frontiers | Frontiers in Cell Death

TYPE Mini Review
PUBLISHED 09 April 2025
DOI 10.3389/fceld.2025.1583221

OPEN ACCESS

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RECEIVED 25 February 2025
ACCEPTED 27 March 2025
PUBLISHED 09 April 2025

CITATION
Macé YM, Bidère N and Douanne T (2025) The good, the bad, and the modified: CYLD's post-translational tale.
Front. Cell Death 4:1583221.
doi: 10.3389/fceld.2025.1583221

The good, the bad, and the modified: CYLD's post-translational tale

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The deubiquitinating enzyme CYLD hydrolyzes Lys63-and Met1-linked ubiquitin chains, playing a crucial role in regulating various cellular processes such as immune cell development, innate and adaptive immunity, spermatogenesis, ciliogenesis, and cell survival. CYLD also functions as a tumor suppressor and is mutated in familial cylindromatosis. This pleiotropic function implies tight regulatory mechanisms. In this review, we summarize the current knowledge on CYLD's molecular characteristics, subcellular location, and binding partners, with a focus on its involvement in life-and-death decisions. In addition, we discuss how post-translational modifications, including phosphorylation, ubiquitination, and proteolysis, shape CYLD's function, unveiling the potential for therapeutic intervention. Finally, we highlight the remaining challenges that need to be overcome to deepen our understanding of this crucial enzyme.

KEYWORDS

CYLD, DUB, cell death, post-translational modifications, phosphorylation, ubiquitin, proteolysis

Introduction

Ubiquitination is a pivotal post-translational modification (PTM) regulating numerous cellular processes in eukaryotes. It consists of the covalent attachment of ubiquitin to substrate proteins, either as a single moiety or in linkage-specific polyubiquitin chains, modulating protein stability, activity, or localization to drive diverse cellular outcomes (Komander and Rape, 2012). Ubiquitination is dynamically reversible through the action of deubiquitinating enzymes (DUBs), a specialized group of proteases that selectively trim ubiquitin from target proteins. CYLD is a member of the DUB family known as ubiquitin-specific proteases (USP). Initially identified as a gene mutated in familial cylindromatosis, an autosomal dominant condition that predisposes individuals to skin appendage tumors (Bignell et al., 2000), CYLD has since been implicated in numerous human diseases, including infectious conditions, neurodegenerative disorders, and various cancers (Marín-Rubio et al., 2023). In most cases, CYLD truncations and mutations found in patients negatively affect its expression. Genetic mouse models have demonstrated the wide functions of CYLD in diverse biological processes such as immune cell development and function, innate immunity, spermatogenesis, hepatocellular homeostasis, osteoclastogenesis, and ciliogenesis (Lork et al., 2017).

CYLD is best known for its role as a negative regulator of NF-κB signaling, where it deubiquitinates key components such as NEMO, TRAF2, TRAF6, TAK1, RIPK1, and Bcl3 (Trompouki et al., 2003; Brummelkamp et al., 2003; Kovalenko et al., 2003; Massoumi et al., 2006; Reiley et al., 2007; Wright et al., 2007). By curbing NF-κB-mediated gene expression following stimulation of cytokine receptors, antigen receptors, and pattern recognition

