From Department of Oncology-Pathology
Karolinska Institutet, Stockholm, Sweden

EXPLORING SIGNALING PATHWAYS IN ENDOTHELIAL MECHANOTRANSDUCTION

Yumeng Zhang

张雨濛

Karolinska Institutet

Stockholm 2024

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2024

ISBN 978-91-8017-235-6
Cover illustration: The Beauty of the Fractal Pattern.

Exploring Signaling Pathways in Endothelial Mechanotransduction

Thesis for Doctoral Degree (Ph.D.)

By

Yumeng Zhang

The thesis will be defended in public at J3:14 Kerstin Hagenfeldt, BioClinicum, Karolinska University Hospital, Solna, Sweden, Tuesday, 13th Febrary, 2024, at 9:30 a.m. CET.

Principal Supervisor:
Yuanyuan Zhang, MD, PhD
Karolinska Institutet
Department of Oncology-Pathology

Co-supervisor:
Professor Lars Holmgren, PhD
Karolinska Institutet
Department of Oncology-Pathology

Opponent:
Assoc. Prof. Stephan Huveneers, PhD
University of Amsterdam
Department of Medical Biochemistry

Examination Board:
Assoc. Prof. Lars Jakobsson, PhD
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Assoc. Prof. Hanna Björck, PhD
Karolinska Institutet
Department of Medicine, Solna

Assoc. Prof. Konstantin Gängel, PhD
Uppsala Universitet
Department of Immunology, Genetics and Pathology

To my family

致我的家人

Abstract

Mechanotransduction, the process by which cells sense and convert mechanical forces from their surroundings, plays a pivotal role in cellular function. For vascular endothelial cells, coping with the continuous and significant shear stress caused by blood flow is essential for the stability of the circulatory system. Dysfunction in this mechanism can lead to various vascular pathologies, including atherosclerosis and aneurysms. Therefore, understanding how endothelial cells form mechanosensory complexes and thereby respond to external forces is crucial.

This thesis aims to study the involvement of the Angiomotin protein family in endothelial mechanotransduction. The Amot protein family, including Angiomotin (Amot), Angiomotin-Like 1 (AmotL1), and Angiomotin-like 2 (AmotL2), shares common structures as well as protein interaction motifs. However, they exhibit significantly different roles in vascular functions. **In Paper I**, we elucidate how Amot binds to Talin within the integrin adhesome and regulates force transmission between fibronectin and the cytoskeleton in migrating endothelial cells. Additionally, we demonstrate that deletion of Amot impairs both physiological and pathological angiogenesis. **In Paper II**, AmotL2 is shown to bind VE-cadherin through p120 catenin and connect to the nuclear membrane via actin filaments in aortic endothelial cells, thereby transmitting junctional mechanical signals. Depletion of AmotL2 resulted in a pro-inflammatory response and, in severe cases, leads to the spontaneous formation abdominal aortic aneurysms (AAAs) in male adult mice. **In Paper III**, it is demonstrated that AmotL1 not only binds to N-cadherin but is also associated with focal adhesion proteins. This suggests that AmotL1 functions may extend beyond endothelial cell junctions to include interactions with the extracellular matrix. Additionally, we provide a comprehensive summary of the protein binding profiles of all Amot proteins, as obtained from BioID-MS analysis, thus offering a global perspective on this protein family.

In conclusion, Amot family proteins, despite their involvement in separate cellular processes, share common connections with a set of junction-related proteins. Furthermore, they exhibit unique, specific binding partners, offering mechanistic insights into the distinct activities of individual Amot proteins.

List of scientific papers

I. The Amot/integrin protein complex transmits mechanical forces required for vascular expansion

   Yuanyuan Zhang, **Yumeng Zhang**, Sumako Kameishi, Giuseppina Barutello, Yujuan Zheng, Nicholas P. Tobin, John Nicosia, Katharina Hennig, David Kung-Chun Chiu, Martial Balland, Thomas H. Barker, Federica Cavallo, Lars Holmgren#

   Cell reports, 2021, 36(8), 109616.

II. The VE-cadherin/AmotL2 mechanosensory pathway suppresses aortic inflammation and the formation of abdominal aortic aneurysms

   Yuanyuan Zhang, **Yumeng Zhang**, Evelyn Hutterer, Sara Hultin, Otto Bergman, Solrun Kolbeinsdottir, Hong Jin, Maria J. Forteza, Daniel F. J. Ketelhuth, Joy Roy, Ulf Hedin, Martin Enge, Ljubica Matic, Per Eriksson & Lars Holmgren#

   Nature Cardiovascular Research, 2023, 2, 629–644.

III. Mapping of the Angiomotin protein family Adhesome by BioID Analysis

   Yumeng Zhang, Lars Holmgren# and Yuanyuan Zhang#

   Manuscript

Other papers not included in this thesis

Deciphering the Role of p60AmotL2 in Epithelial Extrusion and Cell Detachment

Weiyingqi Cui, Aravindh Subramani, Pedro Fonseca, **Yumeng Zhang**, Le Tong, Yuanyuan Zhang, Lars Egevad, Andreas Lundqvist, and Lars Holmgren#

Cells. 2023; 12(17):2158

 # Corresponding authors

Contents

List of abbreviations

AAA	Abdominal Aortic Aneurysm
ABS	Actin–Binding Site
Amot	Angiomotin
AmotL1	Angiomotin-like 1
AmotL2	Angiomotin-like 2
BioID	Proximity-dependent biotin identification
CCL2/5	C-C motif chemokine ligand 2/5
CNS	Central Nervous System
Co-IP	Co-Immunoprecipitation
Crb3	Crumbs protein homolog 3
CVD	Cardiovascular disease
Cx	Connexins
CXCL10	C-X-C motif chemokine 10
DA	Descending Aorta
DHFR	Dihydrofolate Reductase
DN-KASH	Dominant Negative KASH
dyn/cm^2	dyne per square centimeter
EC	Endothelial Cell
ECM	Extracellular Matrix
EMT	Epithelial–Mesenchymal Transition
FAK	Focal Adhesion Kinase
F-actin	Filamentous-actin
Fn	Fibronectin
G-actin	Globular-actin
ICAM1	Intercellular Adhesion Molecule 1
ICM	inner cell mass
IHC	Immunohistochemistry

IVC	Inferior Vena Cava
KASH	Klarsicht, ANC-1, Syne Homology
KLF2	Krüppel-Like Factor 2
KLF4	Krüppel-Like Factor 4
LDL	Low-Density Lipoprotein
LINC	the Linker of Nucleoskeleton and Cytoskeleton
LLC	Lewis Lung Carcinoma
MMP	Matrix Metalloproteinase
MS	Mass Spectrometry
N-cadherin/CDH2	Neural cadherin
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
Par3	Partitioning Defective 3 homolog
PECAM1	Platelet Endothelial Cell Adhesion Molecule 1
PLA	Proximity Ligation Assay
PPE	Porcine Pancreatic Elastase
RNA-seq	RNA sequence
ROCK	Rho-associated protein kinase
SFs	Stress Fibers
TAA	Thoracic Aortic Aneurysm
TAZ	the Transcriptional coactivator with PDZ-binding motif
TFM	Traction Force Microscopy
THD	Talin Head Domain
VBS	Vinculin-Binding Site
Vcam1	Vascular cell adhesion protein 1
VE-cadherin/CDH5	Vascular Endothelial cadherin
VEGF	Vascular Endothelial Growth Factor
YAP	Yes-Associated Protein
ZO-1/2	Zonula occludens-1/2

1 Literature review

In living animals, the growth, movement, and response of cells and tissues are influenced by both chemical signals and the mechanical properties of their environment, such as stiffness and elasticity. Cells possess the ability to detect external mechanical forces, which they convert into biochemical and electrical signals. These signals play a critical role in regulating essential cellular activities, including proliferation, adhesion, and migration. Thus, mechanotransduction is of great importance in developmental and homeostatic processes. Additionally, it influences the onset and progression of diseases, including cancer, where it can impact tumor growth and metastasis, as well as fibrosis, involving the excessive formation of connective tissue. For example, alterations in the stiffness observed in cancerous tissues can influence the mechanical activation of biochemical pathways, thereby affecting epithelial–mesenchymal transition (EMT) [1]. During the initiation of fibrosis, the increasingly rigid fibrotic matrix signals fibroblasts to become more active, thus establishing feedback loops that enhance extracellular matrix (ECM) deposition and facilitate fibrotic tissue remodeling [2]. External mechanical stresses on the heart, such as increased pressure or volume overload, can trigger mechanotransduction pathways. This activation leads to significant changes in the cardiomyocytes, including alterations in their size, shape, and function [3].

1.1 Mechanotransduction in vascular biology

Living cells rely on a vascular system to facilitate blood circulation, transporting essential substances for their survival. Blood is pumped from the heart through arteries, delivering oxygen and nutrients via capillaries, and then is returned to the heart through veins. Endothelial cells (ECs) form a continuous layer lining blood vessels and are responsible for regulating the exchange between blood and surrounding cells. These cells are constantly exposed to shear stress from blood flow and pulsatile stretch from the rhythmic contractions of the heart [4].

1.1.1 Vascular development

The process of vascular development is a continuous and meticulously orchestrated phenomenon, influenced by interactions between cells and biophysical forces [5,6]. Understanding how ECs sense and respond to such mechanical cues is crucial for vascular expansion, maturation and the eventual formation of a functioning blood vessel network.

1.1.1.1 Vascular formation

As multicellular organisms grow, the formation of new blood vessels is triggered to support the tissue expansion. This occurs through two primary processes: vasculogenesis and angiogenesis. Vasculogenesis is characterized by the differentiation of angioblasts from mesodermal progenitor cells or angioblasts and the subsequent formation of primitive blood vessels originating at or near the site of their emergence [7] (**Figure 1A**). In contrast,

angiogenesis describes the process by which new blood vessels form from pre-existing ones [8]. Physiologically, angiogenesis occurs not only during embryonic development but also plays a role in the female reproductive cycle and wound healing processes in adults. Pathologically, angiogenesis is a critical hallmark of cancer progression, as both tumor growth and metastasis rely on vascular expansion.

The migration of ECs is a fundamental component of new blood vessel formation. This process is regulated by chemotaxis, directed by soluble chemical attractants; haptotaxis, guided by immobilized ligands; and mechanotaxis, driven by mechanical forces [9].

ECs establish a selective barrier between the bloodstream and surrounding tissue. In adulthood, these cells typically exist in a quiescent state, with little to no proliferation. However, upon receiving angiogenic signals, such as those from vascular endothelial growth factor (VEGF), a cascade of events is triggered, starting with the degradation of the basement membrane. This degradation enables a designated EC to become a tip cell and migrate towards the source of angiogenic cues. Behind the tip cell, proliferative stalk ECs extend, and through this coordinated process, a new blood vessel structure is assembled. Finally, supporting cells, such as pericytes attracted by cytokines secreted by ECs, stabilize and mature the newly formed capillaries (**Figure 1B**). During these processes, the migration of ECs involves a mechanical interaction with the ECM. The finger-like structures known as filopodia, rich in actin, extend from the tip cells and play a crucial role in guiding their migration and initiating new interactions with the ECM [10]. These filopodia are sensitive to both chemotactic factors, such as VEGF-A, and physical cues from the ECM, actively responding to these signals and facilitating the transmission of mechanical forces between the ECs and the ECM [11].

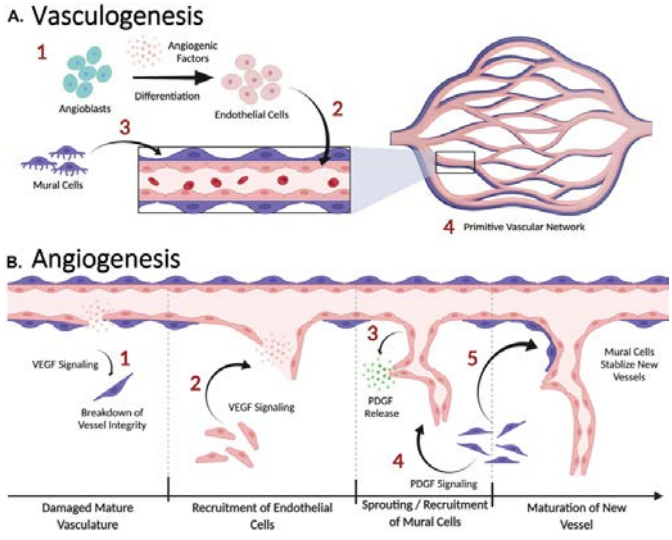

***Figure 1**: Schematics depicting the processes of vasculogenesis (**A**) and angiogenesis (**B**) (adapted from Nelson, R. A. et al., 2022 [12]).*

1.1.1.2 Vascular maturation

Maturation involves the recruitment of supporting mural cells, such as pericytes and vascular smooth muscle cells, which play a vital role in stabilizing blood vessels. This stability is necessary to prevent vessel leakage, maintain structural integrity, and withstand mechanical forces within the circulatory system. A number of studies have illustrated that contractile pericytes play a crucial role in enabling blood vessels to withstand the rising pressures in the central nervous system (CNS) [13-15]. Neural cadherin (N-cadherin, also known as CDH2) associated complex located at the direct contact between ECs and pericytes form a mechanosensory complex that transmits force [16-18]. The importance of integrins in pericytes function has also been studied. For example, β1 integrin knockout pericytes have a rounded morphology, failing to wrap around capillaries, and areas of EC-pericyte contact are smaller compared to those with wild-type pericytes [19]. However, the loss of α5 integrin in pericytes has no distinct effect on normal blood vessel development [20,21].

1.1.2 The circulatory system

The circulatory system comprises three major parts: the heart, blood vessels, and blood. The heart acts as a hydraulic pump, providing the driving force for the circulatory system and consistently maintaining the continuous flow of blood through various vessels.

The circulatory system contains three principal types of blood vessels:

1. **Arteries**. Transport oxygen-rich blood away from the heart to the entire body. They are characterized by thick, elastic walls to withstand high pressure.
2. **Veins**. Return oxygen-depleted blood back to the heart. Equipped with valves that prevent backflow, veins have thinner walls than arteries.
3. **Capillaries**. The smallest blood vessels with semi-permeable walls that form a network for the exchange of nutrients, oxygen, and waste between blood and tissues.

Considering the different roles of arteries and veins in responding to blood flow, they have different corresponding structures. Arteries have a thicker wall and three distinct layers:

Tunica intima. The innermost layer. It is composed of a thin layer of ECs to prevent blood coagulationand reduce turbulence created by blood circulation.

Tunica media. The middle and thickest layer. It is composed of multiple layers of smooth muscle, which are reinforced by connective tissue predominantly consisting of elastic fibers, providing the necessary elasticity for the artery to expand and contract, controlling blood circulation. In contrast, tunica media in veins is significantly thinner, since it contains fewer layers of smooth muscles.

Tunica adventitia. The external layer. It is primarily composed of loosely structured connective tissue with collagen and elastic fibers, contributing to its structural integrity, in

larger arteries, the tunica adventitia can surpass the thickness of the tunica media. It is typically the most substantial layer in veins.

Apart from the structural differences, the level of shear stress that arteries and veins experience differ markedly. The shear stress in the aorta is approximately 10 dynes per square centimeter (dyn/cm^2) reflecting the high pressure of blood directly pumped from the heart. In contrast, the vena cava, a major vein, experiences a lower shear stress, around one dyn/cm², owing to reduced pressure and slower velocity of returning venous blood [22]. Consequently, ECs of arteries and veins exhibit different morphologies. In response to the high shear stress and unidirectional flow of blood in arteries, arterial ECs are typically elongated and align in the direction of blood flow. Conversely, venous ECs often adopt a rounded shape [23].

Not only is the magnitude of shear stress important, but different types of shear stress may also elicit variable responses from ECs. In the circulatory system, the nature of blood flow dictates the type of shear stress exerted on vessel walls. Various types of flow in the circulatory system, primarily laminar flow and disturbed (or turbulent) flow, give rise to different types of shear stress. Laminar flow occurs where vessels are uniform and straight, whereas disturbed flow occurs in areas where vessels branch, have significant curvature, or where there is plaque aggregation in the arteries [24,25]. Responses to laminar flow, described as 'atheroprotective', involve EC alignment in the direction of flow, the formation of stress fibers, and the expression of Krüppel-like Factor 2 (KLF2) and Krüppel-like Factor 4 (KLF4), leading to the upregulation of anti-inflammatory genes [26-30]. On the other hand, responses to disturbed flow, referred to as 'atheroprone', are predominantly inflammatory and include the activation of Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and associated transcription [31].

1.1.3 Vascular diseases

The abnormal response of ECs in blood vessels to shear stress can lead to various vascular dysfunctions, contributing to the progression of multiple vascular diseases such as hypertension, thrombosis, and atherosclerosis [32]. Cardiovascular disease (CVD) is the leading global cause of mortality [33]. Among CVDs, atherosclerosis and abdominal aortic aneurysm (AAA) are particularly prevalent and highly dangerous, posing a significant risk of acute organ ischemia or severe bleeding into the peritoneal cavity.

