Ubiquitin Ligases: guardians of mammalian development

David A. Cruz Walma[1,2], Zhuoyao Chen[2], Alex N. Bullock[2] and Kenneth M. Yamada[1]

[1] Cell Biology Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA

[2] Centre for Medicines Discovery, University of Oxford, Oxford, UK

e-mail(s): david.cruzwalma@ndm.ox.ac.uk, kenneth.yamada@nih.gov

[H1] Abstract
Mammalian development demands precision. Millions of molecules must be properly located in temporal order, and their function regulated, to orchestrate important steps in cell cycle progression, apoptosis, migration and differentiation, to shape developing embryos. Ubiquitin and its associated enzymes act as cellular guardians to ensure precise spatio-temporal control of key molecules during each of these important cellular processes. Loss of precision results in numerous examples of embryological disorders or even cancer. This Review discusses the crucial roles of E3 ubiquitin ligases during key steps of early mammalian development, their roles in human disease, and considers how new methods to manipulate and exploit the ubiquitin regulatory machinery — for example the development of molecular glues and PROTACs — might facilitate clinical therapy.

[H1] Introduction
Life is written in code: from genetic codes to post-translational barcodes, a dynamic, complex network of peptides, nucleotides, and other small molecules translates microenvironmental cues to cellular responses. Cells then undergo growth, migration, division, differentiation or death. Intracellular communication encoded by the highly conserved 76-amino acid ubiquitin molecule[1,2] is a crucial regulator of the many intricate steps of embryonic development. Classically, the modification of proteins by ubiquitin is mediated by enzymes that catalyse ubiquitin activation (E1s), conjugation (E2s) and ligation to protein targets (E3s), as well as deubiquitinating enzymes (DUBs), which remove ubiquitin molecules and chains from the targets[2-4]. E3 ubiquitin ligases determine the precise substrate specificity of ubiquitination. Accordingly, altered E3 activity, and resulting changes to the proteolytic ubiquitin-proteasome system (UPS), protein quality control, protein trafficking, and other ubiquitin-driven pathways, affects fundamental biological processes, several of which are linked to diverse human diseases, elucidating their important physiological roles.

E3 ligases play essential roles in the intricate cell signaling networks that direct embryonic development, which involve growth factors[5], Hh (Hedgehog)[6], Wnt/β-catenin[7], Cyclins–Cdks (cyclin dependent kinases)[8], retinoic acid[9] and many other key molecules. Through these pathways, the ubiquitin code orchestrates cell cycle progression, differentiation, apoptosis, and migration to shape the maturing embryo. Defects in E3 function and ubiquitination can have devastating effects on development. Although recent excellent reviews have highlighted the important roles of ubiquitination in cancer and stem cell biology[10,11], less attention has been paid to the many ways in which ubiquitin ligases govern embryogenesis.

In this review, we describe the key steps of human development that are under the control of E3 ubiquitin ligases. After briefly introducing the molecular machinery of ubiquitination (**Figure 1**), we review the crucial roles of E3 ubiquitin ligases in key processes of the different stages in early mammalian embryonic development. We begin with gametogenesis, proceed through pre-, peri-, and post-implantation embryogenesis, and culminate with organogenesis (**Figures 2, 3, 4, Supplementary table 1**). A variety of E3 pathogenic mutations and variants are known to cause human congenital disorders, many of which are highlighted in our text with the remainder summarized in Supplementary table 2. Even though there are clear associations between E3 ligase mutations and human disease, the underlying molecular mechanisms tying genotype to phenotype are often poorly understood, particularly when non-degradative ubiquitination is

involved. For therapy, ubiquitin-based research has often focused on understanding and harnessing ubiquitin signaling in cancer and for cancer treatments. We thus discuss the current knowledge of E3s in oncogenesis and cancer progression with a focus on mechanistic overlaps between cancer and embryologic disorders. This increasing knowledge of the ubiquitin code and recent development of drugs to target ubiquitination may ultimately translate into innovative therapeutic strategies for human developmental diseases and disorders.

[H1] E3 classes, specificity and catalytic mechanisms

The multistep process of ubiquitination is governed by E1, E2, and E3 enzymes that sequentially activate, conjugate, and ligate ubiquitin to protein substrate targets. With greater than 600 E3 ligases encoded by the human genome[12], the structural diversity of E3s far surpasses that of E1 and E2 enzymes (recent bioinformatics analyses suggest humans express 8 E1s, 41 E2s and 634 E3s[12]).

E3 ligases are categorized into three classes according to their E2 binding domain structure and ubiquitin transfer mechanism. The RING E3s form the largest class and mediate direct transfer of ubiquitin from the charged E2 to the target substrate (**Figure 1**). Of the RING E3s, the Cullin-RING ligases (CRLs) are the largest subfamily, representing almost half of human E3s and responsible for nearly 20% of all cellular ubiquitination[12]. In general, CRLs consist of four components: the core Cullin protein that serves as a scaffold for the complex, a RING finger protein responsible for binding to the E2 enzyme, a substrate receptor capable of recognizing molecular targets, and adaptor proteins that link substrate receptors to the Cullin[13] (**Figure 1**). In contrast, HECT (homologous to the E6-associated protein carboxyl domain) and RBR (RING-between-RING) E3-promoted ubiquitination is a two-step process that first involves the transfer of ubiquitin from an E2 to a reactive cysteine on the C-terminus of HECT E3s or the second RING domain of RBR E3s, creating a thioester intermediate, before transfer from the E3 to the substrate (**Figure 1**)[14]. In addition to these classical ubiquitin transfer mechanisms, a novel E3 mechanism called RING-Cys-relay (RCR) was described for MYCBP2, an E3 involved in axon integrity in neurodevelopment[15,16].

Mechanisms governing E3 substrate specificity vary and often reflect the main functions of E3s in either cellular signaling regulation or protein quality control[17]. E3s involved in protein quality control must target a variety of atypical substrates with relatively low intracellular abundance. Accordingly, many of these quality control E3s have adapted to localize to defined intracellular locations where they ubiquitinate nearby targets[17]. Key quality control E3s include Listerin, an E3 that localizes to the 60S subunit of stalled ribosomes to mediate ubiquitination and degradation of truncated polypeptides[17] as well as the E3s CUL2-KLHDC10 and RCHY1 which target and degrade alanine-tailed products on stalled ribosomes[18]. In contrast, E3s involved in cell signaling cascades target a narrower spectrum of substrates and contain interaction domains that can either bind targets directly or indirectly through an additional interacting protein or non-protein molecule (e.g., the endoplasmic reticulum associated CRL SCF/Fbs1 binding high-mannose oligosaccharides to ubiquitinate N-linked glycoproteins[19,20]. The requirement for additional interacting proteins is exemplified by the RING ligases, which use not only interchangeable substrate-binding proteins (e.g., CRL proteins) to recruit distinct substrates, but can also create E3-E3 super-assemblies with RBR E3 ligases to ubiquitinate diverse substrate lysines that are sterically incompatible with conventional E2-Cullin-RING assembly[20].

The multifunctionality of E3 ligases stems from their ability to add a variety of different ubiquitin-based structures to target substrates, thereby generating a ubiquitin code that can in turn be recognized by different effector proteins. Substrates can be modified with ubiquitin monomers at one (monoubiquitination) or multiple loci (multi-monoubiquitination)[21]. Alternatively, ubiquitin can be conjugated to substrates as structurally distinct polyubiquitin chains formed from isopeptide bonds between the carboxy terminus of one ubiquitin monomer and the α-amino group of the N-terminal methionine (M1) or the ε-amino group of one of the seven internal lysine residues (K6, K11, K27, K29, K33, K48, or K63) of an adjacent ubiquitin monomer[22]. Compared to monoubiquitination, polyubiquitin chains exert more varied downstream signaling effects dependent on linkage type[22,23]. The functional roles of homotypic linkages (links formed from the same ubiquitin acceptor site (e.g., K63 chains)) are better characterized than their heterotypic counterparts (links formed from different ubiquitin acceptor sites (e.g., K48/K63 chains)). Homotypic linkages are known

to regulate DNA damage repair (K6, K27, K33, and K63), endocytosis (K63), protein trafficking (K33), proteasomal protein degradation (K48), Wnt/beta-catenin signaling (K29), NF-kB signaling (M1 and K63), and the innate immune response (M1, K33, and K63), among others[24]. Emerging functions of heterotypic chains include branched K48/K63, K29/48, and K11/48 linkages serving as degradation signals[25,26] as well as branched K48/63 and M1/K63 chains activating NF-kB signaling[23]. Most research on E3 ligase function in development has focused on the functions of ubiquitin linkages to serve as degradation signals. However, the critical roles of polyubiquitin chains and non-degradative ubiquitination in development are beginning to be recognized.

Recent advances in genome and mRNA sequencing have identified several mammalian-specific gene families. These lineage-specific genes are revealing evolutionary adaptation and innovation among specific species. At present, few ubiquitin-signaling related proteins are known to be mammalian specific. One such example is DCAF16, a protein that interacts with CUL4[27]. As highlighted throughout this review, several CUL4–DCAF complexes regulate critical events in embryogenesis (e.g., CUL4-DCAF13 regulating oogenesis and zygotic gene expression, CUL4-DCAF2 regulating zygotic divisions, and others). Although DCAF16's biological functions are poorly characterized, it can be targeted specifically by a small-molecule binder (see section below on PROTACs) to modulate the degradation of nuclear proteins[28]. Such small molecule binders can be used to further probe DCAF16's biological functions. As more inter-species comparisons are reported, some ubiquitin-related protein specificity may emerge as selective targets or evolutionary drivers.

[H1] Regulation of E3 activity

Because E3 ligases regulate nearly every cellular process, their activity must be modulated or checked. Numerous mechanisms, including post-translational modifications, binding of protein partners, alteration in subcellular localizations, and interactions with small molecules, have emerged as means to inhibit, activate, and/or enhance E3 activity[29].

[H2] Post-translational modifications of E3s.

Our understanding of how the ubiquitin code is written has been expanded by the discovery that E3 ligase function is regulated by a variety of post-translational modifications (PTMs) including phosphorylation, neddylation, acetylation, deamidation, and sumoylation[30]. Such modifications are crucial during embryogenesis, as they allow cells to respond rapidly to endogenous and environmental cues by modifying E3 ligase activity, localization, substrate interaction and half-life. Consequently, human developmental diseases can result from mutations not only in E3 ligases, but also in components of their regulatory machinery.

Prominent examples of this regulation in embryonic development involve the E3 ligase MDM2 and its substrate p53. As discussed below, the MDM2–p53 axis coordinates several events throughout post-implantation embryogenesis including neurulation and organogenesis, and human mutations in MDM2 are linked to developmental disorders. Mirroring its widespread involvement in embryogenesis, a host of post-translational modifications control MDM2 stability (phosphorylation[31]), substrate affinity (acetylation[32]), activity (sumoylation[33]), and abundance (autoubiquitination and ubiquitination by several E3s including MARCH7, XIAP, and others[34-37]. Equally important are the phosphatases, deacetylases, and deubiquitinases that reverse these modifications to stabilize MDM2, thus lowering cellular p53 levels[38,32,39]. Indeed, recent studies link human genetic mutations in deubiquitinases to congenital/developmental disorders[40]. As another example of such modifications regulating E3 activity in development, the RING E3 ligase c-CBL is a critical regulator of RAS signaling in development. Phosphorylation activates and stabilizes c-CBL, creating an enzyme with near 1000-fold higher catalytic efficiency than its non-phosphorylated counterpart[41]. Phosphorylation-induced c-CBL activity is critical for human development, as exemplified by loss-of-function mutations in c-CBL causing a Noonan-syndrome like disorder with clinical features including facial dysmorphism, cardiac disease, cognitive deficiency, and various musculoskeletal defects (OMIM 613563).

[H2] Adaptor proteins, co-factors and small molecules.
Beyond post-translational modifications, the binding of specific adapter proteins, co-factors, and small molecules adds another layer of regulation to E3 ligase activity. There are both common and unique regulatory proteins associated with the three major E3 ligase classes. All classes require the binding of an E2-Ub conjugate to successfully ubiquitinate substrates. Examples of additional regulatory interactions include: modulation of HECT E3 catalytic activity by HECT domain oligomerization plus substrate proteins and calcium ions[42] and regulation of RING and RBR catalytic activities by dimerization, substrate interactions, and protein complex formation[43]. It is evident that numerous checks and balances are in place to fine-tune the regulation of E3 ligase expression and activity levels[44]. The ability of E3 ligases to be auto-ubiquitinated and/or ubiquitinated by other E3s for degradation by the proteasome is a well-known and characterized method of regulating E3 expression[44]. However, how upstream transcription factors are regulated to control E3 expression in development is poorly understood and requires further investigation. Recent reports show E3 expression can be induced by various stressors (e.g., nutrient starvation[45]). With E3 ligase expression altered in several pathologies[10], a more comprehensive understanding of the players involved in maintaining intracellular E3 levels will be a crucial step forward in understanding and eventually treating human disease.

The large number of E3 ligases that can be regulated by many intramolecular covalent modifications and intermolecular interactions, as well as their many target proteins, can explain why E3s play such crucial roles in each of the key stages of embryonic development. Of note, their importance and indispensable roles in development makes many E3s difficult to characterize, with homozygous knockout models commonly resulting in embryonic lethality (e.g., CUL1, CUL3, CUL4B, LTN1, and several others[46,47,48,49,17]), as discussed below.

[H1] Gametogenesis
Gametes – eggs and sperm – play critical roles in genetic transmission, diversity and evolution. Errors in gamete formation (gametogenesis) can lead to failed fertilization, miscarriage, or a variety of developmental defects[50-52]. Understandably, a number of quality control mechanisms, many of which involve ubiquitin signaling, regulate the two successive meiotic divisions and series of morphologic events fundamental to gamete maturation (**Figure 2**)[53-56].

[H2] Targeting Histones and DNA-binding proteins.
E3 ligases can be the crucial factors in mammalian fertility by altering germ cell chromatin condensation, alignment, and/or crossover. In humans, histone ubiquitination has both direct physical effects on higher-order chromatin structure, such as promoting chromatin opening and activation, and indirect effects, such as recruiting additional proteins and signaling successive histone modifications[57]. For example, the E3 ligase RYBP ubiquitinates histone H2A to signal subsequent trimethylation of histone H3 to regulate transcriptional activity[57]. Reductions in this RYBP-mediated H2A ubiquitination diminishes global histone H3K9me3 levels in spermatocytes, resulting in increased apoptosis and failed meiosis, potentially through transcriptional repression. Similar changes in spermatocyte meiosis are observed when another E3 ligase, Ring Finger 2 (RNF2), ubiquitinates histone H2A during spermatogenesis[58]. Recently, the initially narrow focus on ubiquitination of histone H2A and H2B has expanded to include analyses of histones H1, H3, and H4 and their roles in DNA double-strand break repair and other critical biological processes (**Supplementary table 1**). Developing female gametes are similarly rigorously regulated by the E3 ligases. For example, CUL4-DCAF13 plays several critical roles during oocyte maturation. Not only does CUL4-DCAF13 bind the core box C/D ribonucleoprotein, fibrillarin, to ensure proper chromatin configuration and follicle development[59], but the E3 ligase also ubiquitinates PTEN phosphatases for degradation, thus controlling the activation of CDK1/Cyclin B1 during oocyte meiosis. Loss of CUL4-DCAF13 prevents oocyte meiosis, arrests follicle development, and results in female infertility[60].

