

The Met1-linked ubiquitin machinery: Emerging themes of (de)regulation

Matous Hrdinka¹ & Mads Gyrd-Hansen^{1,*}

¹ Ludwig Institute for Cancer Research, Nuffield Department of Clinical Medicine, University of Oxford,
Old Road Campus Research Building, Oxford, OX3 7DQ, UK

* Corresponding author: Mads Gyrd-Hansen, mads.gyrd-hansen@ludwig.ox.ac.uk

Character count: 61,218

Abstract

The linear ubiquitin chain assembly complex, LUBAC, is the only known mammalian ubiquitin ligase that makes methionine 1 (Met1) linked polyubiquitin (also referred to as linear ubiquitin). A decade after LUBAC was discovered as a cellular activity of unknown function, there are now many lines of evidence connecting Met1-linked polyubiquitin to NF- κ B signaling, cell death, inflammation, immunity and cancer. We now know that Met1-linked polyubiquitin has potent signaling functions and that its deregulation is connected to disease. Indeed, mutations and deficiencies in several factors involved in conjugation and deconjugation of Met1-linked polyubiquitin have been implicated in immune-related disorders. Here, we discuss current knowledge and recent insights into the role and regulation of Met1-linked polyubiquitin, with an emphasis on the mechanisms controlling the function of LUBAC.

Introduction

Transcription factors in the nuclear factor-kappa B (NF- κ B) family orchestrate inflammatory responses and their activation by immune receptors, such as pattern recognition receptors (PRRs), cytokine receptors and antigen receptors, is important for innate and adaptive immune function. A unifying feature of the signaling processes triggered by these receptors is that they rely on formation of ubiquitin (Ub) chains to transmit the signal from the activated receptor to the nucleus for stimulation of NF- κ B-mediated transcription (**Figure 1**).

The discovery that Ub chains are required for NF- κ B activation was reported more than 20 years ago with the finding that Inhibitor of NF- κ B alpha (I κ B α , also termed NFKBIA) is modified with Ub chains (linked via Lys48; Lys48-Ub) in response to receptor activation, leading to its rapid degradation via the proteasome (Chen et al., 1995; Palombella et al., 1994; Traenckner et al., 1994). Subsequently, a series of studies by Zhijian “James” Chen and colleagues showed that Ub chains linked via Lys63 (Lys63-Ub) play a non-degradative role in kinase signaling and NF- κ B activation by facilitating the activation of TGF β -activated kinase 1 (TAK1) (Deng et al., 2000; Wang et al., 2001). In 2006, Kazuhiro Iwai and colleagues identified a Ub E3 ligase complex that only assembles Ub chains through the N-terminal methionine (Met1-Ub); they called this the linear Ub chain assembly complex (LUBAC), and subsequently discovered that LUBAC stimulates NF- κ B activity by conjugating Met1-Ub (Tokunaga et al., 2009)(Kirisako et al., 2006). Now, after 10 years of research into LUBAC and Met1-Ub biology, it is clear that Met1-Ub harbors potent signaling properties and, together with Lys63-Ub and Lys48-Ub, plays a central role in NF- κ B activation and immune function (**Figure 2**). Met1-Ub is also implicated in signaling by viral nucleotide-sensing receptors, leading to interferon response factor (IRF)-mediated transcription (**Figure 1**) and other signaling processes (reviewed in (Sasaki and Iwai, 2015)). In this review we primarily discuss its role in NF- κ B signaling.

Recent research into the cellular mechanisms that govern LUBAC function, indicate that LUBAC is regulated in multiple ways to ensure accurate control of Met1-Ub levels. In this review, we discuss existing knowledge of the cellular machinery that controls Met1-Ub conjugation and stability, the pathologies associated with deregulation of Met1-Ub, and remaining questions.

The Met1-Ub machinery

The function and regulation of Ub modifications involves, at a minimum, three classes of proteins: i) Ub E3 ligases that, together with E2 and E1 enzymes, conjugate Ub moieties to target proteins; ii) Ub receptors that harbor Ub-binding domains (UBDs) and “read” the Ub modification; and iii) deubiquitinases (DUBs) that disassemble Ub chains and remove Ub modifications from target proteins. Proteins of all three classes specific or highly selective for Met1-Ub have been discovered and the Met1-linkage (and probably other linkages) should therefore be viewed as a posttranslational modification distinct from other types of Ub-modifications.

Met1-Ub conjugation and deconjugation

LUBAC, the only known E3 catalyzing Met1-Ub in vertebrates, is composed of three core subunits (**Figure 3**): HOIL-1-interacting protein (HOIP; also termed RNF31, ZIBRA); Heme-oxidized IRP2 ubiquitin ligase-1 (HOIL-1; also termed RBCK1, RNF54); and Shank-associated RH domain-interacting protein (SHARPIN; also termed hSIPL1) (Gerlach et al., 2011; Ikeda et al., 2011; Kirisako et al., 2006; Tokunaga et al., 2011). Interestingly, an orthologue of LUBAC, termed LUBEL (Linear ubiquitin E3 ligase), has recently been discovered in fruit flies suggesting that the machinery to assemble Met1-Ub is also present in non-vertebrates (Asaoka et al., 2016), although the cellular function of Met1-Ub in flies appears not to be related to immune signaling.

The LUBAC subunits HOIP and HOIL-1 belong to the RING-betweenRING-RING (RBR) family of E3s, which facilitate the transfer of Ub from “charged” E2-Ub complexes to substrates via formation of a Ub-thioester intermediate with the catalytic cysteine in the E3 (Stieglitz et al., 2012; Wenzel et al., 2011) (**Figure 4**). The HOIP RBR has a C-terminal extension termed a “linear ubiquitin chains-determining domain (LDD)” (Smit et al., 2012), which together with the RING2 of the RBR constitutes the catalytic in-between RING (CBR) (Stieglitz et al., 2013) (**Figure 4**). Although HOIL-1 has been shown to have weak Ub ligase activity *in vitro*, HOIP is required for Met1-Ub assembly in cells and is

considered the catalytic subunit of LUBAC (Emmerich et al., 2013; Stieglitz et al., 2012). SHARPIN does not harbor Ub ligase activity but interacts with HOIP and functions as a co-factor in the complex.

HOIP in isolation (in vitro) is auto-inhibited by an intramolecular interaction between the ubiquitin associated (UBA) domain-containing N-terminal part of the protein and the RBR (Stieglitz et al., 2012). However, the E3 activity of HOIP is unleashed by intermolecular interactions with HOIL-1 or SHARPIN (Smit et al., 2012; Stieglitz et al., 2012) (**Figure 4**). In vitro activity measurements indicate that SHARPIN and HOIL-1 are redundant for LUBAC activity, but both are clearly important in a cellular context (see below), and might provide unique properties to LUBAC such as localization or substrate specificity.

In cells, HOIP, HOIL-1, and SHARPIN exist largely in stable LUBAC complexes with an estimated molecular mass of approx. 600 kDa (Kirisako et al., 2006; Tokunaga et al., 2011). The trimeric structure of LUBAC is maintained by interactions mediated by ubiquitin-like (UBL) domains in HOIL-1 and SHARPIN, and the UBA domain and the second nuclear protein localization 4 (Npl4) zinc finger (NZF) domain in HOIP (Gerlach et al., 2011; Ikeda et al., 2011; Kirisako et al., 2006; Tokunaga et al., 2011) (**Figure 4**). Mass spectrometry-based quantitation indicates similar abundance of the three components in cells (MaxQB Database; (Schaab et al., 2012)), suggesting that levels of LUBAC subunits are closely coordinated. In line with this, loss of any of the three subunits leads to destabilization of the other components and impaired LUBAC function (Boisson et al., 2015; Boisson et al., 2012; Gerlach et al., 2011; Ikeda et al., 2011; Peltzer et al., 2014; Tokunaga et al., 2011; Tokunaga et al., 2009).

To date, two DUBs have been found to disassemble LUBAC-conjugated Met1-Ub: OTULIN and CYLD (Keusekotten et al., 2013; Komander et al., 2009; Rivkin et al., 2013; Sato et al., 2015) (**Figure 3**). Their characteristics and activities are discussed in detail in later sections.

Met1-Ub binding proteins

The best characterized Met1-Ub binding protein is the non-catalytic subunit of the I κ B kinase (IKK) complex, NF- κ B essential modifier (NEMO; also termed IKK γ). NEMO binds Met1-Ub through its

UBAN domain (UBAN for A20 binding and inhibitor of NF- κ B (ABIN) and NEMO; also termed Nemo Optineurin Abin (NOA)) (Laplantine et al., 2009; Lo et al., 2009; Rahighi et al., 2009). NEMO also has a C-terminal zinc finger (ZnF) that, together with the UBAN domain, enables NEMO to interact with Lys63-Ub albeit with much lower affinity than Met1-Ub (Ea et al., 2006; Kensche et al., 2012; Laplantine et al., 2009). ABIN proteins (ABIN 1-3) and Optineurin also contain UBAN domains and interact with Met1-Ub as well as Lys63-Ub (Oshima et al., 2009; Wagner et al., 2008), although ABIN1 and ABIN2 show preference for Met1-Ub in binding competition assays (Komander et al., 2009; Rahighi et al., 2009). Other Met1-Ub binding proteins include the LUBAC components HOIL-1 and SHARPIN, cellular Inhibitor of Apoptosis (IAP) 1 (cIAP1), cIAP2, X-linked IAP (XIAP), and A20 (Gyrd-Hansen et al., 2008; Haas et al., 2009; Ikeda et al., 2011; Komander et al., 2009; Tokunaga et al., 2012; Verhelst et al., 2012). A20 (encoded by the *TNFAIP3* gene) belongs to the ovarian tumor (OTU) family of deubiquitinases (DUBs) but does not show activity towards Met1-Ub (A20 cleaves Lys48-Ub in vitro and also facilitates disassembly of Lys63-Ub in cells) (Komander et al., 2009; Wertz et al., 2015; Wertz et al., 2004). A20 binds Met1-Ub and Lys63-Ub via its ZnF7 and ZnF4 domains, respectively (Bosanac et al., 2010; Skaug et al., 2011; Tokunaga et al., 2012; Verhelst et al., 2012; Wertz et al., 2015), and is reported to stabilize Met1-Ub at the tumor necrosis factor (TNF) receptor 1 (TNFR1) complex via its ZnF7 Draber et al. (2015).

Met1-Ub in cell signaling

LUBAC was first described to contribute to signaling in response to TNFR1 activation and has since been found to regulate signal transduction by a wide range of NF- κ B-activating immune receptors, including cytokine receptors, Toll-like receptors (TLRs), NOD-like receptors (NLRs), and antigen receptors (**Figure 1**). Therefore, LUBAC can be considered one of the core components of NF- κ B-activating signaling pathways (Reviewed in (Shimizu et al., 2015)).

Recruitment to signaling complexes

As a general concept, the engagement of NF- κ B-activating immune receptors leads to recruitment of receptor-associated adaptor proteins (such as TRADD and RIPK1 to TNFR1, RIPK2 to NOD2,

MyD88 and IRAK1/4 to IL-1 β R and TLRs) and E3s (such as TRAF2 and cIAP1/2 to TNFR1, XIAP and Pellino 3 to NOD2, TRAF6 and Pellino 1 to IL-1 β R and TLRs), forming an initial receptor complex (Reviewed in (Fiil and Gyrð-Hansen, 2014; Lork et al., 2017)). In the case of antigen receptors, activation leads to formation of a cytosolic CARD11-BCL10-MALT1 (CBM) complex, which recruits TRAF6 and cIAP1/2. Formation of initial receptor complexes facilitates conjugation of Lys63-Ub and other Ub chain types on complex components (**Figure 2**). Current evidence suggests that these Ub modifications facilitate the recruitment of LUBAC to the signaling complexes, as has been demonstrated for TNFR1 and NOD2 (Damgaard et al., 2012; Haas et al., 2009). In line with this, all three core LUBAC proteins contain NZF domains enabling their interaction with Lys63-Ub and Met1-Ub (Emmerich et al., 2013; Gerlach et al., 2011; Haas et al., 2009; Ikeda et al., 2011; Sato et al., 2011; Shimizu et al., 2016). For example, the NZF in SHARPIN is needed for the recruitment of LUBAC to the TNFR1 complex (Shimizu et al., 2016) (**Figure 4**).

Recruitment of LUBAC leads to conjugation of Met1-Ub within the signaling complex, which together with Lys63-Ub facilitates the activation of two ubiquitin-activated kinase complexes. Specifically, the TGF β -activated kinase 1 (TAK1) complex is recruited via its Lys63-Ub-binding subunits called TAK1-binding protein 2 (TAB2) and TAB3 (Kanayama et al., 2004) (here forth referred to as TAB-TAK1), and the I κ B kinase α (IKK α)/IKK β complex is recruited via the non-catalytic subunit NEMO (here forth referred to as NEMO-IKK) through its binding to Met1-Ub (Rahighi et al., 2009) (**Figure 2**). TAK1 phosphorylates IKK β , which primes NEMO-IKK for auto-phosphorylation and full activation (Zhang et al., 2014). In turn, NEMO-IKK phosphorylates the NF- κ B inhibitor, I κ B α , leading to its proteasomal degradation and translocation of NF- κ B to the nucleus where it engages in gene transcription. TAB-TAK1 also mediates activation of the mitogen-activated protein kinase (MAPK) cascade (Wang et al., 2001) (**Figure 2**). It should be noted that there are exceptions where Met1-Ub and Lys63-Ub appear to be entirely or partly dispensable for immune receptor signaling, implying that in some contexts TAB-TAK1 and NEMO-IKK can be engaged by other Ub linkages or even independently of Ub chains (Hrdinka et al., 2016; Ori et al., 2013; Sasaki et al., 2013; Zhang et al., 2017).

In addition to its role in immune signaling that activates gene expression, LUBAC is also involved in regulating cell death after receptor activation. This role for LUBAC is described in several recent reviews (Sasaki and Iwai, 2015; Shimizu et al., 2015) and is not be discussed in detail here.

LUBAC substrates

Several receptor-associated proteins have been reported to be modified by LUBAC, including RIPK1, RIPK2, MyD88, IRAK1/4, NEMO and TNFR1 (Draber et al., 2015; Emmerich et al., 2013; Fiil et al., 2013; Gerlach et al., 2011; Hrdinka et al., 2016; Tokunaga et al., 2009). However, an emerging concept is that LUBAC preferentially conjugates Met1-Ub to existing Lys63-Ub (and possibly other linkages), thereby generating hybrid Lys63/Met1-Ub (**Figure 2**) (Emmerich et al., 2016; Emmerich et al., 2013; Fiil et al., 2013; Hrdinka et al., 2016). Thus, the major substrate for LUBAC might be Lys63-Ub and, as such, not the protein subunits of the complexes to which LUBAC is recruited. The molecular determinants in LUBAC important for generation of hybrid chains have not yet been defined but it is possible that the HOIL-1 NZF, which selectively binds Met1-Ub and contributes to LUBAC-induced NF- κ B activation, could facilitate extension of Met1 linkages on Lys63-Ub (Haas et al., 2009; Sato et al., 2011; Tokunaga et al., 2009). The functional importance of hybrid chains remains to be determined directly but it is plausible that they ensure efficient kinase activation by juxtaposing the TAB-TAK1 and NEMO-IKK complexes to the same Ub chain. Hybrid Lys63/Met1-Ub might also reinforce the recruitment of NEMO-IKK by enabling a bipartite interaction with the UBAN and C-terminal ZnF in NEMO (Laplantine et al., 2009). Alternatively, they could enable proper termination of NF- κ B signaling by providing the optimal recruitment platform for A20 and ABIN1, since these proteins interact with both Met1-Ub and Lys63-Ub, and rely on Ub binding to negatively regulate immune signaling (Lu et al., 2013; Nanda et al., 2011).

