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REVIEW

SUBJECT COLLECTION: UBIQUITIN

Ubiquitin-based modifications in endothelial cell–cell contact and inflammation

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ABSTRACT

Endothelial cell–cell contacts are essential for vascular integrity and physiology, protecting tissues and organs from edema and uncontrolled invasion of inflammatory cells. The vascular endothelial barrier is dynamic, but its integrity is preserved through a tight control at different levels. Inflammatory cytokines and G-protein-coupled receptor agonists, such as histamine, reduce endothelial integrity and increase vascular leakage. This is due to elevated myosin-based contractility, in conjunction with phosphorylation of proteins at cell–cell contacts. Conversely, reducing contractility stabilizes or even increases endothelial junctional integrity. Rho GTPases are key regulators of such cytoskeletal dynamics and endothelial cell–cell contacts. In addition to signaling-induced regulation, the expression of junctional proteins, such as occludin, claudins and vascular endothelial cadherin, also controls endothelial barrier function. There is increasing evidence that, in addition to protein phosphorylation, ubiquitylation (also known as ubiquitination) is an important and dynamic post-translational modification that regulates Rho GTPases, junctional proteins and, consequently, endothelial barrier function. In this Review, we discuss the emerging role of ubiquitylation and deubiquitylation events in endothelial integrity and inflammation. The picture that emerges is one of increasing complexity, which is both fascinating and promising given the clinical relevance of vascular integrity in the control of inflammation, and of tissue and organ damage.

KEY WORDS: Endothelium, Inflammation, Ubiquitin

Introduction

The inner lining of all blood and lymphatic vessels is formed by a monolayer of vascular endothelial cells (ECs), which preserves integrity through dynamic but well-controlled cell–cell contacts. Loss of endothelial integrity, due to increased actomyosin-based contractility and reduced cell–cell contact, is among the first signs of inflammation and is associated with vascular pathology that accompanies chronic disorders, such as diabetes, atherosclerosis or rheumatoid arthritis (Bordy et al., 2018; Huveneers et al., 2015; Saharinen et al., 2017). Because of its clinical relevance, there is much interest in the mechanisms that govern endothelial integrity. This integrity is mainly determined by the adhesive function of vascular endothelial (VE)-cadherin, which acts in complex with F-actin-binding adapter proteins, such as β-catenin, α-catenin and vinculin. The adhesive function of this VE-cadherin (also known as CDH5) complex is mediated and controlled by actin dynamics and tyrosine (de)phosphorylation of VE-cadherin and β-catenin (Hordijk, 2016; Huveneers et al., 2012; Komarova et al., 2017; Orsenigo et al., 2012; Vestweber et al., 2014; Wessel et al., 2014). Recently, there has been growing interest in another post-translational modification that controls endothelial integrity, namely protein ubiquitylation (also known as ubiquitination).

Protein ubiquitylation is a three-step process, in which the 76-amino-acid peptide ubiquitin is transferred from an E1 to an E2 ligase, after which an associated E3 ligase catalyzes covalent linkage of the ubiquitin moiety to the substrate, in most cases on a lysine residue, or to lysine residues of a previously linked ubiquitin. This results in ubiquitin chain formation through, for example, K63 or K48 linkages (Fig. 1) (Rape, 2018). In addition to ubiquitin, cells also use ubiquitin-like proteins such as small ubiquitin-like modifier (SUMO) to modify target proteins (Akutsu et al., 2016; Kirkin and Dikic, 2007). It is estimated that there might be up to 600 ubiquitin E3 ligases, which can be subdivided in several families (Rape, 2018). Homologous to the E6AP C-terminus (HECT) ligases obtain the ubiquitin from the E2 ligase, prior to linkage to the substrate. In contrast, really interesting new gene (RING) ligases, multi-protein complexes comprised of scaffold, adapter and substrate recognition proteins, do not bind ubiquitin directly, but mediate its transfer from the E2 ligase to the substrate (Kirkin and Dikic, 2007; Schaefer et al., 2012). The ring-between-ring (RBR) E3 ligases (for example PARKIN) are a relatively small subgroup (14 members in humans) that combines features of HECT and RING ligases in their mode of ubiquitin binding and transfer to their substrates (Spratt et al., 2014; Walden and Rittinger, 2018). Finally, a new class of ubiquitin ligase was recently identified, designated RING-Cys-relay (RCR), which transfers ubiquitin to its substrate in a unique way, through esterification of a threonine, rather than a lysine, residue (Pao et al., 2018).

In addition to the variety in the types of ubiquitin ligase, there is also considerable complexity in the site-specific modifications with ubiquitin, including mono-, di- or poly-ubiquitylation, as well as linear or branched ubiquitin chains (Akutsu et al., 2016; Swatek and Komander, 2016) (Fig. 1). Moreover, as an example, the linear ubiquitin chain assembly complex (LUBAC) has a preference for sites that are already modified by K63-linked ubiquitin chains. Its addition of linear, methionine-linked ubiquitin results in a K63-polyUb/M1-polyUb hybrid or mixed chains (Cohen and Strickson, 2017; Emmerich et al., 2013).

There is increasing evidence that ubiquitylation not only serves to target the substrate for proteasomal degradation, but in fact controls cellular functions in many ways, including regulation of protein–protein interactions, vesicular trafficking, receptor internalization and subcellular localization of signaling proteins (Rape, 2018; Schaefer et al., 2012). Since ubiquitylation is reversible through the action of deubiquitylating enzymes (DUBs), it qualifies as a bona fide signal transduction event, similar to (de)phosphorylation or (de)acetylation (Kovačević et al., 2018; Li et al., 2018; Lv et al., 2018; Nethe et al., 2010, 2012; Nethe and Hordijk, 2010; Schaefer et al., 2012).

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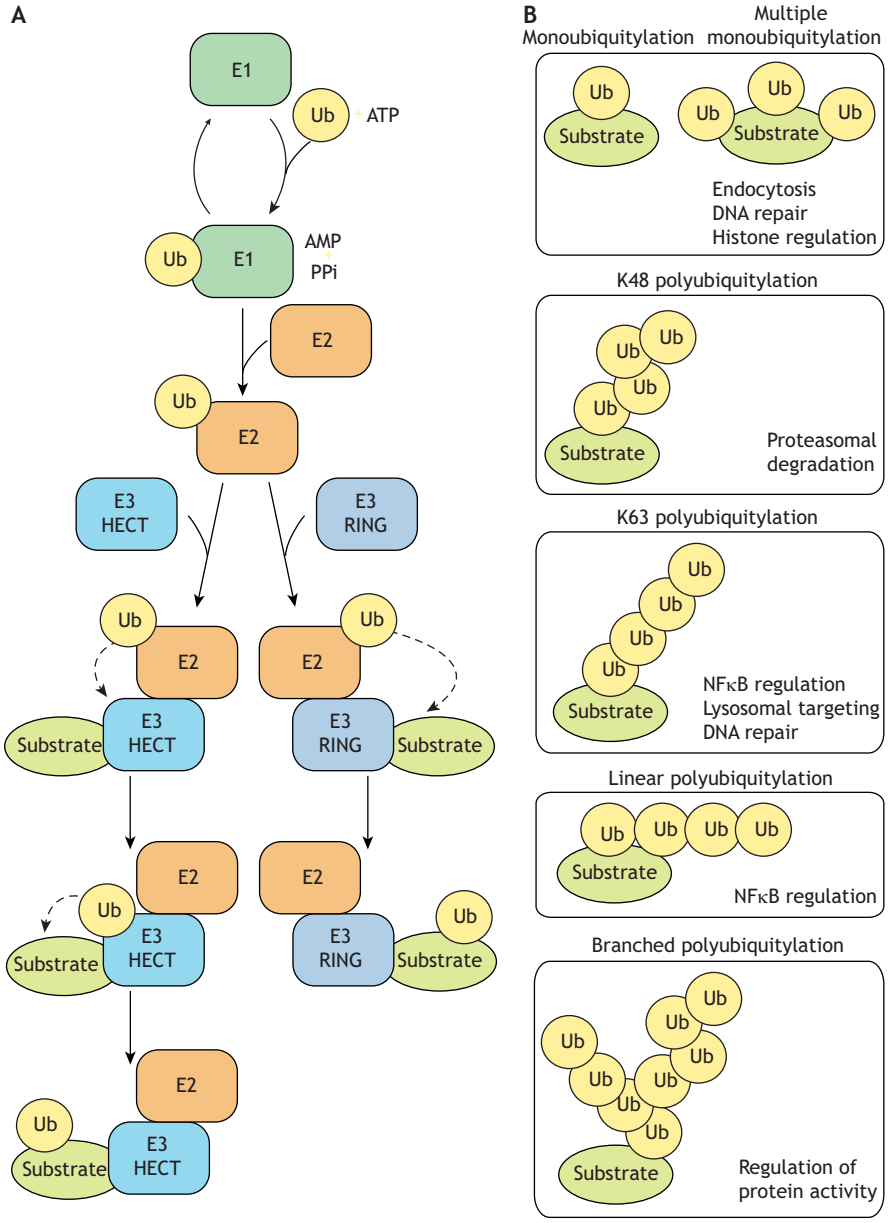

Fig. 1. Protein ubiquitylation complexity. (A) The different steps in protein ubiquitylation through E1, E2 and E3 ligases are depicted. Ubiquitin (Ub) is transferred from the E1 to E2 and subsequently the HECT or RING E3 ligase. The E3 ligase interacts with the substrate for final ubiquitin transfer, either directly to the substrate acceptor site or to a lysine residue in already linked ubiquitin, resulting in chain formation. (B) A selection of different ubiquitin chain elongation and branching products are depicted with their associated cellular responses (e.g. proteasomal degradation or endocytosis) indicated. Importantly, the orientation of ubiquitin moieties is different in K48-linked chains compared to K63-linked chains. This allows different binding partners to associate to either of these poly-ubiquitin chains for signal transmission. Please note that additional linkage types are known, see also main text.

Increasing numbers of ubiquitin E3 ligases and DUBs have recently been linked to endothelial cell–cell contact and inflammation. There is evidence that ubiquitylation regulates proximal signaling induced by inflammatory cytokines, such as tumor necrosis factor (TNF), that increase endothelial permeability, leading to edema and, eventually, tissue damage (Heger et al., 2018; Kovačević et al., 2018; Kwon et al., 2016; Lv et al., 2018; Nanes et al., 2017). Here, we will first briefly address ubiquitin modifications that have been described in inflammatory cytokine signaling, before discussing the regulation of Rho GTPases by ubiquitylation. Rho GTPases act downstream of inflammatory and other activating agonists, and are considered master regulators of endothelial cell–cell contact and inflammation. Finally, we discuss ubiquitylation of junctional proteins and the relevance of ubiquitylation for vascular disease.

