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Review

Title

Role of linear ubiquitination in inflammatory responses and tissue homeostasis

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Abstract

Polyubiquitination is a post-translational modification involved in a wide range of immunological events, including inflammatory responses, immune cell differentiation, and development of inflammatory diseases. The versatile functions of polyubiquitination are based on different types of ubiquitin linkage, which enable various UBD (ubiquitin binding domain)-containing adaptor proteins to associate and induce distinct biological outputs. A unique and atypical type of polyubiquitin chain comprising a conjugation between the N-terminal methionine of the proximal ubiquitin moiety and the C-terminal glycine of the distal ubiquitin moiety, referred to as a linear or M1-linked ubiquitin chain, has been studied exclusively within the field of immunology because it is distinct from other polyubiquitin forms: linear ubiquitin chains are generated predominantly by various inflammatory stimulants, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and act as a critical modulator of transient and optimal signal transduction. Moreover, accumulating evidence suggests that linear ubiquitin chains are of physiological significance. Dysregulation of linear ubiquitination triggers chronic inflammation and immunodeficiency via downregulation of linear ubiquitin-dependent nuclear factor-kappa B (NF-κB) signaling and by triggering TNF-α-induced cell death, suggesting that linear ubiquitination is a homeostatic regulator of tissue-specific functions. In this review, we focus on our current understating of the molecular and cellular mechanisms by which linear ubiquitin chains control inflammatory environments. Furthermore, we review the role of linear ubiquitination on T cell development, differentiation, and function, thereby providing insight into its direct association with maintaining the immune system.

Running title: Optimal inflammation via linear ubiquitination

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Introduction

Ubiquitin was identified originally as a critical modifier of energy-dependent proteasomal degradation of discarded intracellular proteins. Accumulating evidence has shown the versatility of ubiquitin modification during various cellular physiological processes, including the cell cycle, DNA repair, and signal transduction. Ubiquitin conjugation occurs in three sequential steps, which are catalyzed by specialized enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin conjugating enzyme (E2), and a ubiquitin ligase (E3) (1). Binding of a ubiquitin to a substrate protein, followed by elongation to initiate conjugation of another ubiquitin to the substrate, generates a polyubiquitinated protein. A distinct inter-ubiquitin linkage can increase structural diversity of polyubiquitin chains, which allows a variety of ubiquitin chain-specific UBD (ubiquitin binding domain)-containing adaptor proteins to interact with them, resulting in expansion of ubiquitin-dependent biological outputs (2) (Fig. 1A). In general, one of seven Lys residues within ubiquitin (K6, K11, K27, K29, K33, K48, and K63) act as an acceptor for another ubiquitin. However, this review highlights a newly identified atypical form of polyubiquitin generated by conjugation between the N-terminal methionine (M1) of the proximal ubiquitin moiety and the C-terminal glycine of the distal ubiquitin moiety; this is referred to as a linear or M1-linked ubiquitin chain (3) (Fig. 1A).

The well-known K48- or K63-linked ubiquitin chains, which are the main promoters of protein degradation and cellular signaling, respectively, occupy the majority of intracellular ubiquitin chains; linear ubiquitin is hardly detectable under stable (unstimulated) conditions. Notably, linear ubiquitin production is induced by the linear ubiquitin assembly complex (LUBAC), the only recognized E3 ligase that generates linear ubiquitin chains, in response to inflammatory stimulants such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (4) (Fig. 1A). In general, polyubiquitin modification is spatially and temporally controlled by the cooperative reaction between an E3 ligase (as a writer) and a deubiquitinating enzyme (DUB; as an eraser), which cleaves the

conjugated ubiquitin chains. We already know the DUBs responsible for linear ubiquitin cleavage: OTU deubiquitinase with linear linkage specificity (OTULIN), and cylindromatosis (CYLD) (5,6). Such strictly-regulated and reversible linear ubiquitination in specified immune-related cells provides a substantial benefit with respect to both optimal expression of genes encoding cytotoxic inflammatory molecules and immediate remission of undesired inflammatory reactions.

Molecular mechanism underlying linear ubiquitination

LUBAC, the only E3 ligase to catalyze linear ubiquitination, comprises three distinct subunits: HOIL-1L interacting protein (HOIP, also known as RNF31), heme-oxidized IRP2 ligase 1L (HOIL-1L, also known as RBCK1), and SHANK-associated RH domain-interacting protein (SHARPIN) (7-9) (Fig. 1B). Gel filtration studies estimate the molecular mass of LUBAC to be 600 kDa, but the summed mass of the three subunits is actually 218 kDa. Thus, although the molecular mechanism responsible for assembly of the ligase, which is mediated by interactions between their binding domains (the ubiquitin-like domains (UBL) of HOIL-1L and SHARPIN, the ubiquitin-associated (UBA) domain of HOIP, and the LUBAC-tethering motifs (LTM) of HOIL-1L and SHARPIN), has been clarified (10) (Fig. 1B), the exact conformation of intracellular LUBAC remains unknown. Expression of LUBAC components is ubiquitous in humans and rodents. In particular, previous reports show high level expression of LUBAC components in murine splenocytes and thymocytes. According to the genome-wide gene expression analysis across immune cells, Immunological Genome Project (ImmGen), there is almost no difference in the expression among subsets of immune cells including hematopoietic stem cells (HSCs), lymphocytes and myeloid cells. Although there is little information about human immune cells, these are indicative of the major role of LUBAC during generation and maintenance of the adaptive immune system (8).

The catalytic center of LUBAC is the C-terminal RING-IBR-RING (RBR) domain of HOIP (Fig. 1B). Although HOIL-1L and SHARPIN, accessory molecules of LUBAC, are dispensable for linear ubiquitination activity, they stabilize the tripartite LUBAC complex. Loss of either results in rapid degradation of other LUBAC components, including HOIP, and decreases ligase activity for linear ubiquitination. The RBR domain includes two RING domains: N-terminal RING1 and C-terminal RING2. HOIP interacts with ubiquitin-bound E2 at RING1, and transfers the ubiquitin from E2 to the conserved Cys residue (Cys885 in human) in RING2 to form a transient thioester intermediate. Then, C-terminal Gly of ubiquitin is transferred to the N-terminal Met of the acceptor ubiquitin that is docked on the linear ubiquitin chain-determining domain (LDD) at the C-terminus of HOIP (11,12) (Fig. 1B). HOIL-1L also has a similar RBR domain. A recent report shows that HOIL-1L ligase activity catalyzes formation of oxyester bonds between the C-terminal carboxylate of ubiquitin and the Ser and Thr residues of its substrates IRAK1, IRAK2, Myd88, and LUBAC, which accelerates Toll-like receptor signaling (13). In addition, our study revealed a novel regulatory mechanism by which HOIL-1L-catalyzing monoubiquitination of LUBAC subunits regulates LUBAC activity, leading to suppression of the linear ubiquitination activity of HOIP (14).