Regulation of LUBAC by deubiquitinases

Ub chains exist in a dynamic equilibrium of E3-mediated assembly and DUB-mediated disassembly. DUBs may be in the same complex as E3s (Sowa et al., 2009) and can work as an Ub chain editing module (Engel et al., 2016; Iyengar et al., 2015; Taranets et al., 2015; Wertz et al., 2004). Recent evidence suggests this may be the case for LUBAC.

LUBAC-DUB complexes

As mentioned above, there are two DUBs associated with LUBAC: OTULIN and CLYD. OTULIN (formerly known as Fam105B and Gumbly) is an OTU-type DUB and exclusively disassembles Met1-Ub (Keusekotten et al., 2013; Rivkin et al., 2013). In addition to its catalytic OTU domain, OTULIN contains a peptide:N-glycanase/UBA- or UBX-containing proteins (PUB)-interacting motif (PIM) in the unstructured N-terminal part of the protein that facilitates interaction with the N-terminal PUB domain of HOIP (Elliott et al., 2014; Schaeffer et al., 2014) (**Figure 4** and **Figure 5**). Interaction of a PUB domain with a PIM was first reported for the PUB domain of peptide:N-glycanase (PNGase) and the C-terminal PIM of the ATPase p97 (also termed valosin-containing protein (VCP)) (Zhao et al., 2007). Despite similarities between the PUB:PIM interactions of PNGase-p97 and HOIP-OTULIN, the HOIP PUB interacts only weakly with the p97 PIM and the OTULIN PIM binds only the PUB domain of HOIP (Elliott et al., 2014; Zhao et al., 2007). This suggests that the HOIP-OTULIN interaction has evolved to create a specific and high-affinity interaction (Elliott et al., 2014). Nonetheless, the interaction between HOIP and p97 might influence LUBAC function since p97 is among the most abundant proteins in cells (Elliott et al., 2014; Schaeffer et al., 2014; Zeiler et al., 2012).

The cylindromatosis tumor suppressor CYLD is a ubiquitin-specific protease (USP)-type DUB that preferentially disassembles Met1-Ub and Lys63-Ub albeit it also has some activity against other linkages (Komander et al., 2009; Ritorto et al., 2014; Sato et al., 2015). HOIP associates also with CYLD through its PUB domain (Draber et al., 2015; Hrdinka et al., 2016; Takiuchi et al., 2014), but the interaction is indirect and requires Spermatogenesis-associated 2 (SPATA2), which acts as a bridging factor between HOIP and CYLD (Elliott et al., 2016; Kupka et al., 2016; Schlicher et al., 2016; Wagner et al., 2016). SPATA2 contains a PIM that docks into the PIM binding pocket in the HOIP PUB in a manner identical to the OTULIN PIM (Elliott et al., 2016). SPATA2 and CYLD interact through an N-terminal PUB domain in SPATA2 and the USP domain of CYLD (**Figure 4** and **Figure 5**). The interaction is reinforced by CYLD dimerization mediated by a B-box within the USP domain (Elliott et al., 2016) (**Figure 5**). Notably, the PIM-binding pocket of the SPATA2 PUB domain does not interact with “canonical” PIM peptides as it is structurally distinct from the pocket in other PUB domains (Elliott et al., 2016).

OTULIN vs. SPATA2-CYLD

Consistent with the fact that OTULIN and SPATA2-CYLD utilize the same mechanism to dock to LUBAC, the binding of these DUBs is mutually exclusive (Draber et al., 2015; Elliott et al., 2016). This suggests there are at least two distinct pools of LUBAC-DUB complexes in cells, but it remains to be determined if these are stable complexes involved in separate cellular processes or if OTULIN and SPATA2-CYLD are dynamically exchanged at the HOIP PUB interface. Currently, there is evidence supporting both models and further studies are needed to confirm the composition and function of LUBAC complexes.

The absence of OTULIN or OTULIN activity results in a dramatic increase in steady-state levels of cellular Met1-Ub, suggesting it is essential for general disassembly of Met1-Ub (Damgaard et al., 2016; Rivkin et al., 2013). In addition, OTULIN restricts the ubiquitination of receptor-associated proteins and prevents LUBAC from conjugating Met1-Ub on itself (Fiil et al., 2013; Hrdinka et al., 2016; Keusekotten et al., 2013). Control of LUBAC autoubiquitination by OTULIN requires the direct interaction of LUBAC with OTULIN via HOIP (Elliott et al., 2014). Although the effects of LUBAC autoubiquitination are incompletely understood, there is evidence that it affects LUBAC-mediated ubiquitination of receptor complex components and can initiate NF- κ B signaling (Damgaard et al., 2016; Draber et al., 2015; Fiil et al., 2013; Hrdinka et al., 2016).

In contrast to OTULIN, SPATA2-CYLD does not control LUBAC autoubiquitination, even though CYLD readily cleaves Met1-Ub *in vitro* and is stably associated with LUBAC (Hrdinka et al., 2016; Komander et al., 2009; Sato et al., 2015). Furthermore, ablation of CYLD does also not result in a major change in cellular Met1-Ub levels (Draber et al., 2015), implying that CYLD's contribution to regulation of Met1-Ub is more restricted than that of OTULIN. The basis for this difference is unknown, but it is unlikely to be due to differences in intrinsic activity towards Met1-Ub as the activity of recombinant OTULIN is comparable to that reported for the CYLD USP domain (Keusekotten et al., 2013; Sato et al., 2015). A possible explanation is that OTULIN binds Met1-Ub with exceptionally high affinity compared to other Met1-Ub binding proteins, including NEMO (Keusekotten et al., 2013; Komander et al., 2009; Rahighi et al., 2009; Wagner et al., 2008). This might enable OTULIN to displace Ub-binding proteins from Met1-Ub and thereby make the Ub chains accessible to cleavage.

In contrast, Met1-Ub bound by proteins such as NEMO and HOIL-1 might not be accessible to CYLD. This notion is supported by the observation that mouse embryos homozygous for a point mutation in OTULIN (W96R) that interferes with Met1-Ub-binding accumulate excessive Met1-Ub (Keusekotten et al., 2013; Rivkin et al., 2013).

Analyses of receptor complexes (TNFR1 and NOD2) indicate that SPATA2-CYLD is co-recruited with LUBAC to activated receptors (Draber et al., 2015; Elliott et al., 2016; Kupka et al., 2016; Schlicher et al., 2016; Wagner et al., 2016). Since the CYLD-SPATA2-LUBAC complex has the capacity not only to regulate Met1-Ub but also to disassemble Lys63-Ub, it is possible that this complex functions as a Ub chain editing complex in which CYLD trims Lys63-Ub early during signaling, which could assist LUBAC in generating hybrid Lys63/Met1-Ub (Emmerich et al., 2016; Emmerich et al., 2013; Hrdinka et al., 2016).

Curiously, little or no OTULIN appears to be stably associated with receptor complexes suggesting there are regulatory mechanisms directing the selective recruitment of the CYLD-SPATA2-LUBAC complex but not the OTULIN-LUBAC complex (Draber et al., 2015; Elliott et al., 2016; Kupka et al., 2016; Schaeffer et al., 2014; Schlicher et al., 2016; Wagner et al., 2016). Another possibility is that the OTULIN-LUBAC complex is being recruited to the receptor complex but that OTULIN-binding to LUBAC is antagonized, for example through phosphorylation of Tyr56 in the OTULIN PIM, which precludes binding to the HOIP PUB domain (Keusekotten et al., 2013). Phosphorylation of Tyr56 in OTULIN has been detected by mass spectrometry (www.phosphosite.org) but there is no direct experimental evidence that this is a regulatory mechanism in cells. Nonetheless, the observation that OTULIN restricts Met1-Ub of receptor-associated proteins suggests that OTULIN has access to receptor complexes even if it is not stably bound (Fiil et al., 2013; Hrdinka et al., 2016; Keusekotten et al., 2013).

Regulation of LUBAC by proteolysis

An emerging mechanism by which LUBAC is regulated is proteolytic cleavage of LUBAC subunits. Regulation of LUBAC by proteolysis was first observed in cells after stimulation of PKC activity with

phorbol 12-myristate 13-acetate (PMA), which resulted in cleavage of HOIL-1 (Nakamura et al., 2006). More recently, HOIL-1 was found to be cleaved by paracaspase mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) following antigen receptor engagement in B and T cells (Douanne et al., 2016; Elton et al., 2016; Klein et al., 2015). The cleavage site is located between the UBL and NZF domain (**Figure 4**) and cleavage by MALT1 is reported to generate a dominant negative fragment that attenuates LUBAC activity and NF- κ B activity at later stages of signaling (Douanne et al., 2016; Elton et al., 2016; Klein et al., 2015). Interestingly, in activated B cell (ABC) diffuse large B cell like lymphoma (DLBCL) cell lines that rely on MALT1 activity for survival (Ferch et al., 2009), HOIL-1 was found to be constitutively cleaved by MALT1 (Douanne et al., 2016). MALT1 has also been reported to cleave several other Ub signaling-related factors, including CYLD and A20, and thereby might regulate the disassembly of Met1-Ub (Demeyer et al., 2016).

HOIP is also cleaved by caspases in response to induction of apoptosis by TNF and TRAIL (Goto and Tokunaga, 2017; Joo et al., 2016; Lafont et al., 2017). The cleavage occurs between the NZF1 and NZF2 (**Figure 4**) and several caspases (caspase-3, -6, and -8) are reported to be involved (Joo et al., 2016) (Lafont et al., 2017). The functional consequence of HOIP cleavage is not fully understood, although in Jurkat cells it was reported to impair LUBAC function and to sensitize cells to TNF-induced cell death (Joo et al., 2016). It is noteworthy that the cleavage removes the N-terminal part of HOIP containing the PUB domain and thus could be a mechanism to separate OTULIN and CYLD-SPATA2 from the catalytic activity of LUBAC.

Consequences of Met1-Ub deregulation

Given the important role of Met1-Ub in the signaling networks it is unsurprising that genetic and environmental factors that interfere with Met1-Ub conjugation or disassembly can result in severe pathologies (Table 1-3 and Figure 6).

Impaired LUBAC function

Embryos of HOIP knockout mice (Peltzer et al., 2014) or with catalytically inactive HOIP (Emmerich et al., 2013; Shimizu et al., 2016) die at midgestation, displaying abnormal heart development and defective vascularization of the yolk sac, caused in part by excessive TNF-dependent apoptosis of endothelial cells. However, embryonic lethality is not rescued by deletion of TNFR1, suggesting that LUBAC regulates other processes important for embryonal development (**Table 1**) (Peltzer et al., 2014). Met1-Ub has been shown to negatively regulate the Wnt- β -catenin pathway (Rivkin et al., 2013; Takiuchi et al., 2014), but whether this underlies the embryonic lethality remains to be determined. Specific ablation of HOIP in hepatocytes was recently reported to result in late-onset liver tumors, possibly as a consequence of cell death, TNF-driven inflammation and regeneration at the early stages of life (Shimizu et al., 2017). In line with this, silencing of LUBAC subunits in livers of adult mice causes acute liver inflammation and fibrosis (Yamamotoya et al., 2017).

In humans, a hypomorphic mutation in HOIP or truncating mutations in HOIL-1 cause severe pathology, including multiorgan autoinflammation, polyglucosan storage myopathy and cardiomyopathy (also termed muscular amylopectinosis), and immunodeficiency (Boisson et al., 2015; Boisson et al., 2012) (**Table 2**). Notably, the clinical presentation associated with HOIL-1 mutations is variable as some cases present only with muscular amylopectinosis with no obvious immunological defects (Nilsson et al., 2013; Wang et al., 2013). The reason for this variability could be linked to patient-specific genetic landscapes or possibly to differences in environmental exposure to microbial or viral infections (MacDuff et al., 2015). The reported human mutations destabilize LUBAC components and result in impaired NF- κ B responses to receptor stimulation (with TNF, IL-1 β , and CD40 ligand) in patient-derived fibroblasts and immortalized B cells (Boisson et al., 2015; Boisson et al., 2012). Unexpectedly, isolated monocytes from patients were found to be hyper-responsive to IL-1 β stimulation, which possibly contributes to autoinflammation in patients. The underlying reason for this was not further explored but suggests cell type-specific roles for Met1-Ub in immune signaling, at least in humans.

In mice, ablation of HOIL-1 (**Table 1**) gives rise to a relatively mild phenotype compared to HOIL-1-mutations found in humans and HOIP knockout mice. However, HOIL-1-deficient mice have impaired immunity to certain pathogens (MacDuff et al., 2015; Tokunaga et al., 2009). This could reflect that

HOIL-1 and SHARPIN are partly redundant during mouse development (see below). An alternative explanation is that the strategy used to target the HOIL-1 gene (*Rbck1*; targeting of exon 7/8) did not fully ablate the gene, resulting in truncated HOIL-1 with residual function (Tokunaga et al., 2009).

The studies of the third LUBAC component, SHARPIN, have been facilitated by a spontaneous autosomal recessive mutation in the *Sharpin* gene (HogenEsch et al., 1993), which results in loss of SHARPIN and destabilization of LUBAC in homozygous mice (Gerlach et al., 2011; Ikeda et al., 2011; Tokunaga et al., 2011). Phenotypically, SHARPIN-deficiency causes pleomorphic disease characterized by early onset of chronic proliferative dermatitis (*cpdm*; mice are referred to as *Sharpin^{cpdm}* mice) and multi-organ inflammation. *Sharpin^{cpdm}* mice have been extensively studied, in combination with a range of other mutations and, as a resource, **Table 1** provides a summary of the mutations and phenotypes. The viability of SHARPIN-deficient embryos is likely due to residual LUBAC activity, since the combined loss of SHARPIN and HOIL-1 results in embryonic lethality of homozygous embryos at the same stage as HOIP-deficient mice (Shimizu et al., 2016).

The skin inflammation phenotype of *Sharpin^{cpdm}* mice is driven by TNF-TNFR1 signaling, which in the absence of SHARPIN results in increased susceptibility to TRADD-, FADD- and caspase-8-mediated apoptosis of keratinocytes (Gerlach et al., 2011; Kumari et al., 2014; Rickard et al., 2014). IL-1 β -mediated inflammation also contributes to the skin phenotype but interference with IL-1 β function does not provide long-term rescue of the skin phenotype (unlike loss of TNFR1 signaling, which does rescue the skin phenotype) (**Table 1**). The skin phenotype of *Sharpin^{cpdm}* mice appears to be largely cell- or tissue intrinsic as it is maintained after allogeneic transplantation to wildtype mice and does not appear to be mediated by the immune compartment (**Table 1**). TNFR1-mediated cell death also contributes to organ inflammation and immunological disease in *Sharpin^{cpdm}* mice but, in contrast to the skin phenotype, this involves necroptosis as well as apoptosis (Berger et al., 2014; Kumari et al., 2014; Rickard et al., 2014) (**Table 1**).

Enhanced Met1-Ub formation

The first indication that excessive Met1-Ub can have detrimental consequences at the organismal levels came from the phenotypic analysis of mice carrying spontaneous missense mutations in OTULIN that interfere with its catalytic function (Rivkin et al., 2013). Mice homozygous for these mutations die during embryogenesis from aberrant angiogenesis, suggested to be a result of impaired canonical Wnt signaling due to accumulation of Met1-Ub.