Several RNF E3 ligases stand out as key regulators of gametogenesis. They target not only histones, but also TGF-signaling repressors and several other key proteins. Mutations in the human E3s RNF216 and RNF12 result in Gordon Holmes Syndrome (GDHS) and X-linked intellectual disability (XLID), respectively, two

diseases with abnormally small male testes and lowered sex hormone levels (hypogonadism and hypogonadotropic hypogonadism)[61-65] (**Supplementary table 2**). Although RNF ligase mutations are linked to these clinical observations, the molecular mechanisms connecting the mutations to GDHS remain elusive. Nevertheless, combining a recent report implicating non-degradative K63-linked ubiquitination[66] with reports of *Rnf216* mutation locations in the DNA-binding zinc finger domains of RNF216[64] suggest altered RNF216-histone/DNA interactions may link the RNF216 mutations to the observed pathogenicity. Several other E3 ligases play critical roles in ensuring genome stability of developing sperm and eggs as listed in Supplementary table 1.

[H2] Targeting cyclin E in spermatogenesis.
Interestingly, even though E-type cyclins are reportedly non-essential for most of mammalian embryogenesis, they are critical for the development of sperm and the placenta[67,68]. Cyclin E2 serves a unique role in spermatogenesis, with cyclin E2 knockout male mice suffering a 4-fold reduction in sperm count and a 50% infertility rate[68]. Spermatogonial stem cell self-renewal is diminished when cyclin E levels (a critical driver of cell-cycle re-entry from the G_0 state[67]) are too low[54,69]. Through targeting cyclin E and other proteins for ubiquitination and degradation, the E3 ligase complex CUL1-FBXW7 is a critical regulator of spermatogonial stem cell self-renewal[54,70-72].

[H2] Regulating function through structure.
Further highlighting the unique roles of ubiquitin ligases in male gametogenesis, certain E3 ligases maintain structural integrity of developing spermatozoa, including MARCH7, MARCH10, and CUL4[73-76] (**Figure 2**) (**Supplementary table 1**). The roles of CUL4B, the E3-ubiquitin ligase implicated in human Brooks-type X-linked intellectual disability syndromes (XLIDS)[77-80], in spermatogenesis have gained particular interest. CUL4B has been reported to have both cell autonomous and non-autonomous roles. Global knockout of CUL4B results in male mouse infertility due to failed maintenance of the spermatogonial stem cell niche[74]. In contrast, conditional knockout of CUL4B in male germ cells results in male infertility secondary to failed ubiquitination and degradation of the CUL4B substrate insulin-like peptide INSL6, leading to defective insulin-dependent flagellar architecture and deficient mitochondrial ATP production[74,75].

[H1] From fertilization to implantation.
During fertilization, the male and female sperm and egg unite in the fallopian tube to form a single diploid cell, the zygote. As the zygote migrates towards the uterus, cells divide and differentiate to form a multicellular two-layered structure called the blastocyst. The blastocyst contains an inner cell mass that will develop into the embryo and an outer layer of cells called trophoblasts that will differentiate, embed into the lining of the uterus, and form the placenta[81] (**Figures 2 and 3**).

[H2] Activating the embryonic genome.
The zygote relies on maternally provided molecules until the embryonic genome is activated in a series of events known as the "maternal-to-zygotic-transition" (MZT). The MZT starts at fertilization and continues until all maternal mRNAs are degraded and replaced by newly synthesized embryonic molecules[82-84]. Should maternal factors fail to be degraded, zygotic genome activation fails, and embryonic development ceases[85]. After ensuring formation and union of functionally competent male and female sex cells, E3 ligases ensure that maternal proteins are degraded and the embryonic genome takes over the reins. For example, E3 ligase CUL4 with its adaptor DCAF13 polyubiquitinates the methylase SUV39H1 for degradation, thus preventing trimethylation and facilitating removal of histone H3, thereby enabling zygotic gene expression[86]. Conditional knockout of maternal DCAF13 results in transcriptionally inactive embryos[87]. Although research to date has focused heavily on the proteolytic activities of E3 ligases in the MZT (**Supplementary table 1**), it will be intriguing to explore the roles of non-degradative ubiquitination during this transition.

[H2] Establishing the fetus-mother connection.

Upon exiting the fallopian tube, trophoblast cells of the embryo must differentiate into migratory and invasive extravillous cytotrophoblasts capable of degrading the extracellular matrix, invading the lining of the uterus, and breaching uterine arteries to establish the fetal-maternal circulation[88] (**Figures 2 and 3).** Defects in trophoblast migration and invasion result in pre-eclampsia **[G]**, intrauterine growth restriction (IUGR) **[G]**, recurrent spontaneous abortion, gestational trophoblastic disease, and even maternal mortality[88-90]. The timing and depth of this trophoblast invasion is determined by the balance of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) at the fetus-uterus interface[88]. CUL1, CUL4, and SMURF2 tightly control this balance through regulating MMP inhibitor protein abundance[88,91-93]. If any of these E3s fail, embryonic development fails. For example, depletion of trophoblast cell CUL1 or CUL4A elevates levels of TIMP1/2, thereby disrupting trophoblast cell migration and invasion[88,91]. Interestingly, CUL1 can target multiple substrates to exert the same effects on trophoblast MMP expression. Besides regulating TIMP1/2 levels, the MMP pathway inhibitor cryptochrome 2 has recently been added to the list of CUL1 targets for ubiquitin-mediated degradation[92,94].

After degrading the surrounding matrix, trophoblast cells must break their existing cell-matrix adhesions and migrate through the uterine lining to the underlying arteries. By ubiquitinating the focal adhesion **[G]** protein LL5β for degradation, the membrane-associated E3 ubiquitin ligase complex CUL7-OBSL1-CCDC8 (also known as the 3M complex) is critical for this cell migration[90]. Mutations in *cul7*, *obsl1*, or *ccdc8*, many of which disrupt CUL7-OBSL1-CCDC8 formation and/or localization to the cell membrane, result in the intrauterine growth restriction developmental disorders of 3M syndrome and Yakuts short stature syndrome[90,95-97]. *Cul7* mutations account for roughly 65% of 3M syndrome cases (*obsl1* and *ccdc8* mutations account for the other 30% and 5%, respectively[98]), and inhibited trophoblast migration secondary to such mutations likely plays a role in the impaired placental development observed in these syndromes[90,98,99].

[H1] Gastrulation and neurulation

Gastrulation and neurulation are two remarkably complex choreographed, four-dimensional processes with extensive hierarchies of regulatory mechanisms. During gastrulation, an originally relatively simple and unorganized sphere of cells reorganizes into a polarized embryo with discrete ectodermal, mesodermal, and endodermal germ layers[100] (**Figure 2**). During neurulation, a flat sheet of ectoderm morphs into the rudiment of the mature adult nervous system, the neural tube (**Figures 2, 3, 4, 5**)[101]. This complex reorganization requires major cell/tissue movements alongside the coordinated differentiation of germ layer and neural precursors - processes tightly regulated by E3 ligases. Should such tight regulation fail, the result can be embryonic lethality or birth defects associated with gastrulation (e.g., caudal dysgenesis and sirenomelia[102]) or defective neurulation (e.g., spina bifida, anencephaly, and other neural tube defects[103]).

[H2] Extraembryonic tissues driving embryogenesis.

Throughout gastrulation, neurulation, and at later developmental stages, the extraembryonic maternal placenta provides critical structural and biochemical support to the developing embryo. CUL4B, HECTD1, CUL3, and HUWE1 (MULE) are only a few of the key E3 ligase components essential for embryogenesis due to their roles in extraembryonic tissues (**Supplementary tables 1 and 2**). For example, CUL4B targets p21 and cyclin E for ubiquitin-mediated degradation. Extraembryonic silencing of CUL4B elevates levels of p21 and cyclin E, resulting in cell-cycle arrest, growth arrest, apoptosis of extraembryonic tissues, and embryonic lethality[48,104]. A similar outcome of intrauterine growth restriction and embryonic lethality occurs from dysregulated placental-HECTD1-mediated Hsp90 localization, CUL3-mediated cyclin E degradation, and MULE-mediated Mcl-1 and p53 degradation[47,105-107]. In fact, altered MULE expression is characteristic of human pre-eclampsia and intrauterine growth restriction placentae[105]. Additional E3 ligases critical in maintaining placental health throughout embryogenesis are listed in **Supplementary table 1**.

[H2] Establishing the embryonic midline.

Establishing the midline **[G]** during gastrulation is fundamental for creating bilateral symmetry in the developing embryo and is crucial for neurulation and organogenesis. The TRIM E3 ligase family helps to define this midline. Loss-of-function mutations in the B-box of TRIM18 (also known as MID1) are linked to

the midline birth defects of X-linked Opitz G Syndrome (XLOS) (**Supplementary table 2**). In non-pathologic conditions, TRIM18 ubiquitinates both PP2A (protein phosphatase 2A) and alpha 4. Ubiquitinated alpha4 protects PP2A from TRIM18-mediated ubiquitination and degradation. Mutant P151L TRIM18 cannot ubiquitinate alpha4 and instead ubiquitinates non-protected PP2A molecules for degradation, resulting in the pathogenic features of this syndrome such as cleft lip/palate, hypertelorism, and other midline defects[108-110] (**Supplementary tables 1 and 2**). The structural and molecular mechanisms of TRIM18-mutant associated XLOS are relatively well characterized and highlight the importance of non-proteolytic E3 ligase activity. Since PP2A regulates nearly every major cell cycle pathway, multiple human developmental disease-linked mutations impair E3-mediated control of PP2A activity, though their mechanisms need elucidation (e.g., HECT E3 ligase UBE3A mutations in Angelman's syndrome and UBE3B mutations in Kaufman oculocerebrofacial syndrome[111-113])(**Supplementary table 2**). Supplementary table 1 provides additional details about the various E3 ligases that help define the embryonic midline.

[H2] Closing the neural tube.
In most vertebrates, the anterior and posterior regions of the neural tube form by distinct mechanisms termed primary and secondary neurulation, respectively. In the anterior region, a flat plane of cells folds to create two elevated sheets that converge into a hollow tube as the opposing folds fuse at the midline (**Figure 3**). In contrast, the posterior neural tube forms from a cranio-caudally oriented rod of condensed cells that cavitates under the ectodermal sheet to form a hollow tube[95]. Tightly controlled gradients of retinoic acid (RA) and its receptor (RAR) ensure spatiotemporal coordination of these intricate cellular events. Subtypes of the RA receptor have distinct expression profiles in open versus closed neural tubes, with RARγ heavily expressed in the former and RARβ in the latter[114,115]. If these processes go awry, anterior or posterior regions of the neural tube fail to close (causing anencephaly and spina bifida, respectively), which are two of the most common congenital defects[95].

Several E3 ligases help to establish and maintain these gradients by controlling RAR stability through selective ubiquitination. MDM2 and HECTD1 ubiquitinate RARα[116,117] and FBXO30 ubiquitinates RARγ[118] to promote their proteasomal degradation (**Supplementary table 1**). All three of these proteins are critical during neural tube closure, and insufficient E3 ligase activity results in serious downstream regulatory effects[118-120]. When FBXO30 fails to ubiquitinate RARγ for degradation (as observed in human neural tube defect (NTD) embryos and NTD mouse models) abnormally elevated RA/RAR-activity represses BMP-regulated neural progenitor cell differentiation and proliferation[118]. Retinoic acid signaling gradients control transcription of cytoskeletal, kinetochore, and other proteins involved in several signaling pathways (e.g., BMP, Wnt, FGF) to spatiotemporally regulate neural tube closure (**Figure 3**).

Apart from RA–RAR signaling, several E3 ligases (e.g., TRIM36, MID1, and MID2) maintain the intricate balance between cell differentiation, proliferation, and migration throughout neurogenesis and beyond (**Supplementary table 1**)(**Figure 3**). As an example of the detrimental effects of altered E3 ligase function during neurulation, homozygous missense mutations in microtubule-associated E3 ligase TRIM36 cause anencephaly (an embryonic-lethal condition caused by failed closure of the anterior neuropore of the neural tube resulting in failed cerebrum and cerebellum formation), highlighting the importance of this E3 ligase in regulating spindle assembly, chromosome alignment, and cytokinesis during neural cell cycle progression and proliferation[121-123] (**Supplementary table 2**). In fact, many additional E3s cooperate to coordinate retinoic acid signaling, microtubule stability, cytoskeletal dynamics, and other critical cellular events throughout neurulation and organogenesis **(Supplementary table 1**).

[H1] Organogenesis.
Beyond these roles in early embryonic development, ubiquitin signaling is important during morphogenesis of the various tissues derived from the initial three germ layers. Below, we describe some of the best-studied roles of E3 ligases during organogenesis. These roles include signal transduction, maintenance of genome stability and gene expression regulation, which all contribute to maintaining the structural integrity of cells and tissues. We highlight new, interesting results that begin to explain the molecular basis of E3-promoted

human developmental diseases and underscore their central roles as guardians of development. As will be evident, even though E3 mutations are clearly implicated in a variety of developmental disorders, very little is known about the molecular basis of these E3 ligase-associated diseases. The advent of high-throughput expression profiling coupled with gene editing tools has produced useful databases correlating E3 ligase expression with the development of various embryonic tissues in mice[124]. Due to various barriers (for example ethical concerns and accessibility of human tissue samples), we are far from being able to profile human embryonic tissues. Nevertheless, lessons learned from human developmental disease states coupled with insights provided by animal models have allowed us to identify tissue-specific E3 ligases (e.g., RNF183 in the kidneys and testis, RNF186 in the lower GI tract and kidneys, and RNF182 in the nervous system[125]), which continue to shed light on the molecular mechanisms surrounding E3s in human development.

[H2] Genome stability, signal transduction and gene expression.