Linear ubiquitination in response to TNF-α signaling

TNF-α is a pivotal regulator of local immune response and its surrounding inflammatory environment. TNF-α enables to induce canonical nuclear factor-kappa B (NF-κB) activation signaling involving the IκB kinase (IKK) complex (comprising IKK1 (IKKα), IKK2 (IKKβ), and NF-κB essential modulator (NEMO, IKKγ)). The positive effects of LUBAC-producing linear ubiquitin on this pathway have been characterized extensively. Binding of TNF-α to its receptor TNFR1 triggers transient assembly of the signaling complex referred to as TNFR1 complex I, which initiates downstream signaling. TNFR1 complex I comprises multiple adaptor proteins, including TNFR1-associated death domain

(TRADD), TNF-receptor associated factor 2 (TRAF2), cellular inhibitor of apoptosis protein 1 and 2 (cIAP1 and cIAP2), and receptor interacting serine/threonine-protein kinase 1 (RIPK1). The cIAP1/2 E3 ligases conjugate K63-, K11-, and K48-linked ubiquitin chains onto RIPK1 and several components of the TNFR1 complex I. The polyubiquitin chains further serve as a scaffold to recruit other signal intermediate complexes, including LUBAC, through K63 ubiquitin binding via the NZF domains in HOIP and SHARPIN (15,16). Conjugation of LUBAC-generated linear ubiquitin chains to the TNFR1 complex I, cooperatively with other types of polyubiquitin chains, activates signaling cascades.

In addition to LUBAC, the IKK complex and the TAK1-TAB complex, which comprises transforming growth factor-β-activated kinase 1 (TAK1), TAK1-binding protein 1 (TAB1), and either TAB2 or 3, are also recruited to the polyubiquitin structure on the TNFR1 complex I via the C-terminal zinc finger (ZF) domain of NEMO and the Npl4 zinc finger (NZF) domain of TAB2/3, respectively. Linear ubiquitination of TNFR1 complex I components such as RIPK1 facilitates accumulation of other LUBACs via preferential binding of the NZF domains of SHARPIN and HOIL-1L to linear ubiquitin chains (7,17). In addition, LUBAC interacts with NEMO through the HOIP NZF1 domain and generates linear ubiquitin chains on NEMO (18). NEMO also contains ubiquitin binding ABIN and NEMO (UBAN) motifs, which interact with linear ubiquitin with much higher affinity than K63 ubiquitin (19). In addition to IKK2 phosphorylation by TAK1 sequestered onto K63 chains, linear ubiquitin-dependent accumulation of several IKK complexes triggers dimerization of IKK2, followed by its activation by trans-autophosphorylation (15,20). The activated IKK complex then induces phosphorylation of inhibitor of NF-κB proteins (IκB), leading to activation of NF-κB signaling. Since loss of LUBAC dampens expression of NF-κB-inducible genes, LUBAC-mediated linear ubiquitination is critical for amplification of NF-κB signaling in response to TNF-α (18).

OTULIN and CYLD, DUBs responsible for cleavage of linear ubiquitin, negatively regulate TNF-α-induced activation of NF-κB (6). While both is constitutively expressed in most cells including all immune cell subsets, expression of CYLD is further increased by TNF-α and IL-1β in the NF-κB signaling-dependent manner. In addition to the inflammatory cytokines, a variety of NF-κB inducers including peptidoglycan, Gram-negative bacterium *Haemophilus influenzae*, and Gram-positive bacterium *Streptococcus pneumoniae* potentiates expression of CYLD, indicating that CYLD acts as a negative feedback regulator for NF-κB activation upon various inflammatory simulation (21). CYLD cleaves both linear and K63 ubiquitin, whereas OTULIN appears to be specific for linear ubiquitin (5). OTULIN includes an N-terminal PUB-interacting motif (PIM), which interacts with the N-terminal peptide N-glycosidase/ubiquitin-associated (PUB) domain of HOIP (6,22). Phosphorylation of Tyr56 in the PIM of OTULIN negatively regulates binding to HOIP, suggesting that linear ubiquitination is regulated by an unknown tyrosine kinase-dependent mechanism. Although reversible ubiquitination by LUBAC and DUBs coordinately optimizes the strength and duration of TNF-α signals, removal of linear ubiquitin chains by OTULIN maintains the integrity of LUBAC for linear ubiquitination (23).

Regulatory role of LUBAC during extrinsic cell death

SHARPIN is the third component of LUBAC, and a causative gene of spontaneous autosomal recessive mutant mice, referred to as chronic proliferative dermatitis mice (cpdm) (8,9) (Fig. 2). These mice develop severe chronic inflammation of the skin, which is characterized by epidermal hyperplasia, hyperkeratosis, and increased programmed cell death of keratinocytes. Moreover, infiltration of the skin, multiple organs (the lungs and liver), and several joints by granulocytes and macrophages is observed (24). Lymphocytes are dispensable for disease development because lymphocyte-lacking cpdm mice also exhibit a similar phenotype. Skin-specific deletion of the *Sharpin*

gene induces dermatitis, whereas skin-specific deletion of the *Tnfr1* gene ameliorates disease development (25-27). Since loss of SHARPIN results in a marked decrease in expression of HOIL-1L and HOIP, LUBAC activity in keratinocytes is critical for maintenance of skin homeostasis and constitutive TNF-α-mediated responses (Fig. 2). In addition, complete loss of HOIL-1L or HOIP results in embryonic lethality at mid-gestation via increased TNFR1-mediated endothelial cell death (28,29). Thus, these *in vivo* data suggest that LUBAC-mediated suppression of programmed cell death, rather than NF-κB activation, would be more requisite for TNF-α-mediated homeostatic processes.

LUBAC-mediated linear ubiquitination protects against TNF-α-induced apoptotic and necroptotic cell death independent of NF-κB activation. Upon LUBAC deficiency, TNF-α stimulation results in release of RIPK1 from the TNFR1 complex I to yield a cytosolic TNFR1 complex II (30). Generation of complex II is critical for induction of programmed cell death. Complex II comprises RIPK1, Fas-associated death domain protein (FADD), cellular FADD-like IL-1β-converting enzyme (FLICE)-like inhibitory protein (cFLIP), caspase-8, RIPK3, and mixed lineage kinase domain-like protein (MLKL). The complex II exerts two distinct modes of programmed cell death: caspase 8-dependent apoptosis and RIPK3-MLKL-dependent necroptosis, a recently identified form of programmed necrotic cell death. We observed both modes of keratinocyte cell death in autoinflammatory or autoimmune skin disease models; therefore, different types of TNF-α-inducible cell death occur simultaneously *in vivo* (27). Regulation of complex II-dependent cell death pathways in each cell is dependent on expression or activity of cell death executers or suppressors.