1.1.3.1 Atherosclerosis

Atherosclerosis is a progressive vascular disorder characterized by the buildup of lipids and fibrous elements within the arterial walls, leading to plaque formation. These plaques are susceptible to lesions and ruptures, which makes atherosclerosis a major precursor to various cardiovascular diseases [34-37]. Studies have identified endothelial cell dysfunction as a key

factor in the initiation of atherosclerosis, which can be detected by angiography or ultrasound before any structural changes become evident in the vessel wall [38,39]. In more detail, EC behavior is tightly regulated by the dynamic microenvironment. Under normal conditions, vessel walls experience laminar flow, which fosters healthy EC activity. However, in regions where flow is disturbed, such as at vessel bifurcations and curves, ECs may begin to behave abnormally. These cells can become hyperpermeable and adopt a pro-inflammatory phenotype, processes which aid the transmigration of low-density lipoprotein (LDL) and immune cells into the wall of vessels and contribute to the development of atherosclerotic plaques [38]. Additionally, increased stiffness of the blood vessel tissue can aggravate these conditions [40].

1.1.3.2 Abdominal aortic aneurysms (AAA)

An aneurysm occurs when there is a weakening in a blood vessel's wall, leading to an abnormal expansion or ballooning that exceeds 50% of the vessel's normal diameter. Aneurysms can form in any blood vessel but are most frequently detected in the aorta, which is known as aortic aneurysms [41]. Specifically, an aneurysm in the ascending aorta, the part of the aorta closest to the heart, is termed a thoracic aortic aneurysm (TAA). Conversely, an aneurysm in the abdominal aorta, extending from diaphragm to just above the pelvis, is known as an abdominal aortic aneurysm (AAA). Both TAAs and AAAs share certain risk factors, but they also have distinct ones. Analysis of the medical histories of patients with TAAs and AAAs has revealed distinct risk factors for each. For TAAs, the risk factors include male sex, smoking, hypertension, and a bicuspid aortic valve. In contrast, the risk factors for AAAs are advanced age, male sex, and smoking [42].The prevalence of AAA in men over the age of 60 is estimated to be 4-8%, while it is around 0.5-1.5% in women [43]. Although AAAs generally remain asymptomatic, their potential to become life-threatening is significant due to the risk of rupture. This catastrophic event is responsible for approximately 60% of mortalities in patients with AAA [44].

Numerous studies have evidenced that the pathophysiology of AAAs is a complex, multifactorial process that includes inflammation responses, activation of matrix metalloproteinases (MMPs), oxidative stress, intraluminal thrombus formation, apoptosis of vascular smooth muscle cells, and the degeneration of ECM [45-47].

The ECM, mainly composed of elastin and collagen, is vital for maintaining the strength of the aortic wall against blood pressure. Inflammation leads to the release of enzymes that break down the ECM. As the ECM weakens, blood components and inflammatory cells can migrate from the adventitia into the tunica media. This movement, along with the aggregation of platelets and activation of the blood clotting system, promotes the formation of blood clots inside the aorta. These processes contribute to the enlargement of the aorta and increase the risk of an AAA rupturing [48,49].

1.2 Molecular basis of mechanotransduction in endothelial cells

Mechanotransduction in ECs is a sophisticated process that converts mechanical stress into cellular signals. This conversion is facilitated by mediators including integrins and junctional proteins such as VE-Cadherin and PECAM-1. The cytoskeleton plays a crucial role in transmitting the force, activating secondary messengers that modulate EC functions and gene expression. Components like the LINC complex and Lamin A/C, are instrumental in connecting to the nuclear lamina as illustrated in **Figure 2**.

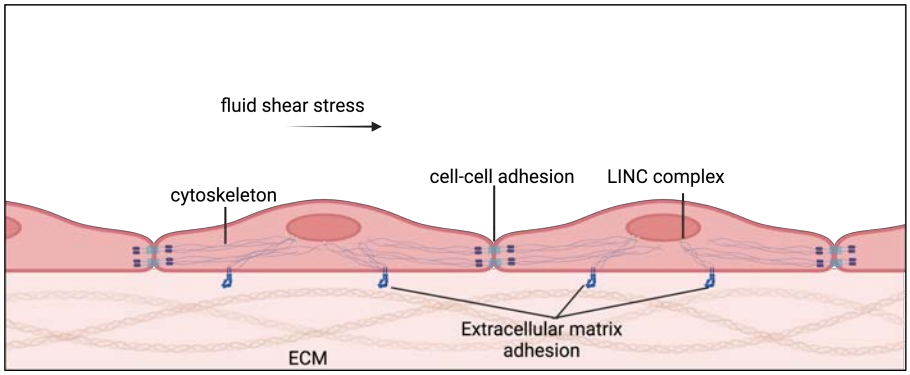

***Figure 2**: Schematics showing extracellular matrix adhesion, cell-cell adhesion, cytoskeleton and LINC complex involved in mechanotransduction at cellular level (modified from José Luis, A. & Wolfgang, H. G, 2016 [50]).*

1.2.1 Primary mechanosensors

1.2.1.1 Extracellular matrix adhesion

Focal adhesions are distinct multiprotein complexes that are formed when cells make contact with the ECM. These structures play a crucial role in mechanosensing and transmitting mechanical force from the ECM to the cytoskeleton. Focal adhesions can be divided into a transmembrane and an intracellular layer. Integrins, transmembrane proteins, physically bind to ECM components, which form heterodimers composed of α- and β-subunits. In humans, 18 alpha (α) and 8 beta (β) subunits have been identified, combining to form 24 distinct αβ heterodimers [51,52]. Integrin activity depends on the alteration of their affinity to the ECM, with shifts to a high-affinity conformation being modulated by external mechanical stimuli or through a process known as 'inside-out signaling' [53,54].

Increased affinity in integrins leads to activation and clustering, thereby strengthening the molecular connections at the cell-matrix interface [55-57]. Subsequently, the extracellular

domain of the integrins engages with the ECM, and the cytoplasmic part connects to the cytoskeleton via docking proteins, forming the core of the focal adhesions.

The integrin-associated focal adhesion complex is essential for EC responsiveness to mechanical forces during angiogenesis. The α5β1 integrin is selectively present in angiogenic blood vessels and supports this process [58-60]. The expression of αvβ3 and αvβ5 integrins is elevated in angiogenic ECs [61-63]. Interestingly, αvβ5 integrin is required for the angiogenic pathways activated by VEGF, which further activates Focal Adhesion Kinase (FAK) and Src kinase [64]. αvβ3 Integrin has been shown to associate with VEGFR2, affecting its recycling events where VEGF signaling is upregulated by linking αvβ3 to its corresponding ECM ligands [65,66]. Knockout mouse models with inducible, endothelial-specific mutations targeting integrins, such as α3, α6 and β3, have shown inhibitory effects on both physiological and tumor-related angiogenesis [67-69]. In addition to their role in angiogenesis, integrins are essential players in inflammatory and infectious processes. Specifically, β2 integrins enhance the recruitment of leukocytes to inflammatory sites by improving their adhesion to vascular endothelium, facilitating transendothelial migration, and supporting the formation of immunological synapses in leukocytes. Moreover, β2 integrins promote inflammatory signaling within the involved cells [70-73].

Mechanical force is relayed to the actin cytoskeletal via F-actin binding proteins, such as talin and Vinculin. Internal cellular force generates mainly from two sources: 1) retrograde actin flow due to actin polymerization at the cell's leading-edge membrane, and 2) the contractile movements of actomyosin, driven by non-muscle myosin II [74,75]. This force exerts a 'pulling' action on the molecular structures within the focal adhesions, effectively propelling the cell body forward.

Talin, a critical protein in this process, has an N-terminal FERM domain (talin head domain, THD), and a C-terminal rod domain structured with 13 helical bundles (R1–R13) ending in a dimerization motif. This THD attaches directly to the β-integrin subunit, while the rod domain can bind to actin through two actin-binding sites (ABS2 and ABS3), 11 vinculin-binding sites (VBSs), and additional sites for regulatory proteins such as RIAM, Kank family proteins, and the Rho GTPase activating protein DLC1. The exposure of cryptic binding sites on talin promotes the assembly of a mechanotransduction complex, linking integrin to the actin cytoskeleton. This linkage is critical for facilitating cellular movement and mechanical signal transmission [76-80].

Vinculin, another mechanosensory protein, connects adhesion receptors like integrins to the actin-myosin cytoskeleton. It binds to talin via its amino-terminal head domain or to paxillin via its rod-like tail domain [81,82]. Vinculin's ability to bind to lipids is facilitated through its tail domain, which is connected to the head by a flexible hinge domain. This domain also includes binding sites for components involved in actin polymerization, such as the Ena/VASP proteins

and Arp2/3 complex [83-85]. Vinculin is not only present in focal adhesion, but also in adherens junctions located between adjacent cells [86].

Overall, cell-matrix adhesions are featured as the large scaffold protein complex composed of hundreds of mechanoresponsive components, which made them profoundly versatile (**Figure 3**).

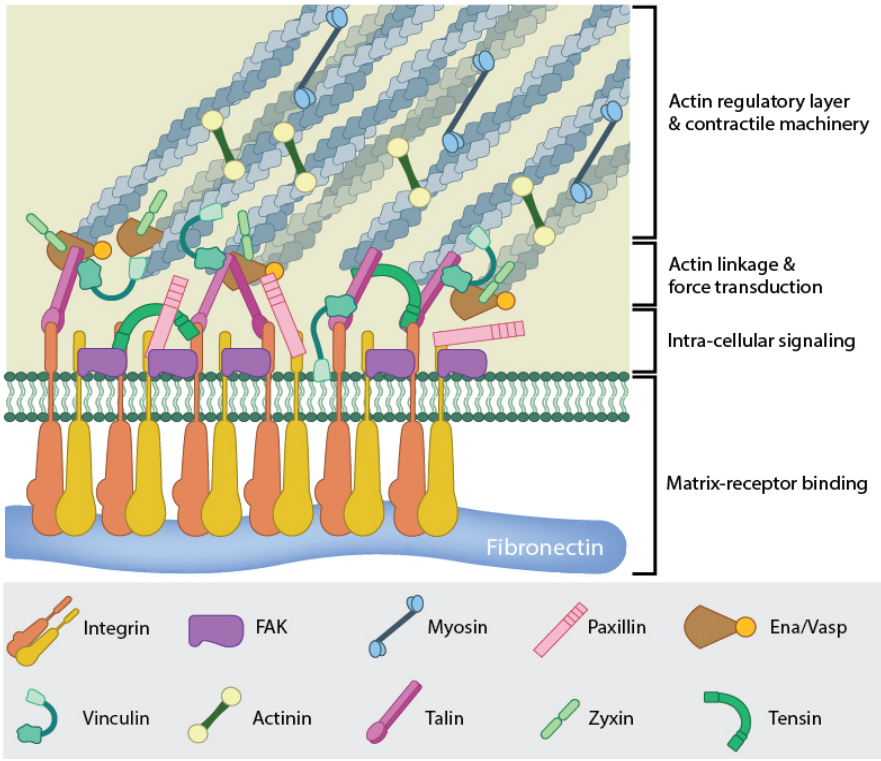

***Figure 3**: Illustration of a mature focal adhesion containing proteins that contribute to ECM binding, linkage to actin cytoskeleton and actin polymerization (adapted from Mechanobiology Institutet, MBI, National University of Singapore, 2023 [87]).*

1.2.1.2 Cell-cell adhesion

In ECs, mechanical connectivity between adjacent cells is established via adherens junctions, tight junctions, and gap junctions (**Figure 4**). Unlike epithelial cells or cardiomyocytes, ECs do not typically form classical desmosomes [88]. However, desmosomal proteins, such as plakoglobin and desmoplakin, are present in ECs. These proteins contribute to the formation of hybrid adherens junctions by associating with VE-cadherin and connecting to intermediate filaments [89].

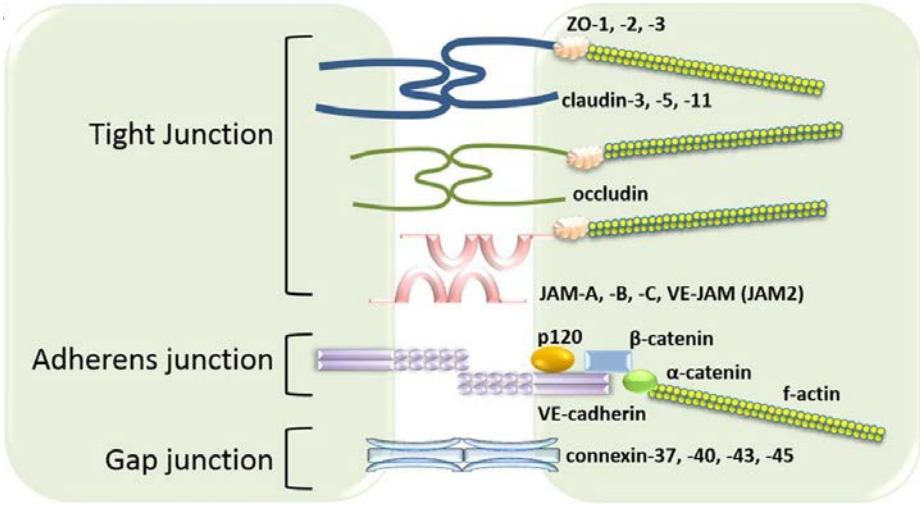

***Figure 4**: Schematics illustrating the cell-cell adhesion in ECs, which encompass adhesion junctions, tight junctions, and gap junctions (adapted from Komarova, Yulia A et al.,2017 [90]).*

Adherens junctions

The adherens junction represents a type of cell-cell junction, characterized by the connection of neighboring cells through the homophilic interactions of cadherin receptors. They play a crucial role in maintaining the physical association and mechanical coupling between cells.

Homotypic interactions between cells initiate the recruitment of transmembrane cadherins, catenins (β-, α-catenins), along with cytoskeletal proteins. The predominant cadherin in endothelial adherens junctions is VE-cadherin, an endothelial specific cadherin [91]. Through its cytoplasmic domain, VE-cadherin connects to p120-catenin and either β-catenin or plakoglobin [92]. Additionally, it promotes cytoskeletal remodeling via its indirect interaction with various actin-binding proteins, including but not limited to α-catenin, vinculin, α-actinin and eplin [93-95].

VE-cadherin, along with platelet endothelial cell adhesion molecule 1 (PECAM1) and VEGFR2/3, forms a mechanosensory complex. Upon mechanical stimulation, PECAM1, a transmembrane immunoglobulin protein, transmits this force, triggering the Src-mediated activation of Src family kinase. This activation results in the phosphorylation and subsequent activation of VEGFR2 [96]. VE-cadherin's transmembrane domain is critical for facilitating the interaction between PECAM-1 and VEGFR2/3 and is essential for the downstream activation of VEGFR2 following the mechanical stimulation of PECAM-1 [97,98]. VE-cadherin is also a key modulator in sensing and responding to the changes in matrix stiffness, potentially regulating the cytoskeleton in response to these variations through the action of small Rho GTPases [99].

As matrix stiffness increases, VE-cadherin-mediated cell-cell adhesions diminish, leading to a reduced barrier integrity and increased permeability of the EC monolayer, as observed in both *in vitro* and *ex vivo* models [100].

In addition to VE-cadherin, N-cadherin is another cadherin expressed in ECs at comparable levels. While VE-cadherin is primarily concentrated at cell-to-cell junctions, N-cadherin is generally absent from these structures [101]. Expressed in various cell types, including mesenchymal and neural cells, N-cadherin has been reported to form a mechanosensory complex transmitting force between ECs and other cells, such as pericytes or astrocytes [102,103].

As mentioned earlier, vinculin has been observed in tensile adherens junctions due to its recruitment by α-catenin in ECs (**Figure 5**). These unique junctions, known as focal adherens junctions, exhibit a spot-like appearance and are linked to oriented actin bundles that are subjected to tension [86,104]. Mechanical pulling experiments on individual α-catenin molecules, using magnetic tweezers, have shown that vinculin is recruited when α-catenin unfolds under tensional forces exceeding 5 pN. This force falls within the physiological range exerted by the cytoskeleton, and vinculin's role is to stabilize the unfolded conformation of α-catenin [105].

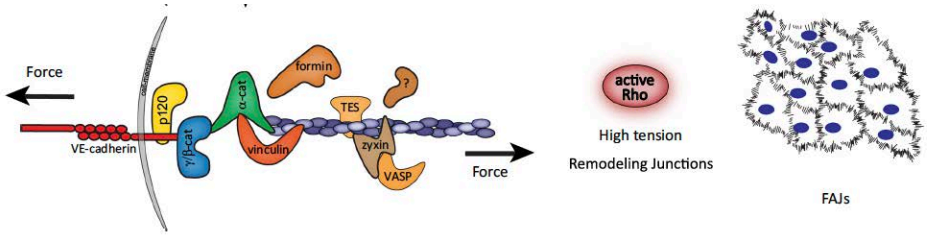

Figure 5*: Schematics of vinculin associated complex at cell-cell junctions in response to contractile force (adapted from Dorland, Yvonne L, and Stephan Huveneers, 2017 [106]).*

Tight junctions

Tight junctions in endothelial monolayers function to seal intercellular spaces, limiting the transportation of ions and solutes [107]. These junctions comprise multiprotein complexes, including transmembrane proteins like claudins, occludin, and junctional adhesion molecules (JAMs), as well as cytoplasmic proteins like ZO1, ZO2, MAGI1, and PATJ [108,109]. Notably, Claudin 5 is exclusively present at endothelial tight junctions [110].