Deregulated ubiquitination can lead to aberrant, or even failed, organ development by influencing DNA damage repair processes (e.g., HUWE1 mutations in neurodevelopmental and craniofacial defects as described below[126,127], gene expression, and signal transduction (**Supplementary table 1**)). Several studies highlight the theme of E3s directing organ growth by creating spatiotemporally regulated gradients of growth factors and other signaling molecules. For example, in the developing murine lung, cycles of high and low FGF10 levels determine if lung branches continue growth (high FGF10 levels) or stop and redirect their growth (transiently lower FGF10 levels)[128]. These FGF10 gradients are created through the transcription factors ETV4 and 5 (E26 transformation-specific Translocation Variant 4 and 5) promoting Shh-mediated local inhibition of FGF10 expression. Through ubiquitinating and degrading ETV4 and ETV5, the E3 ligase RFWD2 is critical for normal lung branching, and conditional knockout of this E3 results in failed branching morphogenesis[129]. Supplementary table 1 lists additional E3s that direct organ growth by creating other molecular gradients in various tissues (e.g., NEDD4, CUL1, CUL3, TRIM67, TRIM9). However, in most of these cases, the molecular mechanisms that tie mutant E3 activity to the observed phenotypes remains to be discovered.

Recent informative examples of pathogenic E3 mutations causing human developmental disorders involve mutations in the E3 ligase HUWE1 (MULE) (**Supplementary tables 1 and 2**). These mutations result in X-linked intellectual disabilities (XLIDs) and craniofacial disorders[127,130-132]. Several *de novo* human mutations in HUWE1 lead to altered genome stability and associated neurodevelopmental and craniofacial defects. Children with HUWE1 mutations can have a variety of dysmorphic facial features, short stature with small hands and feet, and severe difficulties learning or even speaking[127,131]. Using XLID patient-derived cells, Bosshard et al., determined that an overactive HUWE1 mutant decreases DNA repair in response to oxidative stress through lowering DNA polymerase λ activity[126]. Because the developing brain is particularly susceptible to oxygenation status, these results may provide initial insights into the molecular basis of HUWE1-associated developmental disorders.

[H2] Cellular functions affecting tissue structure.

The final structure of developing organs is determined by cell-to-extracellular matrix and cell-to-cell interactions alongside a series of changes in cellular abundance, shape, and position[133]. E3 ligases can drive these changes by regulating cell-cell fusion (e.g., CUL3-KCTD10 in muscle cell fusion[134]), extracellular matrix degradation (e.g., RNF31-SHARPIN in mammary glands[135]), cell migration (e.g., CUL3-BACURD1/2 and cortical neuron migration[136-138]), epithelial-mesenchymal transition (e.g., SMURF2 in mammary glands[139]), cytoskeletal/structural molecule stability (e.g., CUL3 regulating neuronal cytoskeletal dynamics and Rho signaling[140] and CUL3-Kelch proteins in skeletal muscle[141]**),** and cell number (e.g., NEDD4 in the craniofacial complex[142], CUL4 in the heart[143], APC/C and MDM2 in the brain and lungs[144,145])(**Figure 2, 4, 5**).

Mutations in the CUL3 substrate adaptors KBTBD13, KLHL40, and KLHL41 cause various congenital neuromuscular-development disorders (myopathies) with clinical features including muscle weakness, difficulties feeding and swallowing, breathing problems, and joint deformities[146-149](**Supplementary table 2**)(**Figure 2, 5**). Although clear associations of these mutations with

myopathies were identified nearly a decade ago[150], the molecular basis for the CUL3-Kelch-promoted disorder has only recently emerged with most studies focusing on essential proteolytic roles affected by the E3 mutations [141]. Mechanistic studies reveal CUL3-KLHL40 and CUL3-KLHL41 play both proteolytic and non-proteolytic roles in myogenesis. CUL3-KLHL41 ubiquitinates the muscle thin-filament chaperone NRAP for degradation[141]. In contrast, both CUL3-KLHL40 and CUL3-KLHL41 stabilize the scaffold protein NEB (nebulin) and thin filament protein LMOD3 (leiomodin 3) by either blocking ubiquitination and promoting proper protein folding (CUL3-KLHL40 with NEB and LMOD3) or preventing their aggregation and subsequent degradation (CUL3-KLHL41 with NEB)[151,152] to ensure establishment and maintenance of proper muscle structure and function (**Supplementary table 1**)(**Figure 5**). While it is satisfying to learn molecular targets through which CUL3-KLHL40 and CUL3-KLHL41 ensure muscle function, the specific molecular details (e.g., E3-substrates, binding domains/degrons, ubiquitin linkage types, etc.) remain ambiguous and need to be determined in these myopathy patients. Adding to the challenge, each pathogenic mutation in the three proteins may act through distinct molecular mechanisms. For example, elevated levels of non-muscle alpha-actinins ACTN1 and ACTN4 are uniquely observed in the fine thread/rod-like structures (nemaline bodies) causing muscle dysfunction in myopathies associated with mutant CUL3-KBTBD13[153]. Mechanistic studies using mouse models have highlighted the overall importance of CUL3 mediated degradation of ACTN1 for muscle development, but the specific pathogenic mechanisms resulting from KBTBD13 mutation in myopathy remain to be determined[153]. Conceptually, as exemplified by CUL3-KLHL40 and CUL3-KLHL41 stabilizing NEB and LMOD3, it will be critical to take into consideration non-proteolytic E3 functions besides degradative roles as we continue to identify the intermediary steps between the initial genetic mutations and the ultimate human phenotypic observations.

A similar lack of mechanistic details underlying pathogenic E3 mutations is exemplified by recent descriptions of a microcephalic boy carrying a heterozygous *de novo* mutation in the Cdh1/Fzr gene encoding the APC/C E3 ligase adapter protein Cdh1[144] (**Supplementary table 2**). Though details remain elusive, preliminary functional characterizations using patient-derived cell cultures suggest that mutant APC/C-Cdh1 E3s may alter human brain size by regulating neural progenitor cell populations[144]. This hypothesis is supported by a prior report in which mutated Cdh1 abrogates the ability of APC/C to promote neural progenitor cell-cycle exit and neuronal differentiation through an APC/C-Cdh1-Skp2-p27 signaling axis[154].

E3 ligases contribute to determining the size of mature organs through regulating cell abundance. For example, the E3 ligase MDM2 regulates levels of its primary substrate p53 to maintain progenitor cell populations in the developing murine lung[145], inner ear[155], pancreas[156] and kidneys[157] (**Supplementary table 1**). Most genomic studies of the MDM2-p53 pathway have focused on cancer, with only recent identification of pathogenic germline mutations in MDM2 resulting in developmental disorders (e.g., autosomal recessive progeroid syndrome resulting in short stature, small kidneys, pinched facial features, and premature aging[158] (**Supplementary table 2**). Although we are still far from a comprehensive understanding of the pathogenicity of mutant MDM2, such insights will not only improve our understanding of human development, but also cancer progression because more than half of human cancers exhibit polymorphisms and mutations in the MDM2-p53 pathway[159,160].

[H1] E3s in cancer progression and suppression

As post-translational modification by ubiquitination is second only to phosphorylation in terms of prevalence[161], E3s have the potential to modify the localization, activity, interactions, and/or abundance of nearly every cellular protein. With such influence on the proteome, it is no surprise that dysregulated E3 activities have far-reaching effects in cancer in addition to roles in congenital disease. Mutant E3 ligases can hijack similar molecular pathways in both cancer and developmental disease to drive altered tissue growth and maturation. We highlight overlapping roles of ubiquitin E3 ligase function in cancer and embryogenesis, focusing on where they play a part in cell fate decisions: influencing whether cells proliferate, differentiate, migrate/invade or undergo programmed cell death. Comprehensive reviews on the broader topic of E3s in cancer can be found in other recent reviews[10,162]. Since most E3-based targeted drug discovery centers on

cancer treatment[123], highlighting this overlap with development will set the stage for our final section on exploiting E3s for targeted therapeutic protein degradation.

[H2] Genome stability: Proliferation, differentiation, or death

Signals controlling proliferation, differentiation, and coordinated cell death are commonly dysregulated in cancer. E3s serve as tumor suppressors through coordinating the activity of key molecules involved in cell-cycle progression. Examples of important roles of E3s balancing cell proliferation and apoptosis in cancer include regulation of cyclin E and/or c-Myc in breast, neural, gastric, and hematologic cancers by FBXW7[163-167], transcriptional activation of c-Myc in colon cancer by HUWE1[168,169], stress response-related NRF2 abundance in lung and other cancers by CUL3-KEAP1[170-172], and regulation of NRF1-mediated coordinated cell death in breast cancer by SIAH2[173]. Exemplifying the overlap of E3 function in development and cancer, disruptions in cyclin E/c-Myc-dependent cell-cycle progression, proliferation, and apoptosis resulting from altered FBXW7 function is observed in both primary cancers[166] and spermatogenesis[54].

This same overlap between development and tumorigenesis is observed with the MDM2-p53 axis regulating cell proliferation and apoptosis to promote both tumor growth and, as reviewed earlier, normal neural tube closure and growth of organs including lung, pancreas, and kidneys[119,174,175] (**Supplementary table 1**). Recent results focusing on p53-promoted oncogenesis reveal yet another mechanism through which MDM2 may contribute to cancer progression; p53 can target master regulators of the epithelial-to-mesenchymal transition (EMT) through miRNA-dependent mechanisms[176-178], suggesting a link to this major developmental and cancer-associated process.

[H2] Regulation of EMT: cell migration and invasion.

Epithelial–mesenchymal transition **[G]** (EMT) is renowned for its roles in cancer cell migration, invasion and metastasis[179], yet it is also a well-known process driving embryonic development. For example, the same or similar changes in transcription factors (e.g., SNAIL1/2, TWIST1, ZEB1[180,181]), enzymes (e.g., MMPs[182]), cell surface receptors (e.g., various growth factor receptors[183]), and cell-cell/cell-ECM junction/structural protein levels (e.g., E-cadherin, N-cadherin, actin, collagen[184]) that drive tumor cell migration and invasion are also critical in vertebrate embryonic development (e.g., for implantation of the embryo in the uterus[91,92] and migration by neural crest, neural, and other progenitor cells[136,185,186]). In fact, some argue the reactivation of these and similar developmental processes comprise the core of cancer progression, with neoplastic cells introducing only minor changes to normal EMT programs[187]. Through mutating and exploiting upstream E3 ligases instead of downstream substrates/signaling molecules, neoplastic cells take advantage of the far-reaching influence of E3s to maximize changes in cell migration and invasion with relatively minimal effort.

The ability to commandeer E3s to promote invasion and metastasis is exemplified by cancer cell exploitation of CUL7 to invade and metastasize in esophageal[188], breast[189], glial[190], liver[191] and several other types of cancer[192]. As discussed in “Fertilization to implantation,” CUL7’s role in promoting the EMT, invasion, and migration of trophoblast cells during implantation is critical to establishing fetal-maternal circulation to support embryo development[90]. Indeed, several reports hypothesize mutant-CUL7-OBSL1-CCDC8-promoted changes in trophoblast cell migration and invasion are at the core of the severe pre- and post-natal developmental growth disorders 3M syndrome and Yakut short stature syndrome, as described earlier in this review[90,96,193,194]. Epitomizing this overlap between congenital disease and cancer, both patients with 3M syndrome and cell samples obtained from Ewing Sarcoma patients exhibit CUL7 frameshift mutations at Valine 1484[195-197].

Thus, it is clear that E3s can influence critical steps of embryogenesis and cancer through similar molecular mechanisms. Research seeking to redirect protein degradation by modifying E3 ligase activity, affinity and/or substrate specificity has historically centered on cancer therapeutics[123]. Indeed, the top selling oncology product of 2017 was the ‘molecular glue’ compound lenalidomide, which acts as an anti-proliferative, anti-angiogenic and immunomodulatory drug through promoting novel CRBN E3 ligase-substrate interactions[198]. Moreover, several phase I clinical trials are underway that involve a modified E3 ligase targeting the

androgen receptor in prostate cancer and estrogen receptor in breast cancer[199]. Nevertheless, these same techniques could potentially be applied to modify developmental processes for possible future clinical application to alleviate congenital and developmental disorders.

[H1] Exploiting E3s for targeted protein degradation

The realization that ubiquitin-dependent pathways impact virtually every aspect of cell biology has motivated the development of molecules capable of inhibiting, activating, and/or modulating the ubiquitin pathway machinery as both research and therapeutic tools. Successful examples include the combined use of the proteasome inhibitor bortezomib and the E3-targeted immunomodulatory drugs thalidomide **[G]** and lenalidomide, which revolutionized multiple myeloma treatment[200]. Since then, ubiquitin-pathway-modifying small molecules have expanded to include, among several others: peptides that neutralize E1 enzymatic activity[201]; drug-like molecules that stabilize, enhance, inhibit, and/or provide new E3–protein substrate interactions (such as 'molecular glues', proteolysis targeting chimeras (PROTACs), specific and non-genetic inhibitors of apoptosis (IAP)-dependent protein erasers (SNIPERs), and immunomodulatory drugs[202-205]); and ubiquitin variants that modulate activities of a variety of ubiquitin-pathway protein interactions[206]. We summarize common methods to exploit E3 ligases as therapeutic and biological discovery tools, highlight the most promising E3 ligases used successfully in the clinic, and discuss the significance of these tools in developmental biology.

[H2] Strategies exploiting E3 ligases.

Modulation of the ubiquitin-proteasome system (UPS) presents a new opportunity to control the stability of disease-linked proteins for therapeutic effect. Ongoing projects in clinical and pre-clinical development suggest tractability for E3 drug development, as well as for a variety of intervention strategies.

The inhibition of E3 ligases presents a potential strategy to address congenital conditions involving haploinsufficiency or defective upstream/downstream signaling pathways by stabilizing target proteins from degradation. E3 inhibitors in current use have three modes of action (**Figure 6A**). The most common mechanism is represented by the small molecule inhibitors TAME and Apcin, which disrupt substrate recruitment by the APC/C-CDC20 ligase through competitive binding[207]. Combination treatments using both compounds in cancer models has proven more potent than using either alone for blocking mitotic exit to induce cell death[207]. Inhibitors have also been developed to target the catalytic sites of E3 ligases. A covalent molecule binding to the N-lobe ubiquitin-binding site of the HECT E3 NEDD4-1 can inhibit its processive polyubiquitination activity[208]. Similarly, an engineered ubiquitin variant with specificity for the ubiquitin-binding site of APC2, a subunit of the APC/C complex, inhibits K48-linked ubiquitin chain elongation by the APC/C complex[209]. The use of such molecules has often enabled the dissection of novel E3 enzyme mechanisms[208-210], highlighting their value as research tools in addition to any therapeutic potential. Antagonists of cIAP illustrate a third inhibitory mechanism – 'self-destruction.' These molecules stabilize cIAP conformations that promote RING domain dimerization and auto-ubiquitination, resulting in the degradation of cIAP and stabilization of cIAP substrates[211].