We do not know how LUBAC-mediated linear ubiquitination protects from TNF-α-induced apoptotic and necroptotic cell death. In addition to LUBAC deficiency, treatment with cIAP inhibitors promotes programmed cell death in response to TNF-α. Moreover, recent reports show that K63 ubiquitination of RIPK1 is requisite for prevention of TNF-α-induced cell death, and *Ripk1*$^{K376R/K376R}$ knock-in mice, in which K63 ubiquitination of RIPK1 is impaired, show embryonic lethality due to

increased expression of complex II (31). RIPK1 kinase activity regulates transition from TNFR1 complex I to complex II. K63 ubiquitination of RIPK1 recruits TAK1 to phosphorylate RIPK1, leading to inhibition of its kinase activity (32). RIPK1 kinase activity is also controlled by kinases such as the IKK complex and MK2 (33-36). Notably, TBK1 and IKKε are newly identified kinases of RIPK1 (37). Upon TNF-α stimulation, NEMO, which recognizes linear ubiquitin chains via its UBAN domain, recruits TBK1 and IKKε to the TNFR1 complex via adaptor proteins TANK and NAP1. This mechanism demonstrates, at least partly, linear ubiquitin-dependent protection from TNF-α-induced cell death.

Effect of LUBAC on T cell receptor (TCR) signaling and T cell-mediated immunity

LUBAC-compromised mice exhibit severe immunodeficiency, and LUBAC components are highly expressed by lymphocytes, suggesting involvement of LUBAC-mediated linear ubiquitination in immune homeostasis. In this section, we focus specifically on the significance of linear ubiquitin with respect to T cell biology. In general, T cells recognize antigen peptide-bound major histocompatibility complex molecules on the surface of target cells through their variable TCRs. LUBAC is essential for TCR-mediated NF-κB signaling and subsequent T cell activation because LUBAC deficiency in T cell hybridoma and Jurkat cells decreases expression of NF-κB-target genes, as well as secretion of IL-2, upon TCR stimulation (27). In addition, TCR activation-induced phosphorylation of RelA, which is a component of NF-κB transcription factors, and degradation of IκBα, are slightly inhibited in murine T cells isolated from *Sharpin*-deficient mice, resulting in reduced surface expression of CD25 and CD69, both of which are surface markers of T cell activation (27).

After peptide antigen recognition by the TCR, tyrosine kinases such as Lck and ZAP70, as well as adaptor proteins, are recruited to mediate downstream signaling. Then, PKCθ phosphorylates CARMA1 and promotes assembly of the CARMA1-BCL10-MALT1 (CBM) complex, followed by

its recruitment to the cell membrane. The CBM complex binds to HOIP in LUBAC, resulting in linear ubiquitination of CBM components (38). In addition to linear chains, K63 ubiquitin is also conjugated to BCL10 in the CBM complex. Regarding the role of RIPK1 during TNF-α signaling, linear and K63 ubiquitin chains on the CBM complex serve as a platform for recruitment of the IKK complex via the ubiquitin binding ability of NEMO, followed by NF-κB activation (39). However, negative regulation of TCR signaling also occurs. MALT1, which has paracaspase activity, mediates proteolytic cleavage of HOIL-1L to downregulate TCR-mediated activation of NF-κB (40). Notably, and in contrast to previous observations, our data and those of others show that ubiquitin binding, but not the linear ubiquitin ligase ability of LUBAC, is indispensable for full activation of NF-κB signaling upon TCR stimulation (27,41). Thus, LUBAC is a critical signal mediator, although its precise role in TCR-mediated NF-κB activation remains elusive.

TCR signaling contributes to T cell development, differentiation, and effector function. A decrease in the mature Foxp3^{+} regulatory T cell (Treg) population, an anti-inflammatory T cell subset, is found in cpdm and T cell-specific SHARPIN-deficient mice (27,42). Since SHARPIN partially contributes to the stability of the LUBAC conformation, as well as its ligase activity, these observations indicate that Treg development and homeostasis are highly dependent on LUBAC (Fig. 2). The high LUBAC dependency of Tregs is not surprising because Tregs require relatively strong TCR stimulation during development in the thymus and are maintained in peripheral tissues by autocrine IL-2 stimulation. The absence of HOIL-1L or HOIP (resulting in near- or complete loss, respectively, of LUBAC) results in severe depletion of Tregs. Notably, Treg-specific deletion of HOIP-encoding *Rnf31* causes systemic autoimmune disease due to severe Treg loss and hyperactivation of peripheral conventional T cells, which results in all of the phenotypic hallmarks of Foxp3-deficient scurfy mice (27,42). To a lesser extent, development of Foxp3^{-} conventional T cells is also impaired gradually, along with a decline in LUBAC expression. During the late stage of

thymocyte differentiation, LUBAC is required for appropriate gene expression, but not for protection from TNF-α-induced cell death. Additionally, the proinflammatory effector function of T cells is dependent on strong TCR activation; thus LUBAC plays a wide role in T cell mediated immunity.

Our recent publication focused on the function of LUBAC in skin tissue homeostasis (27) (Fig. 2). Specific ablation of *Sharpin* in Tregs mimics the cpdm phenotype characterized by skin inflammation, suggesting that partial activation of autoimmune T cell subset facilitates TNF-α-mediated keratinocyte apoptosis and necroptosis via an innate immune mechanism, despite sufficient expression of LUBAC components in the skin. Moreover, loss of SHARPIN from both Tregs and skin cells results in more severe disruption of skin architecture, accompanied by abundant T cell infiltrates, than that observed in mice lacking SHARPIN in Tregs or keratinocytes. These observations reaffirm that LUBAC plays multiple roles in various cell types, and contributes to maintenance of physiological skin homeostasis in healthy individuals by regulating both T cell-associated immune balance and tissue tolerance to proinflammatory cytokine-induced cell death (Fig. 2).

Role of linear ubiquitin in human immunological diseases

Whole exome sequencing of clinical samples revealed that LUBAC and linear ubiquitin-related genes cause autoinflammatory diseases. Autoinflammation is an inherited, and mostly monogenic, disorder characterized by recurrent fever and sterile systemic inflammation. An early study showed that biallelic loss-of-expression and loss-of-function mutations in HOIL-1L are the cause (43). Such patients develop chronic autoinflammation, invasive bacterial infections, and muscular amylopectinosis. Fibroblasts from patients show impaired NF-κB activation in response to IL-1β. Two cases of homozygous mutations in HOIP have been reported (44,45). The biallelic missense L72P mutation in HOIP destabilizes the LUBAC complex, resulting in severe hypomorphic expression. Patients exhibit multiorgan autoinflammation, combined immunodeficiency, subclinical

amylopectinosis, and systemic lymphangiectasia. Another case of HOIP deficiency due to compound heterozygous mutations in *RNF31* presented with early-onset immune deficiency and autoinflammation. Considering that fibroblasts from these patients show reduced expression of LUBAC coupled with decreased activation of NF-κB upon IL-1β or TNF-α stimulation, systemic accumulation of cytokine-induced cell death is likely the main cause of autoinflammation.

