Survey of the human proteostasis network: the ubiquitin-proteasome system

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SUMMARY

Modification by ubiquitination governs the half-lives of thousands of proteins that are fated for elimination by either the proteasome or autophagy pathways, depending on the intricate architectures of ubiquitin modification. This system mediates quality control for individual proteins, protein complexes, and organelles, as well as myriad purely regulatory functions. Here we provide a comprehensive survey of the ubiquitin-proteasome system (UPS), the scope of which is at present poorly defined. The UPS, with the inclusion of pathways involving ubiquitin-like modifiers, comprises in our estimate over 1400 distinct proteins in humans, a vast set of activities whose collective impact on the biology of the cell is pervasive. The UPS is an integral component of the proteostasis network (PN), the remainder of which we have also surveyed in recent studies. With the addition of molecular chaperones, proteins from autophagy-lysosome pathway, and related activities, the PN includes in total over 3100 components by our estimates. Comprehensive and systematic definition of these pathways should support a range of ongoing investigations in the areas of genomics, proteomics, biochemistry, cell biology, and disease research.

INTRODUCTION

Modification of target proteins with the small protein ubiquitin, first identified 49 years ago[1,2], can target proteins for degradation by the proteasome or by autophagy, and also functions in nonproteolytic signaling. These distinct effects on protein fate and function largely reflect the number of bound ubiquitin groups and their arrangement, typically in multiubiquitin chains of varied architecture. The configuration of the substrate-bound ubiquitin chain is the foundational feature of the ubiquitin code, and arises from highly specific utilization of any of eight ubiquitin acceptor sites in ubiquitin itself, seven of these being lysine residues[3]. As originally conceived[4], the ubiquitin-proteasome system

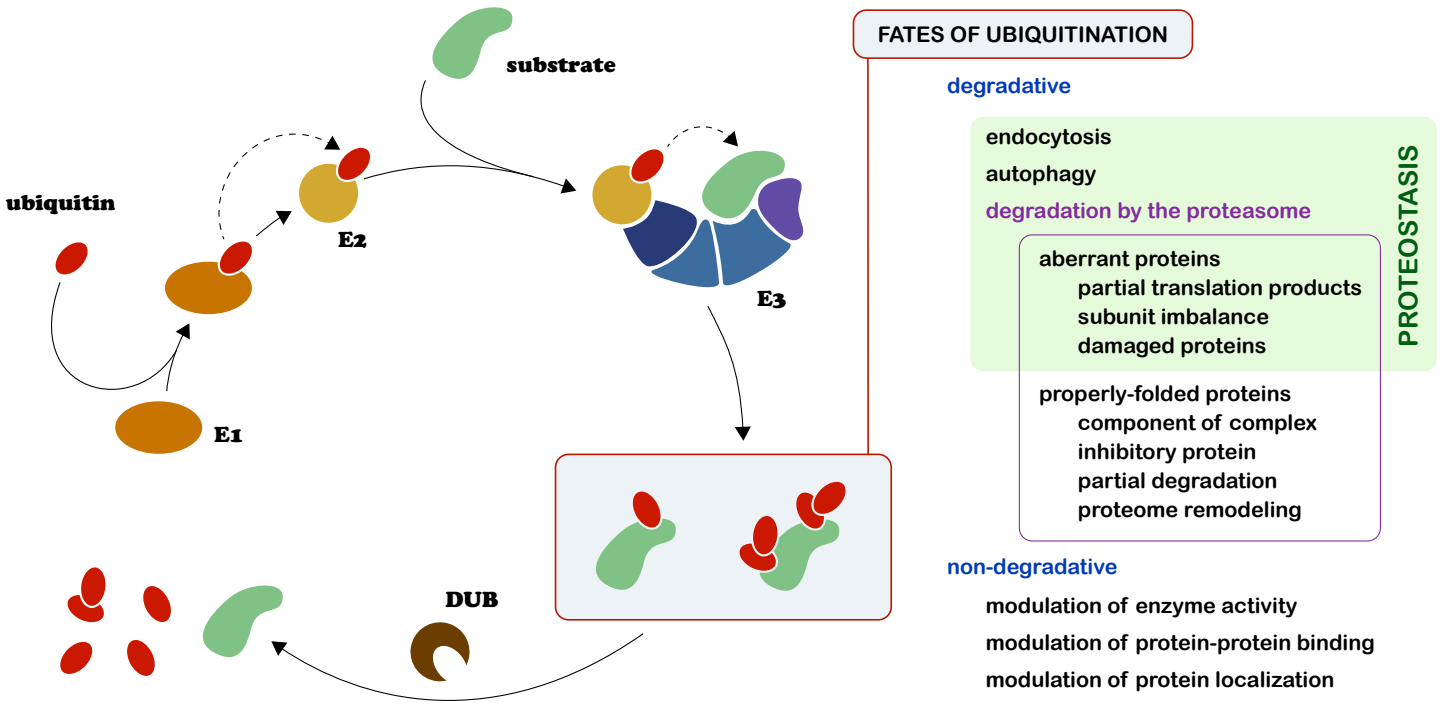

Figure 1. The ubiquitin cycle

Left: Ubiquitin (red) is activated by E1 (orange) in an ATP-dependent reaction which results in ubiquitin forming a thioester adduct with E1. Ubiquitin is transferred to E2 (yellow) in a transthiolation reaction. Charged E2 is recruited by the E3 ligase via an E2 docking element (dark blue). Substrate (green) is recruited by the E3 (blues and purple), and ubiquitin is transferred from E2 to substrate, or from E2 to E3 to substrate, depending on the E3 group. Substrate modification may be reversed by deubiquitinating enzymes (brown). ***Right:*** the fates of substrate ubiquitination, which may include engagement with a number of pathways, resulting in either degradative or non-degradative fates.

consisted of a small handful of enzymes (Fig. 1): ubiquitin-activating enzyme, ubiquitin conjugating enzyme, ubiquitin ligase, a protease later called the proteasome[5], and a deubiquitinating enzyme. Already by 1985, as ubiquitin-affinity chromatography was refined, it became apparent that some of these biochemical activities actually correspond to families of enzymes[6], and a stunning multiplicity of ubiquitin ligases was eventually uncovered[7,8], here estimated to be at least 765 in humans (see Methods). Likewise, ubiquitin recognition was originally attributed to a single receptor on the proteasome[9], but we currently classify more than 420 proteins as likely "interpreters" of the ubiquitin signal in its many forms, including ubiquitin-like modifiers as discussed below. There is also an unexpected functional diversity of deubiquitinating enzymes, which now number well over 100.

With likely UPS members now at 1412 in number, as estimated below, most reviews of the topic have been focused understandably on a specific class of enzymatic activity, often ubiquitin ligases. At the outset of the present study, a systematic accounting of all members of the entire pathway had not been attempted for many years. Therefore, the scope of the UPS is still in this sense lacking in definition, and is not well demarcated as a specific set of gene products. No catalog has been available for querying the entire UPS at the resolution of individual genes, which is a hindrance to many areas of biological research. Given the deep impact of ubiquitination in the biology of the cell, in human health, and in therapeutics[10], the utility of defining the scope of the UPS in terms of its protein components is evident. For example, genomics and proteomics data now being generated on a tremendous scale are typically filtered through bioinformatic pipelines, or classification systems, based on distinct biochemical systems such as ubiquitination. Proper interpretation of such data is limited by the quality of the assignment of system components, impacting the depth and precision of the biological literature in many ways.

We have recently worked to define the protein components of other biochemical systems that are functionally related to the UPS, namely autophagy, protein synthesis machinery, molecular chaperones, systems for trafficking proteins into

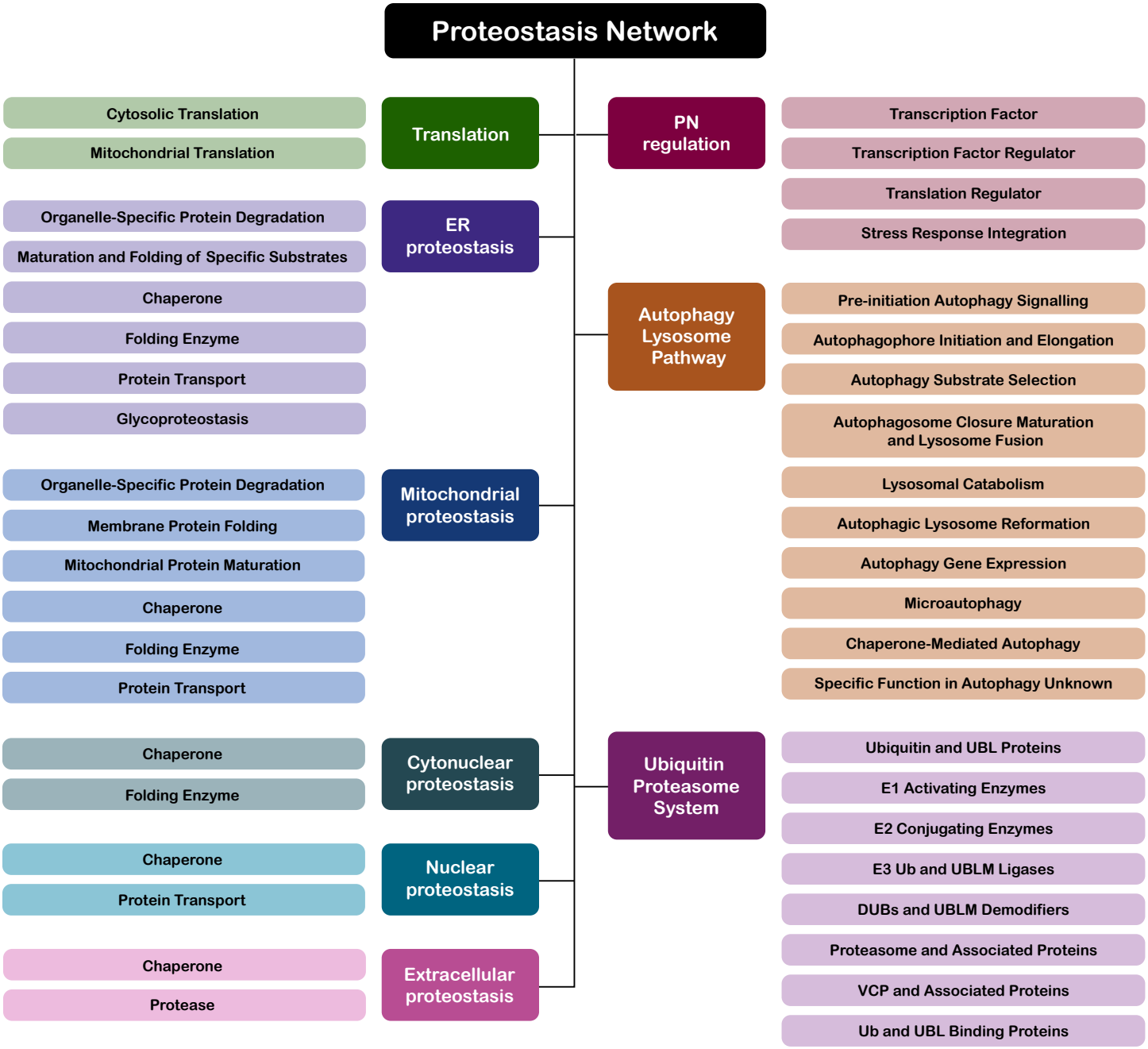

Figure 2. The proteostasis network

The nine Branches of the Proteostasis Network are shown in two center columns. A Branch comprises multiple Classes, shown flanking each Branch. Progressive subdivisions of the Classes include Groups, Types, and Subtypes (not shown; see Table S1). Tallies for each of the Branches and Classes are given in Table S1.

and out of organelles, and organelle-specific protein degradation systems (Fig. 2)[11,12]. In total, these are parsed as nine main branches that form the proteostasis network, or PN[13]. The PN is defined as the entirety of activities that ensure protein homeostasis, and can be viewed most simply, albeit reductively, as a vast pathway for protein quality control. PN pathways ensure accurate protein synthesis, protein folding, and the removal of damaged, mislocalized, or mutant proteins, and the UPS constitutes a central component of this apparatus. In supporting the elimination of aberrant proteins, it works closely with the molecular chaperone machinery and autophagy. Indeed, many proteins and organelles are targeted to autophagy by ubiquitination[14].

While the UPS promotes proteostasis by degrading damaged and aberrant proteins, it also degrades properly formed proteins, particularly in response to

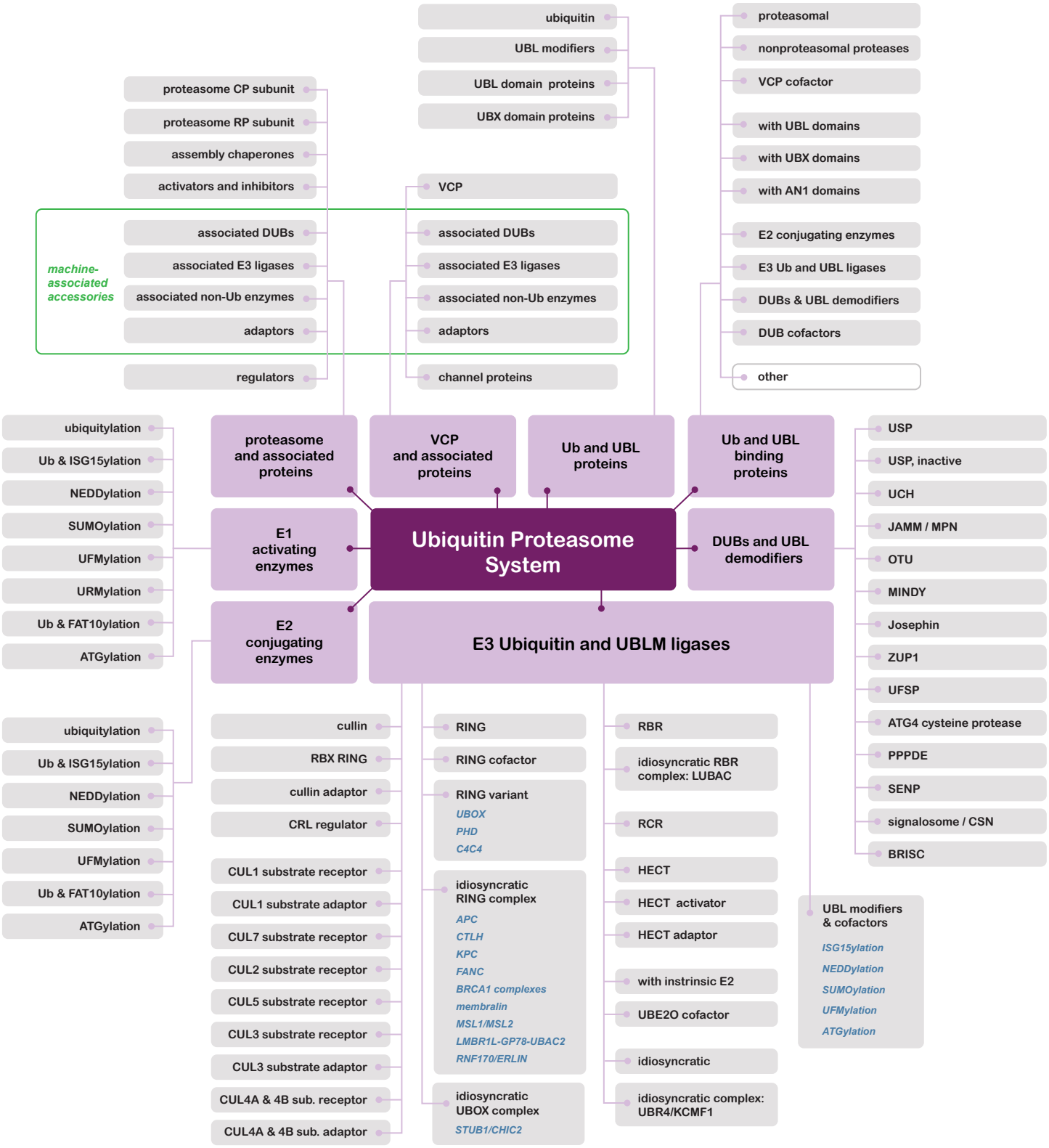

Figure 3. Taxonomic structure of the ubiquitin-proteasome system

The Ubiquitin-Proteasome System is shown with its Classes (fushia) and Groups (grey). For finer divisions–Type and Subtype–see **Table S1**. In a few instances, Types are shown in the figure (blue italics). The molecular machines of the UPS - the proteasome and VCP - associate with comparable suites of enzymes and cofactors, as highlighted by the green frame (upper left).

environmental, developmental, and cell-cycle cues. These more strictly regulatory functions of UPS lie largely outside of proteostasis and the PN per se. However, given the current state of knowledge, we are far from being able to generally classify UPS components as specifically serving either proteostasis or other roles. We therefore aimed to be inclusive of UPS components in our survey, and not to focus specifically on UPS components that support protein or organellar quality control.

For the foreseeable future, any attempt to comprehensively survey the UPS, however carefully done, will carry caveats. For example, hundreds of proteins whose features signify membership in the UPS have not yet been studied in any detail, and the taxonomic weight of their features, such as specific structural domains, is subject to debate in certain instances, as discussed below. Another caveat is that new pathway components presumably remain to be discovered.

To make this survey as accessible as possible, we developed a five-tiered taxonomic system, proceeding from broad to narrow through Branch, Class, Group, Type, and Subtype (**Fig. 3**). The complete survey is presented in tabular form (**Table S1**), and in abbreviated form for easier reading (**Table S2**). The UPS encompasses three Classes that cooperate to write the ubiquitin signal (E1, E2, and E3), one that erases it (deubiquitinating enzymes), one that constitutes the signal itself (ubiquitin and ubiquitin-like proteins), and one that reads it (ubiquitin receptors). The remaining two Classes consist of the molecular machines that determine the fate of proteins carrying the ubiquitin signal (the proteasome and VCP/p97/Cdc48). Overall, we find that within our hierarchy 1180 of 1412 UPS components fall into only one Class. This demonstrates that our taxonomic scheme can organize UPS genes into functionally distinctive and human-interpretable categories that minimize ambiguity while preserving molecular context.

RESULTS

Approach for the survey

To generate a comprehensive accounting of the UPS (**Table S1**), we relied heavily on the modularity inherent in the system. The InterPro database[15] has been a key reference for us in exploring the UPS as a modular system. A strength of InterPro is that it aggregates the output of independent efforts to relate protein sequence to protein function[15]. InterPro uses multiple designations to describe these protein sequence elements, most commonly *Domains*, which correspond to generally to functional or structural elements, and *Families*, which flag evolutionary relationships and frequently span the length of a protein. InterPro *Repeats* are smaller, repeated structural elements, which for the UPS most often correspond to protein-protein interaction sites. InterPro *Homologous Superfamilies* aggregate structurally related groups, often with low sequence homology. We identified InterPro designations, most often *Domains* (**Table S3**), shared by well-characterized UPS enzymes and other proteins, and used these signature domains to identify additional components. Typically, these signature domains correspond to well-understood molecular functions such as ubiquitin-directed enzymatic activities. In the course of searching for signature domains, we also identified InterPro *Domains* or *Repeats* shared by subsets of a given family. These auxiliary domains allowed subdivision of families, giving texture to what in some cases are large protein families. Some of these auxiliary domains recur in a variety of protein families, and are described as distributed domains (**Table S3**). While the majority of the proteins in our survey meet domain-based criteria for inclusion, other components of the UPS are sui generis rather than members of protein families. These are included in the UPS based on positive biochemical, cell biological, or genetic evidence, and we refer to this mode as entity-based inclusion. We used the dual approach of domain-based inclusion and entity-based inclusion in our earlier surveys as well[11,12].

Ubiquitin and ubiquitin-like proteins

Ubiquitin (UB), the foundational component of the UPS, shares its unitary structural motif with ubiquitin-like modifiers (UBLMs), collectively known as Type I ubiquitin-like proteins (UBLs), and with proteins bearing ubiquitin-like domains that do not participate in protein modification (Type II UBLs) (**Fig. 4**)[16]. We include here ubiquitin itself, UBLMs, and all proteins bearing structurally related domains in one Class. The mode of action of Type I UBLs can be quite different from that of ubiquitin. For example, conjugation of ATG8/LC3 (the autophagy-specific ubiquitin-like modifier) promotes protein degradation, as ubiquitin does, but only via autophagy, or the lysosome. However, ATG8/LC3 is not conjugated to proteins but to lipids, primarily phosphotidylethanolamine[17]. This reaction targets it to membranes, where it plays a key role in the genesis and trafficking of the autophagosome[18].

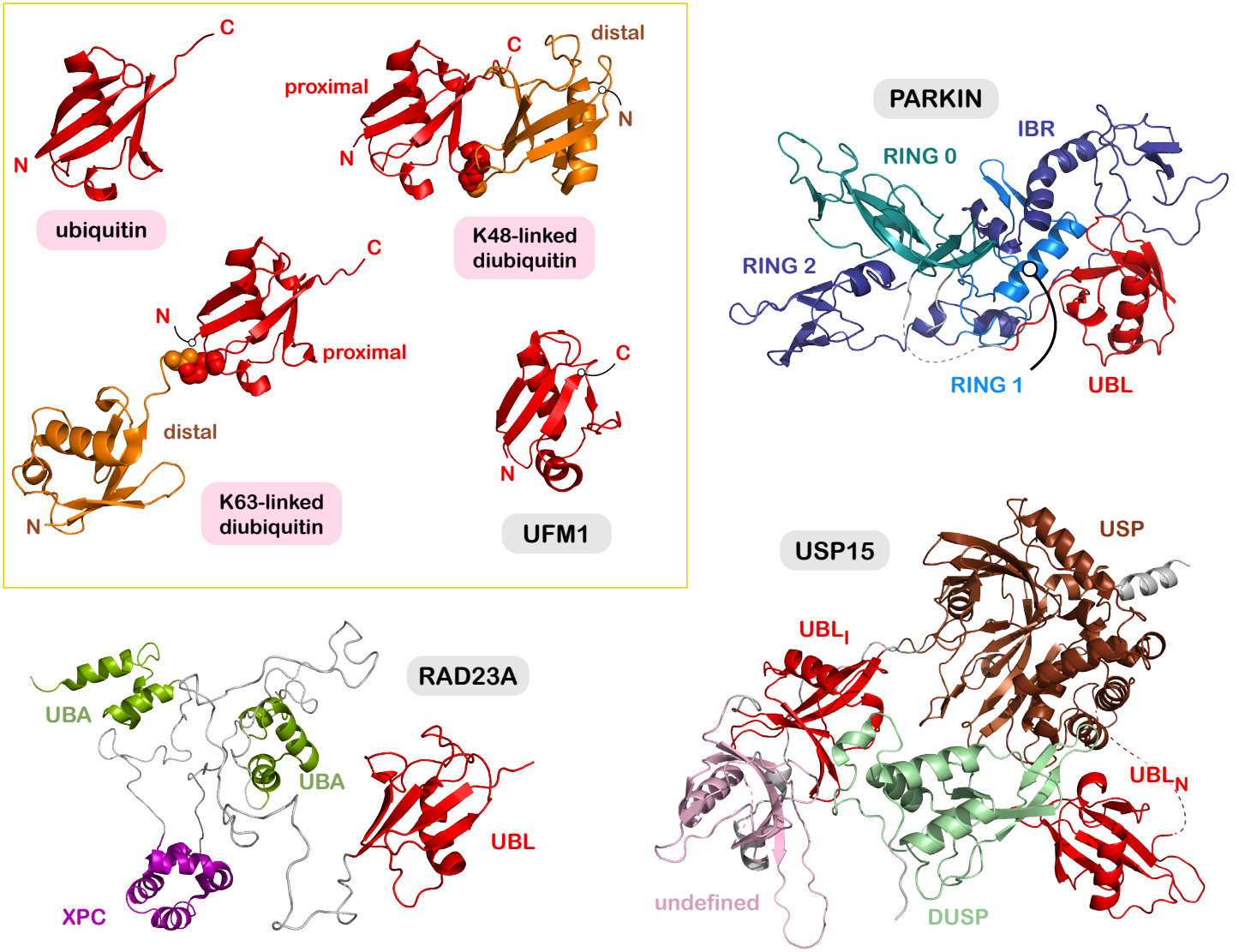

Figure 4. Structures of ubiquitin, UBLM, and UBL proteins

Type I UBLs are presented with in the yellow frame, and Type II UBLs outside of it. Ubiquitin and UBL domains are shown in red. For diubiquitin forms, proximal ubiquitins are shown in red and distal ubiquitins in orange. Isopeptide linkages between two ubiquitins are depicted in sphere format. Free termini for ubiquitin and UFM1 are labeled with N and C. ***Top left***: ubiquitin and two forms of diubiquitin. ***Center***: ubiquitin-like modifier UFM1. ***Top right***: E3 ubiquitin ligase PARKIN. ***Bottom right***: deubiquitinating enzyme USP15, with two UBL domains. ***Bottom left:*** proteasome shuttling factor RAD23A. Structures are referenced in Table S7.

Ubiquitin itself is not expressed in usable form by the cell; ubiquitin precursors in the form of polyubiquitin (UBB and UBC) and ribosomal ubiquitin fusions (RPS27A and UBA52) must be processed to generate an activatable C-terminus (glycine-76). Under growth-permissive conditions, ubiquitin is expressed primarily as ribosomal fusions, yoking the production of ubiquitin to increases in ribosome abundance[19]. FAU/MNSFβ, a ubiquitin-like modifier[20], is likewise produced as a ribosomal fusion protein and FAU itself is absent from assembled ribosomes[21]. Most ubiquitin-like modifiers must be processed prior to ligation to target proteins, resulting in a rapid release of amino acids or small peptides that has historically been considered to be of no consequence. However, deletion of the single-residue C-terminal leaving group from the polyubiquitin gene was recently shown to result in sensitivity to proteotoxic stress[22]. Another extended version of ubiquitin, known as UBB+1, is the product of frameshifting in transcription of the ubiquitin B gene, and its accumulation has been linked to neurodegeneration[23]. Among the Type II ubiquitin-like domains, the UBL and UBX domains are especially significant in that they target to the proteasome and VCP, respectively[24–26], though it is highly unlikely that all Type II UBL domains can confer proteasome association. UBL

UBLM type	Gene Symbols for UBLMs	E1	E2	E3	Demodifiers
ATG8	GABARAP, GABARAPL1, GABARAPL2, GABARAPL3 MAP1LC3A, MAP1LC3B, MAP1LC3B2, MAP1LC3C	ATG7	ATG3	ATG12-modified ATG5 in complex with ATG16L1	ATG4A, ATG4B, ATG4C, ATG4D
ATG12	ATG12	ATG7	ATG10		
FAT10	UBD	UBA6	UBE2Z		
FUBI	FAU				USP36
ISG15	ISG15	UBA7	UBE2E2 UBE2L6	ARIH1, HERC5, HERC6, TRIM25	USP16, USP18
NEDD8	NEDD8	UBA3 NAE1	UBE2F UBE2M	*DCN1 family* DCNL1, DCNL2, DCNL3, DCNL4, DCNL5 *dual specificity ligases* ITCH, SMURF1, SMURF2, HUWE1 SCF^{FBXO11}, SCF^{FBXO41}, and SCF^{FBXW11} CBL, CBLB, CBLL1, XIAP, BIRC2, TRIM40, MDM2, RAD18, RNF111, RNF168	SENP8 Signalosome / CSN complex COPS2, COPS3, COPS4, COPS5, COPS6, COPS7A, COPS7B, COPS8, COPS9, GPS1
SUMO	SUMO1, SUMO2, SUMO3, SUMO4	SAE1 SAE2	UBE2I	*SP-RING / MIZ-type* PIAS1, PIAS2, PIAS3, PIAS4, NSMCE2, ZMIZ1, ZMIZ2 *other* RANBP2, ZNF451, KIAA1586	*ULP1-type* SENP1, SENP2, SENP3, SENP5, SENP6, SENP7 *PPPDE- and USP-type* DESI1, DESI2, USPL1
UBL3	UBL3 *				
UFM1	UFM1	UBA5	UFC1	UFL1, UFBP1, CDK5RAP3	USFP1, USFP2
URM1	URM1	UBA4			

Table 1. Ubiquitin-like modifiers and their cognate enzymes

* UBL3 appears to be conjugated to protein targets via a disulfide linkage[227], rather than an isopeptide, but no responsible enzymes have been identified.

domain proteins also have the potential to associate with any of the hundreds of proteins that have been broadly classified under likely ubiquitin binders in our survey.

While the structure of ubiquitin has been known for decades[27], the ubiquitin and ubiquitin-like proteins are not captured by a single InterPro domain. The InterPro-designated *Ubiquitin-like domain superfamily* (**IPR029071**) is too broad to capture the UB/UBL proteins precisely, as it encompasses domains that are not functionally related to ubiquitin. The *Ubiquitin-like domain* (**IPR000626**) captures 60 family members, representing the most efficient InterPro domain for retrieving members of the UB/UBL Class, though at the same time missing more than it collects. Additional InterPro domains indicating UBLMs and UBL domains were identified by evaluating domains overlapping with **IPR029071**. Proteins described in the literature as containing UBL domains, but not identified as containing **IPR029071**, were also analyzed for the presence of InterPro and Conserved Domain Database (CDD) domains. This collection of signature domains was used to define the members of the UB/UBL Class in the main annotation (**Table S1**) and the domains themselves are presented in **Table S3**.