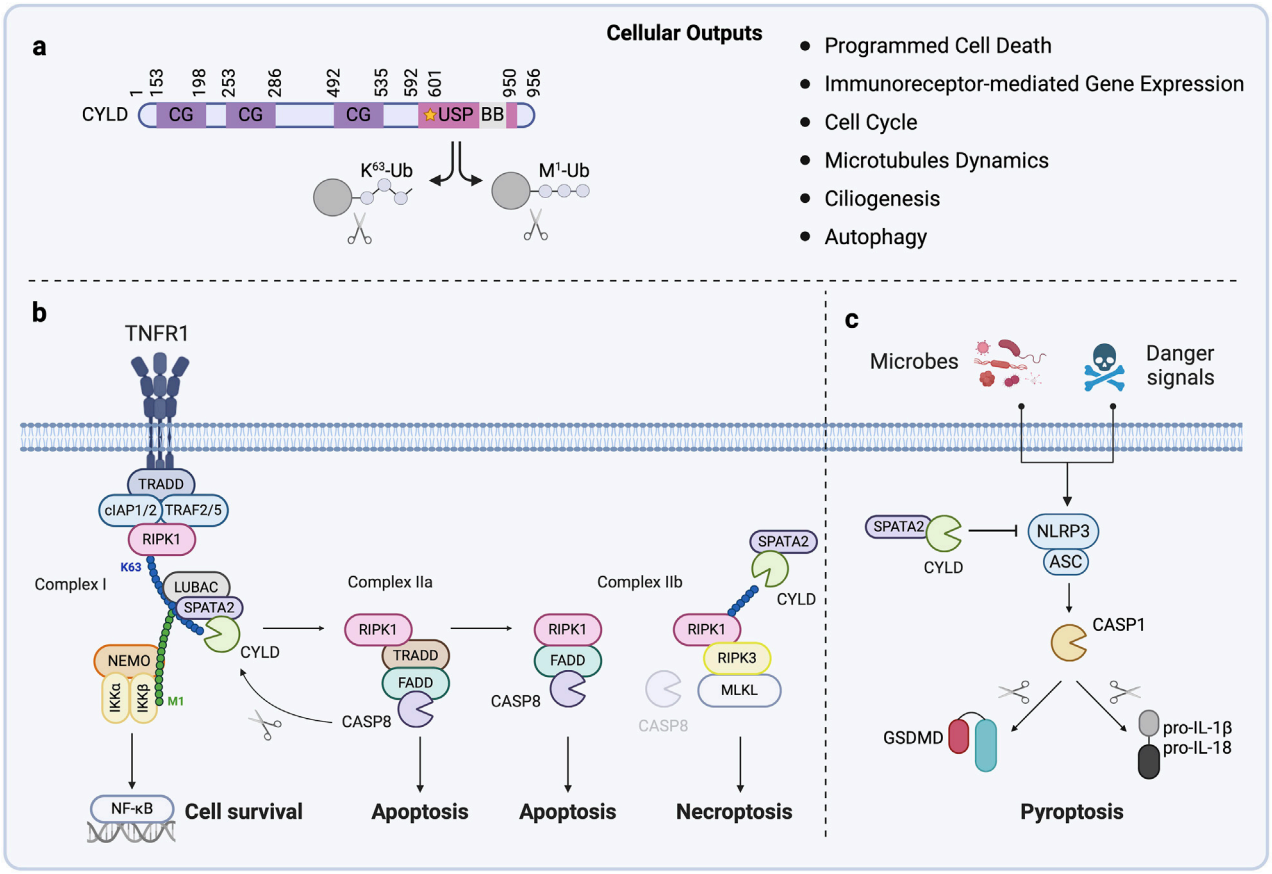

FIGURE 1
CYLD, a key Regulator of Programmed Cell Death **(A)** Domain structure of CYLD. CYLD contains three cytoskeleton-associated protein glycine-rich (CAP-Gly, CG) domains that mediate microtubule binding and the ubiquitin-specific protease domain (USP) harboring its catalytic activity and active cysteine (C601, illustrated by a star). The B box (BB) is embedded within the USP domain. CYLD hydrolyzes Lys63-and Met1-linked ubiquitin chains on substrates, leading to varied cellular outputs. **(B)** The engagement of TNF receptor 1 (TNFR1) triggers the formation of signaling complex I (RIPK1, cIAP1/2, TRADD, and TRAF2/5) at the cell membrane. This leads to the recruitment of the LUBAC together with CYLD and SPATA2, driving the ubiquitin-dependent activation of NF-κB and expression of anti-apoptotic genes. Deubiquitination of RIPK1 by CYLD inhibits NF-κB signaling and leads to the formation of complex IIa (RIPK1, TRADD, caspase-8 (CASP8), and FADD) in the cytosol. Activation of CASP8 within this complex results in apoptosis and also the CASP8-mediated cleavage of CYLD, restoring NF-κB signaling and establishing a finely tuned balance between cell survival and apoptosis. When NF-κB independent checkpoint is disrupted, RIPK1 engages another signaling complex called complex IIb, together with FADD and CASP8, to initiate apoptosis. Blockade of CASP8 drives binding of RIPK1 with RIPK3 and cell death by necroptosis. CYLD-mediated deubiquitination of RIPK1 in the necrosome facilitates necroptosis. **(C)** Various microbial motifs, endogenous and exogenous danger signals trigger the formation of the NLRP3 inflammasome, which serves as a cytosolic platform for the activation of CASP1. Active CASP1 matures the inflammatory cytokines IL-1β and IL-18 and the pore-forming protein Gasdermin D (GSDMD) into biologically active forms. Plasma membrane-assembled GSDMD pores allow the release of IL-1β and IL-18 and cause cell rupture and release of damage-associated molecular patterns leading to pyroptosis. CYLD restricts NLRP3 activity, limiting pyroptosis.

receptors, CYLD prevents excessive inflammation and maintains immune homeostasis. Beyond NF-κB, CYLD also regulates other pathways like MAPK (Liang et al., 2011), Wnt/βCat (Tauriello et al., 2010; Van Andel et al., 2017), TGF-β (Zhao et al., 2011; Tang et al., 2019), and JNK signaling (Reiley et al., 2004; Staal et al., 2011), underscoring its broad influence on cellular physiology. In addition, CYLD is involved in cell cycle progression (Stegmeier et al., 2007), p53 DNA damage response (Fernández-Majada et al., 2016), autophagy (Qi et al., 2020; Colombo et al., 2021; Zajicek et al., 2022), centriolar satellite homeostasis (Douanne et al., 2019; Renaud et al., 2023), and primary cilia formation (Douanne et al., 2019; Eguether et al., 2014; Yang et al., 2014).

