Dev Biol*. 2005;278(2):301-309. doi:10.1016/j.ydbio.2004.10.025
 244. Morioka T, Sakabe M, Ioka T, et al. An important role of endothelial hairy-related transcription factors in mouse vascular development. *Genesis*. 2014;52(11):897-906. doi:10.1002/dvg.22825
 245. Herbert SP, Cheung JYM, Stainier D.Y.R. Determination of endothelial stalk versus tip cell potential during angiogenesis by H2.0-like Homeobox-1. *Curr Biol*. 2012;22(19):1789-1794. doi:10.1016/j.cub.2012.07.037
 246. Testori J, Schweighofer B, Helfrich I, et al. The VEGF-regulated transcription factor HLX controls the expression of guidance cues and negatively regulates sprouting of endothelial cells. *Blood*. 2011;117(9):2735-2744. doi:10.1182/blood-2010-07-293209
 247. Prahst C, Kasaii B, Moraes F, et al. The H2.0-like homeobox transcription factor modulates yolk sac vascular remodeling in mouse embryos. *Arterioscler Thromb Vasc Biol*. 2018;34(7):1468-1476. doi:10.1161/atvbaha.114.303626
 248. Potthoff MJ, Olson EN. MEF2: a central regulator of diverse developmental programs. *Development*. 2007;134(23):4131-4140. doi:10.1242/dev.008367
 249. Lin Q, Lu J, Yanagisawa H, et al. Requirement of the MADS-box transcription factor MEF2C for vascular development. *Development*. 1998;125(22):4565-4574. <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9778514&retmode=ref&cmd=prlinks>
 250. Bi W, Drake CJ, Schwarz JJ. The transcription factor MEF2C-null mouse exhibits complex vascular malformations and reduced cardiac expression of angiotensin 1 and VEGF. *Dev Biol*. 1999;211(2):255-267. doi:10.1006/dbio.1999.9307
 251. Xu Z, Gong J, Maiti D, et al. MEF2C ablation in endothelial cells reduces retinal vessel loss and suppresses pathologic retinal neovascularization in oxygen-induced retinopathy. *Am J Pathol*. 2012;180(6):2548-2560. doi:10.1016/j.ajpath.2012.02.021
 252. Materna SC, Sinha T, Barnes RM, van Bueren KL, Black BL. Cardiovascular development and survival require Mef2c