Unlike epithelial cells, ECs display less separated apical-basal polarity at the cell-cell junctions. Consequently, the arrangement of tight junctions and adherens junctions in ECs differs from that in epithelial cells, where tight junctions are typically situated at the most apical-lateral side, followed by adherens junctions and desmosomes. In ECs, tight junctions are often

interspersed with adherens junctions along the lateral aspect of the cell-cell contact interface [91].

Gap junctions

Gap junctions in the endothelium are formed by connexins (Cx), and four specific types: Cx43, Cx40, Cx45, and Cx37 [111]. These connexins are organized into connexons, which function as channels allowing the intercellular exchange of ions and molecules of small size [112].

1.2.2 Cytoskeleton network

The cytoskeletal network provides mechanical support to the cells and is crucial in controlling their motility, shape, and tension homeostasis [113]. Mechanical forces detected at focal adhesion or cell-cell adhesions are conveyed through the cytoskeleton into intercellular space. This network is composed of actin filaments, intermediate filaments, and microtubules.

1.2.2.1 Actin filaments

Actin is the primary constituent of actin filament structures. Globular-actin (G-actin) polymerizes to form filamentous-actin (F-actin), which comprises two intertwined chains. F-actin and myosin II, bound by crosslinking proteins such as α-actinin, fascin, filamin, form stress fibers (SFs) [114]. These fibers enable F-actin to slide over myosin II, ensuring cytoskeleton contractility. SFs convey force from the ECM to the cell by pulling on focal adhesions and vice versa [113]. The cytoskeleton is therefore essential for the cellular response to external force. Studies in both 2D and 3D cultures have shown that HUVECs seeded on stiffer substrate exhibit more pronounced SFs [115]. Actin filaments interact with cellular junctions, including tight and adherens junctions, as well as focal adhesions. Cell-cell junctions connect to different types of actin structures, including cortical actin (or circumferential actin) and radial actin filaments. Cortical actin, located just beneath the plasma membrane, stabilizes the junction. In contrast, radial actin filaments may facilitate junction weakening and opening in response to mechanical and inflammatory stimuli [116].

Small GTPases from Rho family, such as RhoA, Rac1, and Cdc42, are pivotal in regulating actin cytoskeleton dynamics. RhoA promotes stress fiber formation through actin polymerization [117]. RhoA and Rho kinase (ROCK) signaling are fundamental mediators in mechanotransduction, influencing feedback mechanisms between focal adhesions, cellular shape, stiffness in the microenvironment, and actomyosin contractility [118,119]. Rac is involved in lamellipodia and filopodia formation through the recruitment of integrins, and vinculin signaling [120,121]. Cell shape-induced organization of ECM adhesions may affect the spatial distribution of intracellular Rac, potentially guiding protrusion formation and directional migration [122].

1.2.2.2 Intermediate filaments

Intermediate filaments, 10nm in diameter with an average, are relatively flexible and more stable compared to microtubules and actin filaments. Their primary role is to maintain cellular and tissue integrity, leveraging their mechanical properties and self-assembly capabilities [123]. In ECs, vimentin is the predominant intermediate filaments protein [124,125]. Kreis *et al.* reported that vimentin can directly bind to the tail of recombinant α2/β1 integrin and co-localized with native α2/β1 integrin [126].

1.2.2.3 Microtubules

Microtubules, which are composed of α/β-tubulin polymers, form hollow cylinders approximately 25nm in diameter. As the stiffest components of the cytoskeleton, microtubules play a vital role in essential biological processes, including intracellular trafficking, mitotic spindle formation, and the establishment of cell polarity [113].

1.2.3 Mechanobiology of the cell nucleus

Recent reports have highlighted the notion that extracellular forces can be transmitted to the nucleus through the actin filaments [127-129]. For example, Tajik *et al.* discovered that force-induced stretching of chromatin can enhance gene transcription, such as that of dihydrofolate reductase (DHFR). Disruption of actin filaments or inhibition of actomyosin contraction leads to the transcription abrogation or attenuation [130]. Furthermore, Tkachenko *et al.* have conducted experiments in microfluidic perfusion chambers showing acute application of substantial force to HUVECs causes an immediate displacement of nuclei [131].

The Linker of Nucleoskeleton and Cytoskeleton (LINC) complex functions as a bridge, physically connecting the cytoskeleton to the nucleoskeleton. The primary components of the LINC complex are SUN (Sad1p, UNC-84) and KASH (Klarsicht, ANC-1, Syne Homology) domain proteins, which span the nuclear envelope. SUN domain proteins, organized in trimers, extend through the inner nuclear membrane and bind to the C-terminal of KASH domain proteins that, in turn, pass through the outer nuclear membrane [132]. KASH domain proteins (nesprin1/2) are linked to cytoskeleton proteins, such as actin, dynein and kinesin, while the SUN domain proteins (SUN1/2) are connected to the nuclear lamina [133]. Therefore, the LINC complex forms a mechanical connection, enabling direct transmission of mechanical cues from cytoskeletal to the nuclear surface (**Figure 6**).

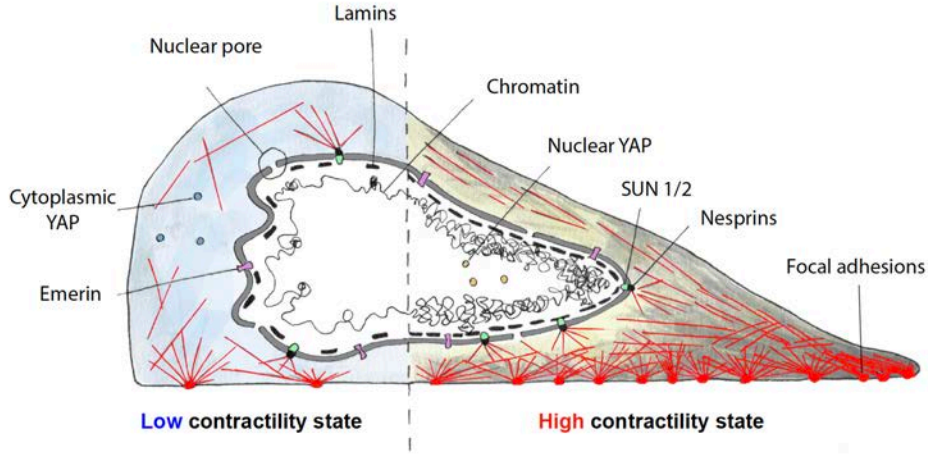

***Figure 6**: Cartoon illustrating the LINC complex physically connecting the cytoskeleton to the nucleus in two states: a low contractility state with weak focal adhesions (left panel), and a high contractility state with strong focal adhesions (right panel) (modified from Pennacchio, Fabrizio A et al., 2021 [134]).*

Chancellor *et al.* demonstrated that the depletion of nesprin 1 in ECs increased the nuclear height, a phenotype similar to that of cells with inhibited myosin. However, no detectable change in non-muscle myosin II activity was observed. This depletion leads to reduced pulling force on the nucleus from cytoskeletons, resulting in a vertically rounded nucleus. Additionally, the number of focal adhesion sites and substrate traction increased, causing abnormal adhesion, migration, and cyclic strain-induced reorientation [135]. King *et al.* reported that depleting nesprin 1 or nesprin 2 in ECs inhibited the migration into a cell-free area in scratch wound assays, and decreased loop formation in angiogenesis assays [136]. Lastly, Denis *et al.* utilized a dominant negative KASH (DN-KASH) peptide in HUVEC, which led to nuclei decoupled from the cytoskeletons. This decoupling resulted in impaired barrier function, decreased cell spreading and adhesion, and defects in endothelial migration, and responsiveness to shear stress [137].

A proposed mechanism for the phenotypes observed following mutation or depletion of LINC complex components is the potential impact on chromatin organization. Alam, *et al.* demonstrated that the disruption of LINC complex impairs the gene mechanosensitivity, thereby inhibiting mechano-regulation of gene expression across the genome [138]. Additionally, research conducted on Drosophila larval muscles lacking a functional LINC complex revealed that the LINC complex prevents excessive chromatin repression by promoting the binding of chromatin to the nuclear envelope [139].

1.2.4 YAP function in mechanotransduction

Yes-associated protein (YAP) and its paralogue, transcriptional coactivator with PDZ-binding motif (TAZ), are key effectors of the Hippo pathway [140]. When the Hippo pathway is on, such as when the cells reach confluency, MST1/2 are activated through direct binding to SAV1. Phosphorylated MST1/2 subsequently induces phosphorylation of MOB1, which in turn phosphorylates LATS1/2. As a result, YAP are phosphorylated and are either retained or degraded in the cytoplasm. Conversely, when the Hippo pathway is off, as seen in sub-confluent cells, YAP are dephosphorylated and translocated into the nucleus. There, it forms complexes with DNA-binding transcription factors, including TEAD, SMADs, and TBX5, to regulate the transcription of a wide range of genes involved in cell proliferation and junction contact [140,141].

Significant research efforts have been dedicated to understanding the role of YAP in EC function and behavior. It has been established that YAP expression is increased in the retinal vasculature during post-natal development and in the processes of EC differentiation in mice [142,143]. Additionally, Wang *et al*. have demonstrated that disturbed flow, compared to laminar flow, leads to decreased YAP phosphorylation and increased expression of downstream genes such as CTGF and CYR61 in both HUVECs and HAoECs. Conversely, inflammatory ECs found in the lesion area of *apoe*$^{-/-}$ mice and in human atherosclerotic aortas exhibit reduced YAP phosphorylation [144].

The subcellular localization and activity of YAP are tightly regulated by mechanical stimuli, such as stress, stiffness of the ECM, and cell geometry. For instance, it has been shown that cellular stretching stimulates the nuclear localization of YAP, which in turn stimulates proliferation [142]. Furthermore, growing ECs on a stiff matrix activates integrin signaling as well as YAP transcriptional activity [145,146].

1.3 Angiomotins: A scaffold protein family involved in mechanotransduction

Angiomotin (Amot) was initially identified by its binding to the angiogenesis inhibitor, angiostatin [147]. Subsequently, Angiomotin-like 1 (AmotL1, also known as JEAP) and Angiomotin-like 2 (AmotL2, also known as LCCP, MASCOT), two highly homological polypeptides of Amot, were identified from GenBank analysis [148,149]. Together, the angiomotin family consists of three members: Amot, AmotL1, and AmotL2.

The structure of Amot family proteins is characterized by distinct features: W-W binding motifs, conserved coiled-coil domains, and C-terminal PDZ motifs (**Figure 7**). Notably, Amot possesses an angiostatin binding domain situated between coiled-coil domains and PDZ motifs. Each member of Amot family has two, isoforms: Amot (p80 and p130), AmotL1 (p90 and p110), and AmotL2 (p60 and p100).

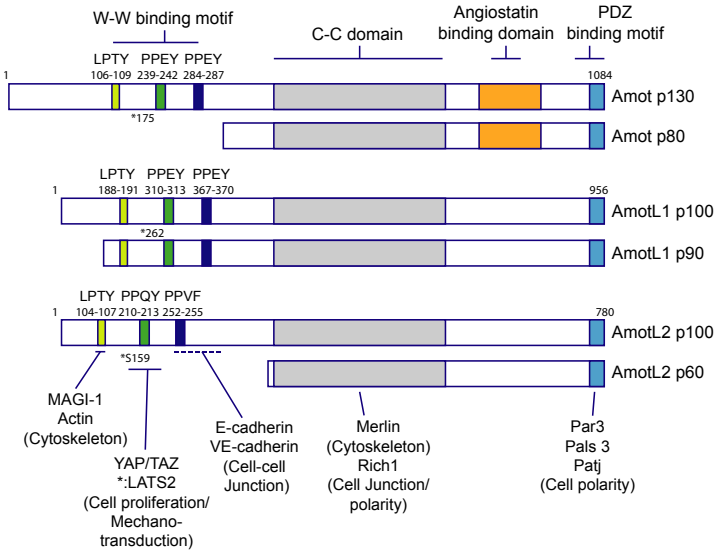

***Figure 7**: Structural binding domains of Angiomotin protein family. The figure illustrates the distinctive binding domains within the Amot scaffold protein family, including Amot, AmotL1 and AmotL2 (adapted from PhD thesis from Yuanyuan Zhang, 2019 [150]).*

Studies have shown that the PDZ binding motif at the C-terminal is important for EC migration. Through PDZ binding motif, Amot, AmotL1 and AmotL2 interact with the apical polarity protein Patj and form a ternary complex with Syx, which control local GTPase activity and regulate EC migration [151]. Additionally, Par3 binds to the PDZ binding motif of AmotL2, promoting its localization with VE-cadherin/E-cadherin in cell-cell adhesions and enables radial actin organization [152].

Coiled-coil domains also play a significant role. For instance, Amot is part of a signaling axis that includes Merlin-Amot-Rich1 and the Rac1 small GTPase, regulating the collective migration of epithelial cells [153]. Amot p130 (but not Amot p80) and AmotL1 bind YAP via LPTY and PPEY motifs of W-W binding domain [154,155]. The PPQY motif of AmotL2 also binds YAP [156,157].

1.3.1 Amot

During mouse embryogenesis, Amot is expressed as early as the inner cell mass (ICM) stage of the blastocyst and plays a crucial role in cellular differentiation, primarily by regulating the localization of YAP [158]. As reported by Shimono *et al.*, most Amot-deficient embryos die shortly after gastrulation due to impaired migration within subregions of the visceral endoderm [159]. However, back crossing of these knockout mice revealed that around 75 % of the embryos die at embryonic day 11 due to vascular insufficiency. Furthermore, depletion of

Amot in zebrafish was shown to inhibit filopodia formation in EC tip cells and thereby affecting migration of intersegmental vessels. Amot depletion has also been implicated in pathological angiogenesis and therefore efforts to inhibit tumor growth by targeting angiogenesis have thus included the development of DNA vaccinations and antibodies against Amot [160,161].

Amot is essential not only for vascular expansion but also for neurogenesis. The p130 isoform of Amot, in particular, plays a crucial role in stabilizing dendritic spines. This isoform is predominantly found in mature dendritic spines, where it stabilizes the actin cytoskeleton by linking F-actin to postsynaptic protein scaffolds [162]. In developing neurons, the function of Amot is contingent upon its interaction with YAP, which is vital for dendrite growth and branching *in vitro*. Conditional depletion of both Amot and YAP in neurons leads to the development of less complex dendritic trees in Purkinje cells and atypical cerebellar morphology [163]. Taken together, Amot plays an important role in development and especially during the morphogenesis of the vascular and neural networks.

1.3.2 AmotL1

Similar to Amot, AmotL1 plays a significant role in EC migration, particularly by influencing the junctional stability in stalk cells during vascular sprouting, as observed in zebrafish angiogenesis models [164]. Additionally, research utilizing EC-specific conditional mouse models have shown that silencing AmotL1 impairs pericyte morphology and blood vessels coverage in a non-autonomous manner [165].

AmotL1 was shown to interact with N-cadherin, forming a complex with MAGI-1 and actin filaments in both pericytes and ECs in the absence of VE-cadherin. This interaction facilitates the formation of homotypic cell-cell interactions involving N-cadherin *in vitro* [165]. Moreover, the influence of AmotL1 on cell migration extends beyond ECs. For example, it interacts with Pdlim2 to regulate the dynamics of the actin cytoskeleton in podocyte foot processes, a finding detailed by Sistani *et al.* [166]. In the context of cancer, an increased expression level of AmotL1 has been observed to enhance cancer cell migration and proliferation. This is achieved through the activation of c-Src in breast cancer, as reported by Couderc *et al.* [167].

1.3.3 AmotL2

Similar to Amot, AmotL2 is expressed during early embryogenesis, with its expression uniquely restricted to the trophectoderm. The inactivation of AmotL2 results in the loss of actin filaments, leading to inhibited hatching from the Zona Pellucida [168]. Predominantly expressed in both developmental and adult tissues, the p100 isoform of AmotL2 is a key component of the VE-cadherin and E-cadherin junctional complexes in endothelial and epithelial cells, respectively. The loss of AmotL2 in these cells leads to the absence of specific actin filaments that connect cells via VE/E-cadherin, crucial for the transmission of mechanical

signals. Beyond its role in blastocyst hatching, the inactivation of AmotL2 results in impaired aortic lumen expansion and embryonic mortality in both mice and zebrafish [169].

As a scaffold protein, AmotL2 facilitates interactions with various proteins. It interacts with MAGI1 and actin through its N-terminal LPTY motif [168]. Mutations in this motif disrupt actin binding, affecting the rescue of AmotL2 depletion phenotypes in zebrafish endothelial and epithelial models [168,169]. Furthermore, molecular studies have revealed that the cell polarity protein Partitioning Defective 3 homolog (Par3) binds to AmotL2's PDZ domains. This binding targets the protein complex to the apical cell junctions, essential for the formation of radial F-actin, a key component of cell structure and organization [152].

Another isoform, p60AmotL2, has been identified. This isoform is transcribed by the c-Fos transcription factor in response to stress signals, such as ischemia. p60AmotL2 binds to p100AmotL2 and acts as a dominant-negative, sequestering proteins in intracellular vesicles. This interaction results in the loss of apical-basal polarity and the disruption of radial actin filament formation. Consequently, it activates the process of apical extrusion *in vitro*, a mechanism crucial for maintaining homeostasis but also potentially exploited by tumor cells for tissue invasion. Consistently, p60AmotL2 has been shown to promote ameboid migration *in vitro* and tumor cell invasion *in vivo* [170].