How CYLD acts on such various processes continues to be investigated. One key factor underlying the DUB pleiotropy is its tight regulation through many PTMs. In this review, we focus on the role of CYLD in programmed cell death, examine its regulation by PTMs, and explore emerging questions in this field.

An atypical DUB

CYLD is a member of the DUB family known as USPs. Together with at least 56 identified members, the USP family represents the largest DUB class and plays critical roles in various cellular processes (Clague et al., 2019). Structurally, CYLD contains three N-terminal cytoskeleton-associated protein-glycine-rich (CAP-Gly) domains and a C-terminal USP catalytic domain (Figure 1A). The CYLD USP domain exhibits distinct structural features compared to other

family members, characterized by several insertions and deletions. Notably, CYLD lacks a Fingers subdomain, enabling it to hydrolyze internal linkages within Lys63 and Met1-linked (also known as linear) polyubiquitin chains. This structural adaptation provides CYLD with a kinetic advantage for these chain types, unlike other USPs that exclusively cleave chains from the distal end. Additionally, differences in the active site, particularly the extended β12/β13 loop, contribute to CYLD's affinity for Lys63 while other differences account for the lack of activity towards Lys48-linked chains. These unique features distinguish CYLD from other USPs, which typically exhibit broader linkage promiscuity (Komander et al., 2008; Komander et al., 2009).

Early biochemical and bioinformatic analysis revealed the presence of a small Zn-binding module inserted within the CYLD catalytic domain bearing similarities to B box domains and RING fingers of E3 ligases (Komander et al., 2008). The presence of this B box in CYLD's USP domain represents another singularity. *In vitro* deubiquitination assay using recombinant CYLD lacking this region demonstrated that this structure is dispensable for the catalytic activity and specificity of the enzyme. Furthermore, the isolated B box neither exhibited E3 ligase activity nor interacted with ubiquitin or ubiquitin chains, excluding it as a ubiquitin-binding domain (UBD) (Komander et al., 2008). Subsequent work identified a role for the B box in CYLD dimerization and its interaction with the canonical partner spermatogenesis-associated protein 2 (SPATA2) (Elliott et al., 2016). Interestingly, in HEK293 T cells, CYLD is predominantly localized in the cytosol, while mutants lacking the B box partially translocate to the nucleus. This suggests the B box is involved in retaining CYLD within the cytosol (Komander et al., 2008; Xie et al., 2017).

Upstream of its C-terminal catalytic domain, CYLD contains three CAP-Gly domains (Komander and Rape, 2012; Bignell et al., 2000). These approximately 70-amino acid SRC homology 3 (SH3)-fold domains mediate interactions with microtubules and microtubule-associated proteins (Yan et al., 2015). The first and second CAP-Gly domains bind directly to microtubules, with CAP-Gly1 displaying the highest binding affinity (Gao et al., 2008; Wickström et al., 2010), while CAP-Gly3 interacts with the non-catalytic subunit of the IKK complex NEMO (Saito et al., 2004). Pre-treatment of *in vitro*-assembled microtubules with subtilisin (also known as PCSK9), a serine protease that removes the C-terminal tails of α-and β-tubulin, blocks the interaction of purified CYLD with microtubules, suggesting that CYLD binds to the C-terminal tails of tubulin (Yang et al., 2015). Of note, the three CYLD CAP-Gly domains possess divergent canonical GKNDG motifs: GFTDG, GNWDG, and GCTDG, respectively, suggesting relatively low microtubule binding affinity. This could explain why CYLD is predominantly localized to the cytoplasm, with only a small portion colocalizing with microtubules. Notably, both CAP-Gly2 and CAP-Gly3 function as UBDs, and CAP-Gly3 is essential for CYLD full catalytic activity *in vitro* and CYLD-mediated NOD2 signaling in cells (Elliott et al., 2021). Interestingly, the ubiquitin-binding properties observed in CYLD CAP-Gly domains do not seem to extend to other CAP-Gly domains. This difference may be attributed to several unique structural features within CYLD's CAP-Gly domains compared to canonical CAP-Gly domains.

