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(54) Title: METHODS AND COMPOSITIONS FOR INHIBITING NEDDYLYATION OF PROTEINS

(57) Abstract: Provided herein is a novel binding pocket within NEDD8 co-E3 proteins that binds NEDD8 E2 enzymes. Methods are provided for screening for compounds that bind to the disclosed E2-binding pocket in NEDD8 co-E3 proteins. Compounds that bind to the E2-binding pocket and optionally inhibit the activity of NEDD8 co-E3 proteins and pharmaceutical compositions comprising the same are further provided. The NEDD8 co-E3 inhibitors find use in inhibiting cell growth and methods for treating cancers, inflammatory disorders, and pathogenic infections.



METHODS AND COMPOSITIONS FOR INHIBITING NEDDYLATION OF PROTEINS

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

The official copy of the sequence listing is submitted electronically via EFS-Web
5 as an ASCII formatted sequence listing with a file named 418048SEQLIST.TXT, created
on May 14, 2012, and having a size of 246 kilobytes and is filed concurrently with the
specification. The sequence listing contained in this ASCII formatted document is part of
the specification and is herein incorporated by reference in its entirety.

10 FIELD OF THE INVENTION

The present invention relates to the inhibition of covalent modifications of proteins
through conjugation with other proteins, particularly, neuronal precursor cell expressed
developmentally downregulated protein 8 (NEDD8).

15 BACKGROUND OF THE INVENTION

Post-translational covalent modifications of proteins through conjugation with
other proteins is an important biological mechanism for regulating protein metabolism
and biological activity. The most well understood post-translational modifier is ubiquitin,
an 8.5 kDa protein, that is covalently attached to lysines in a target protein.
20 Polyubiquitination of a protein targets the protein for degradation.

Ubiquitin is conjugated to its target proteins through an enzymatic cascade
involving a specific E1 activating enzyme, Ubal or Uba6, a conjugating enzyme from the
family of E2s, and a ubiquitin ligase that is typically from either the RING or HECT
classes of E3s (Huang *et al.* (2004) *Oncogene* 23:1958-71). Target specificity is
25 controlled by the particular combination of E2 and E3 proteins. For example, the multi-
protein ubiquitin E3 ligase Skp, cullin, F-box containing complexes (SCFs) ubiquitinate
targets involved in cell-cycle progression, transcription, metabolism, and inflammation,
such as the cyclin-dependent kinase (CDK) inhibitor p27^{Kip1} and NFκB inhibitor, IkappaB
(IκB).

Other proteins that are structurally similar to ubiquitin and are referred to as ubiquitin-like proteins (UBLs) have been identified that covalently modify cellular targets using their own pathways that are parallel to that of ubiquitin. Examples of UBLs include small ubiquitin-like modifier (SUMO) and neuronal precursor cell expressed

5 developmentally downregulated protein 8 (NEDD8). Similar to ubiquitin, UBLs are covalently attached to a lysine on a target protein via an isopeptide linkage with a C-terminal glycine of the UBL, a process that is mediated via an E1 activating enzyme, E2 conjugating enzyme, and an E3 ligase.

The covalent modification of a target protein with the UBL NEDD8 is referred to
10 herein as neddylation. The neddylation of cullin proteins, the best understood targets of NEDD8, is necessary for SCF-mediated ubiquitination and subsequent degradation of SCF target proteins (Podust *et al.* (2000) *Proc Natl Acad Sci USA* 97:4579-4584; Read *et al.* (2000) *Mol Cell Biol* 20:2326-2333). SCFs form a subset of cullin-containing ubiquitin E3s. Members of the large family of cullin-containing E3s are called cullin-RING E3s,
15 and contain CUL1, CUL2, Cul3, CUL4A, CUL5, CUL7, or Parc, which are regulated by covalent ligation of NEDD8. Not surprisingly, the NEDD8 pathway enzymes play an essential role in cell proliferation in organisms ranging from fission yeast to mammals (Osaka *et al.* (2000) *EMBO J* 19:3475-3484; Tateishi *et al.* (2001) *J Cell Biol* 155:571-579). Given the importance of NEDD8 conjugation in cell growth and inflammation,
20 further characterization of NEDD8 pathway enzymes and the domains with which they use to interact with one another is needed in order to develop therapeutics that target the neddylation pathway for the treatment of disorders such as cancer and inflammatory diseases.

25 BRIEF SUMMARY OF THE INVENTION

A novel binding pocket within NEDD8 co-E3 proteins that binds NEDD8 E2 enzymes is provided. Methods are provided for screening for compounds that bind to the disclosed E2-binding pocket in NEDD8 co-E3 proteins, including computational methods and binding assays. In some embodiments, compounds that bind to the pocket inhibit the
30 activity of NEDD8 co-E3 proteins and the subsequent neddylation of protein targets. Compounds (e.g., peptides and small molecules) that bind to the E2-binding pocket and optionally inhibit the activity of NEDD8 co-E3 proteins and pharmaceutical compositions comprising the same are provided. In some embodiments, the inhibitory peptides are amino-terminally acetylated. Methods for inhibiting NEDD8 co-E3 activity and inhibiting

cell growth with the NEDD8 co-E3 inhibitors are provided, as well as methods for treating cancer, inflammatory disorders, or pathogenic infections by administering the disclosed NEDD8 co-E3 inhibitors to a subject in need thereof.

5 These and other aspects of the invention are disclosed in more detail in the description of the invention given below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A provides MaxEnt liquid chromatography-time of flight (LC-TOF) spectra of *S. cerevisiae* yUbc12-His6 purified from WT yeast (left), $\Delta mak3$ yeast
10 (middle), or from coexpression with yeast NatC subunits in *E. coli* (right).

Figure 1B provides a Western blot of yCul1 (aka Cdc53p) from WT yeast and the indicated deletion strains.

Figures 2A and 2B provide results of a pulse-chase assay monitoring [32 P]-yNEDD8 transfer from the indicated variants of yUbc12 to yCul1^{C+}-yRbx1 (Figure
15 2A) or hUbc12 to hCul1^{C+}-hRbx1 (Figure 2B) in the absence (left panel) or presence (right panel) of yDcn1^P (Figure 2A) or hDcn1^P (Figure 2B). Note the differences in times between left and right, with longer times required to observe activity without yDcn1^P.

Figure 3A provides raw isothermal titration calorimetry (ITC) data for titration of hDcn1^P binding to hCul1^{ctd}-hRbx1 (left panel) and thermodynamic parameters for
20 hDcn1^P binding to hCul1^{ctd}-hRbx1 (right panel). The measured binding *K_d* of 1 μ M for human proteins is approximately 30-fold worse than for the yeast counterparts (yCul1^{C+}-yRbx1 : yDcn1^P, *K_d* = 28 nM³).

Figure 3B provides an autoradiograph of pulse-chase assays monitoring [32 P]-hNEDD8 transfer from hUbc12^{AcMet} to 125 nM hCul1^{ctd}-hRbx1 in the absence or
25 presence of the indicated fold molar excess of hDcn1^P. hDcn1^P concentration-dependence in pulse-chase assays demonstrates activation while maintaining the absolute requirement for N-terminal acetylation of hUbc12. At saturating hDcn1^P concentrations based on *K_d* measured above, hDcn1^P demonstrates a striking co-E3 activity for hNEDD8 only with hUbc12^{AcMet}.

30 Figure 3C demonstrates that Cul1 neddylation requires Rbx1 RING domain, even in the presence of Dcn1^P and N-terminally acetylated Ubc12^{AcMet} and provides phosphorimager data for a time course of pulse-chase assays monitoring [32 P]-yNEDD8 transfer from yUbc12^{AcMet} to yCul1^{C+}-yRbx1 and yCul1^{C+}-yRbx1^R in the absence or presence of yDcn1^P (left panel) or [32 P]-hNEDD8 transfer from hUbc12^{AcMet} to hCul1^{C+}-

hRbx1 and hCul1^{C+}-hRbx1^R in the absence or presence of hDcn1^P (right panel). Assays were carried out at a final concentration of 500 nM y or hCul1 or a 500 nM mix of y or hCul1 with y or hDcn1^P. Experiments with yeast proteins were performed at 18°C instead of 4°C as in other experiments. These differences in experimental design were implemented to highlight the necessity of the RING domain for cullin neddylation. Note differences in time-scales as some chase timepoints are on the order of seconds, and others minutes.

Figure 4 provides a sequence alignment of Uba3, the Ubc12-binding subunit of NEDD8's E1 enzyme. The ClustalW sequence alignment was generated in MegAlign of Uba3, the Ubc12-binding subunit of NEDD8's heterodimeric E1 enzyme, from human (SEQ ID NO: 59), mouse (SEQ ID NO: 61), rat (SEQ ID NO: 74), *Xenopus tropicalis* (SEQ ID NO: 75), zebrafish (SEQ ID NO: 76), *S. pombe* (SEQ ID NO: 77), and *S. cerevisiae* (SEQ ID NO: 78). Boxes indicate Ubc12- interacting regions identified in crystal structures of human Uba3 complexes with Ubc12 (Huang *et al.* (2004) *Nat Struct Mol Biol* 11(10):927-935; Huang *et al.* (2007) *Nature* 445(7126):394-398), which are notably absent from *S. cerevisiae* Uba3p. Black boxes encircling the alignment indicate regions of hUba3 interacting with hUbc12's N-terminal extension. Gray boxes encircling the alignment correspond to Uba3's ubiquitin-fold domain, known to bind the catalytic domain of Ubc12.

Figure 5A provides a depiction of the structure of hCul1^{WHB}-hDcn1^P-Acetyl-hUbc12¹⁻¹⁵. hDcn1^P is displayed in surface-mode, colored according to conservation between 5 human and yeast Dcn1^P orthologs (white – not conserved; black – 100% conserved). Acetyl-hUbc12¹⁻¹⁵ peptide is shown as a helix and stick figure. hCul1^{WHB} is not shown for simplification.

Figure 5B provides a close-up depiction of Acetyl-hUbc12¹⁻¹⁵ peptide N-terminus binding to hDcn1^P. Left - interacting residues are shown as sticks with electrostatic interactions as dashes. Right - hUbc12's N-acetyl-methionine and residues 2 and 4 as spheres in a mesh view of the hDcn1^P crevasse.

Figure 5C provides a close-up depiction of Acetyl-yUbc12¹⁻²⁴ peptide N-terminus binding to yDcn1^P. Left - interacting residues are shown as sticks with electrostatic interactions as dashes. Right - yUbc12's N-acetyl-methionine and residues 2 and 4 as spheres in a mesh view of the yDcn1^P crevasse.

Figure 6 provides a sequence alignment of Dcn1^P family members. Structure-based sequence alignment of Dcn1^P (also called "PONY" (Kurz *et al.*

(2007) *Nature* 445(7126):394-398)) domains from yeast Dcn1 (SEQ ID NO: 49) and human Dcn1 family members 1-5 (also called DCUND1-DCUND5; SEQ ID NOs: 34, 37, 40, 43, and 46, respectively). Secondary structural elements are indicated above (Kurz *et al.* (2007) *Nature* 445(7126):394-398). Black asterisks above the alignment indicate contact sites between yDcn1^P and the N-terminally acetylated yUbc12 peptide. Black asterisks below the alignment indicate contact sites between hDcn1^P and the N-terminally acetylated hUbc12 peptide. Gray asterisks below the alignment indicate contact sites between hDcn1^P and the hCul1^{WHB} sub-domain.

Figure 7A provides an autoradiograph of pulse-chase assays monitoring [³²P]-yNEDD8 transfer from yUbc12^{Met} (top) or yUbc12^{AcMet} (bottom) to yCul1^{CTD}-yRbx1 in the absence or presence of the indicated yDcn1^P variants (note differences in times between top and bottom panels).

Figure 7B provides Western blots for yCul1 (aka Cdc53p, top) or HA-tag (bottom) from mid-log whole cell extracts from Δ dcn1 or Δ dcn1/ Δ mak10 yeast harboring empty or WT Dcn1-HA, or Y190A mutant Dcn1-HA expression vectors.

Figure 8A provides an autoradiograph of pulse-chase assays in the absence or presence (500nM) of the indicated DCUN1D family members monitoring transfer of NEDD8 from 40nM Ubc12^{AcMet}-NEDD8 (upper panel) or 40nM Ube2f^{AcMet}-NEDD8 (lower panel) to 500nM Cul1^{CTD}-Rbx1, demonstrating that DCUN1D(1-5) family members stimulate Ubc12^{AcMet} and Ube2f^{AcMet} NEDD8 transfer to Cul1^{CTD}-Rbx1.

Figure 8B provides an autoradiograph of pulse-chase assays in the absence or presence of 500nM DCUN1D1 monitoring transfer of NEDD8 from 40nM Ubc12^{AcMet}-NEDD8 to 500nM Cul(1-5)^{CTD}-Rbx1, demonstrating that DCUN1D1 potentiates NEDD8 transfer from Ubc12^{AcMet} to Cul(1-5) family members.

Figure 9 demonstrates the inhibition of DCUN1D1 potentiated NEDD8 modification of Cul1^{CTD}-Rbx1.

Figure 9A presents phosphoimager data of pulse-chase assays monitoring the transfer of NEDD8 from 40nM Ubc12^{AcMet}-NEDD8 to 125nM Cul1^{CTD}-Rbx1 in the absence or presence of 125nM DCUN1D1^P and the indicated amounts of the peptide inhibitor Acetyl-yUbc12¹⁻¹²(6:10Staple).

Figure 9B provides quantification of Figure 9A. The amount of Cul1^{CTD}-NEDD8 formed at each time point was quantified and normalized with the amount of Cul1^{CTD}-NEDD8 formed at two minutes in the presence of DCUN1D1^P and absence of inhibitor set to one.

DETAILED DESCRIPTION OF THE INVENTION

A novel binding pocket in NEDD8 co-E3 proteins that interacts with the amino-terminal (N-terminal) extension of N-terminally acetylated NEDD8 E2 conjugating enzymes is provided. The disclosed E2-binding pocket of NEDD8 co-E3 proteins finds
5 use in methods for identifying inhibitors of the neddylation cascade. Compounds (e.g., peptides, small molecules) that bind to the disclosed binding pocket and inhibit co-E3 activity are provided herein. These co-E3 inhibitors find use in treating diseases such as cancer, inflammatory disorders, and pathogenic infections.

As used herein, the terms “NEDD8” and “neuronal precursor cell expressed
10 developmentally downregulated protein 8” refer to a member of the family of ubiquitin-like proteins (UBLs) that is covalently attached to target proteins. The human, mouse, and rat NEDD8 sequences are each 81 amino acids in length and are about 6 kDa. The terms “NEDD8” and “neuronal precursor cell expressed developmentally downregulated protein 8” also refer to the *Saccharomyces cerevisiae* Rub1 protein. Nucleotide and
15 amino acid sequences of NEDD8 proteins are known in the art. Non-limiting examples of NEDD8 sequences include *Homo sapiens* NEDD8, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_006156 and NP_006147, respectively (and in SEQ ID NOs: 50 and 51, respectively); *Mus musculus* NEDD8, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos.
20 NM_008683 and NP_032709, respectively (and in SEQ ID NOs: 52 and 53, respectively) (Kamitani *et al.* (1997) *J Biol Chem* 272:28557-28562; Kumar *et al.* (1992) *Biochem Biophys Res Comm* 185:1155-1161, each of which are herein incorporated by reference in its entirety); and *Saccharomyces cerevisiae* Rub1, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. Y16890 and CAA76516,
25 respectively (and in SEQ ID NOs: 72 and 73, respectively).

NEDD8 is conjugated to its protein targets through a series of enzymatic reactions similar to other E1-E2-E3 ubiquitin-like (UBL) conjugation cascades. First, NEDD8 is activated in an ATP-dependent manner by an E1 enzyme, NEDD8 activating enzyme (NAE), which is a heterodimer of NAE1 and UBA3 subunits.

30 As used herein, the terms “NAE,” “NEDD8 activating enzyme,” “NEDD8 E1 activating enzyme,” and “NEDD8 E1 enzyme” refer to a protein capable of catalyzing the transfer of NEDD8’s C terminus to the catalytic cysteine of NEDD8 E2, forming a thiolester-linked E2-NEDD8 intermediate (Gong and Yeh (1999) *J Biol Chem* 274:12036-12042; and Liakopoulos *et al.* (1998) *EMBO J* 17:2208-2214; Osaka *et al.*

(1998) *Genes Dev* 12:2263-2268, each of which are herein incorporated by reference in its entirety).

The only described NEDD8 E1 enzyme is a heterodimer of NAE1 (also referred to as APPBP1; amyloid beta precursor protein binding protein 1; and NEDD8-activating enzyme E1 regulatory subunit) and UBA3 (also referred to as NEDD8-activating enzyme E1 catalytic subunit or UBEC1) subunits. Nucleotide and amino acid sequences of NAE1 proteins are known in the art. Non-limiting examples of NAE1 sequences include *Homo sapiens* NAE1, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_001018159 and NP_001018169, respectively (and in SEQ ID NOs: 54 and 55, respectively); and *Mus musculus* NAE1, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_144931 and NP_659180, respectively (and in SEQ ID NOs: 56 and 57, respectively). In some embodiments of the presently disclosed invention, the NAE1 has the sequence set forth in SEQ ID NO: 55 or 57, or a biologically active fragment or variant thereof that is capable of interacting with UBA3.

Nucleotide and amino acid sequences of UBA3 proteins are also known in the art. Non-limiting examples of UBA3 sequences include *Homo sapiens* UBA3, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_003968 and NP_003959, respectively (and in SEQ ID NOs: 58 and 59, respectively); and *Mus musculus* UBA3, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_011666 and NP_035796, respectively (and in SEQ ID NOs: 60 and 61, respectively). The rat, *Xenopus tropicalis*, zebrafish (*Danio rerio*), *Schizosaccharomyces pombe*, and *Saccharomyces cerevisiae* UBA3 amino acid sequences are set forth in SEQ ID NOs: 74, 75, 76, 77, and 78, respectively. In some embodiments of the presently disclosed invention, the NEDD8 UBA3 protein has the sequence set forth in SEQ ID NO: 59, 61, 74, 75, 76, 77, or 78, or a biologically active variant thereof that is capable of interacting with NAE1 and catalyzing a reaction culminating in the generation of a thiolester-linked E2-NEDD8 intermediate.

Crystal structures of human Uba3 complexes with the NEDD8 E2 enzyme Ubc12 have been solved (Huang *et al.* (2004) *Nat Struct Mol Biol* 11(10):927-935; Huang *et al.* (2007) *Nature* 445(7126):394-398), which aided in identifying the Ubc12-interacting regions of NEDD8 E1 enzymes. Figure 4 provides an alignment of UBA3 proteins from various organisms, highlighting the regions of human Uba3 that interact with human

Ubc12's N-terminal extension and Uba3's ubiquitin-fold domain, which binds the catalytic domain of Ubc12.

NEDD8 E2 enzymes play central roles in the E1-E2-E3 NEDD8 conjugation cascade. As used herein, the terms "NEDD8 conjugating enzyme," and "NEDD8 E2 enzyme" refer to a protein capable of transiently binding a NEDD8 E1 enzyme for generation of a thiolester-linked E2~UBL intermediate (~ denotes covalent complex; - denotes noncovalent complex) and interacting with a NEDD8 E3 ligase as an E2~UBL intermediate. The two known NEDD8 conjugating enzymes are UBC12, which is also known as UBE2M, and UBE2F. Nucleotide and amino acid sequences of UBC12 proteins are known in the art. Non-limiting examples of UBC12 sequences include *Homo sapiens* UBC12 (also known as UBE2M), the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_003969 and NP_003960, respectively (and in SEQ ID NOs: 1 and 2, respectively); *Mus musculus* UBC12, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_145578 and NP_663553, respectively (and in SEQ ID NOs: 62 and 63, respectively); and *Saccharomyces cerevisiae* UBC12, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_001182194 and NP_013409, respectively (and in SEQ ID NOs: 5 and 6, respectively).

Nucleotide and amino acid sequences of UBE2F proteins are known in the art. Non-limiting examples of UBE2F sequences include *Homo sapiens* UBE2F, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_080678 and NP_542409, respectively (and in SEQ ID NOs: 3 and 4, respectively); and *Mus musculus* UBE2F, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_026454 and NP_080730, respectively (and in SEQ ID NOs: 64 and 65, respectively). In some embodiments of the presently disclosed invention, the NEDD8 E2 enzyme has the sequence set forth in SEQ ID NO: 2, 4, 6, 63, or 65, or a biologically active fragment or variant thereof capable of transiently binding a NEDD8 E1 enzyme for generation of a thiolester-linked E2~UBL intermediate and interacting with a NEDD8 E3 ligase as an E2~UBL intermediate.

NEDD8 E2 enzymes have an about 20 to about 29-residue N-terminal extension upstream of its about 150-residue conserved E2 core domain, which contains the E2 catalytic cysteine. Ubc12's N-terminal sequence is conserved across species, but is not found in other E2 enzymes and is therefore, unique to the NEDD8 pathway. NEDD8 E2 enzymes interact with both NEDD8 E1 and co-E3 proteins via its unique N-terminal

extension. As disclosed herein, acetylation of the amino terminus of the N-terminal extension of NEDD8 E2 enzymes enhances binding of yeast Ubc12 to NEDD8 co-E3 proteins and is necessary for binding of mammalian NEDD8 E2 enzymes to co-E3 proteins, but does not affect binding of NEDD8 E2 enzymes to NEDD8 E1 proteins.

5 Unlike other ubiquitin like proteins known to date, NEDD8 transfer from NEDD8 E2 enzymes to target proteins involves a "dual E3" mechanism (Scott *et al.* (2010) *Mol Cell* 39(5):784-796): a RING E3 ligase, Rbx1, is essential for target protein neddylation; an auxiliary co-E3, Dcn1, contains a "potentiating neddylation" domain (Dcn1^P) thought to bind different regions of Ubc12 and target proteins from Rbx1 (Kim *et al.* (2008) *J Biol Chem* 283(48):33211-33220; Scott *et al.* (2010) *Mol Cell* 39(5):784-796; Kurz *et al.* (2005) *Nature* 435(7046):1257-1261; and Huang *et al.* (2011) *J Biol Chem*, each of which is herein incorporated by reference in its entirety).

As used herein, the terms "NEDD8 E3 ligase" and "NEDD8 E3 enzyme" refer to RING domain NEDD8 E3 ligases capable of interacting with an E2~NEDD8 intermediate and catalyzing the transfer of NEDD8 from the E2~NEDD8 intermediate to an E3-associated target's lysine, producing an isopeptide-bonded target~NEDD8 complex; or HECT domain NEDD8 E3 ligases capable of forming an E3~NEDD8 thiolester intermediate and catalyzing the subsequent transfer of NEDD8 onto a target's lysine. The only known NEDD8 E3 ligase is a RING domain E3 ligase. Nucleotide and amino acid sequences of NEDD8 E3 ligases are known in the art. Non-limiting examples of NEDD8 E3 ligase sequences include *Homo sapiens* Rbx1, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_014248 and NP_055063, respectively (and in SEQ ID NOs: 66 and 67, respectively); *Mus musculus* Rbx1, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_019712 and NP_062686, respectively (and in SEQ ID NOs: 68 and 69, respectively); and *Saccharomyces cerevisiae* Hrt1, the amino acid sequence of which is set forth in SEQ ID NO: 86. In some embodiments of the presently disclosed invention, the NEDD8 E3 ligase has the sequence set forth in SEQ ID NO: 67, 69, or 86, or a biologically active fragment or variant thereof that is capable of interacting with an E2~NEDD8 intermediate and catalyzing the transfer of NEDD8 onto a target's lysine.

The amino terminus of Hrt1 recruits Cdc53 (yeast cullin ortholog) and the carboxy terminus of Hrt1 comprises the RING domain that activates Ubc12~Rub1 (Rub1 is a NEDD8 ortholog).

As used herein, the terms “NEDD8 co-E3” and “NEDD8 co-E3 protein” refer to a protein capable of interacting with an E2~NEDD8 intermediate and catalyzing the transfer of NEDD8 from the E2~NEDD8 intermediate to an E3-associated target’s lysine, wherein the protein does not comprise a RING or HECT domain. A structural model of the Dcn1-Cdc53-Hrt1-Ubc12 complex has been prepared and it suggests that Dcn1 restricts the otherwise flexible RING-Ubc12~Rub1 into a catalytically competent orientation, bringing Ubc12’s active site toward Cdc53 (Scott *et al.* (2010) *Mol Cell* 39:784-796, which is herein incorporated by reference in its entirety). Thus, the term “co-E3 activity” refers to the ability to interact with an E2~NEDD8 intermediate and catalyze the transfer of NEDD8 from the E2~NEDD8 intermediate to an E3-associated target’s lysine in the absence of a RING or HECT domain; to enhance the activity of a RING or HECT domain NEDD8 E3 ligase; or to reduce nonspecific E2~NEDD8 discharge, which refers to the transfer of NEDD8 from an E2 catalytic cysteine to a nucleophile, such as a primary amine. Thus, in some embodiments, a NEDD8 co-E3 increases the catalytic rate (k_{cat}) of the NEDD8 E3 ligase reaction. In particular embodiments, the k_{cat} of a NEDD8 E3 ligase reaction is increased by about 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 50-fold, 100-fold, or greater by a NEDD8 co-E3 ligase.

Nucleotide and amino acid sequences of NEDD8 co-E3 proteins are known in the art. Non-limiting examples of NEDD8 co-E3 sequences include *Saccharomyces cerevisiae* Dcn1, the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_001182015 and NP_013229, respectively (and in SEQ ID NOs: 47 and 48, respectively); *Homo sapiens* DCUN1D1 (which is also referred to herein as squamous cell carcinoma-related oncogene or SCCRO or Dcn1), the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_020640 and NP_065691, respectively (and in SEQ ID NOs: 32 and 33, respectively); *Mus musculus* DCUN1D1 (also referred to herein as Dcn1), the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_001205361 and NP_001192290, respectively (and in SEQ ID NOs: 70 and 71, respectively); *Homo sapiens* DCUN1D2 (also referred to herein as Dcn2), the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_001014283 and NP_001014305, respectively (and in SEQ ID NOs: 35 and 36, respectively); *Homo sapiens* DCUN1D3 (also referred to herein as Dcn3), the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_173475.2 and NP_775746.1, respectively (and in SEQ ID NOs: 38 and 39, respectively); *Homo sapiens* DCUN1D4 (also referred to herein as Dcn4), the

nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos. NM_001040402 and NP_001035492, respectively (and in SEQ ID NOs: 41 and 42, respectively); and *Homo sapiens* DCUN1D5 (also referred to herein as Dcn5), the nucleotide and amino acid sequences of which are set forth in GenBank Acc. Nos.

- 5 NM_032299.3 and NP_115675, respectively (and in SEQ ID NOs: 44 and 45, respectively). In some embodiments of the presently disclosed invention, the NEDD8 co-E3 protein has the sequence set forth in SEQ ID NO: 33, 36, 39, 42, 45, 48, or 71, or a biologically active fragment or variant thereof having co-E3 activity.

- Dcn1 and other known NEDD8 co-E3 ligases comprise an amino-terminal
 10 ubiquitin associated (UBA) domain (residues 57-69 of yeast Dcn1, the sequence of which is set forth in SEQ ID NO: 48), followed by a disordered linker and a carboxy-terminal “potentiating neddylation” (PONY) domain. The PONY domain of co-E3 ligases, such as Dcn1, is sufficient for binding a NEDD8 E2 (Ubc12) and Cdc53/cullin proteins and for enhancing Cdc53~Rub1 levels (Kurz *et al.* (2008) *Mol Cell* 29:23-35). The PONY
 15 domain of *Homo sapiens* DCUN1D1, DCUN1D2, DCUN1D3, DCUN1D4, DCUN1D5, and *Saccharomyces cerevisiae* Dcn1 is set forth in SEQ ID NOs: 34, 37, 40, 43, 46, and 49, respectively. An alignment of the potentiating neddylation domain from *S. cerevisiae* Dcn1, and human Dcn1, Dcn2, Dcn3, Dcn4, and Dcn5 is provided in Figure 6, which highlights those residues that form contacts with acetylated *S. cerevisiae* Ubc12¹⁻²⁴ or
 20 acetylated human Ubc12¹⁻¹⁵ or with cullin. A further description of the interactions between NEDD8 E2 and co-E3 ligases is provided elsewhere herein.

- The most well-characterized neddylated proteins are cullin proteins. As used herein, the term “cullin” refers to a protein belonging to a family of hydrophobic proteins that serve as scaffolds for ubiquitin ligases, such as the Skp, cullin, F-box containing
 25 (SCF) ubiquitin ligase. There are 7 known cullin proteins, cullin 1 (also referred to herein as CUL1), cullin 2 (also referred to herein as CUL2), cullin 3 (also referred to herein as CUL3), cullin 4A (also referred to herein as CUL4A), cullin 4B (also referred to herein as CUL4B), cullin 5 (also referred to herein as CUL5), and cullin 7 (also referred to herein as CUL7). The amino acid sequences of human CUL1, CUL2, CUL3, CUL4A, CUL4B,
 30 CUL5, and CUL7 are set forth herein as SEQ ID NOs: 79, 80, 81, 82, 83, 84, and 85, respectively. The term “cullin” also refers to the *S. cerevisiae* Cdc53 ortholog. The yeast Cdc53 protein has a C-terminal domain comprised of several subdomains: a four-helix bundle (4HB) that connects to the N-terminal domain, an α/β -subdomain that binds Rbx1’s N-terminal strand, and a C-terminal winged-helix subdomain (WHB) that contains

the Rub1 acceptor Lys760. Cdc53's N-terminal region binds Skp1-F box protein complexes, which recruit substrates for ubiquitination and the Cdc53's C-terminal region binds the RING protein Hrt1 (Kamura *et al.* (1999) *Science* 284:657-661; Ohta *et al.* (1999) *Mol Cell* 3:535-541; Seol *et al.* (1999) *Genes Dev* 13:1614-1626; Skowyra *et al.* (1999) *Science* 284:662-665; Tan *et al.* (1999) *Mol Cell* 3:527-533; each of which are herein incorporated by reference in its entirety). The WHB subdomain of cullin proteins are important for Dcn1 binding (Kurz *et al.* (2008) *Mol Cell* 29:23-35).

The presently disclosed invention involves biologically active fragments and variants of the various NEDD8 E1, E2, E3, or co-E3 proteins, or of peptides that bind to the E2-binding pocket of co-E3 proteins and optionally inhibit its activity. As used herein "biologically active" fragments or variants of a polypeptide or a nucleotide sequence encoding the same that is useful in the methods of the invention retains substantially the same function as the respective native sequence. For example, a biologically active fragment or variant of a NEDD8 E2 enzyme is one that retains the ability to transiently bind a NEDD8 E1 enzyme for generation of a thiolester-linked E2~UBL intermediate and interact with a NEDD8 E3 ligase as an E2~UBL intermediate. Such nucleotide sequence fragments can comprise at least about 10, at least about 15, at least about 20, at least about 50, at least about 60, at least about 80, at least about 100, at least about 150, at least about 200, at least about 300, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1,000, at least about 1,500, at least about 2,000, at least about 2,500, at least about 3,000, at least about 3,500, at least about 4,000, at least about 4,500, at least about 5,000 contiguous nucleotides or up to the entire contiguous nucleotides of the nucleotide sequence of interest. Biologically active polypeptide fragments can comprise at least about 5, at least about 10, at least about 15, at least about 20, at least about 50, at least about 60, at least about 80, at least about 100, at least about 150, at least about 200, at least about 300, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1,000 contiguous amino acid residues or up to the entire contiguous amino acid residues of the polypeptide. Methods for obtaining such fragments are known in the art and are described in further detail elsewhere herein.

By "variant" is intended substantially similar sequences. Thus, for nucleotide sequences or amino acid sequences, variants include sequences that are functionally equivalent to the nucleotide sequence of interest. Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example,

by site directed mutagenesis but which still retain the function of the native sequence.

Generally, nucleotide sequence variants or amino acid sequence variants of the invention will have at least 40%, at least about 50%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%,
5 at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to its respective native nucleotide or amino acid sequence. As discussed elsewhere herein, variants of the nucleotide sequences can encode amino acid sequences that differ conservatively because of the degeneracy of the genetic code. Methods of determining sequence identity are also
10 discussed elsewhere herein.

With respect to the amino acid sequences for the various full length polypeptides, variants include those polypeptides that are derived from the native polypeptides by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native polypeptide; deletion or addition of one or more amino
15 acids at one or more sites in the native polypeptide; or substitution of one or more amino acids at one or more sites in the native polypeptide. Such variants may result from, for example, genetic polymorphism or from human manipulation. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing
20 Company, New York); Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods Enzymol.* 154:367-382; Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York); U.S. Patent No. 4,873,192; and the references cited therein; herein incorporated by reference. Guidance as to appropriate amino acid substitutions that may not affect
25 biological activity of the various vector polypeptide may be found in the model of Dayhoff *et al.* (1978) *Atlas of Polypeptide Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred. The deletions, insertions, and substitutions of the protein sequences encompassed herein are
30 not expected to produce radical changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays.

By "sequence identity" is intended the same nucleotides or amino acid residues are found within the variant sequence and a reference sequence when a specified, contiguous segment of the nucleotide sequence or amino acid sequence of the variant is aligned and compared to the nucleotide sequence or amino acid sequence of the reference sequence.