Ubiquitylation in cytokine-induced inflammation and vascular integrity

Inflammatory signaling in ECs serves to protect tissues from excessive damage by initiating, directly or indirectly, the removal of infectious or damaging agents. Uncontrolled or low-grade chronic inflammation, however, leads to pathologies, such as rheumatoid arthritis or atherosclerosis. ECs are among the first cells to participate in an inflammatory response. In ECs, this response comprises the production of reactive oxygen species (ROS), upregulation of adhesion molecules that recruit activated leukocytes and lymphocytes, a disruption of the endothelial barrier and increased leukocyte diapedesis (Nourshargh et al., 2010). One of the key pathways that drives these effects downstream from the pro-inflammatory cytokine TNFα, the interleukins IL-1β and IL-17, or Toll-like receptor (TLR) ligands such as lipopolysaccharide (LPS), is the nuclear factor κB (NFκB) pathway (Ebner et al., 2017; Kawasaki and Kawai, 2014). The NFκB pathway is complex, and its activation comprises a series of different components that are regulated by both phosphorylation and ubiquitylation (Grabbe et al., 2011). The first level of ubiquitin-mediated regulation concerns the transmembrane receptors and associated adaptor proteins. These are typified by the E3 ligases cellular inhibitor of apoptosis protein (cIAP)1 and cIAP2 (also

known as BIRC2 and BIRC3, respectively), LUBAC and Itch, which have all been linked to the TNF-induced ubiquitylation of receptor interacting protein 1 (RIP1, also known as RIPK1) (Boisson et al., 2012; Bradley, 2008; Ikeda, 2015; Micheau and Tschopp, 2003). Ubiquitylation by these ligases and regulated deubiquitylation by the DUBs cylindromatosis (CYLD), A20 (also known as TNFAIP3) and Otulin are crucial for steering the TNF pathway towards pro-survival NFκB-dependent signaling (Heger et al., 2018; Keusekotten et al., 2013; Kovalenko et al., 2003; Wertz et al., 2016, 2004). Furthermore, polyubiquitin chains assembled by these E3 ligases are required for the activation of the IκB kinase (IKK)α–IKKβ complex and subsequent phosphorylation of IκBα, the inhibitory subunit of NFκB (Kanarek and Ben-Neriah, 2012). Phosphorylated IκBα is subsequently ubiquitylated by the E3 ligase SCF-βTRCP [the Skp, cullin, F-box-containing complex containing βTRCP (also known as BTRC) as the F-box protein] and degraded by the proteasome. This results in the release and nuclear translocation of NFκB, which then activates the transcription of genes required for cell survival and leukocyte-endothelial cell interactions (Boisson et al., 2012; Christian et al., 2016; Kanarek and Ben-Neriah, 2012).

In TNF-treated retinal ECs, NFκB-dependent loss of vascular integrity has been linked to reduced expression and altered subcellular localization of the tight junction proteins Zonula occludens 1 (ZO-1, also known as TJP1) and claudin-5 (Aveleira et al., 2010). In lymphatic ECs, IL-1β and TNF have been found to decrease the expression of VE-cadherin and activate actomyosin-based contraction, partially through an endothelial nitric oxide synthase (eNOS, also known as NOS3)-dependent mechanism whose molecular details remain to be established (Cromer et al., 2014). Finally, in murine brain ECs, IL-17 induces ROS production and actomyosin contraction, resulting in the downregulation of the tight junction protein occludin and disruption of the endothelial barrier (Huppert et al., 2010).

Together, while ubiquitylation is an abundant post-translational modification (PTM) in cytokine-induced proximal signaling, its regulation of the associated reduced expression of junctional proteins and the loss of endothelial barrier function remains to be further investigated. In this context, the role of actomyosin-based contraction, which may weaken junctions mechanically and thereby indirectly induce junctional protein internalization and possibly degradation, also warrants more detailed analysis.

Rho GTPase ubiquitylation and endothelial integrity

The integrity of the endothelial barrier is dependent on the dynamic stability of cell–cell contacts in the monolayer. Each EC exerts pushing and pulling forces on its neighboring cells, and the net result of these forces determines junctional integrity and permeability. Changes in these forces, for instance due to vascular contraction or relaxation, will determine the response of ECs to maintain and restore the barrier. ECs control the strength of their cell–cell contacts in part through the actin cytoskeleton, for instance by inducing actin (de)polymerization or the formation and bundling of F-actin stress fibers. The key molecular players that control these actin dynamics are members of the family of Rho GTPases.

Rho GTPases switch between GTP-bound ‘on’ and GDP-bound ‘off’ states through the action of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively (Bos et al., 2007). When GTP bound, Rho GTPases are typically located at the plasma membrane where they interact with their effectors, including protein kinases and actin-binding proteins. This way, they affect the local assembly or disassembly of F-actin and allow polarized regulation of cell motility (Sit and Manser, 2011; Bravo-Cordero et al., 2013; Bustelo et al., 2012; Kraynov et al., 2000; Machacek et al., 2009). In their GDP-bound state, most Rho GTPases are bound to a member of the cytosolic Rho GDP dissociation inhibitor (GDI) family, which prevent nucleotide dissociation and thus the activation of Rho GTPases. The GDI also protects the Rho GTPase from degradation (Boulter et al., 2010; Dovas and Couchman, 2005).

More than 20 members of the family of Rho GTPases have been described thus far (Schaefer et al., 2014). The best characterized of these, and which are implicated in endothelial barrier function, are RhoA, RhoB, RhoC, Rac1 and Cdc42, each with distinct roles in cell adhesion (Pronk et al., 2017; Ridley, 2015). Typically, RhoA activity leads to stress fiber formation and actomyosin-based contraction, whereas activation of Rac1 and Cdc42 gives rise to actin polymerization and cell spreading due to the formation of lamellipodia and filopodia, respectively. The mechanical force exerted by contracting F-actin filaments on VE-cadherin complexes, and thereby on cell–cell contacts in an intact monolayer, can lead to barrier disruption. Consequently, tightly coordinated (in)activation of Rho GTPase signaling is essential for the stabilization, disruption and reformation of endothelial junctions (Huveneers et al., 2015). Localization of Rho GTPases is key to their activation and downstream signaling and is regulated by PTMs, including phosphorylation, isoprenylation, palmitoylation and, as identified more recently, sumoylation and ubiquitylation (Hodge and Ridley, 2016).

A clear role for ubiquitylation in the regulation of both Rho GTPase localization, as well as proteasomal degradation has been shown by us and others. Treatment of human umbilical vein endothelial cell (HUVEC) monolayers with the neddylation inhibitor MLN4924, an inhibitor of cullin RING ligase (CRL) activity, induces a rapid loss of endothelial barrier function (Sakaue et al., 2017; Kovačević et al., 2018). Neddylation is the covalent attachment of the ubiquitin-like Nedd8 protein to the cullin scaffold proteins, a modification that is required for their activity. MLN4942-induced loss of barrier function is accompanied by an increase in the levels of RhoB, which results from a decrease in RhoB degradation (Kovačević et al., 2018). This indicates that, in resting conditions, HUVECs constantly ubiquitylate and degrade RhoB through CRLs to preserve endothelial integrity. The de-neddylation inhibitor CSN5i-3 caused similar barrier-disruptive results, which were also accompanied by an increase in RhoB levels, although in this case, this was a result of increased IκB degradation, NFκB activation and a subsequent increase in RhoB transcription (Kovačević et al., 2018; Marcos-Ramiro et al., 2016; Schaefer et al., 2014; Wojciak-Stothard et al., 2001). Thus, CRLs play divergent roles in endothelial integrity, in part direct, by ubiquitylating key signaling molecules, such as RhoB, and indirect, through the activation of inflammatory signaling pathways. Since the role of ubiquitylation in the localization and activity of Rho GTPases has been previously reviewed (Cai et al., 2018; de la Vega et al., 2011; Ding et al., 2011; Hodge and Ridley, 2016), here, we limit the discussion to their regulation of endothelial barrier function (Fig. 2).

RhoA

In human embryonic kidney 233 (HEK293T) and mouse embryonic fibroblast (MEF) cells, RhoA is targeted for ubiquitylation and degradation at K5 and K6 by the HECT ligase Smurf1 (Boyer et al., 2006), an event localized at cellular protrusions (Wang et al., 2003). Smurf1 acts in a complex formed by Cdc42, PAR6 and PKCζ, which induces the ubiquitylation and degradation of RhoA. This in turn

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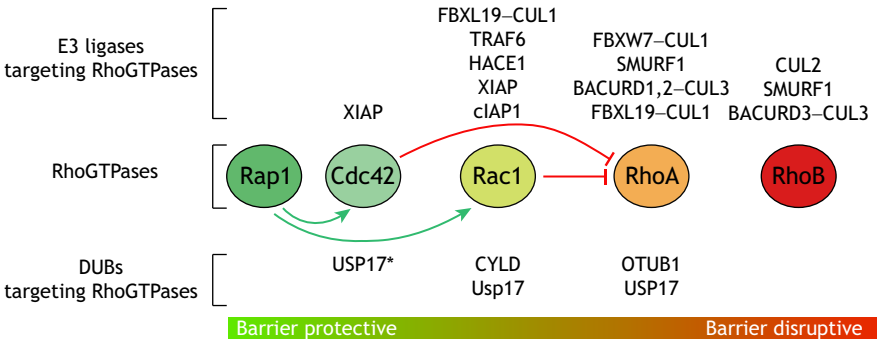

Fig. 2. Ubiquitin E3 ligases and DUBs regulating Rho GTPases. Indicated here are the different ubiquitin ligases as well as several identified DUBs that target the Rho GTPases. Barrier-protecting or -disrupting functions of the different Rho GTPases are highlighted, with RhoA and RhoB being disruptive, and Rac1 and Cdc42 protective. The Ras-like GTPase Rap1 is known for its barrier-stabilizing properties and is therefore included in the figure. See main text for further details. BACURD, BTB-containing adaptor for Cul3-mediated RhoA degradation.

stimulates the localized activation of Rac1 and Cdc42, thereby identifying ubiquitylation as part of the mechanism by which RhoA inhibits signaling by Rac1 and/or Cdc42 (Izzi and Attisano, 2006; Sander et al., 1999). In brain ECs, crebral cavernous malformation protein 2 (CCM2) localizes Smurf1 to the plasma membrane, thereby targeting RhoA for degradation (Crose et al., 2009). To counteract RhoA degradation, the actin-associated protein synaptopodin can directly bind to RhoA, which blocks Smurf1-mediated ubiquitylation and degradation in podocytes (Asanuma et al., 2006). Recently, it was reported that HUVECs express synaptopodin when they are under laminar shear stress, suggesting that similar signaling takes place in the vascular endothelium (Mun et al., 2014).

RhoB

The regulation of RhoB is significantly different from that of RhoA and RhoC. This is mainly due to the difference in their hypervariable C-terminal regions. The hypervariable region of RhoB contains more polar amino acids than that of RhoA and RhoC, and additional isoprenylation modifications have been reported for RhoB (Allal et al., 2002; Baron et al., 2000; Pronk et al., 2019). Unlike RhoA and RhoC, RhoB does not bind the ubiquitously expressed RhoGDI1 (also known as ARHGDIA) and is therefore prone to ubiquitylation and degradation (Garcia-Mata et al., 2011). As a result, ECs typically express only low levels of RhoB in resting conditions (Kovačević et al., 2018; Kroon et al., 2013).

Mechanistically, we found that in HUVECs, knockdown of components of the RING E3 ligase, which comprises the scaffold protein Cullin3, the adapter protein Rbx1 and its RhoB-binding substrate receptor KCTD10, increases the protein level and activation of RhoB, resulting in cell contraction and barrier disruption (Kovačević et al., 2018). We further identified the relevant ubiquitylation acceptor sites in RhoB as K162 and K181, following ectopic expression of RhoB loss-of-ubiquitylation (K-R) mutants in HEK293T cells. In HUVECs, ubiquitylation of RhoB at these two lysine residues leads to its lysosomal targeting and degradation (Kovačević et al., 2018). A recent study showed that KCTD10-mediated RhoB degradation in epithelial cells serves to allow Rac1 activation (Murakami et al., 2018). In HUVECs, Rac1 is a barrier-stabilizing Rho GTPase (see below). This suggests that KCTD10-mediated downregulation of RhoB not only limits contractility, but also promotes cell spreading and endothelial barrier stability. Conversely, RhoB has been shown to drive internalization of Rac1 in TNFα-treated HUVECs, which limits the capacity of Rac1 to stabilize the endothelial barrier (Marcos-Ramiro et al., 2016).