CYLD at the crossroads of life and death decisions

Historically, CYLD has been identified as a negative regulator of signal transduction leading to NF-κB (Trompouki et al., 2003; Brummelkamp et al., 2003; Kovalenko et al., 2003; Regamey et al., 2003) and JNK (Reiley et al., 2004; Nikolaou et al., 2012; Pannem et al., 2014) activation in response to TNF, thereby modulating the balance between cell survival and programmed cell death by apoptosis (Huyghe et al., 2023). Specifically, TNF binding to its receptor 1 (TNFR1) assembles a complex named TNF-RSC (TNF-receptor signaling complex, also called complex I), which comprises TRADD, RIPK1, TRAF2/5, and cIAP1/2 (Figure 1B). Within this complex, Lys63-ubiquitination of RIPK1 facilitates the recruitment of the linear ubiquitin chain assembly complex (LUBAC), a tripartite complex of the E3 ligases HOIP and HOIL-1, and the adaptor SHARPIN, which catalyzes Met1 ubiquitin chains, thereby enabling NF-κB activation (Tokunaga et al., 2009; Haas et al., 2009; Tokunaga et al., 2011; Gerlach et al., 2011). Notably, a subset of CYLD is bound to the LUBAC and consequently recruited to the TNFR1, where it counteracts the ubiquitination of several TNF-RSC components (Takiuchi et al., 2014; Draber et al., 2015). At a later point after following TNFR1 engagement, a secondary complex, termed complex IIa, is formed containing TRADD, FADD, cFLIP, and cysteine aspartyl protease caspase-8 (CASP8). When NF-κB is active, the transient formation of this complex is inhibited. However, if NF-κB is repressed, the inactive CASP8 homolog cFLIP levels are downregulated, driving CASP8 activation and resulting in apoptosis (Figure 1B) (Draber et al., 2015; Wang et al., 2008; Ting and Bertrand, 2016). If the early NF-κB independent checkpoint is disrupted, RIPK1 engages another signaling complex called complex IIb, together with CASP8, to initiate apoptosis (Ting and Bertrand, 2016). Inhibition or absence of CASP8 can cause RIPK1 to associate with RIPK3, activating the pore-forming protein MLKL, and ultimately triggering necroptosis, an inflammatory form of cell death (Figure 1B). In both forms of complex IIb-mediated death, RIPK1 kinase activity seems required. The balance between survival and different death modalities is tightly controlled by DUBs, which dismantle the ubiquitin network associated with these signaling complexes. By deubiquitinating RIPK1, CYLD promotes the formation of complex IIa favoring apoptosis (Figure 1B) (Draber et al., 2015; Wang et al., 2008). Moreover, CYLD's deubiquitinating activity toward RIPK1 induces TNF-mediated necroptosis, while its depletion or cleavage limits cell death (O'Donnell et al., 2011; Hitomi et al., 2008; Vanlangenakker et al., 2011; Legarda et al., 2016; Ganjam et al., 2018; Moquin et al., 2013). Additionally, CYLD downregulation or deficiency has been linked to increased cell survival in gefitinib-treated non-small cell lung cancer (Yuan et al., 2020), enhanced proliferation in skin or cervical cancer (Massoumi et al., 2006; Huang et al., 2023), and attenuated germ cell apoptosis (Wright et al., 2007), as well as TNF-mediated apoptosis in a panel of cancer cell lines (Wang et al., 2008).

Recently, CYLD has been shown to limit pyroptosis, a lytic inflammatory form of cell death triggered by various infectious and sterile insults (Yu et al., 2021). For instance, CYLD deficiency

accelerates LPS-induced pyroptosis in astrocytes (Li et al., 2019). Furthermore, CYLD restricts NLRP3 inflammasome activity by deubiquitinating PLK4 in macrophages (Yang et al., 2020) or WNK1 in an osteoporosis mouse model (Jiang et al., 2024) (Figure 1C). In estrogen receptor-positive and triple-negative breast cancer cell lines, hypoxia drives the RNF213-mediated ubiquitination and subsequent degradation of CYLD, leading to NF-κB-induced gene expression of NLRP3, which, in conjunction with hypoxia-induced endoplasmic reticulum stress, triggers pyroptosis (Bhardwaj et al., 2025). Finally, recent work proposed that CYLD could enhance the sensitivity of prostate cancer cells to ferroptosis *via* YAP deubiquitination, thereby inducing ferroptosis-related gene expression (Gu et al., 2024).

Overall, CYLD balances life and death decisions through its deubiquitinating activity. CYLD's dual role in either promoting or restricting distinct types of cell death seems to also rely on its interactions, modifications, localization, and input signal.

