Biochemical Journal (2022) **479** 1103–1119
https://doi.org/10.1042/BCJ20210735

PORTLAND PRESS

Review Article

Deubiquitinases in cell death and inflammation

Kim Newton[1] **and Alexander D. Gitlin**[1,2]

[1]Department of Physiological Chemistry, Genentech, South San Francisco, CA 94080, U.S.A.; [2]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, U.S.A.

Correspondence: Kim Newton (knewton@gene.com)

Apoptosis, pyroptosis, and necroptosis are distinct forms of programmed cell death that eliminate infected, damaged, or obsolete cells. Many proteins that regulate or are a part of the cell death machinery undergo ubiquitination, a post-translational modification made by ubiquitin ligases that modulates protein abundance, localization, and/or activity. For example, some ubiquitin chains target proteins for degradation, while others function as scaffolds for the assembly of signaling complexes. Deubiquitinases (DUBs) are the proteases that counteract ubiquitin ligases by cleaving ubiquitin from their protein substrates. Here, we review the DUBs that have been found to suppress or promote apoptosis, pyroptosis, or necroptosis.

Introduction

Ubiquitination is the covalent, post-translational modification of a protein with the 8.4 kDa protein ubiquitin. An isopeptide bond is formed between the C-terminus of ubiquitin and a lysine side chain in the target protein, or less commonly, the C-terminal glycine is linked to either the N-terminus or a serine or threonine side chain of the protein [1–3]. Moreover, ubiquitin itself can be modified at its N-terminus or at one of its seven lysines allowing the assembly of polyubiquitin chains. Ubiquitination is mediated by the concerted action of ubiquitin-activating E1, ubiquitin-conjugating E2, and ubiquitin ligase E3 enzymes, resulting in altered protein stability, interactions, or localization. Deubiquitinating enzymes (DUBs) counter ubiquitin ligases by cleaving ubiquitin from their protein substrates (Figure 1). DUBs belong to the USP (ubiquitin-specific protease), UCH (ubiquitin C-terminal hydrolase), OTU (ovarian tumor), MINDY (motif-interacting with ubiquitin-containing novel DUB family), MJD (Machado–Josephin domain-containing), and JAMM (JAB1/MPN/Mov34) protease families [4]. Here we review our current understanding of DUBs that regulate the cell death programs of apoptosis, pyroptosis, and necroptosis.

DUBs regulating caspase-8-dependent cell death and necroptosis

Tumor Necrosis Factor Receptor 1 (TNFR1), Toll-like Receptor 3 (TLR3), and TLR4 contribute to innate immune surveillance and defense against invading pathogens [5–7]. Endosomal TLR3 is activated by viral double-stranded RNA [6], whereas TLR4 on the plasma membrane responds to bacterial lipopolysaccharide [7]. TNFR1 responds to either TNF or lymphotoxin-α [8,9], the former produced by many cell types in response to infection. TNFR1, TLR3, and TLR4 each recruit ubiquitin ligases, including the linear ubiquitin chain assembly complex (LUBAC) [10–12], to build a ubiquitin scaffold for activating the protein kinases TAK1, IKKα/β, and IKKε/TBK1 [13–17]. Activation of these kinases culminates in the transcription of proinflammatory genes, while formation of a secondary, death-inducing signaling complex is suppressed. Genetic [11,12,18–26], small molecule [17,20,27,28], or pathogen-induced perturbations [29] that compromise the assembly of the ubiquitin scaffold or activation of these kinases promotes the formation of the death-inducing complex. The nature of the perturbation governs whether the enzymatic activity of the kinase RIPK1 is required for assembly of the death-inducing complex (reviewed in [30]).

Received: 30 March 2022
Revised: 7 May 2022
Accepted: 10 May 2022

Version of Record published: 24 May 2022

Biochemical Journal (2022) 479 1103–1119
https://doi.org/10.1042/BCJ20210735

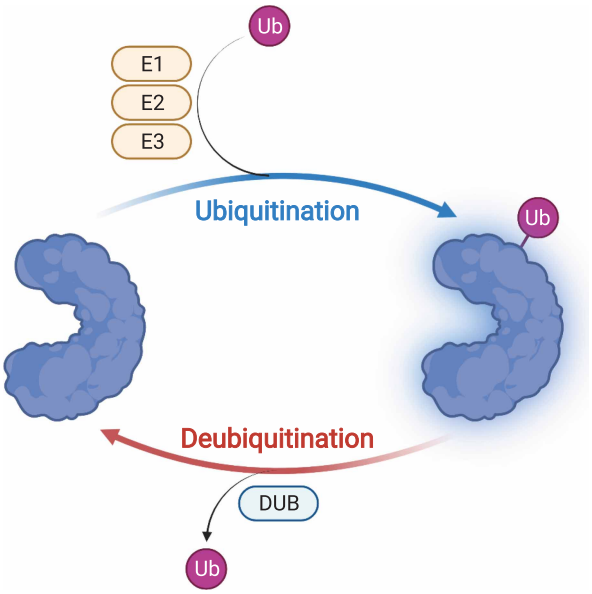

Figure 1. Deubiquitinases (DUBs) counteract protein modifications made by E3 ubiquitin ligase enzymes.
Figure created with BioRender.com.

In the case of the more extensively studied TNFR1, ligation of the receptor triggers the assembly of the TNFR1-associated signaling complex termed complex I [31] (Figure 2). The cytoplasmic death domain (DD) of TNFR1 recruits the DD-containing proteins TRADD and RIPK1 via homotypic interactions, with TRADD recruiting TRAF2, the adaptor for the E3 ubiquitin ligases cellular inhibitor of apoptosis protein 1 (cIAP1) and cIAP2 [32–37]. Subsequently, cIAP1/2 modify themselves and RIPK1 with lysine 63 (K63)-linked polyubiquitin and this contributes to the recruitment of TAB2 and TAB3, ubiquitin-binding adaptors for the kinase TAK1 [13,38]. K63-linked polyubiquitin within complex I also recruits LUBAC, composed of HOIP (also called RNF31), HOIL-1 (also called RBCK1), and SHARPIN, as both HOIP and SHARPIN bind to K63-linked polyubiquitin [10,15,39]. LUBAC then modifies several proteins in TNFR1 complex I with M1-linked polyubiquitin, including TNFR1 itself, TRADD, and RIPK1 [40–43]. Indeed, LUBAC can modify K63-linked polyubiquitin on RIPK1 with M1-linked ubiquitin to create hybrid polyubiquitin chains [16]. The M1-linked polyubiquitin in complex I recruits NEMO (also called IKKγ), the ubiquitin-binding regulatory subunit of the canonical IκB kinase (IKK) [14,44,45]. Hybrid polyubiquitin chains may position TAK1 and IKK to facilitate the activating phosphorylation of IKK by TAK1 [15,16,46]. Collectively, these molecular events stabilize complex I for productive signal transduction.

Many cells are not killed by TNF because the formation of a death-inducing complex II is transient and unproductive [31]. TRADD and RIPK1 move into the cytoplasm where they interact with FADD, caspase-8, and the long isoform of cFLIP (cFLIP$_L$) [31], but cleavage of RIPK1 by the caspase-8/cFLIP$_L$ heterodimer then disrupts the complex [47–50]. Accordingly, heterozygous mutations altering the aspartic acid cleavage site in RIPK1 sensitize cells to TNF killing [48–50]. Notably, these mutations give rise to an autoinflammatory syndrome in humans [49,50]. Loss of the labile protein cFLIP, as in cells treated with the translational inhibitor cycloheximide, also sensitizes to TNF-induced cell death [31]. In this case, cells die because caspase-8 homodimers assemble within a stabilized complex II, autoprocess, and then cleave and activate caspases 3 and 7 to execute the apoptotic program [51–53]. If caspase-8 is eliminated or inactivated, however, cells may still die because RIPK1 in complex II can interact with RIPK3, if it is expressed, to form the necrosome [54–56]. Activation of RIPK3 within the necrosome leads to phosphorylation of the pseudokinase MLKL, which then mediates a lytic form of cell death termed necroptosis [57–61]. TLR3 and TLR4 utilize slightly different combinations of adaptor proteins and E3 ubiquitin ligases when compared with TNFR1, but they elicit similar death-inducing signaling complexes if LUBAC [11] or caspase-8 is compromised [62,63].

Biochemical Journal (2022) **479** 1103–1119
https://doi.org/10.1042/BCJ20210735

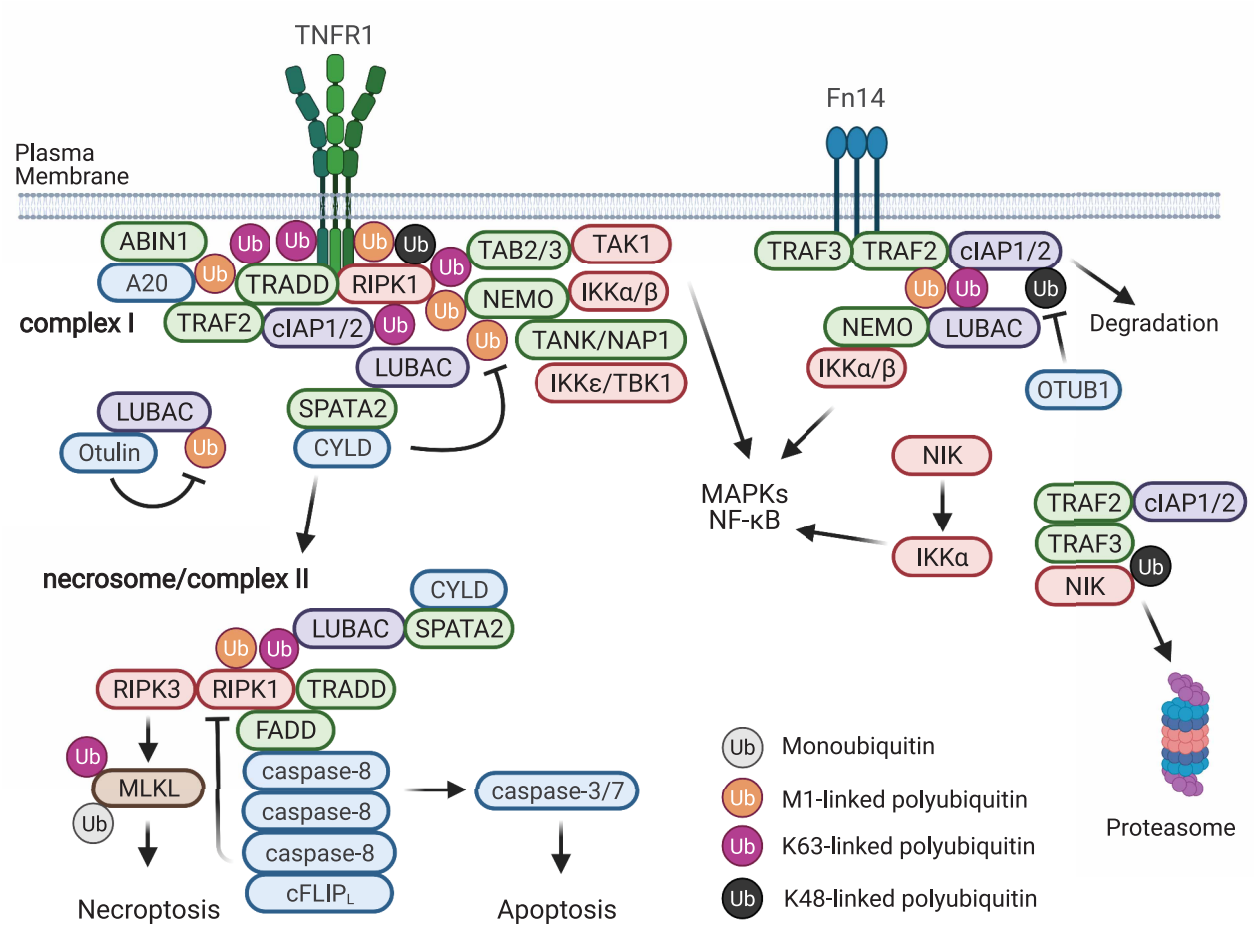

Figure 2. Deubiquitinases OTULIN, CYLD, and OTUB1 modulate sensitivity to TNF-induced cell death.
OTULIN suppresses cell death by removing M1-linked polyubiquitin from LUBAC and preserving its ligase activity. LUBAC modifies components of TNFR1 complex I with M1-linked polyubiquitin, which serves as a scaffold for the recruitment of kinases that activate gene expression via the MAPK and NF-κB pathways. Stabilization of complex I in this manner limits assembly of the death-inducing complex II/necrosome. CYLD promotes cell death by removing K63- and M1-linked polyubiquitin from components of TNFR1 complex I, which facilitates complex II/necrosome assembly. OTUB1 limits sensitization to TNF-induced cell death by TWEAK, which is the ligand for the receptor Fn14. cIAPs recruited to Fn14 modify the signaling complex with K48-linked polyubiquitin and target it for degradation. By removing this polyubiquitin, OTUB1 preserves the pool of cIAP1 available for recruitment into TNFR1 complex I. Within TNFR1 complex I, cIAP1 builds a K63-linked polyubiquitin scaffold that limits activation of the kinase RIPK1 and complex II/necrosome formation. Whether there are DUBs that limit ubiquitination of RIPK1 in the necrosome [216] or ubiquitination of MLKL [214,215] is unclear. Adaptor proteins are colored green, E3 ubiquitin ligases are purple, kinases are red, and proteases, including DUBs, are blue. Figure created with BioRender.com.

A20

The deubiquitinase and ubiquitin-binding protein A20 (also called TNFAIP3) confers a degree of protection against TNF-induced apoptosis [64–66] or necroptosis [43,67,68]. Accordingly, loss of A20 in intestinal epithelial cells sensitizes mice to TNF toxicity that requires, in part, the kinase activity of RIPK1 [66,69,70]. It is worth noting, however, that enforced expression of A20 in intestinal epithelial cells also sensitizes mice to TNF toxicity driven by the kinase activity of RIPK1 [71]. Thus, the expression of *Tnfaip3*, which is an NF-κB-inducible gene [72], must be finely tuned for optimal signal transduction.