The ubiquitin-proteasome system can also be harnessed to destabilize targeted proteins (**Figure 6B**). PROTACs (proteolysis targeting chimeras) are bifunctional small molecules with an E3-binding moiety, a targeted protein-binding moiety and a connecting linker. Molecular glues work in a similar manner to PROTACs, but lack obvious bifunctionality by the absence of a chemical linker. PROTACs and molecular glues are a novel modality to target previously undruggable proteins, including the transcription factors that play important roles in development. For example, thalidomide and its derivatives recruit zinc finger protein Ikaros (IKZF1) to CUL4-CRBN for ubiquitination-mediated degradation to treat myeloma[212]. However, these therapeutics carry safety risks in causing severe birth defects by recruiting the teratogenicity target Sal-like protein 4 (SALL4) to CUL4-CRBN in a similar manner (**Figure 6B**), suggesting a need for structure-based design of safer thalidomide derivatives to improve target selectivity[202]. The progress on small molecule degraders has been extensively reviewed recently[213]. Accordingly, we will limit our discussion of these molecules to the overall theme of mammalian development.

Several studies utilizing a CRISPR-based phenotypic screen coupled to chemoproteomics successfully deconvoluted E3 targets for PROTACs/molecular glues[214-216]. This approach not only expands degrader molecule discovery to the whole ubiquitome, but also profiles the degraders in specific cell contexts most relevant to their activity and raises the possibility of achieving tissue specificity by harnessing specific cell/biological contexts. Through the choice of E3s, PROTACs/molecular glues can enable tissue-specific or stage-specific chemical knock-down during development. Moreover, the targeting of different E3s also offers the potential for inducing different types of ubiquitination to modulate not only the stability, but also the localization, activity or trafficking of target proteins, further expanding the application of these chemical tools in developmental biology research.

[H2] Bench to bedside: clinical application.
The proteasome has been the most successful therapeutic target for clinical exploitation amongst ubiquitin-proteasome pathway components. Several FDA-approved proteasome inhibitors are used to treat cancers including leukemias, non-small-cell lung cancer, pancreatic cancer, hepatocellular carcinoma, multiple myeloma, mantle cell lymphoma, and others[217]. Due to various adverse off-target effects and increasing resistance to proteasome inhibitor drugs by neoplastic cells, molecules targeting upstream components of the ubiquitin-proteasome system are being explored. In recent years, the activity of each enzyme in the E1-E2-E3 cascade has been targeted for therapeutic intervention. Concerning E3 ligases, FDA-approved thalidomide derivatives successfully redirect CUL4-CRBN-mediated degradation to the neosubstrates IKZF1, IKZF3, and ARID2 in multiple myeloma[218,219]. The FDA has also approved the use of dimethyl fumarate (Tecfidera; an activator of the CUL3-KEAP1-NRF2 antioxidant response pathway) for the treatment of relapsing multiple sclerosis[220-222].

A surge in clinical trials has occurred recently involving PROTACs recruiting von Hippel-Lindau E3 ligases to target the androgen and estrogen receptors (ARV-110 and 471)[223,224], novel CUL4-DCAF15 and KEAP1 modulators targeting RBM39 and NRF2[225-229] and novel CUL4-CRBN modulators targeting IKZ1 and IKZ3 in various cancers beyond multiple myeloma (CC-122/avadomide and CC-220/iberdomide)[230-233]. Also under clinical investigation are molecules targeting the E3 ligase MDM2 (APG-115, CGM097, RG7112 and RG7388), the E1 enzyme UBA1 (MLN7243), the deubiquitinase USP1 (Pimozide) and NEDD8 activating enzyme (MLN4924)[234-239]. Though not yet in the clinic, inhibitors/modulators of E3 ligase complexes including FBXW7, SKP2, and APC[240-243], immunomodulatory drugs repurposing CRL4-CRBN to ubiquitinate and degrade a host of zinc-finger neo-substrates[212], specific and non-genetic inhibitors of apoptosis (IAP)-dependent protein erasers (SNIPERs) exploiting IAP ubiquitin ligases[203,244], and PROTACs targeting nearly 50 substrates including protein kinases, nuclear receptors, transcription regulators, regulatory proteins, and others[245] show promise in preclinical studies.

Although E3s in embryos and their substrates are promising candidates for ubiquitin-based therapeutics (e.g., CUL3-KLHL12 and its substrates LNPK and DVL[246,247] besides others highlighted in this review), we are far from therapeutically modifying human E3 activity *in utero* due to obstacles that include organ and tissue accessibility, targeted drug delivery, and ethical concerns[248]. Nevertheless, the critical role of E3s in stem cell biology has become clear, so we are much closer to exploiting E3s to modify stem cell dynamics for tissue regeneration[11]. Armed with this knowledge and new drug modalities to commandeer E3s for targeted protein degradation, we are on the cusp of exploiting E3 ligases for stem-cell based regenerative medicine. For example, the small molecule UM171 acts in a CUL3-KBTBD4 dependent manner to degrade the demethylase KDM1A, resulting in epigenetic changes to produce the expansion of hematopoietic stem cells[249]. The *ex vivo* use of UM171 is currently under clinical investigation to amplify cord blood cells for stem cell transplantation[250].

[H1] Conclusions and perspectives
The field of ubiquitin biology has expanded explosively since the seminal experiments in the 1980s linking ubiquitin to protein turnover[251-253]. With the increasing understanding of the many important roles of E3

ligases in embryonic development has come improved proficiency in exploiting E3 function to 'drug the undruggable'[254] as well as to develop novel biochemical techniques to further elucidate biological mechanisms. We have made great strides in ascribing multiple developmental processes and disorders to specific E3 ligases, but remaining questions abound: What are the direct E3 substrates? Which ubiquitin linkages are involved? How are the E3s regulated at different developmental stages and in each tissue? Is the developmental process regulated by degradative ubiquitination, non-degradative ubiquitination, or a combination of both? Which functions are dispensable and what mechanisms compensate? We have explored the commonly reported proteolytic E3 functions and have highlighted similarly critical, yet understudied, non-proteolytic functions of E3s in development. PROTACs and molecular glues offer the potential to induce different types of ubiquitin modifications and promote degradative and non-degradative ubiquitination in a spatiotemporally controlled manner. Developmental and molecular biology must continue to merge as we tackle the major challenge of using our knowledge of developmental biology and the new tools for altering E3 ligase functions to achieve practical therapeutic applications.

References

1 Hershko, A. & Ciechanover, A. The ubiquitin system. *Annu Rev Biochem* **67**, 425-479, doi:10.1146/annurev.biochem.67.1.425 (1998).

2 Komander, D. & Rape, M. The ubiquitin code. *Annu Rev Biochem* **81**, 203-229, doi:10.1146/annurev-biochem-060310-170328 (2012).

3 Clague, M. J., Urbe, S. & Komander, D. Breaking the chains: deubiquitylating enzyme specificity begets function. *Nat Rev Mol Cell Biol* **20**, 338-352, doi:10.1038/s41580-019-0099-1 (2019).

4 Pinto-Fernandez, A. *et al.* Comprehensive Landscape of Active Deubiquitinating Enzymes Profiled by Advanced Chemoproteomics. *Front Chem* **7**, 592, doi:10.3389/fchem.2019.00592 (2019).

5 Wrana, J. L. Signaling by the TGFbeta superfamily. *Cold Spring Harb Perspect Biol* **5**, a011197, doi:10.1101/cshperspect.a011197 (2013).

6 Jiang, J. & Hui, C. C. Hedgehog signaling in development and cancer. *Dev Cell* **15**, 801-812, doi:10.1016/j.devcel.2008.11.010 (2008).

7 Steinhart, Z. & Angers, S. Wnt signaling in development and tissue homeostasis. *Development* **145**, doi:10.1242/dev.146589 (2018).

8 Lim, S. & Kaldis, P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. *Development* **140**, 3079-3093, doi:10.1242/dev.091744 (2013).

9 Gouti, M. *et al.* A Gene Regulatory Network Balances Neural and Mesoderm Specification during Vertebrate Trunk Development. *Dev Cell* **41**, 243-261 e247, doi:10.1016/j.devcel.2017.04.002 (2017).

10 Deng, L., Meng, T., Chen, L., Wei, W. & Wang, P. The role of ubiquitination in tumorigenesis and targeted drug discovery. *Signal Transduct Target Ther* **5**, 11, doi:10.1038/s41392-020-0107-0 (2020).

11 Werner, A., Manford, A. G. & Rape, M. Ubiquitin-Dependent Regulation of Stem Cell Biology. *Trends Cell Biol* **27**, 568-579, doi:10.1016/j.tcb.2017.04.002 (2017).

12 Liu, L. *et al.* UbiHub: a data hub for the explorers of ubiquitination pathways. *Bioinformatics (Oxford, England)* **35**, 2882-2884, doi:10.1093/bioinformatics/bty1067 (2019).

13 Baek, K. *et al.* NEDD8 nucleates a multivalent cullin-RING-UBE2D ubiquitin ligation assembly. *Nature* **578**, 461-466, doi:10.1038/s41586-020-2000-y (2020).

14 Berndsen, C. E. & Wolberger, C. New insights into ubiquitin E3 ligase mechanism. *Nat Struct Mol Biol* **21**, 301-307, doi:10.1038/nsmb.2780 (2014).

15 Pao, K. C. *et al.* Activity-based E3 ligase profiling uncovers an E3 ligase with esterification activity. *Nature* **556**, 381-385, doi:10.1038/s41586-018-0026-1 (2018).

16 Mabbitt, P. D. *et al.* Structural basis for RING-Cys-Relay E3 ligase activity and its role in axon integrity. *Nat Chem Biol* **16**, 1227-1236, doi:10.1038/s41589-020-0598-6 (2020).

17 Joazeiro, C. A. P. Mechanisms and functions of ribosome-associated protein quality control. *Nat Rev Mol Cell Biol* **20**, 368-383, doi:10.1038/s41580-019-0118-2 (2019).

18 Thrun, A. *et al.* Convergence of mammalian RQC and C-end rule proteolytic pathways via alanine tailing. *Mol Cell* **81**, 2112-2122.e2117, doi:10.1016/j.molcel.2021.03.004 (2021).

19 Mizushima, T. *et al.* Structural basis for the selection of glycosylated substrates by SCF(Fbs1) ubiquitin ligase. *Proc Natl Acad Sci U S A* **104**, 5777-5781, doi:10.1073/pnas.0610312104 (2007).

20 Horn-Ghetko, D. *et al.* Ubiquitin ligation to F-box protein targets by SCF-RBR E3-E3 super-assembly. *Nature* **590**, 671-676, doi:10.1038/s41586-021-03197-9 (2021).

21 Yau, R. & Rape, M. The increasing complexity of the ubiquitin code. *Nat Cell Biol* **18**, 579-586, doi:10.1038/ncb3358 (2016).

22 French, M. E., Koehler, C. F. & Hunter, T. Emerging functions of branched ubiquitin chains. *Cell Discov* **7**, 6, doi:10.1038/s41421-020-00237-y (2021).

23 Ohtake, F., Saeki, Y., Ishido, S., Kanno, J. & Tanaka, K. The K48-K63 Branched Ubiquitin Chain Regulates NF-kappaB Signaling. *Mol Cell* **64**, 251-266, doi:10.1016/j.molcel.2016.09.014 (2016).

24 Akutsu, M., Dikic, I. & Bremm, A. Ubiquitin chain diversity at a glance. *Journal of cell science* **129**, 875-880, doi:10.1242/jcs.183954 (2016).

25 Ohtake, F., Tsuchiya, H., Saeki, Y. & Tanaka, K. K63 ubiquitylation triggers proteasomal degradation by seeding branched ubiquitin chains. *Proc Natl Acad Sci U S A* **115**, E1401-e1408, doi:10.1073/pnas.1716673115 (2018).

26 Meyer, H. J. & Rape, M. Enhanced protein degradation by branched ubiquitin chains. *Cell* **157**, 910-921, doi:10.1016/j.cell.2014.03.037 (2014).

27 Luis Villanueva-Cañas, J. *et al.* New Genes and Functional Innovation in Mammals. *Genome biology and evolution* **9**, 1886-1900, doi:10.1093/gbe/evx136 (2017).

28 Zhang, X., Crowley, V. M., Wucherpfennig, T. G., Dix, M. M. & Cravatt, B. F. Electrophilic PROTACs that degrade nuclear proteins by engaging DCAF16. *Nat Chem Biol* **15**, 737-746, doi:10.1038/s41589-019-0279-5 (2019).

29 Vittal, V., Stewart, M. D., Brzovic, P. S. & Klevit, R. E. Regulating the Regulators: Recent Revelations in the Control of E3 Ubiquitin Ligases. *J Biol Chem* **290**, 21244-21251, doi:10.1074/jbc.R115.675165 (2015).

30 Song, L. & Luo, Z. Q. Post-translational regulation of ubiquitin signaling. *J Cell Biol* **218**, 1776-1786, doi:10.1083/jcb.201902074 (2019).

31 Mayo, L. D. & Donner, D. B. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci U S A* **98**, 11598-11603, doi:10.1073/pnas.181181198 (2001).

32 Nihira, N. T. *et al.* Acetylation-dependent regulation of MDM2 E3 ligase activity dictates its oncogenic function. *Sci Signal* **10**, doi:10.1126/scisignal.aai8026 (2017).

33 Miyauchi, Y., Yogosawa, S., Honda, R., Nishida, T. & Yasuda, H. Sumoylation of Mdm2 by protein inhibitor of activated STAT (PIAS) and RanBP2 enzymes. *J Biol Chem* **277**, 50131-50136, doi:10.1074/jbc.M208319200 (2002).

34 Gu, L. *et al.* Discovery of Dual Inhibitors of MDM2 and XIAP for Cancer Treatment. *Cancer Cell* **30**, 623-636, doi:10.1016/j.ccell.2016.08.015 (2016).

35 Liu, X. *et al.* NAT10 regulates p53 activation through acetylating p53 at K120 and ubiquitinating Mdm2. *EMBO Rep* **17**, 349-366, doi:10.15252/embr.201540505 (2016).

36 Zhao, K. *et al.* Regulation of the Mdm2-p53 pathway by the ubiquitin E3 ligase MARCH7. *EMBO Rep* **19**, 305-319, doi:10.15252/embr.201744465 (2018).

37 Bai, J. *et al.* SCF(FBXO22) targets HDM2 for degradation and modulates breast cancer cell invasion and metastasis. *Proc Natl Acad Sci U S A* **116**, 11754-11763, doi:10.1073/pnas.1820990116 (2019).

38 Carr, M. I., Roderick, J. E., Gannon, H. S., Kelliher, M. A. & Jones, S. N. Mdm2 Phosphorylation Regulates Its Stability and Has Contrasting Effects on Oncogene and Radiation-Induced Tumorigenesis. *Cell Rep* **16**, 2618-2629, doi:10.1016/j.celrep.2016.08.014 (2016).

39 Cetkovská, K., Šustová, H. & Uldrijan, S. Ubiquitin-specific peptidase 48 regulates Mdm2 protein levels independent of its deubiquitinase activity. *Sci Rep* **7**, 43180, doi:10.1038/srep43180 (2017).