Excessive Met1-Ub is harmful also after birth as illustrated by conditional deletion of *Otulin* in adult mice, which is lethal within days due to acute systemic inflammation (Damgaard et al., 2016). The systemic inflammation is mediated by myeloid cells (but not B and T cells) that accumulate high levels of Met1-Ub and display increased NF- κ B activity and cytokine production. This implies that the accumulation of Met1-Ub is sufficient to trigger inflammatory signaling. Curiously, OTULIN-deficient T and B cells showed a concomitant reduction in HOIP and SHARPIN levels, possibly explaining the lack of an overt phenotype in mice with B or T cell-specific ablation of *Otulin* (Damgaard et al., 2016).

The phenotype of *Otulin*-ablated mice largely recapitulates the clinical features of human patients with OTULIN-deficiency, which causes a severe autoinflammatory syndrome termed OTULIN-related autoinflammatory syndrome (ORAS; also termed otulipenia) (Damgaard et al., 2016; Zhou et al., 2016). Patients present with early-onset idiopathic systemic inflammation (**Table 2**), which appears to be well managed by anti-TNF treatment and in some cases IL-1 β R blockade (partial response), consistent with the notion that disease is caused by autoinflammation (Damgaard et al., 2016; Zhou et al., 2016).

The best described function of CYLD is as a tumor suppressor and although most of CYLD's *in vivo* functions have been ascribed to its Lys63-Ub DUB activity, a potential contribution of deregulated Met1-Ub in CYLD-associated pathologies needs to be considered. Germline mutations in *CYLD* underlie autosomal dominant tumor predisposition syndromes (Reviewed in (Kazakov, 2016)) and somatic loss-of-function mutations in *CYLD* have been linked to increased NF- κ B activity and human papilloma virus-associated head and neck squamous cell carcinoma (Hajek et al., 2017). Similarly, ablation of *Cyld* in mice predisposes to the development of inflammation-associated tumorigenesis in skin, intestine and liver (Karatzas et al., 2016; Massoumi et al., 2006; Zhang et al., 2006)(Nikolaou et al., 2012). CYLD-deficiency in mice also affects B and T cell development and innate immune

responses to acute bacterial infection (Jin et al., 2007; Lim et al., 2007; Nishanth et al., 2013; Reiley et al., 2006). Thus, CYLD has bona fide tumor suppressive functions, but also appears to restrict inflammation primarily in response to environmental cues such as tissue damage or infection and, as such, is functionally distinct from OTULIN.

Enhanced LUBAC activity has also been implicated in susceptibility to certain types of blood cancer. Specifically, single nucleotide polymorphisms (SNPs) in HOIP that increase LUBAC activity and enhance NF- κ B signaling, possibly by strengthening the interaction between HOIP and HOIL-1, are enriched ~8-fold in patients with ABC DLBCL relative to the general population (Yang et al., 2014) (Table 2). This is consistent with deregulated pro-survival and proliferative NF- κ B signaling being a hallmark of ABC DLBCL (Davis et al., 2001). Consistent with this, oncogenic mutations in CARD11, one of the components of the CBM complex, have been found promote its spontaneous association with LUBAC and consequently increase Met1-Ub of BCL10, leading to activation of NF- κ B (Yang et al., 2016).

Perturbation of LUBAC function by pathogens

Key proteins in immune signaling pathways are often targeted by pathogens to subvert normal host responses. Several recent studies have revealed that LUBAC and its capacity to stimulate innate immune signaling pathways are perturbed by bacteria and viruses (**Table 3**).

LUBAC is targeted by *Shigella flexneri* as a part of its broad immune evasion strategy facilitated by its type-III secretion system (Ashida et al., 2014). Upon infection, the bacterial Ub ligases IpaH1.4 and IpaH2.5 modify HOIP with Lys48-Ub, targeting it for proteasomal degradation (de Jong et al., 2016). In addition, the *S. flexneri* OspG protein targets LUBAC indirectly by sequestering the E2 UBE2L3 (also termed UbCH7) which LUBAC utilizes for Met1-Ub synthesis (Pruneda et al., 2014).

A slightly different mechanism is employed by Hepatitis C virus (HCV). The HCV-encoded proteins NS3 and NS5B inhibit LUBAC-mediated NF- κ B signaling possibly by interacting with the NZF2 domain in HOIP to prevent the interaction of LUBAC with NEMO (Chen et al., 2015; de Chassey et al., 2008). Physical disruption of the LUBAC complex also appears to be a strategy used by the Porcine

reproductive and respiratory syndrome virus (PRRSV): its virulence factor Nonstructural Protein 1 (NSP1) interferes with the interaction between HOIP and SHARPIN and attenuates NF- κ B signaling (Jing et al., 2017). Whether targeting LUBAC is a virulence mechanism used by other viruses remains to be determined.

In contrast to pathogens that disrupt LUBAC to evade the immune system, the oncogenic Epstein-Barr virus (EBV), responsible for human B cell malignancies, engages LUBAC to evade the cellular anti-viral response and to facilitate NF- κ B-dependent cellular transformation (Wang et al., 2017). The EBV-encoded oncoprotein Latent Membrane Protein 1 (LMP1), a member of the TNFR superfamily, recruits LUBAC to ubiquitinate and inactivate the transcription factor IRF7 and thereby avert anti-viral interferon production (Wang et al., 2017). A similar mechanism is employed by Hepatitis B virus (HBV), which via its HBx protein recruits LUBAC to disrupt assembly of the mitochondrial antiviral signaling protein (MAVS) signalosome and thereby impair IRF3-mediated interferon production (Khan et al., 2016).

EBV-encoded LMP1 also facilitates the interaction of LUBAC with TRAF1/TRAF2 complexes, leading to assembly of Lys63- and Met1-Ub on LMP1 and TRAF1 to drive constitutive activation of the NF- κ B pathway (Greenfield et al., 2015). Likewise, the Tax protein of human T cell leukemia virus type 1 (HTLV-1) has been found to interact with LUBAC to induce the conjugation of Lys63- and Met1-Ub on Tax, resulting in stimulation of IKK-mediated NF- κ B activity (Shibata et al., 2017).

Summary and Future perspectives

Since the discovery of LUBAC, our understanding of the Met1-Ub machinery and its role in cell signaling and physiology has increased dramatically. It is now clear that the cellular factors that conjugate, recognize and disassemble Met1-Ub play an important role in immune function and inflammation, and that defects in the Met1-Ub machinery give rise to immunological disorders and cancer (Figure 6). Our understanding of how the Met1-Ub machinery is regulated is still emerging but, given the potent signaling capacity of Met1-Ub, it is likely that the activities of LUBAC and its associated DUBs are tightly controlled.

LUBAC in cells is constitutively active and the “output” of its activity, i.e. assembly of Met1-Ub, appears largely to be controlled by OTULIN with a lesser contribution by CYLD. Yet, Met1-Ub is virtually undetectable in unstimulated cells and rapidly accumulates after receptor engagement (Draber et al., 2015; Emmerich et al., 2013), suggesting that the rate of Met1-Ub disassembly exceeds the conjugation rate under steady-state conditions but that this balance changes towards Met1-Ub assembly in response to receptor engagement. It may seem energetically “wasteful” to continuously assemble and disassemble Met1-Ub, but this cost is possibly out-weighed by the advantages of cells being able to increase their Met1-Ub levels rapidly when needed, for example to respond appropriately to immune receptor stimulation or bacterial infections.

Similar rapid-response mechanisms have been described for other cellular processes, such as autophagy of damaged mitochondria. The mitochondrial kinase PINK1 (protein phosphatase and tensin homolog (PTEN)-induced kinase 1) is maintained at low levels through proteolysis but is rapidly stabilized upon mitochondrial damage. In turn, PINK1 stimulates Parkin-mediated ubiquitination of mitochondrial surface proteins and autophagic removal of damaged mitochondria (reviewed in (McWilliams and Muqit, 2017)). It will be hugely interesting to identify the sensor mechanisms that control the global and localized accumulation of Met1-Ub in response to immune receptor engagement and possibly other environmental cues. One such mechanism could be the recruitment of LUBAC to signaling complexes containing ubiquitinated proteins (Damgaard et al., 2012; Haas et al., 2009). This brings LUBAC in close proximity to pre-existing Ub chains (in particular Lys63-linked chains) and thereby could stimulate assembly of Met1-Ub into hybrid chains without directly altering the activity of LUBAC.

The relative abundance of LUBAC subunits appears to be coordinated through protein stability and there is emerging evidence that LUBAC and CYLD are regulated by proteolytic cleavage in response to receptor stimulation. However, little is known about how the abundance of LUBAC, OTULIN, CYLD and SPATA2 is regulated, albeit CYLD is reported to be regulated transcriptionally by NF- κ B under some circumstances (Hrdinka et al., 2016; Jono et al., 2004). It will be particularly interesting to determine if the relative abundance of OTULIN, SPATA2-CYLD and LUBAC is regulated by environmental cues or other factors, and how such regulation might influence LUBAC function, Met1-Ub levels and stability, and, ultimately, cellular signaling processes.

Given the potent signaling capacity of Met1-Ub, the Met1-Ub machinery should be an attractive target for therapeutic intervention for immune-regulated pathologies, cancer and possibly other disorders. However, direct interference with the function of core components, i.e. LUBAC and OTULIN, will likely have detrimental consequences. A more feasible strategy to modify Met1-Ub signaling is probably to target the regulatory mechanisms controlling the Met1-Ub machinery. Undoubtedly, we have only just begun to understand the basics of how the Met1-Ub machinery is regulated and it will be fascinating to witness what will be uncovered in the years to follow.

Acknowledgements

We apologize to the many people whose work we could not cite here due to space restrictions. We thank Mary Muers for critical reading of the manuscript and helpful suggestions. M.G-H is supported by the Ludwig Institute for Cancer Research Ltd., a Wellcome Trust Senior Research Fellowship (102894/Z/13/Z), a Sapere Aude: Danish Council for independent Research Starting Grant, the EMBO Young Investigator Programme. The authors declare no conflict of interest.

References

- Asaoka, T., Almagro, J., Ehrhardt, C., Tsai, I., Schleiffer, A., Deszcz, L., Junttila, S., Ringrose, L., Mechtler, K., Kavirayani, A., *et al.* (2016). Linear ubiquitination by LUBEL has a role in *Drosophila* heat stress response. *EMBO Rep* 17, 1624-1640.
- Ashida, H., Kim, M., and Sasakawa, C. (2014). Exploitation of the host ubiquitin system by human bacterial pathogens. *Nat Rev Microbiol* 12, 399-413.
- Berger, S.B., Kasparcova, V., Hoffman, S., Swift, B., Dare, L., Schaeffer, M., Capriotti, C., Cook, M., Finger, J., Hughes-Earle, A., *et al.* (2014). Cutting Edge: RIP1 kinase activity is dispensable for normal development but is a key regulator of inflammation in SHARPIN-deficient mice. *J Immunol* 192, 5476-5480.
- Boisson, B., Laplantine, E., Dobbs, K., Cobat, A., Tarantino, N., Hazen, M., Lidov, H.G., Hopkins, G., Du, L., Belkadi, A., *et al.* (2015). Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J Exp Med* 212, 939-951.
- Boisson, B., Laplantine, E., Prando, C., Giliani, S., Israelsson, E., Xu, Z., Abhyankar, A., Israel, 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.
- Bosanac, I., Wertz, I.E., Pan, B., Yu, C., Kusam, S., Lam, C., Phu, L., Phung, Q., Maurer, B., Arnott, D., *et al.* (2010). Ubiquitin binding to A20 ZnF4 is required for modulation of NF-kappaB signaling. *Mol Cell* 40, 548-557.
- Chen, Y., He, L., Peng, Y., Shi, X., Chen, J., Zhong, J., Chen, X., Cheng, G., and Deng, H. (2015). The hepatitis C virus protein NS3 suppresses TNF-alpha-stimulated activation of NF-kappaB by targeting LUBAC. *Sci Signal* 8, ra118.
- Chen, Z., Hagler, J., Palombella, V.J., Melandri, F., Scherer, D., Ballard, D., and Maniatis, T. (1995). Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. *Genes Dev* 9, 1586-1597.
- Damgaard, R.B., Nachbur, U., Yabal, M., Wong, W.W., Fiil, B.K., Kastirr, M., Rieser, E., Rickard, J.A., Bankovacki, A., Peschel, C., *et al.* (2012). The ubiquitin ligase XIAP recruits LUBAC for NOD2 signaling in inflammation and innate immunity. *Mol Cell* 46, 746-758.
- Damgaard, R.B., Walker, J.A., Marco-Casanova, P., Morgan, N.V., Titheradge, H.L., Elliott, P.R., McHale, D., Maher, E.R., McKenzie, A.N., and Komander, D. (2016). The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. *Cell* 166, 1215-1230 e1220.
- Davis, R.E., Brown, K.D., Siebenlist, U., and Staudt, L.M. (2001). Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med* 194, 1861-1874.
- de Chasse, B., Navratil, V., Tafforeau, L., Hiet, M.S., Aublin-Gex, A., Agaugue, S., Meiffren, G., Pradezynski, F., Faria, B.F., Chantier, T., *et al.* (2008). Hepatitis C virus infection protein network. *Mol Syst Biol* 4, 230.
- de Jong, M.F., Liu, Z., Chen, D., and Alto, N.M. (2016). *Shigella flexneri* suppresses NF-kB activation by inhibiting linear ubiquitin chain ligation. *Nature Microbiology* 1, 16084.
- Demeyer, A., Staal, J., and Beyaert, R. (2016). Targeting MALT1 Proteolytic Activity in Immunity, Inflammation and Disease: Good or Bad? *Trends Mol Med* 22, 135-150.
- Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C., and Chen, Z.J. (2000). Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103, 351-361.

Douanne, T., Gavard, J., and Bidere, N. (2016). The paracaspase MALT1 cleaves the LUBAC subunit HOIL1 during antigen receptor signaling. *J Cell Sci* 129, 1775-1780.

Draber, P., Kupka, S., Reichert, M., Draberova, H., Lafont, E., de Miguel, D., Spilgies, L., Surinova, S., Taraborrelli, L., Hartwig, T., *et al.* (2015). LUBAC-Recruited CYLD and A20 Regulate Gene Activation and Cell Death by Exerting Opposing Effects on Linear Ubiquitin in Signaling Complexes. *Cell Rep* 13, 2258-2272.

Ea, C.K., Deng, L., Xia, Z.P., Pineda, G., and Chen, Z.J. (2006). Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. *Mol Cell* 22, 245-257.

Elliott, P.R., Leske, D., Hrdinka, M., Bagola, K., Fiil, B.K., McLaughlin, S.H., Wagstaff, J., Volkmar, N., Christianson, J.C., Kessler, B.M., *et al.* (2016). SPATA2 Links CYLD to LUBAC, Activates CYLD, and Controls LUBAC Signaling. *Mol Cell*.

Elliott, P.R., Nielsen, S.V., Marco-Casanova, P., Fiil, B.K., Keusekotten, K., Mailand, N., Freund, S.M., Gyrd-Hansen, M., and Komander, D. (2014). Molecular basis and regulation of OTULIN-LUBAC interaction. *Mol Cell* 54, 335-348.

Elton, L., Carpentier, I., Staal, J., Driege, Y., Haegman, M., and Beyaert, R. (2016). MALT1 cleaves the E3 ubiquitin ligase HOIL-1 in activated T cells, generating a dominant negative inhibitor of LUBAC-induced NF-kappaB signaling. *FEBS J* 283, 403-412.

Emmerich, C.H., Bakshi, S., Kelsall, I.R., Ortiz-Guerrero, J., Shpiro, N., and Cohen, P. (2016). Lys63/Met1-hybrid ubiquitin chains are commonly formed during the activation of innate immune signalling. *Biochem Biophys Res Commun* 474, 452-461.