A20 is recruited to TNFR1 complex I by virtue of its ubiquitin-binding zinc finger 4 (ZnF4) and ZnF7 motifs [73–78]. ZnF4 binds to monoubiquitin or K63-linked polyubiquitin [79], while ZnF7 binds to

Biochemical Journal (2022) 479 1103–1119
https://doi.org/10.1042/BCJ20210735

M1-linked polyubiquitin [73,74]. The ubiquitin-binding protein ABIN1 has also been implicated in the recruitment of A20 to TNFR1 complex I [80]. Ubiquitin binding by ZnF7, in particular, appears crucial for A20 to suppress TNF-induced NF-κB activation and cell death [66,73,74,77,78]. In mice, mutation of A20 ZnF7 results in TNF-dependent arthritis [78]. Mutation of A20 ZnF7 and ZnF4, however, results in lethal inflammation soon after birth [77,78] similar to A20 deficiency [65]. It is unclear if aberrant cell death is a major driver of lethality in either model. RIPK3 deficiency, but not MLKL deficiency, prolongs survival of A20-deficient mice [68,81], but the effect of eliminating both MLKL and caspase-8 to disable both caspase-8-dependent cell death and necroptosis has not been reported. When A20 deficiency is restricted to myeloid cells, however, mice develop arthritis that requires TLR4, RIPK3, and MLKL, but not TNFR1 [76,82]. These genetic data indicate that suppression of necroptosis is an important physiological function of A20 not only in the context of TNFR1 signaling. Consistent with A20 also suppressing caspase-8-dependent cell death, lethal inflammation in mice lacking both ABIN1 and A20 in intestinal epithelial cells is prevented by the combined loss of RIPK3 and caspase-8 [83].

Despite having an OTU domain that cleaves K48- or K63-linked polyubiquitin *in vitro* [84], the deubiquitinating activity of A20 appears largely dispensable for suppressing inflammation [43,75,85,86]. Mutation of the OTU catalytic cysteine in mice does not give an overt phenotype [75,85,86], although the mice are more sensitive to TNF toxicity [86]. Whether RIPK1 is a substrate of A20 in this context requires further study. Overall, available data indicate that A20 binding to polyubiquitin in TNFR1 complex I is more important than its DUB activity for suppressing complex II assembly. Recruitment of A20 to TNFR1 complex I may preserve the ubiquitin scaffold by protecting polyubiquitin from cleavage by the death promoting DUB CYLD (described in the next section) [43]. In keeping with this notion, M1-linked polyubiquitin in TNFR1 complex I is reduced by A20 deficiency or mutation of A20 ZnF7 [43,66,76,77].

CYLD

CYLD (encoded by the cylindromatosis gene) promotes assembly of the TNFR1-induced necrosome [87–89]. Accordingly, knockdown, deletion, or inactivation of CYLD renders cells less sensitive to TNF-induced necroptosis [88–91]. CYLD also promotes, to varying degrees, caspase-8-dependent cell death induced by TNF plus cIAP antagonist [87], TNF plus cycloheximide [92], and TNF plus SHARPIN deficiency [93]. The role of CYLD in promoting cell death is evident in mice as well as cell culture. For example, inactivation of CYLD prevents RIPK3- and MLKL-dependent colitis in the FADD-deficient mouse intestine [91], and ameliorates RIPK3-dependent inflammation in the FADD-deficient mouse epidermis [94]. CYLD deficiency ameliorates TNFR1-, FADD- and RIPK1-dependent skin inflammation in SHARPIN-deficient mice [18,19,93,95].

CYLD is recruited to TNFR1 complex I via the adaptor protein SPATA2, which in turn binds to HOIP within LUBAC [96–99]. A PUB domain-interacting motif (PIM) in SPATA2 binds to the same HOIP PUB domain as the PIM in the DUB OTULIN (described in the next section). Consequently, OTULIN and CYLD exhibit mutually exclusive recruitment to LUBAC [43]. CYLD cleaves M1- or K63-linked ubiquitin chains [100], with phosphorylation of CYLD boosting its activity towards K63-linked polyubiquitin [101]. Contrary to expectations, however, ubiquitination of RIPK1 and TNFR1 in complex I is either unchanged or decreased, rather than increased in cells lacking either CYLD or SPATA2 [92,96,99,102]. Although one study reported that SPATA2 deficiency increased M1-linked polyubiquitin in TNFR1 complex I [97], others found that SPATA2 or CYLD deficiency decreased both M1- and K63-linked polyubiquitin in complex I [99,101]. Nonetheless, when whole cell lysates are analyzed, TNF-induced ubiquitination of RIPK1, TNFR1, and TRADD is increased by CYLD or SPATA2 deficiency [43,92,99,102]. M1-linked polyubiquitination, in particular, appears increased on RIPK1 [98]. These alterations, in the context of the necroptosis stimulus TNF plus zVAD, coincide with reduced activation of RIPK1 and reduced necrosome assembly [102]. Thus, the accumulation of polyubiquitin on TNFR1 complex I in cells lacking SPATA2 or CYLD may promote dissociation of complex I, thereby limiting dimerization and autophosphorylation of RIPK1, which in turn limits the ability of RIPK1 to engage RIPK3 [54,55,103,104]. SPATA2 and CYLD have also been observed in the TNF-induced necrosome [102], so deubiquitination of CYLD substrates may be important in both complexes. Whether hybrid polyubiquitin chains on complex I components influence deubiquitination by CYLD is unclear. In the context of interleukin-1 receptor signaling, however, modification of K63-linked ubiquitin chains with K48-linked chains is reported to protect K63-linked polyubiquitin from cleavage by CYLD [105].

Despite SPATA2- or CYLD-deficient cells being less sensitive than their wild-type counterparts to various forms of TNF-induced cell death, SPATA2-deficient mice are actually more sensitive than wild-type mice to

TNF toxicity. Moreover, this toxicity requires the kinase activity of RIPK1 [102]. How SPATA2 suppresses activation of RIPK1 in this context and whether CYLD-deficient mice are also more susceptible to TNF toxicity is unclear. Chronic NF-κB activation in intestinal epithelial cells sensitizes mice to TNF toxicity [106] and an early study identified CYLD as a negative regulator of TNF-induced NF-κB signaling [107]. However, it is unclear if there is aberrant NF-κB activation in SPATA2-deficient mouse intestines. Mouse macrophages, fibroblasts and keratinocytes lacking SPATA2 or CYLD exhibit, at best, a modest enhancement in TNF-induced activation of MAPKs or NF-κB [97,98,102,108–110].

Although SPATA2-deficient mice and several different strains of CYLD-deficient mice are viable [102,108–111], mice expressing inactive CYLD, owing to truncation of the C-terminal USP domain, die soon after birth [112]. Whether this lethality reflects a gain-of-function of the mutant CYLD scaffold remains unclear. In humans, germline mutations in *CYLD* are associated with a predisposition to tumors of skin appendages, with a majority of the disease-causing mutations predicted to C-terminally truncate CYLD [113]. Details of the pathway(s) perturbed in this setting by aberrant ubiquitination are unclear.

OTULIN

OTULIN (OTU DUB with linear linkage specificity; also known as FAM105B or GUMBY) cleaves M1-linked polyubiquitin with exquisite specificity via substrate-assisted catalysis, a mechanism in which selective binding of OTULIN to M1-linked polyubiquitin activates its catalytic triad [41]. Strikingly, patients carrying biallelic loss-of-function *OTULIN* mutations develop a severe autoinflammatory syndrome termed OTULIN-related autoinflammatory syndrome (ORAS; also known as Otulipenia). These patients suffer from recurrent fevers, skin rashes, panniculitis, arthritis, and diarrhea, among other symptoms, and have been successfully treated with TNF-blocking therapeutics [114–118], highlighting the essential role of OTULIN in regulating TNF signaling.

In addition to its OTU domain, OTULIN possesses a PIM domain that interacts with the PUB domain in HOIP, and a PDZ-binding motif that interacts with the PDZ-containing protein SNX27 [41,119–121]. The physiological significance of the OTULIN-SNX27 interaction is unclear. Intriguingly, although OTULIN binds to HOIP, only HOIP is readily detected within TNFR1 complex I [43]. Why the CYLD-SPATA2-HOIP complex associates with complex I, but the OTULIN-HOIP complex does not remains unknown. One study that characterized complex I using mass spectrometry detected a small amount of OTULIN [96], raising the possibility that OTULIN is actively excluded from complex I. Cells lacking OTULIN or expressing catalytically inactive OTULIN contain more total M1-linked polyubiquitin than their wild-type counterparts, but have less M1-linked polyubiquitin in complex I [22,43,117]. The latter may stem from decreased expression of LUBAC components and/or reduced recruitment of LUBAC to complex I [22,115,117,122–124]. In some cell types, however, *OTULIN* mutations have less of an impact on LUBAC levels [117,118]. Thus, distinct *OTULIN* mutations and/or cell types may give rise to variable effects on LUBAC levels.

OTULIN bound to HOIP is thought to sustain LUBAC levels by cleaving M1-linked polyubiquitin attached to LUBAC itself [22,43]. Autoubiquitination of LUBAC is mediated by HOIL-1 monoubiquitinating itself, HOIP, and/or SHARPIN [2,125], and then HOIP modifying this monoubiquitin with M1-linked polyubiquitin [125]. The dynamic exchange of LUBAC components between OTULIN-containing complexes and the complexes assembled by receptors such as TNFR1 is poorly understood. Phosphorylation of tyrosine 56 within the OTULIN PIM limits HOIP binding [119,120], and appears to be increased in cells undergoing TNF-induced necroptosis [126], but whether this post-translational modification is crucial for OTULIN- and LUBAC-dependent functions *in vivo* remains to be shown.

By diminishing LUBAC activity, OTULIN deficiency destabilizes TNF-induced complex I and promotes the formation of complex II, leading to increased cell death [22,117,122–124,127]. Homozygous mutations compromising OTULIN DUB activity in mice cause embryonic lethality owing to excessive cell death, particularly among endothelial cells [22,128]. Embryonic lethality is prevented by the combined loss of RIPK3 and caspase-8, although the mice still die perinatally from RIPK1-dependent inflammation [22]. Systemic inactivation of OTULIN in adult mice [22], or *Otulin* deletion in keratinocytes [123,127], leads to severe inflammation that is ameliorated by *Tnfr1* deletion or the combined loss of RIPK3/MLKL-dependent necroptosis and FADD/caspase-8-dependent cell death. In contrast, while *Otulin* deletion in hepatocytes also produces a severe inflammatory phenotype, this is not ameliorated by *Tnf* or *Tnfr1* deletion [122,124], but is improved by *Fadd* deletion [124]. These data are consistent with OTULIN preventing autoinflammation by suppressing aberrant cell death, with intriguing differences observed in the TNF-dependence of these death programs in different cell types.

Biochemical Journal (2022) 479 1103–1119
https://doi.org/10.1042/BCJ20210735

Similar phenotypes have been reported for mice deficient in HOIL-1 [21], further supporting the notion that LUBAC and OTULIN act in concert to favor signal transduction over cell death in TNF signaling.

In humans, loss of function mutations in LUBAC components cause a syndrome characterized variably by systemic autoinflammation, immunodeficiency, and amylopectinosis [129–131]. Thus, while these conditions partially overlap with those of ORAS, they differ in key features as well. Overall, these findings point to a model in which OTULIN and LUBAC act cooperatively in a linear pathway downstream of TNFR1 that favors complex I-mediated signaling over complex II-driven cell death. Functions of OTULIN and LUBAC that may be independent of one another, and their importance in certain cell types, remain an important area of study. The mechanism by which autoubiquitination destabilizes LUBAC and/or alters its activity also awaits elucidation.

OTUB1

OTU deubiquitinase, ubiquitin aldehyde binding 1 (OTUB1) suppresses TNF-induced cell death in a manner that is distinct from A20 and OTULIN [132]. It functions in the signaling complexes of TNF receptor family members, such as Fn14, that recruit cIAP1/2, TRAF2, and TRAF3 (Figure 2). These receptors activate MAPKs and NF-κB, leading to increased expression of many genes, including *Tnf*. Both the canonical and non-canonical NF-κB pathways are stimulated. The non-canonical pathway is mediated by the kinase NIK, which is freed from constitutive cIAP-dependent ubiquitination and degradation when cIAP1/2, TRAF2, and TRAF3 are sequestered by the ligated receptor [27,28,133,134]. cIAP-dependent ubiquitination of the receptor complex eventually culminates in the degradation of cIAP1/2, which sensitizes cells to TNF-induced apoptosis [135,136]. Cell death is tempered by OTUB1 removing the K48-linked polyubiquitin on cIAP1 that marks it for degradation [132]. Accordingly, loss of OTUB1 in certain cell lines exacerbates apoptosis induced by TWEAK, the ligand for Fn14.

Interestingly, loss of OTUB1 in hepatocytes sensitizes mice to intravenous infection with *Listeria monocytogenes* in an MLKL-dependent manner. Thus, exacerbated pathology is due to aberrant necroptosis rather than apoptosis [137]. However, the use of full body *Mlkl* knockout mice makes it unclear if MLKL acts in Kuppfer cells and/or hepatocytes of the infected liver. The role of RIPK3, which is difficult to detect in healthy hepatocytes [138], was not assessed genetically. Restricting deletion of both *Otub1* and *Mlkl* (or *Otub1* and *Ripk3*) to hepatocytes would be informative. Another study suggested that MLKL inhibits *Listeria* replication in epithelial cells without inducing necroptosis [139]. Thus, the mechanisms underlying MLKL-dependent pathology in *Listeria*-infected *Otub1* hepatocyte-specific knockout mice warrant further study.