Not only does ubiquitin modification support protein turnover, but the attachment of several ubiquitin-like modifiers can likewise support substrate degradation by the proteasome[28–30]. Ubiquitin-like modifiers

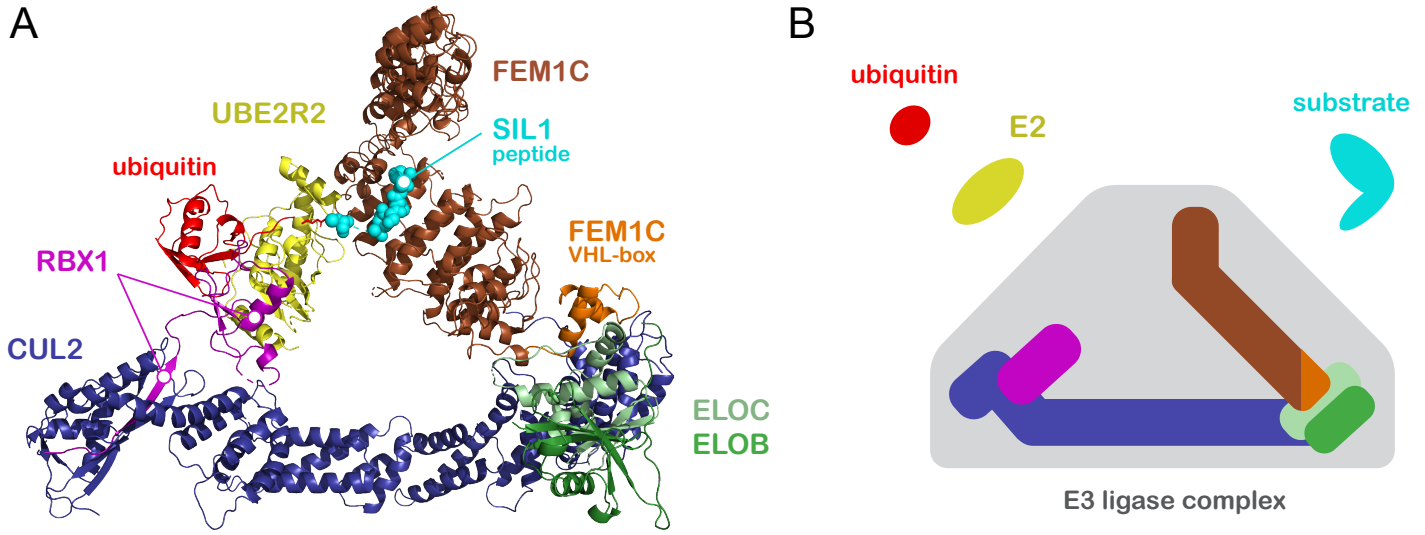

Figure 5. Architecture of cullin RING ligases

Structural model derived from the cryoEM map of a complex containing a cullin RING ligase bound to a ubiquitin-charged E2 poised for transfer to a substrate [PDB:8Q7R][48]. (**A**) CUL2 (blue) with RING subunit RBX1 (magenta), cullin adaptor ELOBC (green), and substrate receptor FEM1C (brown). The adaptor-binding VHL box of FEM1C is shown in orange. UBE2R2 (yellow green), charged with ubiquitin (red), contacts the RING domain of RBX1. Substrate SIL1 (turquoise) is bound by the ankyrin repeat domain of the substrate receptor and positioned in proximity to the charged E2 for ubiquitin transfer. (**B**) A schematic of the $CUL2^{FEM1C}$ ligase.

are also well known to modulate the activity of UPS pathway enzymes–most notably, cullin RING ligases are activated by the attachment of NEDD8 to the cullin subunit[31]. While the consequences of ubiquitin-like protein modification may not strictly fall within UPS processes in every case, as presently understood, they are as a group sufficiently linked in function that we chose to include all ubiquitin-like modifiers and their cognate enzymes in our survey (**Table 1**, **Table S1**)[28,32].

Ubiquitin activating and conjugating enzymes

Ubiquitin enters the conjugation pathway via the action of one of two E1s, or ubiquitin activating enzymes (in humans). Ubiquitin, activated at its C-terminus by thioester formation, proceeds to undergo thiolester transfer onto one of tens of E2s, or ubiquitin conjugating enzymes, and is subsequently ligated to substrate via the action of one of hundreds of E3s, or ubiquitin ligases (**Fig. 1**). The enzyme families at the top of this pyramid are easily defined by canonical domains. While the E1 enzymes for ubiquitin and ubiquitin-like modifiers are multidomain proteins, there is one InterPro entry that unifies them: the *THIF-type NAD/FAD binding fold* (**IPR000594**; **Table S3**). Most of the E2 enzymes share a single stereotypical domain (**IPR000608**), though the E2 enzymes for UFMylation and ATGylation are identified by other domains (**IPR014806** and **IPR007135**, respectively). The E2 enzymes have been divided into 17 families[33], and we have included these as Type designations in our taxonomy (**Table S1**). For the most part, the E2 Class is lightly elaborated beyond the principal domain, typically bearing what seem to be unstructured N termini, unstructured C termini, or both. These tails contribute to the specificity of E2-E3 interactions[34,35]. Two E2s, UBE2J1 and UBE2J2, include transmembrane domains placing them in the ER, two E2s, UBE2Q1 and UBE2Q2, include *RWD-like domains* (**IPR006575**), and one E2, UBE2K, includes a *UBA domain* (**IPR015940**). This UBA domain confers ubiquitin binding and therefore promotes ubiquitin chain formation[36,37].

There are four catalytically inactive E2s (known as ubiquitin E2 variants or UEVs) captured by the **IPR000608** domain, and these are grouped with the active E2s, as well as with the ubiquitin binding proteins. To take the UEV MMS2 as an example, it was once thought to be inactive in that it lacks a catalytic cysteine by which to form a ubiquitin thioester. Yet it is a subunit of a K63-chain forming heterodimer with UBC13 and, by orienting the acceptor ubiquitin with respect to the active site of UBC13, governs linkage specificity[38]. Perhaps the most unusual E2 enzymes are the two that function

E3 ligase group	Related Interpro Domain	Occurrences in survey	Mode of E2 docking	Transthiolation by E3
Cullin RING	IPR016158	9	via associated RBX1 or RBX2 (RINGs)	no
RING			via RING domain	no
most groups, complexes	IPR001841	281		
MARCH	IPR011016	11		
Pellino	IPR048335	3		
IRF2 binding	IPR044882	3		
CTLH complex	IPR044063	3		
FANCL complex	IPR026850	1		
RING variant			via variant RING domain	no
UBOX	IPR003613	9		
select PHDs	IPR001965	6		
C4C4 (TMEM129)	none	1		
HECT	IPR000569	28	via N-lobe of HECT domain	yes
RBR	IPR044066	14	via RING1 of TRIAD domain	yes
RCR	none	1	via RING and loop extension	yes (twice)
UBR4	IPR025704	1	via hemiring-UZI module	no
RZ	IPR046439	2	variable	yes
E2-E3 fusions	none	2	intrinsic E2	no
idiosyncratic	none	13	variably known	variably known

Table 2: E2 recognition components of E3 ligases

essentially as E2-E3 hybrids: UBE2O and BIRC6[39]. In fact, UBE2O also has E4 activity, in that it recognizes substrate-bound ubiquitin and preferentially ubiquitinates ubiquitinated proteins[40]. While the E2-E3 and E2-E3-E4 fusions are arguably models of efficiency, this design has barely proliferated in evolution, and probably has drawbacks such as an inherent lack of modularity.

Properties of E3 enzymes

Once ubiquitin has moved through the tight script of E1 activation and E2 conjugation, ligation to substrate relies on a myriad cast of ubiquitin ligases. The E3s represent the most variably annotated Class, due to the breadth and complexity of this family. We apply both structural and enzymatic criteria in our basis for inclusion. For E3s to be functional as ubiquitin ligases, they must recruit an E2 charged with ubiquitin, recruit substrate, and promote the transfer of ubiquitin to substrate. The recruited substrate must also be positioned in a productive orientation with respect to the E2. Some ubiquitin ligases accomplish this with a single polypeptide, but many are composed of several subunits and when fully assembled can take the shape of arcs or rings[41–47]. CUL2^{FEM1C} serves as an example (Fig. 5)[48]. While only one subunit associates with the E2, and only one with substrate, the remaining three subunits are essential for the E3 activity (and are included in our survey). While E3 enzymes as a whole have been a subject of intense study for decades, many are understudied, and it is likely that some of the smaller ligases function with partners not yet identified.

E2 Recruitment by E3 enzymes

E3s are distinguished by their method of recruiting charged E2s, as well as whether they accept ubiquitin from the E2 by thioester transfer (Table 2, Fig. 6). The cullin RING ligases recruit charged E2s via a small RING-domain subunit specialized for this purpose—either RBX1 or RBX2. The RING-

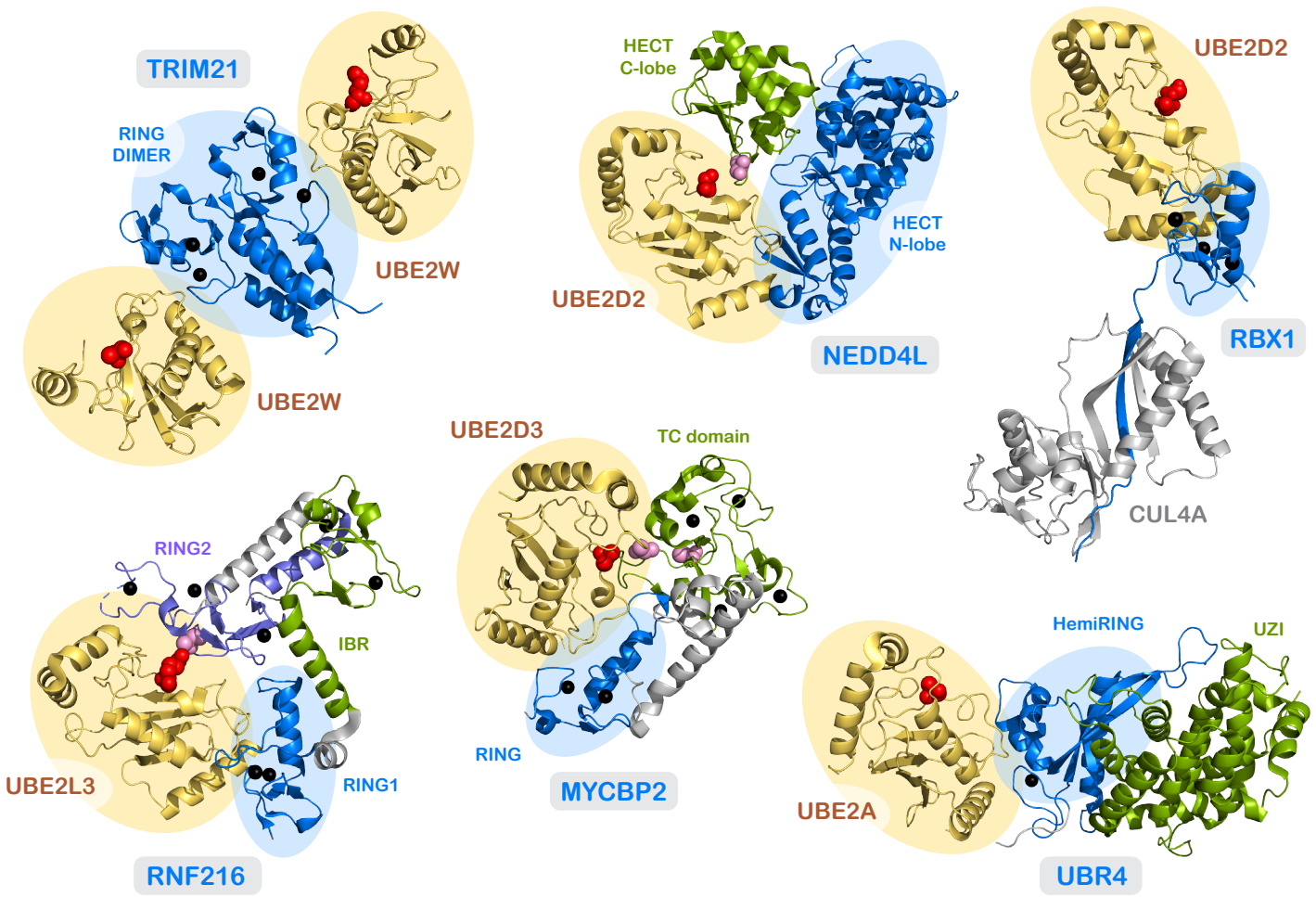

Figure 6. Varieties of E2 engagement

E3s engaging cognate E2s using a variety of motifs are shown. E2s are shadowed in yellow and their active site cysteines are shown in red as a space filling model. Portions of E3s that dock E2s are shadowed in blue. Catalytic cysteines for E3s are depicted in magenta. Zinc ions are rendered as black spheres. Ubiquitin, when present in structures, was omitted for clarity. ***Clockwise from top left:*** TRIM21 engages UBE2W via a dimerized RING domain; HECT ligase NEDD4L engages UBE2D2 via the N-lobe of the HECT domain; RBX1 docks into cullin CUL4A by completing a beta sheet in the RBX1 binding domain, then recruits UBE2D2 via its RING domain; UBR4 contacts UBE2A via a unique hemiRING domain that is embedded in an UZI domain; RCR ligase MYCBP2 engages UBE2D3 via a RING domain which supports transfer of ubiquitin sequentially to two cysteines in the TC domain; and RBR ligase RNF216 docks UBE2L3 via one RING domain, which supports the formation of a covalent adduct with the second RING domain. PDB IDs for figure structures are given in **Table S7**.

domain ligases, in contrast, use integrated RING domains to recruit charged E2s. The variant RING domains UBOX and PHD operate similarly. HECT ligases are mechanistically distinct from all of these, in that they form a thioester adduct with ubiquitin before transferring it to substrate. Their mode of E2 recognition is also distinct: charged E2s are bound by the N lobe of the HECT domain rather than through a RING. The RING-in-between-RING (RBR) ligases likewise form adducts with ubiquitin before transferring ubiquitin to substrate, though they do dock the charged E2 through an intrinsic RING domain[49]. The single member of the RING-Cys-Relay (RCR) Group[50] similarly employs a RING domain for E2 recruitment and forms ubiquitin adducts, though notably it passes ubiquitin through two sequential thioester adducts before completing substrate ubiquitination. RZ ligases RNF213 and ZNFX1 recruit distinct E2s through dissimilar domains, though they each form thioester adducts with ubiquitin via their RZ domains[51–54].

Specificity of E2-E3 pairings

Given that E3s vastly outnumber E2s, at least some E2s must work in concert with many E3 partners. However, insofar as the mapping function is understood, it appears to be most typically a

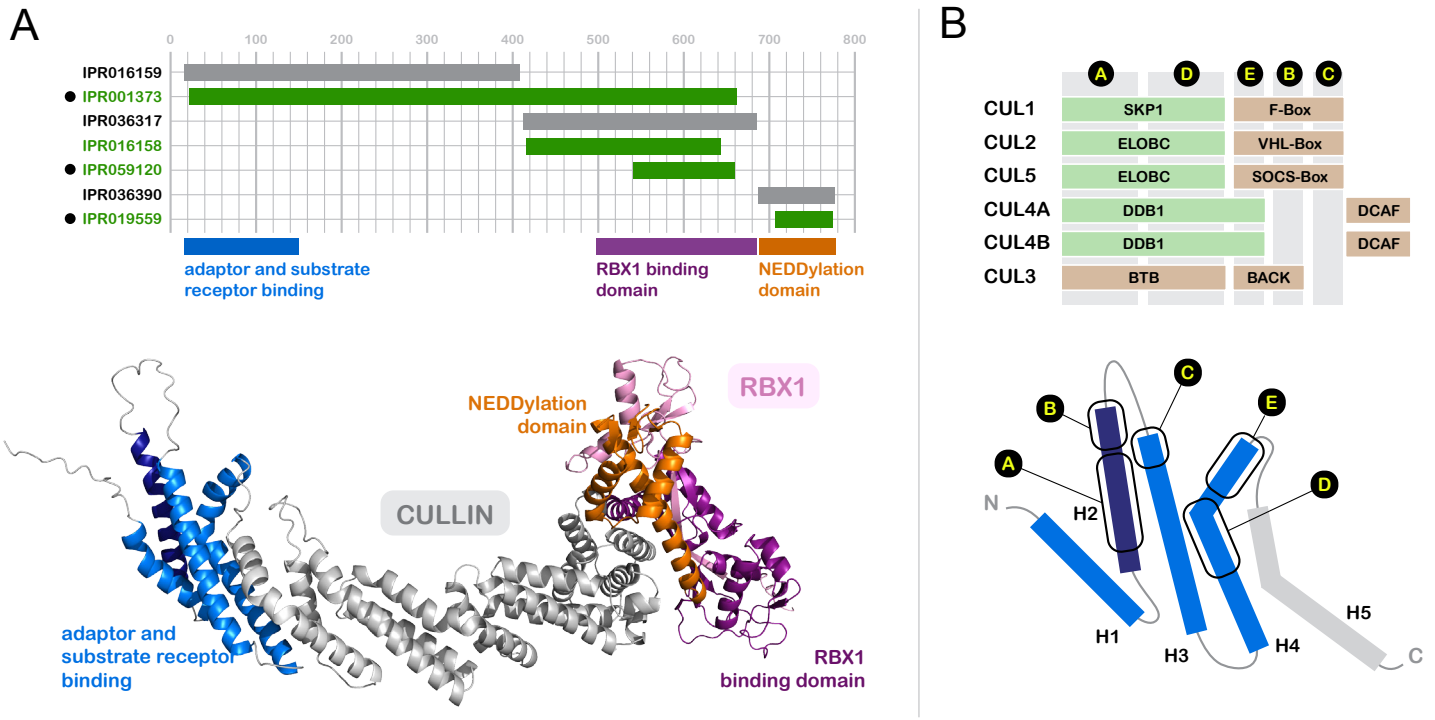

Figure 7. Cullin determinants engaging the cullin adaptors and substrate receptors

(**A**) Structural features of cullins. Top: Linear map of a generic cullin, with grey and green bars depicting InterPro homologous families and domains, respectively. Black dots indicate domains referenced in the main text. Shared features of CUL1, CUL2, CUL3, CUL4A, CUL4B, and CUL5 are shown in colored bars below the diagram. Bottom: shared features mapped onto an AlphaFold3 model of CUL1 in complex with RBX1. (**B**) Comparison of adaptor and substrate receptor docking by cullins. Bottom: a cartoon of a portion of a generic cullin is shown with binding determinants highlighted by letters A-E. Top: a schematic representing engagement of cullin subunits with their cognate adaptors (mint green) and substrate receptors (light brown). CUL1, CUL2, and CUL5 engage adaptors through elements A and D. Substrate receptors associate principally with adaptors, but also form contacts with elements B, C, and E of the cullins. CUL4A and CUL4B use a large adaptor, which holds receptors (DCAFs) at a considerable distance from the cullins. CUL3 does not have a dedicated adaptor such as SKP1, but associates with substrate receptors that have an adaptor-like BTB domain. Like true adaptors, this adaptor-like domain engages the A and D elements of the cullins. The adjacent BACK domain associates with the B and E elements used by other substrate receptors.

many-to-many map rather than one-to-many. Some fairly specific E2-E3 pairs are known[35,55], but they may be atypical. A comprehensive determination of E2-E3 pairings has not been achieved, though many robust biochemical pairings are known[34]. In the course of the Predictomes project[56], about 16% of the 15,000 possible pairings were modeled with AlphaFold Multimer, generating ipTM scores. (These scores reflect the overall confidence with which AlphaFold positions proteins with respect to each other.) This project used machine learning to calibrate AlphaFold Multimer predictions in light of experimental evidence, and the resulting SPOC (Structure Prediction and Omics-informed Classifier) scores reflect this new weighting.

Table S4 lists all E3 components expected to interact with E2s, and both the ipTM and SPOC scores as available. Of the 349 E3 components expected to associate with E2s and also examined via AlphaFold Multimer, roughly half were modeled at high confidence with one or more E2s. In contrast, in the BioPlex 3.0 database, which is compiled from proteome-wide affinity screens, relatively few E2-E3 pairs were recovered. For example, while UBE2D2 shows high-confidence modelling with 126 E3s, only three E3s were identified as BioPlex hits. Similarly, for UBE2L3, high confidence models were obtained with 72 E3s, but only four were recovered in BioPlex 3.0. The transient nature of the E2-E3 interaction presumably hampers the identification of authentic E2-E3 pairings via affinity screens. Beyond the issue of affinity, a number of valid interactions may have been missed because the relevant proteins were not expressed under the experimental conditions of BioPlex.

Cullin	RING	Ancestor	Cullin adaptor	Receptor signature domain	# in group	Associated Interpro domains	Percentage of domain matches included in UPS
CUL1	RBX1	Culα	SKP1	F-box	74	IPR001810, IPR036047	100%
CUL2	RBX1	Culα	Elongin BC	VHL box	45	none	N/A
CUL5	RBX2	Culα	Elongin BC	SOCS box	38	IPR001496	100%
CUL3	RBX1	Culβ	Intrinsic to substrate receptor	BTB	96	IPR000210, IPR003131	51%
CUL4A CUL4B	RBX1	Culγ	DDB1	none	N/A	N/A	N/A

Table 3: Composition of the modular Cullin Ring Ligases

Ubiquitin ligase families and their components

Cullin RING ligases

Cullin RING ligases (CRLs) constitute one of the two largest families of ubiquitin ligases[8,57–59], the other being the RING ligases (Table S1, Table S2). The cullin subunits serve as scaffolds to nucleate multicomponent E3s. Cullins share a variety of overlapping InterPro domains (Table S3, Fig. 7). The Cullin-like, alpha+beta domain (IPR059120) binds the small RBX1-type RING domain proteins[60], which in turn recruit E2 enzymes. The C-terminal *Cullin, neddylation domain* (IPR019559) is modified by NEDD8, a requirement for CRL activation[61]. *The Cullin, N-terminal domain* (IPR001373) is a superhelical domain comprising roughly 20 alpha helices and forms the backbone of the scaffold. The N-terminal four-helix bundle of this domain recruits adaptors which in turn recruit substrate receptors. The metazoan cullins CUL7 and CUL9 lack this four-helix bundle and engage substrates in a manner distinct from that of the canonical cullins. ANAPC2, a component of the Anaphase Promoting Complex (APC), lacks the neddylation domain, and is not considered to be a cullin but a degenerate offshoot of the family. CACUL1 lacks both the neddylation and RING-binding domains and is not expected to support E3 activity. It has been found to stabilize the oxidative-stress-sensitive transcription factor NRF2, probably by suppressing the $CUL3^{KEAP1}$ ligase[62], and is retained in the survey and filed with the cullins.

The cullin family is ancient, and members have been suggested to map to three hypothetical ancestral genes: Culα, Culβ, and Culγ (Table 3)[63]. The grouping of CUL1, CUL2, and CUL5 correspond to Culα, with CUL3 and CUL4 corresponding to Culβ and Culγ, respectively. The mode of substrate recognition used by each of these cullin groups reflects, to a degree, their ancient origin. CUL1 associates with cullin adaptor SKP1, and this complex recruits substrate receptors through the F-box domain. Proteins bearing this motif are characterized by the *F-box domain* (IPR001810) and *F-box-like domain superfamily* (IPR036047), with neither alone found in all F-box proteins (Table S3). The F-box proteins also contain various domains that are known to interact with substrate, the most common being leucine-rich repeats (IPR032675) and WD40 repeats (IPR001680). These are broadly-used protein-protein interaction domains. *The F-box associated (FBA) domain* (IPR007397) is specific to the CUL1 substrate receptors, though it does appear in one protein lacking an F-box.

Both CUL2 and CUL5 recruit their substrate receptors using the heteromeric adaptor ElonginBC[64,65]. Despite employing the same cullin adaptor complex, these two cullins have distinct sets of substrate receptors. CUL2 recruits substrate receptors through VHL boxes, and CUL5 through SOCS boxes. Each of these domains is divided into a BC-box, which contacts ElonginBC, and cullin-box, which distinguishes between the two cullins[66,67]. The SOCS box domain (IPR001496) efficiently identifies the CUL5 substrate receptors, though we propose seven SOCS box proteins that do not have the canonical motif (Table S1). CUL5 substrate receptors most commonly use ankyrin repeats (IPR002110) and SH2 domains (IPR000980) for substrate recruitment, which are of course broadly represented in the proteome. Despite the similarities between CUL5 and CUL2, the substrate receptors of CUL2 are not easily identified using a canonical domain. The CUL2 substrate receptor VHL interacts with CUL2 via the VHL box (IPR024048), but other

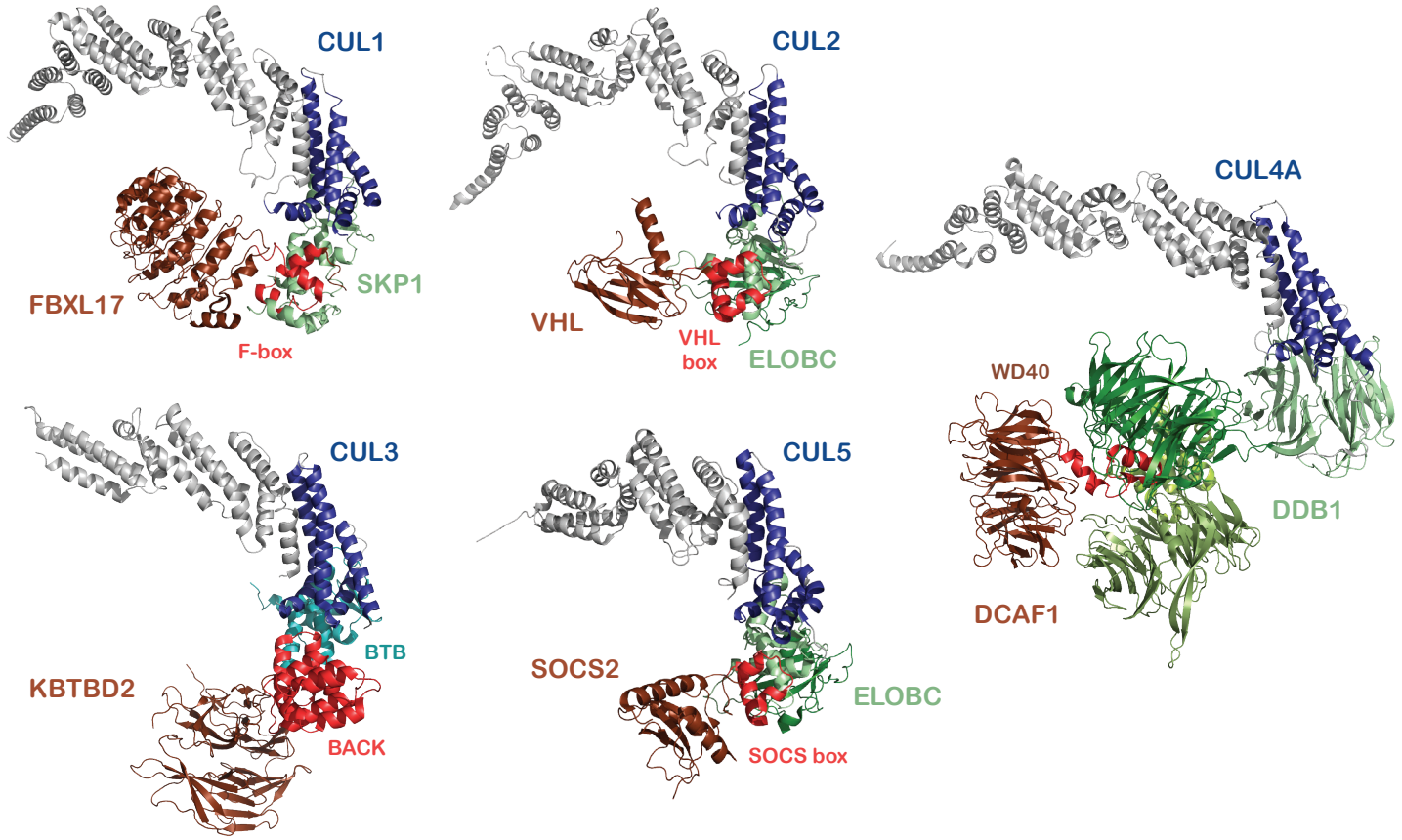

Figure 8. Canonical Cullin Ring Ligases with their receptors

Canonical cullins are shown in complex with their adaptors and representative substrate receptors. Cullin domains are depicted (without RBX1-binding domains or NEDDylation domains) in grey, with the four-helix bundle which binds adaptors and substrate receptors shown in blue. Adaptors for CUL1, CUL2, CUL4A, and CUL5 are shown in shades of green, with the portions that contact receptors shown in light green. For the substrate receptors, adaptor and cullin binding portions shown in red and the presumptive substrate binding domains shown in brown. In the CUL3 assembly, the BTB portion of receptor KBTBD2 is shown in teal, and the BACK domain in red. Structure references are listed in Table S7.

proposed CUL2 receptors do not share this domain, and in fact have no common signature domain. The CUL2 receptors use a variety of substrate interaction domains, with leucine-rich repeats (IPR032675) being dominant.