5 Methods for sequence alignment and for determining identity between sequences are well known in the art. With respect to optimal alignment of two nucleotide sequences, the contiguous segment of the variant nucleotide sequence may have additional nucleotides or deleted nucleotides with respect to the reference nucleotide sequence. Likewise, for purposes of optimal alignment of two amino acid sequences, the contiguous segment of
10 the variant amino acid sequence may have additional amino acid residues or deleted amino acid residues with respect to the reference amino acid sequence. The contiguous segment used for comparison to the reference nucleotide sequence or reference amino acid sequence will comprise at least 20 contiguous nucleotides, or amino acid residues, and may be 30, 40, 50, 100, or more nucleotides or amino acid residues. Corrections for
15 increased sequence identity associated with inclusion of gaps in the variant's nucleotide sequence or amino acid sequence can be made by assigning gap penalties. Methods of sequence alignment are well known in the art.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, percent identity of an amino acid sequence
20 can be determined using the Smith-Waterman homology search algorithm using an affine 6 gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix 62. Alternatively, percent identity of a nucleotide sequence is determined using the Smith-Waterman homology search algorithm using a gap open penalty of 25 and a gap extension penalty of 5. Such a determination of sequence identity can be performed using,
25 for example, the DeCypher Hardware Accelerator from TimeLogic Version G. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) *Adv. Appl. Math* 2:482-489, herein incorporated by reference. Alternatively, the alignment program GCG Gap (Wisconsin Genetic Computing Group, Suite Version 10.1) using the default parameters may be used. The GCG Gap program applies the Needleman
30 and Wunch algorithm and for the alignment of nucleotide sequences with an open gap penalty of 3 and an extend gap penalty of 1 may be used. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an

algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences having sufficient sequence identity. BLAST protein searches can be performed with the

5 XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences having sufficient sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing

10 BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program

15 (version 2.0), which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Provided herein (see Examples 1 and Table 24) and in Scott *et al.* (2011) *Science* 334:674-678 and supporting online material, which can be found at

20 www.sciencemag.org/content/suppl/2011/09/21/science.1209307.DC1.html (Scott *et al.* and its supporting online material is herein incorporated by reference in its entirety), is a description of the production and analysis of crystals of four NEDD8 E2/co-E3 complexes: (1) the PONY domain of *S. cerevisiae* Dcn1 and N-terminally acetylated *S. cerevisiae* Ubc12¹⁻²⁴; (2) human Cul1^{WHB}, the PONY domain of hDcn1 (Dcn1^P), and N-

25 terminally acetylated human Ubc12¹⁻¹⁵; (3) human Cul1^{WHB}, human Dcn1^P, and N-terminally acetylated human Ubc12¹⁻¹² comprising a hydrophobic staple between residues 5 and 9; and (4) the PONY domain of human DCUN1D3 and N-terminally acetylated human Ube2f¹⁻²⁵. The solved structures of (1) the PONY domain of *S. cerevisiae* Dcn1 and N-terminally acetylated *S. cerevisiae* Ubc12¹⁻²⁴; (2) human Cul1^{WHB}, the PONY

30 domain of hDcn1 (Dcn1^P), and N-terminally acetylated human Ubc12¹⁻¹⁵; and (3) human Cul1^{WHB}, human Dcn1^P, and N-terminally acetylated human Ubc12¹⁻¹² comprising a hydrophobic staple between residues 5 and 9 have been deposited in the Structural Bioinformatics Research Collaboratory for Structural Bioinformatics Protein Data Bank as

accession numbers 3TDI, 3TDU, and 3TDZ, respectively, and each of which are herein incorporated by reference in its entirety.

As used herein, the terms "molecular structure" refer to the arrangement of atoms within a particular object (e.g., polypeptide). A three-dimensional molecular structure of a polypeptide is a representation of the tertiary or quaternary structure of the polypeptide.

As used herein, the term "atomic coordinates" refers to mathematical coordinates (represented as "X," "Y" and "Z" values) that describe the positions of atoms in a crystal of a polypeptide with respect to a chosen crystallographic origin. As used herein, the term "crystallographic origin" refers to a reference point in the crystal unit cell with respect to the crystallographic symmetry operation. These atomic coordinates can be used to generate a three-dimensional representation of the molecular structure of the polypeptide. When referring to an E2-binding pocket having a particular set of atomic coordinates of a particular table provided herein, this refers to both the atom itself as well as its "X", "Y", and "Z" values.

Analysis of the four solved three-dimensional molecular structures led to the identification of a novel NEDD8 E2-binding pocket within NEDD8 co-E3 proteins. As used herein, an "E2-binding pocket" or "NEDD8 E2-binding pocket" of a NEDD8 co-E3 protein is comprised of the atoms of the NEDD8 co-E3 protein that interact with a NEDD8 E2 protein, and in some embodiments, additional atoms of the amino acids comprising the interacting atoms. Those atoms of the NEDD8 co-E3 protein that interact with a NEDD8 E2 protein are those atoms that form a bond (e.g., hydrogen bond, van der Waals interactions), with an atom of a NEDD8 E2 protein.

In some embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises the atoms of Table 1, which are found in amino acid residues Ile90, Leu93, Val94, Ile97, Leu104, Glu105, Asp106, Leu107, Thr109, Leu110, Ala113, Leu121, Glu122, Leu173, Ile174, Leu175, Asp176, Gln189, Tyr190, and Leu193 of *S. cerevisiae* Dcn1 (SEQ ID NO: 48), or a structural variant thereof. In particular embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises at least one of the following amino acid residues: an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine

corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, and a leucine corresponding to position 193 of SEQ ID NO: 48.

As used herein, an amino acid residue of a NEDD8 co-E3 protein at the position corresponding to a particular amino acid residue of SEQ ID NO: 33, 36, 39, 42, 45, or 48 refers to the amino acid residue within the NEDD8 co-E3 protein that appears opposite the amino acid residue at a particular position in SEQ ID NO: 33, 36, 39, 42, 45, or 48 when the NEDD8 co-E3 protein sequence is aligned with SEQ ID NO: 33, 36, 39, 42, 45, or 48 for maximum homology using an alignment program, such as one known in the art (e.g., the GAP program in the GCG software package, using either a BLOSUM62 matrix or a PAM250 matrix).

In some of these embodiments, the E2-binding pocket comprises the atomic coordinates of Table 2 or 3, or a structural variant thereof. In particular embodiments, the co-E3 protein has the amino acid sequence set forth in SEQ ID NO: 48.

In certain embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises the atoms of Table 1 and Table 4. The atoms in Table 4 are found in amino acid residues Asn84, Asp89, Asp91, Gly101, Tyr102, Asn103, and Glu186 of SEQ ID NO: 48. Thus, in some embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises at least one of the following amino acid residues: an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine

corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, a leucine corresponding to position 193 of SEQ ID NO: 48, an asparagine corresponding to position 84 of SEQ ID NO: 48; an aspartic acid corresponding to position 89 of SEQ ID NO: 48, an aspartic acid corresponding to position 91 of SEQ ID NO: 48, a glycine corresponding to position 101 of SEQ ID NO: 48, a tyrosine corresponding to position 102 of SEQ ID NO: 48, an asparagine corresponding to position 103 of SEQ ID NO: 48, and a glutamic acid corresponding to position 186 of SEQ ID NO: 48.

In some of these embodiments, the E2-binding pocket comprises the atomic coordinates of Table 2 and 5 or a structural variant thereof. In other embodiments, the E2-binding pocket comprises the atomic coordinates of Table 3 and 6, or a structural variant thereof.

Table 1. Minimal contacts between *S. cerevisiae* Dcn1 and *S. cerevisiae* Ubc12 with yUbc12 Chain C interactions with yDcn1^P Chain A and yUbc12 Chain D interactions with yDcn1^P Chain B shown.

yUbc12 Atom	yDcn1 Atom	Distance (Å)
Acetyl-Met 1C O	Leu 104A CB	4.01
	Leu 104A O	4.1
	Leu 104A CD2	4.17
Acetyl-Met 1C C	Leu 104A O	3.82
	Tyr 190A OH	4.08
Acetyl-Met 1C CA	Leu 104A O	3.58
	Tyr 190A OH	4.44
	Thr 109A OG1	4.21
Acetyl-Met 1C N	Leu 104A C	3.94
	Leu 104A O	2.73
	Thr 109A OG1	3.31
	Thr 109A CB	4.46
	Tyr 190A CE1	3.9
Acetyl-Met 1C CT1	Leu 104A O	3.61
	Tyr 190A OH	4.34
	Thr 109A OG1	3.44
	Leu 110A CD2	4.36
	Leu 110A CG	4.16
Acetyl-Met 1C OT	Tyr 190A CD1	4.33
	Tyr 190A CE1	3.29
	Tyr 190A CZ	3.96

	Tyr 190A OH	3.71
	Thr 109A OG1	4.26
	Leu 110A CD2	3.57
	Leu 110A CG	3.72
	Leu 110A CD1	4.26
Acetyl-Met 1C CT2	Tyr 190A CE1	4.3
	Leu 107A CD2	3.9
	Leu 104A O	3.55
	Glu 105A C	4.32
	Glu 105A O	4.27
	Asp 106A C	4.13
	Asp 106A O	3.63
	Leu 107A N	4.37
	Leu 107A CA	4.1
	Thr 109A OG1	3.29
	Leu 110A CG	4.07
	Leu 110A CD1	4.36
Acetyl-Met 1C CB	Leu 104A CB	4.45
	Leu 104A O	3.65
	Thr 109A OG1	3.9
	Thr 109A CB	4.38
Acetyl-Met 1C CG	Leu 104A O	4.32
	Thr 109A OG1	3.11
	Ile 97A CD1	4.01
	Thr 109A CB	3.36
	Thr 109A CG2	4.25
	Thr 109A C	4.41
	Thr 109A O	4.36
Acetyl-Met 1C SD	Leu 121A CD1	3.76
	Thr 109A OG1	4.44
	Leu 121A CG	4.44
	Leu 121A CD2	3.83
	Thr 109A O	4.39
Acetyl-Met 1C CE	Leu 121A CD1	4.02
	Thr 109A OG1	4.42
	Leu 110A CD2	3.5
	Leu 173A CD1	3.68
	Leu 110A N	4.36
	Leu 110A CA	4.16
	Leu 110A CG	4.29
	Leu 121A CD2	4.07
	Thr 109A C	4.27
	Ala 113A CB	3.74
	Thr 109A O	3.9
Leu 2C N	Tyr 190A CE1	4.48
	Tyr 190A CZ	4.15
	Leu 104A O	4.35
	Tyr 190A OH	3.13
Leu 2C CA	Tyr 190A OH	3.59
Leu 2C CB	Gln 189A NE2	4.2

	Tyr 190A CE1	4.43
	Tyr 190A CZ	3.85
	Tyr 190A CE2	4.43
	Tyr 190A OH	3.31
Leu 2C CG	Gln 189A NE2	4.49
	Tyr 190A CE1	3.97
	Tyr 190A CZ	3.87
	Leu 193A CD1	4.27
	Tyr 190A OH	3.75
Leu 2C CD1	Gln 189A CB	4.19
	Gln 189A NE2	3.5
	Tyr 190A CE1	4.16
	Tyr 190A CZ	4.07
	Tyr 190A CE2	4.35
	Leu 193A CD1	3.87
	Tyr 190A OH	4.38
Leu 2C CD2	Leu 193A CD1	4.35
	Leu 104A O	4.32
	Glu 105A N	4.5
	Glu 105A CA	3.87
	Glu 105A CB	3.94
	Glu 105A CG	3.74
Leu 2C C	Tyr 190A OH	3.89
Lys 3C N	Tyr 190A OH	3.59
Lys 3C CB	Leu 121A CD1	4.45
	Tyr 190A OH	4.29
Lys 3C CD	Ile 174A CA	4.25
	Ile 174A C	4.32
	Ile 174A O	3.82
	Leu 173A O	3.41
	Leu 173A C	4.44
Lys 3C CE	Ile 174A C	4.41
	Ile 174A O	3.77
	Glu 122A OE2	3.24
	Leu 175A O	4.18
	Asp 176A OD1	3.87
	Leu 173A O	3.62
	Glu 122A CD	4.13
Lys 3C NZ	Asp 176A N	4.04
	Asp 176A CB	4.24
	Asp 176A CG	3.45
	Asp 176A OD2	4.25
	Ile 174A C	4.41
	Ile 174A O	3.45
	Glu 122A OE2	3.71
	Leu 175A C	4.28
	Leu 175A O	4.15
	Asp 176A CA	3.81
	Asp 176A OD1	2.45
Leu 4C CB	Val 94A CG2	4.49

	Leu 104A CD2	4.41
Leu 4C CG	Val 94A CG2	4.16
Leu 4C CD1	Leu 121A CD1	3.91
	Ile 90A CG2	3.86
	Leu 121A CB	4.03
	Leu 121A CG	4.3
	Leu 93A CD2	4.49
	Leu 121A CD2	4.38
Leu 4C CD2	Val 94A CG2	4.26
	Leu 93A CD2	4.31
	Ile 97A CD1	4.05
	Leu 93A CG	4.35
Acetyl-Met 1D O	Leu 104B CD2	4.46
	Tyr 190B OH	4.22
	Leu 104B O	4.38
	Leu 104B CB	3.84
	Leu 104B CG	4.35
	Leu 104B CD1	4.14
Acetyl-Met 1D C	Tyr 190B OH	3.32
	Leu 104B C	4.47
	Leu 104B O	3.71
	Leu 104B CB	4.09
	Tyr 190B CZ	4.45
Acetyl-Met 1D CA	Tyr 190B OH	3.45
	Leu 104B O	3.61
	Thr 109B OG1	4.13
Acetyl-Met 1D N	Tyr 190B OH	3.67
	Leu 104B C	4.01
	Leu 104B O	2.84
	Thr 109B OG1	3.38
	Tyr 190B CZ	4.37
	Tyr 190B CE1	4.31
Acetyl-Met 1D CT1	Leu 110B CG	3.8
	Leu 110B CD1	4.17
	Leu 110B CD2	4.41
	Tyr 190B OH	3.58
	Leu 104B O	3.81
	Thr 109B OG1	3.55
	Tyr 190B CZ	3.97
	Tyr 190B CE1	3.52
Acetyl-Met 1D OT	Leu 110B CB	4.48
	Leu 110B CG	3.18
	Leu 110B CD1	3.64
	Leu 110B CD2	3.44
	Tyr 190B OH	3.24
	Thr 109B OG1	4.24
	Tyr 190B CZ	3.66
	Tyr 190B CD1	4.35
	Tyr 190B CE1	3.17
Acetyl-Met 1D CT2	Leu 110B CG	3.84

	Leu 110B CD1	3.84
	Leu 107B CA	3.96
	Leu 104B O	3.81
	Thr 109B OG1	3.62
	Asp 106B C	4.18
	Asp 106B O	3.68
	Leu 107B N	4.31
	Leu 107B CD2	3.95
	Tyr 190B CE1	3.87
	Leu 193B CD1	4.19
Acetyl-Met 1D CB	Thr 109B CB	4.21
	Leu 104B CD2	4.23
	Leu 104B C	4.45
	Leu 104B O	3.72
	Thr 109B OG1	3.58
	Leu 104B CA	4.28
	Leu 104B CB	4.19
Acetyl-Met 1D CG	Thr 109B C	4.33
	Thr 109B O	4.2
	Ile 97B CD1	3.98
	Thr 109B CB	3.55
	Thr 109B OG1	3.19
Acetyl-Met 1D SD	Leu 121B CD2	4.09
	Leu 121B CD1	3.91
Acetyl-Met 1D CE	Ala 113B CB	3.73
	Thr 109B C	4.42
	Thr 109B O	3.96
	Leu 121B CD2	4.21
	Leu 173B CD1	3.7
	Leu 110B CA	4.26
	Leu 110B CG	4.24
	Leu 110B CD2	3.58
	Leu 121B CD1	4.35
Leu 2D N	Tyr 190B OH	2.85
	Leu 104B O	3.88
	Tyr 190B CZ	3.74
	Tyr 190B CE2	4.34
Leu 2D CA	Tyr 190B OH	3.65
	Tyr 190B CZ	4.37
Leu 2D CB	Tyr 190B OH	3.86
	Tyr 190B CZ	4.08
	Tyr 190B CE2	3.87
Leu 2D CG	Tyr 190B OH	4.39
	Tyr 190B CZ	4.31
	Tyr 190B CE2	4.21
	Leu 193B CD1	4.05
Leu 2D CD1	Tyr 190B CE2	4.27
	Tyr 190B CD2	4.41
	Gln 189B O	4.16
	Leu 193B CD1	3.98

	Gln 189B CB	4.05
	Gln 189B C	4.36
Leu 2D CD2	Leu 104B C	4.33
	Leu 104B O	4.17
	Glu 105B CA	4
	Glu 105B N	4.22
	Glu 105B CB	4.46
	Glu 105B CG	3.87
	Leu 193B CD1	4.37
Leu 2D C	Tyr 190B OH	3.82
Lys 3D N	Tyr 190B OH	3.01
	Tyr 190B CZ	3.98
	Tyr 190B CE2	4.04
Lys 3D CA	Tyr 190B OH	3.92
Lys 3D CB	Leu 121B CD1	4.22
	Tyr 190B OH	3.63
Lys 3D CG	Leu 173B O	4.25
	Leu 121B CD1	4
Lys 3D CD	Leu 173B O	4.3
	Ile 174B C	4.29
	Ile 174B CA	4.31
	Ile 174B O	3.58
	Asp 176B OD1	4
Lys 3D CE	Leu 173B O	4.42
	Glu 122B OE2	4.11
	Ile 174B C	4.47
	Ile 174B O	3.64
	Asp 176B CG	4.26
	Asp 176B OD1	3.08
Lys 3D NZ	Leu 173B O	3.4
	Leu 175B O	3.56
	Glu 122B CD	3.93
	Glu 122B OE2	2.93
	Ile 174B C	4.15
	Leu 175B C	4.08
	Ile 174B O	3.64
	Asp 176B N	4.29
	Asp 176B CA	4.18
	Asp 176B OD1	3.72
Leu 4D N	Leu 121B CD1	4.4
Leu 4D CG	Ile 90B CG2	4.02
	Ile 90B O	4.47
	Val 94B CG2	3.81
Leu 4D CD1	Ile 90B CG1	4.29
	Leu 121B CB	4.16
	Leu 93B CD2	4.43
	Leu 121B CD2	4.33
	Ile 90B CB	4.4
	Ile 90B CG2	3.7
	Leu 121B CG	4.39

	Leu 121B CD1	4.07
Leu 4D CD2	Leu 93B CB	4.15
	Leu 93B CG	3.79
	Leu 93B CD2	3.77
	Ile 97B CD1	4.02
	Val 94B N	4.34
	Val 94B CG2	3.86
Leu 4D O	Ile 90B CG2	3.87

Table 2. Atomic coordinates of amino acid residues of yDcnI Chain B essential for binding yUbc12.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
5	ATOM 187	N ILE B 90	-17.000	-3.153	-25.375	1.00	26.78
	ATOM 188	CA ILE B 90	-15.892	-2.790	-24.488	1.00	27.28
	ATOM 189	CB ILE B 90	-14.690	-2.236	-25.299	1.00	27.75
	ATOM 190	CG1 ILE B 90	-14.046	-3.360	-26.125	1.00	28.57
10	ATOM 191	CD1 ILE B 90	-13.276	-2.873	-27.351	1.00	31.07
	ATOM 192	CG2 ILE B 90	-13.656	-1.614	-24.375	1.00	28.76
	ATOM 193	C ILE B 90	-16.331	-1.797	-23.407	1.00	27.14
	ATOM 194	O ILE B 90	-15.941	-1.928	-22.242	1.00	27.17
	ATOM 209	N LEU B 93	-18.305	-3.687	-20.756	1.00	23.07
15	ATOM 210	CA LEU B 93	-17.487	-4.475	-19.843	1.00	22.86
	ATOM 211	CB LEU B 93	-16.484	-5.377	-20.591	1.00	22.77
	ATOM 212	CG LEU B 93	-15.645	-6.359	-19.739	1.00	23.37
	ATOM 213	CD1 LEU B 93	-16.488	-7.155	-18.740	1.00	23.06
	ATOM 214	CD2 LEU B 93	-14.825	-7.312	-20.618	1.00	23.28
20	ATOM 215	C LEU B 93	-16.802	-3.612	-18.784	1.00	22.47
	ATOM 216	O LEU B 93	-16.760	-3.987	-17.614	1.00	22.13
	ATOM 217	N VAL B 94	-16.280	-2.461	-19.207	1.00	22.50
	ATOM 218	CA VAL B 94	-15.661	-1.472	-18.311	1.00	22.44
	ATOM 219	CB VAL B 94	-15.067	-0.282	-19.111	1.00	22.56
25	ATOM 220	CG1 VAL B 94	-14.565	0.820	-18.189	1.00	21.37
	ATOM 221	CG2 VAL B 94	-13.932	-0.771	-20.001	1.00	23.01
	ATOM 222	C VAL B 94	-16.636	-0.969	-17.241	1.00	22.81
	ATOM 223	O VAL B 94	-16.310	-0.957	-16.049	1.00	22.30
	ATOM 244	N ILE B 97	-17.365	-3.889	-14.797	1.00	23.81
30	ATOM 245	CA ILE B 97	-16.271	-4.009	-13.840	1.00	24.10
	ATOM 246	CB ILE B 97	-14.901	-3.980	-14.553	1.00	23.81
	ATOM 247	CG1 ILE B 97	-14.670	-5.292	-15.301	1.00	21.97
	ATOM 248	CD1 ILE B 97	-13.777	-5.147	-16.491	1.00	19.97
	ATOM 249	CG2 ILE B 97	-13.760	-3.721	-13.556	1.00	23.53
35	ATOM 250	C ILE B 97	-16.347	-2.919	-12.769	1.00	24.99
	ATOM 251	O ILE B 97	-16.207	-3.208	-11.577	1.00	24.71
	ATOM 302	N LEU B 104	-9.556	-1.736	-13.282	1.00	30.61
	ATOM 303	CA LEU B 104	-9.073	-2.533	-14.405	1.00	30.52
	ATOM 304	CB LEU B 104	-8.981	-1.689	-15.675	1.00	30.40
40	ATOM 305	CG LEU B 104	-10.217	-0.909	-16.135	1.00	30.20
	ATOM 306	CD1 LEU B 104	-9.854	-0.018	-17.301	1.00	28.88

	ATOM	307	CD2	LEU B 104	-11.395	-1.826	-16.495	1.00	30.08
	ATOM	308	C	LEU B 104	-7.719	-3.189	-14.112	1.00	30.86
	ATOM	309	O	LEU B 104	-7.334	-4.139	-14.789	1.00	31.23
5	ATOM	310	N	GLU B 105	-7.008	-2.677	-13.107	1.00	30.82
	ATOM	311	CA	GLU B 105	-5.735	-3.255	-12.660	1.00	30.75
	ATOM	312	CB	GLU B 105	-4.947	-2.250	-11.801	1.00	31.01
	ATOM	313	CG	GLU B 105	-4.454	-1.002	-12.547	1.00	34.35
	ATOM	314	CD	GLU B 105	-5.495	0.131	-12.635	1.00	38.44
10	ATOM	315	OE1	GLU B 105	-6.630	-0.025	-12.121	1.00	39.33
	ATOM	316	OE2	GLU B 105	-5.166	1.192	-13.223	1.00	39.74
	ATOM	317	C	GLU B 105	-5.939	-4.552	-11.871	1.00	29.84
	ATOM	318	O	GLU B 105	-4.991	-5.313	-11.687	1.00	29.75
	ATOM	319	N	ASP B 106	-7.164	-4.787	-11.393	1.00	28.79
15	ATOM	320	CA	ASP B 106	-7.466	-5.960	-10.575	1.00	27.99
	ATOM	321	CB	ASP B 106	-8.806	-5.800	-9.845	1.00	28.43
	ATOM	322	CG	ASP B 106	-8.778	-4.721	-8.757	1.00	30.42
	ATOM	323	OD1	ASP B 106	-7.717	-4.520	-8.116	1.00	32.67
	ATOM	324	OD2	ASP B 106	-9.836	-4.082	-8.527	1.00	31.35
20	ATOM	325	C	ASP B 106	-7.492	-7.233	-11.420	1.00	27.00
	ATOM	326	O	ASP B 106	-8.260	-7.336	-12.384	1.00	26.52
	ATOM	327	N	LEU B 107	-6.651	-8.199	-11.051	1.00	25.92
	ATOM	328	CA	LEU B 107	-6.592	-9.494	-11.756	1.00	25.10
	ATOM	329	CB	LEU B 107	-5.474	-10.388	-11.204	1.00	25.01
	ATOM	330	CG	LEU B 107	-4.012	-10.030	-11.483	1.00	25.41
25	ATOM	331	CD1	LEU B 107	-3.132	-11.160	-11.002	1.00	26.14
	ATOM	332	CD2	LEU B 107	-3.759	-9.781	-12.962	1.00	25.10
	ATOM	333	C	LEU B 107	-7.926	-10.248	-11.743	1.00	24.17
	ATOM	334	O	LEU B 107	-8.231	-10.968	-12.688	1.00	23.94
	ATOM	340	N	THR B 109	-10.630	-9.254	-12.532	1.00	20.74
30	ATOM	341	CA	THR B 109	-11.358	-8.910	-13.749	1.00	20.00
	ATOM	342	CB	THR B 109	-11.009	-7.506	-14.241	1.00	20.13
	ATOM	343	OG1	THR B 109	-9.607	-7.447	-14.529	1.00	21.02
	ATOM	344	CG2	THR B 109	-11.372	-6.445	-13.185	1.00	19.14
	ATOM	345	C	THR B 109	-11.098	-9.920	-14.870	1.00	19.91
35	ATOM	346	O	THR B 109	-11.932	-10.086	-15.768	1.00	19.52
	ATOM	347	N	LEU B 110	-9.940	-10.587	-14.809	1.00	19.41
	ATOM	348	CA	LEU B 110	-9.612	-11.680	-15.716	1.00	18.82
	ATOM	349	CB	LEU B 110	-8.118	-11.996	-15.665	1.00	18.81
	ATOM	350	CG	LEU B 110	-7.233	-11.177	-16.602	1.00	19.63
40	ATOM	351	CD1	LEU B 110	-5.757	-11.366	-16.250	1.00	18.67
	ATOM	352	CD2	LEU B 110	-7.519	-11.548	-18.066	1.00	18.09
	ATOM	353	C	LEU B 110	-10.410	-12.941	-15.406	1.00	18.59
	ATOM	354	O	LEU B 110	-10.817	-13.646	-16.324	1.00	17.95
	ATOM	369	N	ALA B 113	-13.765	-12.239	-16.984	1.00	18.55
45	ATOM	370	CA	ALA B 113	-13.760	-12.421	-18.436	1.00	18.75
	ATOM	371	CB	ALA B 113	-12.557	-11.717	-19.068	1.00	18.05
	ATOM	372	C	ALA B 113	-13.772	-13.909	-18.784	1.00	19.11
	ATOM	373	O	ALA B 113	-14.497	-14.348	-19.687	1.00	18.94
	ATOM	434	N	LEU B 121	-11.230	-9.617	-26.513	1.00	30.79
50	ATOM	435	CA	LEU B 121	-11.866	-8.449	-25.908	1.00	31.55

	ATOM	436	CB	LEU B 121	-10.860	-7.659	-25.075	1.00	31.43
	ATOM	437	CG	LEU B 121	-10.332	-8.355	-23.817	1.00	31.12
	ATOM	438	CD1	LEU B 121	-9.213	-7.527	-23.191	1.00	30.04
	ATOM	439	CD2	LEU B 121	-11.457	-8.632	-22.807	1.00	30.44
5	ATOM	440	C	LEU B 121	-12.509	-7.548	-26.950	1.00	32.31
	ATOM	441	O	LEU B 121	-13.418	-6.783	-26.638	1.00	32.69
	ATOM	442	N	GLU B 122	-12.036	-7.659	-28.188	1.00	33.07
	ATOM	443	CA	GLU B 122	-12.507	-6.842	-29.293	1.00	33.86
	ATOM	444	CB	GLU B 122	-11.527	-6.931	-30.474	1.00	34.09
10	ATOM	445	CG	GLU B 122	-10.065	-6.525	-30.148	1.00	35.66
	ATOM	446	CD	GLU B 122	-9.214	-7.651	-29.515	1.00	37.96
	ATOM	447	OE1	GLU B 122	-9.750	-8.745	-29.236	1.00	38.95
	ATOM	448	OE2	GLU B 122	-7.993	-7.440	-29.300	1.00	38.39
	ATOM	449	C	GLU B 122	-13.922	-7.244	-29.727	1.00	34.10
15	ATOM	450	O	GLU B 122	-14.689	-6.403	-30.199	1.00	34.37
	ATOM	874	N	LEU B 173	-5.876	-13.081	-24.295	1.00	27.16
	ATOM	875	CA	LEU B 173	-6.626	-11.828	-24.339	1.00	27.91
	ATOM	876	CB	LEU B 173	-7.349	-11.582	-23.011	1.00	27.86
	ATOM	877	CG	LEU B 173	-8.606	-12.381	-22.684	1.00	26.53
20	ATOM	878	CD1	LEU B 173	-9.208	-11.801	-21.423	1.00	23.89
	ATOM	879	CD2	LEU B 173	-9.605	-12.329	-23.848	1.00	25.91
	ATOM	880	C	LEU B 173	-5.762	-10.612	-24.635	1.00	28.87
	ATOM	881	O	LEU B 173	-6.252	-9.630	-25.203	1.00	29.01
	ATOM	882	N	ILE B 174	-4.494	-10.655	-24.222	1.00	29.78
25	ATOM	883	CA	ILE B 174	-3.571	-9.545	-24.473	1.00	30.66
	ATOM	884	CB	ILE B 174	-2.274	-9.654	-23.640	1.00	30.37
	ATOM	885	CG1	ILE B 174	-2.571	-9.533	-22.145	1.00	30.97
	ATOM	886	CD1	ILE B 174	-1.443	-10.039	-21.251	1.00	31.49
	ATOM	887	CG2	ILE B 174	-1.287	-8.569	-24.026	1.00	30.73
30	ATOM	888	C	ILE B 174	-3.230	-9.489	-25.958	1.00	31.61
	ATOM	889	O	ILE B 174	-2.929	-8.415	-26.494	1.00	31.77
	ATOM	890	N	LEU B 175	-3.279	-10.649	-26.616	1.00	32.57
	ATOM	891	CA	LEU B 175	-3.011	-10.740	-28.046	1.00	33.68
	ATOM	892	CB	LEU B 175	-3.232	-12.164	-28.553	1.00	33.40
35	ATOM	893	CG	LEU B 175	-2.038	-13.108	-28.646	1.00	33.23
	ATOM	894	CD1	LEU B 175	-2.475	-14.462	-29.171	1.00	32.15
	ATOM	895	CD2	LEU B 175	-0.973	-12.517	-29.552	1.00	33.47
	ATOM	896	C	LEU B 175	-3.915	-9.793	-28.806	1.00	34.64
	ATOM	897	O	LEU B 175	-5.136	-9.836	-28.634	1.00	35.03
40	ATOM	898	N	ASP B 176	-3.314	-8.918	-29.614	1.00	35.77
	ATOM	899	CA	ASP B 176	-4.084	-8.053	-30.510	1.00	36.75
	ATOM	900	CB	ASP B 176	-3.204	-6.954	-31.120	1.00	37.22
	ATOM	901	CG	ASP B 176	-2.718	-5.939	-30.098	1.00	38.70
	ATOM	902	OD1	ASP B 176	-3.105	-6.022	-28.908	1.00	41.31
45	ATOM	903	OD2	ASP B 176	-1.935	-5.044	-30.492	1.00	40.73
	ATOM	904	C	ASP B 176	-4.670	-8.929	-31.614	1.00	36.97
	ATOM	905	O	ASP B 176	-4.183	-10.046	-31.831	1.00	37.27
	ATOM	1001	N	GLN B 189	3.796	-4.772	-18.447	1.00	20.32
	ATOM	1002	CA	GLN B 189	2.672	-4.221	-17.680	1.00	21.04
50	ATOM	1003	CB	GLN B 189	1.788	-3.313	-18.553	1.00	21.59

	ATOM	1004	CG	GLN B 189	2.471	-2.017	-19.012	1.00	24.86
	ATOM	1005	CD	GLN B 189	1.627	-1.176	-19.980	1.00	30.95
	ATOM	1006	OE1	GLN B 189	0.584	-1.617	-20.484	1.00	32.81
5	ATOM	1007	NE2	GLN B 189	2.091	0.057	-20.240	1.00	34.12
	ATOM	1008	C	GLN B 189	1.828	-5.307	-17.005	1.00	20.86
	ATOM	1009	O	GLN B 189	1.480	-5.169	-15.829	1.00	20.70
	ATOM	1010	N	TYR B 190	1.512	-6.387	-17.725	1.00	20.44
	ATOM	1011	CA	TYR B 190	0.788	-7.510	-17.097	1.00	20.64
	ATOM	1012	CB	TYR B 190	0.072	-8.398	-18.121	1.00	20.85
10	ATOM	1013	CG	TYR B 190	-1.295	-7.875	-18.511	1.00	22.90
	ATOM	1014	CD1	TYR B 190	-2.454	-8.460	-18.007	1.00	24.80
	ATOM	1015	CE1	TYR B 190	-3.720	-7.982	-18.364	1.00	25.31
	ATOM	1016	CZ	TYR B 190	-3.825	-6.906	-19.230	1.00	25.68
	ATOM	1017	OH	TYR B 190	-5.069	-6.425	-19.583	1.00	25.69
15	ATOM	1018	CE2	TYR B 190	-2.681	-6.306	-19.742	1.00	25.12
	ATOM	1019	CD2	TYR B 190	-1.430	-6.789	-19.384	1.00	23.39
	ATOM	1020	C	TYR B 190	1.634	-8.354	-16.138	1.00	20.10
	ATOM	1021	O	TYR B 190	1.133	-8.764	-15.094	1.00	19.57
	ATOM	1045	N	LEU B 193	1.535	-6.975	-12.748	1.00	20.07
20	ATOM	1046	CA	LEU B 193	0.316	-7.313	-12.019	1.00	20.06
	ATOM	1047	CB	LEU B 193	-0.922	-7.167	-12.909	1.00	20.10
	ATOM	1048	CG	LEU B 193	-1.217	-5.770	-13.476	1.00	20.30
	ATOM	1049	CD1	LEU B 193	-2.506	-5.798	-14.258	1.00	19.96
	ATOM	1050	CD2	LEU B 193	-1.263	-4.673	-12.390	1.00	20.81
25	ATOM	1051	C	LEU B 193	0.395	-8.711	-11.423	1.00	20.19
	ATOM	1052	O	LEU B 193	0.190	-8.887	-10.221	1.00	20.50

Table 3. Atomic coordinates of amino acid residues of yDcn1 Chain A essential for binding yUbc12.