2 Research aims

Each member in the Amot protein family functions as a scaffold protein with the ability to associate with various mechanosensitive proteins. It is intriguing to identify their direct binding partners, and to further unveil the mechanism underlying how individual Amot proteins contribute to mechanical force transmission in distinct vascular events.

Paper I

- To investigate how Angiomotin contributes to the transmission of mechanical forces during the process of vascular expansion.

Paper II

-To study the function of the AmotL2/VE-cadherin mechanotransductory complex in adult aorta.

Paper III

-To investigate how AmotL1 associates with N-cadherin to form the complex in EC/pericyte interaction and the transmission of force between ECs and pericytes.

-To develop a comprehensive overview of the binding profiles across the Amot protein family.

3 Materials and methods

3.1 Mouse models

3.1.1 Conditional knockout mouse models (Papers I and II)

3.1.1.1 amot^{flox} mice

The *amot^{flox}* mice, carrying a loxP-flanked *amot* gene, were crossed with Cdh5 (PAC)^{CreERT2} and ROSA26-EYFP double transgenic mice. *amot^{ec+}* and *amot^{ec-}* are the abbreviations used for depicting *amot^{wt}*-Cdh5 (PAC)^{CreERT2}-ROSA26-EYFP and *amot^{flox}*-Cdh5 (PAC)^{CreERT2}-ROSA26-EYFP mice, respectively, as the *amot* gene localizes to the X chromosome. All the mice in this report were in C57BL/6 background. Biopsies from mouse tail-tip and ear were used for genotyping of each mouse included in these studies. Postnatal day 6 (P6) mice were analyzed in this study. Both female and male animals were included. Ethical permission was granted by the North Stockholm Animal Ethical Committee. All animal housing and experiments were carried out in accordance with the guidelines of the Swedish Board of Agriculture.

For retinal angiogenesis study, tamoxifen (400 mg/mouse/day, dissolved in sunflower oil) was administered by intraperitoneal (IP) injection from P1 to P3 and the retinas were harvested at P6.

3.1.1.2 amotl2^{flox} mice

In the same fashion, we established a mouse model for the AmotL2 endothelium-specific KO mouse model. *amotl2$^{ec+/ec+}$* and *amotl2$^{ec-/ec-}$* are the abbreviations for *amotl2$^{wt/wt}$*-Cdh5 (PAC)^{CreERT2}-ROSA26-EYFP and *amotl2$^{flox/flox}$*-Cdh5 (PAC)^{CreERT2}-ROSA26-EYFP mice, respectively.

3.1.2 Tumor transplantation mouse model (Paper I)

The *amot^{ec+}* and *amot^{ec-}* male mice at 6 weeks of age were injected by tamoxifen to induce *amot* depletion. Lewis lung carcinoma cells (LLC, 0.5 × 10^6/mouse) were injected subcutaneously on the last day of tamoxifen injection. Tumor growth was manually inspected twice per week and tumor volumes were calculated according to the formula: 0.52 × length × width × width (cm^3). Starting from 2 weeks after the first injection, the same dosage of tamoxifen was injected into the tumor-bearing mice for 5 days. When tumors reached the diameter of 10 mm, mice were sacrificed according to the ethical permission.

3.1.3 The porcine pancreatic elastase (PPE) mouse model (Paper II)

Tamoxifen injections (5 days) were administered to mice aged 9 weeks to induce endothelial-specific *amotl2* gene inactivation. At week 12, mice were induced with 2 - 3% isoflurane anesthesia in the chamber, placed on and fixed to a heating pad and then maintained with

1.5% isoflurane anesthesia during surgery. The abdominal aorta from just below the left renal vein to the iliac bifurcation was identified and dissected peripherally from about 2 mm below the left renal nerve to the bifurcation. Topical local application of 5 µL of elastase from porcine pancreases (10.1 mg of protein per milliliter, 19 U mg^{-1} protein) was used to the exposed aortic adventitia for 5 min. Afterwards, the aortas were dried with a cotton swab and gently washed with warm 0.9% saline. The intestines were returned to the abdominal cavity, and the laparotomy was closed.

3.1.4 Ethical consideration

The ethic permits for our mouse experiments, including N129/15 (2015-2020), 12931-2020 (2020-2025) and 22902-2021 (2021-2026), were granted by the Stockholm North Animal Experiment Ethics Board. These studies were conducted in strict accordance with the guidelines provided by the Swedish Board of Agriculture, with a commitment to the '3R' principles:

Replacement: Priority was given to *in vitro* experiments using cultured ECs, such as Bovine Aortic Endothelial Cells (BAE cells), MS1 cells, and Human Aortic Endothelial Cells (HAoECs), and Human Umbilical Vein Endothelial Cells (HUVECs). This approach aimed to minimize the use of live animals where possible.

Reduction: Experiments were thoroughly planned in order to reduce the number of mice used to the minimum required to achieve reliable results.

Refinement: Several measures were implemented to enhance the welfare of the mice used in these studies, including the use of individually ventilated cages and gentle handling procedures. Additionally, I underwent proper training before performing any procedures that involved wounding, such as injections.

3.2 Human materials

3.2.1 Human Protein Atlas (Paper I)

The Human Protein Atlas is a Swedish-based project established in 2003 and aiming to map all the human proteins in cells, tissues, and organs. An integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology, has been utilized for exploration of the human proteome (https://www.proteinatlas.org/) [171].

3.2.2 Human aortic aneurysm samples (Paper II)

Aortic samples from patients with AAA were obtained from surgeries performed at Karolinska Hospital in Stockholm, Sweden. Signed consent from patients with AAA was obtained for tissue collection. Control samples were taken from the abdominal aorta of beating-heart,

solid organ transplant donors. Organ donors consented to the use of tissue for research purposes at the time of enlisting to the donation registry. Ethical permission was granted by the Regional Ethical Review Board in Stockholm. No participant compensation was granted.

RNA was extracted from both medial and adventitia layers of the aortic wall and subsequently sequenced on Human Transcriptome Array 2.0–Affymetrix (HTA 2.0) platform [172].

3.2.3 Human primary cell line (Paper II)

HUVECs from ScienCell were cultured in EC medium. HAoECs from PromoCell were cultured with Endothelial Cell Growth Medium MV. The batch of HAoECs used for this study came from a 55-year-old male donor with a Caucasian background.

3.3 Proximity-dependent biotin identification (BioID)

Proximity-dependent biotin identification (BioID) plasmids (Mammalian Gene Expression Lentiviral Vector) were constructed by combining cDNA fragments with N-terminus of E. coli biotin ligase (BirA) (**Table 1**). An empty vector with the same backbone was used as negative control. All constructs (produced by Vector Builder) were verified by restriction enzyme digestion. BioID constructs and the empty vector were packaged into lentivirus using Lipofectamine 3000 Transfection Reagent. MS1 cells, *cdh5* $^{+/+}$ cells or *cdh5* $^{-/-}$ cells were used to establish stable transfected cell lines via lentivirus transfection with the selection of 0.5 mg/mL geneticin. Stable transfected cells were cultured in completed medium without biotin and 0.5 mg/mL geneticin. For the BioID experiment, cells were treated with 50 mM biotin for 16 hr, followed by harvesting in a lysis buffer consisting of 50 mM Tris·HCl pH 7.4, 8 M urea, 1 mM DTT and protease inhibitors. 1% Triton X-100 was added to lysates before sonication. Biotinylated proteins were purified with streptavidin beads overnight at 4 °C. After five washes with 8M urea in 50 mM Tris·HCl (pH 7.4) and one wash with 50 mM Tris·HCl (pH 7.4), the beads were resuspended in PBS and kept ready for further Mass Spec analysis.

Name of BioID	BioID construct
empty vector plasmid	pLV[Exp]-Neo-CMV > Stuffer300
mouse p130-Amot BirA-tagged plasmid	pLV[Exp]-Neo-CMV > {BirA(R118G)}:[NM_153319.3]*
mouse p80-Amot BirA-tagged plasmid	pLV[Exp]-Neo-CMV > {BirA(R118G)}:[NM_133265.2](G449D)
human p100-AmotL2 BirA-tagged plasmid	pLV[Exp]-Neo-CMV>{BirA(R118G)}:hAMOTL2[NM_001278683.1
human p60-AmotL2 BirA-tagged plasmid	pLV[Exp]-Neo-CMV>{BirA(R118G)}:{P60}
mouse AmotL1 BirA-tagged plasmid	pLV[Exp]-Neo-CMV>{BirA(R118G)/HA}:mAmotl1[NM_001081395.1]

***Table 1**: The structure of BioID constructs were used in Papers.*

4 Results and Discussion

4.1 Paper I: The Amot/integrin protein complex transmits mechanical forces required for vascular expansion.

4.1.1 Amot is required for both retinal and tumor angiogenesis.

In this paper, we investigate the role of Amot in vascular expansion. Previous studies, such as Aase *et al.* have demonstrated that Amot is expressed during the physiological angiogenesis of zebrafish and mice [160]. Amot has been considered as a negative regulator of the Hippo pathway. It suggests that Amot appears to promote vascular and endothelial expansion, whereas in epithelial cells, it acts as a suppressor of cell proliferation.

To clarify this apparent inconsistency, the expression pattern of Amot was examined in sections of both healthy and malignant human tissues via IHC [173]. The Amot expression was confined to in the blood vessels of the placenta during the third trimester and was present in adult endometrium. Additionally, in adult tissues, such as the pancreas, brain, prostate, breast, and colon, Amot was not observed in regions external to the blood vessels. Coincidentally, when examining corresponding human cancer tissues, it was found that there was an increase in Amot expression in the blood vessels and stroma of various human cancers, but this upregulation was not observed in the adjacent non-cancerous tissues.

To study the role of Amot in mouse retinal angiogenesis, we induced inactivation of the *amot* gene in ECs using tamoxifen injections from P1-P3. This was achieved with our endothelium-specific knockout mouse model. Whole mount staining analysis of retinas harvested at P6 demonstrated that inactivation of *amot* in ECs led to the inhibition of radial vessel expansion, characterized by asymmetry and partial collapse. Quantitative analysis indicated the number of tip cells filopodial extensions in $amot^{ec-}$ retinas were reduced compared to that in $amot^{ec+}$ retinas.

Observing the upregulation of Amot in the vasculature of human tumors, we aimed to determine whether Amot also contributed to the angiogenesis process in tumors. To this end, we used two distinct transgenic mouse models for breast cancer: the LLC transplantation model and MMTV-PyMT. LLC tumors injected in $amot^{ec-}$ mice were notably smaller in terms of tumor weight, as compared with $amot^{ec+}$ mice. IF staining analysis of tumor sections revealed a substantial reduction in vascular density and a higher rate of necrosis in $amot^{ec-}$ tumor compared to $amot^{ec+}$ tumor. In the MMTV-PyMT transgenic breast tumor model, our analysis discovered that the onset of early tumors in mice lacking *amot* in ECs was delayed by an average of six weeks compared to control mice. Moreover, there was a significant improvement in the overall survival rate of $amot^{ec-}$ mice. Together, these findings substantiate the protective impact of *amot* deletion in reducing tumor occurrence through the inhibition of angiogenesis.

4.1.2 Amot relays force between ECs and the ECM both *in vivo* and *in vitro*.

We hypothesized that Amot played a crucial role for the force transmission between the Fibronectin (Fn) and the cytoskeleton within migrating ECs. Firstly, enhanced resolution confocal microscopy was used to visualize Fn fibrils in the endothelial migrating front. Fn fibrils were stretched and aligned with tip cell filopodia in $amot^{ec+}$ retinas, whereas Fn was localized to punctate and shortened fibrils in $amot^{ec-}$ retinas. Previous research has reported that the assembly of cell-mediated Fn fibrils are regulated by mechanical signals [174-176]. To examine force patterns of Fn *in vivo*, H5-scFv antibody, which allows the detection of cryptic epitopes specifically exposed under mechanical tension, was utilized. By comparing the ratio of the signal derived from the H5-scFv antibody relative to that from a pan-Fn antibody, a decreased ratio was observed in areas where the tip cell filopodia extended in $amot^{ec-}$ retinas. Furthermore, we employed Traction Force Microscopy (TFM) to more accurately measure the forces exerted in the interaction between individual EC and the ECM *in vitro.* As a result, Amot siRNA knockdown cells displayed a marked reduction in the force applied to the Fn-coated polyacrylamide gel.

4.1.3 Amot is identified as a novel partner in the integrin adhesome.

To identify the components in the Amot complex involved in force transmission, we performed Amot co-immunoprecipitation (co-IP) in BAE cells at 40% confluent. Mass Spectrometry (MS) analysis unveiled an enrichment of 'Integrin signaling pathway' Panther pathways and several integrin subtypes, such as β3, α5, and β1, were recognized from the protein list. We verified these findings through co-IP analysis of β1 integrin and αvβ3 integrin in control siRNA and Amot siRNA-treated cells. We found that the p130 isoform of Amot was the specific isoform associated with β1 and αvβ3 integrins, suggesting that the N-terminal domain of Amot mediates this interaction. To further identify potential direct binders to Amot, we utilized BioID technology. The biotin ligase, BirA, fused to a protein of interest, biotinylates the lysines of closely interacting proteins within 10 nm[177]. For this purpose, BirA was fused to the N-terminal end of the p130 and p80 Amot isoforms and cloned into lentiviral expression vectors. Subsequently, these vectors were used to infect MS1 cells. A total of 178 proteins were identified as potential binding partners of Amot p130 and were subsequently analyzed using Panther pathway analysis. 'Integrin signaling pathway' was identified as a top hit. Multiple known focal adhesion-related proteins, such as Talin, Zyxin, Kank2, and Myosin 9/10, were identified. The association of these proteins was verified by co-IP analysis using either Amot-, integrin-, and Talin-specific antibodies.

Our data indicate that Amot binds to actin under subconfluent conditions but not under confluent conditions using Co-IP. Furthermore, analyses on β1 integrin and αvβ3 integrin immunoprecipitations show reduced binding of actin to both β1 integrin and αvβ3 integrin in cells treated with Amot siRNA compared to control cells. Taken together, we proposed the hypothesis that Amot might influence the interaction or polymerization of actin filaments

following the activation of integrins. To assess the organization of focal adhesions and actin filaments, we utilized Y-shaped CYTOO chips to analyze both control and Amot siRNA-depleted cells. Our findings revealed that the depletion of Amot did not alter Vinculin recruitment at focal adhesion sites. However, there was a notable decrease in the intensity of actin staining in the Amot-depleted cells.

4.1.4 Discussion

In this study, we identified that Amot is a component of the integrin adhesome, playing a crucial role in transmitting forces between ECM and cytoskeleton. More importantly, we underscored Amot's significance in aiding essential force transmission for vascular expansion in both normal and pathological situations, using EC-specific gene deletion mice model.

Integrin-mediated mechanotransduction between the ECM and ECs has been extensively studied and plays a crucial role in development and tissue homeostasis. This has been a focus of research for the last several decades, as highlighted by Sun *et al.* [76]. The integrin adhesome is complicated, comprising more than 200 proteins that are involved in cell-ECM interactions, as reported by Horton and Humphries [178]. Our findings provide evidence that Amot is a previously unidentified component of the integrin adhesome. The reason why Amot's role in force transmission has not been previously discovered may be due to the fact that typical cellular adhesion studies are conducted in fibroblasts, which lack Amot expression. Our study, however, demonstrates that Amot is primarily expressed in ECs during both developmental angiogenesis and tumor angiogenesis in mice and humans.

Ernkvist *et al.* have demonstrated that Amot binds to actin and triggers the formation of actin filaments [151]. The results were consistent with our present finding the BioID MS analysis identified actin and actin-associated proteins. Our previous work also revealed the binding of the Rho-GTPase RhoA to Amot, aligning with our findings [151]. Amot has also been reported to regulate Rho-GAP activity, providing a potential explanation for the extensive actin fiber formation upon p130-Amot overexpression [179]. Moreover, Amot is essential for the spatially restricted localization of Rho-GTPase to cellular protrusions or lamellipodia during EC migration via Rho-FRET analysis [151]. Apart from Rho-GTPase, Amot may also activate other GTPases to mediate cytoskeleton reorganization. Amot has been suggested to coordinate directional migration within the Merlin-Amot-Rich1 complex [180]. Furthermore, the binding of Amot has been associated with the hydrolysis and downregulation of the activity of the Rho GTPases Rac1 and Cdc42 [153,181]. However, it remains unclear that how Amot, in conjunction with the mentioned Rho GTPases, collaborates to regulate actin dynamics during EC migration.

Overall, our findings suggest that Amot plays a role in integrating mechanical cues perceived by migrating ECs. These cues are then translated into actomyosin contractility, which is essential for the migration of ECs.

4.2 Paper II: The VE-cadherin/AmotL2 mechanosensory pathway suppresses aortic inflammation and the formation of abdominal aortic aneurysms.

4.2.1 AmotL2 is essential for arterial endothelial alignment in response to flow.

This paper aims to investigate the function of AmotL2 in the adult mouse aorta. Previous research has shown that AmotL2 is associated with VE-cadherin and is essential for aortic lumen expansion during mouse embryogenesis [169]. In this study, we investigated the expression patterns of AmotL2 in major vessels, specifically the descending aorta (DA) and the inferior vena cava (IVC). The findings revealed that AmotL2 was localized to the junctions of aortic ECs. In contrast, ECs in the IVC exhibited a rounder shape, with fewer or no radial actin filaments, and demonstrated reduced expression of AmotL2.