DUBs regulating pyroptosis

Pyroptosis is a lytic form of cell death mediated by members of the gasdermin family [140]. Gasdermins are intracellular proteins expressed in latent form that promote cell death after their pore-forming domain (PFD) is liberated by proteolytic cleavage. For example, gasdermin D (GSDMD) induces pyroptosis after it is cleaved by human caspases 1, 4, and 5 (mouse caspases 1 and 11) (Figure 3). These caspases get activated when cells are exposed to pathogen-derived molecules (examples include toxins, cytoplasmic lipopolysaccharide (LPS), and cytoplasmic DNA) or sterile insults (examples include uric acid and cholesterol crystals, which are associated with gout and atherosclerosis, respectively) [141,142]. The N-terminal PFD of GSDMD, having been released from its C-terminal inhibitory domain, assembles oligomeric pores in the plasma membrane that disrupt the electrochemical gradient and release small proteins such as IL-1α, IL-1β, and IL-18 [143–147]. Subsequent rupture of the plasma membrane through the ill-defined activity of membrane protein NINJ1 then allows larger intracellular components, including lactate dehydrogenase (LDH), to escape the dying cell [148]. In other contexts, caspase-8, neutrophil elastase or cathepsin G may cleave GSDMD to unleash pyroptosis [29,149,150].

Caspase 1 is activated within canonical inflammasome complexes whose makeup is governed by the nature of the cellular insult. The NLPR3-ASC inflammasome activates caspase-1-dependent pyroptosis in response to diverse cellular perturbations, including extracellular ATP, bacterial toxin nigericin, non-canonical caspase-11-dependent pyroptosis, and RIPK3-dependent cell death [141,151–153]. In many cell types, including mouse macrophages and hepatocytes, optimal activation of the NLRP3 inflammasome relies on transcriptional up-regulation of *Nlrp3* gene expression by NF-κB [154,155]. This priming step is satisfied in culture by treatment with TLR agonists, including LPS. Loss of WDR48 (also called UAF1), a cofactor that stimulates the

Biochemical Journal (2022) **479** 1103–1119
https://doi.org/10.1042/BCJ20210735

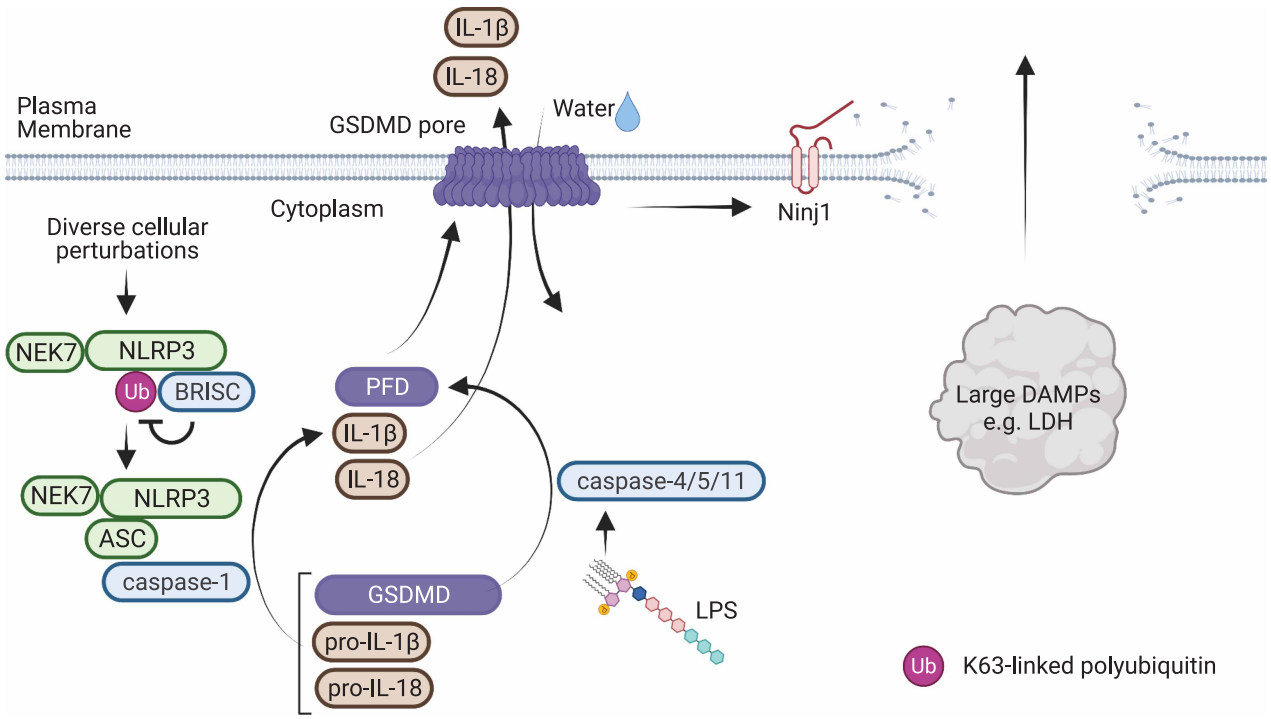

Figure 3. Deubiquitinase BRISC promotes pyroptosis induced by the NLRP3 inflammasome.

Assembly of the NLRP3 inflammasome is triggered by a range of cellular insults. BRISC interacts with NLRP3 in complex with NEK7 and removes K63-linked polyubiquitin from NLRP3. Deubiquitination of NLRP3 is required for NLRP3 to interact with ASC, the adaptor for caspase-1. Activation of caspase-1 within the NLRP3-nucleated complex results in cleavage of its substrates pro-IL-1β and pro-IL-18, yielding the biologically active cytokines. Caspase-1 also cleaves GSDMD, producing an N-terminal fragment that assembles oligomeric pores in the plasma membrane capable of releasing IL-1β and IL-18. GSDMD pores also disrupt the electrochemical gradient and kill the cell. Pyroptosis culminates in large scale rupture of the plasma membrane, which is mediated by the membrane protein NINJ1. Precisely how NINJ1 triggers membrane breakdown and the release larger intracellular proteins such as lactate dehydrogenase (LDH) is unclear. Other proteases that can elicit the pore-forming fragment of GSDMD include human caspase-4 (mouse counterpart caspase-11) and caspase-5, which are activated by cytosolic lipopolysaccharide (LPS).
Figure created with BioRender.com.

DUB activity of USP1, USP12, and USP46 [156–158], impairs LPS-induced up-regulation of NLRP3 in mouse macrophages [159]. Therefore, WDR48-associated DUBs may facilitate pyroptosis indirectly.

BRISC

The K63 linkage-specific DUB BRCC3 (also called BRCC36) [160] promotes activation of the NLRP3 inflammasome by deubiquitinating NLRP3 [161,162] (Figure 3). Belonging to the JAMM DUB family, BRCC3 may cleave K63-linked polyubiquitin that is conjugated to NLRP3 by the ubiquitin ligase RNF125 [163]. BRCC3 is part of the BRCC3 isopeptidase complex (BRISC), wherein the activity of BRCC3 is dependent on interactions with the pseudoDUB ABRAXAS2 (also called KIAA0157 and ABRO1) [164]. Accordingly, ABRAXAS2-deficient mouse macrophages phenocopy BRCC3-deficent macrophages and exhibit impaired NLRP3-dependent processing of caspase-1, despite evidence of normal priming [162]. Aberrant ubiquitination of NLRP3 in ABRAXAS2-deficient cells appears to limit interactions between NLRP3 and ASC, rather than target NLRP3 for degradation.

Biochemical experiments suggest that ABRAXAS2 and BRCC3 associate with NLRP3 after priming. This interaction requires phosphorylation of NLRP3 serine 194 and the NLRP3-interactor NEK7. However, ABRAXAS2- and BRCC3-dependent deubiquitination of NLRP3 also requires an NLRP3 activation stimulus [162,165]. Thus, priming is proposed to recruit BRISC to NLRP3 so that it is poised to deubiquitinate NLRP3 upon receipt of an activation stimulus [162]. Activating stimuli may induce conformational changes in NLRP3 that facilitate its deubiquitination by BRISC. The autoactivating mutant NLRP3 A350V (equivalent to human

Biochemical Journal (2022) 479 1103–1119
https://doi.org/10.1042/BCJ20210735

Muckle–Wells syndrome mutant NLRP3 A352V) is deubiquitinated in macrophages after priming alone [162], consistent with this mutation destabilizing the inactive conformation of NLRP3 [166].

Interestingly, BRISC is bound and inhibited by the inactive form of the metabolic enzyme serine hydroxymethyltransferase 2 (SHMT2) [167,168], suggesting a connection between metabolism and deubiquitination of BRISC substrates. Contrary to what might be expected, however, interactions between SHMT2 and BRISC are required for the latter to deubiquitinate type I interferon (IFN) receptor IFNAR1 at the cell surface [167,169]. Deubiquitination of IFNAR1 limits internalization and lysosomal degradation of the receptor, and thereby promotes IFN signaling. It was suggested that SHMT2 involvement in substrate targeting combined with its reversible inhibition of BRISC, perhaps through its displacement by K63-linked polyubiquitin on BRISC substrates, might prevent non-specific BRISC DUB activity [167]. Deubiquitination of the HIV-1 Tat protein by BRISC has also been shown to require SHMT1 or SHMT2 [170]. Whether deubiquitination of NLRP3 by BRISC is dependent on SHMT enzymes has not been investigated.

Small molecule inhibitors of JAMM DUBs, thiolutin and holomycin, limit pyroptosis and inflammation induced by both wild-type and autoactivating NLRP3 mutants, in large part by inhibiting BRCC3 [171]. Therefore, inhibition of BRISC with more specific inhibitors may represent an alternative strategy to NLRP3 inhibitors for the treatment of NLRP3-driven diseases. However, effects on other BRISC substrates, including IFNAR1 [169] and JAK2 [172] must be considered. Further complicating matters, BRCC3 has been implicated in oligodendrocyte differentiation [173], plus BRCC3 and some of the other BRISC components also function in the nuclear BRCA1-A complex involved in DNA repair [168].

Other DUBs

In contrast with BRCC3, CYLD and its binding partner SPATA2 suppress activation of the NLRP3 inflammasome [174]. Mechanistically, it was suggested that CYLD deubiquitinates centrosomal PLK4, leading to interactions between NEK7 and PLK4 at the centrosome that interfere with inflammasome assembly by preventing interactions between NEK7 and NLRP3. NEK7 serves as a scaffold bridging adjacent NLRP3 subunits [166]. A20 also suppresses activation of the NLRP3 inflammasome [175]. Eliminating A20 from mouse macrophages causes arthritis that involves NLRP3, ASC, IL-1R, RIPK3, and MLKL [76,176]. RIPK3-dependent cell death can activate the NLRP3 inflammasome [152,153]. Therefore, aberrant necroptosis of A20-deficient macrophages is thought to activate the NLRP3-ASC-caspase-1 inflammasome to processes pro-IL-1β into biologically active IL-1β [76]. Cleavage of GSDMD by caspase-1 and subsequent pyroptosis are probably dispensable because MLKL-dependent necroptosis suffices to release proinflammatory IL-1α and IL-1β [76].

DUBs regulating activation of the other inflammasomes have been described, but the details are still emerging. For example, USP21 was shown to deubiquitinate and stabilize AIM2 that is activated by cytosolic double-stranded DNA [177], whereas CYLD may remove K63-linked polyubiquitin from NLRP6 to suppress inflammasome activation in mice infected with *Citrobacter rodentium* [178].

DUBs regulating intrinsic apoptosis

The intrinsic apoptosis pathway is triggered by diverse cellular insults, including DNA damage, oncogene activation, and survival factor withdrawal. The pathway is regulated by members of the BCL-2 protein family, which feature up to four BCL-2 homology domains (BH1-4). Death is unleashed when the BH3-only proteins (BAD, BID, BIK, BIM, BMF, HRK, NOXA, or PUMA) are up-regulated. BH3-only proteins bind to a selection of their pro-survival relatives (BCL-2, BCL-2 related protein A1, BCL-W, BCL-X_L, or MCL-1) and prevent them from sequestering the pro-apoptotic effectors BAK and BAX (Figure 4). Certain BH3-only proteins may also activate BAK and BAX directly. Oligomerization of BAX and BAK leads to permeabilization of the outer mitochondrial membrane and cytochrome c is released into the cytoplasm. Interactions between cytochrome c and cytoplasmic APAF1 lead to assembly of the apoptosome complex that activates caspase-9, the apical caspase in a proteolytic cascade that dismantles the cell (reviewed by [179,180]). Another pro-apoptotic effector, BOK, appears to disrupt mitochondria and trigger apoptosis only when it escapes ubiquitin-dependent proteasomal degradation mediated by the gp78 ubiquitin ligase complex [181]. Whether there is a DUB that can reverse the constitutive ubiquitination of BOK is unclear.