30	Atom				X	Y	Z	Occu-pancy	B-factor
	No.		Atom Name						
	ATOM	1906	N	ILE A 90	-2.222	-45.427	-20.314	1.00	21.48
	ATOM	1907	CA	ILE A 90	-3.339	-45.857	-19.489	1.00	22.02
	ATOM	1908	CB	ILE A 90	-4.434	-46.463	-20.379	1.00	22.47
35	ATOM	1909	CG1	ILE A 90	-5.253	-45.340	-21.002	1.00	23.62
	ATOM	1910	CD1	ILE A 90	-6.077	-44.572	-19.976	1.00	26.80
	ATOM	1911	CG2	ILE A 90	-5.389	-47.303	-19.578	1.00	23.20
	ATOM	1912	C	ILE A 90	-2.935	-46.825	-18.384	1.00	21.85
	ATOM	1913	O	ILE A 90	-3.369	-46.679	-17.243	1.00	21.90
40	ATOM	1928	N	LEU A 93	-1.200	-44.808	-15.777	1.00	19.63
	ATOM	1929	CA	LEU A 93	-2.179	-44.094	-14.947	1.00	19.61
	ATOM	1930	CB	LEU A 93	-3.210	-43.349	-15.811	1.00	19.52
	ATOM	1931	CG	LEU A 93	-4.211	-42.444	-15.074	1.00	18.80
	ATOM	1932	CD1	LEU A 93	-3.517	-41.460	-14.123	1.00	16.51
45	ATOM	1933	CD2	LEU A 93	-5.095	-41.703	-16.067	1.00	18.64
	ATOM	1934	C	LEU A 93	-2.874	-45.028	-13.952	1.00	19.66
	ATOM	1935	O	LEU A 93	-2.998	-44.694	-12.775	1.00	19.34
	ATOM	1936	N	VAL A 94	-3.316	-46.191	-14.436	1.00	20.02
	ATOM	1937	CA	VAL A 94	-3.895	-47.231	-13.576	1.00	20.55

	ATOM	1938	CB	VAL	A	94	-4.275	-48.515	-14.381	1.00	20.23
	ATOM	1939	CG1	VAL	A	94	-4.684	-49.648	-13.453	1.00	19.91
	ATOM	1940	CG2	VAL	A	94	-5.409	-48.223	-15.353	1.00	20.95
5	ATOM	1941	C	VAL	A	94	-2.968	-47.587	-12.408	1.00	21.13
	ATOM	1942	O	VAL	A	94	-3.399	-47.571	-11.247	1.00	21.68
	ATOM	1963	N	ILE	A	97	-2.551	-44.631	-9.955	1.00	19.78
	ATOM	1964	CA	ILE	A	97	-3.723	-44.543	-9.089	1.00	20.30
	ATOM	1965	CB	ILE	A	97	-5.053	-44.675	-9.910	1.00	20.09
10	ATOM	1966	CG1	ILE	A	97	-5.296	-43.410	-10.745	1.00	19.51
	ATOM	1967	CD1	ILE	A	97	-6.192	-43.624	-11.960	1.00	19.12
	ATOM	1968	CG2	ILE	A	97	-6.261	-44.946	-8.997	1.00	20.08
	ATOM	1969	C	ILE	A	97	-3.635	-45.556	-7.938	1.00	21.00
	ATOM	1970	O	ILE	A	97	-3.840	-45.204	-6.761	1.00	20.37
15	ATOM	2021	N	LEU	A	104	-10.173	-47.353	-8.911	1.00	29.83
	ATOM	2022	CA	LEU	A	104	-10.542	-46.612	-10.123	1.00	30.31
	ATOM	2023	CB	LEU	A	104	-10.528	-47.522	-11.352	1.00	30.63
	ATOM	2024	CG	LEU	A	104	-9.261	-48.272	-11.763	1.00	31.62
	ATOM	2025	CD1	LEU	A	104	-8.933	-49.439	-10.812	1.00	33.32
20	ATOM	2026	CD2	LEU	A	104	-9.442	-48.785	-13.180	1.00	32.12
	ATOM	2027	C	LEU	A	104	-11.905	-45.940	-10.024	1.00	30.38
	ATOM	2028	O	LEU	A	104	-12.200	-45.008	-10.772	1.00	30.61
	ATOM	2029	N	GLU	A	105	-12.724	-46.408	-9.089	1.00	30.37
	ATOM	2030	CA	GLU	A	105	-14.089	-45.925	-8.944	1.00	30.32
	ATOM	2031	CB	GLU	A	105	-15.027	-47.060	-8.505	1.00	30.79
25	ATOM	2032	CG	GLU	A	105	-14.852	-48.382	-9.278	1.00	34.03
	ATOM	2033	CD	GLU	A	105	-13.910	-49.374	-8.588	1.00	37.92
	ATOM	2034	OE1	GLU	A	105	-13.067	-48.946	-7.770	1.00	40.04
	ATOM	2035	OE2	GLU	A	105	-14.018	-50.591	-8.863	1.00	39.49
	ATOM	2036	C	GLU	A	105	-14.181	-44.755	-7.969	1.00	29.38
30	ATOM	2037	O	GLU	A	105	-15.248	-44.154	-7.827	1.00	29.38
	ATOM	2038	N	ASP	A	106	-13.076	-44.453	-7.286	1.00	28.35
	ATOM	2039	CA	ASP	A	106	-13.009	-43.299	-6.390	1.00	27.37
	ATOM	2040	CB	ASP	A	106	-11.772	-43.351	-5.483	1.00	27.73
	ATOM	2041	CG	ASP	A	106	-11.780	-44.528	-4.520	1.00	28.81
35	ATOM	2042	OD1	ASP	A	106	-12.877	-44.964	-4.089	1.00	31.71
	ATOM	2043	OD2	ASP	A	106	-10.674	-45.011	-4.184	1.00	28.03
	ATOM	2044	C	ASP	A	106	-12.944	-42.039	-7.238	1.00	26.37
	ATOM	2045	O	ASP	A	106	-12.138	-41.952	-8.177	1.00	26.21
40	ATOM	2046	N	LEU	A	107	-13.788	-41.067	-6.903	1.00	25.02
	ATOM	2047	CA	LEU	A	107	-13.857	-39.806	-7.646	1.00	24.02
	ATOM	2048	CB	LEU	A	107	-15.111	-39.011	-7.251	1.00	24.13
	ATOM	2049	CG	LEU	A	107	-16.464	-39.654	-7.601	1.00	24.37
	ATOM	2050	CD1	LEU	A	107	-17.625	-38.816	-7.098	1.00	24.63
	ATOM	2051	CD2	LEU	A	107	-16.630	-39.928	-9.101	1.00	24.56
45	ATOM	2052	C	LEU	A	107	-12.589	-38.955	-7.544	1.00	23.14
	ATOM	2053	O	LEU	A	107	-12.312	-38.151	-8.430	1.00	23.15
	ATOM	2059	N	THR	A	109	-9.811	-39.869	-8.275	1.00	20.00
	ATOM	2060	CA	THR	A	109	-9.090	-40.122	-9.527	1.00	19.48
	ATOM	2061	CB	THR	A	109	-9.400	-41.516	-10.126	1.00	19.37
50	ATOM	2062	OG1	THR	A	109	-10.783	-41.600	-10.504	1.00	19.30

	ATOM	2063	CG2	THR	A	109	-9.065	-42.606	-9.129	1.00	20.66
	ATOM	2064	C	THR	A	109	-9.367	-39.047	-10.576	1.00	18.77
	ATOM	2065	O	THR	A	109	-8.517	-38.779	-11.418	1.00	18.55
5	ATOM	2066	N	LEU	A	110	-10.557	-38.449	-10.525	1.00	18.32
	ATOM	2067	CA	LEU	A	110	-10.897	-37.323	-11.405	1.00	18.09
	ATOM	2068	CB	LEU	A	110	-12.408	-37.051	-11.408	1.00	18.21
	ATOM	2069	CG	LEU	A	110	-13.322	-38.008	-12.173	1.00	19.07
	ATOM	2070	CD1	LEU	A	110	-14.784	-37.727	-11.833	1.00	19.91
10	ATOM	2071	CD2	LEU	A	110	-13.098	-37.862	-13.661	1.00	20.22
	ATOM	2072	C	LEU	A	110	-10.154	-36.050	-11.012	1.00	17.74
	ATOM	2073	O	LEU	A	110	-9.798	-35.262	-11.877	1.00	17.47
	ATOM	2088	N	ALA	A	113	-6.659	-36.664	-12.409	1.00	16.15
	ATOM	2089	CA	ALA	A	113	-6.724	-36.421	-13.847	1.00	16.33
15	ATOM	2090	CB	ALA	A	113	-7.934	-37.130	-14.448	1.00	15.86
	ATOM	2091	C	ALA	A	113	-6.769	-34.929	-14.143	1.00	16.46
	ATOM	2092	O	ALA	A	113	-6.073	-34.432	-15.042	1.00	16.39
	ATOM	2153	N	LEU	A	121	-8.648	-39.338	-21.818	1.00	26.23
	ATOM	2154	CA	LEU	A	121	-7.960	-40.441	-21.168	1.00	26.53
20	ATOM	2155	CB	LEU	A	121	-8.935	-41.261	-20.341	1.00	26.04
	ATOM	2156	CG	LEU	A	121	-9.410	-40.537	-19.073	1.00	25.47
	ATOM	2157	CD1	LEU	A	121	-10.506	-41.328	-18.368	1.00	23.26
	ATOM	2158	CD2	LEU	A	121	-8.260	-40.242	-18.117	1.00	22.91
	ATOM	2159	C	LEU	A	121	-7.210	-41.300	-22.176	1.00	27.40
25	ATOM	2160	O	LEU	A	121	-6.244	-41.976	-21.832	1.00	27.21
	ATOM	2161	N	GLU	A	122	-7.652	-41.223	-23.429	1.00	28.34
	ATOM	2162	CA	GLU	A	122	-7.059	-41.940	-24.547	1.00	29.67
	ATOM	2163	CB	GLU	A	122	-7.893	-41.694	-25.815	1.00	30.04
	ATOM	2164	CG	GLU	A	122	-9.327	-42.285	-25.788	1.00	32.62
	ATOM	2165	CD	GLU	A	122	-10.340	-41.459	-24.962	1.00	36.03
30	ATOM	2166	OE1	GLU	A	122	-9.945	-40.458	-24.326	1.00	37.15
	ATOM	2167	OE2	GLU	A	122	-11.546	-41.817	-24.951	1.00	37.17
	ATOM	2168	C	GLU	A	122	-5.604	-41.538	-24.807	1.00	29.90
	ATOM	2169	O	GLU	A	122	-4.828	-42.329	-25.341	1.00	29.76
35	ATOM	2593	N	LEU	A	173	-14.257	-36.100	-20.020	1.00	27.24
	ATOM	2594	CA	LEU	A	173	-13.462	-37.319	-20.047	1.00	28.20
	ATOM	2595	CB	LEU	A	173	-12.752	-37.534	-18.702	1.00	27.88
	ATOM	2596	CG	LEU	A	173	-11.564	-36.653	-18.295	1.00	26.35
	ATOM	2597	CD1	LEU	A	173	-10.879	-37.249	-17.070	1.00	24.58
40	ATOM	2598	CD2	LEU	A	173	-10.565	-36.496	-19.415	1.00	23.46
	ATOM	2599	C	LEU	A	173	-14.284	-38.561	-20.404	1.00	29.58
	ATOM	2600	O	LEU	A	173	-13.745	-39.509	-20.986	1.00	30.13
	ATOM	2601	N	ILE	A	174	-15.566	-38.577	-20.028	1.00	30.42
	ATOM	2602	CA	ILE	A	174	-16.469	-39.669	-20.419	1.00	31.46
	ATOM	2603	CB	ILE	A	174	-17.863	-39.576	-19.712	1.00	31.45
45	ATOM	2604	CG1	ILE	A	174	-17.714	-39.524	-18.182	1.00	32.09
	ATOM	2605	CD1	ILE	A	174	-18.987	-39.100	-17.425	1.00	31.01
	ATOM	2606	CG2	ILE	A	174	-18.727	-40.771	-20.069	1.00	31.27
	ATOM	2607	C	ILE	A	174	-16.639	-39.721	-21.958	1.00	32.27
	ATOM	2608	O	ILE	A	174	-16.727	-40.805	-22.548	1.00	32.29
50	ATOM	2609	N	LEU	A	175	-16.662	-38.548	-22.595	1.00	33.10

	ATOM	2610	CA	LEU A 175	-16.824	-38.438	-24.046	1.00	33.94
	ATOM	2611	CB	LEU A 175	-16.591	-37.000	-24.504	1.00	33.73
	ATOM	2612	CG	LEU A 175	-17.754	-36.016	-24.433	1.00	32.38
5	ATOM	2613	CD1	LEU A 175	-17.305	-34.695	-25.015	1.00	31.73
	ATOM	2614	CD2	LEU A 175	-18.961	-36.553	-25.170	1.00	30.45
	ATOM	2615	C	LEU A 175	-15.899	-39.346	-24.836	1.00	35.08
	ATOM	2616	O	LEU A 175	-14.676	-39.224	-24.732	1.00	35.38
	ATOM	2617	N	ASP A 176	-16.491	-40.256	-25.612	1.00	36.30
10	ATOM	2618	CA	ASP A 176	-15.745	-41.072	-26.566	1.00	37.58
	ATOM	2619	CB	ASP A 176	-16.638	-42.160	-27.185	1.00	37.78
	ATOM	2620	CG	ASP A 176	-16.713	-43.424	-26.336	1.00	38.93
	ATOM	2621	OD1	ASP A 176	-15.702	-43.778	-25.687	1.00	40.88
	ATOM	2622	OD2	ASP A 176	-17.783	-44.073	-26.324	1.00	39.22
15	ATOM	2623	C	ASP A 176	-15.208	-40.161	-27.664	1.00	38.32
	ATOM	2624	O	ASP A 176	-15.816	-39.123	-27.955	1.00	38.43
	ATOM	2720	N	GLN A 189	-24.108	-44.371	-14.531	1.00	23.07
	ATOM	2721	CA	GLN A 189	-22.967	-44.901	-13.784	1.00	23.84
	ATOM	2722	CB	GLN A 189	-21.967	-45.602	-14.716	1.00	24.30
20	ATOM	2723	CG	GLN A 189	-22.352	-47.016	-15.106	1.00	26.75
	ATOM	2724	CD	GLN A 189	-21.292	-47.707	-15.960	1.00	31.60
	ATOM	2725	OE1	GLN A 189	-21.601	-48.637	-16.715	1.00	34.94
	ATOM	2726	NE2	GLN A 189	-20.037	-47.268	-15.842	1.00	32.62
	ATOM	2727	C	GLN A 189	-22.245	-43.822	-12.980	1.00	23.49
25	ATOM	2728	O	GLN A 189	-21.936	-44.017	-11.802	1.00	23.77
	ATOM	2729	N	TYR A 190	-21.980	-42.684	-13.609	1.00	22.91
	ATOM	2730	CA	TYR A 190	-21.298	-41.606	-12.912	1.00	22.41
	ATOM	2731	CB	TYR A 190	-20.578	-40.677	-13.886	1.00	22.75
	ATOM	2732	CG	TYR A 190	-19.344	-41.317	-14.475	1.00	25.31
30	ATOM	2733	CD1	TYR A 190	-18.109	-41.216	-13.839	1.00	27.94
	ATOM	2734	CE1	TYR A 190	-16.965	-41.818	-14.389	1.00	30.54
	ATOM	2735	CZ	TYR A 190	-17.072	-42.532	-15.580	1.00	30.87
	ATOM	2736	OH	TYR A 190	-15.967	-43.134	-16.144	1.00	33.77
	ATOM	2737	CE2	TYR A 190	-18.292	-42.651	-16.215	1.00	29.70
35	ATOM	2738	CD2	TYR A 190	-19.416	-42.047	-15.664	1.00	28.05
	ATOM	2739	C	TYR A 190	-22.212	-40.836	-11.971	1.00	21.59
	ATOM	2740	O	TYR A 190	-21.766	-40.423	-10.891	1.00	21.41
40	ATOM	2764	N	LEU A 193	-22.165	-42.386	-8.662	1.00	20.37
	ATOM	2765	CA	LEU A 193	-20.961	-42.081	-7.894	1.00	20.05
	ATOM	2766	CB	LEU A 193	-19.711	-42.257	-8.753	1.00	20.28
	ATOM	2767	CG	LEU A 193	-19.352	-43.673	-9.200	1.00	20.34
	ATOM	2768	CD1	LEU A 193	-18.137	-43.611	-10.088	1.00	20.81
	ATOM	2769	CD2	LEU A 193	-19.125	-44.614	-8.014	1.00	19.15
	ATOM	2770	C	LEU A 193	-21.011	-40.669	-7.325	1.00	19.95
45	ATOM	2771	O	LEU A 193	-20.753	-40.470	-6.136	1.00	20.12

Table 4. Additional contacts that contribute to, but are not essential, for contact between *S. cerevisiae* Dcn1 and *S. cerevisiae* Ubc12 with yUbc12 Chain C interactions with yDcn1^P Chain A and yUbc12 Chain D interactions with yDcn1^P Chain B shown.

yUbc12 Atom	yDcn1 Atom	Distance (Å)
Arg 5C NE	Leu 104A CD2	4.07
Arg 5C CZ	Leu 104A CB	4.43
	Glu 105A CD	4.44
	Glu 105A OE2	3.95
	Leu 104A CD2	4.28
Arg 5C NH1	Glu 105A CG	4.02
	Glu 105A CD	4.28
	Glu 105A OE2	3.96
Arg 5C NH2	Leu 104A CB	4.21
	Glu 105A CG	4.21
	Glu 105A CD	3.64
	Glu 105A OE1	4.3
	Glu 105A OE2	3.05
	Leu 104A CD2	4.39
	Leu 104A CG	4.46
	Leu 104A CD1	4.13
Leu 7C CB	Ile 90A CD1	4.44
	Ile 90A CG2	4.39
Leu 7C CD1	Leu 121A CB	4.19
Leu 7C CD2	Glu 122A OE2	4.34
	Glu 122A CG	3.85
	Glu 122A CD	4.36
Leu 7C C	Ile 90A CD1	3.57
Leu 7C O	Ile 90A CD1	3.4
Gln 8C N	Ile 90A CD1	3.49
Gln 8C CA	Ile 90A CD1	3.4
Gln 8C CB	Ile 90A CD1	4.18
	Asp 91A OD1	4.43
Gln 8C CG	Ile 90A CD1	3.79
	Val 94A CG2	4.28
	Asp 91A OD1	3.49
Gln 8C CD	Val 94A CG1	4.46
	Val 94A CG2	3.58
	Asp 91A OD1	3.87
	Val 94A CB	4.37
Gln 8C OE1	Val 94A CG1	4.46
	Val 94A CG2	3.72
Gln 8C NE2	Val 94A CG1	3.83
	Val 94A CG2	3.53
	Asp 91A OD1	3.24
	Asp 91A CG	4.36
	Asp 91A CA	4.34
	Val 94A CB	3.84
Lys 11C CB	Ile 90A CD1	4.03
Lys 11C CD	Ile 90A CD1	4.14

	Asp 89A OD2	4.33
	Asp 91A CG	4.43
	Asp 91A OD2	3.44
Lys 11C OE	Asp 89A CG	4.47
	Asp 89A OD2	3.48
	Asp 91A OD2	3.64
Lys 11C NZ	Asp 89A CG	4.01
	Asp 89A OD2	2.97
	Asp 91A CG	3.92
	Asp 91A OD2	2.7
	Asn 84A CB	4.47
Arg 5D N	Leu 104B CD1	4.25
Arg 5D CA	Leu 104B CD1	4.37
Arg 5D CB	Leu 104B CD1	3.71
Arg 5D CG	Leu 104B CD1	4.36
Arg 5D NE	Leu 104B CD1	3.9
Arg 5D CZ	Leu 104B CB	4.48
	Leu 104B CD1	3.74
	Glu 105B OE2	4.2
Arg 5D NH1	Leu 104B CD1	4.31
	Glu 105B OE2	4.16
Arg 5D NH2	Leu 104B CB	3.97
	Leu 104B CG	4.04
	Leu 104B CD1	3.67
	Glu 105B OE2	3.4
	Glu 105B CD	4
	Glu 105B OE1	4.12
Gln 6D CD	Glu 186B CD	4.47
	Glu 186B OE2	3.46
Gln 6D OE1	Glu 186B CG	3.8
	Glu 186B CD	3.69
	Glu 186B OE2	2.9
Gln 6D NE2	Asp 176B OD1	4.18
	Glu 186B CD	4.41
	Glu 186B OE2	3.25
Leu 7D CB	Ile 90B CD1	3.77
Leu 7D CG	Ile 90B CD1	4.25
Leu 7D CD1	Leu 121B CB	4.01
	Leu 121B C	4.43
	Ile 90B CD1	3.76
	Glu 122B CG	4.4
Leu 7D CD2	Glu 122B CG	4.4
Gln 8D CG	Ile 90B CG2	4.17
	Asp 91B OD1	4.03
Gln 8D CD	Asp 91B OD1	4.34
	Val 94B CG2	4.02
Gln 8D OE1	Val 94B CG2	4.29
Gln 8D NE2	Asp 91B OD1	3.6
	Val 94B CB	4.02

	Val 94B CG2	3.75
	Val 94B CG1	3.87

Table 5. Atomic coordinates of residues that contribute to, but are not required for, binding of yDcn1 Chain B to yUbc12.

	Atom No.	Atom Name		X	Y	Z	Occu-pancy	B-factor
5	ATOM 136	N ASN B 84		-24.546	-0.988	-25.260	1.00	25.93
	ATOM 137	CA ASN B 84		-24.488	0.253	-26.016	1.00	27.03
	ATOM 138	CB ASN B 84		-23.072	0.833	-25.998	1.00	27.10
	ATOM 139	CG ASN B 84		-22.586	1.127	-24.609	1.00	26.68
10	ATOM 140	OD1 ASN B 84		-23.153	1.954	-23.903	1.00	27.53
	ATOM 141	ND2 ASN B 84		-21.525	0.454	-24.205	1.00	26.15
	ATOM 142	C ASN B 84		-24.887	-0.019	-27.445	1.00	27.74
	ATOM 143	O ASN B 84		-24.248	-0.823	-28.120	1.00	27.97
	ATOM 179	N ASP B 89		-20.480	-4.359	-25.414	1.00	26.21
15	ATOM 180	CA ASP B 89		-19.176	-4.073	-25.996	1.00	26.19
	ATOM 181	CB ASP B 89		-19.314	-2.957	-27.045	1.00	26.08
	ATOM 182	CG ASP B 89		-19.779	-1.625	-26.445	1.00	26.39
	ATOM 183	OD1 ASP B 89		-19.976	-1.535	-25.210	1.00	26.46
	ATOM 184	OD2 ASP B 89		-19.945	-0.655	-27.218	1.00	27.17
20	ATOM 185	C ASP B 89		-18.124	-3.716	-24.930	1.00	26.36
	ATOM 186	O ASP B 89		-18.336	-3.935	-23.735	1.00	25.92
	ATOM 195	N ASP B 91		-17.152	-0.819	-23.793	1.00	26.91
	ATOM 196	CA ASP B 91		-17.667	0.172	-22.850	1.00	26.57
	ATOM 197	CB ASP B 91		-18.385	1.308	-23.586	1.00	27.05
25	ATOM 198	CG ASP B 91		-17.432	2.176	-24.392	1.00	29.32
	ATOM 199	OD1 ASP B 91		-16.274	2.369	-23.951	1.00	31.34
	ATOM 200	OD2 ASP B 91		-17.840	2.667	-25.469	1.00	31.93
	ATOM 201	C ASP B 91		-18.579	-0.476	-21.814	1.00	25.69
	ATOM 202	O ASP B 91		-18.575	-0.092	-20.643	1.00	25.22
30	ATOM 278	N GLY B 101		-17.275	-3.210	-8.645	1.00	29.34
	ATOM 279	CA GLY B 101		-16.450	-2.623	-7.591	1.00	29.68
	ATOM 280	C GLY B 101		-14.959	-2.810	-7.784	1.00	29.86
	ATOM 281	O GLY B 101		-14.203	-2.788	-6.815	1.00	30.12
	ATOM 282	N TYR B 102		-14.530	-2.981	-9.033	1.00	29.95
35	ATOM 283	CA TYR B 102		-13.125	-3.256	-9.329	1.00	29.83
	ATOM 284	CB TYR B 102		-12.976	-4.620	-10.014	1.00	29.64
	ATOM 285	CG TYR B 102		-13.264	-5.790	-9.097	1.00	28.75
	ATOM 286	CD1 TYR B 102		-12.267	-6.325	-8.285	1.00	27.79
	ATOM 287	CE1 TYR B 102		-12.529	-7.385	-7.429	1.00	27.33
40	ATOM 288	CZ TYR B 102		-13.798	-7.929	-7.382	1.00	28.21
	ATOM 289	OH TYR B 102		-14.063	-8.983	-6.538	1.00	28.62
	ATOM 290	CE2 TYR B 102		-14.812	-7.417	-8.175	1.00	28.92
	ATOM 291	CD2 TYR B 102		-14.539	-6.349	-9.029	1.00	29.04
	ATOM 292	C TYR B 102		-12.471	-2.173	-10.169	1.00	30.22
45	ATOM 293	O TYR B 102		-13.155	-1.397	-10.842	1.00	30.20
	ATOM 294	N ASN B 103		-11.141	-2.110	-10.079	1.00	30.62

	ATOM	295	CA	ASN B 103	-10.307	-1.343	-11.000	1.00	30.76
	ATOM	296	CB	ASN B 103	-9.066	-0.790	-10.286	1.00	30.89
	ATOM	297	CG	ASN B 103	-9.406	0.188	-9.160	1.00	32.03
5	ATOM	298	OD1	ASN B 103	-10.086	1.193	-9.372	1.00	34.05
	ATOM	299	ND2	ASN B 103	-8.912	-0.096	-7.960	1.00	32.51
	ATOM	300	C	ASN B 103	-9.874	-2.289	-12.114	1.00	30.71
	ATOM	301	O	ASN B 103	-9.821	-3.509	-11.911	1.00	30.68
	ATOM	980	N	GLU B 186	3.220	-4.286	-23.322	1.00	25.28
	ATOM	981	CA	GLU B 186	1.962	-4.813	-22.773	1.00	25.53
10	ATOM	982	CB	GLU B 186	1.005	-5.255	-23.886	1.00	26.28
	ATOM	983	CG	GLU B 186	0.428	-4.141	-24.769	1.00	30.25
	ATOM	984	CD	GLU B 186	-0.261	-4.683	-26.032	1.00	35.54
	ATOM	985	OE1	GLU B 186	-0.215	-5.914	-26.273	1.00	38.14
	ATOM	986	OE2	GLU B 186	-0.848	-3.877	-26.794	1.00	38.37
15	ATOM	987	C	GLU B 186	2.231	-5.998	-21.836	1.00	24.41
	ATOM	988	O	GLU B 186	1.635	-6.093	-20.756	1.00	24.41

Table 6. Atomic coordinates of residues that contribute to, but are not required for, binding of yDcn1 Chain A to yUbc12.

	Atom No.	Atom Name	X	Y	Z	Occu- pancy	B- factor
20	ATOM	1855 N ASN A 84	5.480	-47.125	-19.771	1.00	24.51
	ATOM	1856 CA ASN A 84	5.351	-48.370	-20.509	1.00	24.71
	ATOM	1857 CB ASN A 84	3.911	-48.902	-20.440	1.00	25.23
25	ATOM	1858 CG ASN A 84	3.565	-49.475	-19.082	1.00	26.13
	ATOM	1859 OD1 ASN A 84	4.293	-50.299	-18.541	1.00	30.17
	ATOM	1860 ND2 ASN A 84	2.447	-49.049	-18.529	1.00	27.12
	ATOM	1861 C ASN A 84	5.729	-48.075	-21.938	1.00	24.19
	ATOM	1862 O ASN A 84	5.204	-47.140	-22.528	1.00	24.25
30	ATOM	1898 N ASP A 89	1.132	-44.006	-20.314	1.00	21.24
	ATOM	1899 CA ASP A 89	-0.152	-44.370	-20.921	1.00	21.52
	ATOM	1900 CB ASP A 89	0.045	-45.548	-21.893	1.00	21.94
	ATOM	1901 CG ASP A 89	0.661	-46.781	-21.218	1.00	23.83
	ATOM	1902 OD1 ASP A 89	0.856	-46.789	-19.967	1.00	27.18
35	ATOM	1903 OD2 ASP A 89	0.951	-47.755	-21.946	1.00	27.23
	ATOM	1904 C ASP A 89	-1.171	-44.759	-19.860	1.00	21.03
	ATOM	1905 O ASP A 89	-0.986	-44.504	-18.677	1.00	20.38
	ATOM	1914 N ASP A 91	-2.097	-47.801	-18.722	1.00	22.01
	ATOM	1915 CA ASP A 91	-1.539	-48.709	-17.724	1.00	21.76
40	ATOM	1916 CB ASP A 91	-0.654	-49.764	-18.384	1.00	22.52
	ATOM	1917 CG ASP A 91	-1.404	-50.617	-19.386	1.00	23.98
	ATOM	1918 OD1 ASP A 91	-2.482	-51.150	-19.045	1.00	25.33
	ATOM	1919 OD2 ASP A 91	-0.903	-50.762	-20.520	1.00	26.75
	ATOM	1920 C ASP A 91	-0.745	-47.940	-16.676	1.00	21.29
45	ATOM	1921 O ASP A 91	-0.916	-48.190	-16.480	1.00	21.45
	ATOM	1997 N GLY A 101	-2.947	-45.057	-3.801	1.00	22.73
	ATOM	1998 CA GLY A 101	-3.770	-45.643	-2.749	1.00	23.50
	ATOM	1999 C GLY A 101	-5.257	-45.694	-3.055	1.00	24.28
	ATOM	2000 O GLY A 101	-6.068	-45.834	-2.140	1.00	24.21

	ATOM	2001	N	TYR A 102	-5.616	-45.604	-4.334	1.00	25.12
	ATOM	2002	CA	TYR A 102	-7.025	-45.504	-4.736	1.00	26.13
	ATOM	2003	CB	TYR A 102	-7.305	-44.158	-5.419	1.00	25.91
	ATOM	2004	CG	TYR A 102	-7.145	-43.006	-4.469	1.00	26.01
5	ATOM	2005	CD1	TYR A 102	-8.209	-42.587	-3.663	1.00	26.06
	ATOM	2006	CE1	TYR A 102	-8.056	-41.536	-2.767	1.00	26.07
	ATOM	2007	CZ	TYR A 102	-6.827	-40.904	-2.664	1.00	25.39
	ATOM	2008	OH	TYR A 102	-6.657	-39.872	-1.780	1.00	25.43
	ATOM	2009	CE2	TYR A 102	-5.758	-41.306	-3.446	1.00	26.78
10	ATOM	2010	CD2	TYR A 102	-5.919	-42.351	-4.346	1.00	25.73
	ATOM	2011	C	TYR A 102	-7.529	-46.640	-5.612	1.00	26.90
	ATOM	2012	O	TYR A 102	-6.748	-47.385	-6.199	1.00	27.17
	ATOM	2013	N	ASN A 103	-8.852	-46.760	-5.649	1.00	28.08
	ATOM	2014	CA	ASN A 103	-9.578	-47.619	-6.565	1.00	29.35
15	ATOM	2015	CB	ASN A 103	-10.792	-48.231	-5.854	1.00	29.88
	ATOM	2016	CG	ASN A 103	-10.399	-49.134	-4.687	1.00	32.31
	ATOM	2017	OD1	ASN A 103	-9.440	-49.903	-4.782	1.00	35.88
	ATOM	2018	ND2	ASN A 103	-11.140	-49.045	-3.585	1.00	33.38
	ATOM	2019	C	ASN A 103	-10.045	-46.753	-7.729	1.00	29.59
20	ATOM	2020	O	ASN A 103	-10.294	-45.555	-7.552	1.00	29.55
	ATOM	2699	N	GLU A 186	-23.763	-44.689	-19.329	1.00	26.90
	ATOM	2700	CA	GLU A 186	-22.562	-44.138	-18.718	1.00	26.47
	ATOM	2701	CB	GLU A 186	-21.512	-43.836	-19.791	1.00	26.96
	ATOM	2702	CG	GLU A 186	-20.845	-45.096	-20.328	1.00	29.85
25	ATOM	2703	CD	GLU A 186	-20.272	-44.939	-21.736	1.00	34.58
	ATOM	2704	OE1	GLU A 186	-20.423	-43.852	-22.339	1.00	37.35
	ATOM	2705	OE2	GLU A 186	-19.666	-45.912	-22.245	1.00	36.39
	ATOM	2706	C	GLU A 186	-22.862	-42.917	-17.846	1.00	25.40
	ATOM	2707	O	GLU A 186	-22.228	-42.733	-16.803	1.00	25.32
30									

In other embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises the atoms of Table 7, which are found in amino acid residues Ile83, Ile86, Gln87, Pro97, Ala98, Ser99, Val102, Leu103, Ala106, Gln114, Cys115, Phe117, Phe164, Asn167, Met177, Ala180, Tyr181, and Leu184 of human Dcn1 (SEQ ID NO: 33), or a structural variant thereof. In particular embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises at least one of the following amino acid residues: an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO:

33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine
 5 corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, and a leucine corresponding to position 184 of SEQ ID NO: 33.

In some of these embodiments, the E2-binding pocket comprises the atomic coordinates of Table 8, 9, 10, or 11, or a structural variant thereof. In particular
 10 embodiments, the co-E3 protein has the amino acid sequence set forth in SEQ ID NO: 33.

In certain embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises the atoms of Tables 7 and 12. The atoms in Table 12 are found in amino acid residues Asp84, Ala94, Leu95, and Asp96 of SEQ ID NO: 33. Thus, in some
 15 embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises at least one of the following amino acid residues: an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of
 20 SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position
 25 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, a leucine corresponding to position 184 of SEQ ID NO: 33, an aspartic acid corresponding to position 84 of SEQ ID NO: 33, an alanine corresponding to position 94 of SEQ ID NO: 33, a leucine corresponding to position 95 of
 30 SEQ ID NO: 33, and an aspartic acid corresponding to position 96 of SEQ ID NO: 33.

In some of these embodiments, the E2-binding pocket comprises the atomic coordinates of Tables 8 and 13; Tables 9 and 14; Tables 10 and 15; or Tables 11 and 16; or a structural variant thereof.