Dysfunction or hypomorphic expression of OTULIN, a linear ubiquitin-specific DUB, also results in TNF-α-induced systemic inflammatory disease in humans. Nine patients carrying homozygous missense or premature stop mutations in the *OTULIN* have been reported, and all suffered from systemic autoinflammation, termed OTULIN-related autoinflammatory syndrome (ORAS) or Otulipenia (46-49). The disease is characterized by recurrent fever, diarrhea, panniculitis, and arthritis, accompanied by an increase in leucocyte and neutrophil numbers during the neonatal period. Fibroblasts and B cells harboring heterozygous missense variants of *OTULIN* exhibit lower expression OTULIN and higher production of linear ubiquitin than normal cells (50). As mentioned above, LUBAC induces cytokine-induced cellular responses and inflammation. Therefore, it has been hypothesized that hyperactivation of a wide range of immune cell types, and increased systemic secretion of inflammatory cytokines, cause sterile autoinflammation in ORAS patients. Intriguingly, OTULIN enables trimming of the linear ubiquitin chains conjugated to LUBAC subunits to maintain its function. Auto-linear ubiquitination of LUBAC subunits is detected in OTULIN-deficient cells, and attenuates its function (23). Although we do not know whether such interruption of LUBAC-mediated linear ubiquitination occurs in ORAS patients, accelerated programmed cell death may contribute to pathogenesis by inducing a mechanism similar to that which causes LUBAC-deficient autoinflammation.

Conclusions and perspectives

Here, we provide an overview of the mechanism(s) underlying linear ubiquitination, and describe its function *in vivo*. In addition to the TNF-α or T cell-specific immune signals mentioned above, linear ubiquitin chains are generated by other extrinsic inflammatory ligands to regulate several physiologic conditions. For a long time, studies on linear ubiquitin and LUBAC subunits focused on inflammatory responses; however, roles including xenophagy, cell cycle, protein homeostasis, and glycogen metabolism have been discovered (51-56). This encouraged us to explore the biological connection between LUBAC ligases and other research fields. A lack of linear ubiquitin chains can cause systemic diseases, suggesting that it plays a significant role in maintenance and protection of physiologic tissue environments with low concentrations of linear ubiquitin-producing cytokines. Although it is obvious that linear ubiquitin is requisite for homeostasis in healthy tissues and organs, its pathogenic contribution to various undesired chronic inflammatory events during autoinflammation or autoimmune disease, chronic infection, and tumorigenesis remains unclear because there are few methods that can detect linear ubiquitin chains *in vivo* in real-time. Multifaced observations of linear ubiquitin chains and their function would allow us to better understand their precise contribution to pathogenesis or remission of inflammatory diseases.

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References

1 Scheffner, M., Nuber, U., and Huibregtse, J. M. 1995. Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. *Nature.* 373:81.

2 Yau, R. and Rape, M. 2016. The increasing complexity of the ubiquitin code. *Nat Cell Biol.* 18:579.

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

4 Sasaki, K. and Iwai, K. 2015. Roles of linear ubiquitinylation, a crucial regulator of NF-κB and cell death, in the immune system. *Immunol Rev.* 266:175.

5 Keusekotten, K., Elliott, P. R., Glockner, L., Fiil, B. K., Damgaard, R. B., Kulathu, Y., Wauer, T., Hospenthal, M. K., Gyrd-Hansen, M., Krappmann, D., Hofmann, K., and Komander, D. 2013. OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell.* 153:1312.

6 Takiuchi, T., Nakagawa, T., Tamiya, H., Fujita, H., Sasaki, Y., Saeki, Y., Takeda, H., Sawasaki, T., Buchberger, A., Kimura, T., and Iwai, K. 2014. Suppression of LUBAC-mediated linear ubiquitination by a specific interaction between LUBAC and the deubiquitinases CYLD and OTULIN. *Genes Cells.* 19:254.

7 Ikeda, F., Deribe, Y. L., Skanland, S. S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M., van Wijk, S. J., Goswami, P., Nagy, V., Terzic, J., Tokunaga, F., Androulidaki, A., Nakagawa, T., Pasparakis, M., Iwai, K., Sundberg, J. P., Schaefer, L., Rittinger, K., Macek, B., and Dikic, I. 2011. SHARPIN forms a linear ubiquitin ligase complex regulating NF-κB activity and apoptosis. *Nature.* 471:637.

8 Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sakata, S., Tanaka,

348 K., Nakano, H., and Iwai, K. 2011. SHARPIN is a component of the NF-κB-activating
349 linear ubiquitin chain assembly complex. *Nature.* 471:633.
350 9 Gerlach, B., Cordier, S. M., Schmukle, A. C., Emmerich, C. H., Rieser, E., Haas, T. L.,
351 Webb, A. I., Rickard, J. A., Anderton, H., Wong, W. W., Nachbur, U., Gangoda, L.,
352 Warnken, U., Purcell, A. W., Silke, J., and Walczak, H. 2011. Linear ubiquitination
353 prevents inflammation and regulates immune signalling. *Nature.* 471:591.
354 10 Fujita, H., Tokunaga, A., Shimizu, S., Whiting, A. L., Aguilar-Alonso, F., Takagi, K.,
355 Walinda, E., Sasaki, Y., Shimokawa, T., Mizushima, T., Ohki, I., Ariyoshi, M., Tochio, H.,
356 Bernal, F., Shirakawa, M., and Iwai, K. 2018. Cooperative Domain Formation by
357 Homologous Motifs in HOIL-1L and SHARPIN Plays A Crucial Role in LUBAC
358 Stabilization. *Cell Rep.* 23:1192.
359 11 Smit, J. J., Monteferrario, D., Noordermeer, S. M., van Dijk, W. J., van der Reijden, B. A.,
360 and Sixma, T. K. 2012. The E3 ligase HOIP specifies linear ubiquitin chain assembly
361 through its RING-IBR-RING domain and the unique LDD extension. *EMBO J.* 31:3833.
362 12 Stieglitz, B., Rana, R. R., Koliopoulos, M. G., Morris-Davies, A. C., Schaeffer, V.,
363 Christodoulou, E., Howell, S., Brown, N. R., Dikic, I., and Rittinger, K. 2013. Structural
364 basis for ligase-specific conjugation of linear ubiquitin chains by HOIP. *Nature.* 503:422.
365 13 Kelsall, I. R., Zhang, J., Knebel, A., Arthur, J. S. C., and Cohen, P. 2019. The E3 ligase
366 HOIL-1 catalyses ester bond formation between ubiquitin and components of the
367 Myddosome in mammalian cells. *Proc Natl Acad Sci U S A.* 116:13293.
368 14 Fuseya, Y., Fujita, H., Kim, M., Ohtake, F., Nishide, A., Sasaki, K., Saeki, Y., Tanaka, K.,
369 Takahashi, R., and Iwai, K. 2020. The HOIL-1L ligase modulates immune signalling and
370 cell death via monoubiquitination of LUBAC. *Nat Cell Biol.* 22:663.
371 15 Fujita, H., Rahighi, S., Akita, M., Kato, R., Sasaki, Y., Wakatsuki, S., and Iwai, K. 2014.