Just as CUL2 and CUL5 share the adaptor ElonginBC, CUL4A and CUL4B share DDB1[68–70]. The central beta propeller of DDB1 contacts the N-terminal four-helical bundle of CUL4A and CUL4B, and the flanking beta propellers form a deep cleft for the binding of the substrate receptors. While SKP1 and ElonginBC are modest in size, and allow the substrate receptors to simultaneously dock with the cullin and cognate adaptor, DDB1 is quite large, interposing at least 40Å between cullin and receptor (Fig. 8). In some cases, the cell exploits the size of this adaptor. CUL4A substrate receptor DDB2 docks into DDB1 via a helical hairpin and one surface of its WD40 domain. Basic residues on the opposite face of the WD40 domain contact UV-damaged DNA, and this large complex sweeps out a "ubiquitination zone" in proximity to DNA photodamage[71]. Putative CUL4A/CUL4B substrate receptors were identified in four proteomic studies[69,70,72,73], and were found to be substantially enriched in WD40 repeats (see Methods). Subsequent studies showed that while the WD40 domains are proximal to DDB1, the most intimate contacts to DDB1 reside in alpha helical elements that are adjacent to the WD40 domains and insert into the DDB1 cleft (Fig. 9). The alpha helical element is likewise a binding determinant for CUL4A/CUL4B substrate receptors lacking a WD40 repeat (Fig. 9). We classified as CUL4A/CUL4B substrate receptors proteins that were identified by DDB1-affinity purification and that also modeled well with DDB1 (Fig. 10). We also included homologs of strong candidates, as well as a few proteins with poor DDB1 modelling that were nonetheless annotated in UniProt as participating in

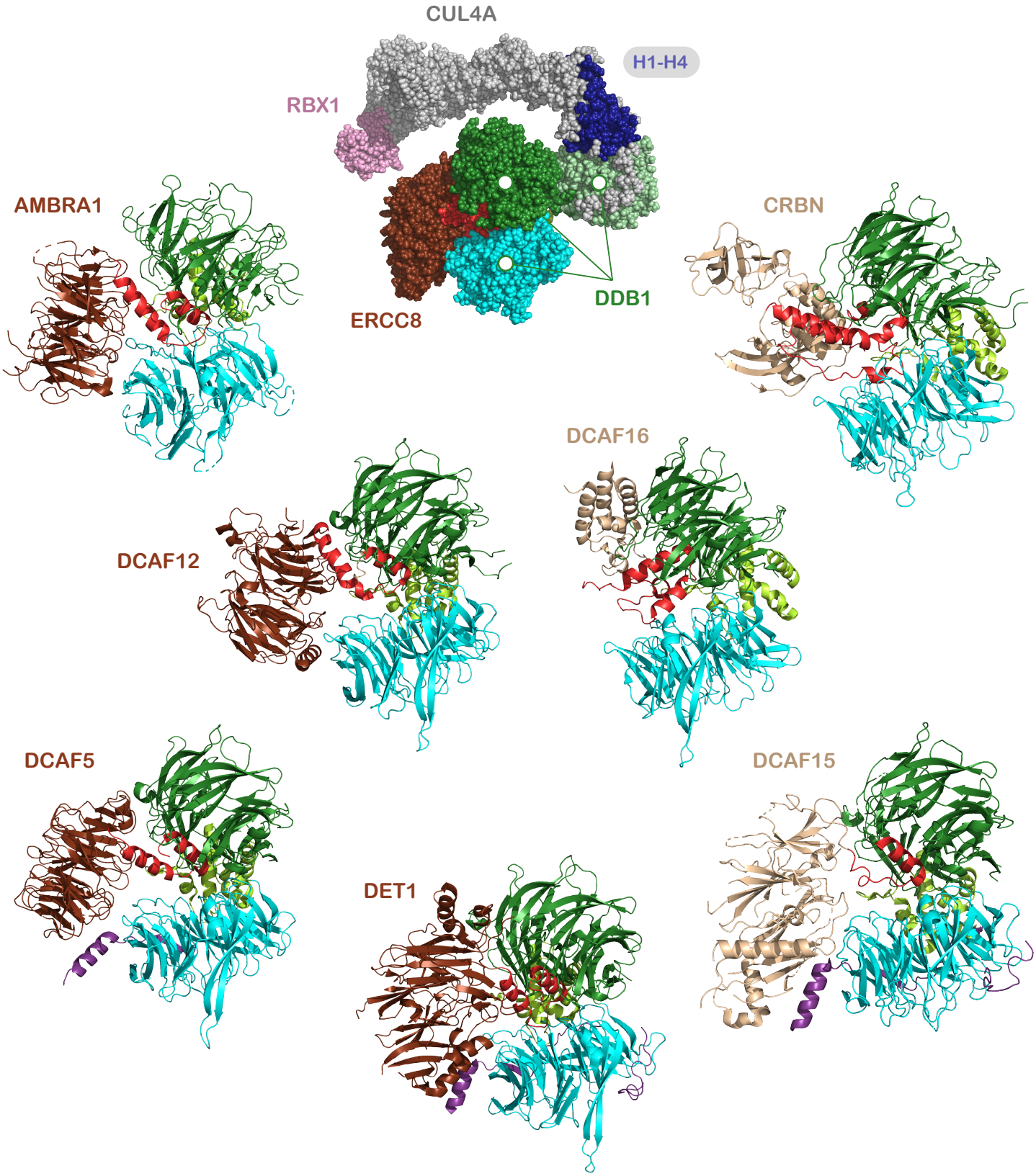

Figure 9. CUL4 substrate adaptors

Top center: CUL4 substrate receptor ERCC8 (brown and red) solved with DDB1 (sage, green, and teal), CUL4A (dark blue and grey), and RBX1 (pink), shown in spacefill. The remaining structures only contain DDB1 and the CUL4 substrate receptors. ***Left and bottom center:*** CUL4 substrate receptors with WD40 domains (brown). ***Right:*** CUL4 substrate receptors without WD40 domains (beige). For all structures, helices in red are the components of CUL4 adaptors that form the majority of contacts between the CUL4 substrate receptors and the flanking beta propellor domains of DDB1. Structure references are listed in **Table S7**.

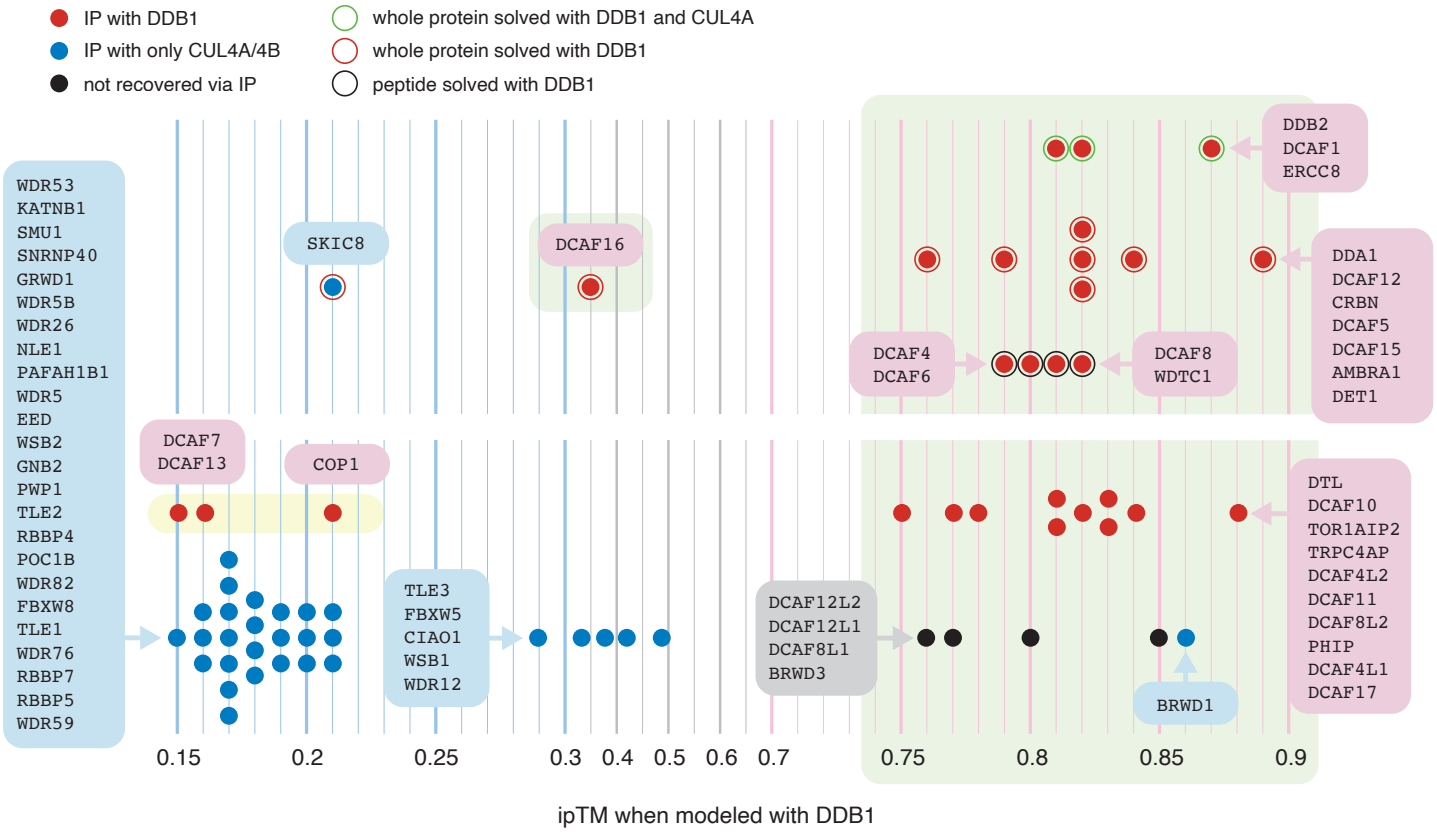

Figure 10. DDB1 and CUL4A/CUL4B associated proteins modeled with DDB1

Proposed CUL4A and CUL4B receptors were modeled with DDB1 using AlphaFold3, and resulting ipTM values are plotted along the x-axis. Red dots indicate recovery in immunoprecipitation with DDB1; blue does indicate recovery in immunoprecipitation with CUL4A/CUL4B; black dots indicate paralogs of proteins recovered by immunoprecipitation[69,70,72,73]. Proteins for which there are structural data supporting a complex with DDB1 are indicated in the top half of the figure. Outline colors indicate the extent of the complex experimentally determined (see figure for details). Green shaded regions indicate proteins with experimentally determined co-complexes with DDB1, high confidence ipTM scores for AlphaFold3-predicted complexes, or both. Yellow shading indicates proteins lacking both but included in our survey. We exclude from our survey all components not shaded in yellow or green.

CRL4 ligase activity. We excluded 30 proteins from the initial studies that modelled poorly with DDB1 and were only recovered in one screen (**Fig. 10**, blue shading)[72,73].

CUL3 recruits substrate receptors not through an intermediate cullin adaptor, but through an adaptor domain intrinsic to CUL3 substrate receptors[74–77], namely the *BTB/POZ domain* (**IPR000210**). This binds the same regions of the cullin four-helix bundle that are recognized by the adaptors for CUL1, CUL2, and CUL5 (**Fig. 7**). The *BTB domain* (**IPR000210** or **IPR011333**) is found in 189 human proteins, but we consider only half of these to be CUL3 receptors, as BTB domains are not necessarily sufficient for productive binding with CUL3[78–80]. Valid CUL3 receptors mostly partition into two groups (**Fig. 11**). The larger group is characterized by a *BACK domain* (**IPR011705**) appended to the BTB domain, with the 3-box element of the BACK domain mimicking the contacts formed between CUL1, CUL2, and CUL5 and their substrate receptors (**Fig. 7**). The BACK domains are not exhaustively captured by **IPR011705**; we identified an additional 20 BACK domains by evaluating each BTB protein for an adjacent BACK domain (see **Table S3**). *KELCH repeats* (**IPR006652**) are found in two-thirds of the BTB-BACK-type CUL3 receptors. All BTB-BACK proteins were modeled with CUL3 using AlphaFold3, and those with weaker scores are included in our survey and flagged as variants.

The second group of CUL3 receptors carries the KCTD-type BTB domains (**IPR003131**). In total, there are fifty KCTD-type BTB domain proteins, partitioning into Type I and Type II. The Type I group was recently evaluated systematically via biochemistry and modelling[81], and only two-thirds were found to associate with CUL3. We adopted those findings wholesale in our survey. Note that

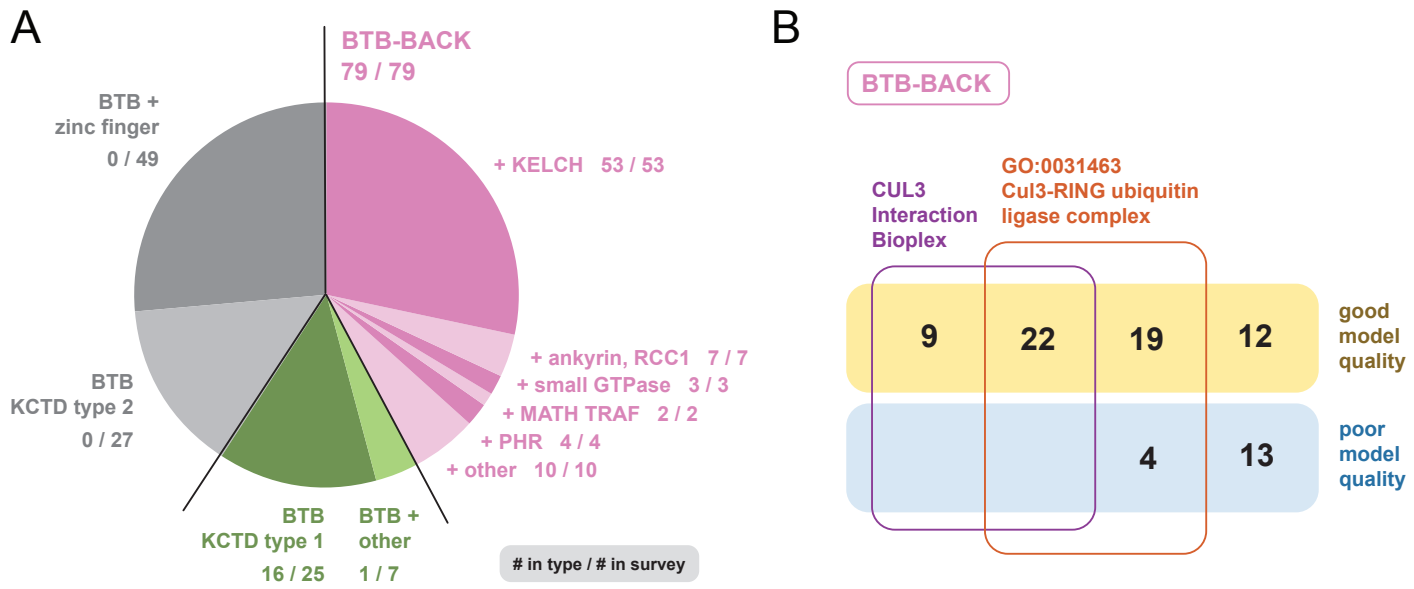

Figure 11. A subset of BTB proteins expected to function as CUL3 partners

(**A**) Pie chart showing complete set of BTB proteins in human, distinguished by context of the BTB domain. Numbers in figure labels indicate proteins included as CUL3 receptors versus the set in each sector. Grey sectors indicate groups of BTB proteins excluded from our survey as CUL3 receptors, green sectors indicate groups of BTB proteins for which a subset are included in our survey, and pink sectors indicate groups of BTB proteins that were fully included. (**B**) Characteristics of BTB-BACK proteins. Members partition into 62 that show possible or likely valid modeling with CUL3 (ipTM of 0.6 or greater) and 17 that show weak modeling. Only BTB-BACK proteins that model well with CUL3 were recovered in the Bioplex 3.0 sets. The GO term for CRL3 primarily, but not exclusively, includes BTB-BACK proteins with strong modeling results.

the KCTD-type BTB proteins that host CUL3 tend to assemble into pentameric forms with CUL3 subunits emanating from BTB rings[82] so as to contact not one but two faces of the BTB domains[81]. The Type II group carries ion transport domains (**IPR005821**) adjacent to the BTB domain, and in at least some cases steric hindrance should prevent association of these proteins with CUL3. The last large group of BTB proteins, which carry between two and fourteen C2H2-type zinc fingers (**IPR013087**) at their C-termini, lack key features for driving CUL3 engagement. Most members of this set are described in UniProt as transcriptional regulators.

RING ligases

While the cullin RING ligases recruit E2s by means of an adaptor subunit, the remaining Groups of ligases recruit charged E2s through intrinsic domains. The RING ligases constitute the largest of these Groups, numbering ~300[83–85]. The RING ligases in our survey are extremely varied, ranging for example in length from about 150 residues to more than 5000. As indicated in **Table 2**, more than 90% of the RING ligases share the *RING-type zinc finger* (**IPR001841**) domain. Within the RING ligase Group, a few Types of RING ligases are designated by other signature domains; the three Pellino ligases share **IPR048335**, the eleven MARCH ligases share **IPR011016**, the three proposed IRF2BP ligases share **IPR044882**. The UBR RING ligases do not share an InterPro domain that corresponds to RING activity, but they have been characterized experimentally[86] and are annotated with RING domains from the CDD. In a few cases, comparison to paralogs allowed the identification of RING domains (RFPL2, PEX12, and VPS1), and these are also annotated with CDD domains. The RING ligase family has been sorted into more than 30 Types with homologous regions beyond the RING domain[85]. We largely adopt these categories, and introduce a few new ones based on shared properties: RING ligases with transmembrane domains, with ubiquitin or UBL binding domains, or bearing additional enzymatic functions.

The RING ligase family contains one exceptionally large family, known as the TRIM ligases. Numbering more than 80, these were parsed previously into 12 subsets[87,88], and we follow that organization as the basis for Type designations for the TRIMs. More than half of the TRIMs contain a PRY-SPRY substrate-recognition domain (**IPR001870**, TRIM

Class IV), but this domain is not exclusive to TRIMs or to RING ligases. Structurally, TRIMs are notable in that their RING domains are separated from their substrate interaction domains by a single alpha helix of typically 100 residues or more, in most cases containing a break in the helix toward its C-terminal end, which allows for the formation of a hairpin structure. These long helices are typically involved in homodimer or heterodimer formation with family members, and some higher order assemblies are known[88–90]. Dimerization places the RING domains in proximity to each other, which promotes the release of ubiquitin from the E2 and transfer of ubiquitin to substrate[91]. Recently, the TRIMs were systematically evaluated for homodimer formation to activate the TRIMs[88]. Some were found to be inactive, at least in these assays, calling into question whether they are true E3s. Nonetheless, we include all of the TRIMs in our survey. RINGless TRIMs, although not expected to recruit E2s, were included in our survey because they may modulate TRIM activity. Interestingly, while we assembled this survey, we noticed that 11 TRIMs are modeled in AlphaFold2 as having folded central helices, which may modulate dimerization (**Table S1**).

Idiosyncratic RING ligase complexes

Of the myriad RING ligases, many function in the context of larger complexes. Annotation efforts typically include only the catalytic portion of these complexes; we include all subunits. While we reference these ligases as idiosyncratic, they are no less interesting for their individuality. CTLH, conserved through eukaryotes, is built around a RING heterodimer and at least three times as many noncatalytic components. The core complex assembles into circular supramolecular complexes of 1 MDa or more, which are capable of ubiquitinating oligomeric structures through avid interactions[92]. The anaphase promoting complex (APC) is built around a catalytic core containing one RING subunit and one degenerate cullin, but this core is embedded in a much larger assembly. Using exchangeable substrate receptors, the APC drives the cell cycle by promoting timed degradation of cyclins, securin, and tens of other proteins. The much smaller KPC complex also controls cell cycle progression, driving the G0 to G1 transition. It is composed of the RNF123 RING subunit, which contains a SPRY domain, and a noncatalytic UBAC subunit. The latter encompasses two UBA domains, which bind the ubiquitin moieties of modified substrates, and a UBL domain, which promotes targeting of substrate to the proteasome.

Several RING complexes are known to engage damaged DNA, and failures of these complexes result in cancers and anemias. The first is the FANC core complex, which promotes the repair of interstrand DNA crosslinks. Mutations in FANC complex components give rise to Fanconi Anemia[93]. The FANC core complex is composed of one RING domain protein, which also contains a ubiquitin binding element, and an additional six noncatalytic subunits. The ubiquitin binding domain of FANCL supports monoubiquitination of pathway-critical FANCD2 and FANCI[94]. The repair of DNA double-strand breaks (DSBs) involves multiple E3 enzymes that target histone H2A for modification in the neighborhood of the DSB, including RNF8, RNF168, and, downstream of them, BRCA1/BARD1[95]. Each subunit of BRCA1/BARD1 contains a RING domain, and the complex dimerizes through helical bundles flanking the RING domain. It may be the case that only recruitment of the E2 through BRCA1 is required for full activity[96], though BRCA1 is strongly activated by BARD1. DNA repair is also promoted by ubiquitination of histone H2B, notably by the Bre1 ligase complex[97–100]. In addition to resolving DNA damage, E3s engage DNA in modulating transcription. The RING ligase MSL2 ubiquitinates histone H2B as well, and this activity is strongly activated by forming a heterodimer with MSL1[101]. Note that this heterodimer is embedded in a larger histone acetylation complex[102].

Misfolded proteins present in the ER are degraded via the ERAD pathway (ER-associated degradation) in which substrates are exported back to the cytosol–a process known as retrotranslocation–before the proteasome can degrade them. In the canonical pathway of retrotranslocation, a ubiquitin ligase, Hrd1, constitutes the primary transmembrane channel in yeast, ubiquitinating the substrate once it is exposed to the cytoplasm[103]. Extraction from the membrane is then driven by VCP[104], which recognizes the ubiquitin groups added by Hrd1. In humans, a variety of ubiquitin ligase complexes are embedded in the ER membrane[105]. To cite one example, the active component of the membralin complex is the RING ligase RNF185, a small protein consisting primarily of a transmembrane hairpin anchoring a RING domain on the cytosolic side of the ER membrane. RNF185 functions in complex with three other membrane proteins, including the much larger TMEM159, which may be involved in substrate recognition in the ER lumen, and TMUB1 and TMUB2, both of which have UBL domains on the cytosolic side. Another includes the LMBR1L-

GP78-UBAC2 complex, which includes the RING ligase GP78/AMFR. The N-terminal portion of AMFR is embedded in the ER membrane, and the C-terminal half projects into the cytoplasm. This portion engages UBE2G2, its cognate E2, not only through a RING domain but also a second domain which promotes high affinity binding. Connecting these two is a CUE domain, which associates with ubiquitin. AMFR associates with two other membrane proteins, UBAC2 and LMBR1L, the former including a UBA-type ubiquitin binding domain and the latter engaging with components of the WNT signaling pathway.

Variant RING ligases

In the RING domain of the RING ligases, two zinc ions coordinate a combination of eight cysteines and histidines to form a cross-brace structure which forms the basis for the RING signature. The 40-60 residue motif contains a small three-strand beta sheet and an alpha helix. Loops connecting these elements provide docking sites for E2 enzymes. To dock the E2s, the *U-box domain* (**IPR003613**) mimics this arrangement through a collection of hydrogen bonds rather than zinc coordination[106]. As compared with true RING domains, the collection of UBOX ligases is small, numbering only eight, and we put them in a distinct Group. There are two proteins, RBBP6 and TRIM21, that have overlapping RING (**IPR001841**) and UBOX (**IPR003613**) motifs, and we place these in the RING rather than UBOX Group. One protein, UBOX5, has distinct UBOX and RING domains, and it is the UBOX that is required for E2 association[107].

Like the RING domain, the PHD domain (**IPR019787**, **IPR001965**) coordinates two zinc ions in a cross-brace configuration[108]. By and large, PHD domains associate with methylated histones rather than E2s[109], but six PHD domain proteins have been found to function as ubiquitin ligases. These include AIRE, G2E3, ING4, JADE2, KMT2A, and KMT2B[110–115]. While lacking a canonical PHD domain, UBR7 bears a PHD-like domain that supports its ubiquitin ligase activity, and it is included as a variant PHD[116]. PHD and RING domains occasionally occur in the same protein. Six RING ligases have predicted PHD domains entirely coincident with predicted RING domains (BAZ1B, NFX1, NSD2, PHRF1, UHRF1, and UHRF2). In contrast, KMT2C and KMT2D have multiple predicted PHD domains that do not fully overlap with predicted RING domains—we categorize these as canonical RING ligases. Five RING ligases have PHD domains that are clearly distinct from their RING domains, and these include SHPRH, PHF7, and the three Class VI TRIMs (TRIM24, TRIM28, and TRIM33).

HECT ligases

Unlike RING ligases, the HECT ligases form thioester adducts with ubiquitin, a property of the HECT domain[117]. This domain is divided structurally into N-terminal and C-terminal lobes connected by a flexible hinge. E2s associate via the N-terminal lobe and donate ubiquitin to a cysteine in the C-terminal lobe. Ubiquitin is transferred from this cysteine to the substrate. The HECT ligases constitute a Group of 28 proteins which are defined by a single domain (*HECT domain*, **IPR000569**). HECT ligases are elaborated by a variety of domains which determine substrate specificity and cellular context. The most common substrate interaction motifs include the *WW* (**IPR001202**) and *RCC1* (**IPR009091**). The smallest of the HECT ligases are roughly 80 kD, and several weigh in around 500 kD: HERC1, HERC2, and HUWE1. The latter adopts a ring-shaped architecture decorated with protein interaction modules, including ubiquitin binding domains[43]. The ubiquitin binding elements provide a means of recruiting soluble substrates and aggregates that carry a high density of ubiquitin chains, and promoting amplification of the ubiquitin signal[118].

RBR, RCR, and RZ ligases

The RBR and RCR ubiquitin ligases combine features of RING and HECT ligases in that they recruit charged E2s via RING or RING-like domains, however they form thioester adducts with ubiquitin before transferring it to substrate[49,50]. For the 14 RBR ligases, the functional trio of an E2-binding RING1 domain, an IBR domain, and a catalytic-cysteine-containing RING2 domain is encompassed by the TRIAD domain (**IPR044066**). In the case of the RBR ligase PARKIN, its activity state is stringently controlled, with both RING1 and RING2 domains being inert in the basal state due to steric occlusion. Activation consists of a complex conformational rearrangement of structural domains (**Fig. 4**) that releases both the E2-binding site and the catalytic cysteine from inhibition[119]. Two of the RBR proteins, RNF31/HOIP and RBCK1/HOIL1, and a third protein, SHARPIN, form the LUBAC complex, which builds linear ubiquitin chains, controlling inflammation, innate immunity, and adaptive immunity[120,121]. HOIL1 has the unusual property of forming oxyesters with the C-terminus of ubiquitin[122]. There is a single RCR ligase in humans, MYCBP2, which as noted above

has the unusual property of forming two sequential adducts with ubiquitin prior to transferring ubiquitin to substrate, employing a unique mediator loop for the formation of the first adduct[50]. As with RCR and the RBRs, the RZ ligases RNF213 and ZNFX1 form thioester adducts with ubiquitin. Adducts are formed via the small RZ finger (**IPR046439**), though this domain does not serve to recruit E2s. For RNF213, the RZ finger is embedded in a so-called E3 shell which, along with and adjacent C-terminal domain, recruits charged E2[51,52]. In contrast, ZNFX1 lacks this structure and uses a variant RING domain to recruit E2[53,54]. The RZ ligases each support innate immunity[51–54].

Idiosyncratic E3 enzymes

The E3s described in this survey generally have known or predicted mechanisms for recruiting E2s. We include fourteen proteins as E3s for which the E2 binding component is atypical or unknown (**Table S1**). In vitro autoubiquitination provides good support for E3 function, if the reaction depends on the addition of ubiquitin, E1, and E2. Identification of substrates stabilized by the deletion of a proposed E3 also provides support, particularly if an interaction between the substrate and proposed E3 has been demonstrated. The idiosyncratic E3 enzymes we propose are supported by some or all of this evidence. We include in this group UBR4, which recruits UBE2A/RAD6 via a hemiRING motif which packs into a novel UZI domain (**Fig. 6**)[123]. UBR4 associates with KMCF1[124], and two recent structural studies have demonstrated that these can combine with calmodulin to form a massive ring structure, recruiting substrates to the interior of the ring[46,47]. In this context, the complex acts as an E4.

E3s for ubiquitin-like modifiers

The E1 and E2 enzymes for the various ubiquitin-like modifiers (UBLMs) are both functionally and structurally analogous to those of ubiquitin. The UBL ligases show impressive variation, as we have seen in ubiquitin ligases. ISG15, induced by interferon in response to viral infection, was the first UBLM to be recognized as such[125]. The principal ligase for ISGylation in humans is HERC5[126,127], although several others may contribute[127–129]. SUMO was first discovered as a ubiquitin-like modifier for RANGAP1, targeting it to the nuclear pore complex[130,131]. Myriad physiological functions for SUMO have since been discovered[132–136] mediated by at least 50 SUMO receptors (**Table S5**)[137,138]. SUMO is ligated to targets via RING variants known as MIZ/SP-RINGs (**Table 1**)[139], and through non-MIZ ligases (**Table 1**)[140]. NEDD8, while serving critically as a modifier of cullin RING ligases[31], can modify many other proteins, depending on conditions of the assay[141]. Modification of the CRLs is promoted by the DCN family (**IPR005176**)[142], and a variety of bifunctional ubiquitin ligases carry out NEDDylation as well (**Table 1**)[143].

As described above, ATG8 is conjugated to lipids rather than proteins[17], which is essential for autophagy. This critical activity, which tethers ATG8 to membranes, is mediated by an E3 complex composed of ATG5, which is itself modified with the ULM ATG12, and ATG16L1[144]. The principal target of UFMylation is ribosomal protein RPL26[145], a modification that promotes the dissociation of ribosomes from the ER translocon, particularly when the translation cycle of secretory proteins is interrupted. This may occur for example as a result of a truncated mRNA, as it will lack a stop codon. This modification is catalyzed by the atypical E3 enzyme UFL1[146], functioning in complex with UFBP1 and CDK5RAP3[147]. Such translational errors are generally resolved by the RQC pathway, involving multiple ubiquitination events, but the RQC pathway is sterically blocked from acting on ribosomes that are complexed with the translocon on the ER membrane. Thus, UFMylation works closely with the RQC pathway to neutralize translational errors[148–150].