Table 7. Minimal contacts between human Dcn1 and human Ubc12 with hUbc12 Chain E interactions with hDcn1^P Chain B and hUbc12 Chain F interactions with hDcn1^P Chain A shown.

hUbc12 Atom	hDcn1 Atom	Distance (Å)
Acetyl-Met 1E O	Pro 97B O	4.28
	Cys 115B SG	4.31
Acetyl-Met 1E C	Gln 114B OE1	3.79
	Tyr 181B OH	4.2
	Pro 97B O	4.19
	Cys 115B SG	4.24
Acetyl-Met 1E CA	Gln 114B OE1	4.13
	Tyr 181B OH	4.11
	Pro 97B C	4.46
	Pro 97B O	3.62
	Cys 115B SG	4.27
	Ala 98B CA	3.95
Acetyl-Met 1E N	Tyr 181B OH	4.3
	Val 102B CG1	4.46
	Pro 97B C	3.72
	Pro 97B O	2.7
	Ala 98B N	4.26
	Phe 164B CE2	4.29
Acetyl-Met 1E CT1	Tyr 181B CE1	4.26
	Tyr 181B CZ	4.38
	Tyr 181B OH	3.64
	Pro 97B O	3.59
Acetyl-Met 1E OT	Leu 103B CD1	4.34
	Phe 164B CE2	3.32
	Phe 164B CD2	4.31
	Gln 114B CB	4.29
	Tyr 181B CE1	3.45
	Tyr 181B CZ	3.44
	Tyr 181B OH	2.61
	Phe 164B CZ	4.04
Acetyl-Met 1E CT2	Leu 103B CD1	4.33
	Ser 99B O	3.61
	Val 102B C	4.33
	Leu 103B N	3.67
	Leu 103B CA	3.91
	Leu 103B CB	3.76
	Ala 98B CA	4.24
	Ala 98B C	4.2
	Ala 98B O	4.47
	Leu 184B CD2	4.07
	Val 102B CB	4.36
	Val 102B CG1	4.25
	Pro 97B O	3.58
	Pro 97B CB	4.35

	Pro 97B C	4.29
	Pro 97B O	3.55
	Cys 115B SG	3.98
	Ile 86B CG2	4.42
Acetyl-Met 1E CG	Val 102B CG1	3.87
	Pro 97B C	4.45
	Pro 97B O	3.53
	Ile 86B CG2	3.63
Acetyl-Met 1E SD	Phe 164B CZ	3.9
	Val 102B CG1	4.47
	Gln 114B CA	4.31
	Gln 114B C	4.36
	Cys 115B N	3.4
	Cys 115B CA	4.02
	Ile 86B CG2	4.1
Acetyl-Met 1E CE	Phe 117B CZ	3.7
	Ala 106B CB	4.48
	Phe 164B CZ	4.24
	Val 102B CG1	3.71
	Phe 117B CE1	4.04
	Phe 117B CE2	4.49
	Ile 86B CD1	4.34
	Ile 86B CG2	4.02
Ile 2E N	Gln 114B CD	4.32
	Gln 114B OE1	3.24
	Tyr 181B CZ	4.13
	Tyr 181B OH	3.28
Ile 2E CA	Gln 114B OE1	3.88
	Tyr 181B OH	4.11
Ile 2E CB	Gln 114B OE1	4.23
	Tyr 181B CZ	3.8
	Tyr 181B OH	3.75
	Tyr 181B CE2	3.7
	Tyr 181B CD2	4.42
	Met 177B SD	4.01
Ile 2E CG1	Tyr 181B CG	4.48
	Tyr 181B CD1	4.32
	Tyr 181B CE1	3.85
	Tyr 181B CZ	3.51
	Tyr 181B OH	3.76
	Tyr 181B CE2	3.69
	Tyr 181B CD2	4.16
Ile 2E CD1	Ala 98B CA	4.32
	Ala 98B CB	3.64
	Ala 98B O	4.16
Ile 2E CG2	Ala 180B CB	3.66
	Met 177B SD	3.73
Ile 2E C	Gln 114B OE1	3.83
Lys 3E N	Gln 114B CD	3.92
	Gln 114B OE1	2.87

	Gln 114B NE2	4.33
	Cys 115B SG	4.07
Lys 3E CA	Gln 114B CD	4.5
	Gln 114B OE1	3.64
	Met 177B CE	4.45
	Cys 115B SG	4.16
Lys 3E CB	Gln 114B CD	3.78
	Gln 114B OE1	3.3
	Gln 114B NE2	3.92
	Met 177B CE	3.86
	Cys 115B SG	4.11
Lys 3E CG	Met 177B CE	3.63
Lys 3E CD	Met 177B CE	3.52
Lys 3E NZ	Asn 167B OD1	4.22
Lys 3E C	Cys 115B SG	3.72
Lys 3E O	Cys 115B SG	4.34
Leu 4E N	Cys 115B SG	3.31
Leu 4E CA	Cys 115B SG	3.54
Leu 4E CB	Cys 115B SG	3.87
Leu 4E CG	Cys 115B SG	4.49
	Ile 83B O	4.35
Leu 4E CD1	Cys 115B CA	4.29
	Cys 115B CB	3.97
	Cys 115B SG	3.82
	Ile 86B CB	4.13
	Ile 86B CG2	4.13
	Ile 83B O	3.71
	Ile 83B CG2	3.74
	Ile 83B CA	3.86
	Ile 83B CB	3.73
	Ile 83B C	4.24
Leu 4E CD2	Pro 97B CB	3.78
	Pro 97B CG	4.09
	Ile 86B CG2	4.22
	Ile 83B O	4.37
	Gln 87B N	4.12
	Gln 87B CA	4.12
	Gln 87B CB	4.21
	Gln 87B OE1	3.57
	Gln 87B CD	4.31
Acetyl-Met 1F O	Pro 97A O	4.39
	Cys 115A SG	4.36
Acetyl-Met 1F C	Pro 97A O	4.33
	Cys 115A SG	4.23
	Gln 114A OE1	3.8
	Tyr 181A OH	4.28
Acetyl-Met 1F CA	Pro 97A C	4.48
	Pro 97A O	3.73
	Cys 115A SG	4.23
	Gln 114A OE1	4.14

		Tyr 181A OH	4.21
Acetyl-Met	1F N	Pro 97A C	3.72
		Pro 97A O	2.79
		Ala 98A N	4.21
		Ala 98A CA	3.88
		Tyr 181A OH	4.37
Acetyl-Met	1F CT1	Pro 97A O	3.65
		Ala 98A CA	4.5
		Tyr 181A CE1	4.26
		Tyr 181A CZ	4.41
		Phe 164A CE2	4.2
		Tyr 181A OH	3.69
Acetyl-Met	1F OT	Leu 103A CD1	4.46
		Tyr 181A CE1	3.54
		Tyr 181A CZ	3.57
		Phe 164A CZ	3.92
		Phe 164A CE2	3.18
		Phe 164A CD2	4.18
		Gln 114A CB	4.17
		Tyr 181A OH	2.74
Acetyl-Met	1F CT2	Ala 98A C	4.16
		Ala 98A O	4.35
		Pro 97A O	3.6
		Ala 98A CA	4.19
		Ser 99A O	3.64
		Val 102A CB	4.44
		Val 102A CG1	4.33
		Leu 103A N	3.83
		Leu 184A CD2	4.05
		Leu 103A CA	4.04
		Leu 103A CB	3.81
		Leu 103A CD1	4.45
		Tyr 181A CE1	4.49
Acetyl-Met	1F CB	Pro 97A C	4.26
		Pro 97A O	3.6
		Pro 97A CB	4.31
		Cys 115A SG	3.95
		Ile 86A CG2	4.45
Acetyl-Met	1F CG	Pro 97A C	4.4
		Pro 97A O	3.53
		Val 102A CG1	3.85
		Ile 86A CG2	3.75
Acetyl-Met	1F SD	Val 102A CG1	4.44
		Phe 164A CZ	3.86
		Ile 86A CG2	4.17
		Cys 115A CA	4
		Gln 114A CA	4.31
		Gln 114A C	4.35
		Cys 115A N	3.38
Acetyl-Met	1F CE	Val 102A CG1	3.7

	Phe 117A CE1	4.13
	Phe 117A CZ	3.69
	Phe 117A CE2	4.4
	Phe 164A CZ	4.26
	Ile 86A CD1	4.29
	Ile 86A CG2	4.09
Ile 2F N	Tyr 181A CZ	4.22
	Gln 114A CD	4.28
	Gln 114A OE1	3.26
	Tyr 181A OH	3.35
Ile 2F CA	Gln 114A OE1	3.92
	Tyr 181A OH	4.16
Ile 2F CB	Tyr 181A CZ	3.88
	Met 177A SD	4.02
	Gln 114A OE1	4.25
	Tyr 181A OH	3.76
	Tyr 181A CE2	3.77
Ile 2F CG1	Tyr 181A CG	4.5
	Tyr 181A CD1	4.36
	Tyr 181A CE1	3.85
	Tyr 181A CZ	3.43
	Tyr 181A CD2	4.11
	Ala 180A CB	4.43
	Tyr 181A OH	3.65
	Tyr 181A CE2	3.58
Ile 2F CD1	Ala 98A CB	3.66
	Ala 98A O	4.19
	Ala 98A CA	4.3
Ile 2F CG2	Met 177A SD	3.78
	Ala 180A CB	3.75
Ile 2F C	Gln 114A OE1	3.86
Lys 3F N	Cys 115A SG	4.11
	Gln 114A CD	3.96
	Gln 114A OE1	2.89
	Gln 114A NE2	4.36
Lys 3F CA	Cys 115A SG	4.14
	Gln 114A OE1	3.66
Lys 3F CB	Cys 115A SG	3.94
	Gln 114A CD	3.88
	Gln 114A OE1	3.27
	Gln 114A NE2	4.16
Lys 3F CG	Met 177A SD	3.99
	Met 177A CE	3.2
	Gln 114A CD	3.91
	Gln 114A OE1	3.67
	Gln 114A NE2	3.69
Lys 3F CD	Met 177A CE	3.67
	Gln 114A CD	4.1
	Gln 114A OE1	4.25
	Gln 114A NE2	3.78

Lys 3F NZ	Asn 167A OD1	3.99
Lys 3F C	Cys 115A SG	3.8
Lys 3F O	Cys 115A SG	4.5
Leu 4F N	Cys 115A SG	3.34
Leu 4F CA	Cys 115A SG	3.58
Leu 4F CB	Cys 115A SG	3.82
Leu 4F CG	Gln 87A OE1	4.29
Leu 4F CD1	Cys 115A SG	4.09
	Ile 86A CB	4.08
	Ile 86A CG2	3.97
	Cys 115A CB	4.28
	Ile 83A C	4.24
	Ile 83A O	3.66
	Ile 83A CA	3.96
	Ile 83A CB	3.75
	Ile 83A CG2	4.05
Leu 4F CD2	Gln 87A CB	4.26
	Gln 87A CD	4.2
	Gln 87A OE1	3.46
	Pro 97A CB	3.63
	Pro 97A CG	3.88
	Gln 87A N	4.16
	Gln 87A CA	4.12
	Ile 86A CG2	4.09

Table 8. Atomic coordinates of amino acid residues of hDcn1 Chain A essential for binding hUbc12 from structure with stapled hUbc12 peptide.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
5	ATOM 198	N ILE A 83	13.031	40.450	4.212	1.00	11.06
	ATOM 199	CA ILE A 83	12.692	41.331	5.343	1.00	11.10
	ATOM 200	CB ILE A 83	12.045	40.580	6.548	1.00	11.11
10	ATOM 201	CG1 ILE A 83	13.025	39.622	7.224	1.00	12.43
	ATOM 202	CD1 ILE A 83	12.509	39.060	8.572	1.00	12.78
	ATOM 203	CG2 ILE A 83	10.770	39.869	6.106	1.00	11.68
	ATOM 204	C ILE A 83	13.839	42.249	5.794	1.00	10.27
	ATOM 205	O ILE A 83	13.610	43.411	6.117	1.00	9.79
15	ATOM 218	N ILE A 86	14.182	44.958	3.139	1.00	9.70
	ATOM 219	CA ILE A 86	13.051	45.879	3.287	1.00	10.08
	ATOM 220	CB ILE A 86	11.691	45.130	3.541	1.00	10.44
	ATOM 221	CG1 ILE A 86	11.201	44.403	2.280	1.00	10.75
	ATOM 222	CD1 ILE A 86	10.930	45.295	1.085	1.00	12.22
20	ATOM 223	CG2 ILE A 86	10.611	46.083	4.067	1.00	10.11
	ATOM 224	C ILE A 86	13.311	46.862	4.423	1.00	9.98
	ATOM 225	O ILE A 86	13.098	48.064	4.264	1.00	9.90
	ATOM 226	N GLN A 87	13.764	46.349	5.567	1.00	10.21
	ATOM 227	CA GLN A 87	14.082	47.199	6.708	1.00	10.45

	ATOM	228	CB	GLN	A	87	14.505	46.369	7.922	1.00	10.47
	ATOM	229	CG	GLN	A	87	14.872	47.217	9.154	1.00	10.24
	ATOM	230	CD	GLN	A	87	13.657	47.835	9.835	0.67	9.76
5	ATOM	231	OE1	GLN	A	87	12.747	47.136	10.263	1.00	11.23
	ATOM	232	NE2	GLN	A	87	13.650	49.151	9.947	1.00	9.80
	ATOM	233	C	GLN	A	87	15.190	48.197	6.361	1.00	10.65
	ATOM	234	O	GLN	A	87	15.079	49.379	6.676	1.00	10.43
	ATOM	306	N	PRO	A	97	8.934	51.727	8.623	1.00	12.49
	ATOM	307	CA	PRO	A	97	8.458	50.775	7.613	1.00	12.43
10	ATOM	308	CB	PRO	A	97	9.098	49.454	8.041	1.00	12.32
	ATOM	309	CG	PRO	A	97	10.354	49.872	8.779	1.00	11.89
	ATOM	310	CD	PRO	A	97	9.949	51.127	9.513	1.00	12.41
	ATOM	311	C	PRO	A	97	6.935	50.663	7.572	1.00	12.50
	ATOM	312	O	PRO	A	97	6.386	50.382	6.524	1.00	12.36
15	ATOM	313	N	ALA	A	98	6.279	50.895	8.710	1.00	12.89
	ATOM	314	CA	ALA	A	98	4.831	50.836	8.815	1.00	13.39
	ATOM	315	CB	ALA	A	98	4.426	50.451	10.252	1.00	13.37
	ATOM	316	C	ALA	A	98	4.138	52.141	8.396	1.00	13.61
	ATOM	317	O	ALA	A	98	2.931	52.264	8.554	1.00	14.04
20	ATOM	318	N	SER	A	99	4.889	53.105	7.864	1.00	13.57
	ATOM	319	CA	SER	A	99	4.318	54.420	7.534	1.00	13.68
	ATOM	320	CB	SER	A	99	5.396	55.509	7.572	1.00	13.92
	ATOM	321	OG	SER	A	99	6.281	55.392	6.464	1.00	14.94
	ATOM	322	C	SER	A	99	3.577	54.467	6.189	1.00	13.30
25	ATOM	323	O	SER	A	99	3.791	53.623	5.310	1.00	13.12
	ATOM	338	N	VAL	A	102	5.854	54.647	2.979	1.00	9.89
	ATOM	339	CA	VAL	A	102	6.354	53.352	2.502	1.00	9.86
	ATOM	340	CB	VAL	A	102	6.884	52.478	3.682	1.00	10.15
	ATOM	341	CG1	VAL	A	102	7.180	51.047	3.233	1.00	9.36
30	ATOM	342	CG2	VAL	A	102	8.139	53.106	4.290	1.00	8.80
	ATOM	343	C	VAL	A	102	5.284	52.604	1.692	1.00	9.91
	ATOM	344	O	VAL	A	102	5.589	51.953	0.692	1.00	10.20
	ATOM	345	N	LEU	A	103	4.031	52.702	2.123	1.00	9.82
	ATOM	346	CA	LEU	A	103	2.926	52.077	1.388	1.00	9.65
35	ATOM	347	CB	LEU	A	103	1.623	52.189	2.187	1.00	9.74
	ATOM	348	CG	LEU	A	103	0.366	51.488	1.645	1.00	9.65
	ATOM	349	CD1	LEU	A	103	0.615	50.022	1.289	1.00	9.76
	ATOM	350	CD2	LEU	A	103	-0.762	51.606	2.661	1.00	8.19
	ATOM	351	C	LEU	A	103	2.767	52.696	-0.006	1.00	9.65
40	ATOM	352	O	LEU	A	103	2.483	51.993	-0.990	1.00	9.39
	ATOM	369	N	ALA	A	106	5.491	51.324	-2.295	1.00	11.19
	ATOM	370	CA	ALA	A	106	5.107	49.981	-2.730	1.00	11.36
	ATOM	371	CB	ALA	A	106	4.610	49.160	-1.550	1.00	11.21
	ATOM	372	C	ALA	A	106	4.047	50.032	-3.832	1.00	11.22
45	ATOM	373	O	ALA	A	106	4.085	49.236	-4.769	1.00	11.65
	ATOM	436	N	GLN	A	114	3.331	42.667	0.750	1.00	11.54
	ATOM	437	CA	GLN	A	114	4.091	43.403	1.767	1.00	11.10
	ATOM	438	CB	GLN	A	114	3.200	43.832	2.955	1.00	10.82
	ATOM	439	CG	GLN	A	114	2.418	42.702	3.641	1.00	9.56
50	ATOM	440	CD	GLN	A	114	1.970	43.064	5.062	1.00	9.99

	ATOM	441	OE1	GLN	A	114	0.868	42.701	5.494	1.00	10.07
	ATOM	442	NE2	GLN	A	114	2.818	43.780	5.787	1.00	5.79
	ATOM	443	C	GLN	A	114	5.289	42.596	2.254	1.00	10.86
	ATOM	444	O	GLN	A	114	5.268	41.370	2.216	1.00	10.95
5	ATOM	445	N	CYS	A	115	6.324	43.312	2.689	1.00	11.28
	ATOM	446	CA	CYS	A	115	7.530	42.756	3.322	1.00	11.28
	ATOM	447	CB	CYS	A	115	7.177	41.891	4.536	1.00	11.40
	ATOM	448	SG	CYS	A	115	6.269	42.812	5.794	1.00	11.83
	ATOM	449	C	CYS	A	115	8.493	42.025	2.382	1.00	11.75
10	ATOM	450	O	CYS	A	115	9.382	41.294	2.836	1.00	11.65
	ATOM	460	N	PHE	A	117	10.183	42.611	-2.011	1.00	11.84
	ATOM	461	CA	PHE	A	117	10.100	43.012	-3.413	1.00	11.80
	ATOM	462	CB	PHE	A	117	10.823	44.337	-3.678	1.00	11.71
	ATOM	463	CG	PHE	A	117	10.223	45.539	-3.003	1.00	11.95
15	ATOM	464	CD1	PHE	A	117	8.855	45.793	-3.052	1.00	12.60
	ATOM	465	CE1	PHE	A	117	8.321	46.933	-2.440	1.00	12.58
	ATOM	466	CZ	PHE	A	117	9.163	47.836	-1.789	1.00	11.89
	ATOM	467	CE2	PHE	A	117	10.532	47.605	-1.757	1.00	12.68
	ATOM	468	CD2	PHE	A	117	11.057	46.465	-2.368	1.00	12.57
20	ATOM	469	C	PHE	A	117	10.803	41.964	-4.252	1.00	11.62
	ATOM	470	O	PHE	A	117	11.867	41.482	-3.871	1.00	11.77
	ATOM	852	N	PHE	A	164	-2.039	43.997	-1.681	1.00	11.20
	ATOM	853	CA	PHE	A	164	-0.671	44.121	-1.150	1.00	11.38
	ATOM	854	CB	PHE	A	164	-0.271	45.597	-1.193	1.00	11.09
25	ATOM	855	CG	PHE	A	164	1.083	45.896	-0.624	1.00	11.82
	ATOM	856	CD1	PHE	A	164	2.238	45.586	-1.333	1.00	10.42
	ATOM	857	CE1	PHE	A	164	3.504	45.881	-0.801	1.00	11.27
	ATOM	858	CZ	PHE	A	164	3.611	46.506	0.436	1.00	10.73
	ATOM	859	CE2	PHE	A	164	2.466	46.842	1.144	1.00	11.29
30	ATOM	860	CD2	PHE	A	164	1.203	46.535	0.612	1.00	10.92
	ATOM	861	C	PHE	A	164	-0.571	43.567	0.276	1.00	11.74
	ATOM	862	O	PHE	A	164	0.378	42.852	0.617	1.00	11.82
	ATOM	877	N	ASN	A	167	-2.074	37.844	2.624	1.00	18.39
	ATOM	878	CA	ASN	A	167	-1.150	36.746	2.375	1.00	20.42
35	ATOM	879	CB	ASN	A	167	-1.347	35.618	3.392	1.00	20.66
	ATOM	880	CG	ASN	A	167	-1.107	36.073	4.821	1.00	22.22
	ATOM	881	OD1	ASN	A	167	-1.802	35.643	5.744	1.00	24.64
	ATOM	882	ND2	ASN	A	167	-0.119	36.949	5.013	1.00	24.02
	ATOM	883	C	ASN	A	167	-1.308	36.220	0.961	1.00	21.22
40	ATOM	884	O	ASN	A	167	-2.432	36.089	0.479	1.00	21.40
	ATOM	951	N	MET	A	177	-4.843	42.866	10.516	1.00	10.02
	ATOM	952	CA	MET	A	177	-4.125	43.512	9.425	1.00	10.50
	ATOM	953	CB	MET	A	177	-3.652	42.449	8.431	1.00	10.73
	ATOM	954	CG	MET	A	177	-2.496	42.886	7.552	1.00	13.40
45	ATOM	955	SD	MET	A	177	-0.990	43.167	8.501	1.00	15.69
	ATOM	956	CE	MET	A	177	-0.624	41.511	9.064	1.00	15.67
	ATOM	957	C	MET	A	177	-5.002	44.547	8.705	1.00	10.32
	ATOM	958	O	MET	A	177	-4.536	45.635	8.370	1.00	10.56
	ATOM	972	N	ALA	A	180	-5.365	47.632	10.862	1.00	9.44
50	ATOM	973	CA	ALA	A	180	-4.089	48.350	10.775	1.00	9.83

	ATOM	974	CB	ALA A 180	-2.915	47.382	10.914	1.00	9.97
	ATOM	975	C	ALA A 180	-3.960	49.171	9.486	1.00	9.72
	ATOM	976	O	ALA A 180	-3.606	50.344	9.539	1.00	9.95
5	ATOM	977	N	TYR A 181	-4.275	48.574	8.341	1.00	9.95
	ATOM	978	CA	TYR A 181	-4.206	49.302	7.069	1.00	10.52
	ATOM	979	CB	TYR A 181	-4.174	48.352	5.862	1.00	10.51
	ATOM	980	CG	TYR A 181	-2.795	47.759	5.628	1.00	10.25
	ATOM	981	CD1	TYR A 181	-1.812	48.485	4.963	1.00	11.37
	ATOM	982	CE1	TYR A 181	-0.533	47.965	4.764	1.00	12.31
10	ATOM	983	CZ	TYR A 181	-0.221	46.695	5.237	1.00	12.09
	ATOM	984	OH	TYR A 181	1.050	46.188	5.040	1.00	10.57
	ATOM	985	CE2	TYR A 181	-1.178	45.951	5.911	1.00	11.41
	ATOM	986	CD2	TYR A 181	-2.466	46.490	6.103	1.00	10.74
	ATOM	987	C	TYR A 181	-5.287	50.388	6.927	1.00	10.90
15	ATOM	988	O	TYR A 181	-5.014	51.471	6.394	1.00	11.09
	ATOM	1011	N	LEU A 184	-4.270	53.352	8.754	1.00	13.42
	ATOM	1012	CA	LEU A 184	-3.243	54.021	7.973	1.00	14.11
	ATOM	1013	CB	LEU A 184	-2.224	52.997	7.460	1.00	14.21
	ATOM	1014	CG	LEU A 184	-0.841	53.458	7.012	1.00	15.69
20	ATOM	1015	CD1	LEU A 184	-0.110	54.189	8.135	1.00	15.46
	ATOM	1016	CD2	LEU A 184	-0.033	52.252	6.522	1.00	15.38
	ATOM	1017	C	LEU A 184	-3.825	54.840	6.810	1.00	14.10
	ATOM	1018	O	LEU A 184	-3.521	56.020	6.690	1.00	14.39

25 Table 9. Atomic coordinates of amino acid residues of hDcn1 Chain B essential for binding hUbc12 from structure with stapled hUbc12 peptide.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
30	ATOM	2412 N	ILE B 83	-30.405	108.348	-21.310	1.00 22.06
	ATOM	2413 CA	ILE B 83	-29.529	107.901	-22.393	1.00 21.85
	ATOM	2414 CB	ILE B 83	-30.307	107.192	-23.536	1.00 21.90
	ATOM	2415 CG1	ILE B 83	-31.335	108.117	-24.185	1.00 21.89
	ATOM	2416 CD1	ILE B 83	-31.892	107.564	-25.477	1.00 21.91
	ATOM	2417 CG2	ILE B 83	-30.979	105.921	-23.005	1.00 21.93
35	ATOM	2418 C	ILE B 83	-28.560	108.983	-22.902	1.00 21.52
	ATOM	2419 O	ILE B 83	-27.395	108.687	-23.175	1.00 21.21
	ATOM	2432 N	ILE B 86	-25.810	109.354	-20.278	1.00 20.01
	ATOM	2433 CA	ILE B 86	-24.882	108.227	-20.411	1.00 19.96
	ATOM	2434 CB	ILE B 86	-25.632	106.873	-20.671	1.00 20.05
40	ATOM	2435 CG1	ILE B 86	-26.467	106.442	-19.450	1.00 20.17
	ATOM	2436 CD1	ILE B 86	-25.690	106.246	-18.153	1.00 19.80
	ATOM	2437 CG2	ILE B 86	-24.662	105.766	-21.113	1.00 19.91
	ATOM	2438 C	ILE B 86	-23.874	108.506	-21.530	1.00 19.93
	ATOM	2439 O	ILE B 86	-22.666	108.343	-21.341	1.00 19.67
45	ATOM	2440 N	GLN B 87	-24.383	108.938	-22.684	1.00 20.03
	ATOM	2441 CA	GLN B 87	-23.539	109.293	-23.826	1.00 20.34
	ATOM	2442 CB	GLN B 87	-24.396	109.721	-25.025	1.00 20.22
	ATOM	2443 CG	GLN B 87	-23.607	110.009	-26.307	1.00 20.12
	ATOM	2444 CD	GLN B 87	-23.008	108.758	-26.930	0.99 20.10

	ATOM	2445	OE1	GLN	B	87	-23.728	107.857	-27.361	1.00	19.31
	ATOM	2446	NE2	GLN	B	87	-21.681	108.704	-26.989	1.00	20.30
	ATOM	2447	C	GLN	B	87	-22.549	110.392	-23.452	1.00	20.46
5	ATOM	2448	O	GLN	B	87	-21.352	110.280	-23.726	1.00	20.60
	ATOM	2520	N	PRO	B	97	-19.269	103.969	-25.674	1.00	22.67
	ATOM	2521	CA	PRO	B	97	-20.254	103.509	-24.684	1.00	22.62
	ATOM	2522	CB	PRO	B	97	-21.551	104.178	-25.141	1.00	22.57
	ATOM	2523	CG	PRO	B	97	-21.104	105.391	-25.892	1.00	22.66
10	ATOM	2524	CD	PRO	B	97	-19.839	104.980	-26.585	1.00	22.59
	ATOM	2525	C	PRO	B	97	-20.426	101.988	-24.639	1.00	22.66
	ATOM	2526	O	PRO	B	97	-20.804	101.448	-23.598	1.00	22.58
	ATOM	2527	N	ALA	B	98	-20.139	101.312	-25.752	1.00	22.54
	ATOM	2528	CA	ALA	B	98	-20.255	99.858	-25.834	1.00	22.70
15	ATOM	2529	CB	ALA	B	98	-20.715	99.437	-27.234	1.00	22.63
	ATOM	2530	C	ALA	B	98	-18.962	99.130	-25.453	1.00	22.71
	ATOM	2531	O	ALA	B	98	-18.874	97.912	-25.585	1.00	23.00
	ATOM	2532	N	SER	B	99	-17.966	99.872	-24.974	1.00	22.72
	ATOM	2533	CA	SER	B	99	-16.659	99.291	-24.670	1.00	22.62
	ATOM	2534	CB	SER	B	99	-15.563	100.356	-24.749	1.00	22.71
20	ATOM	2535	OG	SER	B	99	-15.605	101.204	-23.615	1.00	23.45
	ATOM	2536	C	SER	B	99	-16.627	98.606	-23.306	1.00	22.39
	ATOM	2537	O	SER	B	99	-17.471	98.871	-22.443	1.00	22.27
	ATOM	2552	N	VAL	B	102	-16.397	100.871	-20.002	1.00	20.71
	ATOM	2553	CA	VAL	B	102	-17.675	101.406	-19.530	1.00	20.51
25	ATOM	2554	CB	VAL	B	102	-18.519	101.986	-20.699	1.00	20.67
	ATOM	2555	CG1	VAL	B	102	-19.911	102.406	-20.225	1.00	20.65
	ATOM	2556	CG2	VAL	B	102	-17.805	103.177	-21.332	1.00	20.43
	ATOM	2557	C	VAL	B	102	-18.460	100.357	-18.727	1.00	20.40
	ATOM	2558	O	VAL	B	102	-19.110	100.693	-17.735	1.00	20.38
30	ATOM	2559	N	LEU	B	103	-18.384	99.094	-19.147	1.00	20.15
	ATOM	2560	CA	LEU	B	103	-18.999	97.991	-18.399	1.00	20.02
	ATOM	2561	CB	LEU	B	103	-18.941	96.682	-19.200	1.00	19.79
	ATOM	2562	CG	LEU	B	103	-19.610	95.427	-18.617	1.00	20.45
	ATOM	2563	CD1	LEU	B	103	-21.060	95.686	-18.213	1.00	20.61
35	ATOM	2564	CD2	LEU	B	103	-19.524	94.245	-19.594	1.00	20.05
	ATOM	2565	C	LEU	B	103	-18.351	97.814	-17.017	1.00	19.81
	ATOM	2566	O	LEU	B	103	-19.041	97.559	-16.027	1.00	19.65
	ATOM	2583	N	ALA	B	106	-19.655	100.587	-14.795	1.00	20.13
	ATOM	2584	CA	ALA	B	106	-21.017	100.219	-14.405	1.00	20.18
40	ATOM	2585	CB	ALA	B	106	-21.765	99.659	-15.593	1.00	20.03
	ATOM	2586	C	ALA	B	106	-21.007	99.215	-13.249	1.00	20.37
	ATOM	2587	O	ALA	B	106	-21.790	99.331	-12.297	1.00	20.20
	ATOM	2650	N	GLN	B	114	-28.388	98.507	-17.786	1.00	19.92
	ATOM	2651	CA	GLN	B	114	-27.641	99.225	-18.818	1.00	20.09
45	ATOM	2652	CB	GLN	B	114	-27.253	98.283	-19.977	1.00	19.77
	ATOM	2653	CG	GLN	B	114	-28.425	97.614	-20.687	1.00	18.83
	ATOM	2654	CD	GLN	B	114	-28.066	97.115	-22.078	1.00	18.37
	ATOM	2655	OE1	GLN	B	114	-27.419	97.821	-22.857	1.00	18.08
	ATOM	2656	NE2	GLN	B	114	-28.501	95.900	-22.402	1.00	16.31
50	ATOM	2657	C	GLN	B	114	-28.403	100.440	-19.347	1.00	20.26

	ATOM	2658	O	GLN B 114	-29.638	100.462	-19.325	1.00	20.37
	ATOM	2659	N	CYS B 115	-27.645	101.440	-19.803	1.00	20.71
	ATOM	2660	CA	CYS B 115	-28.163	102.672	-20.438	1.00	20.94
5	ATOM	2661	CB	CYS B 115	-29.037	102.341	-21.650	1.00	21.06
	ATOM	2662	SG	CYS B 115	-28.203	101.301	-22.847	1.00	21.61
	ATOM	2663	C	CYS B 115	-28.878	103.664	-19.512	1.00	21.23
	ATOM	2664	O	CYS B 115	-29.614	104.542	-19.982	1.00	21.16
	ATOM	2674	N	PHE B 117	-28.223	105.377	-15.127	1.00	21.67
10	ATOM	2675	CA	PHE B 117	-27.839	105.322	-13.715	1.00	21.68
	ATOM	2676	CB	PHE B 117	-26.477	105.984	-13.463	1.00	21.63
	ATOM	2677	CG	PHE B 117	-25.319	105.324	-14.166	1.00	22.09
	ATOM	2678	CD1	PHE B 117	-25.134	103.944	-14.111	1.00	21.98
	ATOM	2679	CE1	PHE B 117	-24.050	103.347	-14.754	1.00	22.16
	ATOM	2680	CZ	PHE B 117	-23.127	104.133	-15.437	1.00	21.74
15	ATOM	2681	CE2	PHE B 117	-23.291	105.507	-15.483	1.00	21.59
	ATOM	2682	CD2	PHE B 117	-24.380	106.097	-14.842	1.00	21.55
	ATOM	2683	C	PHE B 117	-28.880	106.059	-12.886	1.00	21.55
	ATOM	2684	O	PHE B 117	-29.336	107.137	-13.271	1.00	21.64
20	ATOM	3066	N	PHE B 164	-27.140	93.194	-15.243	1.00	19.87
	ATOM	3067	CA	PHE B 164	-26.985	94.525	-15.822	1.00	20.00
	ATOM	3068	CB	PHE B 164	-25.500	94.894	-15.809	1.00	20.10
	ATOM	3069	CG	PHE B 164	-25.185	96.227	-16.431	1.00	20.07
	ATOM	3070	CD1	PHE B 164	-25.504	97.413	-15.774	1.00	20.33
	ATOM	3071	CE1	PHE B 164	-25.201	98.652	-16.344	1.00	19.91
25	ATOM	3072	CZ	PHE B 164	-24.556	98.709	-17.579	1.00	19.28
	ATOM	3073	CE2	PHE B 164	-24.227	97.532	-18.241	1.00	20.37
	ATOM	3074	CD2	PHE B 164	-24.542	96.295	-17.662	1.00	20.04
	ATOM	3075	C	PHE B 164	-27.543	94.604	-17.252	1.00	20.03
	ATOM	3076	O	PHE B 164	-28.208	95.577	-17.616	1.00	19.88
30	ATOM	3091	N	ASN B 167	-33.402	93.223	-19.278	1.00	23.56
	ATOM	3092	CA	ASN B 167	-34.452	94.170	-18.910	1.00	25.06
	ATOM	3093	CB	ASN B 167	-35.630	94.094	-19.885	1.00	25.40
	ATOM	3094	CG	ASN B 167	-35.207	94.269	-21.327	1.00	26.53
	ATOM	3095	OD1	ASN B 167	-35.598	93.486	-22.193	1.00	28.48
35	ATOM	3096	ND2	ASN B 167	-34.401	95.293	-21.595	1.00	27.88
	ATOM	3097	C	ASN B 167	-34.945	93.921	-17.488	1.00	25.57
	ATOM	3098	O	ASN B 167	-35.087	92.765	-17.079	1.00	25.68
	ATOM	3165	N	MET B 177	-28.572	90.183	-27.289	1.00	17.20
40	ATOM	3166	CA	MET B 177	-27.921	90.921	-26.216	1.00	17.43
	ATOM	3167	CB	MET B 177	-28.969	91.422	-25.212	1.00	17.67
	ATOM	3168	CG	MET B 177	-28.531	92.623	-24.387	1.00	19.15
	ATOM	3169	SD	MET B 177	-28.144	94.055	-25.410	1.00	21.43
	ATOM	3170	CE	MET B 177	-29.768	94.544	-25.987	1.00	22.65
	ATOM	3171	C	MET B 177	-26.868	90.049	-25.528	1.00	17.09
45	ATOM	3172	O	MET B 177	-25.758	90.510	-25.258	1.00	16.99
	ATOM	3186	N	ALA B 180	-23.753	89.661	-27.733	1.00	17.12
	ATOM	3187	CA	ALA B 180	-22.999	90.911	-27.738	1.00	17.35
	ATOM	3188	CB	ALA B 180	-23.929	92.104	-27.945	1.00	17.41
	ATOM	3189	C	ALA B 180	-22.183	91.081	-26.458	1.00	17.51
50	ATOM	3190	O	ALA B 180	-21.041	91.537	-26.509	1.00	17.37