SPATA2 and CYLD, partners in crime

In 2016, four independent groups used mass spectrometry to characterize either the TNF-RSC or CYLD interactome and concurrently identified SPATA2 as a partner for CYLD (Elliott et al., 2016; Schlicher et al., 2016; Wagner et al., 2016; Kupka et al., 2016). SPATA2 encompasses an N-terminal PUB domain that binds a non-PIM domain in the CYLD C-terminal USP domain. SPATA2 also connects CYLD to the LUBAC *via* a PIM (in SPATA2)-PUB (in HOIP) interaction (Elliott et al., 2016). This interaction facilitates the recruitment of CYLD to the TNF-RSC and NOD2-RSC and likely to other immune signaling complexes (Elliott et al., 2016; Draber et al., 2015; Kupka et al., 2016; Hrdinka et al., 2016). *In vitro*, SPATA2 binding enhances the enzyme activity of CYLD toward Met1-and Lys63-linked ubiquitin substrates, and the loss of SPATA2 results in an increased ubiquitination of CYLD substrates (Elliott et al., 2021; Schlicher et al., 2016; Griewahn et al., 2023). Besides bridging CYLD to the LUBAC, SPATA2 competes with the Met1 DUB OTULIN for binding to HOIP, thereby balancing the activity of the LUBAC (Griewahn et al., 2023). The combined absence of SPATA2 and CYLD causes the overactivation of the LUBAC and uncontrolled inflammation and results in penetrant perinatal lethality (Griewahn et al., 2023). Of note, *Cyld*$^{-/-}$*Spata2*$^{-/-}$ MEFs exhibit reduced TNF-mediated caspase activation and apoptosis (Griewahn et al., 2023).

Whether SPATA2 contributes to all known functions controlled by CYLD is not fully understood. Interestingly, SPATA2 was initially identified through screening a human testis cDNA library expressed in the cytosol of Sertoli cells (Graziotto et al., 1999), echoing CYLD's role during spermatogenesis (Wright et al., 2007). Furthermore, similar to CYLD defect, cells silenced or knocked out for SPATA2 resist RIPK1-dependent apoptosis and necroptosis (Hitomi et al., 2008; Schlicher et al., 2016; Wagner et al., 2016; Kupka et al., 2016; Wei et al., 2017). SPATA2 deficient cells display increased Met1-linked ubiquitination of RIPK1, causing inhibition of RIPK1 kinase activity (Wei et al., 2017). Consequently, SPATA2 deficiency sensitizes mice to systemic inflammatory response syndrome (SIRS) induced by TNF (Wei et al., 2017). The impact of SPATA2 on gene induction is debated, with some reports indicating a role (Schlicher et al., 2016; Wagner et al., 2016) and others not (Elliott et al., 2016; Kupka et al., 2016) in NF-κB signaling. Additionally, SPATA2 was shown to recruit CYLD to the centrosome, thereby limiting NLRP3-dependent inflammation and cell death (Yang et al., 2020).

Phosphorylation modulates CYLD DUB activity

Several groups have reported the phosphorylation of CYLD on multiple Ser/Thr residues within a "phospho-patch" between the CAP-Gly2 and CAP-Gly3 between amino acids 418 and 444 and have highlighted a role for the phospho-acceptor Serine 418. For example, phosphorylation at S418 has been observed in response to various stimuli, including mitogens, antigen receptor ligation, TNF, LPS, stimulation of the C-type lectin receptor DC-SIGN, MDP, and constitutively in some aggressive B-cell lymphoma and Adult T-cell leukemia/lymphoma (T-ALL) samples (Elliott et al., 2021; Reiley et al., 2005; Lork et al., 2018; Xu et al., 2021; Gringhuis et al., 2014; Xu et al., 2020; Ang et al., 2021). However, caution is advised when interpreting these results, as Lork *et al*. found that the commercially used phospho-CYLD antibody also recognizes an unknown protein of the size of CYLD (Lork et al., 2018). Recently, a proteomic analysis by mass spectrometry of TNF-stimulated U2OS cells identified six serine phosphorylation sites, out of which five are within the "phospho-patch" (S392, S418, S422, S439, S444) and one in a linker region between the CAP-Gly3 and the USP domain (S568) (Elliott et al., 2021).

The phosphorylation of CYLD is mediated by both the canonical IKKβ and noncanonical IKK TBK1/IKKε, depending on the nature of the input signal. For instance, IKKβ was associated with CYLD phosphorylation following stimulation with TNF and MDP and in T-ALL (Elliott et al., 2021; Reiley et al., 2005). TBK1 and IKKε phosphorylate CYLD in T lymphocytes upon antigen receptor ligation (Lork et al., 2018), in dendritic cells stimulated with the C-type lectin receptor DC-SIGN (Gringhuis et al., 2014), and in T-ALL (Xu et al., 2020). Moreover, TBK1 and IKKε bind and phosphorylate CYLD at S418 *in vitro* and *in cellulo* (Lork et al., 2018; Friedman et al., 2008; Hutti et al., 2009). Of note, CYLD was proposed to deubiquitinate TBK1 and IKKε (Friedman et al., 2008).