Deubiquitinating enzymes

The median half-life of a ubiquitination event in HeLa cells is estimated to be very short–approximately 12 minutes–with some sites being demodified in under one minute[151]. The rapid reversal of ubiquitin modifications is mediated collectively by over 100 dedicated proteases, which are highly differentiated in their specificity, subcellular localization, and regulation. The domains that govern the deubiquitinating enzymes (DUBs) that remove ubiquitin and ubiquitin-like modifiers from their target proteins are large and well-defined, so construction of the DUB Class is straightforward. The USP-type DUBs constitute the largest family of DUBs, and share the InterPro domain **IPR028889**. This domain also captures PAN2, which functions in mRNA deadenylation rather than deubiquitination. The USP family is elaborated with a variety of other domains, including ubiquitin binding and ubiquitin-like domains, modulating function and substrate specificity. The auxiliary domains that establish Types within the USP Group are listed in **Table S3**. Multiubiquitin chains may be built through any of the

seven lysines of ubiquitin or through its N-terminal methionine. On the whole, the USPs do not show strong linkage preferences in their deconjugating activity[152]. The USP17 group comprises a third of the USP-type DUBs, with group members being at least 90% identical. This family was generated by the expansion of tandem repeats on two chromosomes in humans and within the RS447 megasatellite[153]. As with the E2 enzymes, we include catalytically inactive members, of which there are five in the USP Group, three of which are of the USP17 Type. The other flagship group of deubiquitinating enzymes is the much smaller UCH Group, numbering four and covered by the domains **IPR057254** and **IPR041507**. The UCH family has been viewed as dedicated to the removal of small peptides from the C-terminus of ubiquitin, consistent with leaving group restrictions imposed by an active-site crossover loop[154], but more recently the proteasome-associated UCHL5 has been shown to resolve branched ubiquitin chains[155,156].

Utilization of the *MPN domain* (**IPR037518**) for deubiquitination traces as far back as archaea such as *C. subterraneum*[157]. In eukaryotes, three multisubunit protein complexes[158]—the lid of the proteasome, the COP9 signalosome, and the translation initiation factor EIF3—contain a pair of JAMM/MPN domain proteins. One is active as a DUB in the proteasome, one as a deNEDDylase in the signalosome, and both as DUBs in EIF3. Of the 15 MPNs we include in the UPS, 10 are active as DUBs, four are included as integral members of active complexes, and one is included as a ubiquitin binding protein. The JAMM/MPNs, uniquely among the DUBs, are metalloproteases rather than cysteine proteases. Most commonly, they act on K63-linked ubiquitin[159], beautifully exemplified by AMSH[160], though PSMD14/RPN11, a proteasome subunit, acts at the proximal ubiquitin in a substrate-linked chain[161–163].

The OTU family shares the OTU domain (**IPR003323**), though OTULIN and its inactive counterpart are identified by FAM105 (**IPR023235**). More than half of the OTU family show a strong preference for one or two of the eight ubiquitin-ubiquitin linkages[164]. The MINDY family, a group of four proteins identified by one of two domains (**IPR033979**, **IPR025257**), prefer K48-linked ubiquitin, with the exception of MINDY[159]. ZUP1, in a family of its own, shows strong preference for K63-linked ubiquitin[165]. Uniquely among DUBs, the four members of the Josephin family (**IPR012462**, **IPR049387**) show strong preference for ubiquitin esters over ubiquitin isopeptide linkages, and thus provide a largely dedicated pathway for the reversal of non-lysine-directed ubiquitination, notably modifications of threonine and serine[166].

The remaining DUB families work exclusively or in part on UBLMs. Two of these are structurally related to ZUP1: the UFSP family (**IPR012462**) and the ATG4 family (**IPR046792**)[165]. The USFP family functions in deUFMylation[167], and the ATG4 family is mainly involved in biosynthetic processing of ATG8 family proteins and their delipidation[168]. Most of the SENP family (**IPR003653**) are deSUMOylating enzymes[169], but SENP8 acts on NEDDylated proteins[170,171]. The PPPDE pair (**IPR008580**) are both proposed to be deSUMOylases[172], though DESI2 additionally functions as a deubiquitinating enzyme[173]. The PPPDE family has also been observed to exhibit cysteine palmitoyl thioesterase activity[174].

The physiological effects of DUBs have frequently been linked to specific E3s, and in some cases they populate the same complexes (reviewed in [175]). A DUB can antagonize an E3, or alternatively potentiate an E3 by counteracting suicidal E3 autoubiquitination. A DUB may also fine-tune E3 output by editing ubiquitin chain generated by the E3.

Ubiquitin and ubiquitin-like binding proteins

As shown in **Fig. 1**, ubiquitination promotes protein turnover through both autophagy and the proteasome, and also promotes signaling events in the absence of any proteolytic targeting. In all cases, it is expected that ubiquitin receptors connect protein ubiquitination with protein fate. We organize ubiquitin binding proteins first by molecular or biological context (described at the Group and Type levels), and then by ubiquitin binding domain (Subtype). We also describe the ubiquitin receptors in an auxiliary table (**Table S5**), ordered first by domain then by molecular context and function.

Close to twenty canonical domains characterize the ubiquitin binding motifs, but even so, only about 60% of the ubiquitin binding elements we list map to InterPro domains. The most frequently used element in ubiquitin recognition is the UBA domain, a three-helix bundle of about 40 amino acids described by the *Ubiquitin-associated domain* (**IPR015940**) and its related homologous superfamily (**IPR009060**)[176]. These two InterPro domains, along with an array of niche UBA domains (**Table S5**, Domain Summary tab), identify 92 UBA elements in the human

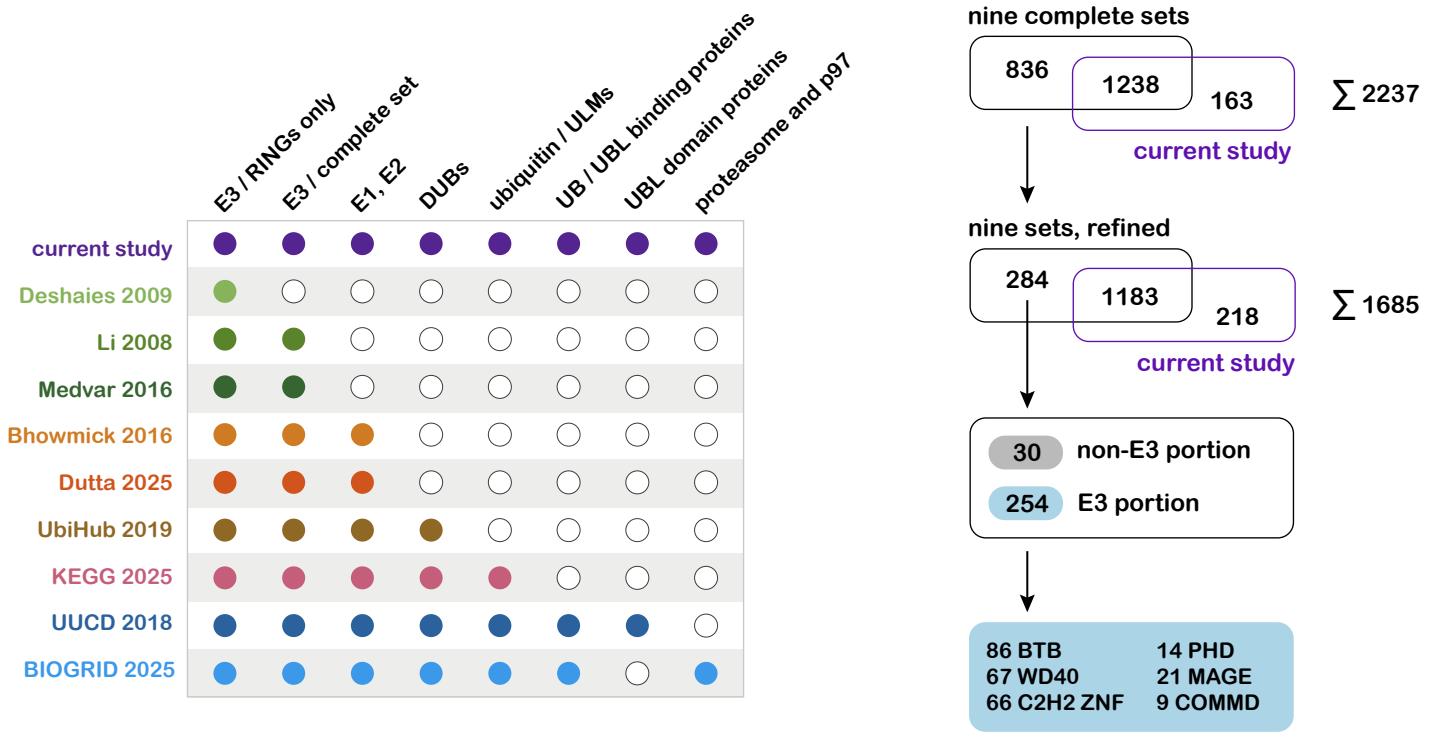

Figure 12. Comparison of UPS annotations including E3 ligases

Nine previous UPS annotations (see **Table S5** [Collections tab]) were chosen for comparison to the current study. ***Left panel***: the scope of each collection. Colored dots represent inclusion of the indicated category in related survey. ***Right panel***: members of the current study are compared with an aggregate of the nine earlier studies (first Venn diagram). The aggregate set was refined, removing proteins that do not carry specific annotations or were computationally derived (see Methods), and again compared with the current study (second Venn diagram). The excluded proteins in the second analysis were partitioned into those proposed to be E3 ligases and not (third diagram), and evaluated for enriched InterPro domains (fourth diagram).

proteome. Given the breadth of this group, members appear in nearly every molecular context and pathway involving ubiquitination. Two contexts are worth special mention: the UBL-UBA proteins that shuttle substrate to the proteasome, and SQSTM1/p62, a critical receptor for autophagy. Like the UBA domain, the CUE (IPR003892), GAT (IPR004152), and VHS (IPR002014) domains are also composed of helical bundles, the last two are principally involved in biosynthetic subcellular trafficking[177,178].

UIM elements are simpler than the domains above, consisting of little more than a short stretch of alpha helix. Fewer than half of UIMs that we find are identified by the *Ubiquitin interacting motif site* (IPR003903), the rest being either validated experimentally or identified by sequence and remaining to be confirmed. Though small, UIM domains embedded in proteasome subunit PSMD4/RPN10 serve as the strongest ubiquitin receptor elements of the proteasome[179,180]. Other simple helical elements involved in ubiquitin recognition include the *UBM* (IPR025527), *UBAN* (IPR032419), and MIU elements, the last having no corresponding InterPro domain. Numerous zinc fingers recognize ubiquitin, in aggregate approaching the size of the UBA family. These include *Zinc finger, RanBP2-type* (IPR001876), *Zinc finger, UBP-type* (IPR001607), *Zinc finger, A20-type* (IPR002653), *CALCOCO1/2, zinc finger UBZ1-type* (IPR041641), *FAAP20, zinc finger UBZ2-type* (IPR031490), *DNA polymerase eta, ubiquitin-binding zinc finger* (IPR041298), *Rad18, zinc finger UBZ4-type* (IPR006642), and *NEMO, Zinc finger* (IPR034735). In addition to these, we identify another 12 zinc fingers that are not covered by these domains.

As mentioned above, catalytically inactive members of the E2 family are retained in our survey. Four such variants–UBE2V1, UBE2V2, AKTIP, and UBE2NL–share the canonical E2 domain (IPR000608), and the first two form heterodimers with UBE2N, imposing chain linkage specificity by orienting the acceptor ubiquitin[181,182], thus supporting DNA repair. The domain IPR000608 falls within a homologous superfamily, *Ubiquitin-conjugating enzyme/RWD-*

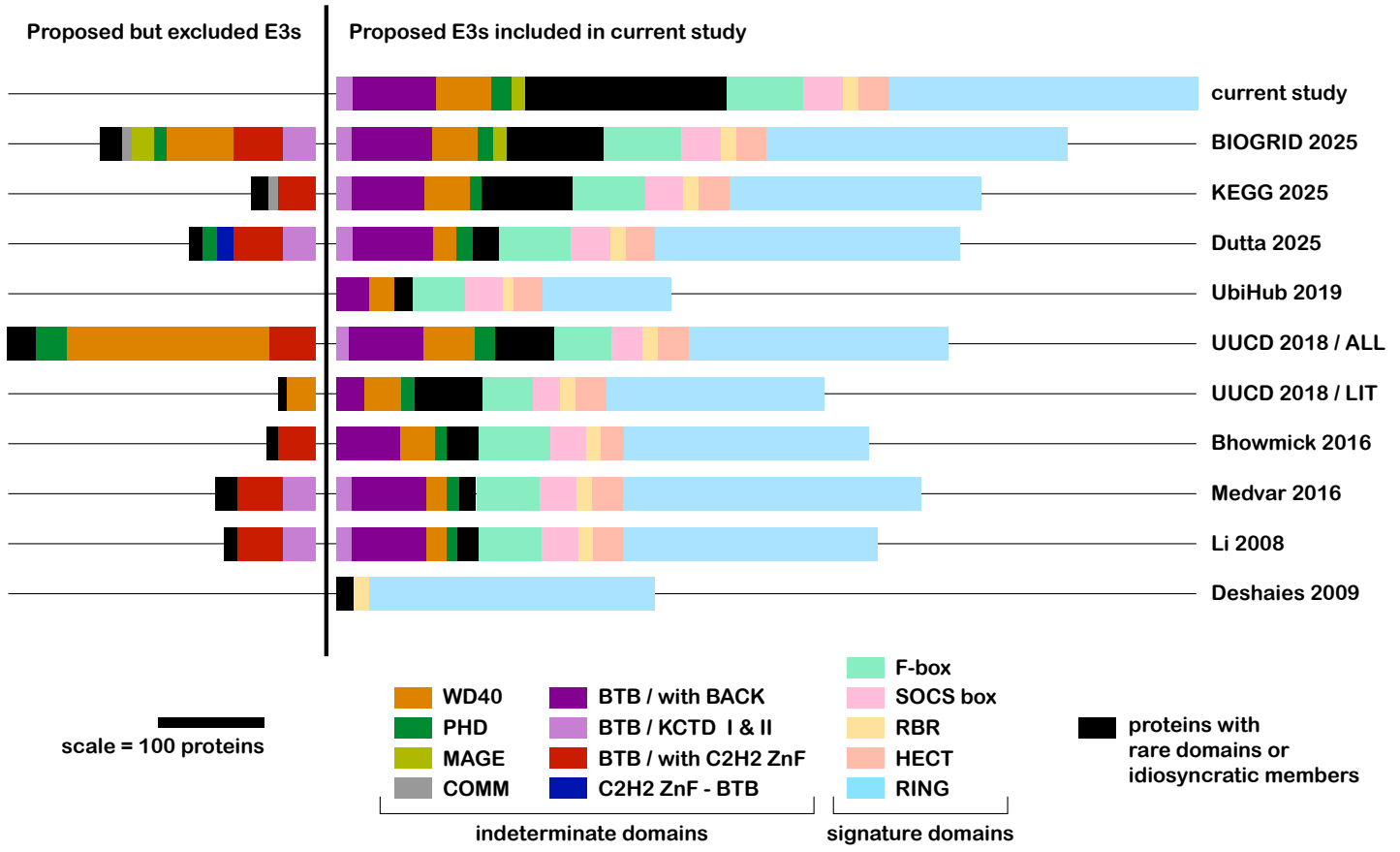

Figure 13. Comparison of the E3 ubiquitin ligase coverage in the Proteostasis Network versus earlier E3 ubiquitin ligase compilations

For each compilation, the number of proteins excluded or included relative to the E3 set in the current study is shown by scaled bars. InterPro domains which are not sufficient for inclusion as E3s (indeterminate domains) are shown in more saturated colors, and InterPro domains that are sufficient for inclusion (signature domains) are shown in pastels. Minor domain types (<9) were omitted from the graph. The current study systematically includes E3s for ubiquitin as well as ubiquitin-like modifiers, and the earlier studies do so to variable extents.

like (IPR016135), and this encompasses the *RWD domain* (IPR006575) and the *Ubiquitin E2 variant, N-terminal* (IPR008883), both of which we also consider to be signatures for ubiquitin binding. Not only does ubiquitin dock into the active site of E2s, but for about a third of the active E2s, ubiquitin binding on the side opposite the active site, termed backside binding, can promote processivity[183].

Several structural domains that are not dedicated ubiquitin binders have acquired this property in certain proteins. The *Armadillo-type fold* (IPR016024) encompasses the ubiquitin binding domain found on PSMD2, but this is to date the lone instance of ubiquitin binding for this domain. Other examples include the *CARD domain* (IPR001315), the *SH3 domain* (IPR001452), and the *WD40 repeats* (IPR001680). Atypical ubiquitin binding is not only seen in minority members of domain families, but also among singleton domains and among structures that have not been described in the domain databases (Table S5).

In addition to ubiquitin binding elements, our classification also includes three proteins involved in URM association, and more than 50 proteins that associate with SUMO via short hydrophobic elements known as SUMO interaction motifs (SIMs)[137]. SUMO binding is also mediated in a few instances by zinc fingers[184], WD40 domains[185], and backside E2 binding[186]. Interestingly, multiple ubiquitin ligases interact with SUMO via SIMs, including RNF4, RNF111, and TOPORSz. These are known as SUMO-targeted ubiquitin ligases and ubiquitinate SUMOylated proteins, forming mixed chains and targeting a subset of substrates for proteasomal degradation[187–194].

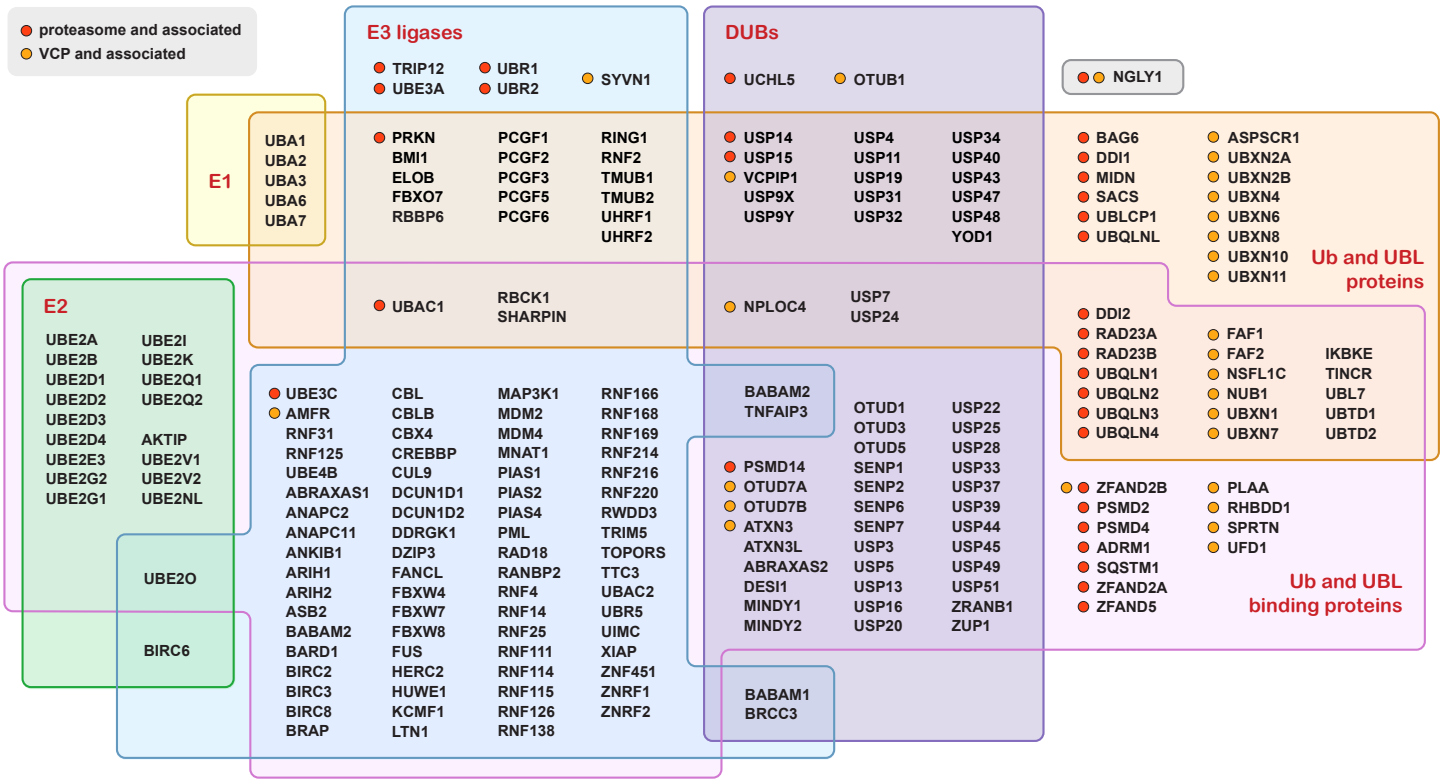

Figure 14. UPS genes with annotations in more than one Class within the UPS Branch

Venn diagram shows six of eight UPS classes: E1 activating enzymes (yellow), E2 conjugating enzymes (green), E3 ligases (blue), deubiquitinating enzymes (purple), ubiquitin and UBL proteins (orange), and ubiquitin and UBL binding proteins (pink). Proteins associated with the molecular machines are indicated with red and orange dots. Only the UPS proteins annotated in more than one Class, comprising 15% of the survey, are shown.

Molecular machines

At the heart of protein turnover by the UPS are two molecular machines, the proteasome[195] and VCP[196–199]. While there are a number of expansive UPS surveys, few of them include the proteasome, its modulators, and its accessory proteins. We annotate each of these molecular machines and their cofactors as two distinct Classes. Fluctuations in the translation machinery, autophagy components, and the proteasome reflect metabolic states, and all of these should be included in a survey used for evaluating the proteostatic capacity of tissues.

A key underlying feature of protein turnover is how destruction is limited to intended targets. The proteolytic sites of the proteasome are sequestered within an internal chamber of the enzyme, whereas in the autophagy pathway the responsible proteases are sequestered behind the membrane of the lysosome.

The proteasome consists of two major subassemblies, the regulatory particle (RP) and the core particle (CP). The RP, a 19-subunit complex, is also known as the 19S particle, and the CP, a 28-subunit complex, is also known as the 20S particle. Subunits of the CP are described in terms of signature domains (**IPR001353**) and assemble into four hetero-heptameric rings, stacked to form a barrel. Gates at the end of the barrel seal the chamber to isolate the proteolytic active sites from the cytosol[200]. These gates can be opened by a variety of activators, the most widely used being the RP. Most importantly, the RP recognizes and prepares substrates for degradation by unfolding them mechanically in the course of injecting them into the CP. The gate-proximal component is a hexameric ring of ATPases (*AAA+ ATPase domain*, **IPR003593**) with a central pore, which contact substrate and drive unfolding, The ATPase ring is ensconced in a larger structure of non-ATPase subunits which help to situate the ring over the CP gate. As mentioned above, the RP subassembly called the lid is structurally similar to the signalosome and eIF3[158]. Like those assemblies, it contains an intrinsic DUB—PSMD14/Rpn11—which is positioned near the pore of the ATPase ring such that it can trim attached ubiquitin from substrate

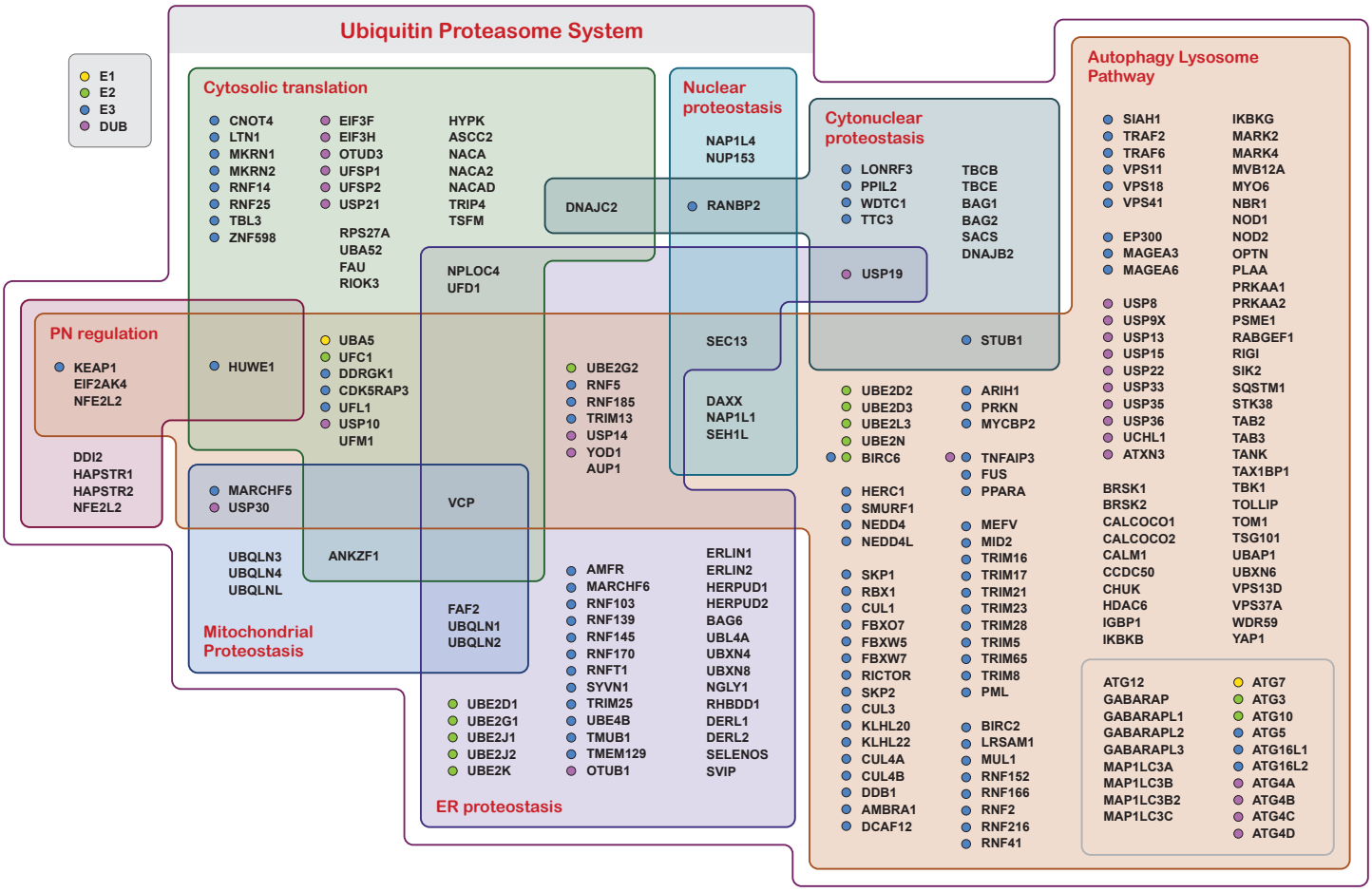

Figure 15. UPS genes annotated in other Proteostasis Network Branches

Proteins with annotations in the Ubiquitin-Proteasome System and in at least one other Proteostasis Network Branch are shown, listed with their official gene symbols. Colored dots indicate functions in the UPS pathway as noted at left. Components without dots fall within the other four UPS classes: ubiquitin and UBL proteins, ubiquitin and UBL binding proteins, proteasome and associated proteins, and VCP and associated proteins). The genes corresponding to the ubiquitin-like modifiers Atg12 and Atg8 (with its homologs) in a grey box at lower right, along with the enzymes that mediate their conjugation and deconjugation.

as it is laced through the ATPase ring and into the chamber of the core particle[163].

Three additional DUBs dock onto the proteasome, each with distinct locations, substrates, and mechanisms of action[155,156,201,202]. The proteasome also recruits a selection of E3 enzymes to it[203,204]. The activity of the proteasome is modulated by proteasome-specific cofactors that open the gate in the absence of the RP and modulate proteasome activity. Substrate engagement is also promoted by various adaptors that deliver substrate to the proteasome, through either ubiquitin-dependent or independent mechanisms, in the latter case most notably by midnolin[26,205,206].

Remarkably, more than 25 kinases, phosphatases, and other enzymes modify the proteasome and promote or suppress its activity[207]. We do not include them as UPS components, as these modifying enzymes are not strictly required for proteasome activity, nor are the enzymes dedicated to the proteasome. Nonetheless, the physiological impact of these modifications can be significant, even if the dynamic range of the activity changes is in the realm of fine-tuning.

Although the proteasome is equipped with the capacity to unfold and translocate proteins into its proteolytic chamber, it cannot extract proteins from intracellular membranes or, typically, protein complexes. VCP fills this void, for example extracting proteins from and through membranes, thus preparing them for degradation by the proteasome. In this way, VCP promotes ERAD and degradation of outer mitochondrial matrix proteins. Soluble proteins can also be poor proteasome substrates due to an inability of the proteasome to

mediate their unfolding, and in such cases VCP may carry out the initial unfolding step.