	ATOM	3191	N	TYR B 181	-22.763	90.710	-25.317	1.00	17.76
	ATOM	3192	CA	TYR B 181	-22.041	90.799	-24.045	1.00	18.12
	ATOM	3193	CB	TYR B 181	-22.995	90.874	-22.845	1.00	18.11
	ATOM	3194	CG	TYR B 181	-23.543	92.269	-22.622	1.00	18.19
5	ATOM	3195	CD1	TYR B 181	-22.789	93.238	-21.952	1.00	18.71
	ATOM	3196	CE1	TYR B 181	-23.283	94.531	-21.749	1.00	19.16
	ATOM	3197	CZ	TYR B 181	-24.544	94.866	-22.224	1.00	19.33
	ATOM	3198	OH	TYR B 181	-25.035	96.146	-22.033	1.00	19.73
	ATOM	3199	CE2	TYR B 181	-25.309	93.921	-22.900	1.00	18.60
10	ATOM	3200	CD2	TYR B 181	-24.805	92.629	-23.094	1.00	18.30
	ATOM	3201	C	TYR B 181	-20.989	89.707	-23.872	1.00	18.34
	ATOM	3202	O	TYR B 181	-19.907	89.973	-23.352	1.00	18.28
	ATOM	3225	N	LEU B 184	-18.023	90.608	-25.720	1.00	21.89
	ATOM	3226	CA	LEU B 184	-17.324	91.670	-25.010	1.00	22.22
15	ATOM	3227	CB	LEU B 184	-18.328	92.723	-24.521	1.00	22.27
	ATOM	3228	CG	LEU B 184	-17.831	94.108	-24.104	1.00	22.26
	ATOM	3229	CD1	LEU B 184	-17.213	94.861	-25.272	1.00	21.91
	ATOM	3230	CD2	LEU B 184	-18.972	94.913	-23.499	1.00	22.24
	ATOM	3231	C	LEU B 184	-16.515	91.123	-23.836	1.00	22.40
20	ATOM	3232	O	LEU B 184	-15.325	91.410	-23.714	1.00	22.83

Table 10. Atomic coordinates of amino acid residues of hDcn1 Chain A essential for binding hUbc12.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
25	ATOM	196 N	ILE A 83	36.977	-14.686	-20.772	1.00 12.90
	ATOM	197 CA	ILE A 83	36.506	-15.088	-22.103	1.00 13.86
	ATOM	198 CB	ILE A 83	37.408	-14.550	-23.249	1.00 14.02
	ATOM	199 CG1	ILE A 83	38.829	-15.121	-23.160	1.00 14.11
30	ATOM	200 CD1	ILE A 83	39.710	-14.759	-24.349	1.00 15.19
	ATOM	201 CG2	ILE A 83	37.426	-13.022	-23.244	1.00 14.59
	ATOM	202 C	ILE A 83	36.282	-16.597	-22.244	1.00 14.48
	ATOM	203 O	ILE A 83	35.350	-17.023	-22.926	1.00 14.64
	ATOM	216 N	ILE A 86	33.068	-17.241	-20.441	1.00 9.57
35	ATOM	217 CA	ILE A 86	31.948	-16.711	-21.229	1.00 9.17
	ATOM	218 CB	ILE A 86	32.238	-15.296	-21.827	1.00 8.85
	ATOM	219 CG1	ILE A 86	32.344	-14.228	-20.724	1.00 9.45
	ATOM	220 CD1	ILE A 86	31.063	-13.939	-19.963	1.00 10.17
	ATOM	221 CG2	ILE A 86	31.154	-14.890	-22.848	1.00 9.18
40	ATOM	222 C	ILE A 86	31.582	-17.703	-22.335	1.00 9.55
	ATOM	223 O	ILE A 86	30.403	-17.993	-22.547	1.00 9.03
	ATOM	224 N	GLN A 87	32.591	-18.224	-23.028	1.00 10.48
	ATOM	225 CA	GLN A 87	32.377	-19.248	-24.049	1.00 11.22
	ATOM	226 CB	GLN A 87	33.700	-19.650	-24.710	1.00 11.32
45	ATOM	227 CG	GLN A 87	33.546	-20.663	-25.841	1.00 12.84
	ATOM	228 CD	GLN A 87	32.742	-20.115	-27.008	0.67 13.76
	ATOM	229 OE1	GLN A 87	33.143	-19.144	-27.646	1.00 16.05
	ATOM	230 NE2	GLN A 87	31.601	-20.736	-27.291	1.00 15.09
	ATOM	231 C	GLN A 87	31.679	-20.479	-23.467	1.00 11.20

5	ATOM	232	O	GLN	A	87	30.738	-21.002	-24.066	1.00	11.47
	ATOM	304	N	PRO	A	97	27.365	-17.205	-28.903	1.00	9.39
	ATOM	305	CA	PRO	A	97	27.770	-16.104	-28.025	1.00	9.60
	ATOM	306	CB	PRO	A	97	29.295	-16.200	-28.024	1.00	9.57
	ATOM	307	CG	PRO	A	97	29.575	-17.639	-28.292	1.00	9.43
10	ATOM	308	CD	PRO	A	97	28.486	-18.109	-29.214	1.00	9.60
	ATOM	309	C	PRO	A	97	27.322	-14.723	-28.507	1.00	9.56
	ATOM	310	O	PRO	A	97	27.104	-13.836	-27.683	1.00	9.53
	ATOM	311	N	ALA	A	98	27.156	-14.566	-29.820	1.00	10.14
	ATOM	312	CA	ALA	A	98	26.780	-13.293	-30.431	1.00	10.80
15	ATOM	313	CB	ALA	A	98	27.526	-13.116	-31.758	1.00	10.99
	ATOM	314	C	ALA	A	98	25.270	-13.144	-30.640	1.00	11.25
	ATOM	315	O	ALA	A	98	24.818	-12.229	-31.340	1.00	12.29
	ATOM	316	N	SER	A	99	24.490	-14.032	-30.029	1.00	11.27
	ATOM	317	CA	SER	A	99	23.039	-14.011	-30.210	1.00	11.21
20	ATOM	318	CB	SER	A	99	22.439	-15.384	-29.919	1.00	11.58
	ATOM	319	OG	SER	A	99	22.566	-15.712	-28.553	1.00	12.43
	ATOM	320	C	SER	A	99	22.370	-12.956	-29.335	1.00	10.50
	ATOM	321	O	SER	A	99	22.918	-12.540	-28.313	1.00	9.98
	ATOM	336	N	VAL	A	102	21.794	-13.908	-25.598	1.00	7.12
25	ATOM	337	CA	VAL	A	102	22.960	-13.673	-24.751	1.00	6.85
	ATOM	338	CB	VAL	A	102	24.260	-14.153	-25.433	1.00	6.88
	ATOM	339	CG1	VAL	A	102	25.492	-13.783	-24.603	1.00	7.40
	ATOM	340	CG2	VAL	A	102	24.198	-15.661	-25.669	1.00	7.91
	ATOM	341	C	VAL	A	102	23.037	-12.205	-24.314	1.00	6.55
30	ATOM	342	O	VAL	A	102	23.311	-11.926	-23.150	1.00	6.96
	ATOM	343	N	LEU	A	103	22.789	-11.276	-25.237	1.00	6.21
	ATOM	344	CA	LEU	A	103	22.751	-9.852	-24.891	1.00	6.05
	ATOM	345	CB	LEU	A	103	22.576	-8.990	-26.146	1.00	6.39
	ATOM	346	CG	LEU	A	103	22.643	-7.472	-25.936	1.00	5.17
35	ATOM	347	CD1	LEU	A	103	23.967	-7.059	-25.301	1.00	6.27
	ATOM	348	CD2	LEU	A	103	22.475	-6.764	-27.258	1.00	6.33
	ATOM	349	C	LEU	A	103	21.661	-9.529	-23.870	1.00	6.49
	ATOM	350	O	LEU	A	103	21.872	-8.721	-22.956	1.00	6.43
	ATOM	367	N	ALA	A	106	22.865	-10.651	-20.508	1.00	7.03
40	ATOM	368	CA	ALA	A	106	23.841	-9.666	-20.041	1.00	6.99
	ATOM	369	CB	ALA	A	106	24.801	-9.293	-21.165	1.00	7.01
	ATOM	370	C	ALA	A	106	23.169	-8.416	-19.464	1.00	6.61
	ATOM	371	O	ALA	A	106	23.605	-7.879	-18.442	1.00	7.01
	ATOM	446	N	GLN	A	114	30.971	-6.245	-22.517	1.00	7.54
45	ATOM	447	CA	GLN	A	114	30.955	-7.504	-23.257	1.00	8.01
	ATOM	448	CB	GLN	A	114	30.775	-7.256	-24.758	1.00	7.85
	ATOM	449	CG	GLN	A	114	31.857	-6.400	-25.406	1.00	7.92
	ATOM	450	CD	GLN	A	114	31.964	-6.653	-26.896	1.00	8.14
	ATOM	451	OE1	GLN	A	114	31.982	-7.801	-27.341	1.00	9.25
50	ATOM	452	NE2	GLN	A	114	32.060	-5.580	-27.674	1.00	7.38
	ATOM	453	C	GLN	A	114	32.186	-8.380	-22.996	1.00	8.45
	ATOM	454	O	GLN	A	114	33.298	-7.883	-22.747	1.00	8.88
	ATOM	455	N	CYS	A	115	31.949	-9.691	-23.029	1.00	9.24
	ATOM	456	CA	CYS	A	115	32.990	-10.723	-22.948	1.00	9.94

	ATOM	457	CB	CYS A 115	34.087	-10.498	-23.996	1.00	10.22
	ATOM	458	SG	CYS A 115	33.482	-10.519	-25.689	1.00	11.14
	ATOM	459	C	CYS A 115	33.596	-10.896	-21.559	1.00	10.57
5	ATOM	460	O	CYS A 115	34.726	-11.387	-21.417	1.00	10.06
	ATOM	470	N	PHE A 117	31.900	-11.315	-17.158	1.00	10.88
	ATOM	471	CA	PHE A 117	31.016	-10.979	-16.047	1.00	10.54
	ATOM	472	CB	PHE A 117	29.925	-12.035	-15.829	1.00	10.31
	ATOM	473	CG	PHE A 117	28.955	-12.191	-16.974	1.00	10.63
	ATOM	474	CD1	PHE A 117	28.370	-11.086	-17.585	1.00	10.88
10	ATOM	475	CE1	PHE A 117	27.466	-11.249	-18.632	1.00	11.40
	ATOM	476	CZ	PHE A 117	27.122	-12.526	-19.065	1.00	10.91
	ATOM	477	CE2	PHE A 117	27.689	-13.637	-18.454	1.00	11.21
	ATOM	478	CD2	PHE A 117	28.595	-13.465	-17.410	1.00	11.19
	ATOM	479	C	PHE A 117	31.842	-10.888	-14.774	1.00	10.24
15	ATOM	480	O	PHE A 117	32.678	-11.748	-14.507	1.00	10.91
	ATOM	868	N	PHE A 164	27.612	-1.423	-22.879	1.00	8.55
	ATOM	869	CA	PHE A 164	28.065	-2.810	-22.824	1.00	8.10
	ATOM	870	CB	PHE A 164	26.836	-3.708	-22.983	1.00	8.03
	ATOM	871	CG	PHE A 164	27.133	-5.176	-22.982	1.00	7.71
20	ATOM	872	CD1	PHE A 164	27.444	-5.836	-21.798	1.00	7.58
	ATOM	873	CE1	PHE A 164	27.695	-7.206	-21.787	1.00	8.30
	ATOM	874	CZ	PHE A 164	27.620	-7.932	-22.970	1.00	8.55
	ATOM	875	CE2	PHE A 164	27.296	-7.287	-24.158	1.00	7.42
	ATOM	876	CD2	PHE A 164	27.062	-5.912	-24.161	1.00	7.45
25	ATOM	877	C	PHE A 164	29.089	-3.107	-23.920	1.00	8.08
	ATOM	878	O	PHE A 164	30.094	-3.790	-23.689	1.00	7.67
	ATOM	893	N	ASN A 167	34.518	0.021	-24.949	1.00	18.51
	ATOM	894	CA	ASN A 167	35.584	-0.198	-23.981	1.00	23.93
	ATOM	895	CB	ASN A 167	36.945	0.137	-24.598	1.00	24.56
30	ATOM	896	CG	ASN A 167	37.254	-0.702	-25.827	1.00	27.69
	ATOM	897	OD1	ASN A 167	37.067	-1.920	-25.830	1.00	30.17
	ATOM	898	ND2	ASN A 167	37.740	-0.051	-26.877	1.00	29.71
	ATOM	899	C	ASN A 167	35.354	0.648	-22.732	1.00	27.76
	ATOM	900	O	ASN A 167	34.860	1.775	-22.833	1.00	27.47
35	ATOM	972	N	MET A 177	32.057	-2.089	-34.346	1.00	9.67
	ATOM	973	CA	MET A 177	31.336	-2.605	-33.182	1.00	8.98
	ATOM	974	CB	MET A 177	32.086	-2.279	-31.885	1.00	9.19
	ATOM	975	CG	MET A 177	31.772	-3.243	-30.739	1.00	10.77
	ATOM	976	SD	MET A 177	32.121	-4.976	-31.150	1.00	13.17
40	ATOM	977	CE	MET A 177	33.883	-4.902	-31.440	1.00	14.81
	ATOM	978	C	MET A 177	29.899	-2.081	-33.121	1.00	8.33
	ATOM	979	O	MET A 177	28.973	-2.845	-32.850	1.00	7.85
	ATOM	993	N	ALA A 180	27.819	-3.852	-35.736	1.00	7.21
	ATOM	994	CA	ALA A 180	27.550	-5.215	-35.276	1.00	7.23
45	ATOM	995	CB	ALA A 180	28.814	-5.842	-34.679	1.00	7.51
	ATOM	996	C	ALA A 180	26.383	-5.261	-34.281	1.00	7.58
	ATOM	997	O	ALA A 180	25.485	-6.095	-34.406	1.00	7.82
	ATOM	998	N	TYR A 181	26.379	-4.344	-33.316	1.00	7.11
	ATOM	999	CA	TYR A 181	25.315	-4.309	-32.316	1.00	6.57
50	ATOM	1000	CB	TYR A 181	25.759	-3.593	-31.036	1.00	7.02

	ATOM	1001	CG	TYR A 181	26.605	-4.491	-30.160	1.00	6.87
	ATOM	1002	CD1	TYR A 181	26.007	-5.394	-29.275	1.00	7.10
	ATOM	1003	CE1	TYR A 181	26.771	-6.228	-28.481	1.00	7.04
	ATOM	1004	CZ	TYR A 181	28.149	-6.176	-28.558	1.00	7.84
5	ATOM	1005	OH	TYR A 181	28.901	-7.006	-27.768	1.00	9.69
	ATOM	1006	CE2	TYR A 181	28.775	-5.298	-29.426	1.00	7.94
	ATOM	1007	CD2	TYR A 181	27.998	-4.463	-30.227	1.00	7.63
	ATOM	1008	C	TYR A 181	24.009	-3.755	-32.876	1.00	6.27
	ATOM	1009	O	TYR A 181	22.947	-4.239	-32.515	1.00	6.90
10	ATOM	1032	N	LEU A 184	22.293	-6.498	-34.554	1.00	8.61
	ATOM	1033	CA	LEU A 184	21.717	-7.379	-33.544	1.00	9.06
	ATOM	1034	CB	LEU A 184	22.740	-7.634	-32.429	1.00	9.47
	ATOM	1035	CG	LEU A 184	22.475	-8.763	-31.427	1.00	10.45
	ATOM	1036	CD1	LEU A 184	22.222	-10.090	-32.134	1.00	11.79
15	ATOM	1037	CD2	LEU A 184	23.652	-8.883	-30.468	1.00	11.28
	ATOM	1038	C	LEU A 184	20.400	-6.841	-32.966	1.00	9.44
	ATOM	1039	O	LEU A 184	19.392	-7.552	-32.958	1.00	10.68

20 Table 11. Atomic coordinates of amino acid residues of hDcn1 Chain B essential for binding hUbc12.

	Atom	Atom			X	Y	Z	Occu-	B-
	No.	Name						pancy	factor
	ATOM	1819	N	ILE B 83	29.948	2.614	6.205	1.00	14.95
25	ATOM	1820	CA	ILE B 83	28.894	3.066	7.119	1.00	15.72
	ATOM	1821	CB	ILE B 83	29.070	2.516	8.566	1.00	15.81
	ATOM	1822	CG1	ILE B 83	30.448	2.860	9.141	1.00	16.94
	ATOM	1823	CD1	ILE B 83	30.633	2.394	10.579	1.00	18.68
	ATOM	1824	CG2	ILE B 83	28.840	1.009	8.592	1.00	16.52
30	ATOM	1825	C	ILE B 83	28.672	4.583	7.111	1.00	15.83
	ATOM	1826	O	ILE B 83	27.533	5.044	7.233	1.00	15.95
	ATOM	1839	N	ILE B 86	26.874	5.426	3.809	1.00	13.37
	ATOM	1840	CA	ILE B 86	25.480	4.984	3.892	1.00	12.69
	ATOM	1841	CB	ILE B 86	25.337	3.542	4.485	1.00	12.54
35	ATOM	1842	CG1	ILE B 86	26.021	2.496	3.586	1.00	12.43
	ATOM	1843	CD1	ILE B 86	25.317	2.196	2.257	1.00	12.53
	ATOM	1844	CG2	ILE B 86	23.859	3.187	4.725	1.00	12.96
	ATOM	1845	C	ILE B 86	24.654	5.989	4.703	1.00	12.56
	ATOM	1846	O	ILE B 86	23.542	6.341	4.310	1.00	12.49
40	ATOM	1847	N	GLN B 87	25.207	6.449	5.822	1.00	12.84
	ATOM	1848	CA	GLN B 87	24.552	7.470	6.634	1.00	13.49
	ATOM	1849	CB	GLN B 87	25.390	7.787	7.878	1.00	13.64
	ATOM	1850	CG	GLN B 87	24.756	8.801	8.836	1.00	15.44
	ATOM	1851	CD	GLN B 87	23.413	8.346	9.383	1.00	16.30
45	ATOM	1852	OE1	GLN B 87	23.328	7.357	10.113	1.00	18.29
	ATOM	1853	NE2	GLN B 87	22.358	9.073	9.039	0.84	17.18
	ATOM	1854	C	GLN B 87	24.278	8.739	5.815	1.00	13.78
	ATOM	1855	O	GLN B 87	23.177	9.286	5.867	0.88	13.69
	ATOM	1927	N	PRO B 97	17.781	5.711	8.137	1.00	11.79
50	ATOM	1928	CA	PRO B 97	18.531	4.583	7.573	1.00	11.68

	ATOM	1929	CB	PRO B	97	19.873	4.646	8.310	1.00	11.76
	ATOM	1930	CG	PRO B	97	20.002	6.061	8.735	1.00	11.97
	ATOM	1931	CD	PRO B	97	18.605	6.494	9.074	1.00	11.71
5	ATOM	1932	C	PRO B	97	17.869	3.225	7.797	1.00	12.06
	ATOM	1933	O	PRO B	97	18.101	2.309	7.017	1.00	12.24
	ATOM	1934	N	ALA B	98	17.049	3.113	8.841	1.00	12.54
	ATOM	1935	CA	ALA B	98	16.374	1.860	9.181	1.00	12.70
	ATOM	1936	CB	ALA B	98	16.361	1.668	10.698	1.00	12.91
10	ATOM	1937	C	ALA B	98	14.952	1.766	8.609	1.00	13.00
	ATOM	1938	O	ALA B	98	14.168	0.906	9.021	1.00	13.88
	ATOM	1939	N	SER B	99	14.622	2.641	7.663	1.00	13.24
	ATOM	1940	CA	SER B	99	13.279	2.672	7.082	1.00	13.23
	ATOM	1941	CB	SER B	99	12.953	4.062	6.531	1.00	13.54
15	ATOM	1942	OG	SER B	99	13.704	4.337	5.363	1.00	14.52
	ATOM	1943	C	SER B	99	13.092	1.629	5.986	1.00	12.89
	ATOM	1944	O	SER B	99	14.056	1.196	5.349	1.00	12.57
	ATOM	1959	N	VAL B	102	14.549	2.518	2.526	1.00	9.33
	ATOM	1960	CA	VAL B	102	15.975	2.245	2.364	1.00	9.28
20	ATOM	1961	CB	VAL B	102	16.792	2.715	3.583	1.00	9.50
	ATOM	1962	CG1	VAL B	102	18.242	2.256	3.483	1.00	9.82
	ATOM	1963	CG2	VAL B	102	16.730	4.243	3.694	1.00	9.83
	ATOM	1964	C	VAL B	102	16.217	0.765	2.040	1.00	8.85
	ATOM	1965	O	VAL B	102	17.042	0.444	1.186	1.00	9.10
25	ATOM	1966	N	LEU B	103	15.477	-0.126	2.694	1.00	8.30
	ATOM	1967	CA	LEU B	103	15.550	-1.554	2.369	1.00	7.93
	ATOM	1968	CB	LEU B	103	14.711	-2.380	3.350	1.00	8.08
	ATOM	1969	CG	LEU B	103	14.821	-3.899	3.221	1.00	7.79
	ATOM	1970	CD1	LEU B	103	16.257	-4.353	3.403	1.00	8.56
30	ATOM	1971	CD2	LEU B	103	13.939	-4.569	4.243	1.00	9.12
	ATOM	1972	C	LEU B	103	15.123	-1.847	0.925	1.00	7.70
	ATOM	1973	O	LEU B	103	15.727	-2.687	0.246	1.00	7.60
	ATOM	1990	N	ALA B	106	17.929	-0.799	-1.331	1.00	7.92
	ATOM	1991	CA	ALA B	106	18.975	-1.823	-1.244	1.00	7.82
35	ATOM	1992	CB	ALA B	106	19.211	-2.221	0.201	1.00	7.95
	ATOM	1993	C	ALA B	106	18.642	-3.054	-2.099	1.00	7.83
	ATOM	1994	O	ALA B	106	19.513	-3.619	-2.767	1.00	7.89
	ATOM	2063	N	GLN B	114	23.763	-5.496	4.444	1.00	10.08
	ATOM	2064	CA	GLN B	114	23.384	-4.238	5.078	1.00	9.77
40	ATOM	2065	CB	GLN B	114	22.454	-4.492	6.267	1.00	9.56
	ATOM	2066	CG	GLN B	114	23.081	-5.287	7.408	1.00	9.34
	ATOM	2067	CD	GLN B	114	22.379	-5.053	8.719	1.00	8.85
	ATOM	2068	OE1	GLN B	114	22.105	-3.910	9.098	1.00	9.42
	ATOM	2069	NE2	GLN B	114	22.098	-6.135	9.437	1.00	8.52
45	ATOM	2070	C	GLN B	114	24.592	-3.416	5.528	1.00	10.01
	ATOM	2071	O	GLN B	114	25.608	-3.968	5.960	1.00	10.08
	ATOM	2072	N	CYS B	115	24.450	-2.095	5.401	1.00	10.57
	ATOM	2073	CA	CYS B	115	25.405	-1.099	5.915	1.00	11.33
	ATOM	2074	CB	CYS B	115	25.797	-1.391	7.369	1.00	11.41
	ATOM	2075	SG	CYS B	115	24.404	-1.367	8.516	1.00	13.01
50	ATOM	2076	C	CYS B	115	26.639	-0.897	5.037	1.00	11.90

	ATOM	2077	O	CYS B 115	27.651	-0.352	5.488	1.00	12.13
	ATOM	2087	N	PHE B 117	27.457	-0.565	0.404	1.00	12.62
	ATOM	2088	CA	PHE B 117	27.233	-0.894	-0.999	1.00	12.39
	ATOM	2089	CB	PHE B 117	26.485	0.223	-1.740	1.00	12.42
5	ATOM	2090	CG	PHE B 117	25.068	0.452	-1.285	1.00	12.77
	ATOM	2091	CD1	PHE B 117	24.193	-0.609	-1.057	1.00	12.77
	ATOM	2092	CE1	PHE B 117	22.874	-0.370	-0.656	1.00	12.83
	ATOM	2093	CZ	PHE B 117	22.423	0.936	-0.498	1.00	12.86
	ATOM	2094	CE2	PHE B 117	23.280	1.999	-0.743	1.00	13.41
10	ATOM	2095	CD2	PHE B 117	24.594	1.752	-1.139	1.00	13.43
	ATOM	2096	C	PHE B 117	28.583	-1.046	-1.667	1.00	11.92
	ATOM	2097	O	PHE B 117	29.497	-0.262	-1.414	1.00	12.30
	ATOM	2485	N	PHE B 164	20.554	-10.249	3.036	1.00	10.13
	ATOM	2486	CA	PHE B 164	21.005	-8.872	3.207	1.00	9.30
15	ATOM	2487	CB	PHE B 164	19.903	-7.944	2.685	1.00	8.96
	ATOM	2488	CG	PHE B 164	20.185	-6.479	2.852	1.00	8.28
	ATOM	2489	CD1	PHE B 164	21.081	-5.824	2.011	1.00	7.37
	ATOM	2490	CE1	PHE B 164	21.325	-4.458	2.152	1.00	7.93
	ATOM	2491	CZ	PHE B 164	20.663	-3.732	3.132	1.00	7.52
20	ATOM	2492	CE2	PHE B 164	19.767	-4.372	3.975	1.00	8.31
	ATOM	2493	CD2	PHE B 164	19.524	-5.739	3.831	1.00	7.61
	ATOM	2494	C	PHE B 164	21.330	-8.579	4.673	1.00	8.93
	ATOM	2495	O	PHE B 164	22.328	-7.921	4.972	1.00	8.93
	ATOM	2510	N	ASN B 167	25.423	-11.883	8.254	1.00	17.88
25	ATOM	2511	CA	AASN B 167	26.822	-11.839	7.864	0.50	20.28
	ATOM	2512	CA	BASN B 167	26.827	-11.858	7.892	0.50	20.24
	ATOM	2513	CB	AASN B 167	27.727	-12.325	8.995	0.50	21.60
	ATOM	2514	CB	BASN B 167	27.634	-12.433	9.057	0.50	21.55
	ATOM	2515	CG	AASN B 167	27.856	-11.307	10.110	0.50	25.80
30	ATOM	2516	CG	BASN B 167	29.119	-12.423	8.809	0.50	25.38
	ATOM	2517	OD1AASN	B 167	28.050	-10.117	9.859	0.50	28.22
	ATOM	2518	OD1BASN	B 167	29.601	-12.981	7.826	0.50	27.79
	ATOM	2519	ND2AASN	B 167	27.750	-11.770	11.350	0.50	28.23
	ATOM	2520	ND2BASN	B 167	29.863	-11.810	9.722	0.50	27.67
35	ATOM	2521	C	ASN B 167	27.010	-12.683	6.608	1.00	20.72
	ATOM	2522	O	ASN B 167	26.555	-13.829	6.549	1.00	20.44
	ATOM	2589	N	MET B 177	18.700	-9.451	15.173	1.00	12.40
	ATOM	2590	CA	MET B 177	18.680	-8.891	13.824	1.00	11.50
	ATOM	2591	CB	MET B 177	19.985	-9.210	13.081	1.00	11.92
40	ATOM	2592	CG	MET B 177	20.314	-8.240	11.947	1.00	13.23
	ATOM	2593	SD	MET B 177	20.326	-6.502	12.455	1.00	13.85
	ATOM	2594	CE	MET B 177	21.758	-6.458	13.517	1.00	14.84
	ATOM	2595	C	MET B 177	17.465	-9.394	13.045	1.00	10.79
	ATOM	2596	O	MET B 177	16.811	-8.624	12.346	1.00	10.53
45	ATOM	2610	N	ALA B 180	14.364	-7.574	14.322	1.00	8.93
	ATOM	2611	CA	ALA B 180	14.340	-6.219	13.771	1.00	8.75
	ATOM	2612	CB	ALA B 180	15.696	-5.561	13.910	1.00	9.14
	ATOM	2613	C	ALA B 180	13.870	-6.189	12.314	1.00	8.69
	ATOM	2614	O	ALA B 180	13.040	-5.350	11.935	1.00	9.10
50	ATOM	2615	N	TYR B 181	14.379	-7.107	11.497	1.00	8.07

	ATOM	2616	CA	TYR B 181	13.968	-7.133	10.097	1.00	7.64
	ATOM	2617	CB	TYR B 181	14.990	-7.869	9.224	1.00	7.89
	ATOM	2618	CG	TYR B 181	16.138	-6.973	8.845	1.00	6.99
	ATOM	2619	CD1	TYR B 181	16.023	-6.068	7.794	1.00	7.98
5	ATOM	2620	CE1	TYR B 181	17.075	-5.234	7.450	1.00	7.54
	ATOM	2621	CZ	TYR B 181	18.255	-5.297	8.173	1.00	8.50
	ATOM	2622	OH	TYR B 181	19.290	-4.473	7.839	1.00	9.59
	ATOM	2623	CE2	TYR B 181	18.390	-6.176	9.224	1.00	8.12
	ATOM	2624	CD2	TYR B 181	17.332	-7.003	9.562	1.00	7.84
10	ATOM	2625	C	TYR B 181	12.540	-7.633	9.894	1.00	7.55
	ATOM	2626	O	TYR B 181	11.829	-7.156	9.006	1.00	8.17
	ATOM	2649	N	LEU B 184	10.286	-4.821	10.528	1.00	10.96
	ATOM	2650	CA	LEU B 184	10.311	-3.937	9.370	1.00	11.71
	ATOM	2651	CB	LEU B 184	11.758	-3.707	8.917	1.00	12.41
15	ATOM	2652	CG	LEU B 184	12.048	-2.593	7.910	1.00	13.96
	ATOM	2653	CD1	LEU B 184	11.550	-1.250	8.429	1.00	14.99
	ATOM	2654	CD2	LEU B 184	13.543	-2.532	7.604	1.00	14.64
	ATOM	2655	C	LEU B 184	9.466	-4.465	8.207	1.00	11.44
20	ATOM	2656	O	LEU B 184	8.546	-3.785	7.754	1.00	12.30

Table 12. Additional contacts that contribute to, but are not essential, for contact between human Dcn1 and human Ubc12 with hUbc12 Chain E interactions with hDcn1^P Chain B and hUbc12 Chain F interactions with hDcn1^P Chain A shown.

hUbc12 Atom	hDcn1 Atom	Distance (Å)
Leu 7E CB	Ile 83B CG2	4.14
	Ile 83B CD1	4.33
Leu 7E CD1	Cys 115B CB	3.61
	Cys 115B SG	3.78
	Ile 83B CG2	3.94
Leu 7E C	Ile 83B CD1	4.08
Leu 7E O	Ile 83B CD1	4.15
Lys 8E N	Ile 83B CD1	3.91
Lys 8E CA	Ile 83B CD1	3.85
Lys 8E CB	Ile 83B CD1	4.35
Lys 8E CG	Ile 83B CB	4.37
	Ile 83B CG1	4.22
	Ile 83B CD1	3.66
Lys 8E CD	Asp 84B OD1	4.18
Lys 8E CE	Gln 87B OE1	4.21
Lys 8E NZ	Gln 87B CB	3.97
	Gln 87B OE1	3.02
	Gln 87B CG	3.92
	Gln 87B CD	3.85
Lys 11E CE	Ile 83B CG1	3.97
	Ile 83B CD1	3.27
Lys 11E NZ	Ile 83B CG1	3.51
	Ile 83B CD1	3.27
	Asp 84B CG	4.36

	Asp 84B OD2	3.58
Leu 7F CB	Ile 83A CD1	4.13
	Ile 83A CG2	4.15
Leu 7F CD1	Cys 115A SG	3.96
	Cys 115A CB	3.83
	Ile 83A CG2	4.06
Leu 7F C	Ile 83A CD1	3.82
Leu 7F O	Ile 83A CD1	3.63
Lys 8F N	Ile 83A CD1	4.02
Lys 8F CA	Ile 83A CD1	4.08
Lys 8F CD	Gln 87A CG	4.46
	Gln 87A CD	4.5
	Gln 87A OE1	3.73
Lys 8F CE	Gln 87A CG	4.42
	Gln 87A CD	3.95
	Gln 87A OE1	2.91
Lys 8F NZ	Gln 87A CB	4.08
	Gln 87A CG	3.4
	Gln 87A CD	2.7
	Gln 87A OE1	1.78
	Gln 87A NE2	3.69
Lys 11F CB	Ile 83A CD1	3.99
Lys 11F CD	Asp 84A OD2	4.28
	Ile 83A CG1	4.26
	Ile 83A CD1	3.82
Lys 11F CE	Asp 84A OD2	3.93
Lys 11F NZ	Asp 84A CG	3.65
	Asp 84A OD1	4.2
	Asp 84A OD2	2.91

Table 13. Atomic coordinates of residues that contribute to, but are not required for, binding of hDcn1 Chain A to hUbc12 from structure with stapled hUbc12 peptide.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy factor
5	ATOM 206	N ASP A 84	15.064	41.735	5.788	1.00 10.17
	ATOM 207	CA ASP A 84	16.219	42.546	6.174	1.00 10.55
	ATOM 208	CB ASP A 84	17.461	41.675	6.366	1.00 11.23
	ATOM 209	CG ASP A 84	17.375	40.805	7.603	1.00 13.04
10	ATOM 210	OD1 ASP A 84	16.581	41.113	8.522	1.00 16.41
	ATOM 211	OD2 ASP A 84	18.111	39.803	7.658	1.00 15.83
	ATOM 212	C ASP A 84	16.489	43.657	5.159	1.00 9.88
	ATOM 213	O ASP A 84	16.754	44.791	5.545	1.00 9.65
	ATOM 285	N ALA A 94	15.031	56.885	5.766	1.00 11.05
15	ATOM 286	CA ALA A 94	14.657	57.464	7.067	1.00 11.12
	ATOM 287	CB ALA A 94	15.239	58.886	7.231	1.00 11.03
	ATOM 288	C ALA A 94	13.133	57.443	7.302	1.00 11.47
	ATOM 289	O ALA A 94	12.545	58.432	7.751	1.00 10.94
	ATOM 290	N LEU A 95	12.504	56.311	6.972	1.00 11.82
20	ATOM 291	CA LEU A 95	11.064	56.133	7.181	1.00 12.39

5	ATOM	292	CB	LEU A	95	10.330	55.975	5.843	1.00	12.25
	ATOM	293	CG	LEU A	95	10.424	57.112	4.823	1.00	12.44
	ATOM	294	CD1	LEU A	95	9.967	56.631	3.452	1.00	12.15
	ATOM	295	CD2	LEU A	95	9.632	58.326	5.278	1.00	12.56
	ATOM	296	C	LEU A	95	10.731	54.935	8.064	1.00	12.66
	ATOM	297	O	LEU A	95	11.381	53.895	7.995	1.00	13.03
	ATOM	298	N	ASP A	96	9.702	55.101	8.883	1.00	13.06
	ATOM	299	CA	ASP A	96	9.059	53.996	9.589	1.00	13.50
10	ATOM	300	CB	ASP A	96	7.901	54.552	10.424	1.00	13.89
	ATOM	301	CG	ASP A	96	7.251	53.509	11.344	1.00	16.05
	ATOM	302	OD1	ASP A	96	7.257	52.290	11.044	1.00	17.16
	ATOM	303	OD2	ASP A	96	6.702	53.934	12.381	1.00	17.91
	ATOM	304	C	ASP A	96	8.539	53.014	8.531	1.00	12.97
15	ATOM	305	O	ASP A	96	7.807	53.421	7.620	1.00	12.93

Table 14. Atomic coordinates of residues that contribute to, but are not required for, binding of hDcn1 Chain B to hUbc12 in structure of stapled hUbc12 peptide.