Rac1

Unlike what is seen for RhoB, the majority of Rac1 in resting ECs is localized in the cytosol, where it is bound to the chaperone RhoGDI and therefore inactive (Boulter et al., 2010; Ren et al., 1999). Upon cell stimulation, Rac1 is released from the GDI to be activated at cellular membranes by a local GEF, followed by its interaction with nearby effectors (Bustelo et al., 2012). These include p21-activated kinases (PAKs), which induce lamellipodia formation (Byrne et al., 2016), partitioning defective (PAR)6, which is important for cell polarity (Lin et al., 2000) and IQGAPs, which increase cell–cell adhesion, proliferation and angiogenesis (Meyer et al., 2008), as well as, specifically, Rac1-associated 1 (SRA1), WASP-family verprolin-homologous (WAVE) proteins and $p67^{phox}$, part of the NADPH oxidase complex that leads to ROS production (Hordijk, 2006). To limit localized Rac1 signaling, active Rac1 is ubiquitylated, which is accompanied by its internalization and leads to its proteasomal degradation (Nethe and Hordijk, 2010; Pop et al., 2004).

Inhibition of Rac1 degradation increases ROS production and disrupts the endothelial barrier; this occurs through various mechanisms, including disruption of the plasma and mitochondrial membrane through membrane lipid peroxidation, which reduces ATP generation and decreases metabolism and cell survival (Daugaard et al., 2013; Farber, 1994; Kovacic et al., 2001; van Wetering et al., 2002). To inhibit ROS production, the HECT E3 ubiquitin ligase HACE1 targets active Rac1 for degradation by ubiquitylation at K147 (Daugaard et al., 2013; Mettouchi and Lemichez, 2012; Torrino et al., 2011; Visvikis et al., 2008). In contrast, Rac1-mediated ROS production is increased by the E3 ligase TRAF6. TRAF6-mediated ubiquitylation of Rac1 occurs in response to H_2O_2 and IL-1β stimulation, and after ischemia-reperfusion injury, all of which also lead to a loss of endothelial barrier function (Li et al., 2006, 2017).

Several other ubiquitin ligases also target Rac1. K147 polyubiquitylation and Rac1 degradation can be mediated by X-linked IAP (XIAP), cIAP1 and cIAP2 in HeLa and HEK293T cells (Oberoi et al., 2012). In addition, the SCF-FBXL19 E3 ligase targets Rac1 K166 for ubiquitylation and degradation, an event which requires AKT-mediated phosphorylation of Rac1 at S71 (Zhao et al., 2013). Through this pathway, FBXL19 negatively regulates Rac1 signaling; this impairs cell migration and reduces endothelial barrier integrity (Pronk et al., 2019).

Collectively, these studies illustrate that Rho GTPases are subject to ubiquitylation by HECT and RING E3 ligases, which, in most cases, alters their localization and limits their abundance and signaling capacities. This is not unique for Rho-like small GTPases, as the activation and output of Ras and Rab GTPases are also controlled through ubiquitylation (Shin et al., 2017; Thurman et al., 2017).

Ubiquitin modifications at endothelial cell–cell junctions

Cell–cell contacts between ECs comprise different types of junctions (adherens, tight and gap junctions) with different cell

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Table 1. Ubiquitin ligases and DUB implicated in the control of endothelial junctional proteins

Ubiquitin ligase or DUB	Target	Output	Reference
Ubiquitin ligases			
Ubr1	ZO-1	Negative regulator of integrity	Chen et al., 2014
K5 (Kaposi sarcoma-ass Herpesvirus) March2, March4, Cullin 3, βTRCP1	VE-cadherin; α,-β-,γ-catenin (indirect)	Negative regulator of integrity	Mansouri et al., 2008; Nanes et al., 2017; Sakaue et al., 2017
Unidentified	VE-cadherin	Bradykinin-induced permeability	Orsenigo et al., 2012
Itch	Occludin	VEGF-induced permeability	Murakami et al., 2009, 2012
βTRCP1, CHIP	VEGFR2	VEFGR2 degradation; inhibition of angiogenesis	Meyer et al., 2011; Shaik et al., 2012; Sun et al., 2015
Cbl	VEGFR2	VEFGR2 degradation; inhibition of VEGF-induced eNOS	Duval et al., 2003
HECW2	AMOTL-1	Stabilization of junctions	Choi et al., 2016
HECTD1	Claudin-5	Disruption of blood–brain barrier	Rui et al., 2018
March3	Junctional proteins (indirect, via FoxO1)	Negative regulator of integrity	Leclair et al., 2016
Cullin3–Rbx1–KCTD10	Notch1	Negative regulator of integrity, stimulation angiogenesis	Ren et al., 2014
DUBs			
USP10	Notch1	Endothelial sprouting	Lim et al., 2019
A20	VE-cadherin	Protection of lung endothelial barrier function	Soni et al., 2018

adhesion molecules and regulators. A large, and growing, number of E3 ligases and some DUBs have been implicated in the control of intercellular contacts, both in epithelial and ECs (Table 1; Fig. 3). A selection of these, i.e. those relevant for endothelial integrity, is discussed below.

Kowalczyk and colleagues provided the first indications that the expression levels of VE-cadherin and its localization at junctions are regulated by controlled, lysosomal degradation in primary microvascular dermal ECs (Xiao et al., 2003). Subsequently, it was shown that VE-cadherin is internalized in a clathrin-dependent fashion in ECs (Chiasson et al., 2009; Xiao et al., 2005) The notion that the clathrin-mediated internalization of VE-cadherin is the result of its ubiquitylation was proposed by Orsenigo et al. (2012). These authors demonstrated that bradykinin induces tyrosine phosphorylation of VE-cadherin at Y658 and Y685, which drives its consequent ubiquitylation, internalization and degradation. Although the responsible ligase was not identified in this study, VE-cadherin ubiquitylation was suggested to occur through K63-linkage, in line with its lysosomal degradation (Orsenigo et al., 2012). Interestingly, VE-cadherin-associated p120 catenin (also

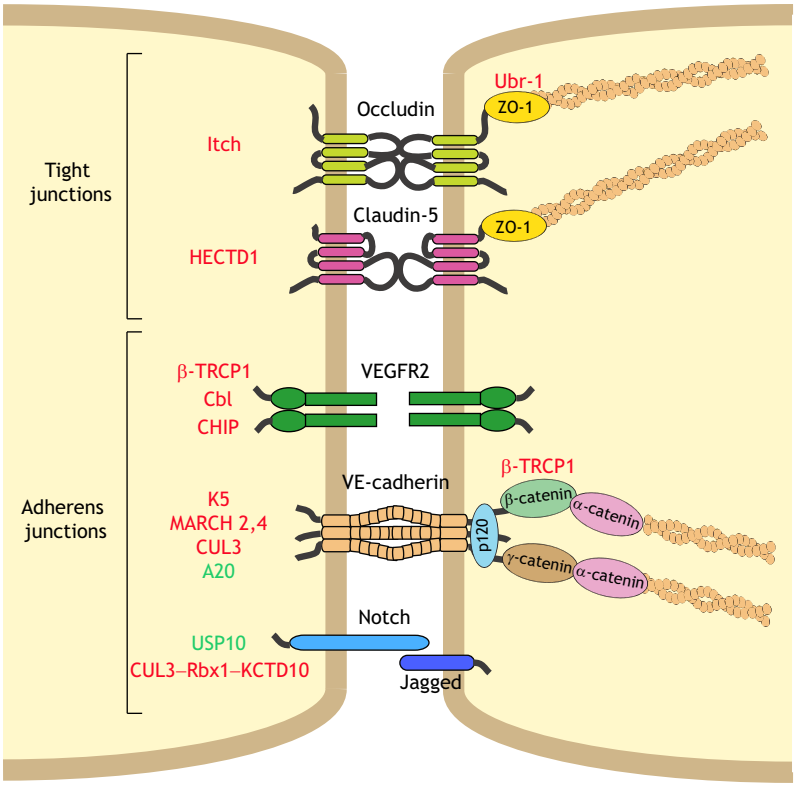

Fig. 3. Ubiquitylation in cell–cell contacts. Overview of the various ubiquitin E3 ligases (in red) and DUBs (in green) that regulate junctional proteins in endothelial cells. In tight junctions, occludin and claudin-5 are regulated by the E3 ligases Itch and HECTD1, respectively, while ZO-1 is regulated by Ubr1. For adherens junctions, several other E3 ligases and their substrates, including both adhesion molecules and cell surface receptors, have been identified. The indicated ligases and DUBs that control endothelial integrity are discussed in more detail in the main text (see also Table 1).

known as CTNND1) protects VE-cadherin from internalization (Chiasson et al., 2009; Xiao et al., 2005); this is caused by the binding of p120 catenin to an endocytic motif in the intracellular part of VE-cadherin, which limits both its internalization as well as ubiquitylation. In Kaposi sarcoma, which is caused by human herpesvirus 8, VE-cadherin is ubiquitylated by the virus-encoded, transmembrane MARCH-family ubiquitin ligase K5 (Mansouri et al., 2008; Nanes et al., 2017) (see Box 1). This E3 ligase thus contributes to the sarcoma-associated vascular leakage and tumorigenesis though its effect on VE-cadherin ubiquitylation and degradation.

Ubiquitylation of the tight junction protein occludin in ECs has been implicated in junctional stability (see Box 1 and Table 1). PKCβ-mediated phosphorylation of occludin at S490 (Murakami et al., 2012) and its subsequent ubiquitylation by the E3 ligase Itch have been linked to vascular endothelial growth factor (VEGF)-induced permeability in primary bovine retinal ECs (Murakami et al., 2012). Interestingly, these authors showed that a C-terminal occluding–ubiquitin chimera was internalized, bypassing the requirement for phosphorylation (Murakami et al., 2009). Thus, VEGF, through PKCβ-mediated phosphorylation, promotes Itch-mediated ubiquitylation of occludin, which is required for its internalization and degradation, thereby enhancing retinal endothelial permeability.

Furthermore, another study has shown that ischemia in the rat brain, induced by permanent middle cerebral artery occlusion, is associated with increased Itch-mediated ubiquitylation of occludin and loss of vascular integrity (Zhang et al., 2013). The ischemia also induced a downregulation of Notch1 and, in line with Notch regulation by γ-secretase, intraventricular treatment with the γ-secretase inhibitor DAPT prevented the ubiquitylation and degradation of occludin. This also reduced Evans Blue leakage of the brain vasculature, indicative of restored barrier function. The notion that Notch may stabilize junctions by limiting occludin ubiquitylation is intriguing, and is in line with the finding that DAPT-mediated inhibition of γ-secretase induces vascular barrier protecting properties (Zhang et al., 2013). Finally, the tight junction protein ZO-1, is targeted for ubiquitylation by the E3 ligase N-recognin-1 (Ubr1) (Chen et al., 2014). This is initiated through the inflammatory cytokine IL-6, which, in turn, is produced following an infection of brain pericytes with Japanese encephalitis virus (Chen et al., 2014). Thus, a growing number of ubiquitin ligases appears to be involved in regulation of junctional integrity. Some of these target junctional proteins directly, or control barrier function indirectly, by ubiquitylating regulatory proteins that, for example, control cytoskeletal dynamics.