MCL-1 is the most labile of the pro-survival proteins, being modified with K48-linked polyubiquitin and targeted for proteasomal degradation by several ubiquitin ligases, including HUWE1 (also called MULE) [182], SCF^{FBW7} [183,184], and MARCH5 [185]. Different ligases appear to act in different contexts. For example, MARCH5 drives degradation of MCL-1 that is bound to NOXA [186,187], HUWE1 promotes degradation of

Biochemical Journal (2022) **479** 1103–1119
https://doi.org/10.1042/BCJ20210735

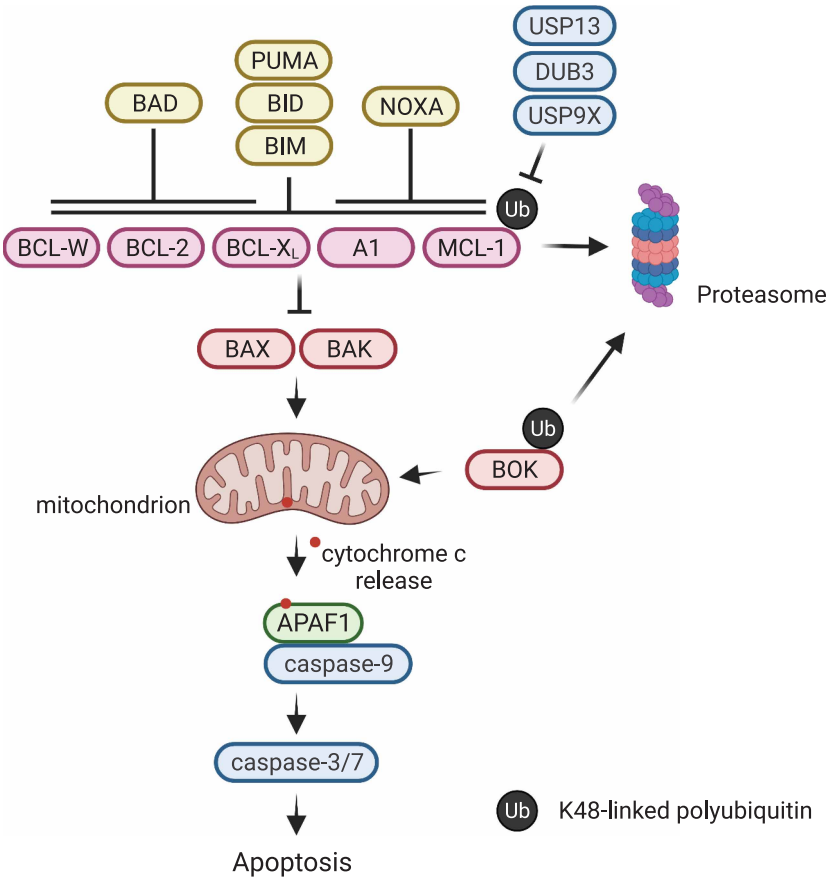

Figure 4. Deubiquitinases USP9X, DUB3, and USP13 promote cell survival by stabilizing MCL-1.
Activation of the intrinsic apoptosis pathway is regulated by members of the BCL2 protein family. The BH3-only pro-apoptotic proteins (colored yellow) bind to and neutralize their pro-survival relatives (colored pink), thereby unleashing the pro-apoptotic effectors BAX and BAK (colored red) to permeabilize the mitochondrial outer membrane. Some BH3-only proteins may also engage BAX and BAK directly. Mitochondrial cytochrome c is released into the cytoplasm, triggering assembly of an APAF1 complex that activates caspase-9. The executioner caspases 3 and 7 are cleaved and activated by caspase-9 resulting in the highly orchestrated proteolytic events that dismantle the cell. Deubiquitinases, including USP9X, DUB3, and USP13, promote cell survival by cleaving K48-linked polyubiquitin from MCL-1 that would otherwise target it for proteasomal degradation. The pro-apoptotic effector BOK is unstable owing to its constitutive ubiquitination. A DUB that removes ubiquitin from BOK has not been described. Figure created with BioRender.com.

MCL-1 in response to DNA damage [182], and SCF^{FBW7} instigates degradation of MCL-1 by antitubulin chemotherapeutics [184]. DUBs shown to enhance cell survival by deubiquitinating MCL-1 and limiting its turnover include USP9X, DUB3 (also called USP17L2), and USP13 [188–190]. Elevated expression of these DUBs correlates with elevated expression of MCL-1 protein in certain patient tumors [188–190]. Thus, aberrantly high expression of DUBs that can stabilize MCL-1 may contribute to tumor development and resistance to chemotherapy. Interactions between MCL-1 and its DUBs may be regulated. For example, phosphorylation of MCL-1 following DNA damage was shown to limit interactions between MCL-1 and USP9X [188].

USP9X is essential for mouse embryogenesis [191–194], but whether MCL-1 instability and activation of the intrinsic apoptosis pathway contributes to lethality in USP9X-deficient embryos is unclear. USP9X has other substrates besides MCL-1 that are essential for normal embryogenesis, including PEG10 and polycomb repressive complex 2 (PRC2) components SUZ12 and EZH2 [194,195]. DUB3 and USP13 also have other substrates that regulate diverse cellular processes [196–198].

BAP1 is a UCH family DUB that suppresses intrinsic apoptosis in some mouse cell types by promoting the expression of *Mcl1* and *Bcl2* [199]. In complex with ASXL1, ASXL2, or ASXL3, BAP1 removes monoubiquitin

Biochemical Journal (2022) 479 1103–1119
https://doi.org/10.1042/BCJ20210735

from histone H2A lysine 119 and thereby counters transcriptional repression mediated by ubiquitin ligases RNF2 and RING1 of the polycomb repressive complex 1 (PRC1) [199–203]. There is considerable interest in understanding gene regulation by BAP1 because it is a potent tumor suppressor. Humans with an inactivating germline mutation in *BAP1* are predisposed to cancer, especially uveal melanoma and mesothelioma (reviewed by [204]). The transcriptional changes that promote tumor development upon loss or inactivation of the other *BAP1* allele remain unclear.

USP7 (also called HAUSP) is another DUB that suppresses intrinsic apoptosis indirectly. In unstressed cells, USP7 deubiquitinates the ubiquitin ligase HDM2 (or its mouse counterpart MDM2) to limit proteasomal degradation of HDM2 and its binding partner HDMX (MDMX in mice) (reviewed by [205]). Stabilization of the HDM2-HDMX ligase promotes ubiquitination and proteasomal degradation of the tumor suppressor and transcription factor p53, whose target genes include *BBC3* (encoding PUMA), *PMAIP1* (encoding NOXA), and *BAX* [206]. Thus, one role of USP7 in healthy cells is to limit p53-dependent expression of pro-apoptotic BCL-2 family members. After DNA damage, however, phosphorylation of the HDM2-HDMX ligase disrupts interactions between HDM2 and USP7, leading to degradation of the ligase and activation of p53-dependent transcription [205]. This regulatory mechanism is reminiscent of how USP9X activity towards MCL-1 is disrupted after DNA damage. Disabling the pro-survival roles of DUBs in stressed cells makes sense because it favors activation of the intrinsic apoptosis pathway if the cells are damaged beyond repair. USP7, like most of the DUBs reviewed here, can deubiquitinate several substrates. For example, it can also deubiquitinate histone H2B [207] and N-MYC [208]. Accordingly, USP7 deficiency in mice has both p53-dependent and p53-independent consequences [209,210].

In contrast with USP7, OTUB1 can stabilize p53 in cells, but this activity does not require its catalytic activity [211]. OTUB1 may instead interfere with ubiquitination of p53 by inhibiting the E2 enzyme UbcH5. Other DUBs implicated in the deubiquitination and stabilization of p53 include USP10 [212] and the MJD DUB Ataxin-3 [213].

Conclusions and future directions

DUBs cleaving either monoubiquitin or polyubiquitin can modulate the cell death machinery directly (for example, USP9X and BRISC) or indirectly (for example, OTULIN and BAP1) by controlling the abundance, conformation, and/or interactions of key cell death proteins. Although this review has discussed some of the DUBs regulating cell death signaling, there are ubiquitination events in these pathways where the ubiquitin ligases and DUBs have yet to be identified. For example, the enzymes controlling ubiquitination of MLKL to either limit [214] or promote necroptosis [215] remain unknown. The mechanisms regulating DUB-substrate interactions are also an area of interest. Relatively little is known about the regulation of DUBs such as OTULIN and BAP1. Finally, most of the DUBs reviewed here do not target a single protein or cell death signaling alone, but have multiple substrates involved in diverse biological processes. Thus, much remains to be uncovered in exploring the therapeutic potential of targeting DUBs to manipulate cell death signaling.

Competing Interests

KN is an employee of Genentech. ADG is a visiting scientist at Genentech.

Abbreviations

BH1-4, four BCL-2 homology domains; BRISC, BRCC3 isopeptidase complex; DD, death domain; DUBs, Deubiquitinases; GSDMD, gasdermin D; IFN, interferon; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; LUBAC, linear ubiquitin chain assembly complex; MJD, Machado–Josephin domain; ORAS, OTULIN-related autoinflammatory syndrome; OTUB1, OTU deubiquitinase, ubiquitin aldehyde binding 1; PFD, pore-forming domain; PIM, PUB domain-interacting motif; SHMT2, serine hydroxymethyltransferase 2; TLR3, Toll-like Receptor 3; TNFR1, Tumor Necrosis Factor Receptor 1; UCH, ubiquitin C-terminal hydrolase; USP, ubiquitin-specific protease.

References

1 Rape, M. (2018) Ubiquitylation at the crossroads of development and disease. *Nat. Rev. Mol. Cell Biol.* **19**, 59–70 https://doi.org/10.1038/nrm.2017.83
2 Kelsall, I.R., Zhang, J., Knebel, A., Arthur, J.S.C. and Cohen, P. (2019) The E3 ligase HOIL-1 catalyses ester bond formation between ubiquitin and components of the myddosome in mammalian cells. *Proc. Natl Acad. Sci. U.S.A.* **116**, 13293–13298 https://doi.org/10.1073/pnas.1905873116

3 Rodriguez Carvajal, A., Grishkovskaya, I., Gomez Diaz, C., Vogel, A., Sonn-Segev, A., Kushwah, M.S. et al. (2021) The linear ubiquitin chain assembly complex (LUBAC) generates heterotypic ubiquitin chains. *eLife* **10**, e60660 https://doi.org/10.7554/eLife.60660
4 Harrigan, J.A., Jacq, X., Martin, N.M. and Jackson, S.P. (2018) Deubiquitylating enzymes and drug discovery: emerging opportunities. *Nat. Rev. Drug Discov.* **17**, 57–78 https://doi.org/10.1038/nrd.2017.152
5 Rothe, J., Lesslauer, W., Lotscher, H., Lang, Y., Koebel, P., Kontgen, F. et al. (1993) Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. *Nature* **364**, 798–802 https://doi.org/10.1038/364798a0
6 Alexopoulou, L., Holt, A.C., Medzhitov, R. and Flavell, R.A. (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* **413**, 732–738 https://doi.org/10.1038/35099560
7 Poltorak, A., He, X., Smirnova, I., Liu, M.Y., Van Huffel, C., Du, X. et al. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* **282**, 2085–2088 https://doi.org/10.1126/science.282.5396.2085
8 Aggarwal, B.B., Eessalu, T.E. and Hass, P.E. (1985) Characterization of receptors for human tumour necrosis factor and their regulation by gamma-interferon. *Nature* **318**, 665–667 https://doi.org/10.1038/318665a0
9 Etemadi, N., Holien, J.K., Chau, D., Dewson, G., Murphy, J.M., Alexander, W.S. et al. (2013) Lymphotoxin alpha induces apoptosis, necroptosis and inflammatory signals with the same potency as tumour necrosis factor. *FEBS J.* **280**, 5283–5297 https://doi.org/10.1111/febs.12419
10 Haas, T.L., Emmerich, C.H., Gerlach, B., Schmukle, A.C., Cordier, S.M., Rieser, E. 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 https://doi.org/10.1016/j.molcel.2009.10.013
11 Zinngrebe, J., Rieser, E., Taraborrelli, L., Peltzer, N., Hartwig, T., Ren, H. et al. (2016) LUBAC deficiency perturbs TLR3 signaling to cause immunodeficiency and autoinflammation. *J. Exp. Med.* **213**, 2671–2689 https://doi.org/10.1084/jem.20160041
12 Sasaki, Y. and Iwai, K. (2018) Crucial role of linear ubiquitin chain assembly complex-mediated inhibition of programmed cell death in TLR4-mediated B cell responses and B1b cell development. *J. Immunol.* **200**, 3438–3449 https://doi.org/10.4049/jimmunol.1701526
13 Kanayama, A., Seth, R.B., Sun, L., Ea, C.K., Hong, M., Shaito, A. et al. (2004) TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol. Cell* **15**, 535–448 https://doi.org/10.1016/j.molcel.2004.08.008
14 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 https://doi.org/10.1016/j.molcel.2006.03.026
15 Emmerich, C.H., Ordureau, A., Strickson, S., Arthur, J.S., Pedrioli, P.G., Komander, D. et al. (2013) Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *Proc. Natl Acad. Sci. U.S.A.* **110**, 15247–15252 https://doi.org/10.1073/pnas.1314715110
16 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 https://doi.org/10.1016/j.bbrc.2016.04.141
17 Lafont, E., Draber, P., Rieser, E., Reichert, M., Kupka, S., de Miguel, D. et al. (2018) TBK1 and IKKepsilon prevent TNF-induced cell death by RIPK1 phosphorylation. *Nat. Cell Biol.* **20**, 1389–1399 https://doi.org/10.1038/s41556-018-0229-6
18 Rickard, J.A., Anderton, H., Etemadi, N., Nachbur, U., Darding, M., Peltzer, N. et al. (2014) TNFR1-dependent cell death drives inflammation in sharpin-deficient mice. *eLife* **3**, e03464 https://doi.org/10.7554/eLife.03464
19 Kumari, S., Redouane, Y., Lopez-Mosqueda, J., Shiraishi, R., Romanowska, M., Lutzmayer, S. et al. (2014) Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. *eLife* **3**, e03422 https://doi.org/10.7554/eLife.03422
20 Dondelinger, Y., Jouan-Lanhouet, S., Divert, T., Theatre, E., Bertin, J., Gough, P.J. et al. (2015) NF-kappaB-independent role of IKKalpha/IKKbeta in preventing RIPK1 kinase-dependent apoptotic and necroptotic cell death during TNF signaling. *Mol. Cell* **60**, 63–76 https://doi.org/10.1016/j.molcel.2015.07.032
21 Peltzer, N., Darding, M., Montinaro, A., Draber, P., Draberova, H., Kupka, S. et al. (2018) LUBAC is essential for embryogenesis by preventing cell death and enabling haematopoiesis. *Nature* **557**, 112–117 https://doi.org/10.1038/s41586-018-0064-8
22 Heger, K., Wickliffe, K.E., Ndoja, A., Zhang, J., Murthy, A., Dugger, D.L. et al. (2018) OTULIN limits cell death and inflammation by deubiquitinating LUBAC. *Nature* **559**, 120–124 https://doi.org/10.1038/s41586-018-0256-2
23 Xu, D., Jin, T., Zhu, H., Chen, H., Ofengeim, D., Zou, C. et al. (2018) TBK1 suppresses RIPK1-driven apoptosis and inflammation during development and in aging. *Cell* **174**, 1477–1491 https://doi.org/10.1016/j.cell.2018.07.041
24 Tang, Y., Tu, H., Zhang, J., Zhao, X., Wang, Y., Qin, J. et al. (2019) K63-linked ubiquitination regulates RIPK1 kinase activity to prevent cell death during embryogenesis and inflammation. *Nat. Commun.* **10**, 4157 https://doi.org/10.1038/s41467-019-12033-8
25 Zhang, X., Zhang, H., Xu, C., Li, X., Li, M., Wu, X. et al. (2019) Ubiquitination of RIPK1 suppresses programmed cell death by regulating RIPK1 kinase activation during embryogenesis. *Nat. Commun.* **10**, 4158 https://doi.org/10.1038/s41467-019-11839-w
26 Kist, M., Komuves, L.G., Goncharov, T., Dugger, D.L., Yu, C., Roose-Girma, M. et al. (2021) Impaired RIPK1 ubiquitination sensitizes mice to TNF toxicity and inflammatory cell death. *Cell Death Differ.* **28**, 985–1000 https://doi.org/10.1038/s41418-020-00629-3
27 Vince, J.E., Wong, W.W., Khan, N., Feltham, R., Chau, D., Ahmed, A.U. et al. (2007) IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. *Cell* **131**, 682–693 https://doi.org/10.1016/j.cell.2007.10.037
28 Varfolomeev, E., Blankenship, J.W., Wayson, S.M., Fedorova, A.V., Kayagaki, N., Garg, P. et al. (2007) IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell* **131**, 669–681 https://doi.org/10.1016/j.cell.2007.10.030
29 Orning, P., Weng, D., Starheim, K., Ratner, D., Best, Z., Lee, B. et al. (2018) Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. *Science* **362**, 1064–1069 https://doi.org/10.1126/science.aau2818
30 Newton, K. (2020) Multitasking kinase RIPK1 regulates cell death and inflammation. *Cold Spring Harb. Perspect. Biol.* **12**, a036368 https://doi.org/10.1101/cshperspect.a036368
31 Micheau, O. and Tschopp, J. (2003) Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* **114**, 181–190 https://doi.org/10.1016/S0092-8674(03)00521-X
32 Shu, H.B., Takeuchi, M. and Goeddel, D.V. (1996) The tumor necrosis factor receptor 2 signal transducers TRAF2 and c-IAP1 are components of the tumor necrosis factor receptor 1 signaling complex. *Proc. Natl Acad. Sci. U.S.A.* **93**, 13973–13978 https://doi.org/10.1073/pnas.93.24.13973
33 Hsu, H., Huang, J., Shu, H.B., Baichwal, V. and Goeddel, D.V. (1996) TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. *Immunity* **4**, 387–396 https://doi.org/10.1016/S1074-7613(00)80252-6