Mechanism underlying IκB kinase activation mediated by the linear ubiquitin chain assembly complex. *Mol Cell Biol.* 34:1322.

16 Shimizu, S., Fujita, H., Sasaki, Y., Tsuruyama, T., Fukuda, K., and Iwai, K. 2016. Differential Involvement of the Npl4 Zinc Finger Domains of SHARPIN and HOIL-1L in Linear Ubiquitin Chain Assembly Complex-Mediated Cell Death Protection. *Mol Cell Biol.* 36:1569.

17 Sato, Y., Fujita, H., Yoshikawa, A., Yamashita, M., Yamagata, A., Kaiser, S. E., Iwai, K., and Fukai, S. 2011. Specific recognition of linear ubiquitin chains by the Npl4 zinc finger (NZF) domain of the HOIL-1L subunit of the linear ubiquitin chain assembly complex. *Proc Natl Acad Sci U S A.* 108:20520.

18 Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K., Nakagawa, T., Kato, M., Murata, S., Yamaoka, S., Yamamoto, M., Akira, S., Takao, T., Tanaka, K., and Iwai, K. 2009. Involvement of linear polyubiquitylation of NEMO in NF-κB activation. *Nat Cell Biol.* 11:123.

19 Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., Kato, R., Kensche, T., Uejima, T., Bloor, S., Komander, D., Randow, F., Wakatsuki, S., and Dikic, I. 2009. Specific recognition of linear ubiquitin chains by NEMO is important for NF-κB activation. *Cell.* 136:1098.

20 Polley, S., Huang, D. B., Hauenstein, A. V., Fusco, A. J., Zhong, X., Vu, D., Schrofelbauer, B., Kim, Y., Hoffmann, A., Verma, I. M., Ghosh, G., and Huxford, T. 2013. A structural basis for IκB kinase 2 activation via oligomerization-dependent trans auto-phosphorylation. *PLoS Biol.* 11:e1001581.

21 Jono, H., Lim, J. H., Chen, L. F., Xu, H., Trompouki, E., Pan, Z. K., Mosialos, G., and Li, J. D. 2004. NF-κB is essential for induction of CYLD, the negative regulator of NF-κB:

396 evidence for a novel inducible autoregulatory feedback pathway. *J Biol Chem.* 279:36171.
397 22 Schaeffer, V., Akutsu, M., Olma, M. H., Gomes, L. C., Kawasaki, M., and Dikic, I. 2014.
398 Binding of OTULIN to the PUB domain of HOIP controls NF-κB signaling. *Mol Cell.*
399 54:349.
400 23 Heger, K., Wickliffe, K. E., Ndoja, A., Zhang, J., Murthy, A., Dugger, D. L., Maltzman, A.,
401 de Sousa, E. M. F., Hung, J., Zeng, Y., Verschueren, E., Kirkpatrick, D. S., Vucic, D., Lee,
402 W. P., Roose-Girma, M., Newman, R. J., Warming, S., Hsiao, Y. C., Komuves, L. G.,
403 Webster, J. D., Newton, K., and Dixit, V. M. 2018. OTULIN limits cell death and
404 inflammation by deubiquitinating LUBAC. *Nature.* 559:120.
405 24 Gijbels, M. J., Zurcher, C., Kraal, G., Elliott, G. R., HogenEsch, H., Schijff, G., Savelkoul,
406 H. F., and Bruijnzeel, P. L. 1996. Pathogenesis of skin lesions in mice with chronic
407 proliferative dermatitis (cpdm/cpdm). *Am J Pathol.* 148:941.
408 25 Rickard, J. A., Anderton, H., Etemadi, N., Nachbur, U., Darding, M., Peltzer, N., Lalaoui,
409 N., Lawlor, K. E., Vanyai, H., Hall, C., Bankovacki, A., Gangoda, L., Wong, W. W., Corbin,
410 J., Huang, C., Mocarski, E. S., Murphy, J. M., Alexander, W. S., Voss, A. K., Vaux, D. L.,
411 Kaiser, W. J., Walczak, H., and Silke, J. 2014. TNFR1-dependent cell death drives
412 inflammation in Sharpin-deficient mice. *Elife.* 3:e03464.
413 26 Kumari, S., Redouane, Y., Lopez-Mosqueda, J., Shiraishi, R., Romanowska, M.,
414 Lutzmayer, S., Kuiper, J., Martinez, C., Dikic, I., Pasparakis, M., and Ikeda, F. 2014.
415 Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis.
416 *Elife.* 3:e03422.
417 27 Sasaki, K., Himeno, A., Nakagawa, T., Sasaki, Y., Kiyonari, H., and Iwai, K. 2019.
418 Modulation of autoimmune pathogenesis by T cell-triggered inflammatory cell death. *Nat*
419 *Commun.* 10:3878.

420 28 Peltzer, N., Darding, M., Montinaro, A., Draber, P., Draberova, H., Kupka, S., Rieser, E.,
421 Fisher, A., Hutchinson, C., Taraborrelli, L., Hartwig, T., Lafont, E., Haas, T. L., Shimizu,
422 Y., Boiers, C., Sarr, A., Rickard, J., Alvarez-Diaz, S., Ashworth, M. T., Beal, A., Enver, T.,
423 Bertin, J., Kaiser, W., Strasser, A., Silke, J., Bouillet, P., and Walczak, H. 2018. LUBAC is
424 essential for embryogenesis by preventing cell death and enabling haematopoiesis. *Nature.*
425 557:112.
426 29 Peltzer, N., Rieser, E., Taraborrelli, L., Draber, P., Darding, M., Pernaute, B., Shimizu,
427 Y., Sarr, A., Draberova, H., Montinaro, A., Martinez-Barbera, J. P., Silke, J., Rodriguez,
428 T. A., and Walczak, H. 2014. HOIP deficiency causes embryonic lethality by aberrant
429 TNFR1-mediated endothelial cell death. *Cell Rep.* 9:153.
430 30 Micheau, O. and Tschopp, J. 2003. Induction of TNF Receptor I-Mediated Apoptosis via
431 Two Sequential Signaling Complexes. *Cell.* 114:181.
432 31 Tang, Y., Tu, H., Zhang, J., Zhao, X., Wang, Y., Qin, J., and Lin, X. 2019. K63-linked
433 ubiquitination regulates RIPK1 kinase activity to prevent cell death during embryogenesis
434 and inflammation. *Nat Commun.* 10:4157.
435 32 Geng, J., Ito, Y., Shi, L., Amin, P., Chu, J., Ouchida, A. T., Mookhtiar, A. K., Zhao, H.,
436 Xu, D., Shan, B., Najafov, A., Gao, G., Akira, S., and Yuan, J. 2017. Regulation of RIPK1
437 activation by TAK1-mediated phosphorylation dictates apoptosis and necroptosis. *Nat*
438 *Commun.* 8:359.
439 33 Jaco, I., Annibaldi, A., Lalaoui, N., Wilson, R., Tenev, T., Laurien, L., Kim, C., Jamal, K.,
440 Wicky John, S., Liccardi, G., Chau, D., Murphy, J. M., Brumatti, G., Feltham, R.,
441 Pasparakis, M., Silke, J., and Meier, P. 2017. MK2 Phosphorylates RIPK1 to Prevent
442 TNF-Induced Cell Death. *Mol Cell.* 66:698.
443 34 Dondelinger, Y., Delanghe, T., Rojas-Rivera, D., Priem, D., Delvaeye, T., Bruggeman, I.,

Van Herreweghe, F., Vandenabeele, P., and Bertrand, M. J. M. 2017. MK2 phosphorylation of RIPK1 regulates TNF-mediated cell death. *Nat Cell Biol.* 19:1237.