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

Engel, K., Rudelius, M., Slawska, J., Jacobs, L., Ahangarian Abhari, B., Altmann, B., Kurutz, J., Rathakrishnan, A., Fernandez-Saiz, V., Brunner, A., *et al.* (2016). USP9X stabilizes XIAP to regulate mitotic cell death and chemoresistance in aggressive B-cell lymphoma. *EMBO Mol Med* 8, 851-862.

Ferch, U., Kloo, B., Gewies, A., Pfander, V., Duwel, M., Peschel, C., Krappmann, D., and Ruland, J. (2009). Inhibition of MALT1 protease activity is selectively toxic for activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med* 206, 2313-2320.

Fiil, B.K., Damgaard, R.B., Wagner, S.A., Keusekotten, K., Fritsch, M., Bekker-Jensen, S., Mailand, N., Choudhary, C., Komander, D., and Gyrd-Hansen, M. (2013). OTULIN restricts Met1-linked ubiquitination to control innate immune signaling. *Mol Cell* 50, 818-830.

Fiil, B.K., and Gyrd-Hansen, M. (2014). Met1-linked ubiquitination in immune signalling. *FEBS J* 281, 4337-4350.

Gerlach, B., Cordier, S.M., Schmukle, A.C., Emmerich, C.H., Rieser, E., Haas, T.L., Webb, A.I., Rickard, J.A., Anderton, H., Wong, W.W., *et al.* (2011). Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* 471, 591-596.

Goto, E., and Tokunaga, F. (2017). Decreased linear ubiquitination of NEMO and FADD on apoptosis with caspase-mediated cleavage of HOIP. *Biochem Biophys Res Commun*.

Greenfield, H., Takasaki, K., Walsh, M.J., Ersing, I., Bernhardt, K., Ma, Y., Fu, B., Ashbaugh, C.W., Cabo, J., Mollo, S.B., *et al.* (2015). TRAF1 Coordinates Polyubiquitin Signaling to Enhance Epstein-Barr Virus LMP1-Mediated Growth and Survival Pathway Activation. *PLoS Pathog* 11, e1004890.

Gyrd-Hansen, M., Darding, M., Miasari, M., Santoro, M.M., Zender, L., Xue, W., Tenev, T., da Fonseca, P.C., Zvelebil, M., Bujnicki, J.M., *et al.* (2008). IAPs contain an evolutionarily conserved

ubiquitin-binding domain that regulates NF-kappaB as well as cell survival and oncogenesis. *Nat Cell Biol* 10, 1309-1317.

Haas, T.L., Emmerich, C.H., Gerlach, B., Schmukle, A.C., Cordier, S.M., Rieser, E., Feltham, R., Vince, J., Warnken, U., Wenger, T., *et al.* (2009). Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. *Mol Cell* 36, 831-844.

Hajek, M., Sewell, A., Kaech, S., Burtess, B., Yarbrough, W.G., and Issaeva, N. (2017). TRAF3/CYLD mutations identify a distinct subset of human papilloma virus-associated head and neck squamous cell carcinoma. *Cancer*.

HogenEsch, H., Gijbels, M.J., Offerman, E., van Hooft, J., van Bekkum, D.W., and Zurcher, C. (1993). A spontaneous mutation characterized by chronic proliferative dermatitis in C57BL mice. *Am J Pathol* 143, 972-982.

Hrdinka, M., Fiil, B.K., Zucca, M., Leske, D., Bagola, K., Yabal, M., Elliott, P.R., Damgaard, R.B., Komander, D., Jost, P.J., *et al.* (2016). CYLD Limits Lys63- and Met1-Linked Ubiquitin at Receptor Complexes to Regulate Innate Immune Signaling. *Cell Rep* 14, 2846-2858.

Ikeda, F., Deribe, Y.L., Skanland, S.S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M., van Wijk, S.J., Goswami, P., Nagy, V., Terzic, J., *et al.* (2011). SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* 471, 637-641.

Iyengar, P.V., Jaynes, P., Rodon, L., Lama, D., Law, K.P., Lim, Y.P., Verma, C., Seoane, J., and Eichhorn, P.J. (2015). USP15 regulates SMURF2 kinetics through C-lobe mediated deubiquitination. *Sci Rep* 5, 14733.

Jin, W., Reiley, W.R., Lee, A.J., Wright, A., Wu, X., Zhang, M., and Sun, S.C. (2007). Deubiquitinating enzyme CYLD regulates the peripheral development and naive phenotype maintenance of B cells. *J Biol Chem* 282, 15884-15893.

Jing, H., Fang, L., Ding, Z., Wang, D., Hao, W., Gao, L., Ke, W., Chen, H., and Xiao, S. (2017). Porcine Reproductive and Respiratory Syndrome Virus nsp1alpha Inhibits NF-kappaB Activation by Targeting the Linear Ubiquitin Chain Assembly Complex. *J Virol* 91.

Jono, H., Lim, J.H., Chen, L.F., Xu, H., Trompouki, E., Pan, Z.K., Mosialos, G., and Li, J.D. (2004). NF-kappaB is essential for induction of CYLD, the negative regulator of NF-kappaB: evidence for a novel inducible autoregulatory feedback pathway. *J Biol Chem* 279, 36171-36174.

Joo, D., Tang, Y., Blonska, M., Jin, J., Zhao, X., and Lin, X. (2016). Regulation of linear ubiquitin chain assembly complex by Caspase-mediated cleavage of RNF31. *Mol Cell Biol*.

Kanayama, A., Seth, R.B., Sun, L., Ea, C.K., Hong, M., Shaito, A., Chiu, Y.H., Deng, L., and Chen, Z.J. (2004). TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol Cell* 15, 535-548.

Karatzas, D.N., Xanthopoulos, K., Kotantaki, P., Pseftogas, A., Teliousis, K., Hatzivassiliou, E.G., Kontoyiannis, D.L., Poutahidis, T., and Mosialos, G. (2016). Inactivation of CYLD in intestinal epithelial cells exacerbates colitis-associated colorectal carcinogenesis - a short report. *Cell Oncol (Dordr)*.

Kazakov, D.V. (2016). Brooke-Spiegler Syndrome and Phenotypic Variants: An Update. *Head Neck Pathol* 10, 125-130.

Kensche, T., Tokunaga, F., Ikeda, F., Goto, E., Iwai, K., and Dikic, I. (2012). Analysis of nuclear factor-kappaB (NF-kappaB) essential modulator (NEMO) binding to linear and lysine-linked ubiquitin chains and its role in the activation of NF-kappaB. *J Biol Chem* 287, 23626-23634.

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.

Khan, M., Syed, G.H., Kim, S.J., and Siddiqui, A. (2016). Hepatitis B Virus-Induced Parkin-Dependent Recruitment of Linear Ubiquitin Assembly Complex (LUBAC) to Mitochondria and Attenuation of Innate Immunity. *PLoS Pathog* 12, e1005693.

Kirisako, T., Kamei, K., Murata, S., Kato, M., Fukumoto, H., Kanie, M., Sano, S., Tokunaga, F., Tanaka, K., and Iwai, K. (2006). A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J* 25, 4877-4887.

Klein, T., Fung, S.Y., Renner, F., Blank, M.A., Dufour, A., Kang, S., Bolger-Munro, M., Scurll, J.M., Priatel, J.J., Schweigler, P., *et al.* (2015). The paracaspase MALT1 cleaves HOIL1 reducing linear ubiquitination by LUBAC to dampen lymphocyte NF-kappaB signalling. *Nat Commun* 6, 8777.

Komander, D., Reyes-Turcu, F., Licchesi, J.D., Odenwaelder, P., Wilkinson, K.D., and Barford, D. (2009). Molecular discrimination of structurally equivalent Lys 63-linked and linear polyubiquitin chains. *EMBO Rep* 10, 466-473.

Kumari, S., Redouane, Y., Lopez-Mosqueda, J., Shiraishi, R., Romanowska, M., Lutzmayer, S., Kuiper, J., Martinez, C., Dikic, I., Pasparakis, M., *et al.* (2014). Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. *Elife* 3.

Kupka, S., De Miguel, D., Draber, P., Martino, L., Surinova, S., Rittinger, K., and Walczak, H. (2016). SPATA2-Mediated Binding of CYLD to HOIP Enables CYLD Recruitment to Signaling Complexes. *Cell Rep* 16, 2271-2280.

Lafont, E., Kantari-Mimoun, C., Draber, P., De Miguel, D., Hartwig, T., Reichert, M., Kupka, S., Shimizu, Y., Taraborrelli, L., Spit, M., *et al.* (2017). The linear ubiquitin chain assembly complex regulates TRAIL-induced gene activation and cell death. *EMBO J*.

Laplantine, E., Fontan, E., Chiaravalli, J., Lopez, T., Lakisic, G., Veron, M., Agou, F., and Israel, A. (2009). NEMO specifically recognizes K63-linked poly-ubiquitin chains through a new bipartite ubiquitin-binding domain. *EMBO J* 28, 2885-2895.

Lim, J.H., Stirling, B., Derry, J., Koga, T., Jono, H., Woo, C.H., Xu, H., Bourne, P., Ha, U.H., Ishinaga, H., *et al.* (2007). Tumor suppressor CYLD regulates acute lung injury in lethal *Streptococcus pneumoniae* infections. *Immunity* 27, 349-360.

Lo, Y.C., Lin, S.C., Rospigliosi, C.C., Conze, D.B., Wu, C.J., Ashwell, J.D., Eliezer, D., and Wu, H. (2009). Structural basis for recognition of diubiquitins by NEMO. *Mol Cell* 33, 602-615.

Lork, M., Verhelst, K., and Beyaert, R. (2017). CYLD, A20 and OTULIN deubiquitinases in NF-kappaB signaling and cell death: so similar, yet so different. *Cell Death Differ*.

Lu, T.T., Onizawa, M., Hammer, G.E., Turer, E.E., Yin, Q., Damko, E., Agelidis, A., Shifrin, N., Advincula, R., Barrera, J., *et al.* (2013). Dimerization and ubiquitin mediated recruitment of A20, a complex deubiquitinating enzyme. *Immunity* 38, 896-905.

MacDuff, D.A., Reese, T.A., Kimmey, J.M., Weiss, L.A., Song, C., Zhang, X., Kambal, A., Duan, E., Carrero, J.A., Boisson, B., *et al.* (2015). Phenotypic complementation of genetic immunodeficiency by chronic herpesvirus infection. *Elife* 4.

Massoumi, R., Chmielarska, K., Hennecke, K., Pfeifer, A., and Fassler, R. (2006). Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. *Cell* 125, 665-677.

McWilliams, T.G., and Muqit, M.M. (2017). PINK1 and Parkin: emerging themes in mitochondrial homeostasis. *Curr Opin Cell Biol* 45, 83-91.

- Nakamura, M., Tokunaga, F., Sakata, S., and Iwai, K. (2006). Mutual regulation of conventional protein kinase C and a ubiquitin ligase complex. *Biochem Biophys Res Commun* 351, 340-347.
- Nanda, S.K., Venigalla, R.K., Ordureau, A., Patterson-Kane, J.C., Powell, D.W., Toth, R., Arthur, J.S., and Cohen, P. (2011). Polyubiquitin binding to ABIN1 is required to prevent autoimmunity. *J Exp Med* 208, 1215-1228.
- Nikolaou, K., Tsagaratou, A., Eftychi, C., Kollias, G., Mosialos, G., and Talianidis, I. (2012). Inactivation of the deubiquitinase CYLD in hepatocytes causes apoptosis, inflammation, fibrosis, and cancer. *Cancer Cell* 21, 738-750.
- Nilsson, J., Schoser, B., Laforet, P., Kalev, O., Lindberg, C., Romero, N.B., Davila Lopez, M., Akman, H.O., Wahbi, K., Iglseder, S., *et al.* (2013). Polyglucosan body myopathy caused by defective ubiquitin ligase RBCK1. *Ann Neurol* 74, 914-919.
- Nishanth, G., Deckert, M., Wex, K., Massoumi, R., Schweitzer, K., Naumann, M., and Schluter, D. (2013). CYLD enhances severe listeriosis by impairing IL-6/STAT3-dependent fibrin production. *PLoS Pathog* 9, e1003455.
- Ori, D., Kato, H., Sanjo, H., Tartey, S., Mino, T., Akira, S., and Takeuchi, O. (2013). Essential roles of K63-linked polyubiquitin-binding proteins TAB2 and TAB3 in B cell activation via MAPKs. *J Immunol* 190, 4037-4045.
- Oshima, S., Turer, E.E., Callahan, J.A., Chai, S., Advincula, R., Barrera, J., Shifrin, N., Lee, B., Benedict Yen, T.S., Woo, T., *et al.* (2009). ABIN-1 is a ubiquitin sensor that restricts cell death and sustains embryonic development. *Nature* 457, 906-909.
- Palombella, V.J., Rando, O.J., Goldberg, A.L., and Maniatis, T. (1994). The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 78, 773-785.
- Peltzer, N., Rieser, E., Taraborrelli, L., Draber, P., Darding, M., Pernaute, B., Shimizu, Y., Sarr, A., Draberova, H., Montinaro, A., *et al.* (2014). HOIP deficiency causes embryonic lethality by aberrant TNFR1-mediated endothelial cell death. *Cell Rep* 9, 153-165.
- Pruneda, J.N., Smith, F.D., Daurie, A., Swaney, D.L., Villen, J., Scott, J.D., Stadnyk, A.W., Le Trong, I., Stenkamp, R.E., Klevit, R.E., *et al.* (2014). E2-Ub conjugates regulate the kinase activity of Shigella effector OspG during pathogenesis. *EMBO J* 33, 437-449.
- Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., Kato, R., Kensche, T., Uejima, T., Bloor, S., Komander, D., *et al.* (2009). Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* 136, 1098-1109.
- Reiley, W.W., Zhang, M., Jin, W., Losiewicz, M., Donohue, K.B., Norbury, C.C., and Sun, S.C. (2006). Regulation of T cell development by the deubiquitinating enzyme CYLD. *Nat Immunol* 7, 411-417.
- Rickard, J.A., Anderton, H., Etemadi, N., Nachbur, U., Darding, M., Peltzer, N., Lalaoui, N., Lawlor, K.E., Vanyai, H., Hall, C., *et al.* (2014). TNFR1-dependent cell death drives inflammation in Sharpin-deficient mice. *Elife* 3.
- Ritorto, M.S., Ewan, R., Perez-Oliva, A.B., Knebel, A., Buhrlage, S.J., Wightman, M., Kelly, S.M., Wood, N.T., Virdee, S., Gray, N.S., *et al.* (2014). Screening of DUB activity and specificity by MALDI-TOF mass spectrometry. *Nat Commun* 5, 4763.
- Rivkin, E., Almeida, S.M., Ceccarelli, D.F., Juang, Y.C., MacLean, T.A., Srikumar, T., Huang, H., Dunham, W.H., Fukumura, R., Xie, G., *et al.* (2013). The linear ubiquitin-specific deubiquitinase gumby regulates angiogenesis. *Nature* 498, 318-324.
- Sasaki, K., and Iwai, K. (2015). Roles of linear ubiquitylation, a crucial regulator of NF-kappaB and cell death, in the immune system. *Immunol Rev* 266, 175-189.