Like the proteasomal ATPases, VCP is a hexameric ring of ATPases formed around a central pore. Each VCP subunit has two iterations of the AAA+ domain, giving rise to a double ring structure. N-terminal domains emanate from each monomer, and serve to recruit adaptor proteins such as UBX domain proteins, a specialized type of UBL-domain protein. Several of these adaptors target ubiquitinated substrates. VCP lacks an intrinsic DUB, but recruits a number of DUBs, as well as a number of E3s. Substrates are unfolded by being passed through VCP's central pore before the proteasome can act on them. In addition to assisting the proteasome, VCP participates in myriad cell processes, including extracting proteins from chromatin[208], promoting ribosome quality control[209], reassembling the Golgi following mitosis[210,211], and promoting the clearance of ruptured lysosomes by autophagy[212].

Comparison of Compendia

The central motivation of our work was to create a reference resource for further studies of the UPS. We therefore designed the survey to be comprehensive, to embed community feedback in its delineation, and to provide a hierarchical structure for organizing UPS genes. Parts of the UPS have already been the focus of prior annotation efforts by other groups. Comparison to those efforts allows us to assess the robustness of our annotations through convergence, as well as to identify conceptual boundaries that emerge from differences in classification.

Our survey is the first to include all eight Classes of the UPS, and it continues a decades-long effort by many in the field to delimit this vast biochemical system. Such efforts, including ours, have relied to variable extents on assessment of the literature, text mining, domain searches, and the elaboration of earlier annotations (**Table S6**, Collections tab). We selected a subset of these for comparison with the current study.

The scopes of prior studies and their relationships to each other are shown in **Fig. 12**, left panel. We compared the aggregate membership of these nine UPS sets to the UPS proteins of the current study (Venn diagram I). In this comparison, there were 163 members unique to our set, roughly half being ubiquitin binding proteins. Surprisingly, 836 proteins previously proposed as UPS components were not included in our collection. The aggregate set was refined by removing proteins that do not carry specific annotations or were computationally derived (see Methods) and again compared to the UPS members in the current study (Venn diagram II). This relatively simple operation accounts for 552 of our exclusions, but this leaves 284 additional exclusions, reached typically through more ad hoc considerations, and 254 of these had been proposed to be ubiquitin ligases.

We conducted a domain analysis on the set of proposed E3s that we excluded from our collection, and found that more than 80% shared at least one of six InterPro domains: MAGE, COMMD, PHD, BTB, C2H2 zinc fingers, and WD40 (**Fig. 12**, lower right). While we do include MAGE RING cofactors in our survey, not all MAGEs are thought to have this function[213]. All proteins with COMMD domains are included in KEGG, but we find it more likely that only COMMD1 is involved in E3 regulation[214]. A handful of the 88 PHD domain proteins have been identified as variant RING ligases, but participation of PHD domains (**IPR001965**) in E2 binding is thought to be the exception rather than the rule[85]. A group of 100 excluded E3s contain BTB domains (**IPR000210**), C2H2-type zinc fingers (**IPR013087**), or both. BTB domains dock into the adaptor-binding region of CUL3, but this domain alone is not necessarily sufficient for docking. In BioPlex 3.0, CUL3 recovered BTB-BACK and KCTD-type I BTB proteins (a third of each group), but was not found with any of the 27 KCTD-type 2 BTB proteins nor with any of the 49 BTB proteins containing zinc fingers. The entire repertoire of BTB proteins was included in an initial catalog (**Fig. 13**)[215], but this now seems overbroad. Moreover, zinc finger clusters found in BTB proteins are structurally distinct from zinc-binding RING domains, but given that they are appended to BTB domains, they may have come to be construed to be E2 recruiting domains over time.

The last group of excluded E3s is the WD40 set, numbering 67 members. The WD40 domain is found among the receptors for most most types of cullin RING ligases, and in the context of F-box proteins, SOCS box proteins, and BTB domain proteins, the WD40 element does not contact the cullin adaptor. For CUL4A/CUL4B substrate receptors, the WD40 domain engages adaptor DDB1 but does not constitute the entire interface. The putative CUL4A/CUL4B receptors were identified in a parallel set of proteomic experiments 20 years ago. Some hits turned up in only one of four screens[72,73], were not subsequently identified by BioPlex, and do not model well with DDB1. Of these, 22 have been

included in previous annotations, but we exclude these give their lack of validation. Even so, we consider these to be borderline cases at present, with reasonable arguments both for and against inclusion. Overall, the inclusion of E3s that we exclude may be rationalized to a large extent as the consequence of overbroad application of domains as sufficient for inclusion as E3s.

To visualize the relationships between the E3 component of our annotation and earlier E3 sets, we applied our domain analysis graphically, and to both the excluded and included portions of the sets (**Fig. 13**). Over time, the scope of E3s identified by canonical domains has expanded (**Fig. 13**), which is consistent with the continuing elaboration of domain assignments in InterPro. The BioGRID E3 collection most closely resembles ours, with 699 shared components and 136 E3s that are unique to our set. Roughly 80% of these differences correspond to E3 components that are idiosyncratic or are included by virtue of rare domains (**Fig. 13**, black bars), with the other 20% corresponding to a broader application of canonical domains. Among the more prominent annotations unique to the present survey are PRAME CUL2 receptors, and noncatalytic subunits of idiosyncratic ubiquitin ligase complexes. In **Table S6**, we compare our annotation to previous annotations on a protein-by-protein basis. Pairwise comparisons between the current study and earlier studies are also made, and can be found in **Table S6**.

Two studies were published contemporaneously with submission of the initial version of this manuscript to bioRxiv: Szulc & Pokrzywa (2026)[216] and Chua et al. (2026)[217]. The former focuses on the cullin RING ligase substrate receptors, the latter on the whole E3 class. We compare the applicable sections of our survey to these catalogs in **Fig. S1**. In comparison to the E3-ome of Chua et al. (2026)[217], the E3 class in our survey is broader by 190 components. Two-thirds of these derive from our more expansive sense of what constitutes an E3. We include all subunits of E3 complexes, rather than a subset, as functional forms of E3s typically require all subunits to be present. We also include all recognized substrate adaptors—components which associate with fully-formed E3 ligases and augment or alter their substrate specificity or affinity. The remaining third of proteins that are unique to our study fall into categories represented in both studies: RING and variant RING ligases, idiosyncratic ligases, UBLM ligases, and substrate receptors for cullin RING ligases. The 27 E3-ome components that we exclude from our study are predominantly proposed CRL substrate receptors (**Fig. S1**, panel B). Likewise, Szulc & Pokrzywa (2026)[216], which catalogues 267 CRL substrate receptors, proposed 33 CRL substrate receptors not present in either this study or in Chua et al. (2026)[217].

We welcome community feedback on the composition of the Proteostasis Network, submitted to pnannotation@gmail.com. The exact composition of the PN will be revised as new data become available, and updates will be posted on the Proteostasis Consortium website (https://proteostasisconsortium.com). An interactive web resource is now available for exploring the contents of the Human Proteostasis Network (https://proteostasisnetwork.streamlit.app/), which allows both user-directed exploration and data downloads.

DISCUSSION

To date, no two surveys of the UPS have arrived at very similar censuses of its components. This reflects the exceptional breadth and complexity of the system, as well as the impact of significant ongoing advances in the field. Even more so perhaps, the methods used to define UPS components have led to different outcomes. At the same time, some surveys of the UPS have drawn from earlier surveys and in doing so have propagated what we consider to be assignment errors. We have avoided metrics such as frequency of annotation to favor inclusion, as this will result in the amplification of early misassignments. Although in many surveys the noncatalytic subunits of E3 enzymes are omitted, they are critical components, and we therefore include them in this survey, placing them in their relevant context. We also include cofactors of E3s that, while not intrinsic subunits, are required for the modification of particular substrates.

We made extensive use of recently developed tools and databases to aid in defining the UPS: the the BioPlex 3.0 database[218], AlphaFold[219], and the Predictomes project[56]. While using these tools, no assignments were made using machine learning per se.

We emphasized a domain-based approach for inclusion in which the functional features of the domains help to undergird and explain why a protein should be considered a component of the UPS. We also note these domains explicitly, and comment on domains that appear frequently among UPS proteins

but do not predict function narrowly. Especially for candidate members lacking obvious domains to associate them within the UPS, we scrutinized the literature before placing a proposed UPS component in our survey via entity-based criteria. In using primarily a domain-based approach for classification, we have included proteins that have not been studied in any detail biochemically–further experimental work on these little-known proteins should be a fertile area for new insights into the UPS and solidify our understanding of their place in the UPS.

In addition to providing a comprehensive survey of UPS components, but we also arrange these in a five-tiered taxonomy that allows for a clear understanding of the position of each protein in the pathway as well as highlighting proteins with similar activities and structural features. This survey is also unusual in covering every aspect of the UPS, including ubiquitin binding proteins, proteasomal proteins, and proteasome-associated cofactors. In covering all aspects of the UPS systematically, we encountered a complexity incompatible with single annotations: for example, UBL domains and ubiquitin binding domains each occur in numerous enzymes, including E2s, E3s, and DUBs. Likewise, while some proteins that associate with molecular machines are dedicated to their maturation and modulation, others also have functions in the UPS beyond their collaboration with molecular machines. These cross-annotations are noted in the **Table S1**, and described in **Fig. 14**.

The present UPS survey was developed in concert with surveys characterizing other parts of the PN: translation, protein folding, organelle-specific proteostasis, and autophagy[11,12]. A remarkable 200 UPS genes, approximately, are cross-annotated in other Branches of the PN (**Fig. 15**). These cross-annotations arise from known features of the pathways involved, most notably the shared ubiquitin-dependence of much proteasomal and autophagic degradation. Consistent annotation of the whole of the PN using a uniform taxonomy should provide a powerful groundwork for the field of proteostasis and its fundamental roles in human health.

It will be important to survey the UPS and more generally the PN in model organisms as well, and this is in progress. Previous work has shown that, remarkably, the UPS was present in a relatively mature form as early in evolution as the Last Eukaryotic Common Ancestor (LECA): It has been estimated that approximately 95% of UPS gene families were represented in the LECA[220]. Therefore, the UPS clearly took shape and assumed great complexity during eukaryogenesis, that is, prior to the LECA. During this unique evolutionary period, the proteome of the cell must have been in tremendous flux, resulting in stringent new demands on both protein and organellar quality control. Adaptation to these conditions during eukaryogenesis may have driven the elaboration and progressive refinement of the UPS and of the proteostasis network as a whole.

METHODS

Counting UPS Genes and Proteins

For the UPS, there are five proteins encoded by more than one gene: Q0WX57 is encoded by 7 genes (USP17L24, USP17L25, USP17L26, USP17L27, USP17L28, USP17L29, USP17L30), C9J1S8 is encoded by 2 genes (TRIM49D1 and TRIM49D2), P43356 is encoded by 2 genes (MAGEA2 and MAGEA2B), Q16637 is encoded by 2 genes (SMN1 and SMN2), Q9GZY0 is encoded by 2 genes (NXF2 and NXF2B). Therefore, the UPS gene count is 10 greater than the UPS protein count, which is 1402. When comparing our survey to previous annotations, we compared Uniprot IDs rather than Gene IDs, so numbers are expressed in terms of proteins rather than genes.

Counting E3 enzymes

To obtain a count of the E3s, we considered all unique E3 complexes. For the cullin RING ligases, each substrate receptor (n=296) and each substrate adaptor (n=10) define a unique E3. CUL4 adaptor AHR-ARNT-TBL3 forms a complex, and is considered to be a single adaptor (n=1). The CUL4 scaffold DDA1 is not counted because it is found in the context of substrate receptors already counted. The cullins themselves, the RBX proteins, the cullin adaptors, and the cullin regulators are all subsumed in this number. We do count metazoan cullins CUL7 and CUL9 as they are not represented by the substrate receptors (n=2).

For the RING ligases, we count the TRIMs (n=74) but not the ringless TRIMs, nor TRIM heterodimers, since it is uncertain how many heterodimers actually form, and so TRIMs may be undercounted. For the remaining RINGs, we count all individual RINGs (n=230). We also count the RING adaptors individually (n=18), as recruitment of each adaptor

function defines a new complex. We do not consider that an adaptor may associate with more than one RING, possibly undercounting. For the idiosyncratic RING complexes, each complex of proteins is counted once (n=11), with the exceptions of the APC, which has two known adaptors (n=2) and CTLH, which has six known adaptors (n=6). The CTLH adaptors dock at multiple sites and may therefore work in numerous combinations, and we do not consider these, which is another likely source of undercounting. The RING variants are counted individually: UBOX (n=8), PHD (n=7), and C4C4 (n=1).

For the smaller classes of E3s, we count the RBRs individually (n=14), and also count LUBAC (n=1), as the two RBR proteins it includes may have individual functions. The RCR (n=1) and the RZ ligases (n=2) are considered individually. The HECT ligases are each counted individually (n=28) and their adaptors are counted additionally (n=13), and each of these is only counted once though adaptors may bind multiple HECTs, representing another undercount. The HECT activators are not counted. The E2-E3 fusions are counted individually (n=2) as well as the UBE2O adaptors (n=2). There are three idiosyncratic E3 enzymes that are counted individually (n=13) and one idiosyncratic complex (n=1). We additionally count the E3s for UBLMs and their adaptors (n=27).

The total for the minimum number of E3 enzymes in human is 770.

Identification of UPS proteins

To generate a comprehensive accounting of the UPS (**Table S1**), we first assessed well-characterized UPS protein families for the presence of shared InterPro domains, which we term signature domains. We also screened the InterPro domain database of more than 50,000 entries for additional signature domains. By querying UniProt with these signature domains (**Table S3**), we identified additional family members. In addition to this domain-based approach, we consulted the literature at length, evaluating review articles and the primary literature with a focus on new UPS developments (**Table S1**). A simple keyword query of UniProt was found to be far less effective than probing with our curated InterPro domains; searching with "ubiquitin OR proteasome" retrieved more than three thousand proteins, including myriad proteins not ultimately included in our survey and missing hundreds that we ultimately included. After the core of our survey was assembled, we consulted a range of existing databases, including BioGRID, KEGG, and Gene Ontology (focusing on the Molecular Function hierarchy). We also evaluated earlier UPS annotations, Bhowmick et al. (2016)[221], Medvar et al. (2016)[222] and Zhou et al. (2018) (UUCD 2.0)[223]. When available, we consulted citations relating to specific genes within these resources. To evaluate E3s lacking signature domains, we weighed most heavily evidence showing ubiquitination in a purified in vitro system dependent on the proposed E3, and these references are included in **Table S1** (Main tab).

Assignment of Signature and Auxiliary Domains

Our survey of more than 1400 proteins has been parsed in terms of protein function, and we link function as often as possible to specific protein domains. InterPro and CDD domains for member proteins were used to develop the taxonomic structure of our survey. To ensure a unified description, the catalog was evaluated at the end of its construction with the profiles present in UniProt 2025_04, (released on October 14, 2025). The InterPro IDs were matched to the entries in InterPro 107.0 (released October 15, 2025), with the last entry being **IPR059259**. In a handful of cases, InterPro domains that had not yet been incorporated into UniProt were identified and included in our annotation.

The Class level is generally too broad to be described by a single unifying InterPro domain (with some exceptions, such as E1 enzymes). Most proteins are annotated with "signature domains" which place them in a particular Class-Group or Class-Group-Type classification. We use "auxiliary domains" to further refine signature domain groupings. We also implement auxiliary domains to note features that appear in multiple Group-Type listings. For VCP-associated proteins and ubiquitin binding proteins, we implement signature domains differently. Rather than using them to designate broader categories, we use signature domains to indicate the functional elements that confer VCP or ubiquitin binding, which we list at the Subtype level.

When InterPro domains are used to represent function, there will occasionally be wholly overlapping domains relating to the same functional element. For a given set of proteins, we chose domains which encompassed the largest number, and then the next largest until we had a complete set of domain designations. We gave preference to InterPro *Domains* and *Repeats* over *Homologous*

Superfamilies and *Families*. *Domains* that were overbroad for our purposes were avoided where possible. Where unavoidable, we show these domains in parentheses. The specificity of each domain is described in **Table S3** by listing the number of appearances in the UPS, the PN, and the human proteome.

Ideally, all entries sharing the same Class-Group or Class-Group-Type annotation would also share the same signature domain, but there are deviations from this ideal in our survey. For example, the F-box proteins nearly all share InterPro domain **IPR001810**, but some are only identified with the cognate homologous superfamily **IPR036047**. In some cases, related homologous superfamilies are overbroad, but not for the F-box proteins. We consider both **IPR001810** and **IPR036047** to be definitional for inclusion as F-box type CUL1 receptors. In both cases, the signature domains relate to a structural element that docks into the CUL1-SKP1 complex. Some annotation groups include members related by function but not structure. RICTOR shows good evidence of being a CUL1 substrate receptor[224], but is not an F-box protein. We include it in the survey, but we do not assign it a signature domain.

F-box proteins are additionally annotated with auxiliary domains, including LRR repeats (**IPR032675**), WD40 repeats (**IPR001680**), and FBA domains (**IPR007397**). We also give auxiliary domains to singleton Subtypes when that domain appears in other parts of the annotation. Only one F-box protein contains a SPRY domain (**IPR001870**), but there are more than 70 proteins in the UPS that contain this domain. Occurrences of auxiliary domains are not always noted. Some of the ATGylation E3s contain WD40 repeats, but the grouping is small enough that we do not apply an auxiliary domain to differentiate its members.

In some cases, auxiliary domains are implemented without signature domains. While most of the CUL4 substrate receptors have WD40 domains, a signature domain for CUL4 substrate receptors should ideally predict the interaction with DDB1, but no InterPro domain captures this feature. Still, the enrichment of WD40 proteins among CUL4 substrate receptors is a significant feature of the grouping (appearing at a frequency of 44-fold higher than in the proteome as a whole) and is described with an auxiliary domain. Idiosyncratic E3 enzyme complexes are frequently described without signature domains for two reasons: first, InterPro domains that predict complex formation for these complexes are rare, and second, characterization with a domain that occurs only once in the proteome is of little use in our taxonomy.

InterPro domain designations are continually expanded, with thousands of entries being added each year. We anticipate that new InterPro domains will be assigned for known functional motifs that are characterized in the literature and in this survey.

Structural Analysis and Modeling

Determinants of cullins involved in docking adaptors and substrate receptors were evaluated by analyzing more than 20 solved structures involving all of the canonical cullins (**Table S7**), though CUL4B was used to represent CUL4A and CUL4B. Structures were evaluated using PyMOL. Residues at the cullin-adaptor and cullin-receptor interfaces were identified by screening for residues in these proteins falling within 4Å of their binding partners. The relative position of binding determinants on helices 1-4 were determined and mapped onto a cartoon of the four-helix bundle (**Fig. 7**).

To establish support for inclusion of the PRAME family as CUL2 receptors, we used AlphaFold3 to model representative PRAMEs with the core components of CRL2. Modeling the CRL2 core complex alone, which contains CUL2 and its heterodimeric adaptor ElonginBC, generated an ipTM score of 0.81. To establish an ipTM range for authentic CRL2 receptors, we modeled all substrate receptors for which structures had been obtained experimentally (VHL [PDB:4WQO], APPBP2 [PDB 8JAL], FEM1B [PDB 8JE1], FEM1C [PDB 8PQL], and LRR1 [PDB 7PLO], resulting in ipTMs ranging from 0.63 to 0.77. PRAME family representatives PRAME, PRAMEF4, and PRAMEF10 modeled with ipTM scores of 0.66, 0.68, and 0.64, respectively, solidly within the range seen for established CRL2 receptors. In these models, the PRAMEs docked at the same surfaces of CUL2-ElonginBC as the established CUL2 receptors, which provides additional support for the PRAMEs as true CRL2 substrate receptors. The PRAME family is strongly conserved in the CUL2/ElonginBC binding element, which spans the first 120 residues.

To evaluate the BTB-BACK proteins for CUL3 association, we consulted the models present in Predictome[56]. Where BTB-BACK-CUL3 models were not already available, we generated models using AlphaFold3 using full length proteins and the default parameters. We construed BTB-BACK proteins as modeling poorly with CUL3 if the modeled pair had

ipTM values lower than 0.6, which AlphaFold3 sets as the lower limit for potentially correct models. In most cases, BTB-BACK proteins that modeled poorly with CUL3 docked at sites other than the N-terminal four-helix bundle.

To evaluate the proposed CUL4 receptors identified through affinity screens[69,70,72,73], each one was modeled with DDB1 using AlphaFold3 using full length proteins and the default parameters. The resulting ipTM scores showed a bimodal distribution, with very few falling between 0.25 and 0.75. The results are presented in **Fig. 10**.

Comparison of current study with earlier annotations

Data set inclusion and preparation

In order to compare the current survey with earlier studies, we distilled our annotation such that all features of each protein were represented in a single table row. Annotations for the E1, E2, E3, DUB, proteasome, and VCP Classes were truncated, then combined into a thumbnail annotation (**Table S6** [Index tab]), and the number of initial annotations was noted. UBL domains and ubiquitin binding domains are noted in separate columns, and also represented in the thumbnail annotations for proteins having only these features. Some resources are static and contain single annotations for each entry, including Li et al. (2008)[215], Deshaies and Joaziero (2009)[85], Bhowmick et al. (2016)[221], Liu et al. (2019) (UbiHub)[225], and Dutta et al. (2025) (Human E3 Ligome)[226]. Medvar et al. (2016)[222] is similar to the aforementioned, though E3s are also annotated as definitive or related, and that information is retained in the relevant tab. Zhou et al. (2018) (UUCD 2.0)[223] includes multiple annotations for some entries, and the scope includes both UBL domain proteins and ubiquitin binding proteins. We compressed that annotation, applying the approach that we used for the current study. The UUCD 2.0 resource distinguishes between annotations that are derived from the literature and those that were arrived at computationally, and this notation is retained. BioGRID is an actively updated resource, and UPS components are profiled as part of a themed curation project (see **Table S6**, [Collections tab] for link). The human component of the collection was downloaded on December 26, 2025. Likewise, the KEGG resource is actively updated, and the UPS is covered by KEGGBRITE ko04121 (see **Table S6** [Collections tab] for link). That collection was downloaded on January 5, 2026, and the human genes were identified and carried forward for analysis. KEGG uses a four-tiered taxonomy for annotation, and compressed annotations were prepared as above. The original four tiers are included in the relevant tab in **Table S6** (though merged for the small number of genes with multiple annotations). Lastly, UniProt was queried with the search string "E3 ubiquitin-protein ligase" on December 26, 2025.

Identification and Classification of Excluded Proteins

We compared the proteins annotated in the current study with an aggregate set comprised of the nine annotation efforts listed above (**Fig. 12**) combined with the results of the UniProt keyword search. There were 1249 proteins found in both sets, 152 proteins found only in the current study, and 952 proteins excluded from the current study. We divided these excluded proteins into four tiers, with Tier 1 encompassing the most frequently annotated genes and Tier 4 the least. Tier 4 proteins (116) had been defined as such solely on the basis of the UniProt keyword search results. Tier 3 proteins (298) in the aggregate set correspond to the "unclassified" and "no annotation" categories from Dutta et al. (2025)[226], and are not included elsewhere. Tier 2 proteins (254) correspond to proteins in the computational portion of the UUCD 2.0 set, and not present elsewhere (except for those discussed above in Dutta et al. [2025][226]). Tier 1 proteins (284) comprise the excluded portion from the narrowest comparison (**Fig. 12**, second Venn diagram). In summary, borderline cases for exclusion are most likely found in Tier 1, whereas stronger arguments for exclusion apply to Tiers 2-4. All 952 of the excluded proteins are listed with tier designations in **Table S6** (Excluded tab). Annotations from the earlier studies are also included for comparison. Note that in **Fig. 12**, the Venn diagram I reflects the status of the comparison after the exclusion of the Tier 4 proteins. Venn diagram II reflects the status of the comparison after the exclusion of the Tier 3 and Tier 2 proteins.

The process of narrowing the aggregate set revealed lightly annotated proteins that we include in our survey. Group A includes 163 proteins that were not annotated in any of the earlier studies we compared. Group B includes 24 proteins that were described in Dutta et al. (2025)[226] as "unclassified" or "no annotation" and do not appear in earlier sets.

Group C includes 31 proteins that are included in the UUCD 2.0[223] computational set but do not carry other annotations beyond Dutta et al. (2025)[226]. The remaining 1183 proteins are included in Group M, each with at least one and typically multiple annotations. The members of each group and their relationship to the current and previous annotations are noted in **Table S6** (Included tab). Borderline cases for inclusion are most likely to be found in Group A, stronger cases for Groups B and C.

Pairwise and Aggregate Comparisons

Each of the resources we chose for analysis is characterized in a unique tab in **Table S6**. Previously published catalogs are presented using the categories used by the authors of each previous study, and the annotations themselves hew as closely to the original while striving for brevity. UniProt IDs and official gene symbols have been updated as appropriate. The Index tab provides the master list for the analysis, and encompasses both proteins included in and excluded from the present survey. The Included and Excluded tabs relate information in the Index tab to earlier studies. The Excluded tab presents all UPS proteins proposed in earlier studies but excluded from our set. In this tab, the 414 proposed E3s are presented first. Of these, 357 contain domains showcased in **Fig. 12** and are sorted by these domains. A group of 12 proposed UBL domain proteins and 103 ubiquitin domain proteins follows the E3 section. Following this is a group of 16 proteins present in BioGRID that do not fall into the above categories. Most of these are ERAD proteins, and they are all included in the ER Proteostasis Branch of the network presented in one of the related Proteostasis surveys[11]. Most ERAD pathway components, such as ubiquitin ligases, are included in the present survey, though some, such as upstream components of the ER lumen, are only given a in the survey of the ER Proteostasis Branch [11]. The remaining 407 proteins are listed last, with a subset of 147 proteins sorted by shared domains.

SUPPLEMENTARY INFORMATION

Table S1. Human Proteostasis Network Annotation

The complete annotation of the Human Proteostasis Network is presented in the Main tab. The annotation of the UPS Branch begins at Row 2872 in the Main tab, and is additionally presented in the UPS_only tab. Detailed information about the tabs and fields is given in the README tab.

Table S2. Overview of the UPS Survey

Classes, Groups, Types, and Subtypes are displayed with gene counts for taxonomic levels. This table is an elaboration of **Fig. 3**, but is more concise than the complete annotation (**Table S1**).

Table S3. InterPro domains of the UPS

Signature domains, distributed domains, and auxiliary domains of the UPS are listed. *Domains* are described in terms of the Classes or Groups they annotate. Frequencies of occurrence in the UPS, the PN, and the proteome are given for each domain.

Table S4. Predictions of E2-E3 pairings

Prediction values for E2-E3 complex formation present in Predictome are listed. SPOC and ipTM scores are given in separate tabs. Higher confidence values are shaded in yellow, and lower confidence values in blue. For each E3, high scores, low scores, and number of models available are shown. Of the 837 E3 components in the survey, only the 414 are expected to recruit E2s were analyzed. The remaining 423 components associate with E2-binding subunits, directly or indirectly.

Table S5. Ubiquitin and UBL binding domain proteins

In **Table S1**, proteins with ubiquitin and UBL binding domains are ordered by their molecular or biological context. In this table, the same set of proteins is arrayed by type of ubiquitin or UBL binding domain.

Table S6. Comparison of current survey to previous catalogs

The current survey is compared with a selection of earlier catalogs. Detailed information about the tabs and fields is given in a README tab.

Table S7. Protein structures

Solved protein structures presented in various figures and used in various analyses. PDB codes are given. For USP15 and PARKIN, AlphaFold2 models are shown, and sections were validated by comparison to the indicated structures.

Figure S1. Comparison of the current study to contemporaneous studies

Current study is compared to Szulc & Pokrzywa (2026)[216] and Chua et al. (2026)[217].

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DISCLOSURE STATEMENT

The authors declare no competing interests.

REFERENCES

(1) Goldknopf, I. L.; Busch, H. Isopeptide Linkage between Nonhistone and Histone 2A Polypeptides of Chromosomal Conjugate-Protein A24. Proc. Natl. Acad. Sci. U. S. A. 1977, 74 (3), 864–868. https://doi.org/10.1073/pnas.74.3.864.

(2) Hunt, L. T.; Dayhoff, M. O. Amino-Terminal Sequence Identity of Ubiquitin and the Nonhistone Component of Nuclear Protein A24. Biochem. Biophys. Res. Commun. 1977, 74 (2), 650–655. https://doi.org/10.1016/0006-291x(77)90352-7.

(3) Komander, D.; Rape, M. The Ubiquitin Code. Annu. Rev. Biochem. 2012, 81, 203–229. https://doi.org/10.1146/annurev-biochem-060310-170328.

(4) Hershko, A.; Ciechanover, A.; Heller, H.; Haas, A. L.; Rose, I. A. Proposed Role of ATP in Protein Breakdown: Conjugation of Protein with Multiple Chains of the Polypeptide of ATP-Dependent Proteolysis. Proc. Natl. Acad. Sci. U. S. A. 1980, 77 (4), 1783–1786. https://doi.org/10.1073/pnas.77.4.1783.

(5) Arrigo, A. P.; Tanaka, K.; Goldberg, A. L.; Welch, W. J. Identity of the 19S "prosome" Particle with the Large Multifunctional Protease Complex of Mammalian Cells (the Proteasome). Nature 1988, 331 (6152), 192–194. https://doi.org/10.1038/331192a0.

(6) Pickart, C. M.; Rose, I. A. Functional Heterogeneity of Ubiquitin Carrier Proteins. J. Biol. Chem. 1985, 260 (3), 1573–1581.