35 Menon, M. B., Gropengiesser, J., Fischer, J., Novikova, L., Deuretzbacher, A., Lafera, J., Schimmeck, H., Czymmeck, N., Ronkina, N., Kotlyarov, A., Aepfelbacher, M., Gaestel, M., and Ruckdeschel, K. 2017. p38(MAPK)/MK2-dependent phosphorylation controls cytotoxic RIPK1 signalling in inflammation and infection. *Nat Cell Biol.* 19:1248.

36 Dondelinger, Y., Jouan-Lanhouet, S., Divert, T., Theatre, E., Bertin, J., Gough, P. J., Giansanti, P., Heck, A. J., Dejardin, E., Vandenabeele, P., and Bertrand, M. J. 2015. NF-κB-Independent Role of IKKα/IKKβ in Preventing RIPK1 Kinase-Dependent Apoptotic and Necroptotic Cell Death during TNF Signaling. *Mol Cell.* 60:63.

37 Lafont, E., Draber, P., Rieser, E., Reichert, M., Kupka, S., de Miguel, D., Draberova, H., von Massenhausen, A., Bhamra, A., Henderson, S., Wojdyla, K., Chalk, A., Surinova, S., Linkermann, A., and Walczak, H. 2018. TBK1 and IKKε prevent TNF-induced cell death by RIPK1 phosphorylation. *Nat Cell Biol.* 20:1389.

38 Oikawa, D., Hatanaka, N., Suzuki, T., and Tokunaga, F. 2020. Cellular and Mathematical Analyses of LUBAC Involvement in T Cell Receptor-Mediated NF-κB Activation Pathway. *Front Immunol.* 11:601926.

39 Wu, C. J. and Ashwell, J. D. 2008. NEMO recognition of ubiquitinated Bcl10 is required for T cell receptor-mediated NF-κB activation. *Proc Natl Acad Sci U S A.* 105:3023.

40 Klein, T., Fung, S. Y., Renner, F., Blank, M. A., Dufour, A., Kang, S., Bolger-Munro, M., Scurll, J. M., Priatel, J. J., Schweigler, P., Melkko, S., Gold, M. R., Viner, R. I., Regnier, C. H., Turvey, S. E., and Overall, C. M. 2015. The paracaspase MALT1 cleaves HOIL1 reducing linear ubiquitination by LUBAC to dampen lymphocyte NF-κB signalling. *Nat Commun.* 6:8777.

468 41 Dubois, S. M., Alexia, C., Wu, Y., Leclair, H. M., Leveau, C., Schol, E., Fest, T., Tarte, K.,
469 Chen, Z. J., Gavard, J., and Bidere, N. 2014. A catalytic-independent role for the LUBAC
470 in NF-κB activation upon antigen receptor engagement and in lymphoma cells. *Blood.*
471 123:2199.
472 42 Teh, C. E., Lalaoui, N., Jain, R., Policheni, A. N., Heinlein, M., Alvarez-Diaz, S., Sheridan,
473 J. M., Rieser, E., Deuser, S., Darding, M., Koay, H. F., Hu, Y., Kupresanin, F., O'Reilly, L.
474 A., Godfrey, D. I., Smyth, G. K., Bouillet, P., Strasser, A., Walczak, H., Silke, J., and Gray,
475 D. H. 2016. Linear ubiquitin chain assembly complex coordinates late thymic T-cell
476 differentiation and regulatory T-cell homeostasis. *Nat Commun.* 7:13353.
477 43 Boisson, B., Laplantine, E., Prando, C., Giliani, S., Israelsson, E., Xu, Z., Abhyankar, A.,
478 Israel, L., Trevejo-Nunez, G., Bogunovic, D., Cepika, A. M., MacDuff, D., Chrabieh, M.,
479 Hubeau, M., Bajolle, F., Debre, M., Mazzolari, E., Vairo, D., Agou, F., Virgin, H. W.,
480 Bossuyt, X., Rambaud, C., Facchetti, F., Bonnet, D., Quartier, P., Fournet, J. C., Pascual,
481 V., Chaussabel, D., Notarangelo, L. D., Puel, A., Israel, A., Casanova, J. L., and Picard, C.
482 2012. Immunodeficiency, autoinflammation and amylopectinosis in humans with
483 inherited HOIL-1 and LUBAC deficiency. *Nat Immunol.* 13:1178.
484 44 Oda, H., Beck, D. B., Kuehn, H. S., Sampaio Moura, N., Hoffmann, P., Ibarra, M.,
485 Stoddard, J., Tsai, W. L., Gutierrez-Cruz, G., Gadina, M., Rosenzweig, S. D., Kastner, D.
486 L., Notarangelo, L. D., and Aksentijevich, I. 2019. Second Case of HOIP Deficiency
487 Expands Clinical Features and Defines Inflammatory Transcriptome Regulated by
488 LUBAC. *Front Immunol.* 10:479.
489 45 Boisson, B., Laplantine, E., Dobbs, K., Cobat, A., Tarantino, N., Hazen, M., Lidov, H. G.,
490 Hopkins, G., Du, L., Belkadi, A., Chrabieh, M., Itan, Y., Picard, C., Fournet, J. C., Eibel,
491 H., Tsitsikov, E., Pai, S. Y., Abel, L., Al-Herz, W., Casanova, J. L., Israel, A., and

Notarangelo, L. D. 2015. Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J Exp Med.* 212:939.

46 Zinngrebe, J., Moepps, B., Monecke, T., Gierschik, P., Schlichtig, F., Barth, T. F. E., Strauss, G., Boldrin, E., Posovszky, C., Schulz, A., Beringer, O., Rieser, E., Jacobsen, E. M., Lorenz, M. R., Schwarz, K., Pannicke, U., Walczak, H., Niessing, D., Schuetz, C., Fischer-Posovszky, P., and Debatin, K. M. 2022. Compound heterozygous variants in OTULIN are associated with fulminant atypical late-onset ORAS. *EMBO Mol Med.* 14:e14901.

47 Damgaard, R. B., Walker, J. A., Marco-Casanova, P., Morgan, N. V., Titheradge, H. L., Elliott, P. R., McHale, D., Maher, E. R., McKenzie, A. N. J., and Komander, D. 2016. The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. *Cell.* 166:1215.