34 Hsu, H., Shu, H.B., Pan, M.G. and Goeddel, D.V. (1996) TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* **84**, 299–308 https://doi.org/10.1016/S0092-8674(00)80984-8
35 Ermolaeva, M.A., Michallet, M.C., Papadopoulou, N., Utermohlen, O., Kranidioti, K., Kollias, G. et al. (2008) Function of TRADD in tumor necrosis factor receptor 1 signaling and in TRIF-dependent inflammatory responses. *Nat. Immunol.* **9**, 1037–1046 https://doi.org/10.1038/ni.1638
36 Pobezinskaya, Y.L., Kim, Y.S., Choksi, S., Morgan, M.J., Li, T., Liu, C. et al. (2008) The function of TRADD in signaling through tumor necrosis factor receptor 1 and TRIF-dependent toll-like receptors. *Nat. Immunol.* **9**, 1047–1054 https://doi.org/10.1038/ni.1639
37 Vince, J.E., Pantaki, D., Feltham, R., Mace, P.D., Cordier, S.M., Schmukle, A.C. et al. (2009) TRAF2 must bind to cellular inhibitors of apoptosis for tumor necrosis factor (tnf) to efficiently activate nf-{kappa}b and to prevent tnf-induced apoptosis. *J. Biol. Chem.* **284**, 35906–35915 https://doi.org/10.1074/jbc.M109.072256
38 Cheung, P.C., Nebreda, A.R. and Cohen, P. (2004) TAB3, a new binding partner of the protein kinase TAK1. *Biochem. J.* **378**, 27–34 https://doi.org/10.1042/bj20031794
39 Ikeda, F., Deribe, Y.L., Skanland, S.S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M. et al. (2011) SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* **471**, 637–641 https://doi.org/10.1038/nature09814
40 Gerlach, B., Cordier, S.M., Schmukle, A.C., Emmerich, C.H., Rieser, E., Haas, T.L. et al. (2011) Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591–596 https://doi.org/10.1038/nature09816
41 Keusekotten, K., Elliott, P.R., Glockner, L., Fiil, B.K., Damgaard, R.B., Kulathu, Y. et al. (2013) OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell* **153**, 1312–1326 https://doi.org/10.1016/j.cell.2013.05.014
42 Fiil, B.K., Damgaard, R.B., Wagner, S.A., Keusekotten, K., Fritsch, M., Bekker-Jensen, S. et al. (2013) OTULIN restricts Met1-linked ubiquitination to control innate immune signaling. *Mol. Cell* **50**, 818–830 https://doi.org/10.1016/j.molcel.2013.06.004
43 Draber, P., Kupka, S., Reichert, M., Draberova, H., Lafont, E., de Miguel, D. 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 https://doi.org/10.1016/j.celrep.2015.11.009
44 Wu, C.J., Conze, D.B., Li, T., Srinivasula, S.M. and Ashwell, J.D. (2006) Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation. *Nat. Cell Biol.* **8**, 398–406 https://doi.org/10.1038/ncb1384
45 Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., Kato, R. et al. (2009) Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* **136**, 1098–1109 https://doi.org/10.1016/j.cell.2009.03.007
46 Zhang, J., Clark, K., Lawrence, T., Peggie, 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 https://doi.org/10.1042/BJ20140444
47 Oberst, A., Dillon, C.P., Weinlich, R., McCormick, L.L., Fitzgerald, P., Pop, C. et al. (2011) Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature* **471**, 363–367 https://doi.org/10.1038/nature09852
48 Newton, K., Wickliffe, K.E., Dugger, D.L., Maltzman, A., Roose-Girma, M., Dohse, M. et al. (2019) Cleavage of RIPK1 by caspase-8 is crucial for limiting apoptosis and necroptosis. *Nature* **574**, 428–431 https://doi.org/10.1038/s41586-019-1548-x
49 Lalaoui, N., Boyden, S.E., Oda, H., Wood, G.M., Stone, D.L., Chau, D. et al. (2020) Mutations that prevent caspase cleavage of RIPK1 cause autoinflammatory disease. *Nature* **577**, 103–108 https://doi.org/10.1038/s41586-019-1828-5
50 Tao, P., Sun, J., Wu, Z., Wang, S., Wang, J., Li, W. et al. (2020) A dominant autoinflammatory disease caused by non-cleavable variants of RIPK1. *Nature* **577**, 109–114 https://doi.org/10.1038/s41586-019-1830-y
51 Lakhani, S.A., Masud, A., Kuida, K., Porter, Jr, G.A., Booth, C.J., Mehal, W.Z. et al. (2006) Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. *Science* **311**, 847–851 https://doi.org/10.1126/science.1115035
52 Oberst, A., Pop, C., Tremblay, A.G., Blais, V., Denault, J.B., Salvesen, G.S. et al. (2010) Inducible dimerization and inducible cleavage reveal a requirement for both processes in caspase-8 activation. *J. Biol. Chem.* **285**, 16632–16642 https://doi.org/10.1074/jbc.M109.095083
53 Fox, J.L., Hughes, M.A., Meng, X., Sarnowska, N.A., Powley, I.R., Jukes-Jones, R. et al. (2021) Cryo-EM structural analysis of FADD:Caspase-8 complexes defines the catalytic dimer architecture for co-ordinated control of cell fate. *Nat. Commun.* **12**, 819 https://doi.org/10.1038/s41467-020-20806-9
54 Cho, Y.S., Challa, S., Moquin, D., Genga, R., Ray, T.D., Guildford, M. et al. (2009) Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell* **137**, 1112–1123 https://doi.org/10.1016/j.cell.2009.05.037
55 He, S., Wang, L., Miao, L., Wang, T., Du, F., Zhao, L. et al. (2009) Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell* **137**, 1100–1111 https://doi.org/10.1016/j.cell.2009.05.021
56 Zhang, D.W., Shao, J., Lin, J., Zhang, N., Lu, B.J., Lin, S.C. et al. (2009) RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* **325**, 332–336 https://doi.org/10.1126/science.1172308
57 Degterev, A., Huang, Z., Boyce, M., Li, Y., Jagtap, P., Mizushima, N. et al. (2005) Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat. Chem. Biol.* **1**, 112–119 https://doi.org/10.1038/nchembio711
58 Sun, L., Wang, H., Wang, Z., He, S., Chen, S., Liao, D. et al. (2012) Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell* **148**, 213–227 https://doi.org/10.1016/j.cell.2011.11.031
59 Zhao, J., Jitkaew, S., Cai, Z., Choksi, S., Li, Q., Luo, J. et al. (2012) Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proc. Natl Acad. Sci. U.S.A.* **109**, 5322–5327 https://doi.org/10.1073/pnas.1200012109
60 Murphy, J.M., Czabotar, P.E., Hildebrand, J.M., Lucet, I.S., Zhang, J.G., Alvarez-Diaz, S. et al. (2013) The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity* **39**, 443–453 https://doi.org/10.1016/j.immuni.2013.06.018
61 Wu, J., Huang, Z., Ren, J., Zhang, Z., He, P., Li, Y. et al. (2013) Mlkl knockout mice demonstrate the indispensable role of Mlkl in necroptosis. *Cell Res.* **23**, 994–1006 https://doi.org/10.1038/cr.2013.91
62 He, S., Liang, Y., Shao, F. and Wang, X. (2011) Toll-like receptors activate programmed necrosis in macrophages through a receptor-interacting kinase-3-mediated pathway. *Proc. Natl Acad. Sci. U.S.A.* **108**, 20054–20059 https://doi.org/10.1073/pnas.1116302108
63 Kaiser, W.J., Sridharan, H., Huang, C., Mandal, P., Upton, J.W., Gough, P.J. et al. (2013) Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. *J. Biol. Chem.* **288**, 31268–31279 https://doi.org/10.1074/jbc.M113.462341
64 Opipari AW, J., Hu, H.M., Yabkowitz, R. and Dixit, V.M. (1992) The A20 zinc finger protein protects cells from tumor necrosis factor cytotoxicity. *J. Biol. Chem.* **267**, 12424–12427 https://doi.org/10.1016/S0021-9258(18)42292-2