- Sasaki, Y., Sano, S., Nakahara, M., Murata, S., Kometani, K., Aiba, Y., Sakamoto, S., Watanabe, Y., Tanaka, K., Kurosaki, T., *et al.* (2013). Defective immune responses in mice lacking LUBAC-mediated linear ubiquitination in B cells. *EMBO J* 32, 2463-2476.
- Sato, Y., Fujita, H., Yoshikawa, A., Yamashita, M., Yamagata, A., Kaiser, S.E., Iwai, K., and Fukai, S. (2011). Specific recognition of linear ubiquitin chains by the Npl4 zinc finger (NZF) domain of the HOIL-1L subunit of the linear ubiquitin chain assembly complex. *Proc Natl Acad Sci U S A* 108, 20520-20525.
- Sato, Y., Goto, E., Shibata, Y., Kubota, Y., Yamagata, A., Goto-Ito, S., Kubota, K., Inoue, J., Takekawa, M., Tokunaga, F., *et al.* (2015). Structures of CYLD USP with Met1- or Lys63-linked diubiquitin reveal mechanisms for dual specificity. *Nat Struct Mol Biol* 22, 222-229.
- Schaab, C., Geiger, T., Stoehr, G., Cox, J., and Mann, M. (2012). Analysis of high accuracy, quantitative proteomics data in the MaxQB database. *Mol Cell Proteomics* 11, M111 014068.
- Schaeffer, V., Akutsu, M., Olma, M.H., Gomes, L.C., Kawasaki, M., and Dikic, I. (2014). Binding of OTULIN to the PUB domain of HOIP controls NF-kappaB signaling. *Mol Cell* 54, 349-361.
- Schlicher, L., Wissler, M., Preiss, F., Brauns-Schubert, P., Jakob, C., Dumit, V., Borner, C., Dengjel, J., and Maurer, U. (2016). SPATA2 promotes CYLD activity and regulates TNF-induced NF-kappaB signaling and cell death. *EMBO Rep*.
- Shibata, Y., Tokunaga, F., Goto, E., Komatsu, G., Gohda, J., Saeki, Y., Tanaka, K., Takahashi, H., Sawasaki, T., Inoue, S., *et al.* (2017). HTLV-1 Tax Induces Formation of the Active Macromolecular IKK Complex by Generating Lys63- and Met1-Linked Hybrid Polyubiquitin Chains. *PLoS Pathog* 13, e1006162.
- Shimizu, S., Fujita, H., Sasaki, Y., Tsuruyama, T., Fukuda, K., and Iwai, K. (2016). Differential Involvement of the Npl4 Zinc Finger Domains of SHARPIN and HOIL-1L in Linear Ubiquitin Chain Assembly Complex-Mediated Cell Death Protection. *Mol Cell Biol* 36, 1569-1583.
- Shimizu, Y., Peltzer, N., Sevko, A., Lafont, E., Sarr, A., Draberova, H., and Walczak, H. (2017). The linear ubiquitin chain assembly complex acts as a liver tumor suppressor and inhibits hepatocyte apoptosis and hepatitis. *Hepatology*.
- Shimizu, Y., Taraborrelli, L., and Walczak, H. (2015). Linear ubiquitination in immunity. *Immunol Rev* 266, 190-207.
- Skaug, B., Chen, J., Du, F., He, J., Ma, A., and Chen, Z.J. (2011). Direct, noncatalytic mechanism of IKK inhibition by A20. *Mol Cell* 44, 559-571.
- Smit, J.J., Monteferrario, D., Noordermeer, S.M., van Dijk, W.J., van der Reijden, B.A., and Sixma, T.K. (2012). The E3 ligase HOIP specifies linear ubiquitin chain assembly through its RING-IBR-RING domain and the unique LDD extension. *EMBO J* 31, 3833-3844.
- Sowa, M.E., Bennett, E.J., Gygi, S.P., and Harper, J.W. (2009). Defining the human deubiquitinating enzyme interaction landscape. *Cell* 138, 389-403.
- Stieglitz, B., Morris-Davies, A.C., Koliopoulos, M.G., Christodoulou, E., and Rittinger, K. (2012). LUBAC synthesizes linear ubiquitin chains via a thioester intermediate. *EMBO Rep* 13, 840-846.
- Stieglitz, B., Rana, R.R., Koliopoulos, M.G., Morris-Davies, A.C., Schaeffer, V., Christodoulou, E., Howell, S., Brown, N.R., Dikic, I., and Rittinger, K. (2013). Structural basis for ligase-specific conjugation of linear ubiquitin chains by HOIP. *Nature* 503, 422-426.
- Takiuchi, T., Nakagawa, T., Tamiya, H., Fujita, H., Sasaki, Y., Saeki, Y., Takeda, H., Sawasaki, T., Buchberger, A., Kimura, T., *et al.* (2014). Suppression of LUBAC-mediated linear ubiquitination by a specific interaction between LUBAC and the deubiquitinases CYLD and OTULIN. *Genes Cells* 19, 254-272.

- Taranets, L., Zhu, J., Xu, W., and Popov, N. (2015). Fbw7 and Usp28 - enemies and allies. *Mol Cell Oncol* 2, e995041.
- Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sakata, S., Tanaka, K., Nakano, H., and Iwai, K. (2011). SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. *Nature* 471, 633-636.
- Tokunaga, F., Nishimasu, H., Ishitani, R., Goto, E., Noguchi, T., Mio, K., Kamei, K., Ma, A., Iwai, K., and Nureki, O. (2012). Specific recognition of linear polyubiquitin by A20 zinc finger 7 is involved in NF-kappaB regulation. *EMBO J* 31, 3856-3870.
- Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K., Nakagawa, T., Kato, M., Murata, S., Yamaoka, S., *et al.* (2009). Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* 11, 123-132.
- Traenckner, E.B., Wilk, S., and Baeuerle, P.A. (1994). A proteasome inhibitor prevents activation of NF-kappa B and stabilizes a newly phosphorylated form of I kappa B-alpha that is still bound to NF-kappa B. *EMBO J* 13, 5433-5441.
- Verhelst, K., Carpentier, I., Kreike, M., Meloni, L., Verstrepen, L., Kensche, T., Dikic, I., and Beyaert, R. (2012). A20 inhibits LUBAC-mediated NF-kappaB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J* 31, 3845-3855.
- Wagner, S., Carpentier, I., Rogov, V., Kreike, M., Ikeda, F., Lohr, F., Wu, C.J., Ashwell, J.D., Dotsch, V., Dikic, I., *et al.* (2008). Ubiquitin binding mediates the NF-kappaB inhibitory potential of ABIN proteins. *Oncogene* 27, 3739-3745.
- Wagner, S.A., Satpathy, S., Beli, P., and Choudhary, C. (2016). SPATA2 links CYLD to the TNF-alpha receptor signaling complex and modulates the receptor signaling outcomes. *EMBO J*.
- Wang, C., Deng, L., Hong, M., Akkaraju, G.R., Inoue, J., and Chen, Z.J. (2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 412, 346-351.
- Wang, K., Kim, C., Bradfield, J., Guo, Y., Toskala, E., Otieno, F.G., Hou, C., Thomas, K., Cardinale, C., Lyon, G.J., *et al.* (2013). Whole-genome DNA/RNA sequencing identifies truncating mutations in RBCK1 in a novel Mendelian disease with neuromuscular and cardiac involvement. *Genome Med* 5, 67.
- Wang, L., Wang, Y., Zhao, J., Ren, J., Hall, K.H., Moorman, J.P., Yao, Z.Q., and Ning, S. (2017). The Linear Ubiquitin Assembly Complex Modulates Latent Membrane Protein 1 Activation of NF-kappaB and Interferon Regulatory Factor 7. *J Virol* 91.
- Wenzel, D.M., Lissounov, A., Brzovic, P.S., and Klevit, R.E. (2011). UBC7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. *Nature* 474, 105-108.
- Wertz, I.E., Newton, K., Seshasayee, D., Kusam, S., Lam, C., Zhang, J., Popovych, N., Helgason, E., Schoeffler, A., Jeet, S., *et al.* (2015). Phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. *Nature* 528, 370-375.
- 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.
- Yamamotoya, T., Nakatsu, Y., Matsunaga, Y., Fukushima, T., Yamazaki, H., Kaneko, S., Fujishiro, M., Kikuchi, T., Kushiya, A., Tokunaga, F., *et al.* (2017). Reduced SHARPIN and LUBAC Formation May Contribute to CCl(4)- or Acetaminophen-Induced Liver Cirrhosis in Mice. *Int J Mol Sci* 18.
- Yang, Y., Schmitz, R., Mitala, J., Whiting, A., Xiao, W., Ceribelli, M., Wright, G.W., Zhao, H., Yang, Y., Xu, W., *et al.* (2014). Essential role of the linear ubiquitin chain assembly complex in lymphoma revealed by rare germline polymorphisms. *Cancer Discov* 4, 480-493.

- Yang, Y.K., Yang, C., Chan, W., Wang, Z., Deibel, K.E., and Pomerantz, J.L. (2016). Molecular Determinants of Scaffold-induced Linear Ubiquitylation of B Cell Lymphoma/Leukemia 10 (Bcl10) during T Cell Receptor and Oncogenic Caspase Recruitment Domain-containing Protein 11 (CARD11) Signaling. *J Biol Chem* **291**, 25921-25936.
- Zeiler, M., Straube, W.L., Lundberg, E., Uhlen, M., and Mann, M. (2012). A Protein Epitope Signature Tag (PrEST) library allows SILAC-based absolute quantification and multiplexed determination of protein copy numbers in cell lines. *Mol Cell Proteomics* **11**, O111 009613.
- Zhang, J., Clark, K., Lawrence, T., Pegg, M.W., and Cohen, P. (2014). An unexpected twist to the activation of IKKbeta: TAK1 primes IKKbeta for activation by autophosphorylation. *Biochem J* **461**, 531-537.
- Zhang, J., Macartney, T., Pegg, M., and Cohen, P. (2017). Interleukin-1 and TRAF6-dependent activation of TAK1 in the absence of TAB2 and TAB3. *Biochem J* **474**, 2235-2248.
- Zhang, J., Stirling, B., Temmerman, S.T., Ma, C.A., Fuss, I.J., Derry, J.M., 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.
- Zhao, G., Zhou, X., Wang, L., Li, G., Schindelin, H., and Lennarz, W.J. (2007). Studies on peptide:N-glycanase-p97 interaction suggest that p97 phosphorylation modulates endoplasmic reticulum-associated degradation. *Proc Natl Acad Sci U S A* **104**, 8785-8790.
- Zhou, Q., Yu, X., Demirkaya, E., Deutch, N., Stone, D., Tsai, W.L., Kuehn, H.S., Wang, H., Yang, D., Park, Y.H., *et al.* (2016). Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc Natl Acad Sci U S A*.

Figure Legends

Figure 1. Model of immune receptor signaling pathways controlled by Ub. Antigen receptors, cytokine receptors and pattern recognition receptors (TLRs, NLRs, CLRs, RLRs, and DSRs) rely on formation of Ub chains for transmission of signals from the activated receptor to nuclear transcriptional responses mediated by transcription factors nuclear factor- κ B (NF- κ B), Activator protein 1 (AP-1) and Interferon Response Factor 3 (IRF3).

Figure 2. A generalized model of innate immune signaling pathways controlled by Met1-Ub, Lys63-Ub and Lys48-Ub. Activation of immune receptors leads to recruitment of adaptor proteins that become ubiquitination targets for E3 Ub ligases. The net outcome of E3 and DUBs activities is the accumulation of Met1-Ub, Lys63-Ub and other linkages at the receptor complex. Notably, Met1-Ub is formed also on pre-existing Lys63-Ub to generate hybrid Ub chains. Lys63- and Met1-Ub modifications are recognized by the Ub-dependent kinase complexes TAB-TAK1 and NEMO-IKK, respectively, which promotes their activation. TAK1 activates MAP kinase cascades and IKK phosphorylates the NF- κ B inhibitor I κ B. I κ B phosphorylation primes it for Lys48-Ub modification and proteasomal degradation. The destruction of I κ B leads to accumulation of NF- κ B in the nucleus, which together with AP-1 facilitate transcription of hundreds of genes, including genes encoding inflammatory mediators, anti-microbial peptides, and pro-survival factors.

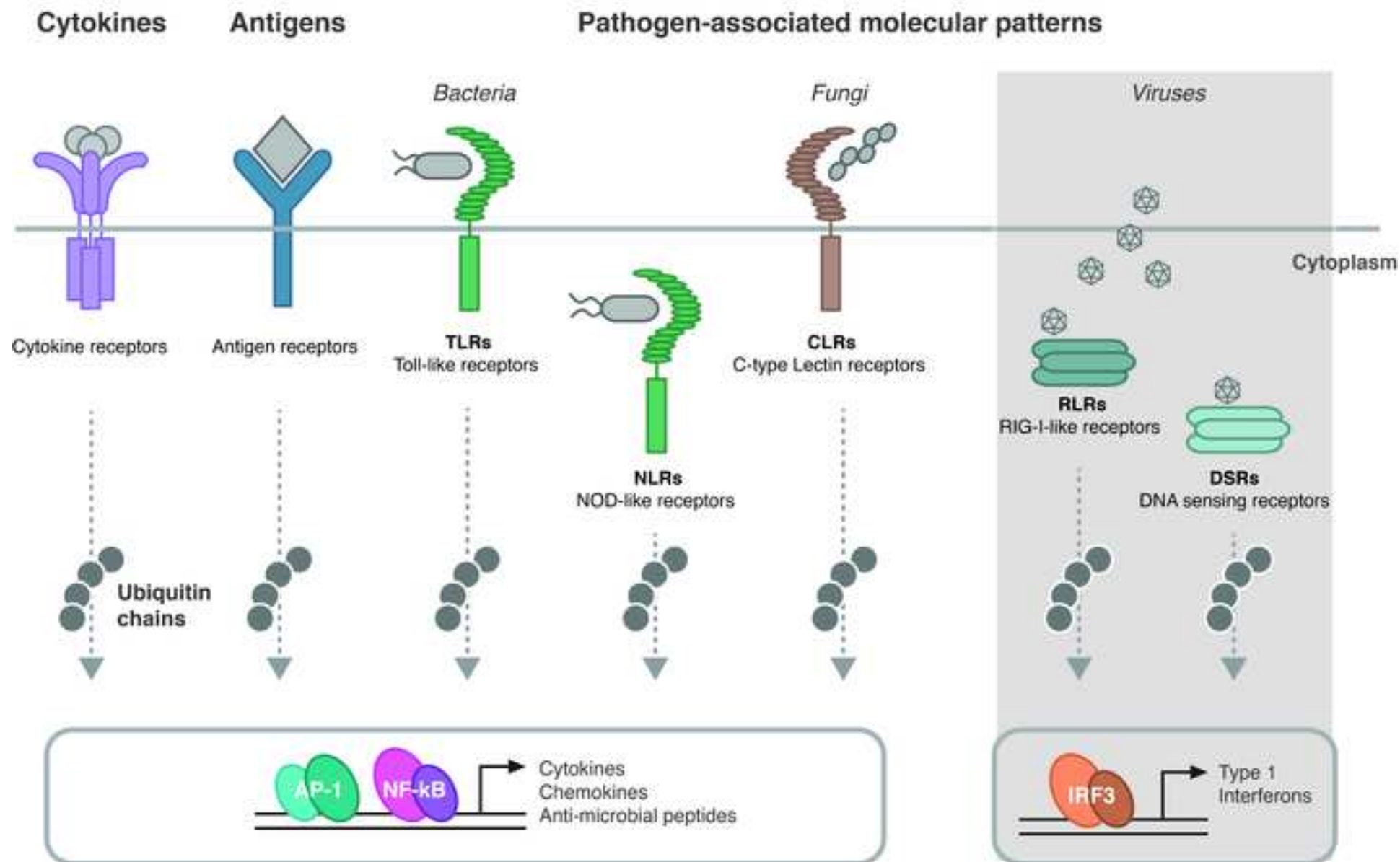
Figure 3. The Met1-Ub machinery. Schematic model of the proteins involved in Met1-Ub conjugation, Met1-Ub binding and Met1-Ub disassembly. LUBAC together with E1 and E2 enzymes conjugates Met1-Ub to existing Ub modifications. The first Ub conjugated to a substrate (shown in grey) does not have a defined linkage as lysine residue (K) is unique to the substrate. Several proteins can bind to Met1-Ub and these are involved in “translating” the biochemical modification of the substrate into cellular responses (see text for details). OTULIN and CYLD (which is in a stable complex with the co-factor SPATA2) are the best described DUBs that disassemble Met1-Ub. OTULIN exclusively disassembles Met1-Ub and therefore will not remove any pre-existing Ub modifications of substrates. CYLD is less specific and in addition to Met1-Ub readily disassembles Lys63-Ub and has activity against some other linkages. For simplicity, this activity is not included in the figure.