40 Basar, M. A., Beck, D. B. & Werner, A. Deubiquitylases in developmental ubiquitin signaling and congenital diseases. *Cell Death Differ* **28**, 538-556, doi:10.1038/s41418-020-00697-5 (2021).

41 Dou, H. *et al.* Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. *Nat Struct Mol Biol* **19**, 184-192, doi:10.1038/nsmb.2231 (2012).

42 Wang, J. *et al.* Calcium activates Nedd4 E3 ubiquitin ligases by releasing the C2 domain-mediated auto-inhibition. *J Biol Chem* **285**, 12279-12288, doi:10.1074/jbc.M109.086405 (2010).

43 Plechanovova, A. *et al.* Mechanism of ubiquitylation by dimeric RING ligase RNF4. *Nat Struct Mol Biol* **18**, 1052-1059, doi:10.1038/nsmb.2108 (2011).

44 Zheng, N. & Shabek, N. Ubiquitin Ligases: Structure, Function, and Regulation. *Annu Rev Biochem* **86**, 129-157, doi:10.1146/annurev-biochem-060815-014922 (2017).

45 Ossareh-Nazari, B. *et al.* Ubiquitylation by the Ltn1 E3 ligase protects 60S ribosomes from starvation-induced selective autophagy. *J Cell Biol* **204**, 909-917, doi:10.1083/jcb.201308139 (2014).

46 Dealy, M. J. *et al.* Loss of Cul1 results in early embryonic lethality and dysregulation of cyclin E. *Nat Genet* **23**, 245-248, doi:10.1038/13886 (1999).

47 Singer, J. D., Gurian-West, M., Clurman, B. & Roberts, J. M. Cullin-3 targets cyclin E for ubiquitination and controls S phase in mammalian cells. *Genes Dev* **13**, 2375-2387, doi:10.1101/gad.13.18.2375 (1999).

48 Jiang, B. *et al.* Lack of Cul4b, an E3 ubiquitin ligase component, leads to embryonic lethality and abnormal placental development. *PLoS One* **7**, e37070, doi:10.1371/journal.pone.0037070 (2012).

49 Chu, J. *et al.* A mouse forward genetics screen identifies LISTERIN as an E3 ubiquitin ligase involved in neurodegeneration. *Proc Natl Acad Sci U S A* **106**, 2097-2103, doi:10.1073/pnas.0812819106 (2009).

50 Fukami, M. *et al.* Catastrophic cellular events leading to complex chromosomal rearrangements in the germline. *Clin Genet* **91**, 653-660, doi:10.1111/cge.12928 (2017).

51 Zitzmann, M. & Rohayem, J. Gonadal dysfunction and beyond: Clinical challenges in children, adolescents, and adults with 47,XXY Klinefelter syndrome. *Am J Med Genet C Semin Med Genet* **184**, 302-312, doi:10.1002/ajmg.c.31786 (2020).

52 Hattori, A. & Fukami, M. Established and Novel Mechanisms Leading to de novo Genomic Rearrangements in the Human Germline. *Cytogenet Genome Res* **160**, 167-176, doi:10.1159/000507837 (2020).

53 Baska, K. M. *et al.* Mechanism of extracellular ubiquitination in the mammalian epididymis. *J Cell Physiol* **215**, 684-696, doi:10.1002/jcp.21349 (2008).

54 Kanatsu-Shinohara, M., Onoyama, I., Nakayama, K. I. & Shinohara, T. Skp1-Cullin-F-box (SCF)-type ubiquitin ligase FBXW7 negatively regulates spermatogonial stem cell self-renewal. *Proc Natl Acad Sci U S A* **111**, 8826-8831, doi:10.1073/pnas.1401837111 (2014).

55 Griswold, M. D. Spermatogenesis: The Commitment to Meiosis. *Physiol Rev* **96**, 1-17, doi:10.1152/physrev.00013.2015 (2016).

56 Ji, S. *et al.* Bam-dependent deubiquitinase complex can disrupt germ-line stem cell maintenance by targeting cyclin A. *Proc Natl Acad Sci U S A* **114**, 6316-6321, doi:10.1073/pnas.1619188114 (2017).

57 Tian, Q., Guo, S. M., Xie, S. M., Yin, Y. & Zhou, L. Q. Rybp orchestrates spermatogenesis via regulating meiosis and sperm motility in mice. *Cell Cycle* **19**, 1492-1501, doi:10.1080/15384101.2020.1754585 (2020).

58 Hasegawa, K. *et al.* SCML2 establishes the male germline epigenome through regulation of histone H2A ubiquitination. *Dev Cell* **32**, 574-588, doi:10.1016/j.devcel.2015.01.014 (2015).

59 Zhang, J. *et al.* Mammalian nucleolar protein DCAF13 is essential for ovarian follicle maintenance and oocyte growth by mediating rRNA processing. *Cell Death Differ* **26**, 1251-1266, doi:10.1038/s41418-018-0203-7 (2019).

60 Zhang, J. *et al.* The CRL4-DCAF13 ubiquitin E3 ligase supports oocyte meiotic resumption by targeting PTEN degradation. *Cell Mol Life Sci* **77**, 2181-2197, doi:10.1007/s00018-019-03280-5 (2020).

61 Alqwaifly, M. & Bohlega, S. Ataxia and Hypogonadotropic Hypogonadism with Intrafamilial Variability Caused by RNF216 Mutation. *Neurol Int* **8**, 6444, doi:10.4081/ni.2016.6444 (2016).

62 Bustos, F. *et al.* RNF12 X-Linked Intellectual Disability Mutations Disrupt E3 Ligase Activity and Neural Differentiation. *Cell Rep* **23**, 1599-1611, doi:10.1016/j.celrep.2018.04.022 (2018).

63 Frints, S. G. M. *et al.* Pathogenic variants in E3 ubiquitin ligase RLIM/RNF12 lead to a syndromic X-linked intellectual disability and behavior disorder. *Mol Psychiatry* **24**, 1748-1768, doi:10.1038/s41380-018-0065-x (2019).

64 Margolin, D. H. *et al.* Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. *N Engl J Med* **368**, 1992-2003, doi:10.1056/NEJMoa1215993 (2013).

65 Melnick, A. F. *et al.* RNF216 is essential for spermatogenesis and male fertilitydagger. *Biol Reprod* **100**, 1132-1134, doi:10.1093/biolre/ioz006 (2019).

66 Seenivasan, R. *et al.* Mechanism and chain specificity of RNF216/TRIAD3, the ubiquitin ligase mutated in Gordon Holmes syndrome. *Hum Mol Genet* **28**, 2862-2873, doi:10.1093/hmg/ddz098 (2019).

67 Geng, Y. *et al.* Kinase-independent function of cyclin E. *Mol Cell* **25**, 127-139, doi:10.1016/j.molcel.2006.11.029 (2007).

68 Geng, Y. *et al.* Cyclin E ablation in the mouse. *Cell* **114**, 431-443, doi:10.1016/s0092-8674(03)00645-7 (2003).

69 Atchison, F. W. & Means, A. R. Spermatogonial depletion in adult Pin1-deficient mice. *Biol Reprod* **69**, 1989-1997, doi:10.1095/biolreprod.103.020859 (2003).

70 Koepp, D. M. *et al.* Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. *Science* **294**, 173-177, doi:10.1126/science.1065203 (2001).

71 Reavie, L. *et al.* Regulation of hematopoietic stem cell differentiation by a single ubiquitin ligase-substrate complex. *Nat Immunol* **11**, 207-215, doi:10.1038/ni.1839 (2010).

72 Tetzlaff, M. T. *et al.* Defective cardiovascular development and elevated cyclin E and Notch proteins in mice lacking the Fbw7 F-box protein. *Proc Natl Acad Sci U S A* **101**, 3338-3345, doi:10.1073/pnas.0307875101 (2004).

73 Iyengar, P. V., Hirota, T., Hirose, S. & Nakamura, N. Membrane-associated RING-CH 10 (MARCH10 protein) is a microtubule-associated E3 ubiquitin ligase of the spermatid flagella. *J Biol Chem* **286**, 39082-39090, doi:10.1074/jbc.M111.256875 (2011).

74 Lin, C. Y. *et al.* Human X-linked Intellectual Disability Factor CUL4B Is Required for Post-meiotic Sperm Development and Male Fertility. *Sci Rep* **6**, 20227, doi:10.1038/srep20227 (2016).

75 Yin, Y. *et al.* Cell Autonomous and Nonautonomous Function of CUL4B in Mouse Spermatogenesis. *J Biol Chem* **291**, 6923-6935, doi:10.1074/jbc.M115.699660 (2016).

76 Zhao, B., Ito, K., Iyengar, P. V., Hirose, S. & Nakamura, N. MARCH7 E3 ubiquitin ligase is highly expressed in developing spermatids of rats and its possible involvement in head and tail formation. *Histochem Cell Biol* **139**, 447-460, doi:10.1007/s00418-012-1043-z (2013).

77 Isidor, B., Pichon, O., Baron, S., David, A. & Le Caignec, C. Deletion of the CUL4B gene in a boy with mental retardation, minor facial anomalies, short stature, hypogonadism, and ataxia. *Am J Med Genet A* **152A**, 175-180, doi:10.1002/ajmg.a.33152 (2010).

78 Kerzendorfer, C. *et al.* CUL4B-deficiency in humans: understanding the clinical consequences of impaired Cullin 4-RING E3 ubiquitin ligase function. *Mech Ageing Dev* **132**, 366-373, doi:10.1016/j.mad.2011.02.003 (2011).

79 Tarpey, P. S. *et al.* Mutations in CUL4B, which encodes a ubiquitin E3 ligase subunit, cause an X-linked mental retardation syndrome associated with aggressive outbursts, seizures, relative macrocephaly, central obesity, hypogonadism, pes cavus, and tremor. *Am J Hum Genet* **80**, 345-352, doi:10.1086/511134 (2007).

80 Zou, Y. *et al.* Mutation in CUL4B, which encodes a member of cullin-RING ubiquitin ligase complex, causes X-linked mental retardation. *Am J Hum Genet* **80**, 561-566, doi:10.1086/512489 (2007).

81 Flores, D., Madhavan, M., Wright, S. & Arora, R. Mechanical and signaling mechanisms that guide pre-implantation embryo movement. *Development* **147**, doi:10.1242/dev.193490 (2020).

82 Flach, G., Johnson, M. H., Braude, P. R., Taylor, R. A. & Bolton, V. N. The transition from maternal to embryonic control in the 2-cell mouse embryo. *EMBO J* **1**, 681-686 (1982).

83 Tadros, W. & Lipshitz, H. D. The maternal-to-zygotic transition: a play in two acts. *Development* **136**, 3033-3042, doi:10.1242/dev.033183 (2009).

84 Toralova, T., Kinterova, V., Chmelikova, E. & Kanka, J. The neglected part of early embryonic development: maternal protein degradation. *Cell Mol Life Sci* **77**, 3177-3194, doi:10.1007/s00018-020-03482-2 (2020).

85 Sha, Q. Q., Zhang, J. & Fan, H. Y. A story of birth and death: mRNA translation and clearance at the onset of maternal-to-zygotic transition in mammalsdagger. *Biol Reprod* **101**, 579-590, doi:10.1093/biolre/ioz012 (2019).

86 Zhang, Y. L. *et al.* DCAF13 promotes pluripotency by negatively regulating SUV39H1 stability during early embryonic development. *EMBO J* **37**, doi:10.15252/embj.201898981 (2018).

87 Liu, Y., Zhao, L. W., Shen, J. L., Fan, H. Y. & Jin, Y. Maternal DCAF13 Regulates Chromatin Tightness to Contribute to Embryonic Development. *Sci Rep* **9**, 6278, doi:10.1038/s41598-019-42179-w (2019).

88 Zhang, Q. *et al.* CUL1 promotes trophoblast cell invasion at the maternal-fetal interface. *Cell Death Dis* **4**, e502, doi:10.1038/cddis.2013.1 (2013).

89 Sun, X. *et al.* Abnormal Cullin1 neddylation-mediated p21 accumulation participates in the pathogenesis of recurrent spontaneous abortion by regulating trophoblast cell proliferation and differentiation. *Mol Hum Reprod* **26**, 327-339, doi:10.1093/molehr/gaaa021 (2020).

90 Wang, P. *et al.* Impaired plasma membrane localization of ubiquitin ligase complex underlies 3-M syndrome development. *J Clin Invest* **129**, 4393-4407, doi:10.1172/JCI129107 (2019).

91 Tan, D., Liang, H., Cao, K., Yi, Q. & Zhang, Q. CUL4A enhances human trophoblast migration and is associated with pre-eclampsia. *Int J Clin Exp Pathol* **10**, 10544-10551 (2017).

92 Wu, L., Liu, Q., Fan, C., Yi, X. & Cheng, B. MALAT1 recruited the E3 ubiquitin ligase FBXW7 to induce CRY2 ubiquitin-mediated degradation and participated in trophoblast migration and invasion. *J Cell Physiol* **236**, 2169-2177, doi:10.1002/jcp.30003 (2021).

93 Yang, Q. *et al.* Smurf2 participates in human trophoblast cell invasion by inhibiting TGF-beta type I receptor. *J Histochem Cytochem* **57**, 605-612, doi:10.1369/jhc.2009.953166 (2009).

94 Wu, L., Cheng, B., Liu, Q., Jiang, P. & Yang, J. CRY2 suppresses trophoblast migration and invasion in recurrent spontaneous abortion. *J Biochem* **167**, 79-87, doi:10.1093/jb/mvz076 (2020).

95 Kandasamy, V. *et al.* A Study on The Incidence of Neural Tube Defects in A Tertiary Care Hospital Over A Period of Five Years. *J Clin Diagn Res* **9**, QC01-04, doi:10.7860/JCDR/2015/14815.6190 (2015).

96 Maksimova, N. *et al.* Clinical, molecular and histopathological features of short stature syndrome with novel CUL7 mutation in Yakuts: new population isolate in Asia. *J Med Genet* **44**, 772-778, doi:10.1136/jmg.2007.051979 (2007).

97 Clayton, P. E. *et al.* Exploring the spectrum of 3-M syndrome, a primordial short stature disorder of disrupted ubiquitination. *Clin Endocrinol (Oxf)* **77**, 335-342, doi:10.1111/j.1365-2265.2012.04428.x (2012).

98 Takatani, T., Shiohama, T., Takatani, R. & Shimojo, N. A novel CUL7 mutation in a Japanese patient with 3M syndrome. *Hum Genome Var* **5**, 30, doi:10.1038/s41439-018-0029-3 (2018).

99 Hu, L. *et al.* Identification of two CUL7 variants in two Chinese families with 3-M syndrome by whole-exome sequencing. *J Clin Lab Anal* **34**, e23265, doi:10.1002/jcla.23265 (2020).

100 Dupont, S. *et al.* Germ-layer specification and control of cell growth by Ectodermin, a Smad4 ubiquitin ligase. *Cell* **121**, 87-99, doi:10.1016/j.cell.2005.01.033 (2005).