Box 1. Degradation of endothelial junction components in virus-induced vascular leakage

Viral infections with the highly pathogenic strains of the influenza A virus, or even more severely with hemorrhagic viruses (e.g. Hanta, Dengue or West Nile Virus) can induce disruption of the endothelial barrier, acute lung injury and shock. Viruses can both directly and indirectly disrupt the endothelial barrier (Armstrong et al., 2012; Basu and Chaturvedi, 2008). Accordingly, strengthening of the endothelial barrier by signaling through Tie2, the receptor for angiopoietin, or by Robo-4, the receptor for the chemorepellent SLIT1, improves lung injury and survival of mice in an influenza model (London et al., 2010). Influenza A virus induces degradation of the tight junction component claudin-5 in primary microvascular ECs and of ZO-1 in primary HUVECs and in mice (Armstrong et al., 2012; Wang et al., 2010). Furthermore, the H1N1 influenza strain enhances hyper-phosphorylation of β-catenin and its proteasomal degradation in primary HUVECs (Hiyoshi et al., 2015). In addition, the E3 ubiquitin ligase Itch was found to be necessary for uncoating of influenza A virus and its transport from endosomes to the nucleus (Su et al., 2013). In epithelial cells, Itch mediates degradation of occludin (Traweger et al., 2002); however, direct interaction partners for Itch that mediate virus-induced loss of endothelial cell–cell contact remain to be identified. Finally, infection of HUVECs or HMECs with dengue virus also causes disruption of adherens and tight junctions, changes in the actin cytoskeleton and reduced expression of several junctional proteins including PECAM-1/CD31 and VE-cadherin (Dewi et al., 2008; Kanlaya et al., 2009; Talavera et al., 2004). Although the molecular mechanisms of this downregulation are currently incompletely understood, examples of virus-encoded ubiquitin ligases do exist; an example is the MARCH-family ubiquitin ligase K5 from herpesvirus (see main text) (Mansouri et al., 2008).

Ubiquitin ligases and junctional regulation

A number of different E3 ligases have been linked to the control of endothelial junctional integrity (Table 1). For instance, ectopic expression of MARCH family of E3 ligases, MARCH2 and MARCH4, whose mRNA is expressed in primary dermal microvascular ECs, impairs VE-cadherin localization to junctions (Nanes et al., 2017). However, this does not exclude a mechanism that involves ubiquitylation of VE-cadherin-associated proteins, which could regulate its internalization. The MARCH3 E3 ligase also negatively regulates endothelial integrity, but in an indirect manner. In human brain ECs in which MARCH3 was downregulated, histamine-induced permeability was reduced (Leclair et al., 2016). In brain ECs transfected with siRNA targeting MARCH3, both the mRNA and protein levels of claudin-5 and occludin were increased, whereas the mRNA and protein levels of VE-cadherin were only increased slightly. Here, MARCH3 was found to act through the transcription repressor FoxO1 to reduce the mRNA expression levels of the tight junction proteins occludin and claudin-5. Thus, MARCH3-mediated reduction of mRNAs that encode tight junction proteins impairs adherens junction stability and endothelial integrity (Leclair et al., 2016). This mechanism is thus clearly different from the ubiquitylation of claudin-5 in human brain microvascular ECs by the HECT domain E3 ubiquitin protein ligase (HECTD)1 (Rui et al., 2018). This pathway was identified following *Streptococcus* infection, which induced the ubiquitylation and degradation of claudin-5, which weakened the blood–brain barrier and promoted further infection with the pathogen (Rui et al., 2018).

Furthermore, it has recently been shown that activation of CRLs by neddylation is required for endothelial integrity (Sakaue et al., 2017). Here, pharmacological inhibition of CRL neddylation by MLN4924 induces a strong increase in the permeability of HUVECs that is associated with the loss of VE-cadherin protein, but not a decrease in its mRNA levels. The study further showed that the cullin 3 scaffold protein is required for the stabilization of VE-cadherin and endothelial integrity. However, it was not established whether the reduced VE-cadherin protein levels were an indirect result of the loss of integrity, or whether CRLs directly control VE-cadherin stability by ubiquitylation (Sakaue et al., 2017).

As discussed above, the cullin3 scaffold, in complex with the adapter protein Rbx1, and the substrate receptor KCTD10, stabilizes the integrity of endothelial monolayers by limiting RhoB levels and signaling output (Kovačević et al., 2018). Intriguingly, KCTD10 also stimulates developmental angiogenesis in mouse embryos, as determined by knockout studies (Ren et al., 2014). Here, KCTD10, in conjunction with cullin 3, was suggested to ubiquitylate Notch1, which reduces Notch signaling in the vasculature. Notch1 positively

regulates endothelial integrity by activating a non-canonical VE-cadherin–Trio–Rac1 signaling pathway (Polacheck et al., 2017). Thus, the role of the Cul3–Rbx1–KCTD10 RING ligase in ECs is complex, as it not only negatively regulates Notch1, thus reducing barrier function, but also downregulates RhoB, which results in barrier stabilization.

Another E3 ligase that negatively regulates endothelial integrity is PDZ domain-containing ring finger 3 (PDZRN3) (Sewduth et al., 2017). PDZRN3 acts downstream of the PAR3 polarity complex and targets the protein discs lost-multi-PDZ domain protein 1 (MUPP1, also known as MPDZ) for poly-ubiquitylation and degradation. This pathway was identified in infarcted mouse brain, thereby implicating PDZRN3 as an important mediator of a compromised blood–brain barrier and tissue damage in acute ischemic stroke (Sewduth et al., 2017).

In contrast to MARCH3 and PDZRN3, the endothelial E3 ligase HECW2 (also known as NEDL2), a member of the NEDD4 family of ligases that also includes Itch, stabilizes endothelial junctions (Choi et al., 2016). Accordingly, siRNA-mediated loss of HECW2 reduces endothelial barrier function and promotes angiogenic sprouting. The authors suggest that this occurs through ubiquitylation-mediated stabilization, rather than destabilization, of the junctional protein AMOTL1 (Choi et al., 2016).

Although there is accumulating data on the role of ubiquitin ligases in endothelial integrity, only very little is known with regard to the role of DUBs in barrier regulation. For instance, the DUB Cezanne (also known as OTUD7B) has been shown to protect against hypoxia-induced NFκB-mediated inflammation in kidney ECs *in vivo*. This occurred through de-ubiquitylation of the E3 ligase TRAF6, which is part of the NFκB pathway (Luong et al., 2013). Another example is the DUB USP40, which is particularly highly expressed in glomerular ECs, as well as in podocytes in rats and mice (Takagi et al., 2017). In *in vivo* experiments in zebrafish, USP40 morpholinos induced cardiac edema and loss of glomerular permeability. Although the USP40 targets were not identified, its association with the intermediate filament protein nestin suggests that it is an endothelial integrity-stabilizing DUB that acts on cell–cell junctions through intermediate filaments (Takagi et al., 2017).

Ubiquitin-dependent modifications in vascular disease

It is not surprising, given the abundance and importance of protein ubiquitylation, that this process (and its deregulation) contributes to a range of disorders, including neurodegenerative and inflammatory diseases, as well as cancer (Lipkowitz and Weissman, 2011; Rape, 2018). Consequently, proteasome inhibition has already been in use as a therapeutic intervention for more than two decades; however, such an approach obviously shows limited specificity, and the use of proteasome inhibitors is accompanied by (cardiovascular) side effects (Cole and Frishman, 2018; Gavazzoni et al., 2018).

Most, if not all, vascular disorders are accompanied by a loss of endothelial integrity, which causes edema and tissue damage owing to elevated interstitial pressure and increased influx of activated leukocytes (Huveneers et al., 2015; Nourshargh et al., 2010). It is therefore imperative to understand in detail the different molecular mechanisms that control stable, as well as disrupted, endothelial barrier function. While the role of ubiquitylation in vascular pathologies is perhaps less well established compared to that in, for instance, cancer, some clear connections exist.

Probably the best-studied pathway involves the regulation of (tumor) angiogenesis through the degradation of hypoxia inducible (HIF) transcription factors by the von Hippel–Lindau (VHL) protein, a substrate receptor that is part of a Cul2–Rbx1-containing RING ubiquitin ligase (Kamura et al., 2004). Loss of VHL promotes VEGF expression and tumor vascularization as a result of HIF1 being stabilized (Robinson and Ohh, 2014). Several other studies have implicated (de-)ubiquitylation in hypoxia and angiogenesis, either through ubiquitin-mediated interactions between VEGFR2 and epsin1, which drives angiogenesis and wound healing (Rahman et al., 2016), or through sumoylation of Notch1, which controls VEGF receptor (VEGFR) signaling and angiogenesis (Zhu et al., 2017). Interestingly, Notch1 ubiquitylation by the FBXW7 RING ligase is required for angiogenesis *in vitro* and *in vivo* (Izumi et al., 2012). In good agreement with this, loss of Usp10, the DUB for Notch1, promotes *in vivo* vessel sprouting (Lim et al., 2019). Finally, VEGFR2 has been identified as a substrate for several ubiquitin ligases, including SCF-βTRCP, CHIP (also known as STUB1) and Cbl, which all regulate angiogenesis through directly targeting VEGFR2 (Duval et al., 2003; Shaik et al., 2012; Sun et al., 2015).

As discussed above, ubiquitin ligases and/or DUBs have been implicated in inflammatory vascular disorders, for example in the lung or the brain (Hartz et al., 2016; Li et al., 2018; Liu et al., 2017; Rape, 2018). Aberrations in the TNF signaling pathway underlie several human pathologies, as has been corroborated by functional studies in animal models. Mutations in TNFR1 or the LUBAC component HOIL-1 (also known as RBCK1) cause inflammation in affected individuals (Boisson et al., 2012; McDermott et al., 1999). Furthermore, genetic depletion of the DUBs A20 or CYLD in mice rendered them more susceptible to inflammatory bowel disease (Vereecke et al., 2014; Zhang et al., 2006). A20 was recently proposed to act as a DUB for VE-cadherin, thereby preserving endothelial barrier function, and its re-expression in A20-deficient mice was found to limit their lung permeability (Soni et al., 2018). Conversely, the RBR E3 ligase Parkin, originally linked to Parkinson disease, was recently shown to mediate vascular permeability both *in vitro* and *in vivo* in a study showing that Parkin-deficient mice are protected from LPS-induced acute inflammation and leakage in the lung (Letsiou et al., 2017).

A deletion in the cullin 3 scaffold (cullin3Δ9, a deletion of 57 amino acids encoding exon 9) drives vessel wall stiffness and hypertension due to impaired turnover of RhoA, resulting in increased smooth muscle cell contractility (Agbor et al., 2016). Conversely, the cullin 3 substrate adapter RhoBTB1 protects from arterial stiffness and hypertension through its ubiquitylation and the consequent degradation of the phosphodiesterase PDE5. Lower amounts of PDE5 lead to increased cGMP levels, which in turn promotes smooth muscle cell relaxation (Mukohda et al., 2019). This important role for cullin 3 in vascular smooth muscle cells is in good agreement with its degradation of RhoB in ECs, which also limits contraction and preserves endothelial integrity (Kovačević et al., 2018; Sakaue et al., 2017). Taken together, a growing number of ubiquitin ligases and DUBs has been implicated in vascular disorders. Targeting these individual ligases, based on detailed structural and mechanistic studies, is of key importance to selectively limit chronic inflammation, together with its associated loss of barrier function and tissue damage.