Figure 4. Schematic representation of domain organization of LUBAC subunits and associated DUBs. CBR, catalytic in-between RING; IBR, in-between-RING; LDD, linear ubiquitin chains-determining domain; NZF, nuclear protein localization 4 (Npl4) zinc finger; OTU, ovarian tumor; PH, pleckstrin homology; PIM, PUB-interacting motif; PUB, peptide:N-glycanase/UBA- or UBX-containing proteins; RBR, RING-betweenRING-RING; RING, really interesting new gene; UBA, ubiquitin associated; UBL, ubiquitin-like; USP; ubiquitin-specific protease; ZnF, zinc finger.

Figure 5. Schematic representation of LUBAC complexes with the proposed configuration for binding of SPATA2 and deubiquitinases, CYLD and OTULIN. The association of LUBAC with DUBs is mediated by a unified interaction between the PUB domain of HOIP and the PUB interacting motif (PIM) in OTULIN and SPATA2. SPATA2 binds CYLD directly through an interaction of its PUB domain and residues within the USP domain of CYLD. CYLD forms stable dimers via its B-box domain and together with SPATA2 forms a hetero-tetrameric complex consisting of two CYLD molecules and two SPATA2 molecules. OTULIN and SPATA2-CYLD do not simultaneously bind the same LUBAC complexes, suggesting that LUBAC exists in at least two configurations.

Figure 6. An overview of human pathologies caused by deregulation of the Met1-Ub machinery. See text for details. ABC-DLBCL, activated B cell diffuse large B cell lymphoma; HPV, human papilloma virus; * an effect on Met1-Ub by CYLD mutations is speculative and has not been shown experimentally.