	Atom No.	Atom Name		X	Y	Z	Occu-pancy	B-factor
20	ATOM	2420	N ASP B 84	-29.031	110.230	-22.980	1.00	21.25
	ATOM	2421	CA ASP B 84	-28.182	111.358	-23.383	1.00	21.22
	ATOM	2422	CB ASP B 84	-29.019	112.619	-23.623	1.00	21.50
	ATOM	2423	CG ASP B 84	-29.913	112.501	-24.842	1.00	22.06
	ATOM	2424	OD1 ASP B 84	-29.581	111.722	-25.760	1.00	23.61
25	ATOM	2425	OD2 ASP B 84	-30.952	113.192	-24.884	1.00	23.44
	ATOM	2426	C ASP B 84	-27.088	111.651	-22.365	1.00	20.88
	ATOM	2427	O ASP B 84	-25.970	112.013	-22.735	1.00	20.81
	ATOM	2499	N ALA B 94	-14.009	109.936	-22.759	1.00	22.67
	ATOM	2500	CA ALA B 94	-13.443	109.599	-24.075	1.00	22.83
30	ATOM	2501	CB ALA B 94	-12.005	110.127	-24.218	1.00	22.75
	ATOM	2502	C ALA B 94	-13.517	108.097	-24.360	1.00	22.93
	ATOM	2503	O ALA B 94	-12.552	107.489	-24.826	1.00	22.99
	ATOM	2504	N LEU B 95	-14.680	107.514	-24.076	1.00	23.01
	ATOM	2505	CA LEU B 95	-14.892	106.079	-24.232	1.00	23.06
35	ATOM	2506	CB LEU B 95	-15.078	105.407	-22.863	1.00	23.00
	ATOM	2507	CG LEU B 95	-13.879	105.257	-21.920	1.00	23.07
	ATOM	2508	CD1 LEU B 95	-14.348	104.751	-20.567	1.00	23.09
	ATOM	2509	CD2 LEU B 95	-12.807	104.331	-22.492	1.00	23.19
	ATOM	2510	C LEU B 95	-16.087	105.753	-25.121	1.00	23.04
40	ATOM	2511	O LEU B 95	-17.128	106.409	-25.048	1.00	23.06
	ATOM	2512	N ASP B 96	-15.912	104.739	-25.966	1.00	23.04
	ATOM	2513	CA ASP B 96	-17.017	104.098	-26.670	1.00	23.21
	ATOM	2514	CB ASP B 96	-16.474	102.949	-27.538	1.00	23.36
	ATOM	2515	CG ASP B 96	-17.524	102.342	-28.477	1.00	24.22
45	ATOM	2516	OD1 ASP B 96	-18.723	102.277	-28.126	1.00	25.07
	ATOM	2517	OD2 ASP B 96	-17.132	101.898	-29.578	1.00	24.66
	ATOM	2518	C ASP B 96	-17.984	103.568	-25.603	1.00	22.87
	ATOM	2519	O ASP B 96	-17.567	102.833	-24.704	1.00	22.78

Table 15. Atomic coordinates of residues that contribute to, but are not required for, binding of hDcn1 Chain A to hUbc12.

	Atom No.	Atom Name			X	Y	Z	Occu-pancy	B-factor
5	ATOM	204	N	ASP A	84	37.117	-17.398	-21.586	1.00 15.30
	ATOM	205	CA	ASP A	84	36.947	-18.847	-21.612	1.00 15.98
	ATOM	206	CB	ASP A	84	38.173	-19.558	-21.028	1.00 17.35
	ATOM	207	CG	ASP A	84	39.362	-19.558	-21.982	1.00 21.48
	ATOM	208	OD1	ASP A	84	39.184	-19.251	-23.181	1.00 24.51
10	ATOM	209	OD2	ASP A	84	40.482	-19.872	-21.530	1.00 24.38
	ATOM	210	C	ASP A	84	35.666	-19.254	-20.888	1.00 14.58
	ATOM	211	O	ASP A	84	34.915	-20.101	-21.374	1.00 14.36
	ATOM	283	N	ALA A	94	23.572	-23.460	-24.936	1.00 12.74
	ATOM	284	CA	ALA A	94	23.354	-23.863	-26.331	1.00 13.46
15	ATOM	285	CB	ALA A	94	22.336	-25.015	-26.429	1.00 13.90
	ATOM	286	C	ALA A	94	22.937	-22.668	-27.195	1.00 13.29
	ATOM	287	O	ALA A	94	22.037	-22.762	-28.036	1.00 13.55
	ATOM	288	N	LEU A	95	23.604	-21.540	-26.966	1.00 12.35
	ATOM	289	CA	LEU A	95	23.344	-20.312	-27.704	1.00 11.64
20	ATOM	290	CB	LEU A	95	22.743	-19.238	-26.788	1.00 11.73
	ATOM	291	CG	LEU A	95	21.370	-19.454	-26.147	1.00 12.12
	ATOM	292	CD1	LEU A	95	21.138	-18.429	-25.053	1.00 12.54
	ATOM	293	CD2	LEU A	95	20.245	-19.399	-27.176	1.00 13.08
	ATOM	294	C	LEU A	95	24.640	-19.790	-28.295	1.00 11.08
25	ATOM	295	O	LEU A	95	25.702	-19.886	-27.675	1.00 11.12
	ATOM	296	N	ASP A	96	24.544	-19.231	-29.496	1.00 10.92
	ATOM	297	CA	ASP A	96	25.671	-18.539	-30.101	1.00 10.77
	ATOM	298	CB	ASP A	96	25.292	-18.026	-31.489	1.00 11.39
	ATOM	299	CG	ASP A	96	26.490	-17.540	-32.278	1.00 13.24
30	ATOM	300	OD1	ASP A	96	27.084	-16.505	-31.903	1.00 14.15
	ATOM	301	OD2	ASP A	96	26.836	-18.197	-33.280	1.00 16.29
	ATOM	302	C	ASP A	96	26.060	-17.373	-29.187	1.00 9.82
	ATOM	303	O	ASP A	96	25.191	-16.644	-28.725	1.00 9.62

Table 16. Atomic coordinates of residues that contribute to, but are not required for, binding of hDcn1 Chain B to hUbc12.

	Atom No.	Atom Name			X	Y	Z	Occu-pancy	B-factor
40	ATOM	1827	N	ASP B	84	29.753	5.342	6.933	1.00 16.27
	ATOM	1828	CA	ASP B	84	29.678	6.800	6.878	1.00 16.75
	ATOM	1829	CB	ASP B	84	31.075	7.421	7.001	1.00 17.47
	ATOM	1830	CG	ASP B	84	31.691	7.209	8.374	1.00 19.75
	ATOM	1831	OD1	ASP B	84	31.009	7.478	9.388	1.00 21.39
	ATOM	1832	OD2	ASP B	84	32.863	6.777	8.441	1.00 21.09
45	ATOM	1833	C	ASP B	84	28.984	7.274	5.602	1.00 16.12
	ATOM	1834	O	ASP B	84	28.155	8.184	5.640	1.00 16.27
	ATOM	1906	N	ALA B	94	16.681	12.075	2.790	1.00 15.25
	ATOM	1907	CA	ALA B	94	15.771	12.480	3.867	1.00 16.29

	ATOM	1908	CB	ALA B	94	14.856	13.631	3.416	1.00	16.50
	ATOM	1909	C	ALA B	94	14.962	11.288	4.394	1.00	16.33
	ATOM	1910	O	ALA B	94	13.767	11.399	4.687	1.00	16.79
5	ATOM	1911	N	LEU B	95	15.642	10.148	4.503	1.00	15.53
	ATOM	1912	CA	LEU B	95	15.058	8.907	5.003	1.00	14.51
	ATOM	1913	CB	LEU B	95	15.049	7.834	3.907	1.00	14.56
	ATOM	1914	CG	LEU B	95	14.170	8.032	2.671	1.00	15.10
	ATOM	1915	CD1	LEU B	95	14.550	7.008	1.608	1.00	15.62
	ATOM	1916	CD2	LEU B	95	12.693	7.925	3.006	1.00	16.31
10	ATOM	1917	C	LEU B	95	15.852	8.393	6.190	1.00	13.79
	ATOM	1918	O	LEU B	95	17.080	8.489	6.215	1.00	13.74
	ATOM	1919	N	ASP B	96	15.143	7.843	7.173	1.00	13.45
	ATOM	1920	CA	ASP B	96	15.789	7.160	8.286	1.00	13.09
15	ATOM	1921	CB	ASP B	96	14.745	6.737	9.323	1.00	13.72
	ATOM	1922	CG	ASP B	96	15.365	6.262	10.624	1.00	15.39
	ATOM	1923	OD1	ASP B	96	16.013	5.196	10.631	1.00	16.12
	ATOM	1924	OD2	ASP B	96	15.186	6.952	11.648	1.00	17.96
	ATOM	1925	C	ASP B	96	16.518	5.939	7.721	1.00	12.21
20	ATOM	1926	O	ASP B	96	15.954	5.217	6.904	1.00	12.01

In still other embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises the atoms of Table 17, which are found in amino acid residues Glu108, Met111, Glu112, Pro122, Thr123, Glu124, Val127, Leu128, Ala131, Met139, Cys140, Phe142, Phe189, Ile204, Ala207, Leu208, and Leu211 of human Dcn3 (SEQ ID NO: 39), or a structural variant thereof. In particular embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises at least one of the following amino acid residues: a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, and a leucine corresponding to position 211 of SEQ ID NO: 39.

In some of these embodiments, the E2-binding pocket comprises the atomic coordinates of Table 18 or 19, or a structural variant thereof. In particular embodiments, the co-E3 protein has the amino acid sequence set forth in SEQ ID NO: 39.

In certain embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises the atoms of Table 17 and 20. The atoms in Table 20 are found in amino acid residues Glu109, Val120, and Asp121. Thus, in some embodiments, the E2-binding pocket of a NEDD8 co-E3 protein comprises at least one of the following amino acid residues: a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, a leucine corresponding to position 211 of SEQ ID NO: 39; a glutamic acid corresponding to position 109 of SEQ ID NO: 39; a valine corresponding to position 120 of SEQ ID NO: 39; and an aspartic acid at position 121 of SEQ ID NO: 39.

In some of these embodiments, the E2-binding pocket comprises the atomic coordinates of Tables 18 and 21; 19 and 22; or a structural variant thereof.

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Table 17. Minimal contacts between human Dcn3 and human Ube2f with hUbe2f Chain F interactions with hDcn3^P Chain A and hUbe2f Chain G interactions with hDcn3^P Chain B shown.

hUbe2f Atom	hDcn3 Atom	Distance (Å)
Acetyl-Met 1F OT	Phe 189A CZ	3.75
	Phe 189A CE2	3.2
	Phe 189A CD2	4.22
	Met 139A CG	3.96
	Met 139A SD	3.8
	Met 139A CE	3.47
Acetyl-Met 1F CT1	Phe 189A CZ	4.37
	Phe 189A CE2	3.94

	Pro 122A O	3.76
	Thr 123A CA	4.33
Acetyl-Met 1F CT2	Phe 189A CE2	4.36
	Glu 124A O	4.33
	Leu 128A N	4.19
	Leu 128A CA	4.42
	Leu 128A CB	4.33
	Leu 211A CD2	3.48
	Thr 123A O	3.78
	Pro 122A O	3.88
	Thr 123A CA	4.03
	Thr 123A C	3.97
Acetyl-Met 1F N	Val 127A CG1	4.18
	Pro 122A C	3.76
	Pro 122A O	2.74
	Thr 123A N	4.26
	Thr 123A CA	3.88
Acetyl-Met 1F CA	Pro 122A O	3.67
Acetyl-Met 1F C	Met 139A SD	4.47
	Cys 140A SG	4.43
	Pro 122A O	4.36
Acetyl-Met 1F O	Pro 122A O	4.34
	Thr 123A CG2	3.93
Acetyl-Met 1F CB	Cys 140A SG	4.24
	Pro 122A CB	4.47
	Pro 122A C	4.36
	Pro 122A O	3.53
Acetyl-Met 1F CG	Met 111A CG	3.84
	Met 111A SD	4.19
	Val 127A CG1	3.64
	Pro 122A O	3.66
Acetyl-Met 1F SD	Met 139A C	4.43
	Phe 189A CE1	4.45
	Phe 189A CZ	3.73
	Met 111A CG	4.27
	Met 111A SD	4.17
	Met 111A CE	4.22
	Cys 140A N	3.38
	Cys 140A CA	3.92
	Val 127A CG1	4.4
Acetyl-Met 1F CE	Ala 131A CB	3.97
	Phe 142A CE1	3.9
	Phe 142A CZ	3.67
	Phe 189A CE1	4.4
	Phe 189A CZ	3.87
	Met 111A SD	3.76
	Met 111A CE	3.86
	Cys 140A N	4.49
	Val 127A O	4.46
	Val 127A CG1	3.89

Leu 2F N	Met 139A SD	4.06
	Thr 123A CG2	4.33
Leu 2F CA	Met 139A SD	4.46
	Thr 123A CG2	4.3
Leu 2F CB	Met 139A SD	3.92
	Met 139A CE	3.91
	Ile 204A CD1	4.16
Leu 2F CG	Met 139A CE	4.47
	Ala 207A CB	4.12
Leu 2F CD1	Leu 211A CD2	4.4
	Thr 123A O	4.2
	Thr 123A CA	4.46
	Thr 123A CB	4.18
Leu 2F CD2	Ile 204A CG2	4.38
	Met 139A CE	3.97
	Ile 204A CD1	4.21
	Ile 204A O	4.08
	Ala 207A CB	3.61
	Ala 207A C	4.4
	Leu 208A N	4.1
	Leu 208A CG	3.56
	Leu 208A CD2	3.57
Thr 3F N	Met 139A SD	4.08
Thr 3F OG1	Met 139A CB	3.82
	Met 139A CG	4.45
	Met 139A SD	3.76
	Cys 140A SG	3.59
Thr 3F CG2	Met 139A SD	4.28
	Ile 204A CD1	3.82
Thr 3F C	Cys 140A SG	3.89
Thr 3F O	Cys 140A SG	4.22
Leu 4F N	Cys 140A SG	3.56
Leu 4F CA	Cys 140A SG	3.65
Leu 4F CB	Cys 140A SG	3.93
Leu 4F CG	Glu 108A O	4.44
Leu 4F CD1	Glu 108A CB	3.63
	Cys 140A CB	4.21
	Glu 108A CA	3.73
	Glu 108A C	3.93
	Met 111A CB	4.3
	Met 111A CG	4.47
	Cys 140A SG	4.04
	Glu 108A O	3.31
Leu 4F CD2	Glu 112A N	4.28
	Glu 112A CA	4.01
	Glu 112A CB	3.92
	Glu 112A CG	4.34
	Pro 122A CB	3.54
	Pro 122A CG	3.87
Acetyl-Met 1G OT	Met 139B CE	3.53

	Phe 189B CZ	3.91
	Phe 189B CE2	3.36
	Phe 189B CD2	4.4
	Met 139B CG	4.22
	Met 139B SD	3.92
Acetyl-Met 1G CT1	Pro 122B O	3.69
	Thr 123B CA	4.42
	Phe 189B CZ	4.46
	Phe 189B CE2	4.03
Acetyl-Met 1G CT2	Pro 122B O	3.91
	Thr 123B CA	4.2
	Thr 123B C	4.12
	Val 127B CG1	4.37
	Thr 123B O	3.97
	Glu 124B O	4.2
	Leu 211B CD2	3.6
	Phe 189B CE2	4.4
	Leu 128B N	4.04
	Leu 128B CB	4.26
	Leu 128B CA	4.32
Acetyl-Met 1G N	Pro 122B C	3.69
	Pro 122B O	2.62
	Thr 123B N	4.23
	Thr 123B CA	3.95
	Val 127B CG1	4.16
Acetyl-Met 1G CA	Pro 122B O	3.5
	Cys 140B SG	4.5
Acetyl-Met 1G C	Thr 123B OG1	4.45
	Pro 122B O	4.19
	Cys 140B SG	4.38
	Met 139B SD	4.49
Acetyl-Met 1G O	Thr 123B OG1	3.96
	Pro 122B O	4.18
	Cys 140B SG	4.47
Acetyl-Met 1G CB	Pro 122B C	4.31
	Pro 122B O	3.34
	Cys 140B SG	4.02
Acetyl-Met 1G CG	Met 111B CG	3.92
	Met 111B SD	4.32
	Pro 122B O	3.43
	Val 127B CG1	3.6
Acetyl-Met 1G SD	Met 111B CG	4.29
	Met 111B SD	4.26
	Met 111B CE	4.35
	Met 139B C	4.46
	Cys 140B N	3.38
	Cys 140B CA	3.84
	Val 127B CG1	4.31
	Phe 189B CZ	3.79
Acetyl-Met 1G CE	Met 111B SD	3.99

	Met 111B CE	4.1
	Val 127B CG1	3.75
	Ala 131B CB	3.9
	Phe 142B CE1	3.96
	Phe 142B CZ	3.83
	Phe 189B CZ	3.76
	Phe 189B CE1	4.3
Leu 2G N	Met 139B CE	4.18
	Met 139B SD	3.68
Leu 2G CA	Thr 123B OG1	4.14
	Met 139B SD	4.37
Leu 2G CB	Met 139B CE	4.09
	Met 139B SD	4.17
Leu 2G CG	Met 139B CE	3.98
	Leu 208B CD2	4.16
Leu 2G CD1	Ala 207B CB	3.61
	Met 139B CE	4.12
	Leu 208B CD2	3.55
	Ala 207B C	4.32
	Ile 204B O	4
	Leu 208B N	4.14
	Leu 208B CG	3.7
Leu 2G CD2	Thr 123B CB	4.48
	Thr 123B OG1	3.87
	Thr 123B CG2	4.41
	Ala 207B CB	4.49
	Thr 123B O	4.27
Thr 3G N	Met 139B SD	4.04
Thr 3G OG1	Met 139B CB	3.84
	Cys 140B SG	3.5
	Met 139B SD	3.9
Thr 3G CG2	Met 139B SD	4.24
Thr 3G C	Cys 140B SG	3.97
Thr 3G O	Cys 140B SG	4.42
Leu 4G N	Cys 140B SG	3.52
Leu 4G CA	Cys 140B SG	3.62
Leu 4G CB	Cys 140B SG	3.85
Leu 4G CG	Cys 140B SG	4.46
Leu 4G CD1	Glu 108B C	4.11
	Glu 108B O	3.6
	Met 111B CB	4.3
	Met 111B CG	4.41
	Glu 108B CA	3.81
	Glu 108B CB	3.67
	Cys 140B CB	4.04
	Cys 140B SG	3.8
Leu 4G CD2	Glu 112B CG	4.47
	Pro 122B CG	3.78
	Glu 112B N	4.42
	Glu 112B CA	4.19

	Glu 112B CB	4.17
	Pro 122B CB	3.39

Table 18. Atomic coordinates of amino acid residues essential for human Den3 Chain A to bind hUbe2F.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
5	ATOM 187	N GLU A 108	16.684	2.422	47.251	1.00	32.01
	ATOM 188	CA GLU A 108	17.898	3.239	47.429	1.00	32.03
	ATOM 189	CB GLU A 108	17.705	4.680	46.928	1.00	32.27
	ATOM 190	CG GLU A 108	16.456	5.392	47.441	1.00	33.27
10	ATOM 191	CD GLU A 108	15.314	5.376	46.434	1.00	35.06
	ATOM 192	OE1 GLU A 108	15.176	4.387	45.679	1.00	35.38
	ATOM 193	OE2 GLU A 108	14.551	6.363	46.399	1.00	36.14
	ATOM 194	C GLU A 108	18.476	3.251	48.851	1.00	31.77
	ATOM 195	O GLU A 108	19.693	3.258	49.018	1.00	31.35
15	ATOM 209	N MET A 111	19.991	-0.224	49.462	1.00	28.11
	ATOM 210	CA MET A 111	21.137	-0.378	48.570	1.00	27.16
	ATOM 211	CB MET A 111	20.793	0.211	47.202	1.00	27.24
	ATOM 212	CG MET A 111	21.843	-0.028	46.128	1.00	27.61
	ATOM 213	SD MET A 111	21.915	-1.768	45.692	1.00	29.58
20	ATOM 214	CE MET A 111	20.390	-1.963	44.779	1.00	25.97
	ATOM 215	C MET A 111	22.400	0.294	49.111	1.00	26.60
	ATOM 216	O MET A 111	23.516	-0.231	48.985	1.00	26.39
	ATOM 217	N GLU A 112	22.213	1.471	49.694	1.00	25.97
	ATOM 218	CA GLU A 112	23.315	2.248	50.250	1.00	25.38
25	ATOM 219	CB GLU A 112	22.847	3.680	50.561	1.00	24.79
	ATOM 220	CG GLU A 112	23.924	4.609	51.094	1.00	24.86
	ATOM 221	CD GLU A 112	24.277	4.342	52.556	1.00	25.41
	ATOM 222	OE1 GLU A 112	23.366	4.048	53.367	1.00	24.01
	ATOM 223	OE2 GLU A 112	25.477	4.428	52.895	1.00	26.77
30	ATOM 224	C GLU A 112	23.899	1.524	51.480	1.00	25.08
	ATOM 225	O GLU A 112	25.118	1.373	51.588	1.00	25.12
	ATOM 299	N PRO A 122	29.322	2.686	46.412	1.00	26.00
	ATOM 300	CA PRO A 122	28.153	2.245	45.628	1.00	25.78
	ATOM 301	CB PRO A 122	27.022	3.162	46.120	1.00	25.58
35	ATOM 302	CG PRO A 122	27.497	3.753	47.390	1.00	25.21
	ATOM 303	CD PRO A 122	28.986	3.786	47.338	1.00	25.69
	ATOM 304	C PRO A 122	28.301	2.388	44.099	1.00	25.85
	ATOM 305	O PRO A 122	27.590	1.718	43.354	1.00	25.98
	ATOM 306	N THR A 123	29.195	3.262	43.644	0.85	26.10
40	ATOM 307	CA THR A 123	29.356	3.551	42.211	1.00	26.12
	ATOM 308	CB THR A 123	29.594	5.050	41.977	1.00	25.83
	ATOM 309	OG1 THR A 123	30.856	5.415	42.536	1.00	25.73
	ATOM 310	CG2 THR A 123	28.509	5.866	42.638	1.00	26.02
	ATOM 311	C THR A 123	30.510	2.765	41.573	1.00	26.40
45	ATOM 312	O THR A 123	30.782	2.871	40.367	1.00	26.51
	ATOM 313	N GLU A 124	31.174	1.964	42.388	1.00	26.52
	ATOM 314	CA GLU A 124	32.391	1.277	41.977	1.00	27.12

	ATOM	315	CB	GLU A 124	33.253	1.064	43.218	1.00	27.17
	ATOM	316	CG	GLU A 124	34.192	-0.090	43.152	1.00	31.54
	ATOM	317	CD	GLU A 124	34.229	-0.832	44.442	1.00	34.97
5	ATOM	318	OE1	GLU A 124	33.153	-0.981	45.040	1.00	38.54
	ATOM	319	OE2	GLU A 124	35.317	-1.249	44.864	1.00	35.84
	ATOM	320	C	GLU A 124	32.137	-0.034	41.195	1.00	26.48
	ATOM	321	O	GLU A 124	31.048	-0.614	41.258	1.00	26.29
	ATOM	344	N	VAL A 127	30.268	-3.281	42.243	1.00	24.00
10	ATOM	345	CA	VAL A 127	28.822	-3.096	42.392	1.00	23.84
	ATOM	346	CB	VAL A 127	28.461	-1.735	43.066	1.00	23.94
	ATOM	347	CG1	VAL A 127	26.957	-1.516	43.092	1.00	23.56
	ATOM	348	CG2	VAL A 127	29.006	-1.696	44.485	1.00	23.26
	ATOM	349	C	VAL A 127	28.117	-3.264	41.046	1.00	23.76
	ATOM	350	O	VAL A 127	27.015	-3.820	40.989	1.00	23.37
15	ATOM	351	N	LEU A 128	28.759	-2.798	39.970	1.00	23.82
	ATOM	352	CA	LEU A 128	28.271	-3.081	38.618	1.00	24.05
	ATOM	353	CB	LEU A 128	29.088	-2.342	37.537	1.00	24.16
	ATOM	354	CG	LEU A 128	28.768	-2.629	36.055	1.00	23.92
	ATOM	355	CD1	LEU A 128	27.382	-2.121	35.666	1.00	23.42
20	ATOM	356	CD2	LEU A 128	29.834	-2.033	35.131	1.00	23.07
	ATOM	357	C	LEU A 128	28.230	-4.597	38.360	1.00	23.85
	ATOM	358	O	LEU A 128	27.226	-5.111	37.861	1.00	24.40
	ATOM	375	N	ALA A 131	25.273	-6.175	40.307	1.00	24.30
	ATOM	376	CA	ALA A 131	24.040	-5.888	39.589	1.00	24.39
25	ATOM	377	CB	ALA A 131	23.979	-4.414	39.234	1.00	23.85
	ATOM	378	C	ALA A 131	23.907	-6.751	38.330	1.00	25.10
	ATOM	379	O	ALA A 131	22.824	-7.264	38.030	1.00	25.10
	ATOM	440	N	MET A 139	18.715	0.516	38.147	1.00	33.32
	ATOM	441	CA	MET A 139	19.661	1.079	39.119	1.00	32.93
30	ATOM	442	CB	MET A 139	20.280	2.368	38.591	1.00	32.99
	ATOM	443	CG	MET A 139	21.560	2.151	37.852	1.00	33.71
	ATOM	444	SD	MET A 139	22.351	3.707	37.450	1.00	35.60
	ATOM	445	CE	MET A 139	23.571	3.131	36.258	1.00	32.49
	ATOM	446	C	MET A 139	19.038	1.357	40.483	1.00	32.55
35	ATOM	447	O	MET A 139	17.924	1.889	40.563	1.00	32.23
	ATOM	448	N	CYS A 140	19.776	1.003	41.540	1.00	31.91
	ATOM	449	CA	CYS A 140	19.395	1.286	42.930	1.00	31.63
	ATOM	450	CB	CYS A 140	19.065	2.773	43.148	1.00	31.56
	ATOM	451	SG	CYS A 140	20.261	3.913	42.469	1.00	31.88
40	ATOM	452	C	CYS A 140	18.235	0.433	43.412	1.00	31.60
	ATOM	453	O	CYS A 140	17.563	0.780	44.391	1.00	31.76
	ATOM	463	N	PHE A 142	17.106	-3.895	43.853	1.00	29.28
	ATOM	464	CA	PHE A 142	17.085	-5.342	43.679	1.00	28.66
	ATOM	465	CB	PHE A 142	18.143	-6.045	44.543	1.00	28.19
45	ATOM	466	CG	PHE A 142	19.576	-5.670	44.235	1.00	27.80
	ATOM	467	CD1	PHE A 142	20.035	-5.556	42.922	1.00	26.71
	ATOM	468	CE1	PHE A 142	21.362	-5.235	42.649	1.00	25.52
	ATOM	469	CZ	PHE A 142	22.250	-5.032	43.693	1.00	26.49
	ATOM	470	CE2	PHE A 142	21.815	-5.161	45.013	1.00	26.54
50	ATOM	471	CD2	PHE A 142	20.487	-5.481	45.277	1.00	27.03

	ATOM	472	C	PHE A 142	15.712	-5.805	44.158	1.00	28.73
	ATOM	473	O	PHE A 142	15.370	-5.604	45.327	1.00	28.41
	ATOM	849	N	PHE A 189	21.039	-1.501	32.229	1.00	30.93
	ATOM	850	CA	PHE A 189	21.160	-1.056	33.617	1.00	31.54
5	ATOM	851	CB	PHE A 189	22.500	-1.553	34.166	1.00	31.55
	ATOM	852	CG	PHE A 189	22.696	-1.333	35.643	1.00	31.93
	ATOM	853	CD1	PHE A 189	21.763	-1.789	36.571	1.00	33.33
	ATOM	854	CE1	PHE A 189	21.968	-1.598	37.943	1.00	33.69
	ATOM	855	CZ	PHE A 189	23.126	-0.973	38.387	1.00	32.63
10	ATOM	856	CE2	PHE A 189	24.069	-0.532	37.470	1.00	32.60
	ATOM	857	CD2	PHE A 189	23.853	-0.718	36.110	1.00	32.46
	ATOM	858	C	PHE A 189	21.058	0.466	33.745	1.00	32.32
	ATOM	859	O	PHE A 189	20.321	0.974	34.589	1.00	32.66
	ATOM	954	N	ILE A 204	25.618	9.706	32.247	1.00	34.61
15	ATOM	955	CA	ILE A 204	25.508	8.298	32.657	1.00	34.09
	ATOM	956	CB	ILE A 204	24.018	7.839	32.683	1.00	34.26
	ATOM	957	CG1	ILE A 204	23.232	8.581	33.776	1.00	34.69
	ATOM	958	CD1	ILE A 204	23.623	8.214	35.217	1.00	35.98
	ATOM	959	CG2	ILE A 204	23.894	6.320	32.839	1.00	34.13
20	ATOM	960	C	ILE A 204	26.319	7.391	31.725	1.00	33.50
	ATOM	961	O	ILE A 204	27.104	6.566	32.178	1.00	33.59
	ATOM	975	N	ALA A 207	29.988	7.778	32.451	1.00	29.61
	ATOM	976	CA	ALA A 207	30.345	7.131	33.705	1.00	29.37
	ATOM	977	CB	ALA A 207	29.407	7.586	34.815	1.00	29.46
25	ATOM	978	C	ALA A 207	30.344	5.607	33.582	1.00	28.94
	ATOM	979	O	ALA A 207	31.266	4.929	34.063	1.00	28.63
	ATOM	980	N	LEU A 208	29.312	5.076	32.934	1.00	28.74
	ATOM	981	CA	LEU A 208	29.198	3.634	32.714	1.00	28.63
	ATOM	982	CB	LEU A 208	27.801	3.262	32.255	1.00	28.66
30	ATOM	983	CG	LEU A 208	26.696	3.311	33.306	1.00	29.25
	ATOM	984	CD1	LEU A 208	25.366	2.970	32.646	1.00	29.90
	ATOM	985	CD2	LEU A 208	26.995	2.351	34.460	1.00	30.23
	ATOM	986	C	LEU A 208	30.233	3.097	31.725	1.00	28.50
	ATOM	987	O	LEU A 208	30.692	1.966	31.874	1.00	28.53
35	ATOM	1011	N	LEU A 211	33.243	2.948	33.888	1.00	28.64
	ATOM	1012	CA	LEU A 211	33.102	1.803	34.773	1.00	28.57
	ATOM	1013	CB	LEU A 211	31.764	1.868	35.513	1.00	28.22
	ATOM	1014	CG	LEU A 211	31.691	1.163	36.865	1.00	27.92
	ATOM	1015	CD1	LEU A 211	32.732	1.716	37.830	1.00	26.69
40	ATOM	1016	CD2	LEU A 211	30.304	1.334	37.429	1.00	26.94
	ATOM	1017	C	LEU A 211	33.255	0.447	34.064	1.00	28.71
	ATOM	1018	O	LEU A 211	34.032	-0.405	34.510	1.00	28.67