Concluding remarks

The ubiquitous nature of protein regulation through controlled degradation makes it obvious that this process is important, and it is also important for endothelial permeability and associated vascular pathologies, such as tumor angiogenesis and inflammation. The above overview aims to highlight our growing knowledge on the role of protein ubiquitylation in endothelial integrity. Although

several of these ubiquitin ligases have already been implicated in vascular permeability, many questions remain unanswered. For instance, what is the ubiquitin ligase for VE-cadherin? Do endothelial-specific ubiquitin ligases or DUBs exist for the control cell–cell adhesion? If so, do these show vascular-bed-specific distribution and how might they be targeted to preserve vascular integrity and limit inflammation? Another interesting aspect is that autophagy, which comprises lysosomal degradation and recycling of proteins, is vascular barrier-protective in both the brain and the lung (Slavin et al., 2018; Yang et al., 2019). Since K48 ubiquitylation drives lysosomal degradation, this type of ubiquitin modification may directly link cellular homeostasis, controlled by autophagy, to endothelial integrity. This may also prove pivotal in the aging-related loss of autophagy, which correlates with an increase in cardiovascular disease (Leidal et al., 2018).

The above overview also underscores how many different ubiquitin ligases are mechanistically, both directly and indirectly, linked to the control of endothelial integrity. This apparent excess of regulators is not unique and is similar to, for example, the large number of RhoGEFs and GAPs (in total over 150) that regulate only ∼20 Rho GTPases (Bos et al., 2007). It is likely that the relatively crude way in which ubiquitin ligases have been studied so far obscures differences in their specific localization or in the conditions during which one or the other ligase or DUB is most relevant. On top of this, cell-type-specific differences in expression, even between EC subtypes, is likely to play a role, in addition to divergent culture conditions or cellular stimulation. The development of specific antibodies for detection of individual E3 ligases or DUBs, as well as of the ubiquitin chains on specific substrates, use of super-resolution imaging and careful definition of the cell type and conditions used, will increase our insight in this complex mode of cellular signaling. Clearly, protein ubiquitylation and its intersection with critical regulatory pathways predicts a busy, but also very interesting future for this field of research.

Competing interests
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References
Agbor, L. N., Ibeawuchi, S.-R. C., Hu, C., Wu, J., Davis, D. R., Keen, H. L., Quelle, F. W. and Sigmund, C. D. (2016). Cullin-3 mutation causes arterial stiffness and hypertension through a vascular smooth muscle mechanism. *JCI Insight* **1**, e91015. doi:10.1172/jci.insight.91015

Akutsu, M., Dikic, I. and Bremm, A. (2016). Ubiquitin chain diversity at a glance. *J. Cell Sci.* **129**, 875-880. doi:10.1242/jcs.183954

Allal, C., Pradines, A., Hamilton, A. D., Sebti, S. M. and Favre, G. (2002). Farnesylated RhoB prevents cell cycle arrest and actin cytoskeleton disruption caused by the geranylgeranyltransferase I inhibitor GGTI-298. *Cell Cycle* **1**, 430-437. doi:10.4161/cc.1.6.272

Armstrong, S. M., Wang, C., Tigdi, J., Si, X., Dumpit, C., Charles, S., Gamage, A., Moraes, T. J. and Lee, W. L. (2012). Influenza infects lung microvascular endothelium leading to microvascular leak: role of apoptosis and claudin-5. *PLoS ONE* **7**, e47323. doi:10.1371/journal.pone.0047323

Asanuma, K., Yanagida-Asanuma, E., Faul, C., Tomino, Y., Kim, K. and Mundel, P. (2006). Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling. *Nat. Cell Biol.* **8**, 485-491. doi:10.1038/ncb1400

Aveleira, C. A., Lin, C.-M., Abcouwer, S. F., Ambrosio, A. F. and Antonetti, D. A. (2010). TNF-alpha signals through PKCzeta/NF-kappaB to alter the tight junction complex and increase retinal endothelial cell permeability. *Diabetes* **59**, 2872-2882. doi:10.2337/db09-1606

Baron, R., Fourcade, E., Lajoie-Mazenc, I., Allal, C., Couderc, B., Barbaras, R., Favre, G., Faye, J.-C. and Pradines, A. (2000). RhoB prenylation is driven by the three carboxyl-terminal amino acids of the protein: evidenced in vivo by an anti-farnesyl cysteine antibody. *Proc. Natl. Acad. Sci. USA* **97**, 11626-11631. doi:10.1073/pnas.97.21.11626

Basu, A. and Chaturvedi, U. C. (2008). Vascular endothelium: the battlefield of dengue viruses. *FEMS Immunol. Med. Microbiol.* **53**, 287-299. doi:10.1111/j.1574-695X.2008.00420.x

Boisson, B., Laplantine, E., Prando, C., Giliani, S., Israelsson, E., Xu, Z., Abhyankar, A., Israël, L., Trevejo-Nunez, G., Bogunovic, D. et al. (2012). Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. *Nat. Immunol.* **13**, 1178-1186. doi:10.1038/ni.2457

Bordy, R., Totoson, P., Prati, C., Marie, C., Wendling, D. and Demougeot, C. (2018). Microvascular endothelial dysfunction in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **14**, 404-420. doi:10.1038/s41584-018-0022-8

Bos, J. L., Rehmann, H. and Wittinghofer, A. (2007). GEFs and GAPs: critical elements in the control of small G proteins. *Cell* **129**, 865-877. doi:10.1016/j.cell.2007.05.018

Boulter, E., Garcia-Mata, R., Guilluy, C., Dubash, A., Rossi, G., Brennwald, P. J. and Burridge, K. (2010). Regulation of Rho GTPase crosstalk, degradation and activity by RhoGDI1. *Nat. Cell Biol.* **12**, 477-483. doi:10.1038/ncb2049

Boyer, L., Turchi, L., Desnues, B., Doye, A., Ponzio, G., Mege, J.-L., Yamashita, M., Zhang, Y. E., Bertoglio, J., Flatau, G. et al. (2006). CNF1-induced ubiquitylation and proteasome destruction of activated RhoA is impaired in Smurf1−/− cells. *Mol. Biol. Cell* **17**, 2489-2497. doi:10.1091/mbc.e05-09-0876

Bradley, J. R. (2008). TNF-mediated inflammatory disease. *J. Pathol.* **214**, 149-160. doi:10.1002/path.2287

Bravo-Cordero, J. J., Sharma, V. P., Roh-Johnson, M., Chen, X., Eddy, R., Condeelis, J. and Hodgson, L. (2013). Spatial regulation of RhoC activity defines protrusion formation in migrating cells. *J. Cell Sci.* **126**, 3356-3369. doi:10.1242/jcs.123547

Bustelo, X. R., Ojeda, V., Barreira, M., Sauzeau, V. and Castro-Castro, A. (2012). Rac-ing to the plasma membrane: the long and complex work commute of Rac1 during cell signaling. *Small GTPases* **3**, 60-66. doi:10.4161/sgtp.19111

Byrne, K. M., Monsefi, N., Dawson, J. C., Degasperi, A., Bukowski-Wills, J.-C., Volinsky, N., Dobrzyński, M., Birtwistle, M. R., Tsyganov, M. A., Kiyatkin, A. et al. (2016). Bistability in the Rac1, PAK, and RhoA signaling network drives actin cytoskeleton dynamics and cell motility switches. *Cell Syst.* **2**, 38-48. doi:10.1016/j.cels.2016.01.003

Cai, J., Culley, M. K., Zhao, Y. and Zhao, J. (2018). The role of ubiquitination and deubiquitination in the regulation of cell junctions. *Protein Cell* **9**, 754-769. doi:10.1007/s13238-017-0486-3

Chen, C.-J., Ou, Y.-C., Li, J.-R., Chang, C.-Y., Pan, H.-C., Lai, C.-Y., Liao, S.-L., Raung, S.-L. and Chang, C.-J. (2014). Infection of pericytes in vitro by Japanese encephalitis virus disrupts the integrity of the endothelial barrier. *J. Virol.* **88**, 1150-1161. doi:10.1128/JVI.02738-13

Chiasson, C. M., Wittich, K. B., Vincent, P. A., Faundez, V. and Kowalczyk, A. P. (2009). p120-catenin inhibits VE-cadherin internalization through a Rho-independent mechanism. *Mol. Biol. Cell* **20**, 1970-1980. doi:10.1091/mbc.e08-07-0735

Choi, K.-S., Choi, H.-J., Lee, J.-K., Im, S., Zhang, H., Jeong, Y., Park, J. A., Lee, I.-K., Kim, Y.-M. and Kwon, Y.-G. (2016). The endothelial E3 ligase HECW2 promotes endothelial cell junctions by increasing AMOTL1 protein stability via K63-linked ubiquitination. *Cell. Signal.* **28**, 1642-1651. doi:10.1016/j.cellsig.2016.07.015

Christian, F., Smith, E. L. and Carmody, R. J. (2016). The regulation of NF-kappaB subunits by phosphorylation. *Cells* **5**. doi:10.3390/cells5010012

Cohen, P. and Strickson, S. (2017). The role of hybrid ubiquitin chains in the MyD88 and other innate immune signalling pathways. *Cell Death Differ.* **24**, 1153-1159. doi:10.1038/cdd.2017.17

Cole, D. C. and Frishman, W. H. (2018). Cardiovascular complications of proteasome inhibitors used in multiple myeloma. *Cardiol. Rev.* **26**, 122-129. doi:10.1097/CRD.0000000000000183

Cromer, W. E., Zawieja, S. D., Tharakan, B., Childs, E. W., Newell, M. K. and Zawieja, D. C. (2014). The effects of inflammatory cytokines on lymphatic endothelial barrier function. *Angiogenesis* **17**, 395-406. doi:10.1007/s10456-013-9393-2

Crose, L. E. S., Hilder, T. L., Sciaky, N. and Johnson, G. L. (2009). Cerebral cavernous malformation 2 protein promotes smad ubiquitin regulatory factor 1-mediated RhoA degradation in endothelial cells. *J. Biol. Chem.* **284**, 13301-13305. doi:10.1074/jbc.C900009200

Daugaard, M., Nitsch, R., Razaghi, B., McDonald, L., Jarrar, A., Torrino, S., Castillo-Lluva, S., Rotblat, B., Li, L., Malliri, A. et al. (2013). Hace1 controls ROS generation of vertebrate Rac1-dependent NADPH oxidase complexes. *Nat. Commun.* **4**, 2180. doi:10.1038/ncomms3180

de la Vega, M., Burrows, J. F. and Johnston, J. A. (2011). Ubiquitination: added complexity in Ras and Rho family GTPase function. *Small GTPases* **2**, 192-201. doi:10.4161/sgtp.2.4.16707

Dewi, B. E., Takasaki, T. and Kurane, I. (2008). Peripheral blood mononuclear cells increase the permeability of dengue virus-infected endothelial cells in association with downregulation of vascular endothelial cadherin. *J. Gen. Virol.* **89**, 642-652. doi:10.1099/vir.0.83356-0

Ding, F., Yin, Z. and Wang, H.-R. (2011). Ubiquitination in Rho signaling. *Curr. Top. Med. Chem.* **11**, 2879-2887. doi:10.2174/156802611798281357

Dovas, A. and Couchman, J. R. (2005). RhoGDI: multiple functions in the regulation of Rho family GTPase activities. *Biochem. J.* **390**, 1-9. doi:10.1042/BJ20050104

Duval, M., Bédard-Goulet, S., Delisle, C. and Gratton, J.-P. (2003). Vascular endothelial growth factor-dependent down-regulation of Flk-1/KDR involves Cbl-mediated ubiquitination. Consequences on nitric oxide production from endothelial cells. *J. Biol. Chem.* **278**, 20091-20097. doi:10.1074/jbc.M301410200