To investigate the role of AmotL2 in aortic function, we targeted the inactivation of *amotl2* in ECs by administering tamoxifen injections. Depletion of *amotl2* in the DA resulted in the loss of radial actin filaments and altered cell shape. Comparable changes in phenotype were noted in arterial ECs but not in venous ECs within urinary bladder. Likewise, in $amotl2^{ec+/ec+}$ mice, the nuclei of ECs in the DA were elongated and aligned to the direction of blood flow, coinciding with cell alignment. In contrast, ECs lacking *amotl2* exhibited rounder and irregularly shaped nuclei, positioned near cell-cell junctions downstream of flow direction. These modifications in nuclear shape and positioning were not detected in vena cava ECs. These findings were also replicated in controlled *in vitro* flow experiments using Ibidi pump system.

4.2.2 AmotL2 links junctional VE-cadherin to the nuclear lamina.

Co-IP analysis was used to identify AmotL2-associated complexes from both MS1 and BAE cells. MS analysis was used to identify several key proteins. These included cellular membrane proteins like VE-cadherin, α-catenin, β-catenin, and p120 catenin, together with nuclear lamina proteins, such as SUN2 and Lamin A. To validate these findings, we plated HUVECs and HAoECs in culture dishes and subjected them to 96-hour flow generated by the orbital shaker. AmotL2 Co-IP in HUVEC revealed that the VE-cadherin/AmotL2/actin complex formation was induced after flow exposure. In contrast, this complex was present in primary aortic endothelial cells independently of flow.

We went on to investigate more precisely how AmotL2 linked with the VE-cadherin complex. We employed the BioID technique as described in Paper I. We identified 121 candidate interactors of AmotL2. Among them, several proteins, such as AmotL1, Magi1, and Mpdz, have been previously reported as direct binders of AmotL2. Further, our KEGG pathway analysis indicated a significant number of proteins related to tight junctions and adherens junctions, including ZO-1, Afadin, and p120 catenin. p120 catenin forms a direct binding connection with the submembrane domain of VE-cadherin, a region separated from the interaction site of β-catenin. Therefore, p120 catenin could potentially link AmotL2 to the junctional complex Subsequently, we examined the interaction between AmotL2 and p120

catenin in EC *in vivo* using Proximity Ligation Assay (PLA). We observed distinct signal (co-localization) in the aortic EC, which was significantly higher than that in *amotL2*$^{ec-/ec-}$ aorta or vena cava. Collectively, our findings propose a model in which the junctional complex of VE-cadherin, p120 catenin, and AmotL2 is linked to actin filaments that extend to connect with the nuclear lamina.

4.2.3 Depletion of *amotl2* promotes vascular inflammation *in vivo*.

It is established that the alignment of ECs and the reorganization of their cytoskeleton in response to laminar blood flow act as protective mechanisms against inflammation. This raises an important question: does the observed lack of alignment in AmotL2 EC-specific knockout (*amotl2*$^{ec-/ec-}$) ECs in the aorta correlate with a pro-inflammatory response? To address this issue, mRNA from the DAs of both *amotl2*$^{ec+/ec+}$ and *amotl2*$^{ec-/ec-}$ mice was extracted and subjected to RNA sequencing (RNA-seq), revealing a significant enrichment of genes associated with immune-related Gene Ontology (GO) terms. The mRNA expression of the identified genes associated with inflammation was confirmed through TaqMan qRT-PCR. Notably, CD68, as a monocyte lineage marker, was predominantly elevated in male *amotl2*-depleted mice, but less obvious in females. Similar trends were detected with cytokines like Tnf, Ccl2, Il6, and Cxcl10. In line with cytokine upregulation, Vcam1, crucial for monocyte adherence to the endothelium, showed increased levels in male *amotl2*$^{ec-/ec-}$ mice.

Following the RNA-seq analysis, we performed immunohistochemistry (IHC) on the DA to determine whether the upregulation of inflammatory markers was associated with the presence of inflammatory cells. As a result, Cd45^{+} cells were detected in the sub-renal region of the DA of *amotl2*$^{ec-/ec-}$ mice. Additionally, we observed monocyte infiltration in the outer curvature of the aortic arch. The morphology of these Cd45^{+} monocytes contrasts with the spindle-like Cd45^{+} macrophages that reside in the inner curvature and at arterial bifurcations.

4.2.4 The formation of AAA is induced in *amotl2*$^{ec-/ec-}$ male mice.

Inflammation in the aortic wall is linked with the emergence of arterial aneurysms. In *amotl2*$^{ec-/ec-}$ mice, we observed the spontaneous formation of AAA near the renal arterial branch. Notably, 20% of male *amotl2*$^{ec-/ec-}$ (5/25) mice exhibited AAA formation, whereas no aneurysms were found in female mice (0/20) or in wild-type mice (0/36). Imaging analyses of a typical AAA exhibited damage to both the endothelium and the vessel wall.

Next, we studied the effects of *amotl2* deletion in a well-established murine model designed for the study of aneurysm formation, the PPE mice model [182,183]. In this model, local application of elastase weakens the vessel wall and induces aneurysm. Ultrasound examinations showed a significantly larger lumen in the *amotl2*$^{ec-/ec-}$ mice. IHC analysis revealed significant intimal thickening, primarily due to the proliferation of α-smooth muscle actin-positive (αSMA^{+}) cells.

4.2.5 The expression of AmotL2 and inflammation in patients with AAA are negative correlated.

Subsequently, we shifted our focus to investigate the association between AmotL2 gene expression and the inflammatory response in AAA patients. We examined mRNA expression levels in samples from both healthy individuals and AAA-diagnosed patients, who received surgical treatment at Karolinska University Hospital. mRNA was extracted from the medial and adventitial layers of the aorta in 13 donors and 35 AAA cases. AMOTL2 expression was markedly reduced in the media of AAA tissues compared to normal samples, after normalization to endothelial markers. An inverse association was found between AMOTL2 expression and the expression of markers for the monocytes/macrophages CD68, and T cell markers CD4 and CD8A, but not with the B cell marker CD19. In line with this, a negative correlation was also observed with the expression of cytokines such as TNF, CCL2, CCL5, CXCL10, and ICAM1, consistent with the links to inflammatory cell markers.

4.2.6 Discussion

ECs play a critical role in responding to hemodynamic forces, and investigating the underlying pathways is key to comprehending the pathogenesis of vascular diseases. In this report, we demonstrate that the VE-cadherin/AmotL2 protein complex forms a linkage between actin and the nuclear lamina. The specific depletion of *amotl2* in ECs led to a notable impairment in EC alignment in response to shear stress, along with disruption in nuclear positioning. These effects contribute to inflammation and the formation of AAAs.

The absence of EC alignment as a result of *amotl2* deficiency, along with the associated irregular shapes of arterial ECs, the concurrent absence of radial actin filaments and disrupted nuclear positioning, had immediate implications for endothelial function. Additionally, the depletion of *amotl2* triggers the activation of pro-inflammatory markers, such as Vcam1 and Icam1, on the surface of ECs, via qPCR analysis and facilitates the extravasation of $Cd45^{+}$ inflammatory cells. We hypothesize that the infiltration of inflammatory cells into the tunica media contributes to the degradation of the ECM, including elastin. Consequently, this process weakens the structural integrity of the aortic wall. The compromised region of the artery may subsequently protrude, elevating the risk of vessel rupture and hemorrhage. Both quantitative PCR analysis of pro-inflammatory markers and the formation of AAA in the $amotl2^{ec-/ec-}$ male mice revealed a sex-specific difference. Interestingly, AAA tends to occur more frequently in males aged 65-79 years compared to females in the same age group [48]. The appearance of sex-specific difference in our *amotl2* endothelial specific depletion mince model implies its potential clinical importance.

The precise genetic link between AmotL2 and AAA is yet to be fully understood. Factors such as epigenetic modifications and environmental influences could also play a role. Therefore, further research is warranted to unravel the complex relationship between AmotL2 and AAA.

4.3 Paper III: Mapping of the Angiomotin protein family Adhesome by BioID Analysis.

4.3.1 AmotL1 adhesome is identified via BioID technique.

Here, we focus on the mechanotransductory complex associated with AmotL1 in endothelium. Previous studies evidenced that VE-cadherin forms homotypic adherens junctions between ECs, whereas N-cadherin is the primary heterotypic adhesive molecule connecting ECs and pericytes [18]. It was observed that N-cadherin was upregulated and relocated to cell junctions in VE-cadherin (*Cdh5*)-deficient cells [184]. Interestingly, AmotL1 was found to associate with N-cadherin through actin filament only when VE-cadherin is depleted [165]. To gain deeper insights into the AmotL1/N-cadherin associated complex, we infected both wild-type endothelial cells ($cdh5^{+/+}$) and $cdh5^{-/-}$ cells with BioID constructs. 399 proteins were identified in $cdh5^{-/-}$ cells but only 64 proteins in $cdh5^{+/+}$ cells. This data suggest that AmotL1 has a more functionally active role in $cdh5^{-/-}$ cells, as indicated by the sheer quantity of associated proteins. An in-depth pathway analysis performed in *Enrichr* indicated that the 'Integrin signaling pathway' was highly enriched in association with AmotL1 in $cdh5^{-/-}$ cells. Conversely, no discernible Panther pathways were significant in $cdh5^{+/+}$ cells. As validation, AmotL1 co-IP in both cell types revealed that the association between AmotL1 and focal adhesion proteins was apparent in $cdh5^{-/-}$ cells compared to that in $cdh5^{+/+}$ cells, whereas the association with p120 catenin markedly declined. Overall, this finding aligns with the results obtained from BioID analysis.

To identify unique binding proteins of AmotL1, the positive hits from both cell types were analyzed using a Venn diagram: 382 proteins identified exclusively in $cdh5^{-/-}$ cells and 47 in $cdh5^{+/+}$ cells. GO Cellular Component analysis of the proteins in $cdh5^{-/-}$ cells revealed significant enrichment in the 'Focal adhesion' pathways, in contrast to the prominent enrichment observed in $cdh5^{+/+}$ cells. Additionally, common positive hits (n=17) in both cell types were analyzed in the same fashion. All the top 10 significantly enriched pathways were related with cytoskeleton or adhesive structure, including ZO-1, Merlin, Jcad, Afadin, Bin1, Twf1 and Shroom4. This indicates that AmotL1 binds to these junctional proteins independently of VE-cadherin.

To demonstrate the interactions between AmotL1 and N-cadherin, we compared AmotL1 binding partners to the previously published N-cadherin adhesome in cardiomyocytes lacking Cdh5 expression [185]. Notably, both proteins were identified using the BioID technique. Interestingly, there was an overlap of 39 shared proteins. This suggested that AmotL1 and Cdh2 might function collaboratively within the same mechanosensitive protein complex. Substantiating this notion, those 39 proteins were subjected to GO Cellular Component analysis. The primary ten enrich pathways predominantly pertained to junctional pathways and cytoskeleton.

4.3.2 Expression Patterns of the Amot Protein Family and Their Associated Adhesomes.

Amot proteins, despite their structural similarities, display distinct phenotypes as shown by gene inactivation studies in mouse and zebrafish. This may be explained by differences in spatiotemporal expression patterns. We therefore investigated the expression of the individual Amot genes in mRNA single cell sequencing databanks of mouse tissues [186]. Firstly, we analyzed expression patterns of Amot genes in the adult aorta. Unlike the absence of Amot, AmotL1 and AmotL2 were highly expressed in distinct EC clusters. Additionally, mRNA single cell sequencing data from human embryos endothelium showed that preferential expression of Amot in the early embryonic stage. In a comparison among endothelial sub-clusters during organogenesis, AmotL1 exhibited relatively higher expression in vascular endothelial cells, while AmotL2 tended to have increased expression in arterial endothelium [187]. In summary, these findings demonstrated the differential expression of Amot genes in ECs, which may explain, at least in part, their distinct roles in vascular functions.

To compare the assembly of protein complexes involving each Amot family member, we conducted a Venn diagram analysis focusing on binding profiles of all Amot adhesomes, as identified by BioID [188,189]. Fifteen proteins were found to be commonly associated to all Amot proteins, including ZO-1, Afadin, Mpdz, Filamin B, Magi1, RhoA, and Talin. Subsequent analysis using the Panther pathway tool revealed that these proteins are involved in cellular structures and processes such as Tight Junctions, Adherens Junctions, and Focal Adhesions. This indicates that all members have the capacity to interact with the actin cytoskeleton and cell adhesion complexes. Specific binding proteins of Amot, AmotL1, and AmotL2 were also subjected to Panther pathway analysis and the results demonstrated that the 'Integrin signaling pathway' was the top hit for both Amot- and AmotL1-specific binding proteins. However, the proteins involved in the focal adhesion complexes were distinguishable. More specifically, AmotL1 was found to interacted with integrin β6, α3, αv and α6, while Amot was bound to caveolin 1, filamin A, Crk, and CrlL.

4.3.3 Discussion

In this study, we have identified AmotL1 adhesome using the BioID technique in both VE-cadherin wild-type and knockout cells.

Zheng *et al.* reported that AmotL1 binds to N-cadherin in the absence of VE-cadherin as evidenced through Co-IP experiments. This binding initiated when N-cadherin translocated to cell junctions in order to compensate for the loss of VE-cadherin [165,184]. Interestingly, when comparing the adhesomes of N-cadherin in mouse cardiomyocytes and AmotL1 in *cdh5*$^{-/-}$ cells using a Venn diagram, it was observed that 39 proteins overlap, representing about 1/10 of the total number of proteins in both adhesomes. Several functional or actin-binding proteins, including ZO-2, Afadin, Talin1, Magi1, have been identified as binding partners between

AmotL1 and N-cadherin. This suggests that N-cadherin and AmotL1 may work together in coordinating cellular junctions and cell-matrix interactions.

The possible explanations include 1) the cadherin-based and integrin-based complexes are not so distinguishable, and 2) AmotL1 associated proteins, such as Magi1, ZO-2, are involved in several junction complexes. During post-natal retinal angiogenesis, EC-specific AmotL1 depletion results in a non-autologous pericyte phenotype characterized by lower pericyte coverage of vessels [165]. This suggests that the EC/pericyte interaction was impaired due to the dissociation of AmotL1 from N-cadherin junction complexes. Interestingly, other research revealed that antagonists of α4β1 integrin resulted in cell death of both ECs and pericytes [190]. It was observed that pericytes depleted of β1 integrin had reduced coverage over capillary vessels [19]. These finding suggest that both integrin and N-cadherin complex are critical for pericyte and EC functions. However, the molecular mechanisms behind these observations are yet to be elucidated.

The distinct functionality of Amot family proteins can, at least in part, be attributed to differences in their spatiotemporal expression pattern. For example, Amot is expressed during both embryonic and post-notal angiogenesis, but it is not detected in adult vessels. Conversely, AmotL1 and AmotL2 are expressed during adulthood, exhibiting distinct patterns in endothelial clusters within the aorta. *In vitro*, however, all three proteins are co-expressed in ECs, yet they still maintain specific functionality. A comparison of their individual adhesome suggests that there are notable differences. For instance, AmotL2, known to interact with VE-cadherin, does not associate with focal adhesion proteins, in contrast to both Amot and AmotL1. Furthermore, they differ in their mechanisms of forming actin complexes: AmotL2 is crucial for formation of actin filaments in response to laminar flow; AmotL1 functions by localizing to the cell membrane and Amot influences actin dynamics by subconfluency.

In conclusion, our study has uncovered that Amot proteins display distinct spatiotemporal expression patterns and form associations with specific adhesomes. This discovery provides a detailed mechanistic insight into the unique roles each Amot proteins plays in vascular biology.

5 Conclusions

Overall, the Amot protein family displays distinct patterns in terms of timing and distribution within the endothelium. Furthermore, while these proteins share some common junction-related proteins as direct binding partners, each member of the Amot family is associated with specific and unique adhesomes. These adhesomes are involved in distinct mechanosensory complexes during various vascular events, as illustrated in **Figure 8**.

- Amot/Talin/Integrin complex, is required for both retinal and tumor angiogenesis, transmitting force between fibronectin and the cytoskeleton.
- AmotL2/p120 catenin/VE-cadherin complex associates actin even to the nuclear membrane in ECs to transmit extracellular mechanical signals. The endothelial deficit of AmotL2 provokes a pro-inflammatory response and AAAs in adult mice fed normal diet.
- AmotL1 not only binds to N-cadherin but also is associated with focal adhesion proteins.