48 Damgaard, R. B., Jolin, H. E., Allison, M. E. D., Davies, S. E., Titheradge, H. L., McKenzie, A. N. J., and Komander, D. 2020. OTULIN protects the liver against cell death, inflammation, fibrosis, and cancer. *Cell Death Differ.* 27:1457.

49 Damgaard, R. B., Elliott, P. R., Swatek, K. N., Maher, E. R., Stepensky, P., Elpeleg, O., Komander, D., and Berkun, Y. 2019. OTULIN deficiency in ORAS causes cell type-specific LUBAC degradation, dysregulated TNF signalling and cell death. *EMBO Mol Med.* 11.

50 Zhou, Q., Yu, X., Demirkaya, E., Deuitch, N., Stone, D., Tsai, W. L., Kuehn, H. S., Wang, H., Yang, D., Park, Y. H., Ombrello, A. K., Blake, M., Romeo, T., Remmers, E. F., Chae, J. J., Mullikin, J. C., Guzel, F., Milner, J. D., Boehm, M., Rosenzweig, S. D., Gadina, M., Welch, S. B., Ozen, S., Topaloglu, R., Abinun, M., Kastner, D. L., and Aksentijevich, I.

2016. Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc Natl Acad Sci U S A.* 113:10127.

51 Noad, J., von der Malsburg, A., Pathe, C., Michel, M. A., Komander, D., and Randow, F. 2017. LUBAC-synthesized linear ubiquitin chains restrict cytosol-invading bacteria by activating autophagy and NF-κB. *Nat Microbiol.* 2:17063.

52 Kelsall, I. R., McCrory, E. H., Xu, Y., Scudamore, C. L., Nanda, S. K., Mancebo-Gamella, P., Wood, N. T., Knebel, A., Matthews, S. J., and Cohen, P. 2022. HOIL-1 ubiquitin ligase activity targets unbranched glucosaccharides and is required to prevent polyglucosan accumulation. *EMBO J.* 41:e109700.

53 Zhang, H., Zhao, X., Guo, Y., Chen, R., He, J., Li, L., Qiang, Z., Yang, Q., Liu, X., Huang, C., Lu, R., Fang, J., Cao, Y., Huang, J., Wang, Y., Huang, J., Chen, G. Q., Cheng, J., and Yu, J. 2021. Hypoxia regulates overall mRNA homeostasis by inducing Met^1-linked linear ubiquitination of AGO2 in cancer cells. *Nat Commun.* 12:5416.

54 van Well, E. M., Bader, V., Patra, M., Sanchez-Vicente, A., Meschede, J., Furthmann, N., Schnack, C., Blusch, A., Longworth, J., Petrasch-Parwez, E., Mori, K., Arzberger, T., Trumbach, D., Angersbach, L., Showkat, C., Sehr, D. A., Berlemann, L. A., Goldmann, P., Clement, A. M., Behl, C., Woerner, A. C., Saft, C., Wurst, W., Haass, C., Ellrichmann, G., Gold, R., Dittmar, G., Hipp, M. S., Hartl, F. U., Tatzelt, J., and Winklhofer, K. F. 2019. A protein quality control pathway regulated by linear ubiquitination. *EMBO J.* 38:e100730.

55 Wu, M., Chang, Y., Hu, H., Mu, R., Zhang, Y., Qin, X., Duan, X., Li, W., Tu, H., Zhang, W., Wang, G., Han, Q., Li, A., Zhou, T., Iwai, K., Zhang, X., and Li, H. 2019. LUBAC controls chromosome alignment by targeting CENP-E to attached kinetochores. *Nat Commun.* 10:273.

56 Nitschke, S., Sullivan, M. A., Mitra, S., Marchioni, C. R., Lee, J. P. Y., Smith, B. H.,

Ahonen, S., Wu, J., Chown, E. E., Wang, P., Petkovic, S., Zhao, X., DiGiovanni, L. F., Perri, A. M., Israelian, L., Grossman, T. R., Kordasiewicz, H., Vilaplana, F., Iwai, K., Nitschke, F., and Minassian, B. A. 2022. Glycogen synthase downregulation rescues the amylopectinosis of murine RBCK1 deficiency. *Brain*. doi: 10.1093/brain/awac017.

Figure legends

Fig. 1. (A) The ubiquitin codes. Polyubiquitin chains are classified according to the type of inter-ubiquitin linkage. Isopeptide bonds formed between the C-terminal carboxyl group of the distal ubiquitin and an ε-amino group of one of seven Lys (K) residues in the proximal ubiquitin results in generation of seven types of linkage (K6, K11, K27, K29, K33, K48, and K63), whereas linear (M1-linked) ubiquitin is formed by peptide bonds formed with the α-amino group of the N-terminal Met residue in ubiquitin. Each type of the chain is recognized specifically by intracellular adaptor proteins, leading to selective physiological outputs. For example, the major intracellular ubiquitin chains K48 and K63 serve as intermediates for proteasomal degradation and homeostatic biological functions, respectively. Linear ubiquitin chains are produced transiently upon extrinsic stimulation, and function to activate NF-κB, protect cells from extrinsic cell death, and stimulate immune cell differentiation. (B) Schematic representation of LUBAC. LUBAC comprises HOIP, HOIL-1L, and SHARPIIN, which interact with each other via their UBL, UBA domain, or LTM motif (indicated by arrows). The catalytic center of LUBAC ligase is present within the C-terminal RBR domain of HOIP. The ZF and NZF domains interact with pre-existing or self-produced polyubiquitin chains. The N-terminal PUB domain of HOIP is associated with OTULIN or CYLD, deubiquitinating enzymes that cleave linear ubiquitin chains.

Fig. 2. Linear ubiquitination-mediated skin homeostasis. A representative picture of SHARPIN-deficient cpdm mice (Left). Loss of SHARPIN destabilizes the LUBAC complex, leading to loss of

HOIL-1L and HOIP. These mice develop severe skin inflammation along with epidermal hyperplasia, hyperkeratosis, parakeratosis, keratinocyte cell death, and infiltration of the skin by immune cells. Extensive investigation of LUBAC and linear ubiquitin functions at the molecular level revealed that the skin disease in cpdm mice is induced by distinct etiologies: autoinflammation and autoimmunity. In an autoinflammatory context, increased susceptibility of keratinocytes to cell death destroys skin tissue architecture directly. Undetectable responses by TNF-α and other death ligands constitutively expressed in the skin is thought to trigger autoinflammation. In addition, LUBAC contributes to T cell receptor (TCR)-mediated thymocyte differentiation and activation of mature T cells. In particular, anti-inflammatory Treg cells depend on LUBAC. LUBAC deficiency disrupts peripheral T cell-mediated immune balance between $Foxp3^{+}$ Tregs and effector subsets of $Foxp3^{-}$ conventional T cells. This autoimmune effect drives death-induced skin inflammation. Thus, LUBAC and linear ubiquitination maintain skin tissue homeostasis by exerting pleiotropic functions in various cell type in healthy individuals.