Ebner, P., Versteeg, G. A. and Ikeda, F. (2017). Ubiquitin enzymes in the regulation of immune responses. *Crit. Rev. Biochem. Mol. Biol.* **52**, 425-460. doi:10.1080/10409238.2017.1325829

Emmerich, C. H., Ordureau, A., Strickson, S., Arthur, J. S. C., Pedrioli, P. G. A., Komander, D. and Cohen, P. (2013). Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *Proc. Natl. Acad. Sci. USA* **110**, 15247-15252. doi:10.1073/pnas.1314715110

Farber, J. L. (1994). Mechanisms of cell injury by activated oxygen species. *Environ. Health Perspect.* **102** Suppl. 10, 17-24. doi:10.1289/ehp.94102s1017

Garcia-Mata, R., Boulter, E. and Burridge, K. (2011). The 'invisible hand': regulation of RHO GTPases by RHOGDIs. *Nat. Rev. Mol. Cell Biol.* **12**, 493-504. doi:10.1038/nrm3153

Gavazzoni, M., Vizzardi, E., Gorga, E., Bonadei, I., Rossi, L., Belotti, A., Rossi, G., Ribolla, R., Metra, M. and Raddino, R. (2018). Mechanism of cardiovascular toxicity by proteasome inhibitors: new paradigm derived from clinical and pre-clinical evidence. *Eur. J. Pharmacol.* **828**, 80-88. doi:10.1016/j.ejphar.2018.03.022

Grabbe, C., Husnjak, K. and Dikic, I. (2011). The spatial and temporal organization of ubiquitin networks. *Nat. Rev. Mol. Cell Biol.* **12**, 295-307. doi:10.1038/nrm3099

Hartz, A. M. S., Zhong, Y., Wolf, A., LeVine, H., III, Miller, D. S. and Bauer, B. (2016). Abeta40 reduces P-glycoprotein at the blood-brain barrier through the ubiquitin-proteasome pathway. *J. Neurosci.* **36**, 1930-1941. doi:10.1523/JNEUROSCI.0350-15.2016

Heger, K., Wickliffe, K. E., Ndoja, A., Zhang, J., Murthy, A., Dugger, D. L., Maltzman, A., de Sousa e Melo, F., Hung, J., Zeng, Y. et al. (2018). OTULIN limits cell death and inflammation by deubiquitinating LUBAC. *Nature* **559**, 120-124. doi:10.1038/s41586-018-0256-2

Hiyoshi, M., Indalao, I. L., Yano, M., Yamane, K., Takahashi, E. and Kido, H. (2015). Influenza A virus infection of vascular endothelial cells induces GSK-3beta-mediated beta-catenin degradation in adherens junctions, with a resultant increase in membrane permeability. *Arch. Virol.* **160**, 225-234. doi:10.1007/s00705-014-2270-5

Hodge, R. G. and Ridley, A. J. (2016). Regulating Rho GTPases and their regulators. *Nat. Rev. Mol. Cell Biol.* **17**, 496-510. doi:10.1038/nrm.2016.67

Hordijk, P. L. (2006). Regulation of NADPH oxidases: the role of Rac proteins. *Circ. Res.* **98**, 453-462. doi:10.1161/01.RES.0000204727.46710.5e

Hordijk, P. L. (2016). Recent insights into endothelial control of leukocyte extravasation. *Cell. Mol. Life Sci.* **73**, 1591-1608. doi:10.1007/s00018-016-2136-y

Huppert, J., Closhen, D., Croxford, A., White, R., Kulig, P., Pietrowski, E. and Kuhlmann, C. R. (2010). Cellular mechanisms of IL-17-induced blood-brain barrier disruption. *FASEB J.* **24**, 1023-1034. doi:10.1096/fj.09-141978

Huveneers, S., Oldenburg, J., Spanjaard, E., van der Krogt, G., Grigoriev, I., Akhmanova, A., Rehmann, H. and de Rooij, J. (2012). Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. *J. Cell Biol.* **196**, 641-652. doi:10.1083/jcb.201108120

Huveneers, S., Daemen, M. J. A. P. and Hordijk, P. L. (2015). 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

Ikeda, F. (2015). Linear ubiquitination signals in adaptive immune responses. *Immunol. Rev.* **266**, 222-236. doi:10.1111/imr.12300

Izumi, N., Helker, C., Ehling, M., Behrens, A., Herzog, W. and Adams, R. H. (2012). Fbxw7 controls angiogenesis by regulating endothelial Notch activity. *PLoS ONE* **7**, e41116. doi:10.1371/journal.pone.0041116

Izzi, L. and Attisano, L. (2006). Ubiquitin-dependent regulation of TGFbeta signaling in cancer. *Neoplasia* **8**, 677-688. doi:10.1593/neo.06472

Kamura, T., Maenaka, K., Kotoshiba, S., Matsumoto, M., Kohda, D., Conaway, R. C., Conaway, J. W. and Nakayama, K. I. (2004). VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. *Genes Dev.* **18**, 3055-3065. doi:10.1101/gad.1252404

Kanarek, N. and Ben-Neriah, Y. (2012). Regulation of NF-kappaB by ubiquitination and degradation of the IkappaBs. *Immunol. Rev.* **246**, 77-94. doi:10.1111/j.1600-065X.2012.01098.x

Kanlaya, R., Pattanakitsakul, S.-N., Sinchaikul, S., Chen, S.-T. and Thongboonkerd, V. (2009). Alterations in actin cytoskeletal assembly and junctional protein complexes in human endothelial cells induced by dengue virus infection and mimicry of leukocyte transendothelial migration. *J. Proteome Res.* **8**, 2551-2562. doi:10.1021/pr900060g

Kawasaki, T. and Kawai, T. (2014). Toll-like receptor signaling pathways. *Front. Immunol.* **5**, 461. doi:10.3389/fimmu.2014.00461

Keusekotten, K., Elliott, P. R., Glockner, L., Fiil, B. K., Damgaard, R. B., Kulathu, Y., Wauer, T., Hospenthal, M. K., Gyrd-Hansen, M., Krappmann, D. et al. (2013). OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell* **153**, 1312-1326. doi:10.1016/j.cell.2013.05.014

Kirkin, V. and Dikic, I. (2007). Role of ubiquitin- and Ubl-binding proteins in cell signaling. *Curr. Opin. Cell Biol.* **19**, 199-205. doi:10.1016/j.ceb.2007.02.002

Komarova, Y. A., Kruse, K., Mehta, D. and Malik, A. B. (2017). Protein interactions at endothelial junctions and signaling mechanisms regulating endothelial permeability. *Circ. Res.* **120**, 179-206. doi:10.1161/CIRCRESAHA.116.306534

Kovačević, I., Sakaue, T., Majoleé, J., Pronk, M. C., Maekawa, M., Geerts, D., Fernandez-Borja, M., Higashiyama, S. and Hordijk, P. L. (2018). The Cullin-3-Rbx1-KCTD10 complex controls endothelial barrier function via K63 ubiquitination of RhoB. *J. Cell Biol.* **217**, 1015-1032. doi:10.1083/jcb.201606055

Kovacic, H. N., Irani, K. and Goldschmidt-Clermont, P. J. (2001). Redox regulation of human Rac1 stability by the proteasome in human aortic endothelial cells. *J. Biol. Chem.* **276**, 45856-45861. doi:10.1074/jbc.M107925200

Kovalenko, A., Chable-Bessia, C., Cantarella, G., Israël, A., Wallach, D. and Courtois, G. (2003). The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature* **424**, 801-805. doi:10.1038/nature01802

Kraynov, V. S., Chamberlain, C., Bokoch, G. M., Schwartz, M. A., Slabaugh, S. and Hahn, K. M. (2000). Localized Rac activation dynamics visualized in living cells. *Science* **290**, 333-337. doi:10.1126/science.290.5490.333

Kroon, J., Tol, S., van Amstel, S., Elias, J. A. and Fernandez-Borja, M. (2013). The small GTPase RhoB regulates TNFalpha signaling in endothelial cells. *PLoS ONE* **8**, e75031. doi:10.1371/journal.pone.0075031

Kwon, D.-H., Eom, G. H., Ko, J. H., Shin, S., Joung, H., Choe, N., Nam, Y. S., Min, H.-K., Kook, T., Yoon, S. et al. (2016). MDM2 E3 ligase-mediated ubiquitination and degradation of HDAC1 in vascular calcification. *Nat. Commun.* **7**, 10492. doi:10.1038/ncomms10492

Leclair, H. M., André-Grégoire, G., Treps, L., Azzi, S., Bidère, N. and Gavard, J. (2016). The E3 ubiquitin ligase MARCH3 controls the endothelial barrier. *FEBS Lett.* **590**, 3660-3668. doi:10.1002/1873-3468.12417

Leidal, A. M., Levine, B. and Debnath, J. (2018). Autophagy and the cell biology of age-related disease. *Nat. Cell Biol.* **20**, 1338-1348. doi:10.1038/s41556-018-0235-8

Letsiou, E., Sammani, S., Wang, H., Belvitch, P. and Dudek, S. M. (2017). Parkin regulates lipopolysaccharide-induced proinflammatory responses in acute lung injury. *Transl. Res.* **181**, 71-82. doi:10.1016/j.trsl.2016.09.002

Li, Q., Harraz, M. M., Zhou, W., Zhang, L. N., Ding, W., Zhang, Y., Eggleston, T., Yeaman, C., Banfi, B. and Engelhardt, J. F. (2006). Nox2 and Rac1 regulate H2O2-dependent recruitment of TRAF6 to endosomal interleukin-1 receptor complexes. *Mol. Cell. Biol.* **26**, 140-154. doi:10.1128/MCB.26.1.140-154.2006

Li, T., Qin, J.-J., Yang, X., Ji, Y.-X., Guo, F., Cheng, W.-L., Wu, X., Gong, F.-H., Hong, Y., Zhu, X.-Y. et al. (2017). The Ubiquitin E3 Ligase TRAF6 Exacerbates Ischemic Stroke by Ubiquitinating and Activating Rac1. *J. Neurosci.* **37**, 12123-12140. doi:10.1523/JNEUROSCI.1751-17.2017

Li, S., Zhao, J., Shang, D., Kass, D. J. and Zhao, Y. (2018). Ubiquitination and deubiquitination emerge as players in idiopathic pulmonary fibrosis pathogenesis and treatment. *JCI Insight* **3**, e120362. doi:10.1172/jci.insight.120362

Lim, R., Sugino, T., Nolte, H., Andrade, J., Zimmermann, B., Shi, C., Doddaballapur, A., Ong, Y. T., Wilhelm, K., Fasse, J. W. D. et al. (2019). Deubiquitinase USP10 regulates Notch signaling in the endothelium. *Science* **364**, 188-193.