65 Lee, E.G. Boone, D.L., Chai, S., Libby, S.L., Chien, M., Lodolce, J.P. et al. (2000) Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* **289**, 2350–2354 https://doi.org/10.1126/science.289.5488.2350
66 Priem, D., Devos, M., Druwe, S., Martens, A., Slowicka, K., Ting, A.T. et al. (2019) A20 protects cells from TNF-induced apoptosis through linear ubiquitin-dependent and -independent mechanisms. *Cell Death Dis.* **10**, 692 https://doi.org/10.1038/s41419-019-1937-y
67 Vanlangenakker, N., Bertrand, M.J., Bogaert, P., Vandenabeele, P. and Vanden Berghe, T. (2011) TNF-induced necroptosis in L929 cells is tightly regulated by multiple TNFR1 complex I and II members. *Cell Death Dis.* **2**, e230 https://doi.org/10.1038/cddis.2011.111
68 Onizawa, M., Oshima, S., Schulze-Topphoff, U., Oses-Prieto, J.A., Lu, T., Tavares, R. et al. (2015) The ubiquitin-modifying enzyme A20 restricts ubiquitination of the kinase RIPK3 and protects cells from necroptosis. *Nat. Immunol.* **16**, 618–627 https://doi.org/10.1038/ni.3172
69 Vereecke, L., Sze, M., Mc Guire, C., Rogiers, B., Chu, Y., Schmidt-Supprian, M. et al. (2010) Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor-induced toxicity and experimental colitis. *J. Exp. Med.* **207**, 1513–1523 https://doi.org/10.1084/jem.20092474
70 Patel, S., Webster, J.D., Varfolomeev, E., Kwon, Y.C., Cheng, J.H., Zhang, J. et al. (2020) RIP1 inhibition blocks inflammatory diseases but not tumor growth or metastases. *Cell Death Differ.* **27**, 161–175 https://doi.org/10.1038/s41418-019-0347-0
71 Garcia-Carbonell, R., Wong, J., Kim, J.Y., Close, L.A., Boland, B.S., Wong, T.L. et al. (2018) Elevated A20 promotes TNF-induced and RIPK1-dependent intestinal epithelial cell death. *Proc. Natl Acad. Sci. U.S.A.* **115**, E9192–E9200 https://doi.org/10.1073/pnas.1810584115
72 Krikos, A., Laherty, C.D. and Dixit, V.M. (1992) Transcriptional activation of the tumor necrosis factor alpha-inducible zinc finger protein, A20, is mediated by kappa B elements. *J. Biol. Chem.* **267**, 17971–17976 https://doi.org/10.1016/S0021-9258(19)37138-8
73 Tokunaga, F., Nishimasu, H., Ishitani, R., Goto, E., Noguchi, T., Mio, K. et al. (2012) Specific recognition of linear polyubiquitin by A20 zinc finger 7 is involved in NF-kappaB regulation. *EMBO J.* **31**, 3856–3870 https://doi.org/10.1038/emboj.2012.241
74 Verhelst, K., Carpentier, I., Kreike, M., Meloni, L., Verstrepen, L., Kensche, T. et al. (2012) A20 inhibits LUBAC-mediated NF-kappaB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J.* **31**, 3845–3855 https://doi.org/10.1038/emboj.2012.240
75 Lu, T.T., Onizawa, M., Hammer, G.E., Turer, E.E., Yin, Q., Damko, E. et al. (2013) Dimerization and ubiquitin mediated recruitment of A20, a complex deubiquitinating enzyme. *Immunity* **38**, 896–905 https://doi.org/10.1016/j.immuni.2013.03.008
76 Polykratis, A., Martens, A., Eren, R.O., Shirasaki, Y., Yamagishi, M., Yamaguchi, Y. et al. (2019) A20 prevents inflammasome-dependent arthritis by inhibiting macrophage necroptosis through its ZnF7 ubiquitin-binding domain. *Nat. Cell Biol.* **21**, 731–742 https://doi.org/10.1038/s41556-019-0324-3
77 Martens, A., Priem, D., Hoste, E., Vetters, J., Rennen, S., Catrysse, L. et al. (2020) Two distinct ubiquitin-binding motifs in A20 mediate its anti-inflammatory and cell-protective activities. *Nat. Immunol.* **21**, 381–387 https://doi.org/10.1038/s41590-020-0621-9
78 Razani, B., Whang, M.I., Kim, F.S., Nakamura, M.C., Sun, X., Advincula, R. et al. (2020) Non-catalytic ubiquitin binding by A20 prevents psoriatic arthritis-like disease and inflammation. *Nat. Immunol.* **21**, 422–433 https://doi.org/10.1038/s41590-020-0634-4
79 Bosanac, I., Wertz, I.E. Pan, B., Yu, C., Kusam, S., Lam, C. et al. (2010) Ubiquitin binding to A20 ZnF4 is required for modulation of NF-kappaB signaling. *Mol. Cell* **40**, 548–557 https://doi.org/10.1016/j.molcel.2010.10.009
80 Dziedzic, S.A., Su, Z., Jean Barrett, V., Najafov, A., Mookhtiar, A.K., Amin, P. et al. (2018) ABIN-1 regulates RIPK1 activation by linking Met1 ubiquitylation with Lys63 deubiquitylation in TNF-RSC. *Nat. Cell Biol.* **20**, 58–68 https://doi.org/10.1038/s41556-017-0003-1
81 Newton, K., Dugger, D.L., Maltzman, A., Greve, J.M., Hedehus, M., Martin-McNulty, B. et al. (2016) RIPK3 deficiency or catalytically inactive RIPK1 provides greater benefit than MLKL deficiency in mouse models of inflammation and tissue injury. *Cell Death Differ.* **23**, 1565–1576 https://doi.org/10.1038/cdd.2016.46
82 Matmati, M., Jacques, P., Maelfait, J., Verheugen, E., Kool, M., Sze, M. et al. (2011) A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat. Genet.* **43**, 908–912 https://doi.org/10.1038/ng.874
83 Rusu, I., Mennillo, E., Bain, J.L., Li, Z., Sun, X., Ly, K.M. et al. (2022) Microbial signals, MyD88, and lymphotoxin drive TNF-independent intestinal epithelial tissue damage. *J. Clin. Invest.* **132**, e154993 https://doi.org/10.1172/JCI154993
84 Wertz, I.E. O'Rourke, K.M., Zhou, H., Eby, M., Aravind, L., Seshagiri, S. et al. (2004) De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* **430**, 694–699 https://doi.org/10.1038/nature02794
85 De, A., Dainichi, T., Rathinam, C.V. and Ghosh, S. (2014) The deubiquitinase activity of A20 is dispensable for NF-kappaB signaling. *EMBO Rep.* **15**, 775–783 https://doi.org/10.15252/embr.201338305
86 Wertz, I.E. Newton, K., Seshasayee, D., Kusam, S., Lam, C., Zhang, J. et al. (2015) Phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. *Nature* **528**, 370–375 https://doi.org/10.1038/nature16165
87 Wang, L., Du, F. and Wang, X. (2008) TNF-alpha induces two distinct caspase-8 activation pathways. *Cell* **133**, 693–703 https://doi.org/10.1016/j.cell.2008.03.036
88 Vanlangenakker, N., Vanden Berghe, T., Bogaert, P., Laukens, B., Zobel, K., Deshayes, K. et al. (2011) cIAP1 and TAK1 protect cells from TNF-induced necrosis by preventing RIP1/RIP3-dependent reactive oxygen species production. *Cell Death Differ.* **18**, 656–665 https://doi.org/10.1038/cdd.2010.138
89 O'Donnell, M.A., Perez-Jimenez, E., Oberst, A., Ng, A., Massoumi, R., Xavier, R. et al. (2011) Caspase 8 inhibits programmed necrosis by processing CYLD. *Nat. Cell Biol.* **13**, 1437–1442 https://doi.org/10.1038/ncb2362
90 Hitomi, J., Christofferson, D.E., Ng, A., Yao, J., Degterev, A., Xavier, R.J. et al. (2008) Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell* **135**, 1311–1323 https://doi.org/10.1016/j.cell.2008.10.044
91 Welz, P.S., Wullaert, A., Vlantis, K., Kondylis, V., Fernandez-Majada, V., Ermolaeva, M. et al. (2011) FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature* **477**, 330–334 https://doi.org/10.1038/nature10273
92 Moquin, D.M., McQuade, T. and Chan, F.K. (2013) CYLD deubiquitinates RIP1 in the TNFalpha-induced necrosome to facilitate kinase activation and programmed necrosis. *PLoS ONE* **8**, e76841 https://doi.org/10.1371/journal.pone.0076841
93 Ang, R.L., Chan, M., Legarda, D., Sundberg, J.P., Sun, S.C., Gillespie, V.L. et al. (2021) Immune dysregulation in SHARPIN-deficient mice is dependent on CYLD-mediated cell death. *Proc. Natl Acad. Sci. U.S.A.* **118**, e2001602118 https://doi.org/10.1073/pnas.2001602118
94 Bonnet, M.C., Preukschat, D., Welz, P.S., van Loo, G., Ermolaeva, M.A., Bloch, W. et al. (2011) The adaptor protein FADD protects epidermal keratinocytes from necroptosis *in vivo* and prevents skin inflammation. *Immunity* **35**, 572–582 https://doi.org/10.1016/j.immuni.2011.08.014
95 Berger, S.B., Kasparcova, V., Hoffman, S., Swift, B., Dare, L., Schaeffer, M. 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 https://doi.org/10.4049/jimmunol.1400499

Biochemical Journal (2022) **479** 1103–1119
https://doi.org/10.1042/BCJ20210735

96 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.* **35**, 1868–1884 https://doi.org/10.15252/embj.201694300
97 Schlicher, L., Wissler, M., Preiss, F., Brauns-Schubert, P., Jakob, C., Dumit, V. et al. (2016) SPATA2 promotes CYLD activity and regulates TNF-induced NF-kappaB signaling and cell death. *EMBO Rep.* **17**, 1485–1497 https://doi.org/10.15252/embr.201642592
98 Kupka, S., De Miguel, D., Draber, P., Martino, L., Surinova, S., Rittinger, K. et al. (2016) SPATA2-Mediated binding of CYLD to HOIP enables CYLD recruitment to signaling complexes. *Cell Rep.* **16**, 2271–2280 https://doi.org/10.1016/j.celrep.2016.07.086
99 Elliott, P.R., Leske, D., Hrdinka, M., Bagola, K., Fiil, B.K., McLaughlin, S.H. et al. (2016) SPATA2 links CYLD to LUBAC, activates CYLD, and controls LUBAC signaling. *Mol. Cell* **63**, 990–1005 https://doi.org/10.1016/j.molcel.2016.08.001
100 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 https://doi.org/10.1038/embor.2009.55
101 Elliott, P.R., Leske, D., Wagstaff, J., Schlicher, L., Berridge, G., Maslen, S. et al. (2021) Regulation of CYLD activity and specificity by phosphorylation and ubiquitin-binding CAP-Gly domains. *Cell Rep.* **37**, 109777 https://doi.org/10.1016/j.celrep.2021.109777
102 Wei, R., Xu, L.W., Liu, J., Li, Y., Zhang, P., Shan, B. et al. (2017) SPATA2 regulates the activation of RIPK1 by modulating linear ubiquitination. *Genes Dev.* **31**, 1162–1176 https://doi.org/10.1101/gad.299776.117
103 Meng, H., Liu, Z., Li, X., Wang, H., Jin, T., Wu, G. et al. (2018) Death-domain dimerization-mediated activation of RIPK1 controls necroptosis and RIPK1-dependent apoptosis. *Proc. Natl. Acad. Sci. U S A* **115**, E2001–E2009 https://doi.org/10.1073/pnas.1722013115
104 Laurien, L., Nagata, M., Schunke, H., Delanghe, T., Wiederstein, J.L., Kumari, S. et al. (2020) Autophosphorylation at serine 166 regulates RIP kinase 1-mediated cell death and inflammation. *Nat. Commun.* **11**, 1747 https://doi.org/10.1038/s41467-020-15466-8
105 Ohtake, F., Saeki, Y., Ishido, S., Kanno, J. and Tanaka, K. (2016) The K48-K63 branched ubiquitin chain regulates NF-kappaB signaling. *Mol. Cell* **64**, 251–266 https://doi.org/10.1016/j.molcel.2016.09.014
106 Wong, J., Garcia-Carbonell, R., Zelic, M., Ho, S.B., Boland, B.S., Yao, S.J. et al. (2020) RIPK1 mediates TNF-induced intestinal crypt apoptosis during chronic NF-kappaB activation. *Cell. Mol. Gastroenterol. Hepatol.* **9**, 295–312 https://doi.org/10.1016/j.jcmgh.2019.10.002
107 Brummelkamp, T.R., Nijman, S.M., Dirac, A.M. and Bernards, R. (2003) Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. *Nature* **424**, 797–801 https://doi.org/10.1038/nature01811
108 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 https://doi.org/10.1016/j.cell.2006.03.041
109 Zhang, J., Stirling, B., Temmerman, S.T., Ma, C.A., Fuss, I.J., Derry, J.M. et al. (2006) Impaired regulation of NF-kappaB and increased susceptibility to colitis-associated tumorigenesis in CYLD-deficient mice. *J. Clin. Invest.* **116**, 3042–3049 https://doi.org/10.1172/JCI28746
110 Reiley, W.W., Zhang, M., Jin, W., Losiewicz, M., Donohue, K.B., Norbury, C.C. et al. (2006) Regulation of T cell development by the deubiquitinating enzyme CYLD. *Nat. Immunol.* **7**, 411–417 https://doi.org/10.1038/ni1315
111 Lim, J.H., Stirling, B., Derry, J., Koga, T., Jono, H., Woo, C.H. et al. (2007) Tumor suppressor CYLD regulates acute lung injury in lethal streptococcus pneumoniae infections. *Immunity* **27**, 349–360 https://doi.org/10.1016/j.immuni.2007.07.011
112 Trompouki, E., Tsagaratou, A., Kosmidis, S.K., Dolle, P., Qian, J., Kontoyiannis, D.L. et al. (2009) Truncation of the catalytic domain of the cylindromatosis tumor suppressor impairs lung maturation. *Neoplasia* **11**, 469–476 https://doi.org/10.1593/neo.81424
113 Blake, P.W. and Toro, J.R. (2009) Update of cylindromatosis gene (CYLD) mutations in Brooke-Spiegler syndrome: novel insights into the role of deubiquitination in cell signaling. *Hum. Mutat.* **30**, 1025–1036 https://doi.org/10.1002/humu.21024
114 Zhou, Q., Yu, X., Demirkaya, E., Deuitch, N., Stone, D., Tsai, W.L. et al. (2016) Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc. Natl Acad. Sci. U.S.A.* **113**, 10127–10132 https://doi.org/10.1073/pnas.1612594113
115 Damgaard, R.B., Walker, J.A., Marco-Casanova, P., Morgan, N.V., Titheradge, H.L., Elliott, P.R. et al. (2016) The deubiquitinase OTULIN Is an essential negative regulator of inflammation and autoimmunity. *Cell* **166**, 1215–1230 https://doi.org/10.1016/j.cell.2016.07.019
116 Nabavi, M., Shahrooei, M., Rokni-Zadeh, H., Vrancken, J., Changi-Ashtiani, M., Darabi, K. et al. (2019) Auto-inflammation in a patient with a novel homozygous OTULIN mutation. *J. Clin. Immunol.* **39**, 138–141 https://doi.org/10.1007/s10875-019-00599-3
117 Damgaard, R.B., Elliott, P.R., Swatek, K.N., Maher, E.R., Stepensky, P., Elpeleg, O. et al. (2019) OTULIN deficiency in ORAS causes cell type-specific LUBAC degradation, dysregulated TNF signalling and cell death. *EMBO Mol. Med.* **11**, e9324 https://doi.org/10.15252/emmm.201809324
118 Zinngrebe, J., Moepps, B., Monecke, T., Gierschik, P., Schlichtig, F., Barth, T.F.E. et al. (2022) Compound heterozygous variants in OTULIN are associated with fulminant atypical late-onset ORAS. *EMBO Mol. Med.* **14**, e14901 https://doi.org/10.15252/emmm.202114901
119 Elliott, P.R., Nielsen, S.V., Marco-Casanova, P., Fiil, B.K., Keusekotten, K., Mailand, N. et al. (2014) Molecular basis and regulation of OTULIN-LUBAC interaction. *Mol. Cell* **54**, 335–348 https://doi.org/10.1016/j.molcel.2014.03.018
120 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 https://doi.org/10.1016/j.molcel.2014.03.016
121 Stangl, A., Elliott, P.R., Pinto-Fernandez, A., Bonham, S., Harrison, L., Schaub, A. et al. (2019) Regulation of the endosomal SNX27-retromer by OTULIN. *Nat. Commun.* **10**, 4320 https://doi.org/10.1038/s41467-019-12309-z
122 Damgaard, R.B., Jolin, H.E., Allison, M.E.D., Davies, S.E., Titheradge, H.L., McKenzie, A.N.J. et al. (2020) OTULIN protects the liver against cell death, inflammation, fibrosis, and cancer. *Cell Death Differ.* **27**, 1457–1474 https://doi.org/10.1038/s41418-020-0532-1
123 Hoste, E., Lecomte, K., Annusver, K., Vandamme, N., Roels, J., Maschalidi, S. et al. (2021) OTULIN maintains skin homeostasis by controlling keratinocyte death and stem cell identity. *Nat. Commun.* **12**, 5913 https://doi.org/10.1038/s41467-021-25944-2
124 Verboom, L., Martens, A., Priem, D., Hoste, E., Sze, M., Vikkula, H. et al. (2020) OTULIN prevents liver inflammation and hepatocellular carcinoma by inhibiting FADD- and RIPK1 kinase-Mediated hepatocyte apoptosis. *Cell Rep.* **30**, 2237–2247 https://doi.org/10.1016/j.celrep.2020.01.028
125 Fuseya, Y., Fujita, H., Kim, M., Ohtake, F., Nishide, A., Sasaki, K. et al. (2020) The HOIL-1L ligase modulates immune signalling and cell death via monoubiquitination of LUBAC. *Nat. Cell Biol.* **22**, 663–673 https://doi.org/10.1038/s41556-020-0517-9
126 Douglas, T. and Saleh, M. (2019) Post-translational modification of OTULIN regulates ubiquitin dynamics and cell death. *Cell Rep.* **29**, 3652–3663 https://doi.org/10.1016/j.celrep.2019.11.014
127 Schunke, H., Gobel, U., Dikic, I. and Pasparakis, M. (2021) OTULIN inhibits RIPK1-mediated keratinocyte necroptosis to prevent skin inflammation in mice. *Nat. Commun.* **12**, 5912 https://doi.org/10.1038/s41467-021-25945-1