(7) Scheffner, M.; Huibregtse, J. M.; Vierstra, R. D.; Howley, P. M. The HPV-16 E6 and E6-AP Complex Functions as a Ubiquitin-Protein Ligase in the Ubiquitination of P53. Cell 1993, 75 (3), 495–505. https://doi.org/10.1016/0092-8674(93)90384-3.

(8) Bai, C.; Sen, P.; Hofmann, K.; Ma, L.; Goebl, M.; Harper, J. W.; Elledge, S. J. SKP1 Connects Cell Cycle Regulators to the Ubiquitin Proteolysis Machinery through a Novel Motif, the F-Box. Cell 1996, 86 (2), 263–274. https://doi.org/10.1016/s0092-8674(00)80098-7.

(9) Deveraux, Q.; Ustrell, V.; Pickart, C.; Rechsteiner, M. A 26 S Protease Subunit That Binds Ubiquitin Conjugates. J. Biol. Chem. 1994, 269 (10), 7059–7061.

(10) Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. Protacs: Chimeric Molecules That Target Proteins to the Skp1-Cullin-F Box Complex for Ubiquitination and Degradation. Proc. Natl. Acad. Sci. U. S. A. 2001, 98 (15), 8554–8559. https://doi.org/10.1073/pnas.141230798.

(11) The Proteostasis Consortium; Overall coordination; Elsasser, S.; Elia, L. P.; Morimoto, R. I.; Powers, E. T.; Harvard Medical School group (analysis); Finley, D.; University of California, San Francisco and Gladstone Institutes group I (chaperones, analysis); Mockler, E.; Lima, L.; Finkbeiner, S.; University of California, San Francisco group II (chaperones, analysis); Gestwicki, J. E.; Northwestern University group (chaperones, analysis); Stoeger, T.; Cao, K.; The Scripps Research Institute group (chaperones, endoplasmic reticulum proteostasis, mitochondrial proteostasis, analysis); Garza, D.; Kelly, J. W.; Stanford University group (chaperones, translation, mitochondrial proteostasis); Collier, M.; Rainbolt, T. K.; Taguwa, S.; Chou, C.-C.; Aviner, R.; Barbosa, N.; Morales-Polanco, F.; Masto, V. B.; Frydman, J. A Comprehensive Enumeration of the Human Proteostasis Network. 1. Components of Translation, Protein Folding, and Organelle-Specific Systems. September 2, 2022. https://doi.org/10.1101/2022.08.30.505920.

(12) The Proteostasis Consortium; Overall coordination; Elsasser, S.; Elia, L. P.; Morimoto, R. I.; Powers, E. T.; Harvard Medical School group; Elsasser, S.; Finley, D.; University of California, San Francisco and Gladstone Institutes group I; Costa, B.; Budron, M.; Tokuno, Z.; Wang, S.; Iyer, R. G.; Barth, B.; Mockler, E.; Elia, L. P.; Finkbeiner, S.; University of California, San Francisco group II; Gestwicki, J. E.; Northwestern University group; Richardson, R. A. K.; Stoeger, T.; Morimoto, R. I.; The Scripps Research Institute group; Tan, E. P.; Xiao, Q.; Cole, C. M.; Massey, L. A.; Garza, D.; Powers, E. T.; Kelly, J. W.; Stanford University group; Rainbolt, T. K.; Chou, C.-C.; Masto, V. B.; Frydman, J.; New York University group; Nixon, R. A. A Comprehensive Enumeration of the Human Proteostasis Network. 2. Components of the Autophagy-Lysosome Pathway. March 24, 2023. https://doi.org/10.1101/2023.03.22.533675.

(13) Balch, W. E.; Morimoto, R. I.; Dillin, A.; Kelly, J. W. Adapting Proteostasis for Disease Intervention. Science 2008, 319 (5865), 916–919. https://doi.org/10.1126/science.1141448.

(14) Vargas, J. N. S.; Hamasaki, M.; Kawabata, T.; Youle, R. J.; Yoshimori, T. The Mechanisms and Roles of Selective Autophagy in Mammals. Nat. Rev. Mol. Cell Biol. 2023, 24 (3), 167–185. https://doi.org/10.1038/s41580-022-00542-2.

(15) Blum, M.; Andreeva, A.; Florentino, L. C.; Chuguransky, S. R.; Grego, T.; Hobbs, E.; Pinto, B. L.; Orr, A.; Paysan-Lafosse, T.; Ponamareva, I.; Salazar, G. A.; Bordin, N.; Bork, P.; Bridge, A.; Colwell, L.; Gough, J.; Haft, D. H.; Letunic, I.; Llinares-López, F.; Marchler-Bauer, A.; Meng-Papaxanthos, L.; Mi, H.; Natale, D. A.; Orengo, C. A.; Pandurangan, A. P.; Piovesan, D.; Rivoire, C.; Sigrist, C. J. A.; Thanki, N.; Thibaud-Nissen, F.; Thomas, P. D.; Tosatto, S. C. E.; Wu, C. H.; Bateman, A. InterPro: The Protein Sequence Classification Resource in 2025. Nucleic Acids Res. 2025, 53 (D1), D444–D456. https://doi.org/10.1093/nar/gkae1082.

(16) Jentsch, S.; Pyrowolakis, G. Ubiquitin and Its Kin: How Close Are the Family Ties? Trends Cell Biol. 2000, 10 (8), 335–342. https://doi.org/10.1016/s0962-8924(00)01785-2.

(17) Ichimura, Y.; Kirisako, T.; Takao, T.; Satomi, Y.; Shimonishi, Y.; Ishihara, N.; Mizushima, N.; Tanida, I.; Kominami, E.; Ohsumi, M.; Noda, T.; Ohsumi, Y. A Ubiquitin-like System Mediates Protein Lipidation. Nature 2000, 408 (6811), 488–492. https://doi.org/10.1038/35044114.

(18) Peña-Martinez, C.; Rickman, A. D.; Heckmann, B. L. Beyond Autophagy: LC3-Associated Phagocytosis and Endocytosis. Sci. Adv. 2022, 8 (43), eabn1702. https://doi.org/10.1126/sciadv.abn1702.

(19) Finley, D.; Bartel, B.; Varshavsky, A. The Tails of Ubiquitin Precursors Are Ribosomal Proteins Whose Fusion to Ubiquitin Facilitates Ribosome Biogenesis. Nature 1989, 338 (6214), 394–401. https://doi.org/10.1038/338394a0.

(20) Nakamura, M.; Watanabe, N.; Notsu, K. Ubiquitin-like Protein MNSFβ Covalently Binds to Cytosolic 10-Formyltetrahydrofolate Dehydrogenase and Regulates Thymocyte Function. Biochem. Biophys. Res. Commun. 2015, 464 (4), 1096–1100. https://doi.org/10.1016/j.bbrc.2015.07.083.

(21) van den Heuvel, J.; Ashiono, C.; Gillet, L. C.; Dörner, K.; Wyler, E.; Zemp, I.; Kutay, U. Processing of the Ribosomal Ubiquitin-like Fusion Protein FUBI-eS30/FAU Is Required for 40S Maturation and Depends on USP36. eLife 2021, 10, e70560. https://doi.org/10.7554/eLife.70560.

(22) Altin, S.; Simões, T.; Behrendt, C.; Anton, V.; Domke, D.; Völtzke, K. M.; Kumar, R.; Nolte, H.; Hermanns, T.; Brocke-Ahmadinejad, N.; Bendrin, K.; Zimmermann, M.; Büttner, R.; Ventura, N.; Krüger, M.; Dohmen, R. J.; Hofmann, K.; Hoppe, T.; Reichert, A. S.; Escobar-Henriques, M. Ubiquitin Precursor with C-Terminal Extension Promotes Proteostasis and Longevity. Mol. Cell 2025, 85 (19), 3677-3693.e7. https://doi.org/10.1016/j.molcel.2025.08.032.

(23) Wagh, A. R.; Glickman, M. H. Molecular Mechanisms Underlying P62-Dependent Secretion of the Alzheimer-Associated Ubiquitin Variant UBB+1. Proc. Natl. Acad. Sci. U. S. A. 2025, 122 (50), e2504528122. https://doi.org/10.1073/pnas.2504528122.

(24) Schuberth, C.; Buchberger, A. UBX Domain Proteins: Major Regulators of the AAA ATPase Cdc48/P97. Cell. Mol. Life Sci. CMLS 2008, 65 (15), 2360–2371. https://doi.org/10.1007/s00018-008-8072-8.

(25) Goldberg, A. L.; Kim, H. T.; Lee, D.; Collins, G. A. Mechanisms That Activate 26S Proteasomes and Enhance Protein Degradation. Biomolecules 2021, 11 (6), 779. https://doi.org/10.3390/biom11060779.

(26) Nardone, C.; Gao, J.; Seo, H.-S.; Mintseris, J.; Ort, L.; Yip, M. C. J.; Negasi, M.; Besschetnova, A. K.; Kamitaki, N.; Gygi, S. P.; Dhe-Paganon, S.; Munshi, N. C.; Fulciniti, M.; Greenberg, M. E.; Shao, S.; Elledge, S. J.; Gu, X. Structural Basis for the Midnolin-Proteasome Pathway and Its Role in Suppressing Myeloma. Mol. Cell 2025, 85 (13), 2597-2609.e11. https://doi.org/10.1016/j.molcel.2025.05.030.

(27) Vijay-Kumar, S.; Bugg, C. E.; Cook, W. J. Structure of Ubiquitin Refined at 1.8 A Resolution. J. Mol. Biol. 1987, 194 (3), 531–544. https://doi.org/10.1016/0022-2836(87)90679-6.

(28) Cappadocia, L.; Lima, C. D. Ubiquitin-like Protein Conjugation: Structures, Chemistry, and Mechanism. Chem. Rev. 2018, 118 (3), 889–918. https://doi.org/10.1021/acs.chemrev.6b00737.

(29) Wang, T.; Jiang, J.; Zhang, X.; Ke, X.; Qu, Y. Ubiquitin-like Modification Dependent Proteasomal Degradation and Disease Therapy. Trends Mol. Med. 2024, 30 (11), 1061–1075. https://doi.org/10.1016/j.molmed.2024.05.005.

(30) Arkinson, C.; Dong, K. C.; Gee, C. L.; Costello, S. M.; Soe, A. C.; Hura, G. L.; Marqusee, S.; Martin, A. NUB1 Traps Unfolded FAT10 for Ubiquitin-Independent Degradation by the 26S Proteasome. Nat. Struct. Mol. Biol. 2025, 32 (9), 1752–1765. https://doi.org/10.1038/s41594-025-01527-3.

(31) Baek, K.; Scott, D. C.; Schulman, B. A. NEDD8 and Ubiquitin Ligation by Cullin-RING E3 Ligases. Curr. Opin. Struct. Biol. 2021, 67, 101–109. https://doi.org/10.1016/j.sbi.2020.10.007.

(32) Cairo, L. V.; Hong, X.; Müller, M. B. D.; Yuste-Checa, P.; Jagadeesan, C.; Bracher, A.; Park, S.-H.; Hayer-Hartl, M.; Hartl, F. U. Stress-Dependent Condensate Formation Regulated by the Ubiquitin-Related Modifier Urm1. Cell 2024, 187 (17), 4656-4673.e28. https://doi.org/10.1016/j.cell.2024.06.009.

(33) Michelle, C.; Vourc'h, P.; Mignon, L.; Andres, C. R. What Was the Set of Ubiquitin and Ubiquitin-like Conjugating Enzymes in the Eukaryote Common Ancestor? J. Mol. Evol. 2009, 68 (6), 616–628. https://doi.org/10.1007/s00239-009-9225-6.

(34) Kleiger, G.; Saha, A.; Lewis, S.; Kuhlman, B.; Deshaies, R. J. Rapid E2-E3 Assembly and Disassembly Enable Processive Ubiquitylation of Cullin-RING Ubiquitin Ligase Substrates. Cell 2009, 139 (5), 957–968. https://doi.org/10.1016/j.cell.2009.10.030.

(35) Chrustowicz, J.; Sherpa, D.; Li, J.; Langlois, C. R.; Papadopoulou, E. C.; Vu, D. T.; Hehl, L. A.; Karayel, Ö.; Beier, V.; von Gronau, S.; Müller, J.; Prabu, J. R.; Mann, M.; Kleiger, G.; Alpi, A. F.; Schulman, B. A. Multisite Phosphorylation Dictates Selective E2-E3 Pairing as Revealed by Ubc8/UBE2H-GID/CTLH Assemblies. Mol. Cell 2024, 84 (2), 293-308.e14. https://doi.org/10.1016/j.molcel.2023.11.027.

(36) Wilson, R. C.; Edmondson, S. P.; Flatt, J. W.; Helms, K.; Twigg, P. D. The E2-25K Ubiquitin-Associated (UBA) Domain Aids in Polyubiquitin Chain Synthesis and Linkage Specificity. Biochem. Biophys. Res. Commun. 2011, 405 (4), 662–666. https://doi.org/10.1016/j.bbrc.2011.01.089.

(37) Lee, J.-G.; Youn, H.-S.; Kang, J. Y.; Park, S.-Y.; Kidera, A.; Yoo, Y. J.; Eom, S. H. Crystal Structure of the Ube2K/E2-25K and K48-Linked Di-Ubiquitin Complex Provides Structural Insight into the Mechanism of K48-Specific Ubiquitin Chain Synthesis. Biochem. Biophys. Res. Commun. 2018, 506 (1), 102–107. https://doi.org/10.1016/j.bbrc.2018.10.067.

(38) Eddins, M. J.; Carlile, C. M.; Gomez, K. M.; Pickart, C. M.; Wolberger, C. Mms2-Ubc13 Covalently Bound to Ubiquitin Reveals the Structural Basis of Linkage-Specific Polyubiquitin Chain Formation. Nat. Struct. Mol. Biol. 2006, 13 (10), 915–920. https://doi.org/10.1038/nsmb1148.

(39) Riechmann, C.; Ellison, C. J.; Anderson, J. W.; Hofmann, K.; Sarkies, P.; Elliott, P. R. UBA6 Specificity for Ubiquitin E2 Conjugating Enzymes Reveals a Priority Mechanism of BIRC6. Nat. Struct. Mol. Biol. 2025. https://doi.org/10.1038/s41594-025-01717-z.

(40) Yip, M. C. J.; Sedor, S. F.; Shao, S. Mechanism of Client Selection by the Protein Quality-Control Factor UBE2O. Nat. Struct. Mol. Biol. 2022, 29 (8), 774–780. https://doi.org/10.1038/s41594-022-00807-6.

(41) Sherpa, D.; Chrustowicz, J.; Qiao, S.; Langlois, C. R.; Hehl, L. A.; Gottemukkala, K. V.; Hansen, F. M.; Karayel, O.; von Gronau, S.; Prabu, J. R.; Mann, M.; Alpi, A. F.; Schulman, B. A. GID E3 Ligase Supramolecular Chelate Assembly Configures Multipronged Ubiquitin Targeting of an Oligomeric Metabolic Enzyme. Mol. Cell 2021, 81 (11), 2445-2459.e13. https://doi.org/10.1016/j.molcel.2021.03.025.

(42) Grabarczyk, D. B.; Petrova, O. A.; Deszcz, L.; Kurzbauer, R.; Murphy, P.; Ahel, J.; Vogel, A.; Gogova, R.; Faas, V.; Kordic, D.; Schleiffer, A.; Meinhart, A.; Imre, R.; Lehner, A.; Neuhold, J.; Bader, G.; Stolt-Bergner, P.; Böttcher, J.; Wolkerstorfer, B.; Fischer, G.; Grishkovskaya, I.; Haselbach, D.; Kessler, D.; Clausen, T. HUWE1 Employs a Giant Substrate-Binding Ring to Feed and Regulate Its HECT E3 Domain. Nat. Chem. Biol. 2021, 17 (10), 1084–1092. https://doi.org/10.1038/s41589-021-00831-5.

(43) Hunkeler, M.; Jin, C. Y.; Ma, M. W.; Monda, J. K.; Overwijn, D.; Bennett, E. J.; Fischer, E. S. Solenoid Architecture of HUWE1 Contributes to Ligase Activity and Substrate Recognition. Mol. Cell 2021, 81 (17), 3468-3480.e7. https://doi.org/10.1016/j.molcel.2021.06.032.

(44) Wang, F.; He, Q.; Zhan, W.; Yu, Z.; Finkin-Groner, E.; Ma, X.; Lin, G.; Li, H. Structure of the Human UBR5 E3 Ubiquitin Ligase. Struct. Lond. Engl. 1993 2023, 31 (5), 541-552.e4. https://doi.org/10.1016/j.str.2023.03.010.

(45) Hodáková, Z.; Grishkovskaya, I.; Brunner, H. L.; Bolhuis, D. L.; Belačić, K.; Schleiffer, A.; Kotisch, H.; Brown, N. G.; Haselbach, D. Cryo-EM Structure of the Chain-Elongating E3 Ubiquitin Ligase UBR5. EMBO J. 2023, 42 (16), e113348. https://doi.org/10.15252/embj.2022113348.

(46) Yang, Z.; Haakonsen, D. L.; Heider, M.; Witus, S. R.; Zelter, A.; Beschauner, T.; MacCoss, M. J.; Rapé, M. Molecular Basis of SIFI Activity in the Integrated Stress Response. Nature 2025, 643 (8073), 1117–1126. https://doi.org/10.1038/s41586-025-09074-z.

(47) Grabarczyk, D. B.; Ehrmann, J. F.; Murphy, P.; Yang, W. S.; Kurzbauer, R.; Bell, L. E.; Deszcz, L.; Neuhold, J.; Schleiffer, A.; Shulkina, A.; Lee, J.; Shin, J. S.; Meinhart, A.; Versteeg, G. A.; Zavodszky, E.; Song, H. K.; Hegde, R. S.; Clausen, T. Architecture of the UBR4 Complex, a Giant E4 Ligase Central to Eukaryotic Protein Quality Control. Science 2025, 389 (6763), 909–914. https://doi.org/10.1126/science.adv9309.

(48) Li, J.; Purser, N.; Liwocha, J.; Scott, D. C.; Byers, H. A.; Steigenberger, B.; Hill, S.; Tripathi-Giesgen, I.; Hinkle, T.; Hansen, F. M.; Prabu, J. R.; Radhakrishnan, S. K.; Kirkpatrick, D. S.; Reichermeier, K. M.; Schulman, B. A.; Kleiger, G. Cullin-RING Ligases Employ Geometrically Optimized Catalytic Partners for Substrate Targeting. Mol. Cell 2024, 84 (7), 1304-1320.e16. https://doi.org/10.1016/j.molcel.2024.01.022.

(49) Wenzel, D. M.; Lissounov, A.; Brzovic, P. S.; Klevit, R. E. UBCH7 Reactivity Profile Reveals Parkin and HHARI to Be RING/HECT Hybrids. Nature 2011,

474 (7349), 105–108. https://doi.org/10.1038/nature09966.

(50) Pao, K.-C.; Wood, N. T.; Knebel, A.; Rafie, K.; Stanley, M.; Mabbitt, P. D.; Sundaramoorthy, R.; Hofmann, K.; van Aalten, D. M. F.; Virdee, S. Activity-Based E3 Ligase Profiling Uncovers an E3 Ligase with Esterification Activity. Nature 2018, 556 (7701), 381–385. https://doi.org/10.1038/s41586-018-0026-1.

(51) Otten, E. G.; Werner, E.; Crespillo-Casado, A.; Boyle, K. B.; Dharamdasani, V.; Pathe, C.; Santhanam, B.; Randow, F. Ubiquitylation of Lipopolysaccharide by RNF213 during Bacterial Infection. Nature 2021, 594 (7861), 111–116. https://doi.org/10.1038/s41586-021-03566-4.

(52) Ahel, J.; Balci, A.; Faas, V.; Grabarczyk, D. B.; Harmo, R.; Squair, D. R.; Zhang, J.; Roitinger, E.; Lamoliatte, F.; Mathur, S.; Deszcz, L.; Bell, L. E.; Lehner, A.; Williams, T. L.; Sowar, H.; Meinhart, A.; Wood, N. T.; Clausen, T.; Virdee, S.; Fletcher, A. J. ATP Functions as a Pathogen-Associated Molecular Pattern to Activate the E3 Ubiquitin Ligase RNF213. Nat. Commun. 2025, 16 (1), 4414. https://doi.org/10.1038/s41467-025-59444-4.

(53) Grabarczyk, D. B.; Aird, E. J.; Reznikow, V.; Kirchgatterer, P. C.; Ehrmann, J. F.; Kurzbauer, R.; Bell, L. E.; Kellner, M. J.; Aggarwal, R.; Schleiffer, A.; Faas, V.; Deszcz, L.; Meinhart, A.; Versteeg, G. A.; Penninger, J. M.; Stelzl, L. S.; Gaidt, M. M.; Tessmer, I.; Corn, J. E.; Clausen, T. A Split-Site E3 Ligase Mechanism Enables ZNFX1 to Ubiquitinate and Cluster Single-Stranded RNA into Ubiquitin-Coated Nucleoprotein Particles. Cell 2025, 188 (21), 5995-6011.e17. https://doi.org/10.1016/j.cell.2025.08.006.

(54) Squair, D. R.; Rivers, E.; Sowar, H.; Balci, A.; Harmo, R.; Wright, D. J.; Beniwal, G.; Soetens, M.; Mathur, S.; Tollervey, A.; Wood, N. T.; Pao, K.-C.; Stanton, C.; Fletcher, A. J.; Virdee, S. ZNFX1 Uses Two-Component Ubiquitin Circuitry to Quarantine Viral RNA. Mol. Cell 2026, 86 (6), 1164-1181.e12. https://doi.org/10.1016/j.molcel.2026.02.015.

(55) Lampert, F.; Stafa, D.; Goga, A.; Soste, M. V.; Gilberto, S.; Olieric, N.; Picotti, P.; Stoffel, M.; Peter, M. The Multi-Subunit GID/CTLH E3 Ubiquitin Ligase Promotes Cell Proliferation and Targets the Transcription Factor Hbp1 for Degradation. eLife 2018, 7, e35528. https://doi.org/10.7554/eLife.35528.

(56) Schmid, E. W.; Walter, J. C. Predictomes, a Classifier-Curated Database of AlphaFold-Modeled Protein-Protein Interactions. Mol. Cell 2025, 85 (6), 1216-1232.e5. https://doi.org/10.1016/j.molcel.2025.01.034.

(57) Skowyra, D.; Craig, K. L.; Tyers, M.; Elledge, S. J.; Harper, J. W. F-Box Proteins Are Receptors That Recruit Phosphorylated Substrates to the SCF Ubiquitin-Ligase Complex. Cell 1997, 91 (2), 209–219. https://doi.org/10.1016/s0092-8674(00)80403-1.

(58) Feldman, R. M.; Correll, C. C.; Kaplan, K. B.; Deshaies, R. J. A Complex of Cdc4p, Skp1p, and Cdc53p/Cullin Catalyzes Ubiquitination of the Phosphorylated CDK Inhibitor Sic1p. Cell 1997, 91 (2), 221–230. https://doi.org/10.1016/s0092-8674(00)80404-3.

(59) Harper, J. W.; Schulman, B. A. Cullin-RING Ubiquitin Ligase Regulatory Circuits: A Quarter Century Beyond the F-Box Hypothesis. Annu. Rev. Biochem. 2021, 90, 403–429. https://doi.org/10.1146/annurev-biochem-090120-013613.

(60) Kamura, T.; Koepp, D. M.; Conrad, M. N.; Skowyra, D.; Moreland, R. J.; Iliopoulos, O.; Lane, W. S.; Kaelin, W. G.; Elledge, S. J.; Conaway, R. C.; Harper, J. W.; Conaway, J. W. Rbx1, a Component of the VHL Tumor Suppressor Complex and SCF Ubiquitin Ligase. Science 1999, 284 (5414), 657–661. https://doi.org/10.1126/science.284.5414.657.

(61) Osaka, F.; Kawasaki, H.; Aida, N.; Saeki, M.; Chiba, T.; Kawashima, S.; Tanaka, K.; Kato, S. A New NEDD8-Ligating System for Cullin-4A. Genes Dev. 1998, 12 (15), 2263–2268. https://doi.org/10.1101/gad.12.15.2263.

(62) Kigoshi, Y.; Fukuda, T.; Endo, T.; Hayasaka, N.; Iemura, S.; Natsume, T.; Tsuruta, F.; Chiba, T. CACUL1/CAC1 Regulates the Antioxidant Response by Stabilizing Nrf2. Sci. Rep. 2015, 5, 12857. https://doi.org/10.1038/srep12857.

(63) Marín, I. Diversification of the Cullin Family. BMC Evol. Biol. 2009, 9, 267. https://doi.org/10.1186/1471-2148-9-267.

(64) Lonergan, K. M.; Iliopoulos, O.; Ohh, M.; Kamura, T.; Conaway, R. C.; Conaway, J. W.; Kaelin, W. G. Regulation of Hypoxia-Inducible mRNAs by the von Hippel-Lindau Tumor Suppressor Protein Requires Binding to Complexes Containing Elongins B/C and Cul2. Mol. Cell. Biol. 1998, 18 (2), 732–741. https://doi.org/10.1128/MCB.18.2.732.

(65) Kamura, T.; Burian, D.; Yan, Q.; Schmidt, S. L.; Lane, W. S.; Querido, E.; Branton, P. E.; Shilatifard, A.; Conaway, R. C.; Conaway, J. W. Muf1, a Novel Elongin BC-Interacting Leucine-Rich Repeat Protein That Can Assemble with Cul5 and Rbx1 to Reconstitute a Ubiquitin Ligase. J. Biol. Chem. 2001, 276 (32), 29748–29753. https://doi.org/10.1074/jbc.M103093200.

(66) Mahrour, N.; Redwine, W. B.; Florens, L.; Swanson, S. K.; Martin-Brown, S.; Bradford, W. D.; Staehling-Hampton, K.; Washburn, M. P.; Conaway, R. C.; Conaway, J. W. Characterization of Cullin-Box Sequences That Direct Recruitment of Cul2-Rbx1 and Cul5-Rbx2 Modules to Elongin BC-Based Ubiquitin Ligases. J. Biol. Chem. 2008, 283 (12), 8005–8013. https://doi.org/10.1074/jbc.

M706987200.

(67) Costessi, A.; Mahrour, N.; Tijchon, E.; Stunnenberg, R.; Stoel, M. A.; Jansen, P. W.; Sela, D.; Martin-Brown, S.; Washburn, M. P.; Florens, L.; Conaway, J. W.; Conaway, R. C.; Stunnenberg, H. G. The Tumour Antigen PRAME Is a Subunit of a Cul2 Ubiquitin Ligase and Associates with Active NFY Promoters. EMBO J. 2011, 30 (18), 3786–3798. https://doi.org/10.1038/emboj.2011.262.

(68) Hu, J.; McCall, C. M.; Ohta, T.; Xiong, Y. Targeted Ubiquitination of CDT1 by the DDB1-CUL4A-ROC1 Ligase in Response to DNA Damage. Nat. Cell Biol. 2004, 6 (10), 1003–1009. https://doi.org/10.1038/ncb1172.

(69) Jin, J.; Arias, E. E.; Chen, J.; Harper, J. W.; Walter, J. C. A Family of Diverse Cul4-Ddb1-Interacting Proteins Includes Cdt2, Which Is Required for S Phase Destruction of the Replication Factor Cdt1. Mol. Cell 2006, 23 (5), 709–721. https://doi.org/10.1016/j.molcel.2006.08.010.

(70) Angers, S.; Li, T.; Yi, X.; MacCoss, M. J.; Moon, R. T.; Zheng, N. Molecular Architecture and Assembly of the DDB1-CUL4A Ubiquitin Ligase Machinery. Nature 2006, 443 (7111), 590–593. https://doi.org/10.1038/nature05175.

(71) Scrima, A.; Konícková, R.; Czyzewski, B. K.; Kawasaki, Y.; Jeffrey, P. D.; Groisman, R.; Nakatani, Y.; Iwai, S.; Pavletich, N. P.; Thomä, N. H. Structural Basis of UV DNA-Damage Recognition by the DDB1-DDB2 Complex. Cell 2008, 135 (7), 1213–1223. https://doi.org/10.1016/j.cell.2008.10.045.

(72) Higa, L. A.; Wu, M.; Ye, T.; Kobayashi, R.; Sun, H.; Zhang, H. CUL4-DDB1 Ubiquitin Ligase Interacts with Multiple WD40-Repeat Proteins and Regulates Histone Methylation. Nat. Cell Biol. 2006, 8 (11), 1277–1283. https://doi.org/10.1038/ncb1490.

(73) He, Y. J.; McCall, C. M.; Hu, J.; Zeng, Y.; Xiong, Y. DDB1 Functions as a Linker to Recruit Receptor WD40 Proteins to CUL4-ROC1 Ubiquitin Ligases. Genes Dev. 2006, 20 (21), 2949–2954. https://doi.org/10.1101/gad.1483206.

(74) Pintard, L.; Willis, J. H.; Willems, A.; Johnson, J.-L. F.; Srayko, M.; Kurz, T.; Glaser, S.; Mains, P. E.; Tyers, M.; Bowerman, B.; Peter, M. The BTB Protein MEL-26 Is a Substrate-Specific Adaptor of the CUL-3 Ubiquitin-Ligase. Nature 2003, 425 (6955), 311–316. https://doi.org/10.1038/nature01959.

(75) Xu, L.; Wei, Y.; Reboul, J.; Vaglio, P.; Shin, T.-H.; Vidal, M.; Elledge, S. J.; Harper, J. W. BTB Proteins Are Substrate-Specific Adaptors in an SCF-like Modular Ubiquitin Ligase Containing CUL-3. Nature 2003, 425 (6955), 316–321. https://doi.org/10.1038/nature01985.