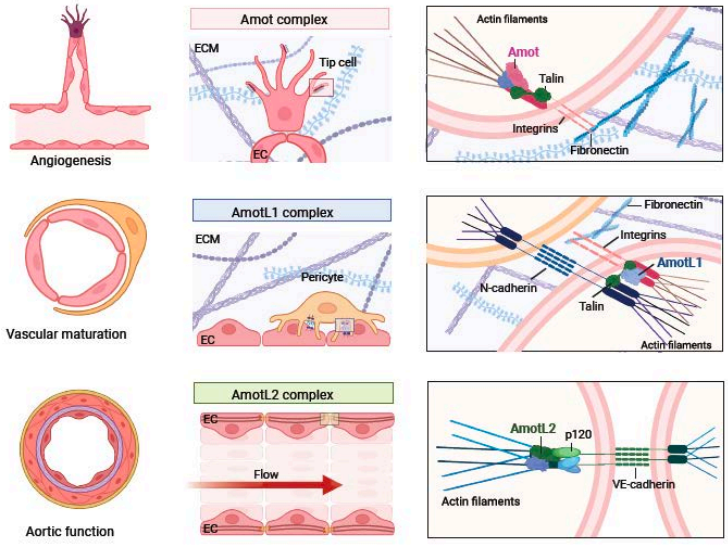

***Figure 8**: Functional overview of the adhesome of Amot proteins in distinct vascular processes.*

6 Future perspectives

The potential effect of Amot on nuclei

Angiogenesis is regulated by multiple factors, including mechanotransduction and chemotransduction. The nucleus, being the largest and most rigid organelle, is particularly responsive to mechanical forces [191]. Nuclear responses to those forces include adaptations in chromatin architecture and transcriptional activities that trigger shifts in cellular states [192]. These mechanically induced alterations also affect the physical characteristics of the chromatin and nucleus, preventing aberrant changes in nuclear shape and maintaining genome integrity. It has been reported that Actin-myosin interactions facilitates the direct transfer of force from integrin-based adhesions to the nuclear region [193]. For example, the application of mechanical forces to the cell nucleus through integrin-mediated cell spreading prompts the polymerization of nuclear F-actin, which subsequently results in the formation, preservation, and activation of the MRTF-SRF complex within the nucleus [194,195]. Integrin/Amot mechanosensory complex is identified for preserving the proper transmission of mechanical forces in vascular expansion and tip cell migration, both in normal physiological conditions and during pathological processes. We may further investigate how deletion of Amot affects the nuclear shape of endothelial tip cells in retinal angiogenesis and relevant mechanism. Since Amot is also required for tumor angiogenesis. Gaining a deeper understanding of the mechanisms how Amot influences tip cells during angiogenesis could offer valuable insights into identifying potential drug targets for cancer treatment.

AmotL2 as a biomarker for AAA diagnosis

Diagnosing an AAA can be challenging because they often grow slowly and don't show noticeable symptoms until they become large or rupture, making them difficult to detect. In clinical practice, abdominal ultrasonography is widely recognized as the golden standard for diagnosing and monitoring asymptomatic AAAs. Its efficacy stems from its near-perfect accuracy in diagnosis, making it a highly reliable tool in these cases [48]. In regions where population-based screening for AAAs in men has been implemented, such as United Kingdom and Sweden, there is a reported decrease in the incidence of ruptured AAAs among men compared with countries that do not have such screening programs [196-199]. However, there has been some debate about the effectiveness of population-based AAA screening programs in recent years. Critics argue that these programs might lead to over-diagnosis by identifying men with AAAs who may never require treatment, raising concerns about the potential drawbacks of widespread screening. Therefore, the identification of a genetic biomarker that could indicate the presence of AAAs in a blood test would be beneficial. This biomarker could be included in regular blood tests, eliminating the need for additional ultrasound tests. We have demonstrated that AMOTL2 expression was markedly reduced in the media of AAA

tissues compared to normal samples, suggesting a potential genetic link between AmotL2 and AAA. Further investigation to determine if AmotL2 can be a genetic biomarker is worthwhile.

Arterialization of veins

Saphenous vein grafts (SVGs) represent the most frequently utilized vessels for coronary artery bypass graft (CABG) surgeries. However, the rates of 10-year vein graft failure (VGF) are around 40-50% [200]. Intimal hyperplasia, which develops from one month to one year after CABG, and accelerated atherosclerosis occurring beyond one year, are the major pathophysiological processes leading to SVG failure over a long period [201]. Exposure of the SVGs to high arterial pressure, a process known as arterialization, results in SVG intimal hyperplasia, which in turn lays the foundation for the development of atherosclerosis. Arterialization initially serves as an adaptive mechanism for venous ECs responding to suddenly increased shear stress. Therefore, it suggests a hypothesis: if venous ECs are engineered to resemble arterial ECs before CABG, this could confer adaptability and anti-inflammatory properties to the venous ECs, potentially preventing intimal hyperplasia and accelerated atherosclerosis. We have demonstrated that the depletion of AmotL2 results in vascular inflammation and abnormal EC alignment. Notably, when HUVECs were exposed to flow conditions, we observed the formation of an AmotL2/VE-cadherin complex, similar to that found in arterial ECs. Taken together, introducing AmotL2 into venous ECs is likely to enhance their compliance to flow and reduce inflammation when these cells are transplanted into an arterial environment. Future plans involve inducing AmotL2 expression in venous ECs through lentivirus transfection in a mouse CABG model. Additionally, we will measure the enhanced junctional connections between VE-cadherin and actin filaments and assess inflammation levels before and after grafting.

7 Acknowledgements

I am profoundly grateful for the opportunity to pursue my PhD studies at Karolinska Institutet. Throughout this journey, I have been incredibly fortunate to receive the generous support and assistance from my supervisors, colleagues, friends, and family.

My supervisors:

Dr. **Yuanyuan Zhang**, my principal supervisor, I extend my deepest gratitude for guiding me with utmost patience through the entire PhD journey. Your mentorship has been instrumental, teaching me not only critical techniques but also instilling in me a professional attitude towards science and work. The times you spent on improving my English communication and presentation skills are indelibly touching in my memory. You have been more than a responsible supervisor, also a warm and comforting friend, I would say. I always recall the kindness you showed by inviting me to your home during the spring festivals and other events, ensuring I did not feel lonely.

Prof. **Lars Holmgren**, my co-supervisor, I am thankful for your decision to take me on as your PhD student. These years under your guidance have been an enriching and invaluable experience. Your expertise and encouragement have played a pivotal role throughout my doctoral studies. I treasure the countless memorable moments, our profound discussions, and all the remarkable experiences we shared during this period.

My lab mates

Pedro, thank you for always stopping your work to help me. I'm grateful for how you share your scientific ideas and advice on my projects. Good luck with your own work and what comes next!

Honglei, I appreciate all the tips and help you've given me, both with work and everyday things. It's been amazing meeting you at the lab and you made my life happier and easier!

My colleagues and friends in Karolinska Institutet

Vicky, your care and support have been a great comfort to me, especially when I was new to Stockholm and needed guidance the most. **Evelyn**, your organizational skills are impressive, making the lab an efficient and orderly environment. Your clarity in labeling has saved me countless times. Thank you! **Aravindh**, I will never forget the evening you dedicated to teaching me the intricacies of live imaging and 3D experiments. Your patience is something I will always be grateful for. **Aarren**, that weekend you came to help me locate the experiment equipment was so memorable. I'm truly thankful for all the help you've provided!

To people at KI who spent time with me: Thank you for all the everyday chats and for helping me when I really needed it. A big thank you to: **Yanhong Su, Yi Li, Hao Shi, Yiyi Xu, Jiawen Lv,**

Yajie Yang, Mingkai Dai, Yanyu Jiang, Simin Zhang, Xiaohao Wang, Mirco Martino, Wei Feng, Chenying Gao, **Huaitao Cheng**, **Le Tong**, **Ying Yang** and **Muyi Yang**.

My friends from outside Sweden

Haixin, I still remember the great time when we traveled around Europe in 2019. I hope we can do that more in future! **Xiao Yi**, I treasure the time we spent in Kiruna. I will not forget the beautiful aurora we watched together during that trip. **Jinrui**, thank you very much for coming to Stockholm and visit me! Those days we supported and comforted each other are so memorable. **Yang**, thank you very much for giving me precious advice about life and career. Hope we can meet again in Shanghai and good luck for your work and family life. **Xuejing**, **Yawen**, **Yanan** and **Hang**, thanks for your kind support during the last five years. Hope we can meet soon when I am back to China.

My family

爸爸和妈妈， 感谢你们一直以来对我的不求回报的支持和爱。在这几年里，每当我感到压力大的时候，你们一直宽慰我，为我托底。**姐姐**，从小开始，你一直是我的人生偶像。非常谢谢你， 在这五年你也一直包容我支持我，经常地开导我，还专门飞到瑞典来看我。希望我们未来都能越来越好！

Fundings

Chinese Scholarship Council (CSC), thanks for granting me 4 years for my PhD study. **Swedish Heart and Lung Foundation**, thanks for sponsoring me attend the 4th International Symposium on Mechanobiology (ISMB) conference in Australia. The projects in this thesis were generously supported by grants received from **Swedish Cancer Society**, **Swedish Heart and Lung Foundation**, **Swedish Childhood Cancer Foundation**, **Cancer Society of Stockholm**, **Swedish Research Council**, and **Radiumhemmets Forskningsfonder**.

8 References

1 Chin, L., Xia, Y., Discher, D. E. & Janmey, P. A. Mechanotransduction in cancer. *Curr Opin Chem Eng* **11**, 77-84, doi:10.1016/j.coche.2016.01.011 (2016).

2 Liu, F. *et al.* Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *J Cell Biol* **190**, 693-706, doi:10.1083/jcb.201004082 (2010).

3 Liao, H. *et al.* Mechanotranduction Pathways in the Regulation of Mitochondrial Homeostasis in Cardiomyocytes. *Front Cell Dev Biol* **8**, 625089, doi:10.3389/fcell.2020.625089 (2020).

4 Charbonier, F. W., Zamani, M. & Huang, N. F. Endothelial Cell Mechanotransduction in the Dynamic Vascular Environment. *Adv Biosyst* **3**, e1800252, doi:10.1002/adbi.201800252 (2019).

5 Majesky, M. W. Vascular Development. *Arterioscler Thromb Vasc Biol* **38**, e17-e24, doi:10.1161/atvbaha.118.310223 (2018).

6 Udan, R. S., Culver, J. C. & Dickinson, M. E. Understanding vascular development. *Wiley Interdiscip Rev Dev Biol* **2**, 327-346, doi:10.1002/wdev.91 (2013).

7 Kolte, D., McClung, J. A. & Aronow, W. S. in *Translational Research in Coronary Artery Disease* (eds Wilbert S. Aronow & John Arthur McClung) 49-65 (Academic Press, 2016).

8 Adair, T. H. & Montani, J. P. in *Angiogenesis* (Morgan & Claypool Life Sciences

Copyright © 2010 by Morgan & Claypool Life Sciences., 2010).

9 Lamalice, L., Le Boeuf, F. & Huot, J. Endothelial cell migration during angiogenesis. *Circ Res* **100**, 782-794, doi:10.1161/01.RES.0000259593.07661.1e (2007).

10 Phng, L. K., Stanchi, F. & Gerhardt, H. Filopodia are dispensable for endothelial tip cell guidance. *Development* **140**, 4031-4040, doi:10.1242/dev.097352 (2013).

11 Schwartz, M. A. Integrins and extracellular matrix in mechanotransduction. *Cold Spring Harb Perspect Biol* **2**, a005066, doi:10.1101/cshperspect.a005066 (2010).

12 Nelson, R. A., Rhee, E., Alaeddine, M. & Nikkhah, M. Advances in Biomaterials for Promoting Vascularization. *Current Stem Cell Reports* **8**, 184 - 196 (2022).

13 Peppiatt, C. M., Howarth, C., Mobbs, P. & Attwell, D. Bidirectional control of CNS capillary diameter by pericytes. *Nature* **443**, 700-704, doi:10.1038/nature05193 (2006).

14 Oishi, K., Kamiyashiki, T. & Ito, Y. Isometric contraction of microvascular pericytes from mouse brain parenchyma. *Microvasc Res* **73**, 20-28, doi:10.1016/j.mvr.2006.08.004 (2007).

15 Fernández-Klett, F., Offenhauser, N., Dirnagl, U., Priller, J. & Lindauer, U. Pericytes in capillaries are contractile in vivo, but arterioles mediate functional hyperemia in the mouse brain. *Proc Natl Acad Sci U S A* **107**, 22290-22295, doi:10.1073/pnas.1011321108 (2010).

16 Gerhardt, H., Wolburg, H. & Redies, C. N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken. *Dev Dyn* **218**, 472-479, doi:10.1002/1097-0177(200007)218:3<472::Aid-dvdy1008>3.0.Co;2-# (2000).

17 Tillet, E. *et al.* N-cadherin deficiency impairs pericyte recruitment, and not endothelial differentiation or sprouting, in embryonic stem cell-derived angiogenesis. *Exp Cell Res* **310**, 392-400, doi:10.1016/j.yexcr.2005.08.021 (2005).

18 Gerhardt, H. & Betsholtz, C. Endothelial-pericyte interactions in angiogenesis. *Cell Tissue Res* **314**, 15-23, doi:10.1007/s00441-003-0745-x (2003).

19 Abraham, S., Kogata, N., Fässler, R. & Adams, R. H. Integrin beta1 subunit controls mural cell adhesion, spreading, and blood vessel wall stability. *Circ Res* **102**, 562-570, doi:10.1161/circresaha.107.167908 (2008).

20 Turner, C. J., Badu-Nkansah, K., Crowley, D., van der Flier, A. & Hynes, R. O. Integrin-$\alpha 5 \beta 1$ is not required for mural cell functions during development of blood vessels but is required for lymphatic-blood vessel separation and lymphovenous valve formation. *Dev Biol* **392**, 381-392, doi:10.1016/j.ydbio.2014.05.006 (2014).

21 Hynes, R. O. Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673-687, doi:10.1016/s0092-8674(02)00971-6 (2002).

22 Papaioannou, T. G. & Stefanadis, C. Vascular wall shear stress: basic principles and methods. *Hellenic J Cardiol* **46**, 9-15 (2005).

23 dela Paz, N. G. & D'Amore, P. A. Arterial versus venous endothelial cells. *Cell Tissue Res* **335**, 5-16, doi:10.1007/s00441-008-0706-5 (2009).

24 Givens, C. & Tzima, E. Endothelial Mechanosignaling: Does One Sensor Fit All? *Antioxid Redox Signal* **25**, 373-388, doi:10.1089/ars.2015.6493 (2016).

25 Chatzizisis, Y. S. *et al.* Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol* **49**, 2379-2393, doi:10.1016/j.jacc.2007.02.059 (2007).

26 Parmar, K. M. *et al.* Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest* **116**, 49-58, doi:10.1172/jci24787 (2006).

27 Baeyens, N. *et al.* Syndecan 4 is required for endothelial alignment in flow and atheroprotective signaling. *Proc Natl Acad Sci U S A* **111**, 17308-17313, doi:10.1073/pnas.1413725111 (2014).

28 Hahn, C. & Schwartz, M. A. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol* **10**, 53-62, doi:10.1038/nrm2596 (2009).

29 Choi, D. *et al.* ORAI1 Activates Proliferation of Lymphatic Endothelial Cells in Response to Laminar Flow Through Krüppel-Like Factors 2 and 4. *Circ Res* **120**, 1426-1439, doi:10.1161/circresaha.116.309548 (2017).

30 Zhou, G. *et al.* Endothelial Kruppel-like factor 4 protects against atherothrombosis in mice. *J Clin Invest* **122**, 4727-4731, doi:10.1172/jci66056 (2012).

31 Tzima, E. *et al.* Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression. *Embo j* **21**, 6791-6800, doi:10.1093/emboj/cdf688 (2002).

32 Ando, J. & Yamamoto, K. Vascular mechanobiology: endothelial cell responses to fluid shear stress. *Circ J* **73**, 1983-1992, doi:10.1253/circj.cj-09-0583 (2009).

33 Roth, G. A. *et al.* Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. *J Am Coll Cardiol* **76**, 2982-3021, doi:10.1016/j.jacc.2020.11.010 (2020).

34 Vaduganathan, M., Mensah, G. A., Turco, J. V., Fuster, V. & Roth, G. A. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. *J Am Coll Cardiol* **80**, 2361-2371, doi:10.1016/j.jacc.2022.11.005 (2022).

35 Libby, P. The changing landscape of atherosclerosis. *Nature* **592**, 524-533, doi:10.1038/s41586-021-03392-8 (2021).

36 Libby, P. *et al.* Atherosclerosis. *Nat Rev Dis Primers* **5**, 56, doi:10.1038/s41572-019-0106-z (2019).

37 Lusis, A. J. Atherosclerosis. *Nature* **407**, 233-241, doi:10.1038/35025203 (2000).

38 Davignon, J. & Ganz, P. Role of endothelial dysfunction in atherosclerosis. *Circulation* **109**, Iii27-32, doi:10.1161/01.CIR.0000131515.03336.f8 (2004).

39 Xu, S. *et al.* Endothelial Dysfunction in Atherosclerotic Cardiovascular Diseases and Beyond: From Mechanism to Pharmacotherapies. *Pharmacol Rev* **73**, 924-967, doi:10.1124/pharmrev.120.000096 (2021).

40 Mitchell, G. F. *et al.* Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* **121**, 505-511, doi:10.1161/circulationaha.109.886655 (2010).

41 Shaw, P. M., Loree, J. & Gibbons, R. C. in *StatPearls* (StatPearls Publishing

Copyright © 2023, StatPearls Publishing LLC., 2023).

42 Butcovan, D. *et al.* Sub-classification of non-inflammatory and inflammatory surgical aortic aneurysms and the association of histological characteristics with potential risk factors. *Exp Ther Med* **18**, 3046-3052, doi:10.3892/etm.2019.7903 (2019).

43 Klink, A. *et al.* Diagnostic and therapeutic strategies for small abdominal aortic aneurysms. *Nat Rev Cardiol* **8**, 338-347, doi:10.1038/nrcardio.2011.1 (2011).

44 Arnaoutakis, D. J. & Upchurch, G. R., Jr. Abdominal Aortic Aneurysm Screening Is Safe yet Lacks Effectiveness. *Circulation* **139**, 1381-1383, doi:10.1161/circulationaha.118.038809 (2019).