128 Rivkin, E., Almeida, S.M., Ceccarelli, D.F., Juang, Y.C., MacLean, T.A., Srikumar, T. et al. (2013) The linear ubiquitin-specific deubiquitinase gumby regulates angiogenesis. *Nature* **498**, 318–324 https://doi.org/10.1038/nature12296
129 Boisson, B., Laplantine, E., Prando, C., Giliani, S., Israelsson, E., Xu, Z. et al. (2012) Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. *Nat. Immunol.* **13**, 1178–1186 https://doi.org/10.1038/ni.2457
130 Boisson, B., Laplantine, E., Dobbs, K., Cobat, A., Tarantino, N., Hazen, M. et al. (2015) Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J. Exp. Med.* **212**, 939–951 https://doi.org/10.1084/jem.20141130
131 Oda, H., Beck, D.B., Kuehn, H.S., Sampaio Moura, N., Hoffmann, P., Ibarra, M. et al. (2019) Second case of HOIP deficiency expands clinical features and defines inflammatory transcriptome regulated by LUBAC. *Front. Immunol.* **10**, 479 https://doi.org/10.3389/fimmu.2019.00479
132 Goncharov, T., Niessen, K., de Almagro, M.C., Izrael-Tomasevic, A., Fedorova, A.V., Varfolomeev, E. et al. (2013) OTUB1 modulates c-IAP1 stability to regulate signalling pathways. *EMBO J.* **32**, 1103–1114 https://doi.org/10.1038/emboj.2013.62
133 Vallabhapurapu, S., Matsuzawa, A., Zhang, W., Tseng, P.H., Keats, J.J., Wang, H. et al. (2008) Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. *Nat. Immunol.* **9**, 1364–1370 https://doi.org/10.1038/ni.1678
134 Zarnegar, B.J., Wang, Y., Mahoney, D.J., Dempsey, P.W., Cheung, H.H., He, J. et al. (2008) Noncanonical NF-kappaB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nat. Immunol.* **9**, 1371–1378 https://doi.org/10.1038/ni.1676
135 Vince, J.E., Chau, D., Callus, B., Wong, W.W., Hawkins, C.J., Schneider, P. et al. (2008) TWEAK-FN14 signaling induces lysosomal degradation of a cIAP1-TRAF2 complex to sensitize tumor cells to TNFalpha. *J. Cell Biol.* **182**, 171–184 https://doi.org/10.1083/jcb.200801010
136 Varfolomeev, E., Goncharov, T., Maecker, H., Zobel, K., Komuves, L.G., Deshayes, K. et al. (2012) Cellular inhibitors of apoptosis are global regulators of NF-kappaB and MAPK activation by members of the TNF family of receptors. *Sci. Signal.* **5**, ra22 https://doi.org/10.1126/scisignal.2001878
137 Koschel, J., Nishanth, G., Just, S., Harit, K., Kroger, A., Deckert, M. et al. (2021) OTUB1 prevents lethal hepatocyte necroptosis through stabilization of c-IAP1 during murine liver inflammation. *Cell Death Differ.* **28**, 2257–2275 https://doi.org/10.1038/s41418-021-00752-9
138 Dara, L., Liu, Z.X. and Kaplowitz, N. (2016) Questions and controversies: the role of necroptosis in liver disease. *Cell Death Discov.* **2**, 16089 https://doi.org/10.1038/cddiscovery.2016.89
139 Sai, K., Parsons, C., House, J.S., Kathariou, S. and Ninomiya-Tsuji, J. (2019) Necroptosis mediators RIPK3 and MLKL suppress intracellular listeria replication independently of host cell killing. *J. Cell Biol.* **218**, 1994–2005 https://doi.org/10.1083/jcb.201810014
140 Newton, K., Dixit, V.M. and Kayagaki, N. (2021) Dying cells fan the flames of inflammation. *Science* **374**, 1076–1080 https://doi.org/10.1126/science.abi5934
141 Kayagaki, N., Stowe, I.B., Lee, B.L., O'Rourke, K., Anderson, K., Warming, S. et al. (2015) Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* **526**, 666–671 https://doi.org/10.1038/nature15541
142 Shi, J., Zhao, Y., Wang, K., Shi, X., Wang, Y., Huang, H. et al. (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* **526**, 660–665 https://doi.org/10.1038/nature15514
143 Ding, J., Wang, K., Liu, W., She, Y., Sun, Q., Shi, J. et al. (2016) Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* **535**, 111–116 https://doi.org/10.1038/nature18590
144 Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V.G., Wu, H. et al. (2016) Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* **535**, 153–158 https://doi.org/10.1038/nature18629
145 Aglietti, R.A., Estevez, A., Gupta, A., Ramirez, M.G., Liu, P.S., Kayagaki, N. et al. (2016) Gsdmd p30 elicited by caspase-11 during pyroptosis forms pores in membranes. *Proc. Natl Acad. Sci. U.S.A.* **113**, 7858–7863 https://doi.org/10.1073/pnas.1607769113
146 Sborgi, L., Ruhl, S., Mulvihill, E., Pipercevic, J., Heilig, R., Stahlberg, H. et al. (2016) GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J.* **35**, 1766–1778 https://doi.org/10.15252/embj.201694696
147 Xia, S., Zhang, Z., Magupalli, V.G., Pablo, J.L., Dong, Y., Vora, S.M. et al. (2021) Gasdermin D pore structure reveals preferential release of mature interleukin-1. *Nature* **593**, 607–611 https://doi.org/10.1038/s41586-021-03478-3
148 Kayagaki, N., Kornfeld, O.S., Lee, B.L., Stowe, I.B., O'Rourke, K., Li, Q. et al. (2021) NINJ1 mediates plasma membrane rupture during lytic cell death. *Nature* **591**, 131–136 https://doi.org/10.1038/s41586-021-03218-7
149 Kambara, H., Liu, F., Zhang, X., Liu, P., Bajrami, B., Teng, Y. et al. (2018) Gasdermin D exerts anti-inflammatory effects by promoting neutrophil death. *Cell Rep.* **22**, 2924–2936 https://doi.org/10.1016/j.celrep.2018.02.067
150 Burgener, S.S., Leborgne, N.G.F., Snipas, S.J., Salvesen, G.S., Bird, P.I. and Benarafa, C. (2019) Cathepsin G inhibition by Serpinb1 and Serpinb6 prevents programmed necrosis in neutrophils and monocytes and reduces GSDMD-driven inflammation. *Cell Rep.* **27**, 3646–3656 https://doi.org/10.1016/j.celrep.2019.05.065
151 Mariathasan, S., Weiss, D.S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M. et al. (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* **440**, 228–232 https://doi.org/10.1038/nature04515
152 Lawlor, K.E., Khan, N., Mildenhall, A., Gerlic, M., Croker, B.A., D'Cruz, A.A. et al. (2015) RIPK3 promotes cell death and NLRP3 inflammasome activation in the absence of MLKL. *Nat. Commun.* **6**, 6282 https://doi.org/10.1038/ncomms7282
153 Conos, S.A., Chen, K.W., De Nardo, D., Hara, H., Whitehead, L., Nunez, G. et al. (2017) Active MLKL triggers the NLRP3 inflammasome in a cell-intrinsic manner. *Proc. Natl Acad. Sci. U.S.A.* **114**, E961–E969 https://doi.org/10.1073/pnas.1613305114
154 Bauernfeind, F.G., Horvath, G., Stutz, A., Alnemri, E.S., MacDonald, K., Speert, D. et al. (2009) Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* **183**, 787–791 https://doi.org/10.4049/jimmunol.0901363
155 Boaru, S.G., Borkham-Kamphorst, E., Van de Leur, E., Lehnen, E., Liedtke, C. and Weiskirchen, R. (2015) NLRP3 inflammasome expression is driven by NF-kappaB in cultured hepatocytes. *Biochem. Biophys. Res. Commun.* **458**, 700–706 https://doi.org/10.1016/j.bbrc.2015.02.029
156 Yin, J., Schoeffler, A.J., Wickliffe, K., Newton, K., Starovasnik, M.A., Dueber, E.C. et al. (2015) Structural insights into WD-Repeat 48 activation of ubiquitin-specific protease 46. *Structure* **23**, 2043–2054 https://doi.org/10.1016/j.str.2015.08.010
157 Li, H., Lim, K.S., Kim, H., Hinds, T.R., Jo, U., Mao, H. et al. (2016) Allosteric activation of ubiquitin-specific proteases by beta-propeller proteins UAF1 and WDR20. *Mol. Cell* **63**, 249–260 https://doi.org/10.1016/j.molcel.2016.05.031