Table 19. Atomic coordinates of amino acid residues essential for human Dcn3 Chain B to bind hUbe2F.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
5	ATOM 1789	N GLU B 108	-2.928	-7.977	13.187	1.00	29.97
	ATOM 1790	CA GLU B 108	-2.667	-8.790	11.989	1.00	30.11
	ATOM 1791	CB GLU B 108	-2.220	-10.221	12.340	1.00	30.21
	ATOM 1792	CG GLU B 108	-3.139	-10.971	13.304	1.00	32.70
	ATOM 1793	CD GLU B 108	-2.758	-10.746	14.765	1.00	35.39
10	ATOM 1794	OE1 GLU B 108	-1.786	-9.993	15.029	1.00	36.06
	ATOM 1795	OE2 GLU B 108	-3.431	-11.324	15.646	1.00	35.26
	ATOM 1796	C GLU B 108	-3.813	-8.795	10.968	1.00	29.58
	ATOM 1797	O GLU B 108	-3.574	-8.641	9.771	1.00	29.17
15	ATOM 1811	N MET B 111	-3.873	-5.424	9.369	1.00	26.60
	ATOM 1812	CA MET B 111	-2.622	-5.223	8.640	1.00	26.08
	ATOM 1813	CB MET B 111	-1.434	-5.761	9.444	1.00	25.97
	ATOM 1814	CG MET B 111	-0.084	-5.508	8.793	1.00	26.18
	ATOM 1815	SD MET B 111	0.323	-3.749	8.840	1.00	28.45
	ATOM 1816	CE MET B 111	0.542	-3.511	10.610	1.00	25.99
20	ATOM 1817	C MET B 111	-2.676	-5.912	7.274	1.00	25.72
	ATOM 1818	O MET B 111	-2.175	-5.375	6.272	1.00	25.80
	ATOM 1819	N GLU B 112	-3.269	-7.106	7.250	1.00	24.77
	ATOM 1820	CA GLU B 112	-3.437	-7.860	6.011	1.00	24.17
	ATOM 1821	CB GLU B 112	-3.954	-9.276	6.294	1.00	23.71
25	ATOM 1822	CG GLU B 112	-3.966	-10.185	5.077	1.00	23.79
	ATOM 1823	CD GLU B 112	-5.214	-10.001	4.225	1.00	24.84
	ATOM 1824	OE1 GLU B 112	-6.315	-9.845	4.793	1.00	23.82
	ATOM 1825	OE2 GLU B 112	-5.100	-10.013	2.982	1.00	25.84
	ATOM 1826	C GLU B 112	-4.352	-7.104	5.033	1.00	23.85
30	ATOM 1827	O GLU B 112	-4.036	-6.998	3.851	1.00	24.18
	ATOM 1901	N PRO B 122	2.307	-8.239	1.798	1.00	23.06
	ATOM 1902	CA PRO B 122	2.578	-7.735	3.172	1.00	23.39
	ATOM 1903	CB PRO B 122	1.678	-8.608	4.056	1.00	23.19
	ATOM 1904	CG PRO B 122	0.682	-9.236	3.122	1.00	23.52
35	ATOM 1905	CD PRO B 122	1.369	-9.376	1.804	1.00	23.09
	ATOM 1906	C PRO B 122	4.037	-7.894	3.620	1.00	23.35
	ATOM 1907	O PRO B 122	4.438	-7.374	4.661	1.00	23.55
	ATOM 1908	N THR B 123	4.802	-8.590	2.790	1.00	23.31
	ATOM 1909	CA THR B 123	6.142	-9.068	3.052	1.00	23.09
40	ATOM 1910	CB THR B 123	6.134	-10.589	2.697	1.00	23.23
	ATOM 1911	OG1 THR B 123	6.033	-11.371	3.890	1.00	23.77
	ATOM 1912	CG2 THR B 123	7.327	-11.017	1.891	1.00	22.66
	ATOM 1913	C THR B 123	7.189	-8.294	2.211	1.00	23.32
	ATOM 1914	O THR B 123	8.400	-8.483	2.374	1.00	23.40
45	ATOM 1915	N GLU B 124	6.737	-7.419	1.314	1.00	23.26
	ATOM 1916	CA GLU B 124	7.663	-6.765	0.384	1.00	23.57
	ATOM 1917	CB GLU B 124	7.014	-6.506	-0.974	1.00	23.45
	ATOM 1918	CG GLU B 124	6.055	-5.334	-1.019	1.00	25.47
	ATOM 1919	CD GLU B 124	5.787	-4.870	-2.441	1.00	28.88

	ATOM	1920	OE1	GLU B 124	4.946	-5.505	-3.136	1.00	29.24
	ATOM	1921	OE2	GLU B 124	6.427	-3.870	-2.854	1.00	30.26
	ATOM	1922	C	GLU B 124	8.329	-5.505	0.945	1.00	23.68
5	ATOM	1923	O	GLU B 124	7.937	-4.985	1.999	1.00	23.52
	ATOM	1946	N	VAL B 127	6.523	-2.296	2.424	1.00	24.05
	ATOM	1947	CA	VAL B 127	5.875	-2.439	3.725	1.00	23.81
	ATOM	1948	CB	VAL B 127	5.124	-3.796	3.862	1.00	23.86
	ATOM	1949	CG1	VAL B 127	4.638	-4.015	5.282	1.00	22.40
10	ATOM	1950	CG2	VAL B 127	3.946	-3.842	2.892	1.00	23.69
	ATOM	1951	C	VAL B 127	6.911	-2.240	4.829	1.00	23.92
	ATOM	1952	O	VAL B 127	6.634	-1.571	5.839	1.00	24.19
	ATOM	1953	N	LEU B 128	8.107	-2.786	4.619	1.00	23.58
	ATOM	1954	CA	LEU B 128	9.239	-2.482	5.489	1.00	23.70
15	ATOM	1955	CB	LEU B 128	10.511	-3.223	5.059	1.00	23.73
	ATOM	1956	CG	LEU B 128	11.749	-2.865	5.898	1.00	23.84
	ATOM	1957	CD1	LEU B 128	11.663	-3.458	7.290	1.00	23.49
	ATOM	1958	CD2	LEU B 128	13.010	-3.333	5.209	1.00	25.14
	ATOM	1959	C	LEU B 128	9.495	-0.971	5.567	1.00	23.52
	ATOM	1960	O	LEU B 128	9.653	-0.440	6.668	1.00	23.85
20	ATOM	1977	N	ALA B 131	6.644	0.572	7.623	1.00	21.94
	ATOM	1978	CA	ALA B 131	6.866	0.327	9.054	1.00	21.30
	ATOM	1979	CB	ALA B 131	7.166	-1.143	9.295	1.00	20.73
	ATOM	1980	C	ALA B 131	7.991	1.213	9.620	1.00	21.62
	ATOM	1981	O	ALA B 131	7.889	1.731	10.736	1.00	21.42
25	ATOM	2042	N	MET B 139	6.269	-6.221	14.687	1.00	31.52
	ATOM	2043	CA	MET B 139	5.752	-6.772	13.433	1.00	30.64
	ATOM	2044	CB	MET B 139	6.474	-8.062	13.065	1.00	30.37
	ATOM	2045	CG	MET B 139	7.683	-7.835	12.225	1.00	30.22
	ATOM	2046	SD	MET B 139	8.256	-9.376	11.518	1.00	30.12
30	ATOM	2047	CE	MET B 139	9.770	-8.814	10.743	1.00	29.99
	ATOM	2048	C	MET B 139	4.250	-7.036	13.490	1.00	30.11
	ATOM	2049	O	MET B 139	3.747	-7.573	14.480	1.00	29.85
	ATOM	2050	N	CYS B 140	3.555	-6.639	12.421	1.00	29.34
	ATOM	2051	CA	CYS B 140	2.118	-6.876	12.238	1.00	28.75
35	ATOM	2052	CB	CYS B 140	1.772	-8.366	12.411	1.00	28.65
	ATOM	2053	SG	CYS B 140	2.841	-9.467	11.455	1.00	29.16
	ATOM	2054	C	CYS B 140	1.239	-5.994	13.126	1.00	28.39
	ATOM	2055	O	CYS B 140	0.065	-6.302	13.357	1.00	28.43
	ATOM	2065	N	PHE B 142	0.389	-1.667	13.943	1.00	25.66
40	ATOM	2066	CA	PHE B 142	0.537	-0.217	14.050	1.00	25.09
	ATOM	2067	CB	PHE B 142	0.094	0.503	12.767	1.00	24.60
	ATOM	2068	CG	PHE B 142	0.887	0.145	11.545	1.00	23.64
	ATOM	2069	CD1	PHE B 142	2.264	-0.020	11.606	1.00	21.72
	ATOM	2070	CE1	PHE B 142	2.995	-0.341	10.471	1.00	21.40
45	ATOM	2071	CZ	PHE B 142	2.353	-0.493	9.244	1.00	21.81
	ATOM	2072	CE2	PHE B 142	0.971	-0.315	9.159	1.00	23.34
	ATOM	2073	CD2	PHE B 142	0.246	0.003	10.312	1.00	24.04
	ATOM	2074	C	PHE B 142	-0.358	0.260	15.188	1.00	25.17
	ATOM	2075	O	PHE B 142	-1.573	0.061	15.140	1.00	25.12
50	ATOM	2451	N	PHE B 189	12.757	-4.136	14.489	1.00	27.50

	ATOM	2452	CA	PHE B 189	11.486	-4.532	13.896	1.00	27.79
	ATOM	2453	CB	PHE B 189	11.384	-3.922	12.498	1.00	27.46
	ATOM	2454	CG	PHE B 189	10.085	-4.177	11.802	1.00	26.05
5	ATOM	2455	CD1	PHE B 189	8.886	-3.711	12.333	1.00	25.09
	ATOM	2456	CE1	PHE B 189	7.680	-3.937	11.675	1.00	24.46
	ATOM	2457	CZ	PHE B 189	7.668	-4.619	10.463	1.00	24.31
	ATOM	2458	CE2	PHE B 189	8.867	-5.087	9.917	1.00	25.07
	ATOM	2459	CD2	PHE B 189	10.064	-4.855	10.588	1.00	24.93
10	ATOM	2460	C	PHE B 189	11.335	-6.053	13.847	1.00	28.94
	ATOM	2461	O	PHE B 189	10.249	-6.580	14.093	1.00	29.14
	ATOM	2559	N	ILE B 204	14.094	-15.322	10.184	1.00	29.32
	ATOM	2560	CA	ILE B 204	13.693	-13.913	10.146	1.00	28.78
	ATOM	2561	CB	ILE B 204	13.179	-13.406	11.518	1.00	28.99
15	ATOM	2562	CG1	ILE B 204	11.731	-13.823	11.745	1.00	29.30
	ATOM	2563	CD1	ILE B 204	11.591	-15.090	12.517	1.00	31.74
	ATOM	2564	CG2	ILE B 204	13.236	-11.883	11.596	1.00	28.88
	ATOM	2565	C	ILE B 204	14.856	-13.037	9.677	1.00	28.15
	ATOM	2566	O	ILE B 204	14.692	-12.219	8.781	1.00	28.26
20	ATOM	2580	N	ALA B 207	15.521	-13.559	5.951	1.00	25.24
	ATOM	2581	CA	ALA B 207	14.484	-12.882	5.189	1.00	25.15
	ATOM	2582	CB	ALA B 207	13.102	-13.349	5.634	1.00	25.21
	ATOM	2583	C	ALA B 207	14.607	-11.362	5.271	1.00	25.20
	ATOM	2584	O	ALA B 207	14.416	-10.670	4.265	1.00	25.68
25	ATOM	2585	N	LEU B 208	14.945	-10.844	6.451	1.00	24.96
	ATOM	2586	CA	LEU B 208	15.113	-9.394	6.626	1.00	24.85
	ATOM	2587	CB	LEU B 208	15.102	-9.001	8.100	1.00	24.71
	ATOM	2588	CG	LEU B 208	13.746	-9.038	8.809	1.00	25.14
	ATOM	2589	CD1	LEU B 208	13.923	-8.622	10.264	1.00	25.27
	ATOM	2590	CD2	LEU B 208	12.710	-8.147	8.123	1.00	24.28
30	ATOM	2591	C	LEU B 208	16.355	-8.848	5.923	1.00	24.52
	ATOM	2592	O	LEU B 208	16.313	-7.779	5.329	1.00	24.45
	ATOM	2616	N	LEU B 211	15.564	-8.585	2.472	1.00	25.74
	ATOM	2617	CA	LEU B 211	14.620	-7.487	2.359	1.00	25.81
	ATOM	2618	CB	LEU B 211	13.474	-7.660	3.359	1.00	25.74
35	ATOM	2619	CG	LEU B 211	12.239	-6.840	3.033	1.00	25.44
	ATOM	2620	CD1	LEU B 211	11.726	-7.220	1.637	1.00	25.06
	ATOM	2621	CD2	LEU B 211	11.181	-7.070	4.099	1.00	26.22
	ATOM	2622	C	LEU B 211	15.257	-6.101	2.507	1.00	26.06
40	ATOM	2623	O	LEU B 211	15.008	-5.230	1.689	1.00	26.06

Table 20. Additional contacts that contribute to, but are not essential, for contact between human Dcn3 and human Ube2f with hUbe2f Chain F interactions with hDcn3^P Chain A and hUbe2f Chain G interactions with hDcn3^P Chain B shown.

hUbe2f Atom	hDcn3 Atom	Distance (Å)
Lys 7F CB	Glu 108A CB	4.3
	Glu 108A CD	4.2
	Glu 108A OE2	4.14
	Glu 108A CG	4.31
Lys 7F CD	Glu 108A OE1	4.42
	Cys 140A CB	4.12
	Cys 140A SG	3.84
Lys 7F CE	Glu 108A CD	4.17
	Glu 108A OE1	3.79
	Glu 108A OE2	4.26
Lys 7F O	Glu 108A CD	4.41
	Glu 108A OE2	3.84
	Glu 108A CG	4.2
Leu 8F CG	Glu 108A CB	4.38
Leu 8F CD1	Glu 108A O	4.29
	Glu 112A CB	3.96
	Glu 112A CG	3.98
Leu 8F CD2	Glu 108A CB	4.03
	Glu 108A CG	3.88
	Glu 108A C	4.11
	Glu 109A N	4.18
	Glu 109A CA	4.26
	Glu 109A CG	4.16
	Glu 108A O	4.12
Lys 7G CB	Glu 108B CG	4.3
	Glu 108B CD	4.48
	Glu 108B CB	4.29
Lys 7G CD	Glu 108B OE1	4.02
	Cys 140B CB	4.12
	Cys 140B SG	4.04
Lys 7G NZ	Glu 108B OE1	4.21
	Met 139B O	3.95
Lys 7G O	Glu 108B CG	4.36
Leu 8G CG	Glu 108B CB	4.41
Leu 8G CD1	Glu 112B CG	3.84
	Glu 112B CB	3.89
Leu 8G CD2	Glu 109B CG	4.39
	Glu 109B CA	3.94
	Glu 109B N	3.88
	Glu 108B CG	4.02
	Glu 108B C	3.95
	Glu 108B O	4.01
	Glu 108B CB	4.12

Table 21. Atomic coordinates of amino acid residues of hDcn3 Chain A that contribute to, but are not required, for binding hUbe2F.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
5	ATOM 196	N GLU A 109	17.607	3.250	49.863	1.00	31.59
	ATOM 197	CA GLU A 109	18.060	3.299	51.247	1.00	31.75
	ATOM 198	CB GLU A 109	16.902	3.575	52.213	1.00	32.09
	ATOM 199	CG GLU A 109	16.229	4.933	52.026	1.00	34.47
	ATOM 200	CD GLU A 109	15.075	4.915	51.007	1.00	37.77
10	ATOM 201	OE1 GLU A 109	14.960	3.957	50.200	1.00	37.79
	ATOM 202	OE2 GLU A 109	14.273	5.878	51.023	1.00	39.47
	ATOM 203	C GLU A 109	18.775	1.997	51.598	1.00	31.35
	ATOM 204	O GLU A 109	19.923	2.016	52.059	1.00	31.47
	ATOM 284	N VAL A 120	32.343	-1.152	50.143	1.00	28.04
15	ATOM 285	CA VAL A 120	32.370	-0.816	48.732	1.00	27.63
	ATOM 286	CB VAL A 120	31.772	-2.024	47.945	1.00	27.52
	ATOM 287	CG1 VAL A 120	30.862	-1.607	46.834	1.00	26.88
	ATOM 288	CG2 VAL A 120	32.884	-2.959	47.468	1.00	27.58
	ATOM 289	C VAL A 120	31.644	0.511	48.420	1.00	27.54
20	ATOM 290	O VAL A 120	30.512	0.733	48.872	1.00	27.62
	ATOM 291	N ASP A 121	32.307	1.388	47.662	1.00	26.97
	ATOM 292	CA ASP A 121	31.673	2.594	47.134	1.00	26.54
	ATOM 293	CB ASP A 121	32.683	3.431	46.341	1.00	26.32
	ATOM 294	CG ASP A 121	32.118	4.781	45.877	1.00	26.75
25	ATOM 295	OD1 ASP A 121	32.719	5.831	46.203	1.00	25.81
	ATOM 296	OD2 ASP A 121	31.087	4.801	45.167	1.00	27.68
	ATOM 297	C ASP A 121	30.539	2.125	46.236	1.00	26.35
	ATOM 298	O ASP A 121	30.760	1.257	45.389	1.00	26.75

Table 22. Atomic coordinates of amino acid residues of hDcn3 Chain B that contribute to, but are not required, for binding hUbe2F.

	Atom No.	Atom Name	X	Y	Z	Occu-pancy	B-factor
	ATOM 1798	N GLU B 109	-5.041	-8.978	11.450	1.00	29.25
35	ATOM 1799	CA GLU B 109	-6.225	-8.988	10.595	1.00	29.12
	ATOM 1800	CB GLU B 109	-7.510	-9.163	11.425	1.00	29.53
	ATOM 1801	CG GLU B 109	-7.538	-10.384	12.392	1.00	31.83
	ATOM 1802	CD GLU B 109	-6.793	-10.146	13.734	1.00	34.71
	ATOM 1803	OE1 GLU B 109	-6.585	-8.973	14.138	1.00	34.75
40	ATOM 1804	OE2 GLU B 109	-6.423	-11.148	14.392	1.00	36.82
	ATOM 1805	C GLU B 109	-6.295	-7.707	9.753	1.00	28.56
	ATOM 1806	O GLU B 109	-6.361	-7.773	8.523	1.00	28.30
	ATOM 1886	N VAL B 120	0.008	-4.308	-2.141	1.00	21.20
	ATOM 1887	CA VAL B 120	1.355	-4.805	-1.885	1.00	20.97
45	ATOM 1888	CB VAL B 120	2.209	-3.786	-1.090	1.00	21.14
	ATOM 1889	CG1 VAL B 120	2.557	-2.574	-1.957	1.00	21.30
	ATOM 1890	CG2 VAL B 120	1.518	-3.371	0.210	1.00	20.07
	ATOM 1891	C VAL B 120	1.321	-6.131	-1.124	1.00	21.03

ATOM	1892	O	VAL B 120	0.463	-6.332	-0.273	1.00	20.30
ATOM	1893	N	ASP B 121	2.243	-7.032	-1.462	1.00	21.30
ATOM	1894	CA	ASP B 121	2.501	-8.218	-0.657	1.00	21.87
ATOM	1895	CB	ASP B 121	3.604	-9.058	-1.304	1.00	21.84
ATOM	1896	CG	ASP B 121	3.835	-10.394	-0.597	1.00	22.12
ATOM	1897	OD1	ASP B 121	4.155	-10.394	0.603	1.00	25.73
ATOM	1898	OD2	ASP B 121	3.727	-11.446	-1.239	1.00	19.92
ATOM	1899	C	ASP B 121	2.939	-7.731	0.719	1.00	22.56
ATOM	1900	O	ASP B 121	3.844	-6.897	0.811	1.00	22.97

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As will be apparent to those of ordinary skill in the art, the atomic structures presented herein are independent of their orientation, and the atomic co-ordinates identified herein merely represent one possible orientation of a particular E2 or E3 polypeptide. The atomic coordinates are a relative set of points that define a shape in three dimensions. Thus, it is possible that a different set of coordinates could define a similar or identical shape. Therefore, slight variations in the individual coordinates will have little effect on overall shape. It is apparent, therefore, that the atomic co-ordinates identified herein may be mathematically rotated, translated, scaled, or a combination thereof, without changing the relative positions of atoms or features of the respective structure. The variations in coordinates discussed may be generated because of mathematical manipulations of the structure coordinates. For example, the structure coordinates could be manipulated by crystallographic permutations of the structure coordinates, fractionalization of the structure coordinates, integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any combination of the above.

As used herein, a "structural variant" comprises a three-dimensional molecular structure that is similar to another three-dimensional molecular structure. In some embodiments, the structural variant comprises a root mean square deviation (RMSD) from the back-bone atoms of the amino acids of listed in a particular table (e.g., Table 2, 3, 5, 6, 8, 9, 10, 11, 13, 14, 15, 16, 18, 19, 21, 22) of not more than about 4 Å, including but not limited to about 3.5 Å, 3 Å, 2.5 Å, 2 Å, 1.9 Å, 1.8 Å, 1.7 Å, 1.6 Å, 1.5 Å, 1.4 Å, 1.3 Å, 1.2 Å, 1.1 Å, 1.0 Å, 0.9 Å, 0.8 Å, 0.7 Å, 0.6 Å, 0.5 Å, 0.4 Å, 0.3 Å, 0.2 Å, and 0.1 Å. In some of these embodiments, the structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids listed in a particular table (e.g., Table 2, 3, 5, 6, 8, 9, 10, 11, 13, 14, 15, 16, 18, 19, 21, 22) of not more than about 2.0 Å. Modifications in the crystal structure due to mutations, additions, substitutions and/or deletions of amino

acids, or other changes in any of the components that make up the crystal could also account for variations in the structure coordinates.

The novel E2-binding pocket of NEDD8 co-E3 proteins can be exploited to discover compounds that bind to the pocket and in some embodiments, inhibit the activity of the NEDD8 co-E3 protein. Such compounds can be identified using computational techniques or various *in vitro* or *in vivo* binding assays.

Computational techniques can be used to screen, identify, select and/or design compounds (*i.e.*, small molecules, peptides) capable of associating with the E2-binding pocket of co-E3 proteins. Knowledge of the structure coordinates for the E2-binding pocket of co-E3 proteins permits the design and/or identification of synthetic compounds and/or other molecules which have a shape complementary to the conformation of the E2-binding pocket of co-E3 proteins. In particular, computational techniques can be used to identify or design compounds, such as inhibitors (also referred to herein as antagonists), that associate with a co-E3 protein E2-binding pocket. Potential inhibitors may bind to or interfere with all or a portion of the E2-binding pocket, and can be competitive, non-competitive, or uncompetitive inhibitors. Once identified and screened for biological activity, these inhibitors may be used therapeutically or prophylactically to inhibit co-E3 activity and, thus, prevent the onset and/or further progression of diseases or conditions associated with, for example, cell hyperproliferation (*e.g.*, cancer, inflammatory disorders). Structure-activity data for inhibitors that bind to or interfere with the E2-binding pocket of co-E3 proteins can also be obtained computationally.

The design of compounds with particular structural relationships to part of a protein molecule are well established and described in the literature (see for example Cochran (2000) *Chem. Biol.* 7:85-94; Grzybowski *et al.* (2002) *Acc. Chem. Res* 35:261-269; Vejasquez-Campoy *et al.* (2001) *Arch. Biochem. Biophys.* 380:169-175; D'Aquino *et al.* (2000) *Proteins: Struct. Func. Genet. Suppl.* 4:93-107). Any of these "molecular modeling" methods for rational drug design can be used to find antagonists of the E2-binding pocket of co-E3 proteins. Most of these molecular modeling methods take into consideration the shape, charge distribution and the distribution of hydrophobic groups, ionic groups and hydrogen bonds in the binding site of the protein molecule. Using this information derived from the crystal structure of proteins and protein complexes, these methods suggest improvements to existing proposed compounds, construct new compounds on their own that are expected to have good binding affinity, screen through virtual compound libraries for such molecules, or otherwise support the interactive design

of new drug compounds *in silico*. Programs such as GOLD (Jones *et al.* (1997) *J. Mol. Biol.* 267:727-748); FLEXX (Kramer *et al.* (1999) *Structure, Functions, and Genetics* 37:228-241); FLEXE (Rarey *et al.* (1996) *JMB* 261:470-489) DOCK (Kuntz (1992) *Science* 257:1078-1082); and AUTODOCK (Morris *et al.* (1998) *J. Computational Chemistry* 19:1639-1662) are virtual screening programs designed to calculate the binding position and conformation as well as the corresponding binding energy of an organic compound to a protein. These programs are specially trimmed to allow a great number of “dockings”, that is, calculations of the conformation with the highest binding energy of a compound to a binding site, per time unit. The binding energy is not always a real value, but can be statistically related to a real binding energy through a validation procedure.

In one embodiment, the method for identifying compounds that bind to the presently disclosed E2-binding pocket of NEDD8 co-E3 proteins comprises comparing the three-dimensional structure of a test compound and the three-dimensional molecular structure of the E2-binding pocket to determine if at least one atom of the test compound will interact with at least one atoms of the E2-binding pocket. As used herein, “interact” refers to both covalent and non-covalent interactions, including but not limited to hydrogen bonds, van der Waals interactions, hydrophobic interactions, and electrostatic interactions.

The term “test compound” refers to the molecule that is being assayed using the presently disclosed methods to determine if the test compound is capable of binding the E2-binding pocket of a NEDD8 co-E3 protein. The test compound can be naturally-occurring or non-naturally occurring and can be purified from a biological sample or synthetically derived. The structure of the test compound may be known or unknown. The test compound need not have a known biological activity or a known ability to interact with proteins. The compound may be solubilized in a solution, such as an aqueous solution or an organic solvent (e.g., dimethyl sulfoxide) prior to addition of the molecule to a sample comprising a NEDD8 co-E3 protein.

In some embodiments, the test compound is a member of a chemical library. A chemical library refers to a plurality of molecules. The components of the chemical library can be well-defined, containing known mixtures of molecules. For example, each molecule of a well-defined chemical library can be catalogued. Alternatively, the components of the library can be poorly defined, as is often the case with combinatorial libraries. Likewise, the structures of the molecules within the chemical library can be known or unknown.

In particular embodiments, the test compound is a member of a combinatorial chemical library. A combinatorial chemical library is a plurality of molecules or compounds which are formed by selectively combining a particular set of chemical building blocks. Combinatorial libraries can be constructed according to methods familiar to those skilled in the art. For example, see Rapoport *et al.*, (1995) *Immunology Today* 16:43-49; Sepetov, N. F. *et al.*, (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92:5426-5430; Gallop, M. A. *et al.*, (1994) *J. Med. Chem.* 9:1233-1251; Gordon, E. M. *et al.*, (1994) *J. Med. Chem.* 37:1385-1401; Stankova, M. *et al.*, (1994) *Peptide Res.* 7:292-298; Erb, E. *et al.*, (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:11422-11426; DeWitt, S. H. *et al.*, (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909-6913; Barbas, C. F. *et al.*, (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89:4457-4461; Brenner, S. *et al.* (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89:5381-5383; Lam, K. S. *et al.*, (1991) *Nature* 354:82-84; Devlin, J. J. *et al.*, (1990) *Science* 245:404-406; Cwirla, S. E. *et al.*, (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:6378-6382; Scott, J. K. *et al.*, (1990) *Science* 249:386-390, and U.S. Pat. No. 5,463,564, each of which is herein incorporated by reference in its entirety.

To be a viable drug candidate, the compound identified or designed according to the method must be capable of structurally associating with at least part of the E2-binding pocket on co-E3 proteins, and must be able, sterically and energetically, to assume a conformation that allows it to associate with the E2-binding pocket of co-E3 proteins. Non-covalent molecular interactions important in this association include hydrogen bonding, van der Waals interactions, hydrophobic interactions, and electrostatic interactions. Conformational considerations include the overall three-dimensional structure and orientation of the compound in relation to the binding pocket, and the spacing between various functional groups of a compound that directly interact with the E2-binding pocket.

One embodiment of the method involves evaluating the potential association of a known compound with the E2-binding pocket of co-E3 proteins. The method of drug design thus includes computationally evaluating the potential of a selected compound to associate with any of the molecules or molecular complexes set forth above. This method includes the steps of (a) employing computational means to perform a fitting operation between the selected chemical entity and the E2-binding pocket; and (b) analyzing the results of said fitting operation to quantify the association between the compound and the binding pocket.

Specialized computer programs may also assist in the process of selecting compounds. Examples include GRID (Goodford (1985) *J. Med. Chem.* 28:849-57); available from Oxford University, Oxford, UK); MCSS (Miranker *et al.* (1991) *Proteins: Struct. Funct. Gen.* 11:29-34); available from Molecular Simulations, San Diego, Calif.);

5 AUTODOCK (Goodsell *et al.* (1990) *Proteins: Struct. Funct. Genet.* 8:195-202; available from Scripps Research Institute, La Jolla, Calif.); and DOCK (Kuntz *et al.* (1982) *J. Mol. Biol.* 161:269-88); available from University of California, San Francisco, Calif.).

If these computational experiments suggest insufficient interaction and association between the test compound and the E2-binding pocket of co-E3 proteins, testing of the

10 compound is obviated. However, if computer modeling indicates a strong interaction, the molecule may then be synthesized and tested for its ability to bind to or interfere with the E2-binding pocket of co-E3 proteins as disclosed elsewhere herein. In some cases, it may be advantageous to develop new inhibitors *de novo*, *i.e.* not on the basis and as a modification of a pre-existing compound. The term "*de novo* compound design" refers to

15 a process whereby the binding pocket of the target macromolecule (e.g., the E2-binding pocket of co-E3 proteins) is determined; and its surfaces is used as a platform or basis for the rational design of compounds that will interact with those surfaces. The molecular modeling steps used in the methods may use the atomic coordinates of the E2-binding pocket disclosed herein and models or structures derived therefrom, to determine binding

20 surfaces. Any such structure will preferably reveal van der Waals contacts, electrostatic interactions, and/or hydrogen bonding opportunities. Said binding surfaces will typically be used by grid-based techniques (e.g. GRID, CERIUSt, [Goodford (1985) *J. Med. Chem.* 28: 849-857]) and/or multiple copy simultaneous search (MCSS) techniques to map favorable interaction positions for functional groups. This preferably reveals positions in

25 the E2-binding pocket of the co-E3 protein for interactions such as, but not limited to, those with protons, hydroxyl groups, amine groups, hydrophobic groups (e.g. methyl, ethyl, benzyl) and/or divalent cations.

Computer-based approaches to *de novo* compound design that can be used with the atomic co-ordinates of the E2-binding pocket of co-E3 proteins include LUDI(15 Bohm

30 (1992) *J. Comp. X ed Molec. Design* 6: 61-78), SPROUT (Available from chem.leeds.ac.uk/ICAMS/SPROUT.html) and LEAPFROG (available from Tripos Inc (www.tripos.com)). Suitable *in silico* libraries include the Available Chemical Directory (MDL Inc), the Derwent World Drug Index (WDI), BioByteMasterFile, the National Cancer Institute database (NCI), and the Maybridge catalog. Compounds in these *in silico*

libraries can also be screened for their ability to interact with the E2-binding pocket of co-E3 proteins by using their respective atomic coordinates in automated docking algorithms. An automated docking algorithm is one which permits the prediction of interactions of a number of compounds with a molecule having a given atomic structure. Suitable docking algorithms include: DOCK (Kuntz *et al.* (1982) *J. Mol. Biol.* 161: 269-288)),
5 AUTODOCK (Goodsell *et al.* (1990) *Proteins: Structure, Function and Genetics* 8: 195-202), MOE-DOCK (Available from Chemical Computing Group Inc. (www.chemcomp.com/)) or FLEXX (Available from Tripos Inc (www.tripos.com)).

Docking algorithms can also be used to verify interactions with ligands designed *de novo*.

10 Another approach encompassed by methods provided herein is the computational screening of small molecule databases for compounds that can bind in whole, or in part, to the E2-binding pocket of co-E3 proteins. In this screening, the quality of fit of such entities to the binding site may be judged either by shape complementarity or by estimated interaction energy (Meng *et al.* (1992) *J. Comp. Chem.* 13:505-24).

15 A compound that is identified or designed as a result of any of these methods can be obtained (or synthesized) and tested for its biological activity, e.g., inhibition of co-E3 activity. Binding assays to determine if a compound (e.g., an inhibitor) actually binds to or interferes with the E2-binding pocket on co-E3 enzymes can also be performed and are well known in the art and are described herein.

20 Further methods for identifying compounds that bind to an E2-binding pocket in a NEDD8 co-E3 protein disclosed herein comprise contacting a NEDD8 co-E3 protein with a test compound and determining if the test compound binds to the E2-binding pocket. The test compound may be contacted with the NEDD8 co-E3 protein in a cell-free system, in cells, or *in vivo*.