45 Yuan, Z. *et al.* Abdominal Aortic Aneurysm: Roles of Inflammatory Cells. *Front Immunol* **11**, 609161, doi:10.3389/fimmu.2020.609161 (2020).

46 Sakalihasan, N., Limet, R. & Defawe, O. D. Abdominal aortic aneurysm. *Lancet* **365**, 1577-1589, doi:10.1016/s0140-6736(05)66459-8 (2005).

47 Piacentini, L. *et al.* Genome-Wide Expression Profiling Unveils Autoimmune Response Signatures in the Perivascular Adipose Tissue of Abdominal Aortic Aneurysm. *Arterioscler Thromb Vasc Biol* **39**, 237-249, doi:10.1161/atvbaha.118.311803 (2019).

48 Sakalihasan, N. *et al.* Abdominal aortic aneurysms. *Nat Rev Dis Primers* **4**, 34, doi:10.1038/s41572-018-0030-7 (2018).

49 Cameron, S. J., Russell, H. M. & Owens, A. P., 3rd. Antithrombotic therapy in abdominal aortic aneurysm: beneficial or detrimental? *Blood* **132**, 2619-2628, doi:10.1182/blood-2017-08-743237 (2018).

50 José Luis, A. & Wolfgang, H. G. Cellular mechanotransduction. *AIMS Biophysics* **3**, 50-62, doi:10.3934/biophy.2016.1.50 (2016).

51 Plow, E. F., Meller, J. & Byzova, T. V. Integrin function in vascular biology: a view from 2013. *Curr Opin Hematol* **21**, 241-247, doi:10.1097/moh.0000000000000042 (2014).

52 Chastney, M. R., Conway, J. R. W. & Ivaska, J. Integrin adhesion complexes. *Curr Biol* **31**, R536-r542, doi:10.1016/j.cub.2021.01.038 (2021).

53 Chen, W., Lou, J., Evans, E. A. & Zhu, C. Observing force-regulated conformational changes and ligand dissociation from a single integrin on cells. *J Cell Biol* **199**, 497-512, doi:10.1083/jcb.201201091 (2012).

54 Takagi, J., Petre, B. M., Walz, T. & Springer, T. A. Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. *Cell* **110**, 599-511, doi:10.1016/s0092-8674(02)00935-2 (2002).

55 Oria, R. *et al.* Force loading explains spatial sensing of ligands by cells. *Nature* **552**, 219-224, doi:10.1038/nature24662 (2017).

56 Pang, X. *et al.* Targeting integrin pathways: mechanisms and advances in therapy. *Signal Transduction and Targeted Therapy* **8**, 1, doi:10.1038/s41392-022-01259-6 (2023).

57 Shattil, S. J., Kim, C. & Ginsberg, M. H. The final steps of integrin activation: the end game. *Nature Reviews Molecular Cell Biology* **11**, 288-300, doi:10.1038/nrm2871 (2010).

58 Muether, P. S. *et al.* The role of integrin alpha5beta1 in the regulation of corneal neovascularization. *Experimental eye research* **85**, 356-365, doi:10.1016/j.exer.2007.06.004 (2007).

59 Ruoslahti, E. Specialization of tumour vasculature. *Nat Rev Cancer* **2**, 83-90, doi:10.1038/nrc724 (2002).

60 Kim, S., Bell, K., Mousa, S. A. & Varner, J. A. Regulation of angiogenesis in vivo by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. *Am J Pathol* **156**, 1345-1362, doi:10.1016/s0002-9440(10)65005-5 (2000).

61 Magnon, C. *et al.* Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with alphavbeta3 and alphavbeta5 integrins. *Cancer research* **65**, 4353-4361, doi:10.1158/0008-5472.Can-04-3536 (2005).

62 Eliceiri, B. P. & Cheresh, D. A. Adhesion events in angiogenesis. *Curr Opin Cell Biol* **13**, 563-568, doi:10.1016/s0955-0674(00)00252-0 (2001).

63 Friedlander, M. *et al.* Definition of two angiogenic pathways by distinct α v integrins. *Science* **270**, 1500-1502 (1995).

64 Hood, J. D., Frausto, R., Kiosses, W. B., Schwartz, M. A. & Cheresh, D. A. Differential alphav integrin-mediated Ras-ERK signaling during two pathways of angiogenesis. *The Journal of cell biology* **162**, 933-943, doi:10.1083/jcb.200304105 (2003).

65 Mahabeleshwar, G. H., Feng, W., Reddy, K., Plow, E. F. & Byzova, T. V. Mechanisms of integrin-vascular endothelial growth factor receptor cross-activation in angiogenesis. *Circulation research* **101**, 570-580, doi:10.1161/circresaha.107.155655 (2007).

66 Somanath, P. R., Malinin, N. L. & Byzova, T. V. Cooperation between integrin alphavbeta3 and VEGFR2 in angiogenesis. *Angiogenesis* **12**, 177-185, doi:10.1007/s10456-009-9141-9 (2009).

67 da Silva, R. G. *et al.* Endothelial alpha3beta1-integrin represses pathological angiogenesis and sustains endothelial-VEGF. *Am J Pathol* **177**, 1534-1548, doi:10.2353/ajpath.2010.100043 (2010).

68 Bouvard, C. *et al.* Tie2-dependent deletion of α 6 integrin subunit in mice reduces tumor growth and angiogenesis. *Int J Oncol* **45**, 2058-2064, doi:10.3892/ijo.2014.2631 (2014).

69 Steri, V. *et al.* Acute depletion of endothelial β 3-integrin transiently inhibits tumor growth and angiogenesis in mice. *Circ Res* **114**, 79-91, doi:10.1161/circresaha.114.301591 (2014).

70 von Andrian, U. H. *et al.* Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and the leukocyte beta 2 integrins in vivo. *Proc Natl Acad Sci U S A* **88**, 7538-7542, doi:10.1073/pnas.88.17.7538 (1991).

71 Lämmermann, T. *et al.* Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature* **453**, 51-55, doi:10.1038/nature06887 (2008).

72 Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N. & Kupfer, A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395**, 82-86, doi:10.1038/25764 (1998).

73 Szukiewicz, D. *et al.* CX3CL1 (fractalkine) and TNF α production by perfused human placental lobules under normoxic and hypoxic conditions in vitro: the importance of CX3CR1 signaling. *Inflamm Res* **63**, 179-189, doi:10.1007/s00011-013-0687-z (2014).

74 Yamashiro, S. & Watanabe, N. A new link between the retrograde actin flow and focal adhesions. *The Journal of Biochemistry* **156**, 239-248, doi:10.1093/jb/mvu053 (2014).

75 Dasanayake, N. L., Michalski, P. J. & Carlsson, A. E. General mechanism of actomyosin contractility. *Phys Rev Lett* **107**, 118101, doi:10.1103/PhysRevLett.107.118101 (2011).

76 Sun, Z., Guo, S. S. & Fässler, R. Integrin-mediated mechanotransduction. *J Cell Biol* **215**, 445-456, doi:10.1083/jcb.201609037 (2016).

77 Goult, B. T. *et al.* RIAM and vinculin binding to talin are mutually exclusive and regulate adhesion assembly and turnover. *J Biol Chem* **288**, 8238-8249, doi:10.1074/jbc.M112.438119 (2013).

78 Goult, B. T., Yan, J. & Schwartz, M. A. Talin as a mechanosensitive signaling hub. *J Cell Biol* **217**, 3776-3784, doi:10.1083/jcb.201808061 (2018).

79 Li, G. *et al.* Full activity of the deleted in liver cancer 1 (DLC1) tumor suppressor depends on an LD-like motif that binds talin and focal adhesion kinase (FAK). *Proc Natl Acad Sci U S A* **108**, 17129-17134, doi:10.1073/pnas.1112122108 (2011).

80 Zacharchenko, T. *et al.* LD Motif Recognition by Talin: Structure of the Talin-DLC1 Complex. *Structure* **24**, 1130-1141, doi:10.1016/j.str.2016.04.016 (2016).

81 Mierke, C. T. *et al.* Mechano-coupling and regulation of contractility by the vinculin tail domain. *Biophys J* **94**, 661-670, doi:10.1529/biophysj.107.108472 (2008).

82 Nuckolls, G. H., Turner, C. E. & Burridge, K. Functional studies of the domains of talin. *J Cell Biol* **110**, 1635-1644, doi:10.1083/jcb.110.5.1635 (1990).

83 DeMali, K. A., Barlow, C. A. & Burridge, K. Recruitment of the Arp2/3 complex to vinculin: coupling membrane protrusion to matrix adhesion. *J Cell Biol* **159**, 881-891, doi:10.1083/jcb.200206043 (2002).

84 Reinhard, M., Rüdiger, M., Jockusch, B. M. & Walter, U. VASP interaction with vinculin: a recurring theme of interactions with proline-rich motifs. *FEBS Lett* **399**, 103-107, doi:10.1016/s0014-5793(96)01295-1 (1996).

85 Brindle, N. P., Holt, M. R., Davies, J. E., Price, C. J. & Critchley, D. R. The focal-adhesion vasodilator-stimulated phosphoprotein (VASP) binds to the proline-rich domain in vinculin. *Biochem J* **318 (Pt 3)**, 753-757, doi:10.1042/bj3180753 (1996).

86 Huveneers, S. *et al.* Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. *J Cell Biol* **196**, 641-652, doi:10.1083/jcb.201108120 (2012).

87 Mechanobiology Institute, N. U. o. S. *What are mature focal adhesions composed of?*, <https://www.mbi.nus.edu.sg/mbinfo/what-are-mature-focal-adhesions-composed-of/> (2023).

88 Dejana, E., Corada, M. & Lampugnani, M. G. Endothelial cell-to-cell junctions. *Faseb j* **9**, 910-918 (1995).

89 Kowalczyk, A. P. *et al.* VE-cadherin and desmoplakin are assembled into dermal microvascular endothelial intercellular junctions: a pivotal role for plakoglobin in the recruitment of desmoplakin to intercellular junctions. *J Cell Sci* **111 (Pt 20)**, 3045-3057, doi:10.1242/jcs.111.20.3045 (1998).

90 Komarova, Y. A., Kruse, K., Mehta, D. & Malik, A. B. Protein Interactions at Endothelial Junctions and Signaling Mechanisms Regulating Endothelial Permeability. *Circ Res* **120**, 179-206, doi:10.1161/circresaha.116.306534 (2017).

91 Bazzoni, G. & Dejana, E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiol Rev* **84**, 869-901, doi:10.1152/physrev.00035.2003 (2004).

92 Grimsley-Myers, C. M. *et al.* VE-cadherin endocytosis controls vascular integrity and patterning during development. *J Cell Biol* **219**, doi:10.1083/jcb.201909081 (2020).

93 Abe, K. & Takeichi, M. EPLIN mediates linkage of the cadherin catenin complex to F-actin and stabilizes the circumferential actin belt. *Proc Natl Acad Sci U S A* **105**, 13-19, doi:10.1073/pnas.0710504105 (2008).

94 Weiss, E. E., Kroemker, M., Rüdiger, A. H., Jockusch, B. M. & Rüdiger, M. Vinculin is part of the cadherin-catenin junctional complex: complex formation between alpha-catenin and vinculin. *J Cell Biol* **141**, 755-764, doi:10.1083/jcb.141.3.755 (1998).

95 Knudsen, K. A., Soler, A. P., Johnson, K. R. & Wheelock, M. J. Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J Cell Biol* **130**, 67-77, doi:10.1083/jcb.130.1.67 (1995).

96 Coon, B. G. *et al.* Intramembrane binding of VE-cadherin to VEGFR2 and VEGFR3 assembles the endothelial mechanosensory complex. *J Cell Biol* **208**, 975-986, doi:10.1083/jcb.201408103 (2015).

97 Kutys, M. L. & Chen, C. S. Forces and mechanotransduction in 3D vascular biology. *Curr Opin Cell Biol* **42**, 73-79, doi:10.1016/j.ceb.2016.04.011 (2016).

98 Du Toit, A. VE-cadherin lets it flow. *Nature Reviews Molecular Cell Biology* **16**, 268-268, doi:10.1038/nrm3985 (2015).

99 Murakami, M. & Simons, M. Regulation of vascular integrity. *J Mol Med (Berl)* **87**, 571-582, doi:10.1007/s00109-009-0463-2 (2009).

100 Huynh, J. *et al.* Age-related intimal stiffening enhances endothelial permeability and leukocyte transmigration. *Sci Transl Med* **3**, 112ra122, doi:10.1126/scitranslmed.3002761 (2011).

101 Navarro, P., Ruco, L. & Dejana, E. Differential localization of VE- and N-cadherins in human endothelial cells: VE-cadherin competes with N-cadherin for junctional localization. *J Cell Biol* **140**, 1475-1484, doi:10.1083/jcb.140.6.1475 (1998).

102 Takeichi, M. The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development* **102**, 639-655, doi:10.1242/dev.102.4.639 (1988).

103 Kruse, K. *et al.* N-cadherin signaling via Trio assembles adherens junctions to restrict endothelial permeability. *J Cell Biol* **218**, 299-316, doi:10.1083/jcb.201802076 (2019).

104 Yonemura, S., Wada, Y., Watanabe, T., Nagafuchi, A. & Shibata, M. alpha-Catenin as a tension transducer that induces adherens junction development. *Nat Cell Biol* **12**, 533-542, doi:10.1038/ncb2055 (2010).

105 Yao, M. *et al.* Force-dependent conformational switch of α-catenin controls vinculin binding. *Nature Communications* **5**, 4525, doi:10.1038/ncomms5525 (2014).

106 Dorland, Y. L. & Huveneers, S. Cell-cell junctional mechanotransduction in endothelial remodeling. *Cell Mol Life Sci* **74**, 279-292, doi:10.1007/s00018-016-2325-8 (2017).

107 Zihni, C., Mills, C., Matter, K. & Balda, M. S. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat Rev Mol Cell Biol* **17**, 564-580, doi:10.1038/nrm.2016.80 (2016).

108 Guillemot, L., Paschoud, S., Pulimeno, P., Foglia, A. & Citi, S. The cytoplasmic plaque of tight junctions: a scaffolding and signalling center. *Biochim Biophys Acta* **1778**, 601-613, doi:10.1016/j.bbamem.2007.09.032 (2008).

109 Heinemann, U. & Schuetz, A. Structural Features of Tight-Junction Proteins. *Int J Mol Sci* **20**, doi:10.3390/ijms20236020 (2019).

110 Nitta, T. *et al.* Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J Cell Biol* **161**, 653-660, doi:10.1083/jcb.200302070 (2003).

111 Hautefort, A., Pfenniger, A. & Kwak, B. R. Endothelial connexins in vascular function. *Vasc Biol* **1**, H117-h124, doi:10.1530/vb-19-0015 (2019).

112 Simon, A. M. & Goodenough, D. A. Diverse functions of vertebrate gap junctions. *Trends Cell Biol* **8**, 477-483, doi:10.1016/s0962-8924(98)01372-5 (1998).

113 Fletcher, D. A. & Mullins, R. D. Cell mechanics and the cytoskeleton. *Nature* **463**, 485-492, doi:10.1038/nature08908 (2010).

114 Khan, A. U., Qu, R., Fan, T., Ouyang, J. & Dai, J. A glance on the role of actin in osteogenic and adipogenic differentiation of mesenchymal stem cells. *Stem Cell Research & Therapy* **11**, 283, doi:10.1186/s13287-020-01789-2 (2020).

115 Byfield, F. J., Reen, R. K., Shentu, T. P., Levitan, I. & Gooch, K. J. Endothelial actin and cell stiffness is modulated by substrate stiffness in 2D and 3D. *Journal of biomechanics* **42**, 1114-1119, doi:10.1016/j.jbiomech.2009.02.012 (2009).

116 Millán, J. *et al.* Adherens junctions connect stress fibres between adjacent endothelial cells. *BMC Biol* **8**, 11, doi:10.1186/1741-7007-8-11 (2010).

117 Ridley, A. J. Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. *Trends Cell Biol* **16**, 522-529, doi:10.1016/j.tcb.2006.08.006 (2006).

118 Bhadriraju, K. *et al.* Activation of ROCK by RhoA is regulated by cell adhesion, shape, and cytoskeletal tension. *Exp Cell Res* **313**, 3616-3623, doi:10.1016/j.yexcr.2007.07.002 (2007).

119 Huveneers, S., Daemen, M. J. & Hordijk, P. L. Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. *Circ Res* **116**, 895-908, doi:10.1161/circresaha.116.305720 (2015).

120 Kiosses, W. B., Shattil, S. J., Pampori, N. & Schwartz, M. A. Rac recruits high-affinity integrin alphavbeta3 to lamellipodia in endothelial cell migration. *Nat Cell Biol* **3**, 316-320, doi:10.1038/35060120 (2001).

121 Goldmann, W. H. & Ingber, D. E. Intact vinculin protein is required for control of cell shape, cell mechanics, and rac-dependent lamellipodia formation. *Biochem Biophys Res Commun* **290**, 749-755, doi:10.1006/bbrc.2001.6243 (2002).

122 Xia, N. *et al.* Directional control of cell motility through focal adhesion positioning and spatial control of Rac activation. *Faseb j* **22**, 1649-1659, doi:10.1096/fj.07-090571 (2008).