158 Dharadhar, S., Clerici, M., van Dijk, W.J., Fish, A. and Sixma, T.K. (2016) A conserved two-step binding for the UAF1 regulator to the USP12 deubiquitinating enzyme. *J. Struct. Biol.* **196**, 437–447 https://doi.org/10.1016/j.jsb.2016.09.011
159 Song, H., Zhao, C., Yu, Z., Li, Q., Yan, R., Qin, Y. et al. (2020) UAF1 deubiquitinase complexes facilitate NLRP3 inflammasome activation by promoting NLRP3 expression. *Nat. Commun.* **11**, 6042 https://doi.org/10.1038/s41467-020-19939-8
160 Cooper, E.M., Cutcliffe, C., Kristiansen, T.Z., Pandey, A., Pickart, C.M. and Cohen, R.E. (2009) K63-specific deubiquitination by two JAMM/MPN+ complexes: BRISC-associated Brcc36 and proteasomal Poh1. *EMBO J.* **28**, 621–631 https://doi.org/10.1038/emboj.2009.27
161 Py, B.F., Kim, M.S., Vakifahmetoglu-Norberg, H. and Yuan, J. (2013) Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. *Mol. Cell* **49**, 331–338 https://doi.org/10.1016/j.molcel.2012.11.009
162 Ren, G., Zhang, X., Xiao, Y., Zhang, W., Wang, Y., Ma, W. et al. (2019) ABRO1 promotes NLRP3 inflammasome activation through regulation of NLRP3 deubiquitination. *EMBO J.* **38**, e100376 https://doi.org/10.15252/embj.2018100376
163 Tang, J., Tu, S., Lin, G., Guo, H., Yan, C., Liu, Q. et al. (2020) Sequential ubiquitination of NLRP3 by RNF125 and Cbl-b limits inflammasome activation and endotoxemia. *J. Exp. Med.* **217**, e20182091 https://doi.org/10.1084/jem.20182091
164 Zeqiraj, E., Tian, L., Piggott, C.A., Pillon, M.C., Duffy, N.M., Ceccarelli, D.F. et al. (2015) Higher-order assembly of BRCC36-KIAA0157 is required for DUB activity and biological function. *Mol. Cell* **59**, 970–983 https://doi.org/10.1016/j.molcel.2015.07.028
165 Niu, T., De Rosny, C., Chautard, S., Rey, A., Patoli, D., Groslambert, M. et al. (2021) NLRP3 phosphorylation in its LRR domain critically regulates inflammasome assembly. *Nat. Commun.* **12**, 5862 https://doi.org/10.1038/s41467-021-26142-w
166 Sharif, H., Wang, L., Wang, W.L., Magupalli, V.G., Andreeva, L., Qiao, Q. et al. (2019) Structural mechanism for NEK7-licensed activation of NLRP3 inflammasome. *Nature* **570**, 338–343 https://doi.org/10.1038/s41586-019-1295-z
167 Walden, M., Tian, L., Ross, R.L., Sykora, U.M., Byrne, D.P., Hesketh, E.L. et al. (2019) Metabolic control of BRISC-SHMT2 assembly regulates immune signalling. *Nature* **570**, 194–199 https://doi.org/10.1038/s41586-019-1232-1
168 Rabl, J., Bunker, R.D., Schenk, A.D., Cavadini, S., Gill, M.E., Abdulrahman, W. et al. (2019) Structural basis of BRCC36 function in DNA repair and immune regulation. *Mol. Cell* **75**, 483–497 https://doi.org/10.1016/j.molcel.2019.06.002
169 Zheng, H., Gupta, V., Patterson-Fortin, J., Bhattacharya, S., Katlinski, K., Wu, J. et al. (2013) A BRISC-SHMT complex deubiquitinates IFNAR1 and regulates interferon responses. *Cell Rep.* **5**, 180–193 https://doi.org/10.1016/j.celrep.2013.08.025
170 Xu, M., Moresco, J.J., Chang, M., Mukim, A., Smith, D., Diedrich, J.K. et al. (2018) SHMT2 and the BRCC36/BRISC deubiquitinase regulate HIV-1 Tat K63-ubiquitylation and destruction by autophagy. *PLoS Pathog.* **14**, e1007071 https://doi.org/10.1371/journal.ppat.1007071
171 Ren, G.M., Li, J., Zhang, X.C., Wang, Y., Xiao, Y., Zhang, X.Y. et al. (2021) Pharmacological targeting of NLRP3 deubiquitination for treatment of NLRP3-associated inflammatory diseases. *Sci. Immunol.* **6**, eabe2933 https://doi.org/10.1126/sciimmunol.abe2933
172 Donaghy, R., Han, X., Rozenova, K., Lv, K., Jiang, Q., Doepner, M. et al. (2019) The BRISC deubiquitinating enzyme complex limits hematopoietic stem cell expansion by regulating JAK2 K63-ubiquitination. *Blood* **133**, 1560–1571 https://doi.org/10.1182/blood-2018-10-877563
173 Wang, C.Y., Deneen, B. and Tzeng, S.F. (2019) BRCA1/BRCA2-containing complex subunit 3 controls oligodendrocyte differentiation by dynamically regulating lysine 63-linked ubiquitination. *Glia* **67**, 1775–1792 https://doi.org/10.1002/glia.23660
174 Yang, X.D., Li, W., Zhang, S., Wu, D., Jiang, X., Tan, R. et al. (2020) PLK4 deubiquitination by Spata2-CYLD suppresses NEK7-mediated NLRP3 inflammasome activation at the centrosome. *EMBO J.* **39**, e102201 https://doi.org/10.15252/embj.2019102201
175 Duong, B.H., Onizawa, M., Oses-Prieto, J.A., Advincula, R., Burlingame, A., Malynn, B.A. et al. (2015) A20 restricts ubiquitination of pro-interleukin-1beta protein complexes and suppresses NLRP3 inflammasome activity. *Immunity* **42**, 55–67 https://doi.org/10.1016/j.immuni.2014.12.031
176 Vande Walle, L., Van Opdenbosch, N., Jacques, P., Fossoul, A., Verheugen, E., Vogel, P. et al. (2014) Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. *Nature* **512**, 69–73 https://doi.org/10.1038/nature13322
177 Hong, Y., Lee, S.O., Oh, C., Kang, K., Ryoo, J., Kim, D. et al. (2021) USP21 deubiquitinase regulates AIM2 inflammasome activation. *J. Immunol.* **207**, 1926–1936 https://doi.org/10.4049/jimmunol.2100449
178 Mukherjee, S., Kumar, R., Tsakem Lenou, E., Basrur, V., Kontoyiannis, D.L., Ioakeimidis, F. et al. (2020) Deubiquitination of NLRP6 inflammasome by cyld critically regulates intestinal inflammation. *Nat. Immunol.* **21**, 626–635 https://doi.org/10.1038/s41590-020-0681-x
179 Adams, J.M. and Cory, S. (2018) The BCL-2 arbiters of apoptosis and their growing role as cancer targets. *Cell Death Differ.* **25**, 27–36 https://doi.org/10.1038/cdd.2017.161
180 Diepstraten, S.T., Anderson, M.A., Czabotar, P.E., Lessene, G., Strasser, A. and Kelly, G.L. (2021) The manipulation of apoptosis for cancer therapy using BH3-mimetic drugs. *Nat. Rev. Cancer* **22**, 45–64 https://doi.org/10.1038/s41568-021-00407-4
181 Llambi, F., Wang, Y.M., Victor, B., Yang, M., Schneider, D.M., Gingras, S. et al. (2016) BOK is a non-canonical BCL-2 family effector of apoptosis regulated by ER-associated degradation. *Cell* **165**, 421–433 https://doi.org/10.1016/j.cell.2016.02.026
182 Zhong, Q., Gao, W., Du, F. and Wang, X. (2005) Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. *Cell* **121**, 1085–1095 https://doi.org/10.1016/j.cell.2005.06.009
183 Inuzuka, H., Shaik, S., Onoyama, I., Gao, D., Tseng, A., Maser, R.S. et al. (2011) SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. *Nature* **471**, 104–109 https://doi.org/10.1038/nature09732
184 Wertz, I.E. Kusam, S., Lam, C., Okamoto, T., Sandoval, W., Anderson, D.J. et al. (2011) Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. *Nature* **471**, 110–114 https://doi.org/10.1038/nature09779
185 Subramanian, A., Andronache, A., Li, Y.C. and Wade, M. (2016) Inhibition of MARCH5 ubiquitin ligase abrogates MCL1-dependent resistance to BH3 mimetics via NOXA. *Oncotarget* **7**, 15986–16002 https://doi.org/10.18632/oncotarget.7558
186 Djajawi, T.M., Liu, L., Gong, J.N., Huang, A.S., Luo, M.J., Xu, Z. et al. (2020) MARCH5 requires MTCH2 to coordinate proteasomal turnover of the MCL1:NOXA complex. *Cell Death Differ.* **27**, 2484–2499 https://doi.org/10.1038/s41418-020-0517-0
187 Arai, S., Varkaris, A., Nouri, M., Chen, S., Xie, L. and Balk, S.P. (2020) MARCH5 mediates NOXA-dependent MCL1 degradation driven by kinase inhibitors and integrated stress response activation. *eLife* **9**, e54954 https://doi.org/10.7554/eLife.54954
188 Schwickart, M., Huang, X., Lill, J.R., Liu, J., Ferrando, R., French, D.M. et al. (2010) Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature* **463**, 103–107 https://doi.org/10.1038/nature08646
189 Zhang, S., Zhang, M., Jing, Y., Yin, X., Ma, P., Zhang, Z. et al. (2018) Deubiquitinase USP13 dictates MCL1 stability and sensitivity to BH3 mimetic inhibitors. *Nat. Commun.* **9**, 215 https://doi.org/10.1038/s41467-017-02693-9

190 Wu, X., Luo, Q., Zhao, P., Chang, W., Wang, Y., Shu, T. et al. (2019) MGMT-activated DUB3 stabilizes MCL1 and drives chemoresistance in ovarian cancer. *Proc. Natl Acad. Sci. U.S.A.* **116**, 2961–2966 https://doi.org/10.1073/pnas.1814742116
191 Nagai, H., Noguchi, T., Homma, K., Katagiri, K., Takeda, K., Matsuzawa, A. et al. (2009) Ubiquitin-like sequence in ASK1 plays critical roles in the recognition and stabilization by USP9X and oxidative stress-induced cell death. *Mol. Cell* **36**, 805–818 https://doi.org/10.1016/j.molcel.2009.10.016
192 Cox, B.J., Vollmer, M., Tamplin, O., Lu, M., Biechele, S., Gertsenstein, M. et al. (2010) Phenotypic annotation of the mouse X chromosome. *Genome Res.* **20**, 1154–1164 https://doi.org/10.1101/gr.105106.110
193 Naik, E., Webster, J.D., DeVoss, J., Liu, J., Suriben, R. and Dixit, V.M. (2014) Regulation of proximal T cell receptor signaling and tolerance induction by deubiquitinase Usp9X. *J. Exp. Med.* **211**, 1947–1955 https://doi.org/10.1084/jem.20140860
194 Macrae, T.A. and Ramalho-Santos, M. (2021) The deubiquitinase Usp9x regulates PRC2-mediated chromatin reprogramming during mouse development. *Nat. Commun.* **12**, 1865 https://doi.org/10.1038/s41467-021-21910-0
195 Abed, M., Verschueren, E., Budayeva, H., Liu, P., Kirkpatrick, D.S., Reja, R. et al. (2019) The Gag protein PEG10 binds to RNA and regulates trophoblast stem cell lineage specification. *PLoS ONE* **14**, e0214110 https://doi.org/10.1371/journal.pone.0214110
196 Pereg, Y., Liu, B.Y., O'Rourke, K.M., Sagolla, M., Dey, A., Komuves, L. et al. (2010) Ubiquitin hydrolase Dub3 promotes oncogenic transformation by stabilizing Cdc25A. *Nat. Cell Biol.* **12**, 400–406 https://doi.org/10.1038/ncb2041
197 Zhao, X., Fiske, B., Kawakami, A., Li, J. and Fisher, D.E. (2011) Regulation of MITF stability by the USP13 deubiquitinase. *Nat. Commun.* **2**, 414 https://doi.org/10.1038/ncomms1421
198 Zhang, Q., Zhang, Z.Y., Du, H., Li, S.Z., Tu, R., Jia, Y.F. et al. (2019) DUB3 deubiquitinates and stabilizes NRF2 in chemotherapy resistance of colorectal cancer. *Cell Death Differ.* **26**, 2300–2313 https://doi.org/10.1038/s41418-019-0303-z
199 He, M., Chaurushiya, M.S., Webster, J.D., Kummerfeld, S., Reja, R., Chaudhuri, S. et al. (2019) Intrinsic apoptosis shapes the tumor spectrum linked to inactivation of the deubiquitinase BAP1. *Science* **364**, 283–285 https://doi.org/10.1126/science.aav4902
200 Scheuermann, J.C., de Ayala Alonso, A.G., Oktaba, K., Ly-Hartig, N., McGinty, R.K., Fraterman, S. et al. (2010) Histone H2A deubiquitinase activity of the polycomb repressive complex PR-DUB. *Nature* **465**, 243–247 https://doi.org/10.1038/nature08966
201 Sahtoe, D.D., van Dijk, W.J., Ekkebus, R., Ovaa, H. and Sixma, T.K. (2016) BAP1/ASXL1 recruitment and activation for H2A deubiquitination. *Nat Commun.* **7**, 10292 https://doi.org/10.1038/ncomms10292
202 Campagne, A., Lee, M.K., Zielinski, D., Michaud, A., Le Corre, S., Dingli, F. et al. (2019) BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation. *Nat. Commun.* **10**, 348 https://doi.org/10.1038/s41467-018-08255-x
203 Fursova, N.A., Turberfield, A.H., Blackledge, N.P., Findlater, E.L., Lastuvkova, A., Huseyin, M.K. et al. (2021) BAP1 constrains pervasive H2AK119ub1 to control the transcriptional potential of the genome. *Genes Dev.* **35**, 749–770 https://doi.org/10.1101/gad.347005.120
204 Masclef, L., Ahmed, O., Estavoyer, B., Larrivee, B., Labrecque, N., Nijnik, A. et al. (2021) Roles and mechanisms of BAP1 deubiquitinase in tumor suppression. *Cell Death Differ.* **28**, 606–625 https://doi.org/10.1038/s41418-020-00709-4
205 Wertz, I.E. and Murray, J.M. (2019) Structurally-defined deubiquitinase inhibitors provide opportunities to investigate disease mechanisms. *Drug Discov. Today Technol.* **31**, 109–123 https://doi.org/10.1016/j.ddtec.2019.02.003
206 Fischer, M. (2017) Census and evaluation of p53 target genes. *Oncogene* **36**, 3943–3956 https://doi.org/10.1038/onc.2016.502
207 van der Knaap, J.A., Kumar, B.R., Moshkin, Y.M., Langenberg, K., Krijgsveld, J., Heck, A.J. et al. (2005) GMP synthetase stimulates histone H2B deubiquitylation by the epigenetic silencer USP7. *Mol. Cell* **17**, 695–707 https://doi.org/10.1016/j.molcel.2005.02.013
208 Tavana, O., Li, D., Dai, C., Lopez, G., Banerjee, D., Kon, N. et al. (2016) HAUSP deubiquitinates and stabilizes N-Myc in neuroblastoma. *Nat. Med.* **22**, 1180–1186 https://doi.org/10.1038/nm.4180
209 Kon, N., Kobayashi, Y., Li, M., Brooks, C.L., Ludwig, T. and Gu, W. (2010) Inactivation of HAUSP *in vivo* modulates p53 function. *Oncogene* **29**, 1270–1279 https://doi.org/10.1038/onc.2009.427
210 Kon, N., Zhong, J., Kobayashi, Y., Li, M., Szabolcs, M., Ludwig, T. et al. (2011) Roles of HAUSP-mediated p53 regulation in central nervous system development. *Cell Death Differ.* **18**, 1366–1375 https://doi.org/10.1038/cdd.2011.12
211 Sun, X.X., Challagundla, K.B. and Dai, M.S. (2012) Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1. *EMBO J.* **31**, 576–592 https://doi.org/10.1038/emboj.2011.434
212 Yuan, J., Luo, K., Zhang, L., Cheville, J.C. and Lou, Z. (2010) USP10 regulates p53 localization and stability by deubiquitinating p53. *Cell* **140**, 384–396 https://doi.org/10.1016/j.cell.2009.12.032
213 Liu, H., Li, X., Ning, G., Zhu, S., Ma, X., Liu, X. et al. (2016) The Machado-Joseph disease deubiquitinase ataxin-3 regulates the stability and apoptotic function of p53. *PLoS Biol.* **14**, e2000733 https://doi.org/10.1371/journal.pbio.2000733
214 Liu, Z., Dagley, L.F., Shield-Artin, K., Young, S.N., Bankovacki, A., Wang, X. et al. (2021) Oligomerization-driven MLKL ubiquitylation antagonizes necroptosis. *EMBO J.* **40**, e103718 https://doi.org/10.15252/embj.2019103718
215 Garcia, L.R., Tenev, T., Newman, R., Haich, R.O., Liccardi, G., John, S.W. et al. (2021) Ubiquitylation of MLKL at lysine 219 positively regulates necroptosis-induced tissue injury and pathogen clearance. *Nat. Commun.* **12**, 3364 https://doi.org/10.1038/s41467-021-23474-5
216 de Almagro, M.C., Goncharov, T., Newton, K. and Vucic, D. (2015) Cellular IAP proteins and LUBAC differentially regulate necrosome-associated RIP1 ubiquitination. *Cell Death Dis.* **6**, e1800 https://doi.org/10.1038/cddis.2015.158