The impact of phosphorylation on CYLD's DUB activity remains disputed, with reports of both downregulation and upregulation. For instance, chemical inhibition of IKKβ modulates TRAF2 ubiquitination, JNK activation, and cell death in response to TNF stimulation (Reiley et al., 2005; Ang et al., 2021). In T-ALL, CYLD phosphorylation by IKKβ and TBK1/IKKε results in its inactivation, promoting cell survival (Xu et al., 2020). A similar mechanism was proposed to explain IKKε-mediated cellular transformation (Hutti et al., 2009). Moreover, IKKε-mediated phosphorylation of CYLD at S418 also limits its DUB activity in dendritic cells after stimulation of the C-type lectin receptor DC-SIGN (Gringhuis et al., 2014). By contrast, one study shows that phosphorylation of CYLD at S418 by IKKβ increases its DUB activity (Thein et al., 2014a), while another reports that it is dispensable (Elliott et al., 2021). Interestingly, Elliott *et al.* discovered that IKKβ can phosphorylate a truncated (436–956) mutant of CYLD lacking S418 *in vitro*, increasing the Lys63 DUB

10.3389/fceld.2025.1583221

TABLE 1 CYLD regulations by PTMs shape Cell Fate.

PTM	Targeted residue	Effector	Proposed functions	References
Phosphorylation	Ser418	IKKβ	Optimal TRAF2 ubiquitination and JNK activation in HeLa and Jurkat cells	Reiley et al. (2005)
			Increased Lys63 DUB activity in hippocampal neurons	Thein et al. (2014a)
			Regulation of TNF-mediated cell death in adult dermal fibroblasts	Ang et al. (2021)
		IKKε	Limits DUB activity and promotes cellular transformation	Hutti et al. (2009)
			Reduces DUB activity in dendritic cells stimulated with DC-SIGN	Gringhuis et al. (2014)
		IKKβ, TBK1/ IKKε	CYLD is inactivated by phosphorylation in T-ALL, favoring cell survival	Xu et al. (2020)
	Ser568	IKKβ	Ser568Ala mutant exhibits reduced Lys63 DUB activity *in vitro* and *in cellulo*. Phosphorylation on Ser418 and Ser568 cooperate to promote RIPK2 deubiquitination in NOD2-stimulated U2OS cells	Elliott et al. (2021)
	Ser362, Ser418, Ser772	CAMKII	Phosphorylation promotes CYLD DUB activity in hippocampal neuron cultures	Thein et al. (2014b)
	Ser392, Ser418, Ser422, Ser439, Ser444, Ser568		Phosphorylation detected by a mass spectrometry analysis of TNF-stimulated U2OS	Elliott et al. (2021)
Ubiquitination		$SCF^{\beta-TRCP}$	Decreases CYLD abundance and participates in osteoclasts differentiation	Wu et al. (2014)
	Lys338, Lys530	MIB2	Proteasomal degradation, leading to increased TNF-mediated NF-κB activation	Uematsu et al. (2019)
		RNF213	Lysosomal degradation driving pyroptosis under hypoxia	Bhardwaj et al. (2025)
SUMOylation	Lys40	UBC9	Decreased Lys63 DUB activity in neuroblastoma cells and GSCs	Kobayashi et al. (2015), Chen et al. (2022)
Proteolysis	Asp215	CASP8	Inactivation and proteasomal degradation of CYLD C-terminal fragment, blocking TNFα-induced necroptosis in fibroblasts	O'Donnell et al. (2011)
			CYLD is removed in TLR4-stimulated macrophages, preventing TNFα-mediated necroptosis	Legarda et al. (2016)
			CYLD inactivation, increased RIG-I and TAK1 ubiquitination, enhancing innate antiviral immunity in Influenza A virus-infected epithelial cells	Yu et al. (2024)
			Generation of a CYLD N-terminal fragment (25 kDa)	O'Donnell et al. (2011), Legarda et al. (2016), Yu et al. (2024)
			Loss of CYLD, reduced Met1-deubiquitination on P65, increased NF-κB activation, enhancing LPS-induced endotoxic shock in mice	Liu et al. (2024)
			CYLD N-terminal fragment is secreted in the serum of septic mice, serving as an inflammatory biomarker	
	Arg324	MALT1	CYLD inactivation, required for TCR-triggered JNK activation in lymphocytes	Staal et al. (2011)
			Proteolysis observed in lymphocytes following TCR engagement	Douanne et al. (2016), Gewies et al. (2014)

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TABLE 1 (*Continued*) CYLD regulations by PTMs shape Cell Fate.