Lin, D., Edwards, A. S., Fawcett, J. P., Mbamalu, G., Scott, J. D. and Pawson, T. (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nat. Cell Biol.* **2**, 540-547. doi:10.1038/35019582

Lipkowitz, S. and Weissman, A. M. (2011). RINGs of good and evil: RING finger ubiquitin ligases at the crossroads of tumour suppression and oncogenesis. *Nat. Rev. Cancer.* **11**, 629-643. doi:10.1038/nrc3120

Liu, B., Lu, H., Li, D., Xiong, X., Gao, L., Wu, Z. and Lu, Y. (2017). Aberrant expression of FBXO2 disrupts glucose homeostasis through ubiquitin-mediated degradation of insulin receptor in obese mice. *Diabetes* **66**, 689-698. doi:10.2337/db16-1104

London, N. R., Zhu, W., Bozza, F. A., Smith, M. C. P., Greif, D. M., Sorensen, L. K., Chen, L., Kaminoh, Y., Chan, A. C., Passi, S. F. et al. (2010). Targeting Robo4-dependent Slit signaling to survive the cytokine storm in sepsis and influenza. *Sci. Transl. Med.* **2**, 23ra19. doi:10.1126/scitranslmed.3000678

Luong, L. A., Fragiadaki, M., Smith, J., Boyle, J., Lutz, J., Dean, J. L. and Maxwell, P. H. (2013). Cezanne regulates inflammatory responses to hypoxia in endothelial cells by targeting TRAF6 for deubiquitination. *Circ. Res.* **112**, 1583-1591. doi:10.1161/circresaha.111.300119

Lv, Y., Kim, K., Sheng, Y., Cho, J., Qian, Z., Zhao, Y.-Y., Hu, G., Pan, D., Malik, A. B. and Hu, G. (2018). YAP controls endothelial activation and vascular inflammation through TRAF6. *Circ. Res.* **123**, 43-56. doi:10.1161/CIRCRESAHA.118.313143

Machacek, M., Hodgson, L., Welch, C., Elliott, H., Pertz, O., Nalbant, P., Abell, A., Johnson, G. L., Hahn, K. M. and Danuser, G. (2009). Coordination of Rho GTPase activities during cell protrusion. *Nature* **461**, 99-103. doi:10.1038/nature08242

Mansouri, M., Rose, P. P., Moses, A. V. and Fruh, K. (2008). Remodeling of endothelial adherens junctions by Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **82**, 9615-9628. doi:10.1128/JVI.02633-07

Marcos-Ramiro, B., García-Weber, D., Barroso, S., Feito, J., Ortega, M. C., Cernuda-Morollón, E., Reglero-Real, N., Fernández-Martín, L., Durán, M. C., Alonso, M. A. et al. (2016). RhoB controls endothelial barrier recovery by inhibiting Rac1 trafficking to the cell border. *J. Cell Biol.* **213**, 385-402. doi:10.1083/jcb.201504038

McDermott, M. F., Aksentijevich, I., Galon, J., McDermott, E. M., Ogunkolade, B. W., Centola, M., Mansfield, E., Gadina, M., Karenko, L., Pettersson, T. et al. (1999). Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* **97**, 133-144. doi:10.1016/S0092-8674(00)80721-7

Mettouchi, A. and Lemichez, E. (2012). Ubiquitylation of active Rac1 by the E3 ubiquitin-ligase HACE1. *Small GTPases* **3**, 102-106. doi:10.4161/sgtp.19221

Meyer, R. D., Sacks, D. B. and Rahimi, N. (2008). IQGAP1-dependent signaling pathway regulates endothelial cell proliferation and angiogenesis. *PLoS ONE* **3**, e3848. doi:10.1371/journal.pone.0003848

Meyer, R. D., Srinivasan, S., Singh, A. J., Mahoney, J. E., Gharahassanlou, K. R. and Rahimi, N. (2011). PEST motif serine and tyrosine phosphorylation controls vascular endothelial growth factor receptor 2 stability and downregulation. *Mol. Cell. Biol.* **31**, 2010-2025. doi:10.1128/MCB.01006-10

Micheau, O. and Tschopp, J. (2003). Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* **114**, 181-190. doi:10.1016/S0092-8674(03)00521-X

Mukohda, M., Fang, S., Wu, J., Agbor, L. N., Nair, A. R., Ibeawuchi, S.-R. C., Hu, C., Liu, X., Lu, K.-T., Guo, D.-F. et al. (2019). RhoBTB1 protects against hypertension and arterial stiffness by restraining phosphodiesterase 5 activity. *J. Clin. Invest.* **130**, 2318-2332. doi:10.1172/JCI123462

Mun, G. I., Park, S., Kremerskothen, J. and Boo, Y. C. (2014). Expression of synaptopodin in endothelial cells exposed to laminar shear stress and its role in endothelial wound healing. *FEBS Lett.* **588**, 1024-1030. doi:10.1016/j.febslet.2014.02.012

Murakami, T., Felinski, E. A. and Antonetti, D. A. (2009). Occludin phosphorylation and ubiquitination regulate tight junction trafficking and vascular endothelial growth factor-induced permeability. *J. Biol. Chem.* **284**, 21036-21046. doi:10.1074/jbc.M109.016766

Murakami, T., Frey, T., Lin, C. and Antonetti, D. A. (2012). Protein kinase cbeta phosphorylates occludin regulating tight junction trafficking in vascular endothelial growth factor-induced permeability in vivo. *Diabetes* **61**, 1573-1583. doi:10.2337/db11-1367

Murakami, A., Maekawa, M., Kawai, K., Nakayama, J., Araki, N., Semba, K., Taguchi, T., Kamei, Y., Takada, Y. and Higashiyama, S. (2018). Cullin-3/KCTD10 E3 complex is essential for Rac1 activation through RhoB degradation in HER2-positive breast cancer cells. *Cancer Sci.* **110**, 650-661. doi:10.1111/cas.13899

Nanes, B. A., Grimsley-Myers, C. M., Cadwell, C. M., Robinson, B. S., Lowery, A. M., Vincent, P. A., Mosunjac, M., Früh, K. and Kowalczyk, A. P. (2017). p120-catenin regulates VE-cadherin endocytosis and degradation induced by the Kaposi sarcoma-associated ubiquitin ligase K5. *Mol. Biol. Cell* **28**, 30-40. doi:10.1091/mbc.e16-06-0459

Nethe, M. and Hordijk, P. L. (2010). The role of ubiquitylation and degradation in RhoGTPase signalling. *J. Cell Sci.* **123**, 4011-4018. doi:10.1242/jcs.078360

Nethe, M., Anthony, E. C., Fernandez-Borja, M., Dee, R., Geerts, D., Hensbergen, P. J., Deelder, A. M., Schmidt, G. and Hordijk, P. L. (2010). Focal-adhesion targeting links caveolin-1 to a Rac1-degradation pathway. *J. Cell Sci.* **123**, 1948-1958. doi:10.1242/jcs.062919

Nethe, M., de Kreuk, B.-J., Tauriello, D. V. F., Anthony, E. C., Snoek, B., Stumpel, T., Salinas, P. C., Maurice, M. M., Geerts, D., Deelder, A. M. et al. (2012). Rac1 acts in conjunction with Nedd4 and dishevelled-1 to promote maturation of cell-cell contacts. *J. Cell Sci.* **125**, 3430-3442. doi:10.1242/jcs.100925

Nourshargh, S., Hordijk, P. L. and Sixt, M. (2010). Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat. Rev. Mol. Cell Biol.* **11**, 366-378. doi:10.1038/nrm2889

Oberoi, T. K., Dogan, T., Hocking, J. C., Scholz, R.-P., Mooz, J., Anderson, C. L., Karreman, C., Meyer zu Heringdorf, D., Schmidt, G., Ruonala, M. et al. (2012). IAPs regulate the plasticity of cell migration by directly targeting Rac1 for degradation. *EMBO J.* **31**, 14-28. doi:10.1038/emboj.2011.423

Orsenigo, F., Giampietro, C., Ferrari, A., Corada, M., Galaup, A., Sigismund, S., Ristagno, G., Maddaluno, L., Koh, G. Y., Franco, D. et al. (2012). Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo. *Nat. Commun.* **3**, 1208. doi:10.1038/ncomms2199

Pao, K.-C., Wood, N. T., Knebel, A., Rafie, K., Stanley, M., Mabbitt, P. D., Sundaramoorthy, R., Hofmann, K., van Aalten, D. M. F. and Virdee, S. (2018). Activity-based E3 ligase profiling uncovers an E3 ligase with esterification activity. *Nature* **556**, 381-385. doi:10.1038/s41586-018-0026-1

Polacheck, W. J., Kutys, M. L., Yang, J., Eyckmans, J., Wu, Y., Vasavada, H., Hirschi, K. K. and Chen, C. S. (2017). A non-canonical Notch complex regulates adherens junctions and vascular barrier function. *Nature* **552**, 258-262. doi:10.1038/nature24998

Pop, M., Aktories, K. and Schmidt, G. (2004). Isotype-specific degradation of Rac activated by the cytotoxic necrotizing factor 1. *J. Biol. Chem.* **279**, 35840-35848. doi:10.1074/jbc.M404346200

Pronk, M. C. A., van Bezu, J. S. M., van Nieuw Amerongen, G. P., van Hinsbergh, V. W. M. and Hordijk, P. L. (2017). RhoA, RhoB and RhoC differentially regulate endothelial barrier function. *Small GTPases* **277**, 1-19. doi:10.1080/21541248.2017.1339767

Pronk, M. C. A., Majolée, J., Loregger, A., van Bezu, J. S. M., Zelcer, N., Hordijk, P. L. and Kovačević, I. (2019). FBXW7 regulates endothelial barrier function by suppression of the cholesterol synthesis pathway and prenylation of RhoB. *Mol. Biol. Cell* **30**, 607-621. doi:10.1091/mbc.E18-04-0259

Rahman, H. N. A., Wu, H., Dong, Y., Pasula, S., Wen, A., Sun, Y., Brophy, M. L., Tessneer, K. L., Cai, X., McManus, J. et al. (2016). Selective targeting of a novel Epsin-VEGFR2 interaction promotes VEGF-mediated angiogenesis. *Circ. Res.* **118**, 957-969. doi:10.1161/CIRCRESAHA.115.307679

Rape, M. (2018). Ubiquitylation at the crossroads of development and disease. *Nat. Rev. Mol. Cell Biol.* **19**, 59-70. doi:10.1038/nrm.2017.83

Ren, X.-D., Kiosses, W. B. and Schwartz, M. A. (1999). Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J.* **18**, 578-585. doi:10.1093/emboj/18.3.578

Ren, K., Yuan, J., Yang, M., Gao, X., Ding, X., Zhou, J., Hu, X., Cao, J., Deng, X., Xiang, S. et al. (2014). KCTD10 is involved in the cardiovascular system and Notch signaling during early embryonic development. *PLoS ONE* **9**, e112275. doi:10.1371/journal.pone.0112275

Ridley, A. J. (2015). Rho GTPase signalling in cell migration. *Curr. Opin. Cell Biol.* **36**, 103-112. doi:10.1016/j.ceb.2015.08.005

Robinson, C. M. and Ohh, M. (2014). The multifaceted von Hippel-Lindau tumour suppressor protein. *FEBS Lett.* **588**, 2704-2711. doi:10.1016/j.febslet.2014.02.026

Rui, L., Weiyi, L., Yu, M., Hong, Z., Jiao, Y., Zhe, M. and Hongjie, F. (2018). The serine/threonine protein kinase of Streptococcus suis serotype 2 affects the ability of the pathogen to penetrate the blood-brain barrier. *Cell. Microbiol.* **20**, e12862. doi:10.1111/cmi.12862

Saharinen, P., Eklund, L. and Alitalo, K. (2017). Therapeutic targeting of the angiopoietin-TIE pathway. *Nat. Rev. Drug Discov.* **16**, 635-661. doi:10.1038/nrd.2016.278

Sakaue, T., Fujisaki, A., Nakayama, H., Maekawa, M., Hiyoshi, H., Kubota, E., Joh, T., Izutani, H. and Higashiyama, S. (2017). Neddylated Cullin 3 is required for vascular endothelial-cadherin-mediated endothelial barrier function. *Cancer Sci.* **108**, 208-215. doi:10.1111/cas.13133