(76) Geyer, R.; Wee, S.; Anderson, S.; Yates, J.; Wolf, D. A. BTB/POZ Domain Proteins Are Putative Substrate Adaptors for Cullin 3 Ubiquitin Ligases. Mol. Cell 2003, 12 (3), 783–790. https://doi.org/10.1016/s1097-2765(03)00341-1.

(77) Furukawa, M.; He, Y. J.; Borchers, C.; Xiong, Y. Targeting of Protein Ubiquitination by BTB-Cullin 3-Roc1 Ubiquitin Ligases. Nat. Cell Biol. 2003, 5 (11), 1001–1007. https://doi.org/10.1038/ncb1056.

(78) Zhuang, M.; Calabrese, M. F.; Liu, J.; Waddell, M. B.; Nourse, A.; Hammel, M.; Miller, D. J.; Walden, H.; Duda, D. M.; Seyedin, S. N.; Hoggard, T.; Harper, J. W.; White, K. P.; Schulman, B. A. Structures of SPOP-Substrate Complexes: Insights into Molecular Architectures of BTB-Cul3 Ubiquitin Ligases. Mol. Cell 2009, 36 (1), 39–50. https://doi.org/10.1016/j.molcel.2009.09.022.

(79) Canning, P.; Cooper, C. D. O.; Krojer, T.; Murray, J. W.; Pike, A. C. W.; Chaikuad, A.; Keates, T.; Thangaratnarajah, C.; Hojzan, V.; Marsden, B. D.; Gileadi, O.; Knapp, S.; von Delft, F.; Bullock, A. N. Structural Basis for Cul3 Protein Assembly with the BTB-Kelch Family of E3 Ubiquitin Ligases. J. Biol. Chem. 2013, 288 (11), 7803–7814. https://doi.org/10.1074/jbc.M112.437996.

(80) Ji, A. X.; Privé, G. G. Crystal Structure of KLHL3 in Complex with Cullin3. PloS One 2013, 8 (4), e60445. https://doi.org/10.1371/journal.pone.0060445.

(81) Balasco, N.; Esposito, L.; Smaldone, G.; Salvatore, M.; Vitagliano, L. A Comprehensive Analysis of the Structural Recognition between KCTD Proteins and Cullin 3. Int. J. Mol. Sci. 2024, 25 (3), 1881. https://doi.org/10.3390/ijms25031881.

(82) Ji, A. X.; Chu, A.; Nielsen, T. K.; Benlekbir, S.; Rubinstein, J. L.; Privé, G. G. Structural Insights into KCTD Protein Assembly and Cullin3 Recognition. J. Mol. Biol. 2016, 428 (1), 92–107. https://doi.org/10.1016/j.jmb.2015.08.019.

(83) Potuschak, T.; Stary, S.; Schlögelhofer, P.; Becker, F.; Nejinskaia, V.; Bachmair, A. PRT1 of Arabidopsis Thaliana Encodes a Component of the Plant N-End Rule Pathway. Proc. Natl. Acad. Sci. U. S. A. 1998, 95 (14), 7904–7908. https://doi.org/10.1073/pnas.95.14.7904.

(84) Joazeiro, C. A.; Weissman, A. M. RING Finger Proteins: Mediators of Ubiquitin Ligase Activity. Cell 2000, 102 (5), 549–552. https://doi.org/10.1016/s0092-8674(00)00077-5.

(85) Deshaies, R. J.; Joazeiro, C. A. P. RING Domain E3 Ubiquitin Ligases. Annu. Rev. Biochem. 2009, 78, 399–434. https://doi.org/10.1146/annurev.biochem.78.101807.093809.

(86) Tasaki, T.; Mulder, L. C. F.; Iwamatsu, A.; Lee, M. J.; Davydov, I. V.; Varshavsky, A.; Muesing, M.; Kwon, Y. T. A Family of Mammalian E3 Ubiquitin Ligases That Contain the UBR Box Motif and Recognize N-Degrons. Mol. Cell. Biol. 2005, 25 (16), 7120–7136. https://doi.org/10.1128/MCB.25.16.7120-7136.2005.

(87) Shen, Z.; Wei, L.; Yu, Z.-B.; Yao, Z.-Y.; Cheng, J.; Wang, Y.-T.; Song, X.-T.; Li, M. The Roles of TRIMs in Antiviral Innate Immune Signaling. Front. Cell. Infect. Microbiol. 2021, 11, 628275. https://doi.org/10.3389/fcimb.2021.628275.

(88) Dudley-Fraser, J.; Esposito, D.; McPhie, K. A.; Morley-Williams, C.; Auchynnikava, T.; Rittinger, K. Identification of RING E3 Pseudoligases in the TRIM Protein Family. Nat. Commun. 2025, 16 (1), 3456. https://doi.org/10.1038/s41467-025-58807-1.

(89) Sanchez, J. G.; Okreglicka, K.; Chandrasekaran, V.; Welker, J. M.; Sundquist, W. I.; Pornillos, O. The Tripartite Motif Coiled-Coil Is an Elongated Antiparallel Hairpin Dimer. Proc. Natl. Acad. Sci. U. S. A. 2014, 111 (7), 2494–2499. https://doi.org/10.1073/pnas.1318962111.

(90) Fiorentini, F.; Esposito, D.; Rittinger, K. Does It Take Two to Tango? RING Domain Self-Association and Activity in TRIM E3 Ubiquitin Ligases. Biochem. Soc. Trans. 2020, 48 (6), 2615–2624. https://doi.org/10.1042/BST20200383.

(91) Stevens, R. V.; Esposito, D.; Rittinger, K. Characterisation of Class VI TRIM RING Domains: Linking RING Activity to C-Terminal Domain Identity. Life Sci. Alliance 2019, 2 (3), e201900295. https://doi.org/10.26508/lsa.201900295.

(92) Alpi, A. F.; Chrustowicz, J.; Sherpa, D.; Schulman, B. A. The Many Faces of the GID/CTLH E3 Ligase Complex. Biochem. Soc. Trans. 2025, 53 (5), 1351–1364. https://doi.org/10.1042/BST20253074.

(93) Niraj, J.; Färkkilä, A.; D'Andrea, A. D. The Fanconi Anemia Pathway in Cancer. Annu. Rev. Cancer Biol. 2019, 3, 457–478. https://doi.org/10.1146/annurev-cancerbio-030617-050422.

(94) Miles, J. A.; Frost, M. G.; Carroll, E.; Rowe, M. L.; Howard, M. J.; Sidhu, A.; Chaugule, V. K.; Alpi, A. F.; Walden, H. The Fanconi Anemia DNA Repair Pathway Is Regulated by an Interaction between Ubiquitin and the E2-like Fold Domain of FANCL. J. Biol. Chem. 2015, 290 (34), 20995–21006. https://doi.org/10.1074/jbc.M115.675835.

(95) Hu, Q.; Zhao, D.; Cui, G.; Bhandari, J.; Thompson, J. R.; Botuyan, M. V.; Mer, G. Mechanisms of RNF168 Nucleosome Recognition and Ubiquitylation. Mol. Cell 2024, 84 (5), 839-853.e12. https://doi.org/10.1016/j.molcel.2023.12.036.

(96) Stewart, M. D.; Duncan, E. D.; Coronado, E.; DaRosa, P. A.; Pruneda, J. N.; Brzovic, P. S.; Klevit, R. E. Tuning BRCA1 and BARD1 Activity to Investigate RING Ubiquitin Ligase Mechanisms. Protein Sci. Publ. Protein Soc. 2017, 26 (3), 475–483. https://doi.org/10.1002/pro.3091.

(97) Gallego, L. D.; Schneider, M.; Mittal, C.; Romanauska, A.; Gudino Carrillo, R. M.; Schubert, T.; Pugh, B. F.; Köhler, A. Phase Separation Directs Ubiquitination of Gene-Body Nucleosomes. Nature 2020, 579 (7800), 592–597. https://doi.org/10.1038/s41586-020-2097-z.

(98) Zhao, F.; Hicks, C. W.; Wolberger, C. Mechanism of Histone H2B Monoubiquitination by Bre1. Nat. Struct. Mol. Biol. 2023, 30 (11), 1623–1627. https://doi.org/10.1038/s41594-023-01137-x.

(99) Onishi, S.; Uchiyama, K.; Sato, K.; Okada, C.; Kobayashi, S.; Hamada, K.; Nishizawa, T.; Nureki, O.; Ogata, K.; Sengoku, T. Structure of the Human Bre1 Complex Bound to the Nucleosome. Nat. Commun. 2024, 15 (1), 2580. https://doi.org/10.1038/s41467-024-46910-8.

(100) Shi, M.; Wang, X.; Zhang, H.; Wen, Y.; Liu, Q.; Chen, P.; Chen, X.; Xiang, S. Structural Insights into the Bre1-Lge1 and RNF20/RNF40-WAC Interactions Critical for H2B Ubiquitination. Nucleic Acids Res. 2026, 54 (2), gkaf1514. https://doi.org/10.1093/nar/gkaf1514.

(101) Wu, L.; Zee, B. M.; Wang, Y.; Garcia, B. A.; Dou, Y. The RING Finger Protein MSL2 in the MOF Complex Is an E3 Ubiquitin Ligase for H2B K34 and Is Involved in Crosstalk with H3 K4 and K79 Methylation. Mol. Cell 2011, 43 (1), 132–144. https://doi.org/10.1016/j.molcel.2011.05.015.

(102) Smith, E. R.; Cayrou, C.; Huang, R.; Lane, W. S.; Côté, J.; Lucchesi, J. C. A Human Protein Complex Homologous to the Drosophila MSL Complex Is Responsible for the Majority of Histone H4 Acetylation at Lysine 16. Mol. Cell. Biol. 2005, 25 (21), 9175–9188. https://doi.org/10.1128/MCB.25.21.9175-9188.2005.

(103) Wu, X.; Siggel, M.; Ovchinnikov, S.; Mi, W.; Svetlov, V.; Nudler, E.; Liao, M.; Hummer, G.; Rapoport, T. A. Structural Basis of ER-Associated Protein Degradation Mediated by the Hrd1 Ubiquitin Ligase Complex. Science 2020, 368 (6489), eaaz2449. https://doi.org/10.1126/science.aaz2449.

(104) Ji, Z.; Li, H.; Peterle, D.; Paulo, J. A.; Ficarro, S. B.; Wales, T. E.; Marto, J. A.; Gygi, S. P.; Engen, J. R.; Rapoport, T. A. Translocation of Polyubiquitinated Protein Substrates by the Hexameric Cdc48 ATPase. Mol. Cell 2022, 82 (3), 570-584.e8. https://doi.org/10.1016/j.molcel.2021.11.033.

(105) Christianson, J. C.; Carvalho, P. Order through Destruction: How ER-Associated Protein Degradation Contributes to Organelle Homeostasis. EMBO J. 2022, 41 (6), e109845. https://doi.org/10.15252/embj.2021109845.

(106) Koegl, M.; Hoppe, T.; Schlenker, S.; Ulrich, H. D.; Mayer, T. U.; Jentsch, S. A Novel Ubiquitination Factor, E4, Is Involved in Multiubiquitin Chain Assembly. Cell 1999, 96 (5), 635–644. https://doi.org/10.1016/s0092-8674(00)80574-7.

(107) Pringa, E.; Martinez-Noel, G.; Muller, U.; Harbers, K. Interaction of the Ring Finger-Related U-Box Motif

of a Nuclear Dot Protein with Ubiquitin-Conjugating Enzymes. J. Biol. Chem. 2001, 276 (22), 19617–19623. https://doi.org/10.1074/jbc.M100192200.

(108) Kosarev, P.; Mayer, K. F. X.; Hardtke, C. S. Evaluation and Classification of RING-Finger Domains Encoded by the Arabidopsis Genome. Genome Biol. 2002, 3 (4), RESEARCH0016. https://doi.org/10.1186/gb-2002-3-4-research0016.

(109) Black, J. C.; Kutateladze, T. G. Atypical Histone Targets of PHD Fingers. J. Biol. Chem. 2023, 299 (4), 104601. https://doi.org/10.1016/j.jbc.2023.104601.

(110) Uchida, D.; Hatakeyama, S.; Matsushima, A.; Han, H.; Ishido, S.; Hotta, H.; Kudoh, J.; Shimizu, N.; Doucas, V.; Nakayama, K. I.; Kuroda, N.; Matsumoto, M. AIRE Functions as an E3 Ubiquitin Ligase. J. Exp. Med. 2004, 199 (2), 167–172. https://doi.org/10.1084/jem.20031291.

(111) Bottomley, M. J.; Stier, G.; Pennacchini, D.; Legube, G.; Simon, B.; Akhtar, A.; Sattler, M.; Musco, G. NMR Structure of the First PHD Finger of Autoimmune Regulator Protein (AIRE1). Insights into Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) Disease. J. Biol. Chem. 2005, 280 (12), 11505–11512. https://doi.org/10.1074/jbc.M413959200.

(112) Brooks, W. S.; Helton, E. S.; Banerjee, S.; Venable, M.; Johnson, L.; Schoeb, T. R.; Kesterson, R. A.; Crawford, D. F. G2E3 Is a Dual Function Ubiquitin Ligase Required for Early Embryonic Development. J. Biol. Chem. 2008, 283 (32), 22304–22315. https://doi.org/10.1074/jbc.M803238200.

(113) Hou, Y.; Zhang, Z.; Xu, Q.; Wang, H.; Xu, Y.; Chen, K. Inhibitor of Growth 4 Induces NFκB/P65 Ubiquitin-Dependent Degradation. Oncogene 2014, 33 (15), 1997–2003. https://doi.org/10.1038/onc.2013.135.

(114) Ali, M.; Hom, R. A.; Blakeslee, W.; Ikenouye, L.; Kutateladze, T. G. Diverse Functions of PHD Fingers of the MLL/KMT2 Subfamily. Biochim. Biophys. Acta 2014, 1843 (2), 366–371. https://doi.org/10.1016/j.bbamcr.2013.11.016.

(115) Han, X.; Gui, B.; Xiong, C.; Zhao, L.; Liang, J.; Sun, L.; Yang, X.; Yu, W.; Si, W.; Yan, R.; Yi, X.; Zhang, D.; Li, W.; Li, L.; Yang, J.; Wang, Y.; Sun, Y. E.; Zhang, D.; Meng, A.; Shang, Y. Destabilizing LSD1 by Jade-2 Promotes Neurogenesis: An Antibraking System in Neural Development. Mol. Cell 2014, 55 (3), 482–494. https://doi.org/10.1016/j.molcel.2014.06.006.

(116) Adhikary, S.; Chakravarti, D.; Terranova, C.; Sengupta, I.; Maitituoheti, M.; Dasgupta, A.; Srivastava, D. K.; Ma, J.; Raman, A. T.; Tarco, E.; Sahin, A. A.; Bassett, R.; Yang, F.; Tapia, C.; Roy, S.; Rai, K.; Das, C. Atypical Plant Homeodomain of UBR7 Functions as an H2BK120Ub Ligase and Breast Tumor Suppressor. Nat. Commun. 2019, 10 (1), 1398. https://doi.org/10.1038/s41467-019-08986-5.

(117) Huibregtse, J. M.; Scheffner, M.; Beaudenon, S.; Howley, P. M. A Family of Proteins Structurally and Functionally Related to the E6-AP Ubiquitin-Protein Ligase. Proc. Natl. Acad. Sci. U. S. A. 1995, 92 (11), 5249. https://doi.org/10.1073/pnas.92.11.5249-b.

(118) Zhou, M.; Fang, R.; Colson, L.; Donovan, K. A.; Hunkeler, M.; Song, Y.; Zhang, C.; Chen, S.; Lee, D.; Bradshaw, G. A.; Eisert, R.; Ye, Y.; Kalocsay, M.; Goldberg, A.; Fischer, E. S.; Lu, Y. HUWE1 Amplifies Ubiquitin Modifications to Broadly Stimulate Clearance of Proteins and Aggregates. bioRxiv May 30, 2023. https://doi.org/10.1101/2023.05.30.542866.

(119) Sauvé, V.; Sung, G.; Soya, N.; Kozlov, G.; Blaimschein, N.; Miotto, L. S.; Trempe, J.-F.; Lukacs, G. L.; Gehring, K. Mechanism of Parkin Activation by Phosphorylation. Nat. Struct. Mol. Biol. 2018, 25 (7), 623–630. https://doi.org/10.1038/s41594-018-0088-7.

(120) Kirisako, T.; Kamei, K.; Murata, S.; Kato, M.; Fukumoto, H.; Kanie, M.; Sano, S.; Tokunaga, F.; Tanaka, K.; Iwai, K. A Ubiquitin Ligase Complex Assembles Linear Polyubiquitin Chains. EMBO J. 2006, 25 (20), 4877–4887. https://doi.org/10.1038/sj.emboj.7601360.

(121) Gerlach, B.; Cordier, S. M.; Schmukle, A. C.; Emmerich, C. H.; Rieser, E.; Haas, T. L.; Webb, A. I.; Rickard, J. A.; Anderton, H.; Wong, W. W.-L.; Nachbur, U.; Gangoda, L.; Warnken, U.; Purcell, A. W.; Silke, J.; Walczak, H. Linear Ubiquitination Prevents Inflammation and Regulates Immune Signalling. Nature 2011, 471 (7340), 591–596. https://doi.org/10.1038/nature09816.

(122) Kelsall, I. R.; Zhang, J.; Knebel, A.; Arthur, J. S. C.; Cohen, P. 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. 2019, 116 (27), 13293–13298. https://doi.org/10.1073/pnas.1905873116.

(123) Barnsby-Greer, L.; Mabbitt, P. D.; Dery, M.-A.; Squair, D. R.; Wood, N. T.; Lamoliatte, F.; Lange, S. M.; Virdee, S. UBE2A and UBE2B Are Recruited by an Atypical E3 Ligase Module in UBR4. Nat. Struct. Mol. Biol. 2024, 31 (2), 351–363. https://doi.org/10.1038/s41594-023-01192-4.

(124) Hong, J. H.; Kaustov, L.; Coyaud, E.; Srikumar, T.; Wan, J.; Arrowsmith, C.; Raught, B. KCMF1 (Potassium Channel Modulatory Factor 1) Links RAD6 to UBR4 (Ubiquitin N-Recognin Domain-Containing E3 Ligase 4) and Lysosome-Mediated Degradation. Mol. Cell. Proteomics MCP 2015, 14 (3), 674–685. https://doi.org/10.1074/mcp.M114.042168.

(125) Loeb, K. R.; Haas, A. L. The Interferon-Inducible 15-kDa Ubiquitin Homolog Conjugates to Intracellular Proteins. J. Biol. Chem. 1992, 267 (11), 7806–7813.

(126) Mathieu, N. A.; Paparisto, E.; Barr, S. D.; Spratt, D. E. HERC5 and the ISGylation Pathway: Critical Modulators of the Antiviral Immune Response. Viruses 2021, 13 (6), 1102. https://doi.org/10.3390/v13061102.

(127) Zhao, X.; Perez, J. M.; Faull, P. A.; Chan, C.; Munting, F. W.; Canadeo, L. A.; Cenik, C.; Huibregtse, J. M. Cellular Targets and Lysine Selectivity of the HERC5 ISG15 Ligase. iScience 2024, 27 (2), 108820. https://doi.org/10.1016/j.isci.2024.108820.

(128) Zou, W.; Zhang, D.-E. The Interferon-Inducible Ubiquitin-Protein Isopeptide Ligase (E3) EFP Also Functions as an ISG15 E3 Ligase. J. Biol. Chem. 2006, 281 (7), 3989–3994. https://doi.org/10.1074/jbc.M510787200.

(129) Xiong, T.-C.; Wei, M.-C.; Li, F.-X.; Shi, M.; Gan, H.; Tang, Z.; Dong, H.-P.; Liuyu, T.; Gao, P.; Zhong, B.; Zhang, Z.-D.; Lin, D. The E3 Ubiquitin Ligase ARIH1 Promotes Antiviral Immunity and Autoimmunity by Inducing Mono-ISGylation and Oligomerization of cGAS. Nat. Commun. 2022, 13 (1), 5973. https://doi.org/10.1038/s41467-022-33671-5.

(130) Matunis, M. J.; Coutavas, E.; Blobel, G. A Novel Ubiquitin-like Modification Modulates the Partitioning of the Ran-GTPase-Activating Protein RanGAP1 between the Cytosol and the Nuclear Pore Complex. J. Cell Biol. 1996, 135 (6 Pt 1), 1457–1470. https://doi.org/10.1083/jcb.135.6.1457.

(131) Mahajan, R.; Delphin, C.; Guan, T.; Gerace, L.; Melchior, F. A Small Ubiquitin-Related Polypeptide Involved in Targeting RanGAP1 to Nuclear Pore Complex Protein RanBP2. Cell 1997, 88 (1), 97–107. https://doi.org/10.1016/s0092-8674(00)81862-0.

(132) Hendriks, I. A.; D'Souza, R. C. J.; Yang, B.; Verlaan-de Vries, M.; Mann, M.; Vertegaal, A. C. O. Uncovering Global SUMOylation Signaling Networks in a Site-Specific Manner. Nat. Struct. Mol. Biol. 2014, 21 (10), 927–936. https://doi.org/10.1038/nsmb.2890.

(133) Hendriks, I. A.; D'Souza, R. C.; Chang, J.-G.; Mann, M.; Vertegaal, A. C. O. System-Wide Identification of Wild-Type SUMO-2 Conjugation Sites. Nat. Commun. 2015, 6, 7289. https://doi.org/10.1038/ncomms8289.

(134) Hendriks, I. A.; Vertegaal, A. C. O. A Comprehensive Compilation of SUMO Proteomics. Nat. Rev. Mol. Cell Biol. 2016, 17 (9), 581–595. https://doi.org/10.1038/nrm.2016.81.

(135) Hendriks, I. A.; Lyon, D.; Young, C.; Jensen, L. J.; Vertegaal, A. C. O.; Nielsen, M. L. Site-Specific Mapping of the Human SUMO Proteome Reveals Co-Modification with Phosphorylation. Nat. Struct. Mol. Biol. 2017, 24 (3), 325–336. https://doi.org/10.1038/nsmb.3366.

(136) Hendriks, I. A.; Lyon, D.; Su, D.; Skotte, N. H.; Daniel, J. A.; Jensen, L. J.; Nielsen, M. L. Site-Specific Characterization of Endogenous SUMOylation across Species and Organs. Nat. Commun. 2018, 9 (1), 2456. https://doi.org/10.1038/s41467-018-04957-4.

(137) González-Prieto, R.; Eifler-Olivi, K.; Claessens, L. A.; Willemstein, E.; Xiao, Z.; Talavera Ormeno, C. M. P.; Ovaa, H.; Ulrich, H. D.; Vertegaal, A. C. O. Global Non-Covalent SUMO Interaction Networks Reveal SUMO-Dependent Stabilization of the Non-Homologous End Joining Complex. Cell Rep. 2021, 34 (4), 108691. https://doi.org/10.1016/j.celrep.2021.108691.

(138) Claessens, L. A.; Verlaan-de Vries, M.; Broekman, N.; Luijsterburg, M. S.; Vertegaal, A. C. O. Interaction Networks of SIM-Binding Groove Mutants Reveal Alternate Modes of SUMO Binding and Profound Impact on SUMO Conjugation. Sci. Adv. 2025, 11 (20), eadp2643. https://doi.org/10.1126/sciadv.adp2643.

(139) Pichler, A.; Fatouros, C.; Lee, H.; Eisenhardt, N. SUMO Conjugation - a Mechanistic View. Biomol. Concepts 2017, 8 (1), 13–36. https://doi.org/10.1515/bmc-2016-0030.

(140) Vertegaal, A. C. O. Signalling Mechanisms and Cellular Functions of SUMO. Nat. Rev. Mol. Cell Biol. 2022, 23 (11), 715–731. https://doi.org/10.1038/s41580-022-00500-y.

(141) Lobato-Gil, S.; Heidelberger, J. B.; Maghames, C.; Bailly, A.; Brunello, L.; Rodriguez, M. S.; Beli, P.; Xirodimas, D. P. Proteome-Wide Identification of NEDD8 Modification Sites Reveals Distinct Proteomes for Canonical and Atypical NEDDylation. Cell Rep. 2021, 34 (3), 108635. https://doi.org/10.1016/j.celrep.2020.108635.

(142) He, Z.; Yuan, Z.; Yang, F.; Zhang, J.; Zhao, W.; Qin, T.; Zheng, X.; Ma, L. A Comprehensive Review on DCN1 Protein, Inhibitors and Their Therapeutic Applications. Int. J. Biol. Macromol. 2024, 277 (Pt 4), 134541. https://doi.org/10.1016/j.ijbiomac.2024.134541.

(143) Zhang, S.; Yu, Q.; Li, Z.; Zhao, Y.; Sun, Y. Protein Neddylation and Its Role in Health and Diseases. Signal Transduct. Target. Ther. 2024, 9 (1), 85. https://doi.org/10.1038/s41392-024-01800-9.

(144) Boada-Romero, E.; Letek, M.; Fleischer, A.; Pallauf, K.; Ramón-Barros, C.; Pimentel-Muiños, F. X. TMEM59 Defines a Novel ATG16L1-Binding Motif That Promotes Local Activation of LC3. EMBO J. 2013, 32 (4), 566–582. https://doi.org/10.1038/emboj.2013.8.

(145) Scavone, F.; Kopito, R. R. Evaluating Evidence for UFMylation Client Diversity. Nat. Rev. Mol. Cell Biol. 2026. https://doi.org/10.1038/s41580-026-00951-7.

(146) Tatsumi, K.; Sou, Y.; Tada, N.; Nakamura, E.;

Iemura, S.; Natsume, T.; Kang, S. H.; Chung, C. H.; Kasahara, M.; Kominami, E.; Yamamoto, M.; Tanaka, K.; Komatsu, M. A Novel Type of E3 Ligase for the Ufm1 Conjugation System. J. Biol. Chem. 2010, 285 (8), 5417–5427. https://doi.org/10.1074/jbc.M109.036814.

(147) Peter, J. J.; Magnussen, H. M.; DaRosa, P. A.; Millrine, D.; Matthews, S. P.; Lamoliatte, F.; Sundaramoorthy, R.; Kopito, R. R.; Kulathu, Y. A Non-Canonical Scaffold-Type E3 Ligase Complex Mediates Protein UFMylation. EMBO J. 2022, 41 (21), e111015. https://doi.org/10.15252/embj.2022111015.

(148) DaRosa, P. A.; Penchev, I.; Gumbin, S. C.; Scavone, F.; Wąchalska, M.; Paulo, J. A.; Ordureau, A.; Peter, J. J.; Kulathu, Y.; Harper, J. W.; Becker, T.; Beckmann, R.; Kopito, R. R. UFM1 E3 Ligase Promotes Recycling of 60S Ribosomal Subunits from the ER. Nature 2024, 627 (8003), 445–452. https://doi.org/10.1038/s41586-024-07073-0.

(149) Penchev, I.; Gumbin, S.; Scavone, F.; Berninghausen, O.; Becker, T.; Kopito, R.; Beckmann, R. UFMylation Orchestrates Spatiotemporal Coordination of RQC at the ER. Sci. Adv. 2025, 11 (18), eadv0435. https://doi.org/10.1126/sciadv.adv0435.

(150) Makhlouf, L.; Peter, J. J.; Magnussen, H. M.; Thakur, R.; Millrine, D.; Minshull, T. C.; Harrison, G.; Varghese, J.; Lamoliatte, F.; Foglizzo, M.; Macartney, T.; Calabrese, A. N.; Zeqiraj, E.; Kulathu, Y. The UFM1 E3 Ligase Recognizes and Releases 60S Ribosomes from ER Translocons. Nature 2024, 627 (8003), 437–444. https://doi.org/10.1038/s41586-024-07093-w.

(151) Prus, G.; Satpathy, S.; Weinert, B. T.; Narita, T.; Choudhary, C. Global, Site-Resolved Analysis of Ubiquitylation Occupancy and Turnover Rate Reveals Systems Properties. Cell 2024, 187 (11), 2875-2892.e21. https://doi.org/10.1016/j.cell.2024.03.024.

(152) Faesen, A. C.; Luna-Vargas, M. P. A.; Geurink, P. P.; Clerici, M.; Merkx, R.; van Dijk, W. J.; Hameed, D. S.; El Oualid, F.; Ovaa, H.; Sixma, T. K. The Differential Modulation of USP Activity by Internal Regulatory Domains, Interactors and Eight Ubiquitin Chain Types. Chem. Biol. 2011, 18 (12), 1550–1561. https://doi.org/10.1016/j.chembiol.2011.10.017.

(153) Burrows, J. F.; Scott, C. J.; Johnston, J. A. The DUB/USP17 Deubiquitinating Enzymes: A Gene Family within a Tandemly Repeated Sequence, Is Also Embedded within the Copy Number Variable Beta-Defensin Cluster. BMC Genomics 2010, 11, 250. https://doi.org/10.1186/1471-2164-11-250.

(154) Johnston, S. C.; Larsen, C. N.; Cook, W. J.; Wilkinson, K. D.; Hill, C. P. Crystal Structure of a Deubiquitinating Enzyme (Human UCH-L3) at 1.8 A Resolution. EMBO J. 1997, 16 (13), 3787–3796. https://doi.org/10.1093/emboj/16.13.3787.

(155) Deol, K. K.; Crowe, S. O.; Du, J.; Bisbee, H. A.; Guenette, R. G.; Strieter, E. R. Proteasome-Bound UCH37/UCHL5 Debranches Ubiquitin Chains to Promote Degradation. Mol. Cell 2020, 80 (5), 796-809.e9. https://doi.org/10.1016/j.molcel.2020.10.017.