PTM	Targeted residue	Effector	Proposed functions	References
			Proteolysis observed in DLBCL	Fontan et al. (2012), Douanne et al. (2016), Minderman et al. (2023), Wimberger et al. (2023)
			Proteolysis observed in MCL	Dai et al. (2017), Minderman et al. (2023)
			Proteasomal degradation of CYLD N- and C-terminal fragments, suggesting increased NF-κB activation and lymphoma cell proliferation	Minderman et al. (2023)
			Proteolysis observed in GSCs	Jacobs et al. (2020)
			Thrombin-mediated CYLD cleavage by MALT1 initiating microtubule disruption, altering endothelial structural integrity	Klei et al. (2016)

activity of CYLD (Elliott et al., 2021). Conversely, a S568A mutant exhibited reduced Lys63 DUB activity. Nonetheless, the phosphorylation of both S418 and S568 is required for optimal signaling, and their combined mutation caused the accumulation of ubiquitinated species of RIPK2 in cells stimulated with NOD2. As a consequence, the production of cytokines was increased (Elliott et al., 2021) (Table 1).

CYLD, a DUB ubiquitinated itself

Though specialized in removing ubiquitin chains, CYLD is itself subject to ubiquitination. While examining the interaction between CYLD and a panel of E3 ligases in an overexpression system in HEK293T, CYLD was found to interact with the beta-transducin repeat-containing protein Skp1-Cullin-F-box protein ($SCF^{\beta-TRCP}$). Further analysis in this system revealed that $SCF^{\beta-TRCP}$ ubiquitinates CYLD to modulate its abundance, though the precise ubiquitination sites are not identified. Depletion of endogenous IKKα or IKKβ by shRNAs led to an accumulation of CYLD, while $SCF^{\beta-TRCP}$-mediated CYLD ubiquitination was lost on S432A/S436A mutants, suggesting that phosphorylation is a prerequisite to ubiquitination (Wu et al., 2014). This regulatory mechanism was further explored in the context of bone differentiation, where $SCF^{\beta-TRCP}$ was found to regulate osteoclast differentiation by modulating CYLD levels, thereby influencing NF-κB activation in response to receptor activator NF-κB ligand (RANKL) stimulation (Wu et al., 2014).

CYLD stability is also controlled by the mind bomb homologue 2 (MIB2) E3 ligase (Uematsu et al., 2019). Overexpression experiments in HEK293T identified that the ankyrin repeat in MIB2 interacts with the third CAP-Gly domain of CYLD. Immunoprecipitation combined with the use of ubiquitin linkage-specific antibodies revealed that MIB2 decorated CYLD with Lys48-linked chains, driving its proteasomal degradation. Mutagenesis identified the acceptor lysines as Lys338 and Lys530. MIB2-dependent CYLD degradation led to an increase in NF-κB activation in response to TNF stimulation. In keeping with this, MIB2-knockout mice exhibited suppressed inflammatory responses in a K/BxN serum transfer arthritis model (Uematsu et al., 2019). A recent study identified the E3 ligase RNF213 as responsible for ubiquitinating CYLD and SPATA2 in hypoxic tumors (Bhardwaj et al., 2025). The use of a recombinant Lys63Arg mutant, which resists Lys63 ubiquitination, confirmed the involvement of this linkage, though the precise modification sites were not determined. This ubiquitination promoted the lysosomal degradation of CYLD and SPATA2, leading to decreased levels of both proteins, initiating pyroptotic cell death (Bhardwaj et al., 2025).

In addition to ubiquitination, CYLD undergoes SUMOylation. Small ubiquitin-like modifiers (SUMOs) are members of the ubiquitin-like protein family that, similar to ubiquitin, can be covalently conjugated to substrate proteins, altering their fate. In neuroblastoma cells, retinoic acid treatment induces CYLD SUMOylation (Kobayashi et al., 2015). Analysis using a SUMO predictor program combined with mutagenesis revealed that CYLD is modified on Lys40 outside of the USP domain. *In vitro* DUB assays and immunoprecipitation experiments revealed that CYLD SUMOylation impairs its catalytic activity against both free ubiquitin and substrate proteins, including TRAF6 and TRAF2. Interestingly, overexpression of a non-SUMOylatable CYLD mutant impaired retinoic acid-induced NF-κB activation and differentiation in neuroblastoma cells, instead promoting cell death (Kobayashi et al., 2015). More recently, CYLD was shown to be SUMOylated by UBC9 at Lys40 in a glioblastoma stem-like cells (GSCs) model. Consistent with previous findings, this modification impaired CYLD activity, resulting in excessive Lys63-linked polyubiquitination of NF-κB signaling intermediaries (TRAF2, TRAF6, RIPK1, and NEMO). This hyperactivation of NF-κB promoted the proneural-to-mesenchymal transition (PMT), a critical phenotypic shift in GSCs (Chen et al., 2022) (Table 1).