Sander, E. E., ten Klooster, J. P., van Delft, S., van der Kammen, R. A. and Collard, J. G. (1999). Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. *J. Cell Biol.* **147**, 1009-1022. doi:10.1083/jcb.147.5.1009

Schaefer, A., Nethe, M. and Hordijk, P. L. (2012). Ubiquitin links to cytoskeletal dynamics, cell adhesion and migration. *Biochem. J.* **442**, 13-25. doi:10.1042/BJ20111815

Schaefer, A., Reinhard, N. R. and Hordijk, P. L. (2014). Toward understanding RhoGTPase specificity: structure, function and local activation. *Small GTPases* **5**, e968004. doi:10.4161/21541248.2014.968004

Sewduth, R. N., Kovacic, H., Jaspard-Vinassa, B., Jecko, V., Wavasseur, T., Fritsch, N., Pernot, M., Jeaningros, S., Roux, E., Dufourcq, P. et al. (2017). PDZRN3 destabilizes endothelial cell-cell junctions through a PKCzeta-containing polarity complex to increase vascular permeability. *Sci. Signal.* **10**, eaag3209. doi:10.1126/scisignal.aag3209

Shaik, S., Nucera, C., Inuzuka, H., Gao, D., Garnaas, M., Frechette, G., Harris, L., Wan, L., Fukushima, H., Husain, A. et al. (2012). SCF(beta-TRCP) suppresses angiogenesis and thyroid cancer cell migration by promoting ubiquitination and destruction of VEGF receptor 2. *J. Exp. Med.* **209**, 1289-1307. doi:10.1084/jem.20112446

Shin, D., Na, W., Lee, J.-H., Kim, G., Baek, J., Park, S. H., Choi, C. Y. and Lee, S. (2017). Site-specific monoubiquitination downregulates Rab5 by disrupting effector binding and guanine nucleotide conversion. *eLife* **6**, e29154. doi:10.7554/eLife.29154

Sit, S.-T. and Manser, E. (2011). Rho GTPases and their role in organizing the actin cytoskeleton. *J. Cell Sci.* **124**, 679-683. doi:10.1242/jcs.064964

Slavin, S. A., Leonard, A., Grose, V., Fazal, F. and Rahman, A. (2018). Autophagy inhibitor 3-methyladenine protects against endothelial cell barrier dysfunction in acute lung injury. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **314**, L388-L396. doi:10.1152/ajplung.00555.2016

Soni, D., Wang, D.-M., Regmi, S. C., Mittal, M., Vogel, S. M., Schlüter, D. and Tiruppathi, C. (2018). Deubiquitinase function of A20 maintains and repairs endothelial barrier after lung vascular injury. *Cell Death Discov.* **4**, 60. doi:10.1038/s41420-018-0056-3

Spratt, D. E., Walden, H. and Shaw, G. S. (2014). RBR E3 ubiquitin ligases: new structures, new insights, new questions. *Biochem. J.* **458**, 421-437. doi:10.1042/BJ20140006

Su, W.-C., Chen, Y.-C., Tseng, C.-H., Hsu, P. W.-C., Tung, K.-F., Jeng, K.-S. and Lai, M. M. C. (2013). Pooled RNAi screen identifies ubiquitin ligase Itch as crucial for influenza A virus release from the endosome during virus entry. *Proc. Natl. Acad. Sci. USA* **110**, 17516-17521. doi:10.1073/pnas.1312374110

Sun, C., Li, H.-L., Chen, H.-R., Shi, M.-L., Liu, Q.-H., Pan, Z.-Q., Bai, J. and Zheng, J.-N. (2015). Decreased expression of CHIP leads to increased angiogenesis via VEGF-VEGFR2 pathway and poor prognosis in human renal cell carcinoma. *Sci. Rep.* **5**, 9774. doi:10.1038/srep09774

Swatek, K. N. and Komander, D. (2016). Ubiquitin modifications. *Cell Res.* **26**, 399-422. doi:10.1038/cr.2016.39

Takagi, H., Nishibori, Y., Katayama, K., Katada, T., Takahashi, S., Kiuchi, Z., Takahashi, S.-I., Kamei, H., Kawakami, H., Akimoto, Y. et al. (2017). USP40 gene knockdown disrupts glomerular permeability in zebrafish. *Am. J. Physiol. Renal. Physiol.* **312**, F702-F715. doi:10.1152/ajprenal.00197.2016

Talavera, D., Castillo, A. M., Dominguez, M. C., Gutierrez, A. E. and Meza, I. (2004). IL8 release, tight junction and cytoskeleton dynamic reorganization conducive to permeability increase are induced by dengue virus infection of microvascular endothelial monolayers. *J. Gen. Virol.* **85**, 1801-1813. doi:10.1099/vir.0.19652-0

Thurman, R., Siraliev-Perez, E. and Campbell, S. L. (2017). RAS ubiquitylation modulates effector interactions. *Small GTPases* **13**, 1-6. doi:10.1080/21541248.2017.1371267

Torrino, S., Visvikis, O., Doye, A., Boyer, L., Stefani, C., Munro, P., Bertoglio, J., Gacon, G., Mettouchi, A. and Lemichez, E. (2011). The E3 ubiquitin-ligase HACE1 catalyzes the ubiquitylation of active Rac1. *Dev. Cell* **21**, 959-965. doi:10.1016/j.devcel.2011.08.015

Traweger, A., Fang, D., Liu, Y.-C., Stelzhammer, W., Krizbai, I. A., Fresser, F., Bauer, H.-C. and Bauer, H. (2002). The tight junction-specific protein occludin is a functional target of the E3 ubiquitin-protein ligase itch. *J. Biol. Chem.* **277**, 10201-10208. doi:10.1074/jbc.M111384200

van Wetering, S., van Buul, J. D., Quik, S., Mul, F. P., Anthony, E. C., ten Klooster, J. P., Collard, J. G. and Hordijk, P. L. (2002). Reactive oxygen species mediate Rac-induced loss of cell-cell adhesion in primary human endothelial cells. *J. Cell Sci.* **115**, 1837-1846.

Vereecke, L., Vieira-Silva, S., Billiet, T., van Es, J. H., Mc Guire, C., Slowicka, K., Sze, M., van den Born, M., De Hertogh, G., Clevers, H. et al. (2014). A20 controls intestinal homeostasis through cell-specific activities. *Nat. Commun.* **5**, 5103. doi:10.1038/ncomms6103

Vestweber, D., Wessel, F. and Nottebaum, A. F. (2014). Similarities and differences in the regulation of leukocyte extravasation and vascular permeability. *Semin. Immunopathol.* **36**, 177-192. doi:10.1007/s00281-014-0419-7

Visvikis, O., Lorès, P., Boyer, L., Chardin, P., Lemichez, E. and Gacon, G. (2008). Activated Rac1, but not the tumorigenic variant Rac1b, is ubiquitinated on Lys 147 through a JNK-regulated process. *FEBS J.* **275**, 386-396. doi:10.1111/j.1742-4658.2007.06209.x

Walden, H. and Rittinger, K. (2018). RBR ligase-mediated ubiquitin transfer: a tale with many twists and turns. *Nat. Struct. Mol. Biol.* **25**, 440-445. doi:10.1038/s41594-018-0063-3

Wang, H.-R., Zhang, Y., Ozdamar, B., Ogunjimi, A. A., Alexandrova, E., Thomsen, G. H. and Wrana, J. L. (2003). Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. *Science* **302**, 1775-1779. doi:10.1126/science.1090772

Wang, S., Le, T. Q., Kurihara, N., Chida, J., Cisse, Y., Yano, M. and Kido, H. (2010). Influenza virus-cytokine-protease cycle in the pathogenesis of vascular hyperpermeability in severe influenza. *J. Infect. Dis.* **202**, 991-1001. doi:10.1086/656044

Wertz, I. E., O'Rourke, K. M., Zhou, H., Eby, M., Aravind, L., Seshagiri, S., Wu, P., Wiesmann, C., Baker, R., Boone, D. L. et al. (2004). De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* **430**, 694-699. doi:10.1038/nature02794

Wertz, I. E., Newton, K., Seshasayee, D., Kusam, S., Lam, C., Zhang, J., Popovych, N., Helgason, E., Schoeffler, A., Jeet, S. et al. (2016). Erratum: phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. *Nature* **532**, 402. doi:10.1038/nature16541

Wessel, F., Winderlich, M., Holm, M., Frye, M., Rivera-Galdos, R., Vockel, M., Linnepe, R., Ipe, U., Stadtmann, A., Zarbock, A. et al. (2014). Leukocyte extravasation and vascular permeability are each controlled in vivo by different tyrosine residues of VE-cadherin. *Nat. Immunol.* **15**, 223-230. doi:10.1038/ni.2824

Wojciak-Stothard, B., Potempa, S., Eichholtz, T. and Ridley, A. J. (2001). Rho and Rac but not Cdc42 regulate endothelial cell permeability. *J. Cell Sci.* **114**, 1343-1355.

Xiao, K., Allison, D. F., Kottke, M. D., Summers, S., Sorescu, G. P., Faundez, V. and Kowalczyk, A. P. (2003). Mechanisms of VE-cadherin processing and degradation in microvascular endothelial cells. *J. Biol. Chem.* **278**, 19199-19208. doi:10.1074/jbc.M211746200

Xiao, K., Garner, J., Buckley, K. M., Vincent, P. A., Chiasson, C. M., Dejana, E., Faundez, V. and Kowalczyk, A. P. (2005). p120-Catenin regulates clathrin-dependent endocytosis of VE-cadherin. *Mol. Biol. Cell* **16**, 5141-5151. doi:10.1091/mbc.e05-05-0440

Yang, Z., Huang, C., Wu, Y., Chen, B., Zhang, W. and Zhang, J. (2019). Autophagy protects the blood-brain barrier through regulating the dynamic of claudin-5 in short-term starvation. *Front. Physiol.* **10**, 2. doi:10.3389/fphys.2019.00002

Zhang, J., Stirling, B., Temmerman, S. T., Ma, C. A., Fuss, I. J., Derry, J. M. J. and Jain, A. (2006). Impaired regulation of NF-kappaB and increased susceptibility to colitis-associated tumorigenesis in CYLD-deficient mice. *J. Clin. Invest.* **116**, 3042-3049. doi:10.1172/JCI28746

Zhang, G.-S., Tian, Y., Huang, J.-Y., Tao, R.-R., Liao, M.-H., Lu, Y.-M., Ye, W.-F., Wang, R., Fukunaga, K., Lou, Y.-J. et al. (2013). The γ-secretase blocker DAPT reduces the permeability of the blood-brain barrier by decreasing the ubiquitination and degradation of occludin during permanent brain ischemia. *CNS Neurosci. Ther.* **19**, 53-60. doi:10.1111/cns.12032

Zhao, J., Mialki, R. K., Wei, J., Coon, T. A., Zou, C., Chen, B. B., Mallampalli, R. K. and Zhao, Y. (2013). SCF E3 ligase F-box protein complex SCF(FBXL19) regulates cell migration by mediating Rac1 ubiquitination and degradation. *FASEB J.* **27**, 2611-2619. doi:10.1096/fj.12-223099

Zhu, X., Ding, S., Qiu, C., Shi, Y., Song, L., Wang, Y., Wang, Y., Li, J., Wang, Y., Sun, Y. et al. (2017). SUMOylation negatively regulates angiogenesis by targeting endothelial NOTCH signaling. *Circ. Res.* **121**, 636-649. doi:10.1161/CIRCRESAHA.117.310696