- function in the myocardial but not the endothelial lineage. *Dev Biol.* 2019;445(2):170-177. doi:10.1016/j.ydbio.2018.12.002
253. Liu N, Nelson BR, Bezprozvannaya S, et al. Requirement of MEF2A, C, and D for skeletal muscle regeneration. *Proc Natl Acad Sci U S A.* 2014;111(11):4109-4114. doi:10.1073/pnas.1401732111
 254. Lu YW, Martino N, Gerlach BD, et al. MEF2 (myocyte enhancer factor 2) is essential for endothelial homeostasis and the athero-protective gene expression program. *Arterioscler Thromb Vasc Biol.* 2021;41(3):1105-1123. doi:10.1161/atvbaha.120.314978
 255. Kumar A, Lin Z, SenBanerjee S, Jain MK. Tumor necrosis factor alpha-mediated reduction of KLF2 is due to inhibition of MEF2 by NF- κ B and histone deacetylases. *Mol Cell Biol.* 2005;25(14):5893-5903. doi:10.1128/mcb.25.14.5893-5903.2005
 256. Sohn SJ, Li D, Lee LK, Winoto A. Transcriptional regulation of tissue-specific genes by the ERK5 mitogen-activated protein kinase. *Mol Cell Biol.* 2005;25(19):8553-8566. doi:10.1128/mcb.25.19.8553-8566.2005
 257. Chang S, Young BD, Li S, Qi X, Richardson JA, Olson EN. Histone deacetylase 7 maintains vascular integrity by repressing matrix metalloproteinase 10. *Cell.* 2006;126(2):321-334. doi:10.1016/j.cell.2006.05.040
 258. Bejjani F, Evanno E, Zibara K, Piechaczyk M, Jariel-Encontre I. The AP-1 transcriptional complex: local switch or remote command? *Biochim Biophys Acta.* 2019;1872(1):11-23. doi:10.1016/j.bbcan.2019.04.003
 259. Yanagida K, Engelbrecht E, Niaudet C, et al. Sphingosine 1-phosphate receptor signaling establishes AP-1 gradients to allow for retinal endothelial cell specialization. *Dev Cell.* 2020;52(6):779-793.e7. doi:10.1016/j.devcel.2020.01.016
 260. Strassheim D, Karoor V, Nijmeh H, et al. c-Jun, Foxo3a, and c-Myc transcription factors are key regulators of ATP-mediated angiogenic responses in pulmonary artery vasa vasorum endothelial cells. *Cells.* 2020;9(2):416. doi:10.3390/cells9020416
 261. Jeong HW, Hernández-Rodríguez B, Kim J, et al. Transcriptional regulation of endothelial cell behavior during sprouting angiogenesis. *Nat Commun.* 2017;8(1):726. doi:10.1038/s41467-017-00738-7
 262. Koltowska K, Paterson S, Bower NI, et al. mafba is a downstream transcriptional effector of Vegfc signaling essential for embryonic lymphangiogenesis in zebrafish. *Genes Dev.* 2015;29(15):1618-1630. <http://www.ncbi.nlm.nih.gov/pubmed/?term=26253536>
 263. Rondon-Galeano M, Skoczylas R, Bower NI, et al. MAFB modulates the maturation of lymphatic vascular networks in mice. *Dev Dyn.* 2020;249(10):1201-1216. doi:10.1002/dvdy.209
 264. Dieterich LC, Klein S, Mathelier A, et al. DeepCAGE transcriptomics reveal an important role of the transcription factor MAFB in the lymphatic endothelium. *Cell Rep.* 2015;13(7):1493-1504. doi:10.1016/j.celrep.2015.10.002
 265. Dieterich LC, Tacconi C, Menzi F, et al. Lymphatic MAFB regulates vascular patterning during developmental and pathological lymphangiogenesis. *Angiogenesis.* 2020;23(3):411-423. doi:10.1007/s10456-020-09721-1
 266. Zhou Y, Huang T, Cheng ASL, Yu J, Kang W, To KF. The TEAD family and its oncogenic role in promoting tumorigenesis. *Int J Mol Sci.* 2016;17(1):138. doi:10.3390/ijms17010138
 267. Varelas X. The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. *Development.* 2014;141(8):1614-1626. doi:10.1242/dev.102376
 268. Maugeri-Saccà M, Maria RD. The Hippo pathway in normal development and cancer. *Pharmacol Ther.* 2018;186:60-72. doi:10.1016/j.pharmthera.2017.12.011
 269. Kim J, Kim YH, Kim J, et al. YAP/TAZ regulates sprouting angiogenesis and vascular barrier maturation. *J Clin Invest.* 2017;127(9):3441-3461. doi:10.1172/jci93825
 270. Wang X, Valls AF, Schermann G, et al. YAP/TAZ orchestrate VEGF signaling during developmental angiogenesis. *Dev Cell.* 2017;42(5):462-478.e7. doi:10.1016/j.devcel.2017.08.002
 271. Ong YT, Andrade J, Armbruster M, et al. A YAP/TAZ-TEAD signalling module links endothelial nutrient acquisition to angiogenic growth. *Nat Metab.* 2022;4(6):672-682. doi:10.1038/s42255-022-00584-y
 272. Sakabe M, Fan J, Odaka Y, et al. YAP/TAZ-CDC42 signaling regulates vascular tip cell migration. *Proc Natl Acad Sci U S A.* 2017;114(41):10918-10923. doi:10.1073/pnas.1704030114
 273. Kim K, Kim IK, Yang JM, et al. SoxF transcription factors are positive feedback regulators of VEGF signaling novelty and significance. *Circ Res.* 2016;119(7):839-852. doi:10.1161/circresaha.116.308483
 274. Lilly AJ, Mazan A, Scott DA, Lacaud G, Kouskoff V. SOX7 expression is critically required in FLK1-expressing cells for vasculogenesis and angiogenesis during mouse embryonic development. *Mech Dev.* 2017;146:31-41. doi:10.1016/j.mod.2017.05.004
 275. Wareing S, Eliades A, Lacaud G, Kouskoff V. ETV2 expression marks blood and endothelium precursors, including hemogenic endothelium, at the onset of blood development. *Dev Dyn.* 2012;241(9):1454-1464. doi:10.1002/dvdy.23825
 276. Sakamoto Y, Hara K, Kanai-Azuma M, et al. Redundant roles of Sox17 and Sox18 in early cardiovascular development of mouse embryos. *Biochem Biophys Res Commun.* 2007;360(3):539-544. doi:10.1016/j.bbrc.2007.06.093
 277. Corada M, Orsenigo F, Morini MF, et al. Sox17 is indispensable for acquisition and maintenance of arterial identity. *Nat Commun.* 2013;4:2609. doi:10.1038/ncomms3609
 278. François M, Caprini A, Hosking B, et al. Sox18 induces development of the lymphatic vasculature in mice. *Nature.* 2008;456(7222):643-647. doi:10.1038/nature07391
 279. Lee SH, Lee S, Yang H, et al. Notch pathway targets proangiogenic regulator Sox17 to restrict angiogenesis. *Circ Res.* 2014;115(2):215-226. doi:10.1161/circresaha.115.303142
 280. Hosking B, François M, Wilhelm D, et al. Sox7 and Sox17 are strain-specific modifiers of the lymphangiogenic defects caused by Sox18 dysfunction in mice. *Development.* 2009;136(14):2385-2391. doi:10.1242/dev.034827
 281. Zhou Y, Williams J, Smallwood PM, Nathans J. Sox7, Sox17, and Sox18 cooperatively regulate vascular development in the mouse retina. *PLoS One.* 2015;10(12):e0143650. doi:10.1371/journal.pone.0143650
 282. Yang H, Lee S, Lee S, et al. Sox17 promotes tumor angiogenesis and destabilizes tumor vessels in mice. *J Clin Invest.* 2013;123(1):418-431. doi:10.1172/jci64547
 283. Chiang IKN, Fritzsche M, Pichol-Thievend C, et al. SoxF factors induce Notch1 expression via direct transcriptional regulation during early arterial development. *Development.* 2017;144(14):2629-2639. doi:10.1242/dev.146241
 284. Corada M, Orsenigo F, Bhat GP, et al. Fine-tuning of Sox17 and canonical Wnt coordinates the permeability properties of

- the blood-brain barrier. *Circ Res.* 2019;124(4):511-525. doi:[10.1161/circresaha.118.313316](https://doi.org/10.1161/circresaha.118.313316)
285. Schachterle W, Badwe CR, Palikuqi B, et al. Sox17 drives functional engraftment of endothelium converted from non-vascular cells. *Nat Commun.* 2017;8(1):13963-13912. doi:[10.1038/ncomms13963](https://doi.org/10.1038/ncomms13963)

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