CYLD irreversible modification by proteolysis

Proteolysis, unlike other PTMs, is irreversible, leading to significant effects on activity, interaction, and localization of the targeted protein. In 2011, the Ting group first reported CYLD cleavage by CASP8 at aspartic residue 215 (between CAP-Gly1 and 2) following TNF stimulation in fibroblasts. This proteolysis inactivated CYLD and promoted degradation of its C-terminal fragment, consequently blocking cell death by necroptosis. Mutation of the CYLD cleavage site promotes RIPK1 deubiquitination and interaction with the necrosome complex, leading to cell death (O'Donnell et al., 2011). A few

years later, the same group extended this knowledge to macrophages, showing that CASP8-mediated CYLD cleavage following TLR4 stimulation prevents TNF-mediated necroptosis (Legarda et al., 2016). However, knock-in mice expressing non-cleavable CYLD D215A are viable, suggesting that CYLD cleavage by CASP8 is dispensable for necroptosis (Newton et al., 2019). More recently, CYLD processing by CASP8 was observed upon Influenza A virus infection in epithelial cells, likely enhancing innate antiviral immunity (Yu et al., 2024). A preprint highlighted a new role for CYLD processing by CASP8, showing that mice expressing the CASP8-resistant CYLD mutant present resistance to LPS-induced endotoxic shock by inhibiting NF-κB pathway activation and transcription of NLRP3 inflammasome. Interestingly, this work also suggests that CYLD N-terminal fragment following CASP8 cleavage is secreted outside the cells and may serve as a biomarker (Liu et al., 2024).

CYLD is also processed at the evolutionary conserved site Arg324 (between CAP-Gly2 and CAP-Gly3) by the MALT1 paracaspase, a caspase-like protein that regulates the activation of immune cells (Jaworski and Thome, 2016; Thys et al., 2018). CYLD is cleaved by MALT1 in lymphocytes following antigen receptor activation, in a subset of aggressive B-cell diffuse large B-cell lymphoma (DLBCL), in mantle cell lymphoma (MCL) (Staal et al., 2011; Fontan et al., 2012; Douanne et al., 2016; Dai et al., 2017; Minderman et al., 2023; Wimberger et al., 2023), and in GSC patient-derived samples (Jacobs et al., 2020). However, the exact role of CYLD cleavage by MALT1 remains elusive. Jurkat T lymphocytes overexpressing a MALT1-resistant CYLD mutant (Arg324Ala) display reduced JNK signaling and interleukin-2 production upon T-cell receptor ligation (Staal et al., 2011). However, JNK is normally activated in cells from mice expressing catalytically dead MALT1 (Gewies et al., 2014). MALT1 proteolysis has also been proposed to favor the degradation of N-terminal and C-terminal fragments, inhibiting CYLD activity, and suggesting a role in lymphoma cell growth (Minderman et al., 2023). Besides this role in immune cells, thrombin-induced MALT1-dependent CYLD cleavage has been presented to initiate microtubule disruption, altering endothelial barrier integrity (Klei et al., 2016). Altogether, CYLD proteolysis appears to depend on cell type and input signal. Nevertheless, the fate of the cleavage fragments is still enigmatic in terms of localization, interaction, and activity (Table 1).

Future perspectives

CYLD is a versatile DUB involved in numerous cellular processes. In this regard, CYLD is tightly regulated at multiple levels, including subcellular localization, interaction with various partners, and both translational and post-translational stages. However, several crucial questions remain unanswered. For instance, the complete landscape of CYLD's PTMs has yet to be mapped. Specific cell death programs, in which the role of CYLD has not been assigned, are known to involve palmitoylation, oxidation, or caspase-independent proteolysis. Assessing the contribution of lysosomal proteases, calpains, or granzymes may yield interesting findings. Another important aspect to explore is the precise impact of PTMs on CYLD's enzyme activity, proximitome, or location. In addition, understanding the potential interplay between PTMs and the cognate CYLD partner SPATA2 represents a compelling avenue for further exploration. Introducing an additional layer of complexity, PTMs may operate hierarchically, where one modification serves as a prerequisite for another. This seems to be the case for certain CYLD ubiquitination events that depend on prior phosphorylation. Investigating these cooperative interactions between PTMs will undoubtedly enhance our understanding of CYLD function and unveil novel layers of regulation.

Author contributions

YM: Writing – original draft, Writing – review and editing. NB: Funding acquisition, Writing – original draft, Writing – review and editing. TD: Writing – original draft, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was funded by Fondation ARC contre le Cancer (NB), Institut National Du Cancer (INCa_18384), Ligue nationale contre le cancer (Equipe labellisée) et comités de Loire-Atlantique, Maine et Loire, Vendée, Ille-et-Vilaine, Mayenne, and Finistère (NB). YM received a fellowship from Ligue Nationale contre le Cancer. The team is part of the SIRIC ILIAD (INCA-DGOS-INSERM-ITMO Cancer_18011). TD received support from the Bettencourt Schueller Foundation as an awardee of the Young Researcher Prize.

Acknowledgments

We apologize to those whose important work could not be cited owing to space constraints.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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