123 Shakhov, A. S. & Alieva, I. B. The "Third Violin" in the Cytoskeleton Orchestra-The Role of Intermediate Filaments in the Endothelial Cell's Life. *Biomedicines* **10**, doi:10.3390/biomedicines10040828 (2022).

124 Bruneel, A. *et al.* Proteomic study of human umbilical vein endothelial cells in culture. *Proteomics* **3**, 714-723, doi:10.1002/pmic.200300409 (2003).

125 Liu, T. *et al.* Regulation of vimentin intermediate filaments in endothelial cells by hypoxia. *Am J Physiol Cell Physiol* **299**, C363-373, doi:10.1152/ajpcell.00057.2010 (2010).

126 Kreis, S., Schönfeld, H. J., Melchior, C., Steiner, B. & Kieffer, N. The intermediate filament protein vimentin binds specifically to a recombinant integrin alpha2/beta1 cytoplasmic tail complex and co-localizes with native alpha2/beta1 in endothelial cell focal adhesions. *Exp Cell Res* **305**, 110-121, doi:10.1016/j.yexcr.2004.12.023 (2005).

127 Haase, K. *et al.* Extracellular Forces Cause the Nucleus to Deform in a Highly Controlled Anisotropic Manner. *Sci Rep* **6**, 21300, doi:10.1038/srep21300 (2016).

128 Sinha, B., Biswas, A. & Soni, G. V. Cellular and Nuclear Forces: An Overview. *Methods Mol Biol* **1805**, 1-29, doi:10.1007/978-1-4939-8556-2_1 (2018).

129 Ingber, D. E. Cellular mechanotransduction: putting all the pieces together again. *Faseb j* **20**, 811-827, doi:10.1096/fj.05-5424rev (2006).

130 Tajik, A. *et al.* Transcription upregulation via force-induced direct stretching of chromatin. *Nature materials* **15**, 1287-1296, doi:10.1038/nmat4729 (2016).

131 Tkachenko, E. *et al.* The nucleus of endothelial cell as a sensor of blood flow direction. *Biol Open* **2**, 1007-1012, doi:10.1242/bio.20134622 (2013).

132 Starr, D. A. & Fridolfsson, H. N. Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. *Annual review of cell and developmental biology* **26**, 421-444, doi:10.1146/annurev-cellbio-100109-104037 (2010).

133 Yu, J. *et al.* KASH protein Syne-2/Nesprin-2 and SUN proteins SUN1/2 mediate nuclear migration during mammalian retinal development. *Human molecular genetics* **20**, 1061-1073, doi:10.1093/hmg/ddq549 (2011).

134 Pennacchio, F. A., Nastały, P., Poli, A. & Maiuri, P. Tailoring Cellular Function: The Contribution of the Nucleus in Mechanotransduction. *Front Bioeng Biotechnol* **8**, 596746, doi:10.3389/fbioe.2020.596746 (2020).

135 Chancellor, T. J., Lee, J., Thodeti, C. K. & Lele, T. Actomyosin tension exerted on the nucleus through nesprin-1 connections influences endothelial cell adhesion, migration, and cyclic strain-induced reorientation. *Biophys J* **99**, 115-123, doi:10.1016/j.bpj.2010.04.011 (2010).

136 King, S. J. *et al.* Nesprin-1 and nesprin-2 regulate endothelial cell shape and migration. *Cytoskeleton (Hoboken)* **71**, 423-434, doi:10.1002/cm.21182 (2014).

137 Denis, K. B. *et al.* The LINC complex is required for endothelial cell adhesion and adaptation to shear stress and cyclic stretch. *Mol Biol Cell* **32**, 1654-1663, doi:10.1091/mbc.E20-11-0698 (2021).

138 Alam, S. G. *et al.* The mammalian LINC complex regulates genome transcriptional responses to substrate rigidity. *Sci Rep* **6**, 38063, doi:10.1038/srep38063 (2016).

139 Amiad Pavlov, D. *et al.* The LINC Complex Inhibits Excessive Chromatin Repression. *Cells* **12**, doi:10.3390/cells12060932 (2023).

140 Meng, Z., Moroishi, T. & Guan, K. L. Mechanisms of Hippo pathway regulation. *Genes & development* **30**, 1-17, doi:10.1101/gad.274027.115 (2016).

141 Lopez-Hernandez, A., Sberna, S. & Campaner, S. Emerging Principles in the Transcriptional Control by YAP and TAZ. *Cancers* **13**, 4242 (2021).

142 Neto, F. *et al.* YAP and TAZ regulate adherens junction dynamics and endothelial cell distribution during vascular development. *eLife* **7**, doi:10.7554/eLife.31037 (2018).

143 Choi, H. J. *et al.* Yes-associated protein regulates endothelial cell contact-mediated expression of angiopoietin-2. *Nature communications* **6**, 6943, doi:10.1038/ncomms7943 (2015).

144 Wang, L. *et al.* Integrin-YAP/TAZ-JNK cascade mediates atheroprotective effect of unidirectional shear flow. *Nature* **540**, 579-582, doi:10.1038/nature20602 (2016).

145 Dupont, S. *et al.* Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179-183, doi:10.1038/nature10137 (2011).

146 Matsuo, E. *et al.* Substrate stiffness modulates endothelial cell function via the YAP-Dll4-Notch1 pathway. *Experimental Cell Research* **408**, 112835, doi:https://doi.org/10.1016/j.yexcr.2021.112835 (2021).

147 Troyanovsky, B., Levchenko, T., Månsson, G., Matvijenko, O. & Holmgren, L. Angiomotin: an angiostatin binding protein that regulates endothelial cell migration and tube formation. *The Journal of cell biology* **152**, 1247-1254, doi:10.1083/jcb.152.6.1247 (2001).

148 Bratt, A. *et al.* Angiomotin belongs to a novel protein family with conserved coiled-coil and PDZ binding domains. *Gene* **298**, 69-77, doi:10.1016/s0378-1119(02)00928-9 (2002).

149 Nishimura, M. *et al.* JEAP, a novel component of tight junctions in exocrine cells. *J Biol Chem* **277**, 5583-5587, doi:10.1074/jbc.M110154200 (2002).

150 Zhang, Y. *Ready to pull : force transmission during vascular development*, Karolinska Institutet, (2019).

151 Ernkvist, M. *et al.* The Amot/Patj/Syx signaling complex spatially controls RhoA GTPase activity in migrating endothelial cells. *Blood* **113**, 244-253, doi:10.1182/blood-2008-04-153874 (2009).

152 Hultin, S. *et al.* AmotL2 integrates polarity and junctional cues to modulate cell shape. *Sci Rep* **7**, 7548, doi:10.1038/s41598-017-07968-1 (2017).

153 Yi, C. *et al.* A tight junction-associated Merlin-angiomotin complex mediates Merlin's regulation of mitogenic signaling and tumor suppressive functions. *Cancer Cell* **19**, 527-540, doi:10.1016/j.ccr.2011.02.017 (2011).

154 Mana-Capelli, S., Paramasivam, M., Dutta, S. & McCollum, D. Angiomotins link F-actin architecture to Hippo pathway signaling. *Mol Biol Cell* **25**, 1676-1685, doi:10.1091/mbc.E13-11-0701 (2014).

155 Moleirinho, S., Guerrant, W. & Kissil, J. L. The Angiomotins--from discovery to function. *FEBS letters* **588**, 2693-2703, doi:10.1016/j.febslet.2014.02.006 (2014).

156 Zhao, B. *et al.* Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. *Genes Dev* **25**, 51-63, doi:10.1101/gad.2000111 (2011).

157 Wang, W., Huang, J. & Chen, J. Angiomotin-like proteins associate with and negatively regulate YAP1. *J Biol Chem* **286**, 4364-4370, doi:10.1074/jbc.C110.205401 (2011).

158 Leung, C. Y. & Zernicka-Goetz, M. Angiomotin prevents pluripotent lineage differentiation in mouse embryos via Hippo pathway-dependent and -independent mechanisms. *Nat Commun* **4**, 2251, doi:10.1038/ncomms3251 (2013).

159 Shimono, A. & Behringer, R. R. Angiomotin regulates visceral endoderm movements during mouse embryogenesis. *Current biology : CB* **13**, 613-617, doi:10.1016/s0960-9822(03)00204-5 (2003).

160 Aase, K. *et al.* Angiomotin regulates endothelial cell migration during embryonic angiogenesis. *Genes Dev* **21**, 2055-2068, doi:10.1101/gad.432007 (2007).

161 Holmgren, L. *et al.* A DNA vaccine targeting angiomotin inhibits angiogenesis and suppresses tumor growth. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 9208-9213, doi:10.1073/pnas.0603110103 (2006).

162 Wigerius, M. *et al.* The polarity protein Angiomotin p130 controls dendritic spine maturation. *J Cell Biol* **217**, 715-730, doi:10.1083/jcb.201705184 (2018).

163 Rojek, K. O. *et al.* Amot and Yap1 regulate neuronal dendritic tree complexity and locomotor coordination in mice. *PLoS Biol* **17**, e3000253, doi:10.1371/journal.pbio.3000253 (2019).

164 Zheng, Y. *et al.* Angiomotin-like protein 1 controls endothelial polarity and junction stability during sprouting angiogenesis. *Circ Res* **105**, 260-270, doi:10.1161/circresaha.109.195156 (2009).

165 Zheng, Y. *et al.* Angiomotin like-1 is a novel component of the N-cadherin complex affecting endothelial/pericyte interaction in normal and tumor angiogenesis. *Sci Rep* **6**, 30622, doi:10.1038/srep30622 (2016).

166 Sistani, L. *et al.* Pdlim2 is a novel actin-regulating protein of podocyte foot processes. *Kidney Int* **80**, 1045-1054, doi:10.1038/ki.2011.231 (2011).

167 Couderc, C. *et al.* AMOTL1 Promotes Breast Cancer Progression and Is Antagonized by Merlin. *Neoplasia* **18**, 10-24, doi:10.1016/j.neo.2015.11.010 (2016).

168 Hildebrand, S. *et al.* The E-cadherin/AmotL2 complex organizes actin filaments required for epithelial hexagonal packing and blastocyst hatching. *Scientific Reports* **7**, 9540, doi:10.1038/s41598-017-10102-w (2017).

169 Hultin, S. *et al.* AmotL2 links VE-cadherin to contractile actin fibres necessary for aortic lumen expansion. *Nat Commun* **5**, 3743, doi:10.1038/ncomms4743 (2014).

170 Mojallal, M. *et al.* AmotL2 disrupts apical-basal cell polarity and promotes tumour invasion. *Nature communications* **5**, 4557, doi:10.1038/ncomms5557 (2014).

171 Uhlén, M. *et al.* Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419, doi:10.1126/science.1260419 (2015).

172 Lindquist Liljeqvist, M. *et al.* Tunica-Specific Transcriptome of Abdominal Aortic Aneurysm and the Effect of Intraluminal Thrombus, Smoking, and Diameter Growth Rate. *Arterioscler Thromb Vasc Biol* **40**, 2700-2713, doi:10.1161/atvbaha.120.314264 (2020).

173 Levchenko, T., Aase, K., Troyanovsky, B., Bratt, A. & Holmgren, L. Loss of responsiveness to chemotactic factors by deletion of the C-terminal protein interaction site of angiomotin. *J Cell Sci* **116**, 3803-3810, doi:10.1242/jcs.00694 (2003).

174 Zhong, C. *et al.* Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. *J Cell Biol* **141**, 539-551, doi:10.1083/jcb.141.2.539 (1998).

175 Leiss, M., Beckmann, K., Girós, A., Costell, M. & Fässler, R. The role of integrin binding sites in fibronectin matrix assembly in vivo. *Curr Opin Cell Biol* **20**, 502-507, doi:10.1016/j.ceb.2008.06.001 (2008).

176 Dallas, S. L. *et al.* Fibronectin regulates latent transforming growth factor-beta (TGF beta) by controlling matrix assembly of latent TGF beta-binding protein-1. *J Biol Chem* **280**, 18871-18880, doi:10.1074/jbc.M410762200 (2005).

177 Roux, K. J., Kim, D. I., Burke, B. & May, D. G. BioID: A Screen for Protein-Protein Interactions. *Curr Protoc Protein Sci* **91**, 19.23.11-19.23.15, doi:10.1002/cpps.51 (2018).

178 Horton, E. R. *et al.* The integrin adhesome network at a glance. *J Cell Sci* **129**, 4159-4163, doi:10.1242/jcs.192054 (2016).

179 Ernkvist, M. *et al.* p130-angiomotin associates to actin and controls endothelial cell shape. *Febs j* **273**, 2000-2011, doi:10.1111/j.1742-4658.2006.05216.x (2006).

180 Das, T. *et al.* A molecular mechanotransduction pathway regulates collective migration of epithelial cells. *Nat Cell Biol* **17**, 276-287, doi:10.1038/ncb3115 (2015).

181 Wells, C. D. *et al.* A Rich1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells. *Cell* **125**, 535-548, doi:10.1016/j.cell.2006.02.045 (2006).

182 Busch, A. *et al.* Translating mouse models of abdominal aortic aneurysm to the translational needs of vascular surgery. *JVS-Vascular Science* **2**, 219-234, doi:https://doi.org/10.1016/j.jvssci.2021.01.002 (2021).

183 Berman, A. G. *et al.* Experimental aortic aneurysm severity and growth depend on topical elastase concentration and lysyl oxidase inhibition. *Scientific Reports* **12**, 99, doi:10.1038/s41598-021-04089-8 (2022).

184 Giampietro, C. *et al.* Overlapping and divergent signaling pathways of N-cadherin and VE-cadherin in endothelial cells. *Blood* **119**, 2159-2170, doi:10.1182/blood-2011-09-381012 (2012).

185 Li, Y. *et al.* The N-cadherin interactome in primary cardiomyocytes as defined using quantitative proximity proteomics. *J Cell Sci* **132**, doi:10.1242/jcs.221606 (2019).

186 Kalluri, A. S. *et al.* Single-Cell Analysis of the Normal Mouse Aorta Reveals Functionally Distinct Endothelial Cell Populations. *Circulation* **140**, 147-163, doi:10.1161/circulationaha.118.038362 (2019).

187 Xu, Y. *et al.* A single-cell transcriptome atlas profiles early organogenesis in human embryos. *Nat Cell Biol* **25**, 604-615, doi:10.1038/s41556-023-01108-w (2023).

188 Zhang, Y. *et al.* The VE-cadherin/AmotL2 mechanosensory pathway suppresses aortic inflammation and the formation of abdominal aortic aneurysms. *Nature Cardiovascular Research* **2**, 629-644, doi:10.1038/s44161-023-00298-8 (2023).

189 Zhang, Y. *et al.* The Amot/integrin protein complex transmits mechanical forces required for vascular expansion. *Cell Rep* **36**, 109616, doi:10.1016/j.celrep.2021.109616 (2021).

190 Garmy-Susini, B. *et al.* Integrin alpha4beta1-VCAM-1-mediated adhesion between endothelial and mural cells is required for blood vessel maturation. *J Clin Invest* **115**, 1542-1551, doi:10.1172/jci23445 (2005).

191 Kalukula, Y., Stephens, A. D., Lammerding, J. & Gabriele, S. Mechanics and functional consequences of nuclear deformations. *Nat Rev Mol Cell Biol* **23**, 583-602, doi:10.1038/s41580-022-00480-z (2022).

192 Miroshnikova, Y. A. & Wickström, S. A. Mechanical Forces in Nuclear Organization. *Cold Spring Harb Perspect Biol* **14**, doi:10.1101/cshperspect.a039685 (2022).

193 Isermann, P. & Lammerding, J. Nuclear mechanics and mechanotransduction in health and disease. *Curr Biol* **23**, R1113-1121, doi:10.1016/j.cub.2013.11.009 (2013).

194 Baarlink, C., Wang, H. & Grosse, R. Nuclear actin network assembly by formins regulates the SRF coactivator MAL. *Science* **340**, 864-867, doi:10.1126/science.1235038 (2013).

195 Plessner, M. & Grosse, R. Extracellular signaling cues for nuclear actin polymerization. *Eur J Cell Biol* **94**, 359-362, doi:10.1016/j.ejcb.2015.05.009 (2015).

196 Oliver-Williams, C. *et al.* Lessons learned about prevalence and growth rates of abdominal aortic aneurysms from a 25-year ultrasound population screening programme. *Br J Surg* **105**, 68-74, doi:10.1002/bjs.10715 (2018).

197 Ashton, H. A. *et al.* Fifteen-year follow-up of a randomized clinical trial of ultrasonographic screening for abdominal aortic aneurysms. *Br J Surg* **94**, 696-701, doi:10.1002/bjs.5780 (2007).

198 Golledge, J. & Powell, J. T. Medical management of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* **34**, 267-273, doi:10.1016/j.ejvs.2007.03.006 (2007).

199 Wanhainen, A. *et al.* Outcome of the Swedish Nationwide Abdominal Aortic Aneurysm Screening Program. *Circulation* **134**, 1141-1148, doi:10.1161/circulationaha.116.022305 (2016).

200 Caliskan, E. *et al.* Saphenous vein grafts in contemporary coronary artery bypass graft surgery. *Nat Rev Cardiol* **17**, 155-169, doi:10.1038/s41569-019-0249-3 (2020).

201 Xenogiannis, I. *et al.* Saphenous Vein Graft Failure: From Pathophysiology to Prevention and Treatment Strategies. *Circulation* **144**, 728-745, doi:10.1161/circulationaha.120.052163 (2021).