101 Werner, J. M. *et al.* Hallmarks of primary neurulation are conserved in the zebrafish forebrain. *Commun Biol* **4**, 147, doi:10.1038/s42003-021-01655-8 (2021).

102 Ferrer-Vaquer, A. & Hadjantonakis, A. K. Birth defects associated with perturbations in preimplantation, gastrulation, and axis extension: from conjoined twinning to caudal dysgenesis. *Wiley Interdiscip Rev Dev Biol* **2**, 427-442, doi:10.1002/wdev.97 (2013).

103 Greene, N. D. & Copp, A. J. Neural tube defects. *Annu Rev Neurosci* **37**, 221-242, doi:10.1146/annurev-neuro-062012-170354 (2014).

104 Liu, L. *et al.* Essential role of the CUL4B ubiquitin ligase in extra-embryonic tissue development during mouse embryogenesis. *Cell Res* **22**, 1258-1269, doi:10.1038/cr.2012.48 (2012).

105 Rolfo, A., Garcia, J., Todros, T., Post, M. & Caniggia, I. The double life of MULE in preeclamptic and IUGR placentae. *Cell Death Dis* **3**, e305, doi:10.1038/cddis.2012.44 (2012).

106 Sarkar, A. A. *et al.* Hectd1 is required for development of the junctional zone of the placenta. *Dev Biol* **392**, 368-380, doi:10.1016/j.ydbio.2014.05.007 (2014).

107 Sarkar, A. A., Sabatino, J. A., Sugrue, K. F. & Zohn, I. E. Abnormal labyrinthine zone in the Hectd1-null placenta. *Placenta* **38**, 16-23, doi:10.1016/j.placenta.2015.12.002 (2016).

108 Nicholson, L. K. Mechanism of midline defect-causing mutation P151L in MID1 revealed. *FEBS J* **284**, 2167-2169, doi:10.1111/febs.14149 (2017).

109 Trockenbacher, A. *et al.* MID1, mutated in Opitz syndrome, encodes an ubiquitin ligase that targets phosphatase 2A for degradation. *Nat Genet* **29**, 287-294, doi:10.1038/ng762 (2001).

110 Wright, K. M., Du, H. & Massiah, M. A. Structural and functional observations of the P151L MID1 mutation reveal alpha4 plays a significant role in X-linked Opitz Syndrome. *FEBS J* **284**, 2183-2193, doi:10.1111/febs.14121 (2017).

111 Buiting, K., Williams, C. & Horsthemke, B. Angelman syndrome - insights into a rare neurogenetic disorder. *Nat Rev Neurol* **12**, 584-593, doi:10.1038/nrneurol.2016.133 (2016).

112 Wang, J. *et al.* UBE3A-mediated PTPA ubiquitination and degradation regulate PP2A activity and dendritic spine morphology. *Proc Natl Acad Sci U S A* **116**, 12500-12505, doi:10.1073/pnas.1820131116 (2019).

113 Wlodarchak, N. & Xing, Y. PP2A as a master regulator of the cell cycle. *Crit Rev Biochem Mol Biol* **51**, 162-184, doi:10.3109/10409238.2016.1143913 (2016).

114 Chen, W. H., Morriss-Kay, G. M. & Copp, A. J. Genesis and prevention of spinal neural tube defects in the curly tail mutant mouse: involvement of retinoic acid and its nuclear receptors RAR-beta and RAR-gamma. *Development* **121**, 681-691 (1995).

115 Lara-Ramirez, R., Zieger, E. & Schubert, M. Retinoic acid signaling in spinal cord development. *Int J Biochem Cell Biol* **45**, 1302-1313, doi:10.1016/j.biocel.2013.04.002 (2013).

116 Ying, M. *et al.* The E3 ubiquitin protein ligase MDM2 dictates all-trans retinoic acid-induced osteoblastic differentiation of osteosarcoma cells by modulating the degradation of RARalpha. *Oncogene* **35**, 4358-4367, doi:10.1038/onc.2015.503 (2016).

117 Sugrue, K. F., Sarkar, A. A., Leatherbury, L. & Zohn, I. E. The ubiquitin ligase HECTD1 promotes retinoic acid signaling required for development of the aortic arch. *Dis Model Mech* **12**, doi:10.1242/dmm.036491 (2019).

118 Cheng, X. *et al.* F-box protein FBXO30 mediates retinoic acid receptor gamma ubiquitination and regulates BMP signaling in neural tube defects. *Cell Death Dis* **10**, 551, doi:10.1038/s41419-019-1783-y (2019).

119 Li, H., Zhang, J. & Niswander, L. Zinc deficiency causes neural tube defects through attenuation of p53 ubiquitylation. *Development* **145**, doi:10.1242/dev.169797 (2018).

120 Zohn, I. E., Anderson, K. V. & Niswander, L. The Hectd1 ubiquitin ligase is required for development of the head mesenchyme and neural tube closure. *Dev Biol* **306**, 208-221, doi:10.1016/j.ydbio.2007.03.018 (2007).

121 Miyajima, N., Maruyama, S., Nonomura, K. & Hatakeyama, S. TRIM36 interacts with the kinetochore protein CENP-H and delays cell cycle progression. *Biochem Biophys Res Commun* **381**, 383-387, doi:10.1016/j.bbrc.2009.02.059 (2009).

122 Singh, N. *et al.* A homozygous mutation in TRIM36 causes autosomal recessive anencephaly in an Indian family. *Hum Mol Genet* **26**, 1104-1114, doi:10.1093/hmg/ddx020 (2017).

123 Schapira, M., Calabrese, M. F., Bullock, A. N. & Crews, C. M. Targeted protein degradation: expanding the toolbox. *Nat Rev Drug Discov* **18**, 949-963, doi:10.1038/s41573-019-0047-y (2019).

124 Baldarelli, R. M. *et al.* The mouse Gene Expression Database (GXD): 2021 update. *Nucleic acids research* **49**, D924-d931, doi:10.1093/nar/gkaa914 (2021).

125 Okamoto, T., Imaizumi, K. & Kaneko, M. The Role of Tissue-Specific Ubiquitin Ligases, RNF183, RNF186, RNF182 and RNF152, in Disease and Biological Function. *International journal of molecular sciences* **21**, doi:10.3390/ijms21113921 (2020).

126 Bosshard, M. *et al.* Impaired oxidative stress response characterizes HUWE1-promoted X-linked intellectual disability. *Sci Rep* **7**, 15050, doi:10.1038/s41598-017-15380-y (2017).

127 Muthusamy, B. *et al.* Exome sequencing reveals a novel splice site variant in HUWE1 gene in patients with suspected Say-Meyer syndrome. *Eur J Med Genet* **63**, 103635, doi:10.1016/j.ejmg.2019.02.007 (2020).

128 Herriges, J. C. *et al.* FGF-Regulated ETV Transcription Factors Control FGF-SHH Feedback Loop in Lung Branching. *Dev Cell* **35**, 322-332, doi:10.1016/j.devcel.2015.10.006 (2015).

129 Zhang, Y. *et al.* E3 ubiquitin ligase RFWD2 controls lung branching through protein-level regulation of ETV transcription factors. *Proc Natl Acad Sci U S A* **113**, 7557-7562, doi:10.1073/pnas.1603310113 (2016).

130 Friez, M. J. *et al.* HUWE1 mutations in Juberg-Marsidi and Brooks syndromes: the results of an X-chromosome exome sequencing study. *BMJ Open* **6**, e009537, doi:10.1136/bmjopen-2015-009537 (2016).

131 Moortgat, S. *et al.* HUWE1 variants cause dominant X-linked intellectual disability: a clinical study of 21 patients. *Eur J Hum Genet* **26**, 64-74, doi:10.1038/s41431-017-0038-6 (2018).

132 Taylor, J. C. *et al.* Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nat Genet* **47**, 717-726, doi:10.1038/ng.3304 (2015).

133 Walma, D. A. C. & Yamada, K. M. The extracellular matrix in development. *Development* **147**, doi:10.1242/dev.175596 (2020).

134 Rodriguez-Perez, F. *et al.* Ubiquitin-dependent remodeling of the actin cytoskeleton drives cell fusion. *Dev Cell* **56**, 588-601 e589, doi:10.1016/j.devcel.2021.01.016 (2021).

135 Peuhu, E. *et al.* SHARPIN regulates collagen architecture and ductal outgrowth in the developing mouse mammary gland. *EMBO J* **36**, 165-182, doi:10.15252/embj.201694387 (2017).

136 Gladwyn-Ng, I. *et al.* Bacurd1/Kctd13 and Bacurd2/Tnfaip1 are interacting partners to Rnd proteins which influence the long-term positioning and dendritic maturation of cerebral cortical neurons. *Neural Dev* **11**, 7, doi:10.1186/s13064-016-0062-1 (2016).

137 Gladwyn-Ng, I. E. *et al.* Bacurd2 is a novel interacting partner to Rnd2 which controls radial migration within the developing mammalian cerebral cortex. *Neural Dev* **10**, 9, doi:10.1186/s13064-015-0032-z (2015).

138 Lin, G. N. *et al.* Spatiotemporal 16p11.2 protein network implicates cortical late mid-fetal brain development and KCTD13-Cul3-RhoA pathway in psychiatric diseases. *Neuron* **85**, 742-754, doi:10.1016/j.neuron.2015.01.010 (2015).

139 Chandhoke, A. S. *et al.* The ubiquitin ligase Smurf2 suppresses TGFbeta-induced epithelial-mesenchymal transition in a sumoylation-regulated manner. *Cell Death Differ* **23**, 876-888, doi:10.1038/cdd.2015.152 (2016).

140 Amar, M. *et al.* Autism-linked Cullin3 germline haploinsufficiency impacts cytoskeletal dynamics and cortical neurogenesis through RhoA signaling. *Mol Psychiatry*, doi:10.1038/s41380-021-01052-x (2021).

141 Jirka, C., Pak, J. H., Grosgogeat, C. A., Marchetii, M. M. & Gupta, V. A. Dysregulation of NRAP degradation by KLHL41 contributes to pathophysiology in nemaline myopathy. *Hum Mol Genet* **28**, 2549-2560, doi:10.1093/hmg/ddz078 (2019).

142 Wiszniak, S., Harvey, N. & Schwarz, Q. Cell autonomous roles of Nedd4 in craniofacial bone formation. *Dev Biol* **410**, 98-107, doi:10.1016/j.ydbio.2015.12.001 (2016).

143 Zhao, X. *et al.* Zebrafish cul4a, but not cul4b, modulates cardiac and forelimb development by upregulating tbx5a expression. *Hum Mol Genet* **24**, 853-864, doi:10.1093/hmg/ddu503 (2015).

144 Rodriguez, C. *et al.* A novel human Cdh1 mutation impairs anaphase promoting complex/cyclosome activity resulting in microcephaly, psychomotor retardation, and epilepsy. *J Neurochem* **151**, 103-115, doi:10.1111/jnc.14828 (2019).

145 Sui, P. *et al.* E3 ubiquitin ligase MDM2 acts through p53 to control respiratory progenitor cell number and lung size. *Development* **146**, doi:10.1242/dev.179820 (2019).

146 Garibaldi, M. *et al.* Core-rod myopathy due to a novel mutation in BTB/POZ domain of KBTBD13 manifesting as late onset LGMD. *Acta neuropathologica communications* **6**, 94, doi:10.1186/s40478-018-0595-0 (2018).

147 Gupta, V. A. *et al.* Identification of KLHL41 Mutations Implicates BTB-Kelch-Mediated Ubiquitination as an Alternate Pathway to Myofibrillar Disruption in Nemaline Myopathy. *Am J Hum Genet* **93**, 1108-1117, doi:10.1016/j.ajhg.2013.10.020 (2013).

148 Ravenscroft, G. *et al.* Mutations in KLHL40 are a frequent cause of severe autosomal-recessive nemaline myopathy. *Am J Hum Genet* **93**, 6-18, doi:10.1016/j.ajhg.2013.05.004 (2013).

149 Sambuughin, N. *et al.* Dominant mutations in KBTBD13, a member of the BTB/Kelch family, cause nemaline myopathy with cores. *Am J Hum Genet* **87**, 842-847, doi:10.1016/j.ajhg.2010.10.020 (2010).

150 Gupta, V. A. & Beggs, A. H. Kelch proteins: emerging roles in skeletal muscle development and diseases. *Skeletal muscle* **4**, 11, doi:10.1186/2044-5040-4-11 (2014).

151 Garg, A. *et al.* KLHL40 deficiency destabilizes thin filament proteins and promotes nemaline myopathy. *J Clin Invest* **124**, 3529-3539, doi:10.1172/jci74994 (2014).

152 Ramirez-Martinez, A. *et al.* KLHL41 stabilizes skeletal muscle sarcomeres by nonproteolytic ubiquitination. *eLife* **6**, doi:10.7554/eLife.26439 (2017).

153 Blondelle, J. *et al.* Cullin-3 dependent deregulation of ACTN1 represents a new pathogenic mechanism in nemaline myopathy. *JCI insight* **5**, doi:10.1172/jci.insight.125665 (2019).

154 Delgado-Esteban, M., Garcia-Higuera, I., Maestre, C., Moreno, S. & Almeida, A. APC/C-Cdh1 coordinates neurogenesis and cortical size during development. *Nat Commun* **4**, 2879, doi:10.1038/ncomms3879 (2013).

155 Laos, M., Sulg, M., Herranen, A., Anttonen, T. & Pirvola, U. Indispensable role of Mdm2/p53 interaction during the embryonic and postnatal inner ear development. *Sci Rep* **7**, 42216, doi:10.1038/srep42216 (2017).

156 Zhang, Y., Zeng, S. X., Hao, Q. & Lu, H. Monitoring p53 by MDM2 and MDMX is required for endocrine pancreas development and function in a spatio-temporal manner. *Dev Biol* **423**, 34-45, doi:10.1016/j.ydbio.2017.01.014 (2017).

157 Hilliard, S. A., Yao, X. & El-Dahr, S. S. Mdm2 is required for maintenance of the nephrogenic niche. *Dev Biol* **387**, 1-14, doi:10.1016/j.ydbio.2014.01.009 (2014).

158 Lessel, D. *et al.* Dysfunction of the MDM2/p53 axis is linked to premature aging. *J Clin Invest* **127**, 3598-3608, doi:10.1172/JCI92171 (2017).

159 Levine, A. J. & Oren, M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer* **9**, 749-758, doi:10.1038/nrc2723 (2009).

160 Albrechtsen, N. *et al.* Maintenance of genomic integrity by p53: complementary roles for activated and non-activated p53. *Oncogene* **18**, 7706-7717, doi:10.1038/sj.onc.1202952 (1999).

161 Duan, G. & Walther, D. The roles of post-translational modifications in the context of protein interaction networks. *PLoS Comput Biol* **11**, e1004049, doi:10.1371/journal.pcbi.1004049 (2015).