(156) Song, A.; Hazlett, Z.; Abeykoon, D.; Dortch, J.; Dillon, A.; Curtiss, J.; Martinez, S. B.; Hill, C. P.; Yu, C.; Huang, L.; Fushman, D.; Cohen, R. E.; Yao, T. Branched Ubiquitin Chain Binding and Deubiquitination by UCH37 Facilitate Proteasome Clearance of Stress-Induced Inclusions. eLife 2021, 10, e72798. https://doi.org/10.7554/eLife.72798.

(157) Fuchs, A. C. D.; Maldoner, L.; Wojtynek, M.; Hartmann, M. D.; Martin, J. Rpn11-Mediated Ubiquitin Processing in an Ancestral Archaeal Ubiquitination System. Nat. Commun. 2018, 9 (1), 2696. https://doi.org/10.1038/s41467-018-05198-1.

(158) Glickman, M. H.; Rubin, D. M.; Coux, O.; Wefes, I.; Pfeifer, G.; Cjeka, Z.; Baumeister, W.; Fried, V. A.; Finley, D. A Subcomplex of the Proteasome Regulatory Particle Required for Ubiquitin-Conjugate Degradation and Related to the COP9-Signalosome and eIF3. Cell 1998, 94 (5), 615–623. https://doi.org/10.1016/s0092-8674(00)81603-7.

(159) Snyder, N. A.; Silva, G. M. Deubiquitinating Enzymes (DUBs): Regulation, Homeostasis, and Oxidative Stress Response. J. Biol. Chem. 2021, 297 (3), 101077. https://doi.org/10.1016/j.jbc.2021.101077.

(160) Sato, Y.; Yoshikawa, A.; Yamagata, A.; Mimura, H.; Yamashita, M.; Ookata, K.; Nureki, O.; Iwai, K.; Komada, M.; Fukai, S. Structural Basis for Specific Cleavage of Lys 63-Linked Polyubiquitin Chains. Nature 2008, 455 (7211), 358–362. https://doi.org/10.1038/nature07254.

(161) Verma, R.; Aravind, L.; Oania, R.; McDonald, W. H.; Yates, J. R.; Koonin, E. V.; Deshaies, R. J. Role of Rpn11 Metalloprotease in Deubiquitination and Degradation by the 26S Proteasome. Science 2002, 298 (5593), 611–615. https://doi.org/10.1126/science.1075898.

(162) Yao, T.; Cohen, R. E. A Cryptic Protease Couples Deubiquitination and Degradation by the Proteasome. Nature 2002, 419 (6905), 403–407. https://doi.org/10.1038/nature01071.

(163) Worden, E. J.; Dong, K. C.; Martin, A. An AAA Motor-Driven Mechanical Switch in Rpn11 Controls Deubiquitination at the 26S Proteasome. Mol. Cell 2017, 67 (5), 799-811.e8. https://doi.org/10.1016/j.molcel.2017.07.023.

(164) Mevissen, T. E. T.; Hospenthal, M. K.; Geurink, P. P.; Elliott, P. R.; Akutsu, M.; Arnaudo, N.; Ekkebus, R.; Kulathu, Y.; Wauer, T.; El Oualid, F.; Freund, S. M.

V.; Ovaa, H.; Komander, D. OTU Deubiquitinases Reveal Mechanisms of Linkage Specificity and Enable Ubiquitin Chain Restriction Analysis. Cell 2013, 154 (1), 169–184. https://doi.org/10.1016/j.cell.2013.05.046.

(165) Hermanns, T.; Pichlo, C.; Woiwode, I.; Klopffleisch, K.; Witting, K. F.; Ovaa, H.; Baumann, U.; Hofmann, K. A Family of Unconventional Deubiquitinases with Modular Chain Specificity Determinants. Nat. Commun. 2018, 9 (1), 799. https://doi.org/10.1038/s41467-018-03148-5.

(166) De Cesare, V.; Carbajo Lopez, D.; Mabbitt, P. D.; Fletcher, A. J.; Soetens, M.; Antico, O.; Wood, N. T.; Virdee, S. Deubiquitinating Enzyme Amino Acid Profiling Reveals a Class of Ubiquitin Esterases. Proc. Natl. Acad. Sci. U. S. A. 2021, 118 (4), e2006947118. https://doi.org/10.1073/pnas.2006947118.

(167) Kang, S. H.; Kim, G. R.; Seong, M.; Baek, S. H.; Seol, J. H.; Bang, O. S.; Ovaa, H.; Tatsumi, K.; Komatsu, M.; Tanaka, K.; Chung, C. H. Two Novel Ubiquitin-Fold Modifier 1 (Ufm1)-Specific Proteases, UfSP1 and UfSP2. J. Biol. Chem. 2007, 282 (8), 5256–5262. https://doi.org/10.1074/jbc.M610590200.

(168) Kirisako, T.; Ichimura, Y.; Okada, H.; Kabeya, Y.; Mizushima, N.; Yoshimori, T.; Ohsumi, M.; Takao, T.; Noda, T.; Ohsumi, Y. The Reversible Modification Regulates the Membrane-Binding State of Apg8/Aut7 Essential for Autophagy and the Cytoplasm to Vacuole Targeting Pathway. J. Cell Biol. 2000, 151 (2), 263–276. https://doi.org/10.1083/jcb.151.2.263.

(169) Mukhopadhyay, D.; Dasso, M. Modification in Reverse: The SUMO Proteases. Trends Biochem. Sci. 2007, 32 (6), 286–295. https://doi.org/10.1016/j.tibs.2007.05.002.

(170) Gan-Erdene, T.; Nagamalleswari, K.; Yin, L.; Wu, K.; Pan, Z.-Q.; Wilkinson, K. D. Identification and Characterization of DEN1, a Deneddylase of the ULP Family. J. Biol. Chem. 2003, 278 (31), 28892–28900. https://doi.org/10.1074/jbc.M302890200.

(171) Wu, K.; Yamoah, K.; Dolios, G.; Gan-Erdene, T.; Tan, P.; Chen, A.; Lee, C.-G.; Wei, N.; Wilkinson, K. D.; Wang, R.; Pan, Z.-Q. DEN1 Is a Dual Function Protease Capable of Processing the C Terminus of Nedd8 and Deconjugating Hyper-Neddylated CUL1. J. Biol. Chem. 2003, 278 (31), 28882–28891. https://doi.org/10.1074/jbc.M302888200.

(172) Shin, E. J.; Shin, H. M.; Nam, E.; Kim, W. S.; Kim, J.-H.; Oh, B.-H.; Yun, Y. DeSUMOylating Isopeptidase: A Second Class of SUMO Protease. EMBO Rep. 2012, 13 (4), 339–346. https://doi.org/10.1038/embor.2012.3.

(173) Xie, X.; Wang, X.; Jiang, D.; Wang, J.; Fei, R.; Cong, X.; Wei, L.; Wang, Y.; Chen, H. PPPDE1 Is a Novel Deubiquitinase Belonging to a Cysteine Isopeptidase Family. Biochem. Biophys. Res. Commun. 2017, 488 (2), 291–296. https://doi.org/10.1016/j.bbrc.2017.04.161.

(174) Luebben, A. V.; Bender, D.; Becker, S.; Crowther, L. M.; Erven, I.; Hofmann, K.; Söding, J.; Klemp, H.; Bellotti, C.; Stäuble, A.; Qiu, T.; Kathayat, R. S.; Dickinson, B. C.; Gärtner, J.; Sheldrick, G. M.; Krätzner, R.; Steinfeld, R. Cln5 Represents a New Type of Cysteine-Based S-Depalmitoylase Linked to Neurodegeneration. Sci. Adv. 2022, 8 (15), eabj8633. https://doi.org/10.1126/sciadv.abj8633.

(175) Bolhuis, D. L.; Emanuele, M. J.; Brown, N. G. Friend or Foe? Reciprocal Regulation between E3 Ubiquitin Ligases and Deubiquitinases. Biochem. Soc. Trans. 2024, 52 (1), 241–267. https://doi.org/10.1042/BST20230454.

(176) Hofmann, K.; Bucher, P. The UBA Domain: A Sequence Motif Present in Multiple Enzyme Classes of the Ubiquitination Pathway. Trends Biochem. Sci. 1996, 21 (5), 172–173.

(177) Lohi, O.; Lehto, V. P. VHS Domain Marks a Group of Proteins Involved in Endocytosis and Vesicular Trafficking. FEBS Lett. 1998, 440 (3), 255–257. https://doi.org/10.1016/s0014-5793(98)01401-x.

(178) Shiba, T.; Kawasaki, M.; Takatsu, H.; Nogi, T.; Matsugaki, N.; Igarashi, N.; Suzuki, M.; Kato, R.; Nakayama, K.; Wakatsuki, S. Molecular Mechanism of Membrane Recruitment of GGA by ARF in Lysosomal Protein Transport. Nat. Struct. Biol. 2003, 10 (5), 386–393. https://doi.org/10.1038/nsb920.

(179) Elsasser, S.; Gali, R. R.; Schwickart, M.; Larsen, C. N.; Leggett, D. S.; Müller, B.; Feng, M. T.; Tübing, F.; Dittmar, G. A. G.; Finley, D. Proteasome Subunit Rpn1 Binds Ubiquitin-like Protein Domains. Nat. Cell Biol. 2002, 4 (9), 725–730. https://doi.org/10.1038/ncb845.

(180) Martinez-Fonts, K.; Davis, C.; Tomita, T.; Elsasser, S.; Nager, A. R.; Shi, Y.; Finley, D.; Matouschek, A. The Proteasome 19S Cap and Its Ubiquitin Receptors Provide a Versatile Recognition Platform for Substrates. Nat. Commun. 2020, 11 (1), 477. https://doi.org/10.1038/s41467-019-13906-8.

(181) Moraes, T. F.; Edwards, R. A.; McKenna, S.; Pastushok, L.; Xiao, W.; Glover, J. N.; Ellison, M. J. Crystal Structure of the Human Ubiquitin Conjugating Enzyme Complex, hMms2-hUbc13. Nat. Struct. Biol. 2001, 8 (8), 669–673. https://doi.org/10.1038/90373.

(182) Hofmann, R. M.; Pickart, C. M. Noncanonical MMS2-Encoded Ubiquitin-Conjugating Enzyme Functions in Assembly of Novel Polyubiquitin Chains for DNA Repair. Cell 1999, 96 (5), 645–653. https://doi.org/10.1016/s0092-8674(00)80575-9.

(183) Stewart, M. D.; Ritterhoff, T.; Klevit, R. E.; Brzovic,

P. S. E2 Enzymes: More than Just Middle Men. Cell Res. 2016, 26 (4), 423–440. https://doi.org/10.1038/cr.2016.35.

(184) Danielsen, J. R.; Povlsen, L. K.; Villumsen, B. H.; Streicher, W.; Nilsson, J.; Wikström, M.; Bekker-Jensen, S.; Mailand, N. DNA Damage-Inducible SUMOylation of HERC2 Promotes RNF8 Binding via a Novel SUMO-Binding Zinc Finger. J. Cell Biol. 2012, 197 (2), 179–187. https://doi.org/10.1083/jcb.201106152.

(185) Pashkova, N.; Gakhar, L.; Winistorfer, S. C.; Yu, L.; Ramaswamy, S.; Piper, R. C. WD40 Repeat Propellers Define a Ubiquitin-Binding Domain That Regulates Turnover of F Box Proteins. Mol. Cell 2010, 40 (3), 433–443. https://doi.org/10.1016/j.molcel.2010.10.018.

(186) Knipscheer, P.; van Dijk, W. J.; Olsen, J. V.; Mann, M.; Sixma, T. K. Noncovalent Interaction between Ubc9 and SUMO Promotes SUMO Chain Formation. EMBO J. 2007, 26 (11), 2797–2807. https://doi.org/10.1038/sj.emboj.7601711.

(187) Lallemand-Breitenbach, V.; Jeanne, M.; Benhenda, S.; Nasr, R.; Lei, M.; Peres, L.; Zhou, J.; Zhu, J.; Raught, B.; de Thé, H. Arsenic Degrades PML or PML-RARalpha through a SUMO-Triggered RNF4/Ubiquitin-Mediated Pathway. Nat. Cell Biol. 2008, 10 (5), 547–555. https://doi.org/10.1038/ncb1717.

(188) Tatham, M. H.; Geoffroy, M.-C.; Shen, L.; Plechanovova, A.; Hattersley, N.; Jaffray, E. G.; Palvimo, J. J.; Hay, R. T. RNF4 Is a Poly-SUMO-Specific E3 Ubiquitin Ligase Required for Arsenic-Induced PML Degradation. Nat. Cell Biol. 2008, 10 (5), 538–546. https://doi.org/10.1038/ncb1716.

(189) Kumar, R.; González-Prieto, R.; Xiao, Z.; Verlaan-de Vries, M.; Vertegaal, A. C. O. The STUbL RNF4 Regulates Protein Group SUMOylation by Targeting the SUMO Conjugation Machinery. Nat. Commun. 2017, 8 (1), 1809. https://doi.org/10.1038/s41467-017-01900-x.

(190) Sriramachandran, A. M.; Meyer-Teschendorf, K.; Pabst, S.; Ulrich, H. D.; Gehring, N. H.; Hofmann, K.; Praefcke, G. J. K.; Dohmen, R. J. Arkadia/RNF111 Is a SUMO-Targeted Ubiquitin Ligase with Preference for Substrates Marked with SUMO1-Capped SUMO2/3 Chain. Nat. Commun. 2019, 10 (1), 3678. https://doi.org/10.1038/s41467-019-11549-3.

(191) Jansen, N. S.; Vertegaal, A. C. O. A Chain of Events: Regulating Target Proteins by SUMO Polymers. Trends Biochem. Sci. 2021, 46 (2), 113–123. https://doi.org/10.1016/j.tibs.2020.09.002.

(192) Chang, Y.-C.; Oram, M. K.; Bielinsky, A.-K. SUMO-Targeted Ubiquitin Ligases and Their Functions in Maintaining Genome Stability. Int. J. Mol. Sci. 2021, 22 (10), 5391. https://doi.org/10.3390/ijms22105391.

(193) Liu, J. C. Y.; Ackermann, L.; Hoffmann, S.; Gál, Z.; Hendriks, I. A.; Jain, C.; Morlot, L.; Tatham, M. H.; McLelland, G.-L.; Hay, R. T.; Nielsen, M. L.; Brummelkamp, T.; Haahr, P.; Mailand, N. Concerted SUMO-Targeted Ubiquitin Ligase Activities of TOPORS and RNF4 Are Essential for Stress Management and Cell Proliferation. Nat. Struct. Mol. Biol. 2024, 31 (9), 1355–1367. https://doi.org/10.1038/s41594-024-01294-7.

(194) Jaffray, E. G.; Tatham, M. H.; Mojsa, B.; Plechanovová, A.; Rojas-Fernandez, A.; Liu, J. C. Y.; Mailand, N.; Ibrahim, A. F. M.; Ball, G.; Porter, I. M.; Hay, R. T. PML Mutants from Arsenic-Resistant Patients Reveal SUMO1-TOPORS and SUMO2/3-RNF4 Degradation Pathways. J. Cell Biol. 2025, 224 (6), e202407133. https://doi.org/10.1083/jcb.202407133.

(195) Arkinson, C.; Dong, K. C.; Gee, C. L.; Martin, A. Mechanisms and Regulation of Substrate Degradation by the 26S Proteasome. Nat. Rev. Mol. Cell Biol. 2025, 26 (2), 104–122. https://doi.org/10.1038/s41580-024-00778-0.

(196) Ahlstedt, B. A.; Ganji, R.; Raman, M. The Functional Importance of VCP to Maintaining Cellular Protein Homeostasis. Biochem. Soc. Trans. 2022, 50 (5), 1457–1469. https://doi.org/10.1042/BST20220648.

(197) Meyer, H.; van den Boom, J. Targeting of Client Proteins to the VCP/P97/Cdc48 Unfolding Machine. Front. Mol. Biosci. 2023, 10, 1142989. https://doi.org/10.3389/fmolb.2023.1142989.

(198) Pontifex, C. S.; Zaman, M.; Fanganiello, R. D.; Shutt, T. E.; Pfeffer, G. Valosin-Containing Protein (VCP): A Review of Its Diverse Molecular Functions and Clinical Phenotypes. Int. J. Mol. Sci. 2024, 25 (11), 5633. https://doi.org/10.3390/ijms25115633.

(199) Ram, J.; Glickman, M. H. The Many Faces of P97/Cdc48 in Mitochondrial Homeostasis. Essays Biochem. 2025, 69 (5), EBC20253045. https://doi.org/10.1042/EBC20253045.

(200) Groll, M.; Bajorek, M.; Köhler, A.; Moroder, L.; Rubin, D. M.; Huber, R.; Glickman, M. H.; Finley, D. A Gated Channel into the Proteasome Core Particle. Nat. Struct. Biol. 2000, 7 (11), 1062–1067. https://doi.org/10.1038/80992.

(201) Lee, B.-H.; Lu, Y.; Prado, M. A.; Shi, Y.; Tian, G.; Sun, S.; Elsasser, S.; Gygi, S. P.; King, R. W.; Finley, D. USP14 Deubiquitinates Proteasome-Bound Substrates That Are Ubiquitinated at Multiple Sites. Nature 2016, 532 (7599), 398–401. https://doi.org/10.1038/nature17433.

(202) Levi, S.; Glaser, F.; Wagh, A. R.; Filimonov, E.; Reis, N.; Mali, S.; Singh, S. K.; Cohen, N.; Byun, I.; Lee, M. J.; Kleifeld, O.; Brik, A.; Glickman, M. H.; Sahu, I. USP15: The Fourth Proteasome-Associated DUB. Biochemistry August 13, 2025. https://doi.org/10.1101/2025.08.13.670075.

(203) Crosas, B.; Hanna, J.; Kirkpatrick, D. S.; Zhang, D. P.; Tone, Y.; Hathaway, N. A.; Buecker, C.; Leggett, D. S.; Schmidt, M.; King, R. W.; Gygi, S. P.; Finley, D. Ubiquitin Chains Are Remodeled at the Proteasome by Opposing Ubiquitin Ligase and Deubiquitinating Activities. Cell 2006, 127 (7), 1401–1413. https://doi.org/10.1016/j.cell.2006.09.051.

(204) Buel, G. R.; Chen, X.; Chari, R.; O'Neill, M. J.; Ebelle, D. L.; Jenkins, C.; Sridharan, V.; Tarasov, S. G.; Tarasova, N. I.; Andresson, T.; Walters, K. J. Structure of E3 Ligase E6AP with a Proteasome-Binding Site Provided by Substrate Receptor hRpn10. Nat. Commun. 2020, 11 (1), 1291. https://doi.org/10.1038/s41467-020-15073-7.

(205) Gu, X.; Nardone, C.; Kamitaki, N.; Mao, A.; Elledge, S. J.; Greenberg, M. E. The Midnolin-Proteasome Pathway Catches Proteins for Ubiquitination-Independent Degradation. Science 2023, 381 (6660), eadh5021. https://doi.org/10.1126/science.adh5021.

(206) Peddada, N.; Zhong, X.; Yin, Y.; Lazaro, D. R.; Wang, J.; Lyon, S.; Choi, J. H.; Bai, X.-C.; Moresco, E. M. Y.; Beutler, B. Structural Insights into the Ubiquitin-Independent Midnolin-Proteasome Pathway. Proc. Natl. Acad. Sci. U. S. A. 2025, 122 (19), e2505345122. https://doi.org/10.1073/pnas.2505345122.

(207) Kors, S.; Geijtenbeek, K.; Reits, E.; Schipper-Krom, S. Regulation of Proteasome Activity by (Post-)Transcriptional Mechanisms. Front. Mol. Biosci. 2019, 6, 48. https://doi.org/10.3389/fmolb.2019.00048.

(208) Meyer, H.; Weihl, C. C. The VCP/P97 System at a Glance: Connecting Cellular Function to Disease Pathogenesis. J. Cell Sci. 2014, 127 (Pt 18), 3877–3883. https://doi.org/10.1242/jcs.093831.

(209) Verma, R.; Oania, R. S.; Kolawa, N. J.; Deshaies, R. J. Cdc48/P97 Promotes Degradation of Aberrant Nascent Polypeptides Bound to the Ribosome. eLife 2013, 2, e00308. https://doi.org/10.7554/eLife.00308.

(210) Acharya, U.; Jacobs, R.; Peters, J. M.; Watson, N.; Farquhar, M. G.; Malhotra, V. The Formation of Golgi Stacks from Vesiculated Golgi Membranes Requires Two Distinct Fusion Events. Cell 1995, 82 (6), 895–904. https://doi.org/10.1016/0092-8674(95)90269-4.

(211) Rabouille, C.; Levine, T. P.; Peters, J. M.; Warren, G. An NSF-like ATPase, P97, and NSF Mediate Cisternal Regrowth from Mitotic Golgi Fragments. Cell 1995, 82 (6), 905–914. https://doi.org/10.1016/0092-8674(95)90270-8.

(212) Papadopoulos, C.; Kirchner, P.; Bug, M.; Grum, D.; Koerver, L.; Schulze, N.; Poehler, R.; Dressler, A.; Fengler, S.; Arhzaouy, K.; Lux, V.; Ehrmann, M.; Weihl, C. C.; Meyer, H. VCP/P97 Cooperates with YOD1, UBXD1 and PLAA to Drive Clearance of Ruptured Lysosomes by Autophagy. EMBO J. 2017, 36 (2), 135–150. https://doi.org/10.15252/embj.201695148.

(213) Florke Gee, R. R.; Chen, H.; Lee, A. K.; Daly, C. A.; Wilander, B. A.; Fon Tacer, K.; Potts, P. R. Emerging Roles of the MAGE Protein Family in Stress Response Pathways. J. Biol. Chem. 2020, 295 (47), 16121–16155. https://doi.org/10.1074/jbc.REV120.008029.

(214) Mao, X.; Gluck, N.; Chen, B.; Starokadomskyy, P.; Li, H.; Maine, G. N.; Burstein, E. COMMD1 (Copper Metabolism MURR1 Domain-Containing Protein 1) Regulates Cullin RING Ligases by Preventing CAND1 (Cullin-Associated Nedd8-Dissociated Protein 1) Binding. J. Biol. Chem. 2011, 286 (37), 32355–32365. https://doi.org/10.1074/jbc.M111.278408.

(215) Li, W.; Bengtson, M. H.; Ulbrich, A.; Matsuda, A.; Reddy, V. A.; Orth, A.; Chanda, S. K.; Batalov, S.; Joazeiro, C. A. P. Genome-Wide and Functional Annotation of Human E3 Ubiquitin Ligases Identifies MULAN, a Mitochondrial E3 That Regulates the Organelle's Dynamics and Signaling. PloS One 2008, 3 (1), e1487. https://doi.org/10.1371/journal.pone.0001487.

(216) Szulc, N. A.; Pokrzywa, W. Cullin-RING Receptors in Rare Disease Biology. Trends Cell Biol. 2026, S0962-8924(26)00003-6. https://doi.org/10.1016/j.tcb.2026.01.003.

(217) Chua, N. K.; González-Robles, T. J.; Reddington, C. J.; Dudley-Fraser, J.; Birkinshaw, R. W.; Han, J.; Solano, A.; Wong, S. W.; Kochańczyk, T.; Peter, J. J.; Nakasone, M. A.; Aust, F.; Munro, J.; Tong, Y. H.; Iskander, J.; Abeysekera, W.; Garnham, A.; Huckstep, H.; Ritchie, M. E.; Wertz, I.; Hymowitz, S.; Kumar, S.; Conaway, R. C.; Privé, G. G.; Bullock, A. N.; Babon, J. J.; Klevit, R. E.; Lorenz, S.; Ciulli, A.; Fischer, E. S.; Thomä, N. H.; Nowak, R. P.; Schulman, B. A.; Rapé, M.; Rittinger, K.; Pagan, J. K.; Bahlo, M.; Mackay, J. P.; Mace, P. D.; Lima, C. D.; Hay, R. T.; Komander, D.; Lechtenberg, B. C.; Joazeiro, C. A. P.; Pagano, M.; Hofmann, K.; Feltham, R. The E3-Ome Gene-Centric Compendium Reveals the Human E3 Ligase Landscape. Cell 2026, S0092-8674(26)00116-9. https://doi.org/10.1016/j.cell.2026.01.029.

(218) Huttlin, E. L.; Bruckner, R. J.; Paulo, J. A.; Cannon, J. R.; Ting, L.; Baltier, K.; Colby, G.; Gebreab, F.; Gygi, M. P.; Parzen, H.; Szpyt, J.; Tam, S.; Zarraga, G.; Pontano-Vaites, L.; Swarup, S.; White, A. E.; Schweppe, D. K.; Rad, R.; Erickson, B. K.; Obar, R. A.; Guruharsha, K. G.; Li, K.; Artavanis-Tsakonas, S.; Gygi, S. P.; Harper, J. W. Architecture of the Human Interactome Defines Protein Communities and Disease Networks. Nature 2017, 545 (7655), 505–509. https://doi.org/10.1038/nature22366.

(219) Abramson, J.; Adler, J.; Dunger, J.; Evans, R.;

Green, T.; Pritzel, A.; Ronneberger, O.; Willmore, L.; Ballard, A. J.; Bambrick, J.; Bodenstein, S. W.; Evans, D. A.; Hung, C.-C.; O'Neill, M.; Reiman, D.; Tunyasuvunakool, K.; Wu, Z.; Žemgulytė, A.; Arvaniti, E.; Beattie, C.; Bertolli, O.; Bridgland, A.; Cherepanov, A.; Congreve, M.; Cowen-Rivers, A. I.; Cowie, A.; Figurnov, M.; Fuchs, F. B.; Gladman, H.; Jain, R.; Khan, Y. A.; Low, C. M. R.; Perlin, K.; Potapenko, A.; Savy, P.; Singh, S.; Stecula, A.; Thillaisundaram, A.; Tong, C.; Yakneen, S.; Zhong, E. D.; Zielinski, M.; Žídek, A.; Bapst, V.; Kohli, P.; Jaderberg, M.; Hassabis, D.; Jumper, J. M. Accurate Structure Prediction of Biomolecular Interactions with AlphaFold 3. Nature 2024, 630 (8016), 493–500. https://doi.org/10.1038/s41586-024-07487-w.

(220) Grau-Bové, X.; Sebé-Pedrós, A.; Ruiz-Trillo, I. The Eukaryotic Ancestor Had a Complex Ubiquitin Signaling System of Archaeal Origin. Mol. Biol. Evol. 2015, 32 (3), 726–739. https://doi.org/10.1093/molbev/msu334.

(221) Bhowmick, P.; Pancsa, R.; Guharoy, M.; Tompa, P. Functional Diversity and Structural Disorder in the Human Ubiquitination Pathway. PloS One 2013, 8 (5), e65443. https://doi.org/10.1371/journal.pone.0065443.

(222) Medvar, B.; Raghuram, V.; Pisitkun, T.; Sarkar, A.; Knepper, M. A. Comprehensive Database of Human E3 Ubiquitin Ligases: Application to Aquaporin-2 Regulation. Physiol. Genomics 2016, 48 (7), 502–512. https://doi.org/10.1152/physiolgenomics.00031.2016.

(223) Zhou, J.; Xu, Y.; Lin, S.; Guo, Y.; Deng, W.; Zhang, Y.; Guo, A.; Xue, Y. iUUCD 2.0: An Update with Rich Annotations for Ubiquitin and Ubiquitin-like Conjugations. Nucleic Acids Res. 2018, 46 (D1), D447–D453. https://doi.org/10.1093/nar/gkx1041.

(224) Gao, D.; Wan, L.; Inuzuka, H.; Berg, A. H.; Tseng, A.; Zhai, B.; Shaik, S.; Bennett, E.; Tron, A. E.; Gasser, J. A.; Lau, A.; Gygi, S. P.; Harper, J. W.; DeCaprio, J. A.; Toker, A.; Wei, W. Rictor Forms a Complex with Cullin-1 to Promote SGK1 Ubiquitination and Destruction. Mol. Cell 2010, 39 (5), 797–808. https://doi.org/10.1016/j.molcel.2010.08.016.

(225) Liu, L.; Damerell, D. R.; Koukouflis, L.; Tong, Y.; Marsden, B. D.; Schapira, M. UbiHub: A Data Hub for the Explorers of Ubiquitination Pathways. Bioinforma. Oxf. Engl. 2019, 35 (16), 2882–2884. https://doi.org/10.1093/bioinformatics/bty1067.

(226) Dutta, A.; Cristiani, A.; Nikte, S. V.; Eisert, J.; Matthess, Y.; Markusic, B.; Tudose, C.; Becht, C.; Cetin, R.; Shah, V. J.; Mosler, T.; Husnjak, K.; Dikic, I.; Kaulich, M.; Bhaskara, R. M. Multi-Scale Classification Decodes the Complexity of the Human E3 Ligome. Nat. Commun. 2025, 16 (1), 11382. https://doi.org/10.1038/s41467-025-67450-9.

(227) Ageta, H.; Shimada, Y.; Nagaoka, T.; Takenaka, K.; Yoshioka, Y.; Konno, K.; Amemiya, R.; Nagase, K.; Hitachi, K.; Onouchi, T.; Watanabe, M.; Ochiya, T.; Tsuchida, K. Statins Attenuate PD-L1 Sorting to Small Extracellular Vesicles Dependent on Ubiquitin-like 3 Modification. Sci. Rep. 2025, 15 (1), 43802. https://doi.org/10.1038/s41598-025-27789-x.