FIGURE 1



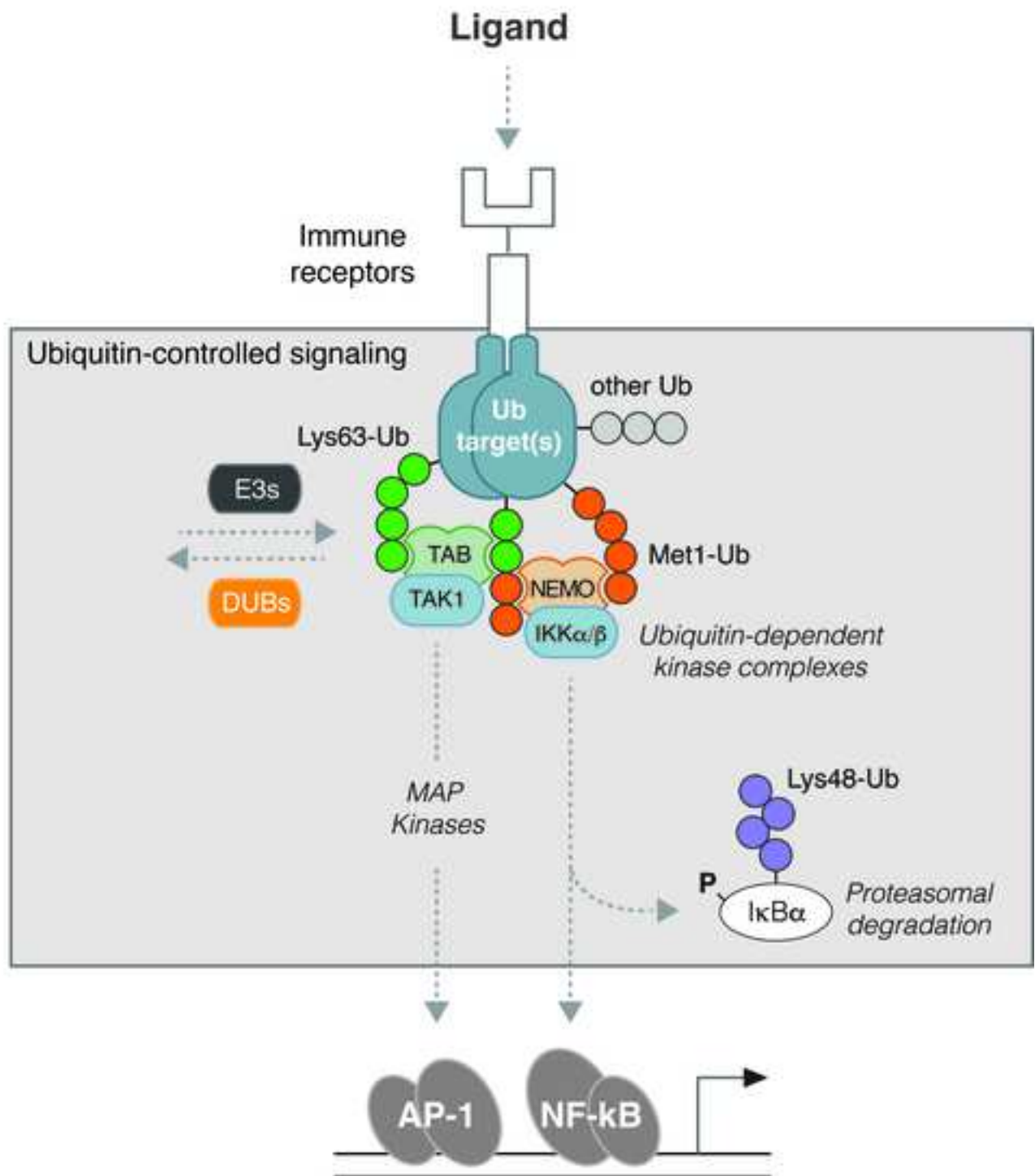


FIGURE 3

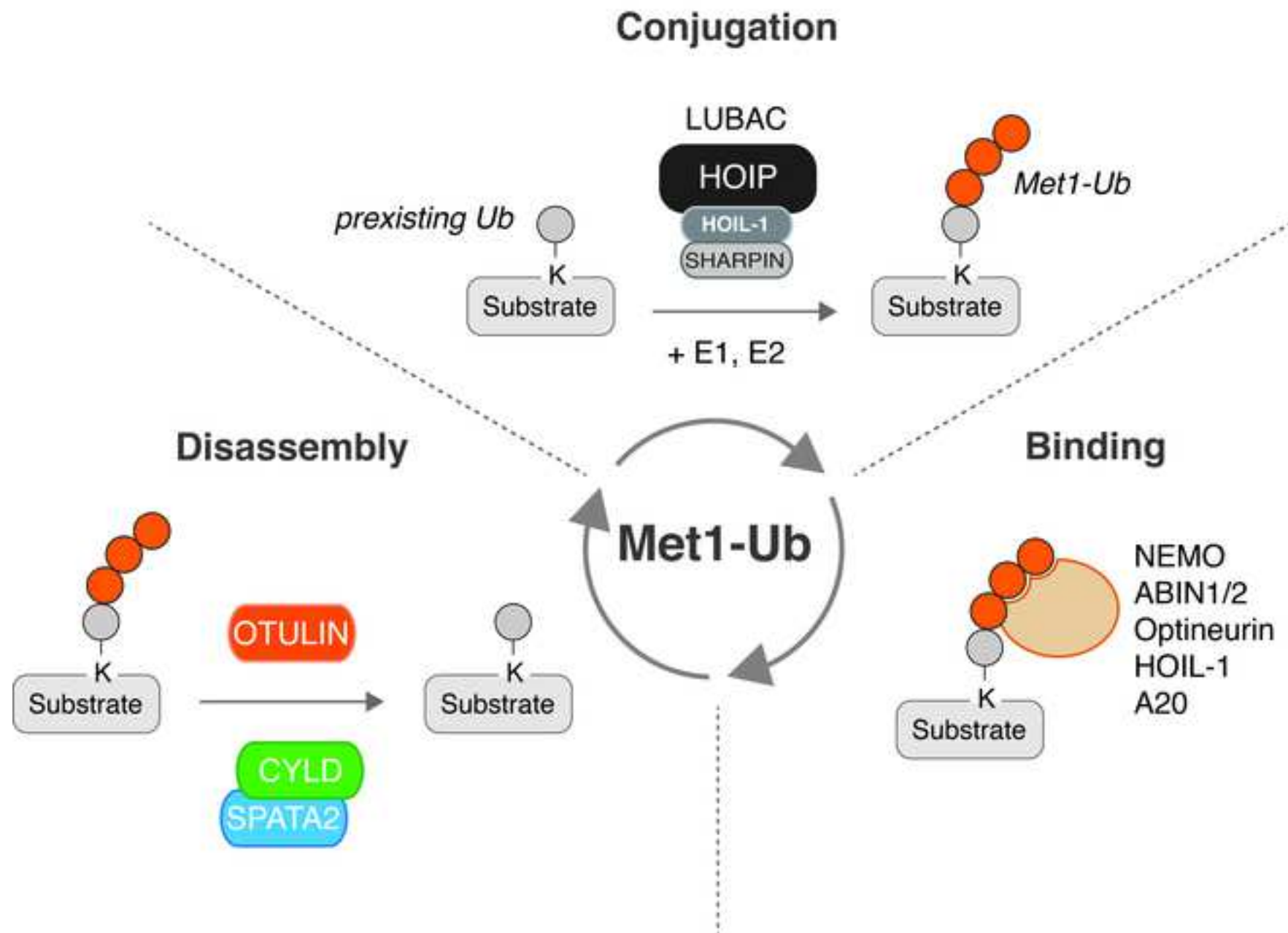


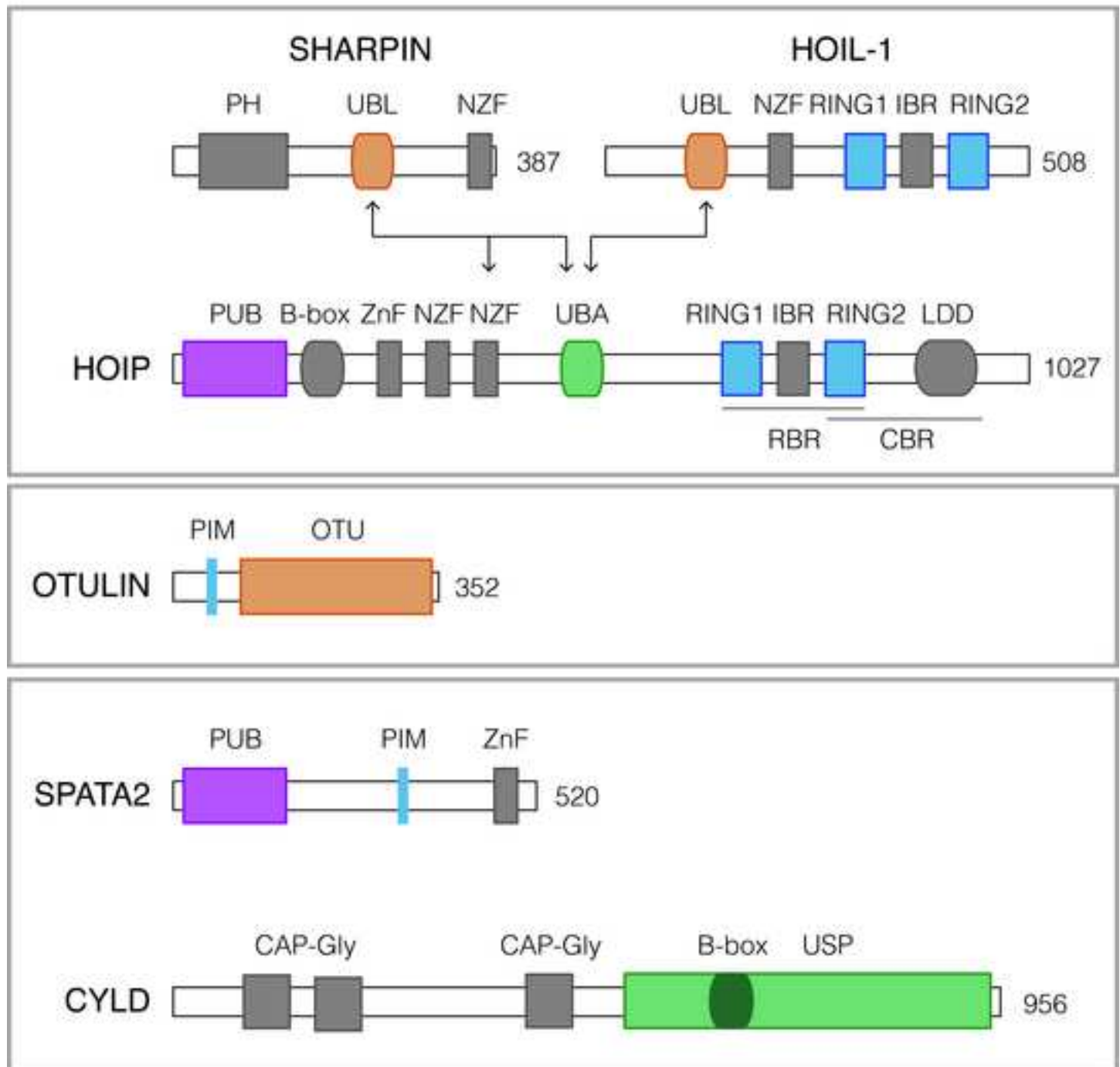
FIGURE 4

FIGURE 5

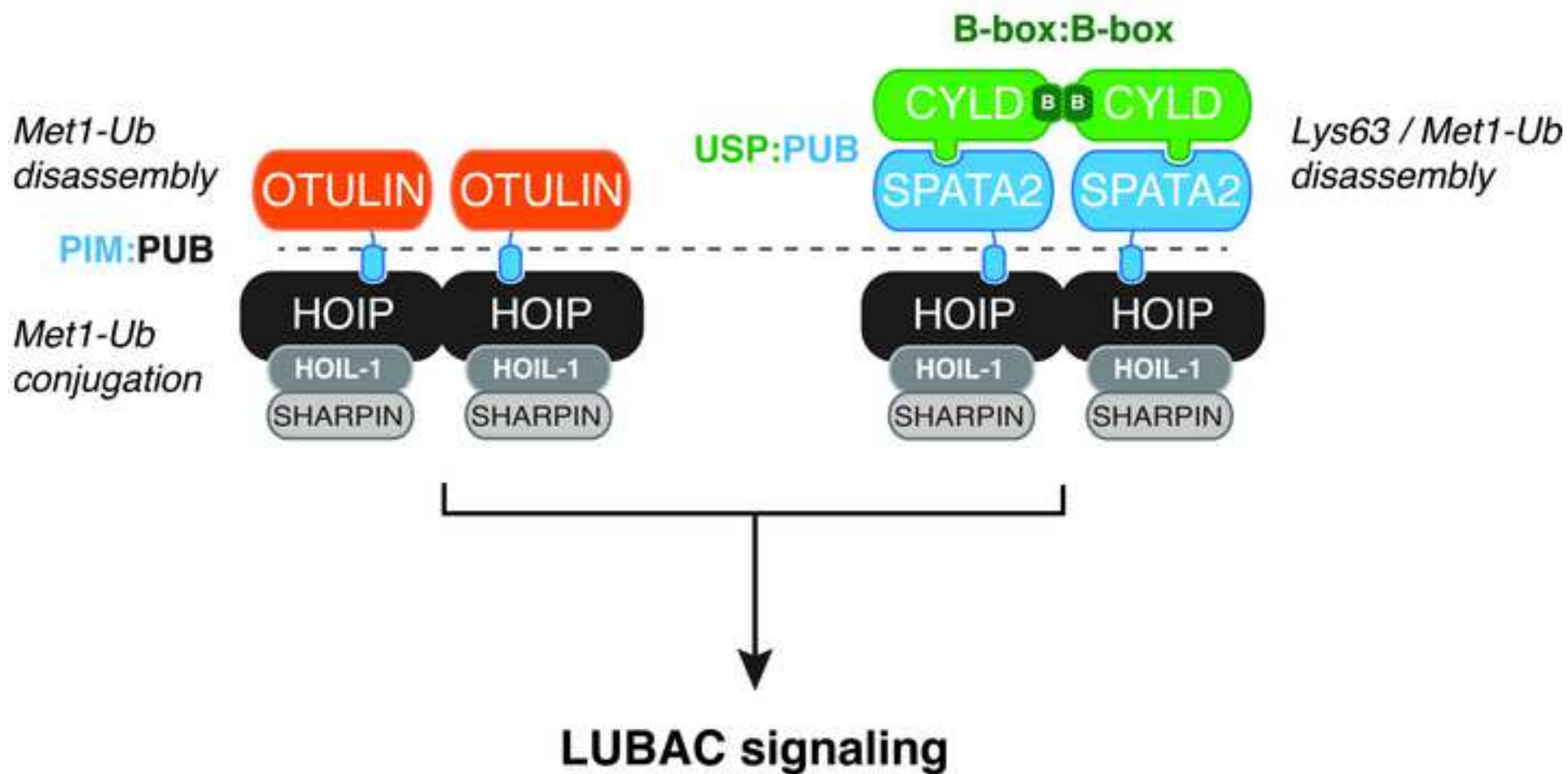


FIGURE 6

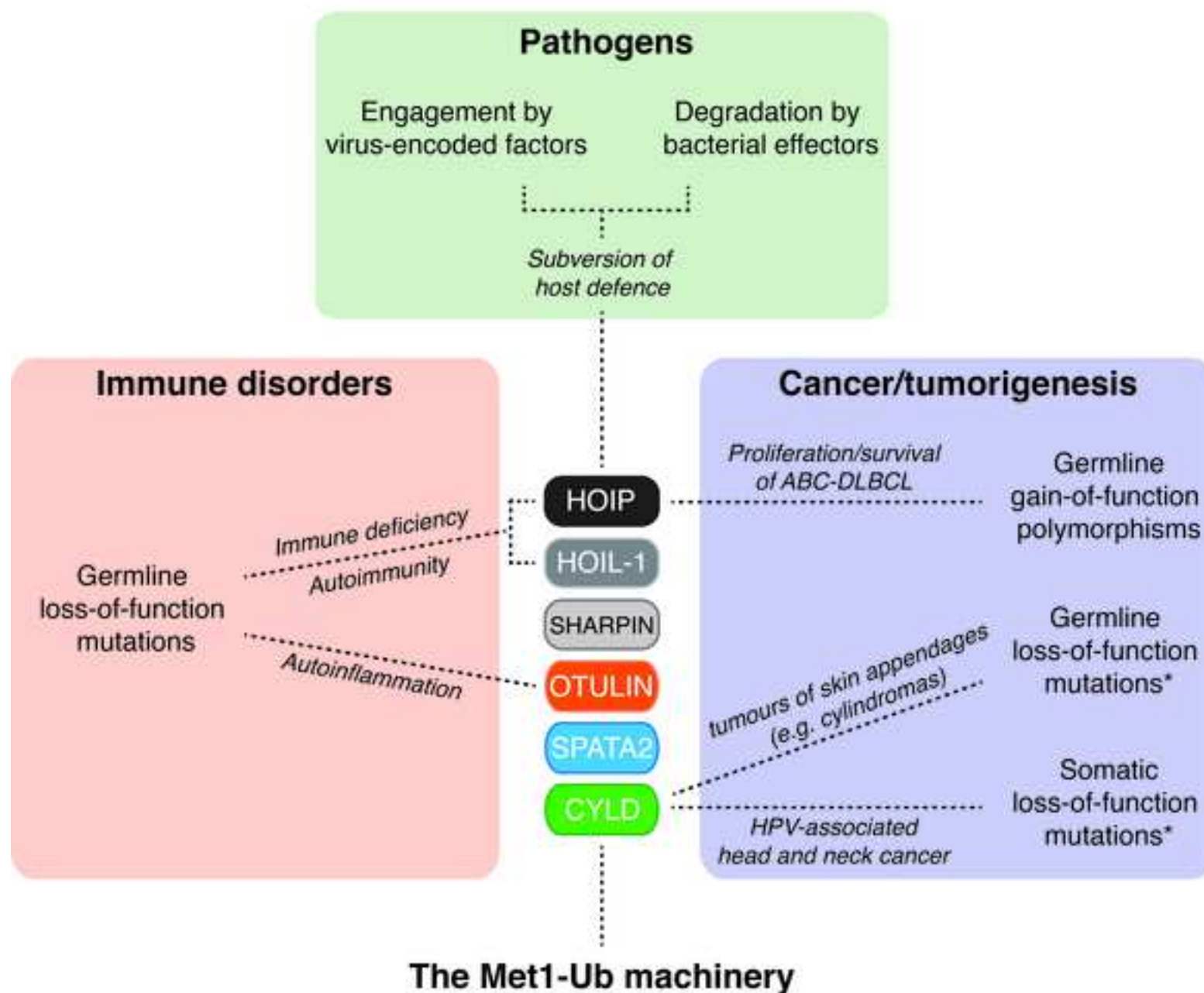


TABLE 1: Mouse genetic alterations and phenotypes

PROTEIN	GENOTYPE	NOTE	PHENOTYPE	REFERENCE
HOIP	<i>Hoip</i> ^{-/-}		Lethal at E10.5, apoptosis in the yolk sac	(Peltzer et al., 2014)
	<i>Hoip</i> ^{-/-} ; <i>Tnf</i> ^{-/-}		Lethal at E15.5, alleviated apoptosis in the yolk sac as compared to <i>Hoip</i> ^{-/-}	
	<i>Hoip</i> ^{-/-} ; <i>Tnfr1</i> ^{-/-}		Lethal at E17.5	
	<i>Hoip</i> ^{fl/fl} ; <i>Tie2-Cre</i> ⁺	Endothelial KO	Lethal at E10.5, recapitulates <i>Hoip</i> ^{-/-}	(Sasaki et al., 2013)
	<i>Hoip</i> ^{fl/fl} ; <i>Mb1-Cre</i> ⁺	B cell KO	Defective development of B1 cells, decreased CD40L signaling in B cells	
	<i>Hoip</i> ^{Δlinear/Δlinear}	Deletion of RBR-LDD region	Embryonic lethality	
	<i>Hoip</i> ^{C879S/C879S}	Mutation of catalytic cysteine	Lethal at E10.5, no Met1 ubiquitin in MEFs	(Emmerich et al., 2013)
	<i>Hoip</i> ^{C879S/C879S} ; <i>Tnfr1</i> ^{-/-}		Extended embryonic life compared to <i>Hoip</i> ^{C879S/C879S}	(Emmerich et al., 2016)
HOIL-1	<i>Hoil-1</i> ^{-/-}		Viable, decreased NF-κB signaling upon TNF and IL1β, increased TNF-induced apoptosis in liver, downregulated HOIP protein	(Tokunaga et al., 2009)
	<i>Hoil-1</i> ^{-/-}		Reduced NLRP3 inflammasome activation and IL1β secretion	(Rodgers et al., 2014)
	<i>Hoil-1</i> ^{-/-}		Immunodeficiency to acute bacterial infection. Protection against mycobacterium and viral infection accompanied by hyper-inflammation	(MacDuff et al., 2015)
	<i>Hoil-1</i> ^{-/-} ; <i>Sharpin</i> ^{+/cpdm}		Recapitulates <i>Hoil-1</i> ^{-/-} phenotype	(Shimizu et al., 2016)
	<i>Hoil-1</i> ^{-/-} ; <i>Sharpin</i> ^{cpdm/cpdm}		Lethal at E10.5	
SHARPIN	<i>Sharpin</i> ^{cpdm/cpdm}	Spontaneous loss-of-function mutation in <i>Sharpin</i>	Chronic proliferative dermatitis from 4-6 weeks of age, severe systemic inflammation (skin, gut, lung, liver, joint), excessive apoptosis (skin, lung, liver), splenomegaly, loss of Peyer's patch formation, increased serum IgM	(Gerlach et al., 2011; HogenEsch et al., 1993; HogenEsch et al., 1999; Ikeda et al., 2011; Liang et al., 2011; Seymour et al., 2007; Tokunaga et al., 2011)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>IL5</i> ^{-/-}		Recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	(Renninger et al., 2010)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Tnf</i> ^{-/-}		No skin phenotype, Peyer's patches absent	(Gerlach et al., 2011)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Tnfr1</i> ^{-/-}		No skin phenotype, Peyer's patches absent	(Rickard et al., 2014) (Kumari et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Tnfrsf1a</i> ^{-/-} ; <i>K14-Cre</i> ⁺	KO in keratinocytes	No skin phenotype, organ inflammation, splenomegaly	(Kumari et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Tnfr2</i> ^{-/-}		Recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	(Rickard et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Il1r1</i> ^{-/-}		Dermatitis from 12 weeks of age recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	(Gurung et al., 2016; Rickard et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Il1rap</i> ^{-/-}		Mild dermatitis	(Liang et al., 2011)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>IL1α</i> ^{-/-}		Recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	(Gurung et al., 2016)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>IL1β</i> ^{-/-}		Dermatitis from 9 weeks of age recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	(Gurung et al., 2016)

	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Rip1</i> ^{K45A/K45A}	Mutation of catalytic lysine	No phenotype	(Berger et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Rip3</i> ^{-/-}		Dermatitis from 10 weeks of age recapitulates <i>Sharpin</i> ^{cpdm/cpdm} , mild organ inflammation	(Kumari et al., 2014; Rickard et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Rip3</i> ^{-/-} ; <i>Fadd</i> ^{fl/fl} ; <i>K14-Cre</i> ⁺	<i>Fadd</i> KO in keratinocytes	No skin phenotype, organ inflammation, splenomegaly	(Kumari et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Tradd</i> ^{fl/fl} ; <i>K14-Cre</i> ⁺	<i>Tradd</i> KO in keratinocytes	No skin phenotype, organ inflammation, splenomegaly	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Mkl1</i> ^{-/-}		Dermatitis recapitulates <i>Sharpin</i> ^{cpdm/cpdm} , mild organ inflammation	(Rickard et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Casp8</i> ^{wt/-}		Dermatitis from 12 weeks of age recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Casp8</i> ^{wt/-} ; <i>Rip3</i> ^{-/-}		No phenotype (late onset dermatitis in one mouse)	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Casp8</i> ^{-/-} ; <i>Rip3</i> ^{-/-}		Perinatal lethality, no skin phenotype	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Bid</i> ^{-/-}		Recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Rag1</i> ^{-/-}		Dermatitis recapitulates <i>Sharpin</i> ^{cpdm/cpdm} , mild organ inflammation	(Potter et al., 2014)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Il4ra</i> ^{-/-}		Exacerbated granulocytic dermatitis, acute system inflammation, hepatic necrosis and mineralization	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Hoil1</i> ^{wt/-}		Recapitulates <i>Sharpin</i> ^{cpdm/cpdm} with exacerbated inflammation	(Shimizu et al., 2016)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Hoil1</i> ^{-/-}		Lethal at E10.5	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Casp1</i> ^{-/-}		No skin phenotype, no inflammation, Peyer's patches absent	(Nastase et al., 2016)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Casp11</i> ^{-/-}		Recapitulates <i>Sharpin</i> ^{cpdm/cpdm}	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Casp1</i> ^{-/-} ; <i>Casp11</i> ^{-/-}		No skin phenotype, no inflammation, Peyer's patches absent	
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Ice</i> ^{-/-}	KO of <i>Sharpin</i> , <i>Casp1</i> and <i>Casp11</i>	Dermatitis from 10 weeks of age, milder than in <i>Sharpin</i> ^{cpdm/cpdm} , system inflammation	(Douglas et al., 2015)
	<i>Sharpin</i> ^{cpdm/cpdm} ; <i>Nlrp3</i> ^{-/-}		Dermatitis from 10 weeks of age, milder than in <i>Sharpin</i> ^{cpdm/cpdm}	
OTULIN	<i>Otulin</i> ^{W96A/W96A}	Spontaneous mutation. Gene termed <i>Gumby</i>	Lethal at E12.5-E14, abnormal facial nerve sprouting, vascularization defect, Wnt signaling defect	(Rivkin et al., 2013)
	<i>Otulin</i> ^{D336E/D336E}	Spontaneous mutation. Gene termed <i>Gumby</i>	Lethal at E12.5-E14, vascularization defect, Wnt signaling defect	
	<i>Otulin</i> ^{LacZ/fl} ; <i>ERT2-Cre</i> ⁺	Tamoxifen-inducible Cre	Mice moribund within a day of tamoxifen administration	(Damgaard et al., 2016)
	<i>Otulin</i> ^{LacZ/fl} ; <i>Mb1-Cre</i> ⁺	KO in B cells	No phenotype	
	<i>Otulin</i> ^{LacZ/fl} ; <i>Cd4-Cre</i> ⁺	KO in T cells	No phenotype	

	<i>Otulin^{LacZfl/-}; LysM-Cre⁺</i>	KO in myeloid cells	Chronic inflammatory phenotype and autoimmunity	
--	--------------------------------------------------------	---------------------	-------------------------------------------------	--

Abbreviations:

KO, knockout; wt, wild type

References

- Berger, S.B., Kasparcova, V., Hoffman, S., Swift, B., Dare, L., Schaeffer, M., Capriotti, C., Cook, M., Finger, J., Hughes-Earle, A., *et al.* (2014). Cutting Edge: RIP1 kinase activity is dispensable for normal development but is a key regulator of inflammation in SHARPIN-deficient mice. *J Immunol* **192**, 5476-5480.
- Damgaard, R.B., Walker, J.A., Marco-Casanova, P., Morgan, N.V., Titheradge, H.L., Elliott, P.R., McHale, D., Maher, E.R., McKenzie, A.N., and Komander, D. (2016). The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. *Cell* **166**, 1215-1230.
- Douglas, T., Champagne, C., Morizot, A., Lapointe, J.M., and Saleh, M. (2015). The Inflammatory Caspases-1 and -11 Mediate the Pathogenesis of Dermatitis in Sharpin-Deficient Mice. *J Immunol* **195**, 2365-2373.
- Emmerich, C.H., Bakshi, S., Kelsall, I.R., Ortiz-Guerrero, J., Shpiro, N., and Cohen, P. (2016). Lys63/Met1-hybrid ubiquitin chains are commonly formed during the activation of innate immune signalling. *Biochem Biophys Res Commun* **474**, 452-461.
- Emmerich, C.H., Ordureau, A., Strickson, S., Arthur, J.S., Pedrioli, P.G., Komander, D., and Cohen, P. (2013). Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *Proc Natl Acad Sci U S A* **110**, 15247-15252.
- Gerlach, B., Cordier, S.M., Schmukle, A.C., Emmerich, C.H., Rieser, E., Haas, T.L., Webb, A.I., Rickard, J.A., Anderton, H., Wong, W.W., *et al.* (2011). Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591-596.
- Gurung, P., Sharma, B.R., and Kanneganti, T.D. (2016). Distinct role of IL-1beta in instigating disease in Sharpincpdm mice. *Sci Rep* **6**, 36634.
- HogenEsch, H., Gijbels, M.J., Offerman, E., van Hooft, J., van Bekkum, D.W., and Zurcher, C. (1993). A spontaneous mutation characterized by chronic proliferative dermatitis in C57BL mice. *Am J Pathol* **143**, 972-982.
- HogenEsch, H., Janke, S., Boggess, D., and Sundberg, J.P. (1999). Absence of Peyer's patches and abnormal lymphoid architecture in chronic proliferative dermatitis (cpdm/cpdm) mice. *J Immunol* **162**, 3890-3896.
- Ikeda, F., Deribe, Y.L., Skanland, S.S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M., van Wijk, S.J., Goswami, P., Nagy, V., Terzic, J., *et al.* (2011). SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* **471**, 637-641.
- Kumari, S., Redouane, Y., Lopez-Mosqueda, J., Shiraishi, R., Romanowska, M., Lutzmayer, S., Kuiper, J., Martinez, C., Dikic, I., Pasparakis, M., *et al.* (2014). Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. *Elife* **3**.