162 Dang, F., Nie, L. & Wei, W. Ubiquitin signaling in cell cycle control and tumorigenesis. *Cell Death Differ* **28**, 427-438, doi:10.1038/s41418-020-00648-0 (2021).

163 Kim, H. S. *et al.* Gliomagenesis arising from Pten- and Ink4a/Arf-deficient neural progenitor cells is mediated by the p53-Fbxw7/Cdc4 pathway, which controls c-Myc. *Cancer Res* **72**, 6065-6075, doi:10.1158/0008-5472.CAN-12-2594 (2012).

164 Li, M. R. *et al.* FBXW7 expression is associated with prognosis and chemotherapeutic outcome in Chinese patients with gastric adenocarcinoma. *BMC Gastroenterol* **17**, 60, doi:10.1186/s12876-017-0616-7 (2017).

165 Lin, J. *et al.* FBW7 is associated with prognosis, inhibits malignancies and enhances temozolomide sensitivity in glioblastoma cells. *Cancer Sci* **109**, 1001-1011, doi:10.1111/cas.13528 (2018).

166 Meyer, A. E., Furumo, Q., Stelloh, C., Minella, A. C. & Rao, S. Loss of Fbxw7 triggers mammary tumorigenesis associated with E2F/c-Myc activation and Trp53 mutation. *Neoplasia* **22**, 644-658, doi:10.1016/j.neo.2020.07.001 (2020).

167 Valliyammai, N., Nancy, N. K., Sagar, T. G. & Rajkumar, T. Study of NOTCH1 and FBXW7 Mutations and Its Prognostic Significance in South Indian T-Cell Acute Lymphoblastic Leukemia. *J Pediatr Hematol Oncol* **40**, e1-e8, doi:10.1097/MPH.0000000000001006 (2018).

168 Adhikary, S. *et al.* The ubiquitin ligase HectH9 regulates transcriptional activation by Myc and is essential for tumor cell proliferation. *Cell* **123**, 409-421, doi:10.1016/j.cell.2005.08.016 (2005).

169 Myant, K. B. *et al.* HUWE1 is a critical colonic tumour suppressor gene that prevents MYC signalling, DNA damage accumulation and tumour initiation. *EMBO Mol Med* **9**, 181-197, doi:10.15252/emmm.201606684 (2017).

170 Drainas, A. P. *et al.* Genome-wide Screens Implicate Loss of Cullin Ring Ligase 3 in Persistent Proliferation and Genome Instability in TP53-Deficient Cells. *Cell Rep* **31**, 107465, doi:10.1016/j.celrep.2020.03.029 (2020).

171 Ohta, T. *et al.* Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. *Cancer Res* **68**, 1303-1309, doi:10.1158/0008-5472.CAN-07-5003 (2008).

172 Taguchi, K., Motohashi, H. & Yamamoto, M. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. *Genes Cells* **16**, 123-140, doi:10.1111/j.1365-2443.2010.01473.x (2011).

173 Ma, B. *et al.* The SIAH2-NRF1 axis spatially regulates tumor microenvironment remodeling for tumor progression. *Nat Commun* **10**, 1034, doi:10.1038/s41467-019-08618-y (2019).

174 Li, Y. *et al.* WDR74 modulates melanoma tumorigenesis and metastasis through the RPL5-MDM2-p53 pathway. *Oncogene* **39**, 2741-2755, doi:10.1038/s41388-020-1179-6 (2020).

175 Liu, S., Tackmann, N. R., Yang, J. & Zhang, Y. Disruption of the RP-MDM2-p53 pathway accelerates APC loss-induced colorectal tumorigenesis. *Oncogene* **36**, 1374-1383, doi:10.1038/onc.2016.301 (2017).

176 Chang, C. J. *et al.* p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. *Nat Cell Biol* **13**, 317-323, doi:10.1038/ncb2173 (2011).

177 Parfenyev, S. *et al.* Interplay between p53 and non-coding RNAs in the regulation of EMT in breast cancer. *Cell Death Dis* **12**, 17, doi:10.1038/s41419-020-03327-7 (2021).

178 Sun, L. *et al.* KLF5 regulates epithelial-mesenchymal transition of liver cancer cells in the context of p53 loss through miR-192 targeting of ZEB2. *Cell Adh Migr* **14**, 182-194, doi:10.1080/19336918.2020.1826216 (2020).

179 Ribatti, D., Tamma, R. & Annese, T. Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. *Transl Oncol* **13**, 100773, doi:10.1016/j.tranon.2020.100773 (2020).

180 Barrallo-Gimeno, A. & Nieto, M. A. The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* **132**, 3151-3161, doi:10.1242/dev.01907 (2005).

181 Yang, J. *et al.* Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* **117**, 927-939, doi:10.1016/j.cell.2004.06.006 (2004).

182 Akhtar, N. Hijacking a Morphogenesis Proteinase for Cancer Cell Invasion. *Dev Cell* **47**, 135-137, doi:10.1016/j.devcel.2018.10.007 (2018).

183 Shao, G. *et al.* The E3 ubiquitin ligase NEDD4 mediates cell migration signaling of EGFR in lung cancer cells. *Mol Cancer* **17**, 24, doi:10.1186/s12943-018-0784-2 (2018).

184 Na, T. Y., Schecterson, L., Mendonsa, A. M. & Gumbiner, B. M. The functional activity of E-cadherin controls tumor cell metastasis at multiple steps. *Proc Natl Acad Sci U S A* **117**, 5931-5937, doi:10.1073/pnas.1918167117 (2020).

185 Wiszniak, S. *et al.* The ubiquitin ligase Nedd4 regulates craniofacial development by promoting cranial neural crest cell survival and stem-cell like properties. *Dev Biol* **383**, 186-200, doi:10.1016/j.ydbio.2013.09.024 (2013).

186 Wiszniak, S. & Schwarz, Q. Notch signalling defines dorsal root ganglia neuroglial fate choice during early neural crest cell migration. *BMC Neurosci* **20**, 21, doi:10.1186/s12868-019-0501-0 (2019).

187 Brabletz, T., Kalluri, R., Nieto, M. A. & Weinberg, R. A. EMT in cancer. *Nat Rev Cancer* **18**, 128-134, doi:10.1038/nrc.2017.118 (2018).

188 Tian, P., Liu, D., Sun, L. & Sun, H. Cullin7 promotes epithelialmesenchymal transition of esophageal carcinoma via the ERKSNAI2 signaling pathway. *Mol Med Rep* **17**, 5362-5367, doi:10.3892/mmr.2018.8503 (2018).

189 Qiu, N. *et al.* Cullin 7 is a predictor of poor prognosis in breast cancer patients and is involved in the proliferation and invasion of breast cancer cells by regulating the cell cycle and microtubule stability. *Oncol Rep* **39**, 603-610, doi:10.3892/or.2017.6106 (2018).

190 Xu, J. *et al.* Cullin-7 (CUL7) is overexpressed in glioma cells and promotes tumorigenesis via NF-kappaB activation. *J Exp Clin Cancer Res* **39**, 59, doi:10.1186/s13046-020-01553-7 (2020).

191 Zhang, D., Yang, G., Li, X., Xu, C. & Ge, H. Inhibition of Liver Carcinoma Cell Invasion and Metastasis by Knockdown of Cullin7 In Vitro and In Vivo. *Oncol Res* **23**, 171-181, doi:10.3727/096504016X14519995067562 (2016).

192 Shi, L., Du, D., Peng, Y., Liu, J. & Long, J. The functional analysis of Cullin 7 E3 ubiquitin ligases in cancer. *Oncogenesis* **9**, 98, doi:10.1038/s41389-020-00276-w (2020).

193 Sasaki, K. *et al.* Maternal uniparental isodisomy and heterodisomy on chromosome 6 encompassing a CUL7 gene mutation causing 3M syndrome. *Clin Genet* **80**, 478-483, doi:10.1111/j.1399-0004.2010.01599.x (2011).

194 Simsek-Kiper, P. O. *et al.* Further expanding the mutational spectrum and investigation of genotype-phenotype correlation in 3M syndrome. *Am J Med Genet A* **179**, 1157-1172, doi:10.1002/ajmg.a.61154 (2019).

195 Ghandi, M. *et al.* Next-generation characterization of the Cancer Cell Line Encyclopedia. *Nature* **569**, 503-508, doi:10.1038/s41586-019-1186-3 (2019).

196 Huber, C. *et al.* A large-scale mutation search reveals genetic heterogeneity in 3M syndrome. *Eur J Hum Genet* **17**, 395-400, doi:10.1038/ejhg.2008.200 (2009).

197 Huber, C. *et al.* Identification of mutations in CUL7 in 3-M syndrome. *Nat Genet* **37**, 1119-1124, doi:10.1038/ng1628 (2005).

198 Urquhart, L. Market watch: Top drugs and companies by sales in 2017. *Nat Rev Drug Discov* **17**, 232, doi:10.1038/nrd.2018.42 (2018).

199 Mullard, A. First targeted protein degrader hits the clinic. *Nat Rev Drug Discov*, doi:10.1038/d41573-019-00043-6 (2019).

200 Richardson, P. G. *et al.* Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. *Blood* **116**, 679-686, doi:10.1182/blood-2010-02-268862 (2010).

201 Cathcart, A. M. *et al.* Targeting a helix-in-groove interaction between E1 and E2 blocks ubiquitin transfer. *Nat Chem Biol* **16**, 1218-1226, doi:10.1038/s41589-020-0625-7 (2020).

202 Furihata, H. *et al.* Structural bases of IMiD selectivity that emerges by 5-hydroxythalidomide. *Nat Commun* **11**, 4578, doi:10.1038/s41467-020-18488-4 (2020).

203 Naito, M., Ohoka, N. & Shibata, N. SNIPERs-Hijacking IAP activity to induce protein degradation. *Drug Discov Today Technol* **31**, 35-42, doi:10.1016/j.ddtec.2018.12.002 (2019).

204 Simonetta, K. R. *et al.* Prospective discovery of small molecule enhancers of an E3 ligase-substrate interaction. *Nat Commun* **10**, 1402, doi:10.1038/s41467-019-09358-9 (2019).

205 Smith, B. E. *et al.* Differential PROTAC substrate specificity dictated by orientation of recruited E3 ligase. *Nat Commun* **10**, 131, doi:10.1038/s41467-018-08027-7 (2019).

206 Gabrielsen, M. *et al.* Identification and Characterization of Mutations in Ubiquitin Required for Non-covalent Dimer Formation. *Structure* **27**, 1452-1459 e1454, doi:10.1016/j.str.2019.06.008 (2019).

207 Sackton, K. L. *et al.* Synergistic blockade of mitotic exit by two chemical inhibitors of the APC/C. *Nature* **514**, 646-649, doi:10.1038/nature13660 (2014).

208 Kathman, S. G. *et al.* A Small Molecule That Switches a Ubiquitin Ligase From a Processive to a Distributive Enzymatic Mechanism. *J Am Chem Soc* **137**, 12442-12445, doi:10.1021/jacs.5b06839 (2015).

209 Watson, E. R. *et al.* Protein engineering of a ubiquitin-variant inhibitor of APC/C identifies a cryptic K48 ubiquitin chain binding site. *Proc Natl Acad Sci U S A* **116**, 17280-17289, doi:10.1073/pnas.1902889116 (2019).

210 Brown, N. G. *et al.* Dual RING E3 Architectures Regulate Multiubiquitination and Ubiquitin Chain Elongation by APC/C. *Cell* **165**, 1440-1453, doi:10.1016/j.cell.2016.05.037 (2016).

211 Dueber, E. C. *et al.* Antagonists induce a conformational change in cIAP1 that promotes autoubiquitination. *Science* **334**, 376-380, doi:10.1126/science.1207862 (2011).

212 Sievers, Q. L. *et al.* Defining the human C2H2 zinc finger degrome targeted by thalidomide analogs through CRBN. *Science* **362**, doi:10.1126/science.aat0572 (2018).

213 Schneider, M. *et al.* The PROTACtable genome. *Nat Rev Drug Discov*, doi:10.1038/s41573-021-00245-x (2021).

214 Ege, N., Bouguenina, H., Tatari, M. & Chopra, R. Phenotypic screening with target identification and validation in the discovery and development of E3 ligase modulators. *Cell chemical biology* **28**, 283-299, doi:10.1016/j.chembiol.2021.02.011 (2021).

215 Hundley, F. V. *et al.* A comprehensive phenotypic CRISPR-Cas9 screen of the ubiquitin pathway uncovers roles of ubiquitin ligases in mitosis. *Mol Cell* **81**, 1319-1336.e1319, doi:10.1016/j.molcel.2021.01.014 (2021).

216 Mayor-Ruiz, C. *et al.* Rational discovery of molecular glue degraders via scalable chemical profiling. *Nat Chem Biol* **16**, 1199-1207, doi:10.1038/s41589-020-0594-x (2020).

217 Hajek, R., Bryce, R., Ro, S., Klencke, B. & Ludwig, H. Design and rationale of FOCUS (PX-171-011): a randomized, open-label, phase 3 study of carfilzomib versus best supportive care regimen in patients with relapsed and refractory multiple myeloma (R/R MM). *BMC Cancer* **12**, 415, doi:10.1186/1471-2407-12-415 (2012).

218 Gandhi, A. K. *et al.* Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4(CRBN.). *Br J Haematol* **164**, 811-821, doi:10.1111/bjh.12708 (2014).

219 Yamamoto, J. *et al.* ARID2 is a pomalidomide-dependent CRL4(CRBN) substrate in multiple myeloma cells. *Nat Chem Biol* **16**, 1208-1217, doi:10.1038/s41589-020-0645-3 (2020).

220 US National Library of Medicine. *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT02959658. (2016).

221 US National Library of Medicine. *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT01930708. (2013).

222 Venci, J. V. & Gandhi, M. A. Dimethyl fumarate (Tecfidera): a new oral agent for multiple sclerosis. *Ann Pharmacother* **47**, 1697-1702, doi:10.1177/1060028013509232 (2013).

223 US National Library of Medicine. *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT04072952. (2019).

224 US National Library of Medicine. *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT03888612. (2019).

225 Assi, R. *et al.* Final results of a phase 2, open-label study of indisulam, idarubicin, and cytarabine in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome. *Cancer* **124**, 2758-2765, doi:10.1002/cncr.31398 (2018).

226 Han, T. *et al.* Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science* **356**, doi:10.1126/science.aal3755 (2017).

227 Nangaku, M. *et al.* Randomized Clinical Trial on the Effect of Bardoxolone Methyl on GFR in Diabetic Kidney Disease Patients (TSUBAKI Study). *Kidney Int Rep* **5**, 879-890, doi:10.1016/j.ekir.2020.03.030 (2020).

228 Lynch, D. R. *et al.* Safety and Efficacy of Omaveloxolone in Friedreich Ataxia (MOXIe Study). *Ann Neurol* **89**, 212-225, doi:10.1002/ana.25934 (2021).