Fig. 1

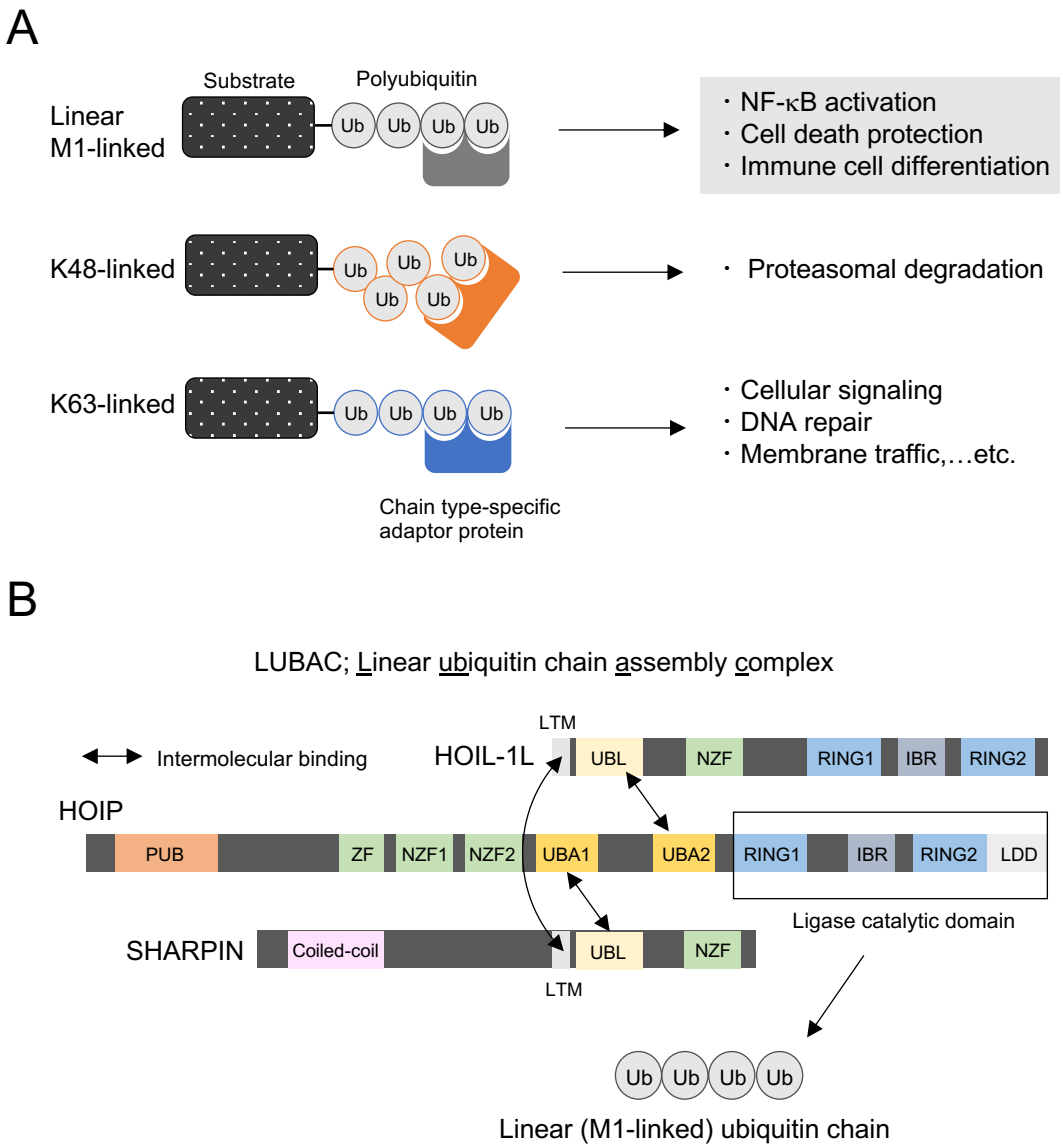
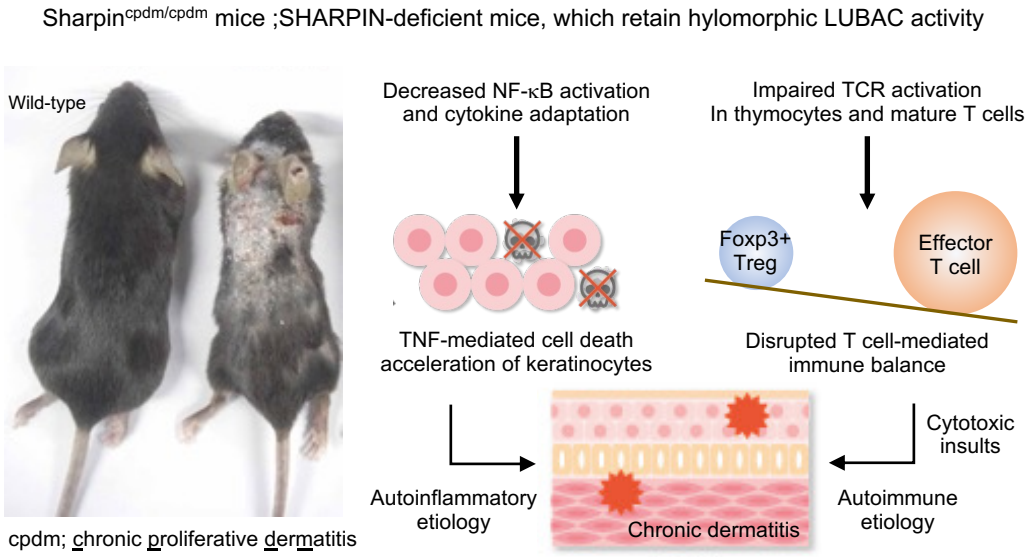

A
Substrate
Polyubiquitin
Linear
M1-linked
Ub
・NF-κB activation
・Cell death protection
・Immune cell differentiation
K48-linked
・ Proteasomal degradation
K63-linked
・Cellular signaling
・DNA repair
・Membrane traffic,…etc.
Chain type-specific
adaptor protein
B
LUBAC; Linear ubiquitin chain assembly complex
Intermolecular binding
LTM
HOIL-1L
UBL
NZF
RING1
IBR
RING2
HOIP
PUB
ZF
NZF1
NZF2
UBA1
UBA2
LDD
Ligase catalytic domain
SHARPIN
Coiled-coil
Linear (M1-linked) ubiquitin chain

Fig. 2


Sharpin$^{cpdm/cpdm}$ mice ;SHARPIN-deficient mice, which retain hylomorphic LUBAC activity
Wild-type
cpdm; chronic proliferative dermatitis
Decreased NF-κB activation
and cytokine adaptation
TNF-mediated cell death
acceleration of keratinocytes
Impaired TCR activation
In thymocytes and mature T cells
Foxp3+
Treg
Effector
T cell
Disrupted T cell-mediated
immune balance
Autoinflammatory
etiology
Chronic dermatitis
Cytotoxic
insults
Autoimmune
etiology