- Liang, Y., Seymour, R.E., and Sundberg, J.P. (2011). Inhibition of NF-kappaB signaling retards eosinophilic dermatitis in SHARPIN-deficient mice. *J Invest Dermatol* 131, 141-149.
- MacDuff, D.A., Reese, T.A., Kimmey, J.M., Weiss, L.A., Song, C., Zhang, X., Kambal, A., Duan, E., Carrero, J.A., Boisson, B., *et al.* (2015). Phenotypic complementation of genetic immunodeficiency by chronic herpesvirus infection. *Elife* 4.
- Nastase, M.V., Zeng-Brouwers, J., Frey, H., Hsieh, L.T., Poluzzi, C., Beckmann, J., Schroeder, N., Pfeilschifter, J., Lopez-Mosqueda, J., Mersmann, J., *et al.* (2016). An Essential Role for SHARPIN in the Regulation of Caspase 1 Activity in Sepsis. *Am J Pathol* 186, 1206-1220.
- Peltzer, N., Rieser, E., Taraborrelli, L., Draber, P., Darding, M., Pernaute, B., Shimizu, Y., Sarr, A., Draberova, H., Montinaro, A., *et al.* (2014). HOIP deficiency causes embryonic lethality by aberrant TNFR1-mediated endothelial cell death. *Cell Rep* 9, 153-165.
- Potter, C.S., Wang, Z., Silva, K.A., Kennedy, V.E., Stearns, T.M., Burzenski, L., Shultz, L.D., Hogenesch, H., and Sundberg, J.P. (2014). Chronic proliferative dermatitis in Sharpin null mice: development of an autoinflammatory disease in the absence of B and T lymphocytes and IL4/IL13 signaling. *PLoS One* 9, e85666.
- Renninger, M.L., Seymour, R.E., Whiteley, L.O., Sundberg, J.P., and Hogenesch, H. (2010). Anti-IL5 decreases the number of eosinophils but not the severity of dermatitis in Sharpin-deficient mice. *Exp Dermatol* 19, 252-258.
- Rickard, J.A., Anderton, H., Etemadi, N., Nachbur, U., Darding, M., Peltzer, N., Lalaoui, N., Lawlor, K.E., Vanyai, H., Hall, C., *et al.* (2014). TNFR1-dependent cell death drives inflammation in Sharpin-deficient mice. *Elife* 3.
- Rivkin, E., Almeida, S.M., Ceccarelli, D.F., Juang, Y.C., MacLean, T.A., Srikumar, T., Huang, H., Dunham, W.H., Fukumura, R., Xie, G., *et al.* (2013). The linear ubiquitin-specific deubiquitinase gumby regulates angiogenesis. *Nature* 498, 318-324.
- Rodgers, M.A., Bowman, J.W., Fujita, H., Orazio, N., Shi, M., Liang, Q., Amatya, R., Kelly, T.J., Iwai, K., Ting, J., *et al.* (2014). The linear ubiquitin assembly complex (LUBAC) is essential for NLRP3 inflammasome activation. *J Exp Med* 211, 1333-1347.
- Sasaki, Y., Sano, S., Nakahara, M., Murata, S., Kometani, K., Aiba, Y., Sakamoto, S., Watanabe, Y., Tanaka, K., Kurosaki, T., *et al.* (2013). Defective immune responses in mice lacking LUBAC-mediated linear ubiquitination in B cells. *EMBO J* 32, 2463-2476.
- Seymour, R.E., Hasham, M.G., Cox, G.A., Shultz, L.D., Hogenesch, H., Roopenian, D.C., and Sundberg, J.P. (2007). Spontaneous mutations in the mouse Sharpin gene result in multiorgan inflammation, immune system dysregulation and dermatitis. *Genes Immun* 8, 416-421.
- Shimizu, S., Fujita, H., Sasaki, Y., Tsuruyama, T., Fukuda, K., and Iwai, K. (2016). Differential Involvement of the Npl4 Zinc Finger Domains of SHARPIN and HOIL-1L in Linear Ubiquitin Chain Assembly Complex-Mediated Cell Death Protection. *Mol Cell Biol* 36, 1569-1583.
- Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sakata, S., Tanaka, K., Nakano, H., and Iwai, K. (2011). SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. *Nature* 471, 633-636.
- Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K., Nakagawa, T., Kato, M., Murata, S., Yamaoka, S., *et al.* (2009). Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* 11, 123-132.

TABLE 2: Human genetic alterations and phenotypes

GENE	DNA	PROTEIN	MOLECULAR PATHOLOGY	DISEASE	REFERENCE
<i>RNF31</i> (HOIP)	SNP,rs184184005	p.Q584H	increased LUBAC activity	Enhanced BCR mediated NF-κB activity in ABC DLBCL	(Yang et al., 2014)
	SNP,rs149481717	p.Q622L	increased LUBAC activity		
	c.215T>C (ex 2)	p.L72P	Reduced HOIP protein	Multiorgan autoinflammation and immunodeficiency	(Boisson et al., 2015)
<i>RBCK1</i> (HOIL-1)	c.121_122delCT	p.L41fsX7	no HOIL-1 protein, less HOIP and SHARPIN	Pyogenic infections, systemic autoimmunity, muscular amylopectinosis	(Boisson et al., 2012)
	c.553 C>T	p.Q185X	no HOIL-1 protein, less HOIP and SHARPIN		
	del: TRIB3: g.-1272_HOIL1:g.9780del	N/A	Chr. 20 deletion of 31.799kb		
	N/A	p.Q222X	truncated HOIL-1	Muscular weakness, cardiomyopathy	(Wang et al., 2013)
	N/A	p.E190fs	truncated HOIL-1		
	c.456+1G>C	N/A	predicted aberrant splicing		
	SNP,rs11698154	N/A	predicted aberrant splicing	Muscular amylopectinosis, cardiomyopathy, skeletal myopathy	(Nilsson et al., 2013)
	c.727G>T	p.E243X	predicted HOIL-1 truncation		
	c.1160A>G	p.N387S	missense mutation in HOIL-1		
	c.896_899delAGTG	p.E299VfsX18	predicted HOIL-1 truncation		
	c.722delC	p.A241GfsX34	predicted HOIL-1 truncation		
	c.52G>C	p.A18P	missense mutation in HOIL-1		
	c.727_728insGGCG	p.E243GfsX114	predicted HOIL-1 truncation		
	c.ex1_ex4del	N/A	predicted HOIL-1 truncation		
	c.1054C>T	p.R352X	missense mutation in HOIL-1		
	c.917+3_917+4insG	p.R298RfsX40	predicted HOIL-1 truncation		
	c.494delG	p.R165RfsX111	predicted HOIL-1 truncation		
<i>OTULIN</i> (OTULIN)	c.815T>C	p.L272P	OTULIN destabilisation, impaired catalytic activity	Systemic inflammation; Recurrent fever, diarrhea, arthritis, panniculitis, neutrophilia, increased blood immunoglobulin levels, and autoantibodies	(Damgaard et al., 2016; Zhou et al., 2016)
	c.731A>G	p.Y244C	OTULIN destabilisation		
	c.517delC	p.G174DfsX2	OTULIN truncation		

Abbreviations:
BCR, B cell receptor; del, deletion; ex, exon; fs, frameshift; ins, insertion; N/A, not available or information not available; SNP, single-nucleotide polymorphism; ABC DLBCL, activated B cell diffuse large B cell lymphoma

References

- Boisson, B., Laplantine, E., Dobbs, K., Cobat, A., Tarantino, N., Hazen, M., Lidov, H.G., Hopkins, G., Du, L., Belkadi, A., *et al.* (2015). Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J Exp Med* 212, 939-951.
- Boisson, B., Laplantine, E., Prando, C., Giliani, S., Israelsson, E., Xu, Z., Abhyankar, A., Israel, 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.
- Damgaard, R.B., Walker, J.A., Marco-Casanova, P., Morgan, N.V., Titheradge, H.L., Elliott, P.R., McHale, D., Maher, E.R., McKenzie, A.N., and Komander, D. (2016). The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. *Cell*.
- Nilsson, J., Schoser, B., Laforet, P., Kalev, O., Lindberg, C., Romero, N.B., Davila Lopez, M., Akman, H.O., Wahbi, K., Iglseider, S., *et al.* (2013). Polyglucosan body myopathy caused by defective ubiquitin ligase RBCK1. *Ann Neurol* 74, 914-919.
- Wang, K., Kim, C., Bradfield, J., Guo, Y., Toskala, E., Otieno, F.G., Hou, C., Thomas, K., Cardinale, C., Lyon, G.J., *et al.* (2013). Whole-genome DNA/RNA sequencing identifies truncating mutations in RBCK1 in a novel Mendelian disease with neuromuscular and cardiac involvement. *Genome Med* 5, 67.
- Yang, Y., Schmitz, R., Mitala, J., Whiting, A., Xiao, W., Ceribelli, M., Wright, G.W., Zhao, H., Yang, Y., Xu, W., *et al.* (2014). Essential role of the linear ubiquitin chain assembly complex in lymphoma revealed by rare germline polymorphisms. *Cancer discovery* 4, 480-493.
- Zhou, Q., Yu, X., Demirkaya, E., Deutch, N., Stone, D., Tsai, W.L., Kuehn, H.S., Wang, H., Yang, D., Park, Y.H., *et al.* (2016). Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc Natl Acad Sci U S A*.

TABLE 3: Pathogen effectors targeting the Met1-Ub machinery

Pathogen	Effector	Mechanism	References
Bacteria			
<i>Salmonella typhimurium</i>	SopE	Activates LUBAC	(Fiskin et al., 2016)
<i>Shigella flexneri</i>	IpaH1.4 IpaH2.5	Lys48 ubiquitination of HOIP followed by proteasomal degradation	(de Jong et al., 2016)
	Ospl	Interferes with LUBAC recruitment	(Sanada et al., 2012)
Viruses			
Hepatitis B virus (HBV)	HBx	Blocks MAVS signalling by recruiting Parkin-associated LUBAC	(Khan et al., 2016)
Hepatitis C virus (HCV)	NS3	Interacts with HOIP and prevents NF- κ B signalling	(Chen et al., 2015)
Porcine reproductive and respiratory syndrome virus (PRRSV)	NSP1	Disruption of HOIP interaction with SHARPIN	(Jing et al., 2017)
Epstein-Barr virus (EBV)	LMP1	Recruits LUBAC to ubiquitinate and inactivate IRF7. Averts anti-viral interferon production	(Wang et al., 2017)
		Recruits LUBAC to activate NF- κ B oncogenic signaling	(Greenfeld et al., 2015)
Human T cell leukemia virus type 1 (HTLV-1)	Tax	Recruits LUBAC to activate NF- κ B signaling in leukemogenesis	(Shibata et al., 2017)

Abbreviations:

HBx, Hepatitis B virus X protein; IpaH1.4, Invasion plasmid antigen H1.4; IpaH2.5, Invasion plasmid antigen H2.5; IRF7, Interferon regulatory factor 7; LMP1, Latent membrane protein 1; MAVS, Mitochondrial antiviral-signaling protein; NS3, Nonstructural protein 3; NSP1, Nonstructural protein 1; Ospl, Outer Shigella protein I; SopE, Salmonella outer protein E

References

- Chen, Y., He, L., Peng, Y., Shi, X., Chen, J., Zhong, J., Chen, X., Cheng, G., and Deng, H. (2015). The hepatitis C virus protein NS3 suppresses TNF- α -stimulated activation of NF- κ B by targeting LUBAC. *Sci Signal* 8, ra118.
- de Jong, M.F., Liu, Z., Chen, D., and Alto, N.M. (2016). *Shigella flexneri* suppresses NF- κ B activation by inhibiting linear ubiquitin chain ligation. *Nature Microbiology* 1, 16084.
- Fiskin, E., Bionda, T., Dikic, I., and Behrends, C. (2016). Global Analysis of Host and Bacterial Ubiquitinome in Response to *Salmonella Typhimurium* Infection. *Mol Cell* 62, 967-981.
- Greenfeld, H., Takasaki, K., Walsh, M.J., Ersing, I., Bernhardt, K., Ma, Y., Fu, B., Ashbaugh, C.W., Cabo, J., Mollo, S.B., et al. (2015). TRAF1 Coordinates Polyubiquitin Signaling to Enhance Epstein-Barr Virus LMP1-Mediated Growth and Survival Pathway Activation. *PLoS Pathog* 11, e1004890.
- Jing, H., Fang, L., Ding, Z., Wang, D., Hao, W., Gao, L., Ke, W., Chen, H., and Xiao, S. (2017). Porcine Reproductive and Respiratory Syndrome Virus nsp1 α Inhibits NF- κ B Activation by Targeting the Linear Ubiquitin Chain Assembly Complex. *J Virol* 91.
- Khan, M., Syed, G.H., Kim, S.J., and Siddiqui, A. (2016). Hepatitis B Virus-Induced Parkin-Dependent Recruitment of Linear Ubiquitin Assembly Complex (LUBAC) to Mitochondria and Attenuation of Innate Immunity. *PLoS Pathog* 12, e1005693.
- Sanada, T., Kim, M., Mimuro, H., Suzuki, M., Ogawa, M., Oyama, A., Ashida, H., Kobayashi, T., Koyama, T., Nagai, S., et al. (2012). The *Shigella flexneri* effector Ospl deamidates UBC13 to dampen the inflammatory response. *Nature* 483, 623-626.
- Shibata, Y., Tokunaga, F., Goto, E., Komatsu, G., Gohda, J., Saeki, Y., Tanaka, K., Takahashi, H., Sawasaki, T., Inoue, S., et al. (2017). HTLV-1 Tax Induces Formation of the Active Macromolecular IKK Complex by Generating Lys63- and Met1-Linked Hybrid Polyubiquitin Chains. *PLoS Pathog* 13, e1006162.

Wang, L., Wang, Y., Zhao, J., Ren, J., Hall, K.H., Moorman, J.P., Yao, Z.Q., and Ning, S. (2017). The Linear Ubiquitin Assembly Complex Modulates Latent Membrane Protein 1 Activation of NF-kappaB and Interferon Regulatory Factor 7. *J Virol* 91.