229 US National Library of Medicine, *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT04702997. (2021).

230 Rasco, D. W. *et al.* A First-in-Human Study of Novel Cereblon Modulator Avadomide (CC-122) in Advanced Malignancies. *Clin Cancer Res* **25**, 90-98, doi:10.1158/1078-0432.CCR-18-1203 (2019).

231 US National Library of Medicine. *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT03834623. (2019).

232 US National Library of Medicine. *ClinicalTrials.gov*, https://www.clinicaltrials.gov/ct2/show/NCT04564703. (2021).

233 US National Library of Medicine. *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT04776395. (2021).

234 Aguilar, A. *et al.* Discovery of 4-((3'R,4'S,5'R)-6''-Chloro-4'-(3-chloro-2-fluorophenyl)-1'-ethyl-2''-oxodispiro[cyclohexane-1,2'-pyrrolidine-3',3''-indoline]-5'-carboxamido)bicyclo[2.2.2]octane -1-carboxylic Acid (AA-115/APG-115): A Potent and Orally Active Murine Double Minute 2 (MDM2) Inhibitor in Clinical Development. *J Med Chem* **60**, 2819-2839, doi:10.1021/acs.jmedchem.6b01665 (2017).

235 Aubry, A., Yu, T. & Bremner, R. Preclinical studies reveal MLN4924 is a promising new retinoblastoma therapy. *Cell Death Discov* **6**, 2, doi:10.1038/s41420-020-0237-8 (2020).

236 Barghout, S. H. *et al.* Preclinical evaluation of the selective small-molecule UBA1 inhibitor, TAK-243, in acute myeloid leukemia. *Leukemia* **33**, 37-51, doi:10.1038/s41375-018-0167-0 (2019).

237 Holzer, P. *et al.* Discovery of a Dihydroisoquinolinone Derivative (NVP-CGM097): A Highly Potent and Selective MDM2 Inhibitor Undergoing Phase 1 Clinical Trials in p53wt Tumors. *J Med Chem* **58**, 6348-6358, doi:10.1021/acs.jmedchem.5b00810 (2015).

238 Lee, J. K. *et al.* USP1 targeting impedes GBM growth by inhibiting stem cell maintenance and radioresistance. *Neuro Oncol* **18**, 37-47, doi:10.1093/neuonc/nov091 (2016).

239 US National Library of Medicine. *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/NCT03816319. (2021).

240 Chan, C. H. *et al.* Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression. *Cell* **154**, 556-568, doi:10.1016/j.cell.2013.06.048 (2013).

241 Huang, H. *et al.* Oridonin Triggers Chaperon-mediated Proteasomal Degradation of BCR-ABL in Leukemia. *Sci Rep* **7**, 41525, doi:10.1038/srep41525 (2017).

242 Shoji, S. *et al.* The zinc-binding region (ZBR) fragment of Emi2 can inhibit APC/C by targeting its association with the coactivator Cdc20 and UBE2C-mediated ubiquitylation. *FEBS Open Bio* **4**, 689-703, doi:10.1016/j.fob.2014.06.010 (2014).

243 Vadhan, A. *et al.* EMI2 expression as a poor prognostic factor in patients with breast cancer. *Kaohsiung J Med Sci* **36**, 640-648, doi:10.1002/kjm2.12208 (2020).

244 Tamanini, E. *et al.* Discovery of a Potent Nonpeptidomimetic, Small-Molecule Antagonist of Cellular Inhibitor of Apoptosis Protein 1 (cIAP1) and X-Linked Inhibitor of Apoptosis Protein (XIAP). *J Med Chem* **60**, 4611-4625, doi:10.1021/acs.jmedchem.6b01877 (2017).

245 Zeng, S. *et al.* Proteolysis targeting chimera (PROTAC) in drug discovery paradigm: Recent progress and future challenges. *Eur J Med Chem* **210**, 112981, doi:10.1016/j.ejmech.2020.112981 (2021).

246 Yuniati, L. *et al.* Ubiquitylation of the ER-Shaping Protein Lunapark via the CRL3(KLHL12) Ubiquitin Ligase Complex. *Cell Rep* **31**, 107664, doi:10.1016/j.celrep.2020.107664 (2020).

247 Funato, Y. *et al.* Nucleoredoxin sustains Wnt/β-catenin signaling by retaining a pool of inactive dishevelled protein. *Current biology : CB* **20**, 1945-1952, doi:10.1016/j.cub.2010.09.065 (2010).

248 Rosner, M., Reithofer, M., Fink, D. & Hengstschläger, M. Human Embryo Models and Drug Discovery. *International journal of molecular sciences* **22**, doi:10.3390/ijms22020637 (2021).

249 Chagraoui, J. *et al.* UM171 Preserves Epigenetic Marks that Are Reduced in Ex Vivo Culture of Human HSCs via Potentiation of the CLR3-KBTBD4 Complex. *Cell stem cell* **28**, 48-62.e46, doi:10.1016/j.stem.2020.12.002 (2021).

250 Cohen, S. *et al.* Hematopoietic stem cell transplantation using single UM171-expanded cord blood: a single-arm, phase 1-2 safety and feasibility study. *The Lancet. Haematology* **7**, e134-e145, doi:10.1016/s2352-3026(19)30202-9 (2020).

251 Hershko, A., Eytan, E., Ciechanover, A. & Haas, A. L. Immunochemical analysis of the turnover of ubiquitin-protein conjugates in intact cells. Relationship to the breakdown of abnormal proteins. *J Biol Chem* **257**, 13964-13970 (1982).
252 Hershko, A., Heller, H., Elias, S. & Ciechanover, A. Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. *J Biol Chem* **258**, 8206-8214 (1983).
253 Waxman, L., Fagan, J. M. & Goldberg, A. L. Demonstration of two distinct high molecular weight proteases in rabbit reticulocytes, one of which degrades ubiquitin conjugates. *J Biol Chem* **262**, 2451-2457 (1987).
254 Scudellari, M. Protein-slaying drugs could be the next blockbuster therapies. *Nature* **567**, 298-300, doi:10.1038/d41586-019-00879-3 (2019).

Acknowledgements
Research conducted by K.M.Y. and D.A.C.W. is supported by the Intramural Research Program of the National Institutes of Health, National Institute of Dental and Craniofacial Research [ZIA-DE000525 and ZIA-DE000719]. Z.C. and A.N.B. receive funding from Cancer Research UK (grant reference DRCNPG\100002) as well as the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 875510. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and Ontario Institute for Cancer Research, Royal Institution for the Advancement of Learning McGill University, Kungliga Tekniska Hoegskolan, Diamond Light Source Limited.

Author contributions
D.A.C.W., K.M.Y., A.N.B., and Z.C. contributed to the conceptualisation, writing, and revisions of the manuscript. D.A.C.W. and K.M.Y. wrote the first draft and final revisions of the manuscript. Z.C. and A.N.B. reviewed and added to the first draft and revisions of the manuscript. D.A.C.W. and Z.C. prepared the figures.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Molecular Cell Biology thanks Izabela Sumara; Yogesh Kulathu, who co-reviewed with Matthew McFarland; and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Figure 1 | **E3 ligase classes and ubiquitin transfer mechanisms. a** | Scheme for Cullin RING E3 ligase (CRL) multi-subunit complex assembled with substrate and ubiquitin-charged E2. The table lists the interchangeable substrate receptors for each Cullin scaffold. **b** | The molecular mechanism of ubiquitin transfer is illustrated for each E3 class by their domain architecture and available structural models. RING E3s promote a one-step ubiquitin transfer directly from E2 to substrate. In contrast, HECT and RBR E3s sequentially transfer Ub first from the E2 to a catalytic cysteine on the E3 then from the E3 to the substrate, as shown in steps (1) and (2). E2s and E3s are colored orange and blue, respectively. Catalytic cysteines forming a thioester bond with ubiquitin are marked by a red star. The arrows indicate the direction of ubiquitin transfer. Structural models show ubiquitin in ribbon and E2 and E3 in surface representation. PDB codes: RING E3- 6TTU; HECT E3- 3JW0, 4BBN; RBR E3- 5EDV, 4LJO. N8, NEDD8.

Figure 2 | **E3 ligases at key stages of human development.** Ubiquitin ligases target histones, transcription factors, cyclins, structural proteins, and a variety of enzymes to orchestrate the many complex events of human development. Shown adjacent to specific developmental processes are non-comprehensive lists of the well-characterized E3 ligase proteins regulating these critical events. **a** | During gametogenesis, E3s including CUL4-DCAF1, RYBP, and RNF2 regulate the sequential meiotic divisions of germ cell precursors as they mature into functionally competent sperm or eggs. CUL1-FBXW7 and CUL4B maintain germ cell precursor populations and CUL4B regulates flagellar architecture and mitochondrial ATP production as sperm cells traverse the fallopian tube to fertilize the egg. **b** | From fertilization through implantation, CUL4-DDB1-DCAF2 and other E3s regulate the zygotic divisions required to produce the bilayered blastocyst, which then implants into the lining of the uterus. Throughout this process, RNF114, CUL4-DDB1-DCAF13, and several other E3s ensure maternal mRNAs are degraded and the embryonic genome is activated in what is known as the maternal-to-zygotic transition (MZT). Figure 3a provides a more detailed schematic of implantation. **c |** Following implantation, the developing embryo relies on nutrients and other molecules provided by the placenta as it proceeds through gastrulation, germ layer specification, and neurulation. CUL3, HECTD1, HUWE1, and CUL4B target cyclin E, p21, p53, HSP90, and other molecules to ensure placental, and thus embryo, health. As depicted in Supplementary tables 1 and 2, various intrauterine growth restriction defects can result should placental health decline. Figure 3b outlines neural tube closure in greater detail. **d |** Numerous E3s direct organ formation by creating spaciotemporally precise molecular gradients of transcription factors, growth factors, and other morphogens, assisting in DNA repair, promoting ECM degradation, and driving cell-cell fusion, cell migration, proliferation, and shape changes. Supplementary table 1 outlines the roles of the E3s listered here in the development of specific tissues including the heart, lungs, pancreas, kidneys, adrenal glands, mammary glands, craniofacial complex, musculoskeletal system and nervous system. Roles of E3s in musculoskeletal and cortical/neural development have received considerable attention and are outlined in greater detail in Figure 4. MZT, maternal-to-zygotic transition; NT, neural tube; HSP; heat shock protein, ECM; extracellular matrix.

Figure 3 | **E3 ligases throughout human development: trophoblast cell invasion and neural tube closure.** **a** | Trophoblast progenitor cells proliferate and differentiate into cytotrophoblasts and synctiotrophoblasts that degrade their surrounding matrix, migrate, and breach the maternal spinal arteries to establish maternal-fetal circulation. The E3 ligase proteins listed here regulate levels of the transcription factors, growth factors, and cytokines that control trophoblast cell activity. **b** | After establishing the midline, antero-posterior and dorso-ventral gradients of various morphogens induce the cell shape changes and movements required for neural tube closure. E3 ligases employ non-degradative ubiquitination of microtubule proteins and morphogen transcription factors, as well as degradative ubiquitination of morphogen receptors, to establish morphogen gradients and induce the cell shape changes and movements required for proper neural tube closure. GF, growth factor; MMP, matrix metalloproteinase; CDK, cyclin-dependent kinase; RA, retinoic acid; RD, retinoid degrader; FGF, fibroblast growth factor; RAR, retinoic acid receptor; BMP, bone morphogenic protein; Shh, sonic hedgehog.

Figure 4 | **E3 ligases throughout human development: nervous system development. a|** Progenitor cell populations in the SVZ differentiate into various cell types, including multipolar neurons, as the embryonic cortex develops. The E3 ligases listed here maintain neural progenitor pools by modifying cyclin, DNA polymerase, and other protein activities. Equally importantly, E3s control Rho-related GTP-binding and other protein activities to ensure that neurons transition from multipolar to bipolar states, migrate along radial glial cells, and reach their final destination as differentiated neurons. **b|** Neural crest cells (purple) and myoblasts (red) migrate from their initial positions adjacent to the neural tube (blue) to form the various tissues of neural crest origin and skeletal muscles as depicted in this 5-week fetus. PP, preplate; SP, subplate; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate; MZ, marginal zone; RND2/3, Rho-related GTP-binding protein RhoN 2/3; PTPA, serine/threonine-protein phosphatase 2A activator; BCKDK, branched-chain alpha-ketoacid dehydrogenase kinase.

Figure 5 | E3 ligases throughout human development: muscle development and innervation

a| In this 6-week fetus, throughout the developing embryonic myotomes, myoblasts migrate, align, fuse, and differentiate into multinucleated skeletal muscle. **b**| E3 ligases such as CUL3-KCTD10 control actin bundling at cell-cell interfaces during myoblast fusion. Since sarcomeres serve as the fundamental units of skeletal muscle, sarcomere stability is critical to ensure proper muscle function. Several E3 ligases control the stability of nebulin, actin, alpha-actinin, and other proteins to ensure proper muscle function. PP, preplate; SP, subplate; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate; MZ, marginal zone; RND2/3, Rho-related GTP-binding protein RhoN 2/3; PTPA, serine/threonine-protein phosphatase 2A activator; BCKDK, branched-chain alpha-ketoacid dehydrogenase kinase.

Figure 6 | **Chemical approaches to exploit E3 ligases. a** | Alternative strategies to inhibit E3 function. **b** | Schematics illustrating the molecular mechanisms of PROTACs and molecular glues used for targeted protein degradation. Schematic and structural models in the blue shading illustrate recruitment of myeloma therapeutic target IKZF1 or teratogenicity-causing target SALL4 to the E3 ligase cereblon (CRBN) by the molecular glue Pomalidomide. PDB codes: IKZF1 - 6H0F, SALL4 – 6UML.

Glossary

Pre-eclampsia: a toxic medical condition during late pregnancy characterized by high blood pressure, edema and protein in the urine.

Intrauterine growth restriction: abnormally slow growth of the fetus defined as less than 10 percent of predicted body weight for gestational age.

Focal adhesion: a subcellular structure containing protein complexes mediating the adhesion of cells to the extracellular matrix.

Midline: a topographical line formed during gastrulation defined by the formation of the notochord that extends from the anterior to the posterior of the embryo that helps define the future embryonic axes.

Epithelial-to-mesenchymal transition: conversion of epithelial cells to migratory fibroblast-like mesenchymal cells by an intracellular regulatory process.

Thalidomide: a small molecule drug originally intended for use as a sedative and to relieve pregnancy-induced nausea but was found to cause birth defects, particularly limb malformations.

ToC

E3 ubiquitin ligases, ensure the precise spatio-temporal control of key molecules during important cellular processes .This Review discusses the crucial roles of E3 ligases during early mammalian development, their roles in human disease, and considers how new methods to manipulate the ubiquitin regulatory machinery — for example the development of molecular glues and PROTACs — might facilitate clinical therapy.