25 Binding assays to determine if the test compound binds the NEDD8 co-E3 protein may employ kinetic or thermodynamic methodology using a wide variety of techniques including, but not limited to, microcalorimetry, circular dichroism, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy, fluorescence spectroscopy, and combinations thereof. One can determine if a test compound binds to the E2-binding
30 pocket of a co-E3 enzyme using, for example, assays such as mutational analyses wherein residues that comprise the E2-binding pocket are mutated and the effect on test compound binding is assessed; and competitive binding assays, wherein a compound known to bind to the E2-binding pocket (for example, one of the peptides disclosed herein) is added to a binding reaction with the test compound to determine if the test compound is able to

compete with the peptide for binding to the co-E3 protein. In some of these embodiments, the compound known to bind to the E2-binding pocket can be coupled with a detectable label, such as a radioisotope, fluorescent label, or enzymatic label, such that binding of the test compound to the E2-binding pocket can be determined by detecting a reduction in the labeled compound in a complex. For example, compounds known to bind to the E2-binding pocket (e.g., one of the peptides disclosed herein) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds known to bind to the E2-binding pocket can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

As another example, the compound known to bind to an E2-binding pocket can be coupled with a fluorescent label to allow for detection of binding using, for example, fluorescence polarization. In some embodiments, the NEDD8 co-E3 protein is also fluorescently labeled. The fluorescent molecules (i.e., fluorophores) on the compound and the NEDD8 co-E3 protein can have an overlapping excitation and emission spectra such as those generally used in fluorescence resonance energy transfer (FRET) technology assays, wherein the excitation of a first fluorescent molecule (donor fluorophore) at a first wavelength of light causes the first fluorescent molecule to emit light at a second wavelength, and wherein the second fluorescent molecule (acceptor fluorophore) is excited by the second wavelength of light if the two fluorescent molecules are in close enough proximity to one another, and subsequently, the second fluorescent molecule emits light at a third wavelength, which can be detected using any method or apparatus known in the art. Non-limiting examples of fluorophores that can be conjugated to antibodies include Cy3, Cy5, Cy5.5, Cy7, Alexa488, Alexa555, FITC, and rhodamine (TRITC). It is to be noted that the selection of the donor fluorophore depends on the excitation and emission spectra of the acceptor fluorophore and vice versa. Frequently used fluorophore pairs for FRET include but are not limited to, Cy3 and Cy5, Alexa488 and Alexa555, Alexa488 and Cy3, and FITC and rhodamine. In such an assay, the compound known to bind to an E2-binding pocket can be coupled to a donor fluorophore (in an alternative embodiment, to an acceptor fluorophore) and the NEDD8 co-E3 protein (e.g., at or near the E2-binding pocket) can be coupled to an acceptor fluorophore (in the alternative embodiment, to a donor fluorophore). If the two come in close contact with another, the acceptor fluorophore will emit light. One can determine if the test compound that is added

to the assay binds to the E2-binding pocket of the NEDD8 co-E3 protein if the emission of the acceptor fluorophore is diminished due to competition with the labeled compound known to bind to the pocket.

In some assays, it may be desirable to immobilize either the NEDD8 co-E3 protein or a portion thereof or the compound known to bind to the E2-binding pocket to facilitate automation of the assay. For example, the NEDD8 co-E3 protein could be produced as a fusion protein that adds a domain that allows the NEDD8 co-E3 protein to be bound to a matrix. A non-limiting example of such a domain is glutathione-S-transferase, which can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione-derivatized microtitre plates. In other embodiments, the co-E3 protein or compounds can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated co-E3 proteins or compounds can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of a plate.

This invention further pertains to novel compounds identified by the above-described screening assays and uses thereof as described herein.

Further, the three-dimensional molecular structure of the test compound bound to the co-E3 protein can be determined using methods known in the art to determine if the test compound is binding within the presently disclosed E2-binding pocket of the NEDD8 co-E3 protein.

Methods known in the art and disclosed elsewhere herein can be used to determine if the test compound inhibits the co-E3 activity of NEDD8 co-E3 proteins (see Examples 1 and 4). Such binding assays can also be used to screen compounds or compound libraries for those that bind to the E2-binding pocket. Compounds that are found to bind can be further characterized for inhibition of co-E3 activity.

Compounds (e.g., small molecules and peptides) that bind to the E2-binding pocket of co-E3 proteins are provided herein. The term "compound" refers to any chemical molecule, including organic and inorganic molecules. In some embodiments, the compound is a small molecule. As used herein, the term "small molecule" refers to a chemical molecule that in some embodiments is a small organic compound, having a molecular weight of more than 100 and less than about 2,500 Daltons, including but not limited to less than 2000, or less than 1500 or less than 1000 or less than 500 D. The term "small molecule" does not encompass a peptide.

As used herein, the terms "peptide," "polypeptide," or "protein" are used interchangeably herein and are intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The terms "peptide" and "polypeptide" refer to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "peptide" and "polypeptide". The terms apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Non-limiting examples of artificial amino acid residues include norleucine and selenomethionine. An amino acid residue is a molecule having a carboxyl group, an amino group, and a side chain and having the generic formula $H_2NCH(R)COOH$, where R is an organic substituent, forming the side chain. An amino acid residue, whether it is artificial or naturally occurring, is capable of forming a peptide bond with a naturally occurring amino acid residue.

An "isolated" or "purified" peptide is substantially or essentially free from components that normally accompany or interact with the peptide as found in its naturally occurring environment. Thus, an isolated or purified peptide is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. A peptide that is substantially free of cellular material includes preparations of peptide having less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of contaminating protein. When the peptide is recombinantly produced, optimally culture medium represents less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of chemical precursors or non-peptide-of-interest chemicals.

In some embodiments of the presently disclosed invention, peptides that bind to the E2-binding pocket of a NEDD8 co-E3 protein comprise the first 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or greater amino acid residues of a naturally-occurring NEDD8 E2 protein or a variant thereof. In particular embodiments, the peptide has the sequence set forth in SEQ ID NO: 7, 12, 13, 14, 15, or 16 or a fragment or variant thereof capable of binding to the E2-binding pocket, including but not limited to an amino acid sequence having at least 40%, at least about 50%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%,

at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 7, 12, 13, 14, 15, or 16. In certain embodiments, the variant peptide may differ from SEQ ID NO: 7, 12, 13, 14, 15, or 16 by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 amino acid residues or greater.

It was disclosed herein that N-terminal acetylation of yeast Ubc12 enhances its interaction with Dcn1 and that N-terminal acetylation of human Ubc12 is essential for its interaction with Dcn1. Acetylation of the nitrogen atom of the N-terminal methioinine of Ubc12 contributed to the binding of Ubc12 to Dcn1 by eliminating the N-terminal positive charge that otherwise would impede burial in the Dcn1 hydrophobic pocket, and the methyl group of the acetyl provides hydrophobic interactions with the hydrophobic pocket. Therefore, in some embodiments, the peptides of the invention that bind to the E2-binding pocket of a NEDD8 co-E3 protein are N-terminally acetylated; that is, the nitrogen atom in the amino-terminal amino acid residue is covalently bound to an acetyl group. An acetyl group has the formula $\text{CH}_3\overset{\text{O}}{\underset{\parallel}{\text{C}}}-$. Amino acid residues referred to herein with N-acetyl- preceding the name of the amino acid residue comprise an acetyl group covalently bound to the backbone nitrogen atom. In other embodiments, the nitrogen atom of the amino-terminal amino acid residue of the peptide is covalently bound to at least one -R group so that the nitrogen is not ionizable, wherein the -R group is any functional group that is not a hydrogen and is uncharged or non-ionizable under physiological conditions when bound to the backbone nitrogen atom. Non-limiting examples of appropriate -R groups include an alkyl (including, but not limited to, a C₂ to a C₂₀ chain), alkenyl, alkynyl, aryl, or heteroaryl, optionally substituted.

Alkyl refers to both straight, branched carbon chains and cyclic hydrocarbon groups; references to individual alkyl groups are specific for the straight chain (e.g. butyl = n-butyl). In one embodiment of alkyl, the number of carbons atoms is 1-20, in other embodiments of alkyl, the number of carbon atoms is 1-12, 1-10 or 1-8 carbon atoms. In yet another embodiment of alkyl, the number of carbon atoms is 1-4 carbon atoms. Other ranges of carbon numbers are also contemplated depending on the location of the alkyl moiety on the molecule;

Examples of C₁-C₁₀ alkyl include, but are not limited to, methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, pentyl, 1-

methylbutyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl, 1-ethylpropyl, hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, heptyl, octyl, 2-ethylhexyl, nonyl and decyl and their isomers. C₁-C₄-alkyl means for example methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylpropyl or 1,1-dimethylethyl.

Cyclic alkyl groups, which are encompassed by alkyls, may be referred to as "cycloalkyl" and include those with 3 to 10 carbon atoms having single or multiple fused rings. Non-limiting examples of cycloalkyl groups include adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like.

The alkyl and cycloalkyl groups described herein can be unsubstituted or substituted with one or more moieties selected from the group consisting of alkyl, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, alkyl- or dialkylamino, amido, arylamino, alkoxy, aryloxy, nitro, cyano, azido, thiol, imino, sulfonic acid, sulfate, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrozine, carbamate, phosphonic acid, phosphate, phosphonate, or any other viable functional group that does not inhibit the biological activity of the compounds of the invention, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., *Protective Groups in Organic Synthesis*, John Wiley and Sons, Third Edition, 1999, hereby incorporated by reference.

Alkenyl refers to both straight and branched carbon chains which have at least one carbon-carbon double bond. In one embodiment of alkenyl, the number of double bonds is 1-3, in another embodiment of alkenyl, the number of double bonds is one. In one embodiment of alkenyl, the number of carbon atoms is 2-20, in other embodiments of alkenyl, the number of carbon atoms is 2-12, 2-10 or 2-8. In yet another embodiment of alkenyl, the number of carbon atoms is 2-4. Other ranges of carbon-carbon double bonds and carbon numbers are also contemplated depending on the location of the alkenyl moiety on the molecule;

"C₂-C₁₀-alkenyl" groups may include more than one double bond in the chain. Examples include, but are not limited to, ethenyl, 1-propenyl, 2-propenyl, 1-methylethenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-methyl-1-propenyl, 2-methyl-1-propenyl, 1-methyl-2-propenyl, 2-methyl-2-propenyl; 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl,

1-methyl-1-butenyl, 2-methyl-1-butenyl, 3-methyl-1-butenyl, 1-methyl-2-butenyl, 2-methyl-2-butenyl, 3-methyl-2-butenyl, 1-methyl-3-butenyl, 2-methyl-3-butenyl, 3-methyl-3-butenyl, 1,1-dimethyl-2-propenyl, 1,2-dimethyl-1-propenyl, 1,2-dimethyl-2-propenyl, 1-ethyl-1-propenyl, 1-ethyl-2-propenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 1-methyl-1-pentenyl, 2-methyl-1-pentenyl, 3-methyl-1-pentenyl, 4-methyl-1-pentenyl, 1-methyl-2-pentenyl, 2-methyl-2-pentenyl, 3-methyl-2-pentenyl, 4-methyl-2-pentenyl, 1-methyl-3-pentenyl, 2-methyl-3-pentenyl, 3-methyl-3-pentenyl, 4-methyl-3-pentenyl, 1-methyl-4-pentenyl, 2-methyl-4-pentenyl, 3-methyl-4-pentenyl, 4-methyl-4-pentenyl, 1,1-dimethyl-2-butenyl, 1,1-dimethyl-3-butenyl, 1,2-dimethyl-1-butenyl, 1,2-dimethyl-2-butenyl, 1,2-dimethyl-3-butenyl, 1,3-dimethyl-1-butenyl, 1,3-dimethyl-2-butenyl, 1,3-dimethyl-3-butenyl, 2,2-dimethyl-3-butenyl, 2,3-dimethyl-1-butenyl, 2,3-dimethyl-2-butenyl, 2,3-dimethyl-3-butenyl, 3,3-dimethyl-1-butenyl, 3,3-dimethyl-2-butenyl, 1-ethyl-1-butenyl, 1-ethyl-2-butenyl, 1-ethyl-3-butenyl, 2-ethyl-1-butenyl, 2-ethyl-2-butenyl, 2-ethyl-3-butenyl, 1,1,2-trimethyl-2-propenyl, 1-ethyl-1-methyl-2-propenyl, 1-ethyl-2-methyl-1-propenyl and 1-ethyl-2-methyl-2-propenyl.

Alkynyl refers to both straight and branched carbon chains which have at least one carbon-carbon triple bond. In one embodiment of alkynyl, the number of triple bonds is 1-3; in another embodiment of alkynyl, the number of triple bonds is one. In one embodiment of alkynyl, the number of carbon atoms is 2-20, in other embodiments of alkynyl, the number of carbon atoms is 2-12, 2-10 or 2-8. In yet another embodiment of alkynyl, the number of carbon atoms is 2-4. Other ranges of carbon-carbon double bonds and carbon numbers are also contemplated depending on the location of the alkenyl moiety on the molecule;

For example, the term "C₂-C₁₀-alkynyl" as used herein refers to a straight-chain or branched unsaturated hydrocarbon group having 2 to 10 carbon atoms and containing at least one triple bond, such as ethynyl, prop-1-yn-1-yl, prop-2-yn-1-yl, n-but-1-yn-1-yl, n-but-1-yn-3-yl, n-but-1-yn-4-yl, n-but-2-yn-1-yl, n-pent-1-yn-1-yl, n-pent-1-yn-3-yl, n-pent-1-yn-4-yl, n-pent-1-yn-5-yl, n-pent-2-yn-1-yl, n-pent-2-yn-4-yl, n-pent-2-yn-5-yl, 3-methylbut-1-yn-3-yl, 3-methylbut-1-yn-4-yl, n-hex-1-yn-1-yl, n-hex-1-yn-3-yl, n-hex-1-yn-4-yl, n-hex-1-yn-5-yl, n-hex-1-yn-6-yl, n-hex-2-yn-1-yl, n-hex-2-yn-4-yl, n-hex-2-yn-5-yl, n-hex-2-yn-6-yl, n-hex-3-yn-1-yl, n-hex-3-yn-2-yl, 3-methylpent-1-yn-1-yl, 3-methylpent-1-yn-3-yl, 3-methylpent-1-yn-4-yl, 3-methylpent-1-yn-5-yl, 4-methylpent-1-yn-1-yl, 4-methylpent-2-yn-4-yl or 4-methylpent-2-yn-5-yl and the like.

Aryl refers to a C₆-C₁₄ aromatic carbocyclic ring structure having a single ring or multiple fused rings. Aryl groups include, but are not limited to, phenyl, biphenyl, and naphthyl. In some embodiments aryl includes tetrahydronaphthyl, phenylcyclopropyl and indanyl. Aryl groups may be unsubstituted or substituted by one or more moieties selected

5 from halogen, cyano, nitro, hydroxy, mercapto, amino, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, haloalkyl, haloalkenyl, haloalkynyl, halocycloalkyl, halocycloalkenyl, alkoxy, alkenyloxy, alkynyloxy, haloalkoxy, haloalkenyloxy, haloalkynyloxy, cycloalkoxy, cycloalkenyloxy, halocycloalkoxy, halocycloalkenyloxy, alkylthio, haloalkylthio, arylthio, cycloalkylthio, halocycloalkylthio, alkylsulfinyl, alkenylsulfinyl,

10 alkynyl-sulfinyl, haloalkylsulfinyl, haloalkenylsulfinyl, haloalkynylsulfinyl, alkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, haloalkyl-sulfonyl, haloalkenylsulfonyl, haloalkynylsulfonyl, alkylcarbonyl, haloalkylcarbonyl, alkylamino, alkenylamino, alkynylamino, di(alkyl)amino, di(alkenyl)-amino, di(alkynyl)amino, or SF₅. In one embodiment of aryl, the moiety is phenyl, naphthyl, tetrahydronaphthyl, phenylcyclopropyl

15 and indanyl; in another embodiment of aryl, the moiety is phenyl. Arylo refers to an aryl substituted at two adjacent sites.

Heteroaryl refers to a monovalent aromatic group of from 1 to 15 carbon atoms, preferably from 1 to 10 carbon atoms, having one or more oxygen, nitrogen, and sulfur heteroatoms within the ring, preferably 1 to 4 heteroatoms, or 1 to 3 heteroatoms. The

20 nitrogen and sulfur heteroatoms may optionally be oxidized. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple fused rings provided that the point of attachment is through a heteroaryl ring atom. Preferred heteroaryls include pyridyl, piridaziny, pyrimidinyl, triazinyl, pyrrolyl, quinolinyl, isoquinolinyl, quinazolinyl, quinoxalinyl, furanyl, thienyl, furyl, imidazolyl, oxazolyl, isoxazolyl, isothiazolyl,

25 pyrazolyl, benzofuranyl, and benzothienyl. Heteroaryl rings may be unsubstituted or substituted by one or more moieties as described for aryl above.

Exemplary monocyclic heterocyclic or heteroaryl groups also include, but are not limited to, pyrrolidinyl, oxetanyl, pyrazolinyl, imidazolinyl, imidazolidinyl, oxazolidinyl, isoxazolinyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolidinyl, tetrahydrofuryl,

30 thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, 4-piperidonyl, pyridinyl, pyrazinyl, pyridazinyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, triazolyl, and the like.

In all embodiments, it is preferred that the R group is uncharged under physiological conditions.

Preferred hydrophobic moieties include substituted and unsubstituted alkyl, alkenyl, alkynyl, aryl and heteroaryl groups. More preferably, the hydrophobic moiety is a C₄-C₂₀ alkyl, alkenyl or alkynyl group. Most preferably, the hydrophobic moiety is a C₆-C₁₂ alkyl, alkenyl or alkynyl group.

In those embodiments wherein the nitrogen is bound to more than one -R group (e.g., 2 -R groups), the -R groups may be the same or distinct from one another. In particular embodiments, the -R group is not an acetyl group. In other embodiments, the -

R group has Formula (I), wherein Formula (I) is: $\text{R}_2\overset{\text{O}}{\underset{\parallel}{\text{C}}}-$; wherein the -R₂ group is any functional group that is uncharged under physiological conditions and is not a hydrogen. Non-limiting examples of appropriate -R₂ groups include an alkyl (including, but not limited to, a C₂ to a C₂₀ chain), aryl, or heteroaryl. In certain embodiments, the -R group is a formyl group, wherein a formyl group has the formula $\text{HC}\overset{\text{O}}{\underset{\parallel}{-}}$.

In particular embodiments, the amino-terminal methionine (at position 1) of the peptide that binds to the E2-binding pocket of a NEDD8 co-E3 protein (e.g., SEQ ID NO: 7, 12, 13, 14, 15, or 16) is substituted with an artificial amino acid residue having an unbranched hydrophobic side chain. As used herein, the term "hydrophobic" is a physical property of a molecule that is repelled from a mass of water and can be referred to as "nonpolar," or "apolar," all of which are terms that can be used interchangeably with "hydrophobic." Non-limiting examples of an unbranched hydrophobic side chain include an alkyl (e.g., C₂ to C₂₀), an alkenyl, or alkynyl. In particular embodiments, peptides of the invention that bind to the E2-binding pocket of a NEDD8 co-E3 protein comprise a norleucine or selenomethionine at the first position of the peptide in place of a methioinine.

Analysis of the presently disclosed crystal structures revealed that in order to place the hydrophobic side chains of amino acid residues at positions 2 and 4 within the Ubc12 N-terminal extension on opposite sides of the amino-terminal acetylated methioinine to aid in binding, a helical structure is required. Thus, in certain embodiments, the peptide that binds to the E2-binding pocket of a NEDD8 co-E3 protein has an alpha-helical structure. As used herein, an "alpha helix" refers to the most abundant helical conformation found in

globular proteins and the term is used in accordance with the standard meaning of the art. In an alpha helix, all amide protons point toward the N-terminus and all carbonyl oxygens point toward the C-terminus. Hydrogen bonds within an alpha helix also display a repeating pattern in which the backbone C=O of residue X (wherein X refers to any amino acid) hydrogen bonds to the backbone H-N of residue X+4. The alpha helix is a coiled structure characterized by 3.6 residues per turn, and translating along its axis 1.5 Å per amino acid. Thus the pitch is 3.6x1.5 or 5.4 Å. The screw sense of alpha helices is always right-handed.

In some of those embodiments wherein the peptide has an alpha helical structure, the peptide comprises a hydrophobic staple (such as those described in Bird *et al.* (2008) *Methods Enzymol* 446:369-386; Houston *et al.* (1995) *J Pept Sci* 1(4):274-282; Taylor (2002) *Biopolymers* 66(1):49-75; and Houston *et al.* (1996) *Biochemistry* 35(31):10041-50, each of which is herein incorporated by reference in its entirety), which is herein incorporated by reference in its entirety. As used herein, a “hydrophobic staple” refers to the presence of two amino acid residues having hydrophobic side chains that are covalently bound to one another. A hydrophobic staple is generated when the peptide comprises artificial amino acid residues comprising hydrophobic staple-forming amino acid residues at positions X and X+4 within the peptide, wherein X is any amino acid residue within any position of the peptide. One of skill in the art will appreciate that in order for a hydrophobic staple-forming amino acid residue to be present at positions X and X+4, X can not be any of the last four residues (i.e., the four most carboxy residues) within a peptide chain. In particular embodiments, the hydrophobic staple-forming amino acid residues are present at positions 4 and 8, 5 and 9, 6 and 10, or 7 and 11 of the peptide. Additional hydrophobic staple-forming amino acid residues can be present within the peptide as long as they are at least four amino acid residues away from another hydrophobic staple-forming amino acid residue.

As used herein a “hydrophobic staple-forming amino acid residue” is an amino acid residue as defined herein that comprises a side chain capable of forming a covalent bond with the side chain of another amino acid residue to form a hydrophobic staple. Non-limiting examples of hydrophobic staple-forming amino acid residues include (S)-2-(4-pentenyl)alanine, (R)-2(4-pentenyl)alanine, (S)-2-(3-butenyl)alanine, (S)-2-(7-octenyl)alanine, lysine, and glutamic acid.

Further, in particular embodiments, the carboxy terminus of the peptide that binds to the E2-binding pocket of a NEDD8 co-E3 protein is amidated, wherein the carbon atom

of the backbone carboxyl group in the most carboxyl amino acid residue is covalently bound to an optionally substituted nitrogen. In other embodiments, the carbon atom of the carboxyl group in the most carboxyl amino acid residue is bound to at least one -R group, as defined elsewhere herein.

5 In particular embodiments, the peptide that binds to the E2-binding pocket has the sequence set forth in SEQ ID NO: 10, 11, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 87, 88, 89, 90, 91, 92, 93, or 94, or a fragment or variant thereof.

 In some embodiments, the small molecule or peptide that binds to the E2-binding pocket of a NEDD8 co-E3 protein inhibits the co-E3 activity of the co-E3 protein.

10 Therefore, the small molecule or peptide is capable of inhibiting the neddylation of at least one NEDD8 target proteins, including but not limited to, a cullin protein. Such a molecule is referred to herein as an antagonist or an inhibitor. In some embodiments, the co-E3 inhibitor inhibits the co-E3 activity of the co-E3 protein by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least
15 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater. The ability of a small molecule or peptide to inhibit the activity of a co-E3 protein can be measured *in vitro* or *in vivo* or in cells in culture using any method known in the art, including those assays disclosed elsewhere herein (see, for example, Example 4). The compound may be assessed for its
20 ability to inhibit co-E3 activity by measuring co-E3 activity directly or indirectly by assaying downstream effects of NEDD8 co-E3 proteins (e.g., inhibition of cullin-dependent ubiquitination and proteolysis). For example, activity may be assessed by detection of downstream protein substrate stabilization (e.g., stabilization of p27, of IκB, NFκB or p27 reporter assays).

25 In addition to NEDD8 co-E3 proteins, the amino terminal extension of NEDD8 E2 enzymes (e.g., Ubc12) also binds to NEDD8 E1 proteins (Huang *et al.* (2004) *Nat Struct Mol Biol* 11:927-935, which is herein incorporated by reference in its entirety). Therefore, in some embodiments, the NEDD8 co-E3 inhibitor that binds to the E2-binding pocket of the co-E3 protein also inhibits the activity of a NEDD8 E1 protein. In order to generate an
30 inhibitor molecule that is more specific for co-E3 proteins, in some embodiments, the phenylalanine corresponding to position 5 and leucine corresponding to position 7 of Ubc12 (SEQ ID NO: 2), which contribute to the bulk of the binding energy of Ubc12 to E1, are substituted with another amino acid residue, such as a hydrophobic staple-forming

residue. Further, the addition of a hydrophobic staple to a peptide, locking it into an alpha helical conformation, prevents binding to NEDD8 E1 proteins (see Example 1).

Further, the peptide binding assays disclosed elsewhere herein (see Example 3 and Table 13) demonstrate that the assayed peptides exhibit binding specificity for particular co-E3 proteins (e.g., Dcn1, Dcn2, Dcn3, Dcn4, Dcn5). For example, SEQ ID NO: 8
5 (hUbc12Ac1-26) and SEQ ID NO: 87 (yUbc12Ac1-24) have greatest affinity for Dcn1 and 2, whereas SEQ ID NO: 88 (hUbc2fAc1-25) has greatest affinity for Dcn3 and is fairly specific for this particular co-E3 protein. The affinity of hUbc12Ac1-12 for Dcn3 and 5 can be enhanced by substituting amino acid residues at positions 6 and 10 with (S)-
10 2-(3-butenyl)alanine and (S)-2-(7-octenyl)alanine, respectively, to form a hydrophobic staple. Similarly, the affinity of yUbc12Ac1-24 for Dcn1, 2, and 3 can be enhanced by substituting the first methionine with norleucine and amino acid residues at positions 6 and 10 with (S)-2-(4-pentenyl)alanine to form a hydrophobic staple. Interestingly, the affinity of yUbc12Ac1-24 for Dcn4 and Dcn5 is reduced with these modifications. Using this
15 information, in combination with the disclosed crystal structures of the E2-binding pocket, one of skill in the art could generate a peptide that has enhanced specificity for one NEDD8 co-E3 protein over another.

The presently disclosed NEDD8 co-E3 inhibitors may be used to inhibit co-E3 activity and cellular growth and therefore, find use in treating disorders associated with
20 unregulated cell growth. Further, as I κ B, the inhibitor of the inflammatory mediator NF κ B, is a target of the cullin-comprising SCF complex, the presently disclosed NEDD8 co-E3 inhibitors also find use in treating inflammatory disorders.

NEDD8 co-E3 proteins can be inhibited by the presently disclosed NEDD8 co-E3 inhibitors by contacting a NEDD8 co-E3 protein (e.g., Dcn1, Dcn2, Dcn3, Dcn4, Dcn5)
25 with a NEDD8 co-E3 inhibitor, such as those described herein, *in vivo* or *in vitro* or in cells in culture.

In some embodiments, the NEDD8 co-E3 protein is contacted by a NEDD8 co-E3 inhibitor in a biological sample. As used herein, the phrase "biological sample" refers to a sample obtained from or comprising a cell, tissue, organ, or organism. Non-limiting
30 examples of biological samples include cellular organelles, cells (e.g., mammalian cells, bacterial cells, cultured cells), a biological fluid, such as blood, plasma, serum, urine, bile, saliva, tears, cerebrospinal fluid, aqueous or vitreous humor, or any bodily secretion), a transudate or exudate (e.g. fluid obtained from an abscess or other site of infection or inflammation), a fluid obtained from a joint (e.g. a normal joint or a joint affected by

disease such as rheumatoid arthritis, osteoarthritis, gout or septic arthritis), or the like, and a lysate or extract of an organelle, cell, tissue, organ, or organism.

Biological samples may be obtained from any organ or tissue (normal or diseased, including a biopsy or autopsy specimen) or may comprise cells or a lysate or extract thereof (including primary cells, passaged or cultured primary cells, cell lines, cells conditioned by a specific medium or grown under a particular set of environmental conditions) or medium conditioned by cells. In some embodiments, the biological sample (e.g., cell, tissue, organism or extract thereof) after exposure to a hormone or other biological or pharmacological agent. If desired, the biological sample may be subjected to processing, such as lysis, extraction, subcellular fractionation, or other standard biochemical procedures known in the art to solubilize proteins. See, Deutscher (ed.) (1990) *Methods in Enzymology* 182:147-238, which is herein incorporated by reference in its entirety. For example, tissues or cells can be ground and homogenized in buffers appropriate for solubilizing proteins and retaining their native conformations, followed by clarification by centrifugation. Other methods known in the art can be used to process the biological sample to obtain the protein with co-E3 activity, including but not limited to osmotic lysis, detergent lysis, sonication, heat, and rapid decompression. In general, methods used to obtain the Nedd8 co-E3 protein are performed under non-denaturing conditions, allowing the majority of proteins to retain their native conformations.

The Nedd8 pathway is involved in the regulation of cell cycle progression and cell proliferation. Thus, the various inhibitors of the E2 binding pocket of the co-E3 enzymes provided herein may be useful for inhibiting cell growth and in the treatment of diseases or disorders that are associated with hyperproliferation (e.g., cancers, inflammatory disorders, pathogenic infections). As used herein, "cell growth" refers to cell proliferation, cell division, or progression through the cell cycle.

Methods for inhibiting cell growth comprise introducing into a cell a NEDD8 co-E3 inhibitor, such as those disclosed herein. As used herein, the terms "introduce" and "introducing" when referring to a compound refers to the presentation of the compound to a cell in such a manner that the compound gains access to the intracellular region of the cell. The compound can be introduced into the cell via any means known in the art.

Any method known in the art can be used to measure the growth rate of a cell, including, but not limited to, optical density (OD₆₀₀), CO₂ production, O₂ consumption, assays that measure mitochondrial function, such as those utilizing tetrazolium salts (e.g., MTT, XTT), or other colorimetric reagents (e.g., the WST-1 reagent available from

Roche), assays that measure or estimate DNA content, including, but not limited to
fluoremetric assays such as those utilizing the fluorescent dye Hoechst 33258, assays that
measure or estimate protein content, including, but not limited to, the sulforhodamine B
(SRB) assay, manual or automated cell counts (with or without the Trypan Blue stain to
5 distinguish live cells), and clonogenic assays with manual or automated colony counts. In
some embodiments, the growth rate of a cell is inhibited by a NEDD co-E3 inhibitor by at
least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%,
at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least
98%, at least 99%, or greater.

10 The NEDD8 co-E3 inhibitors disclosed herein can be used to treat disorders that
are associated with hyperproliferation, such as cancers, inflammatory disorders, and
pathogenic infections.

As used herein, "treatment" is an approach for obtaining beneficial or desired
clinical results. For purposes of this invention, beneficial or desired clinical results
15 include, but are not limited to, alleviation of symptoms, partial or complete restoration of
eyesight (e.g., central vision, visual acuity), diminishment of extent of the disorder,
stabilized (i.e., not worsening) state of the disorder (e.g., degeneration of cone
photoreceptors), delaying or slowing of progression of the disorder, amelioration or
palliation of the disorder, and prevention of, inhibition of, or reduction of risk of
20 developing a retinal disorder. "Treatment" refers to both therapeutic treatment and
prophylactic or preventative measures. Those in need of treatment include those already
with the disorder (to prevent further degeneration) as well as those in which the disorder is
to be prevented. "Palliating" a disorder means that the extent and/or undesirable clinical
manifestations of the disorder are lessened and/or the time course of the progression is
25 slowed or lengthened, as compared to a situation without treatment.

Methods for treating a disorder associated with hyperproliferation (e.g., cancer,
inflammatory disorder, pathogenic infection) comprise administering a therapeutically
effective amount of a NEDD co-E3 inhibitor, such as those disclosed herein, to a subject
in need thereof.

30 By "therapeutically effective amount" is intended an amount that is useful in the
treatment, prevention or diagnosis of a disease or condition. As used herein, a
therapeutically effective amount of a NEDD8 co-E3 enzyme inhibitor is an amount which,
when administered to a subject, is sufficient to achieve a desired effect, such as inhibiting
NEDD8 co-E3 activity or cell growth in a subject being treated with that composition.

The effective amount of a NEDD8 co-E3 protein inhibitor useful for inhibiting cell growth will depend on the subject being treated, the severity of the affliction, and the manner of administration of the NEDD8 co-E3 protein inhibitor.

By "subject" is intended an animal, including but not limited to, mammals, e.g.,
5 primates, humans, agricultural and domesticated animals such as, but not limited to, dogs, cats, cattle, horses, pigs, sheep, and the like. In some embodiments, the subject undergoing treatment with the pharmaceutical formulations of the invention is a human.

Given the involvement of NEDD8 in the regulation of cell cycle progression, NEDD8 co-E3 inhibitors, such as those disclosed herein, can be used to treat disorders that
10 are associated with unregulated cell growth, such as cancer. Components of the Nedd8 pathway have been implicated in various types of cancer, including, but not limited to, gliomas, cancers of the lung, head and neck, esophagus, tongue, urinary tract, cervix, vulva, vagina and ovary (Broderick *et al.* (2010) *Neoplasia* 12(6):476-84; Sarkaria *et al.* (2006) *Cancer Res* 66(19): 9437-44; and Sarkaria *et al.* (2004) *Ann Thorac Surg* 78:1734-
15 41, each of which are herein incorporated by reference in its entirety). In fact, human DCUN1D1 is also known as squamous cell carcinoma-related oncogene (SCCRO) due to its overexpression in various squamous cell carcinomas of mucosal origin, including, for example, oral tongue squamous cell carcinoma (Estilo *et al.* (2003) *Clin Can Res* 9:2300-06, which is herein incorporated by reference in its entirety).

20 The term "cancer" refers to the condition in a subject that is characterized by unregulated cell growth, wherein the cancerous cells are capable of local invasion and/or metastasis to noncontiguous sites. As used herein, "cancer cells," "cancerous cells," or "tumor cells" refer to the cells that are characterized by this unregulated cell growth and invasive property. The term "cancer" encompasses all types of cancers, including, but not
25 limited to, all forms of carcinomas, melanomas, sarcomas, gliomas, lymphomas and leukemias, including without limitation, lung cancer, head and neck cancer, esophageal cancer, tongue cancer, cancers of the urinary tract, cervical cancer, vulval cancer, vaginal cancer, ovarian cancer. In particular embodiments, the cancer that is being treated with the presently disclosed NEDD8 co-E3 inhibitors is a squamous cell carcinoma, such as an
30 oral tongue squamous cell carcinoma.

The neddylation of cullin proteins promotes the ubiquitination of I κ B and subsequent activation of the inflammatory mediator NF κ B. Therefore, NEDD8 co-E3 inhibitors can be used to treat inflammatory disorders.

As used herein, an "inflammatory disorder" is a condition characterized by inflammation and tissue destruction, or a combination thereof. An "inflammatory disorder" includes any inflammatory immune-mediated process where the initiating event or target of the immune response involves non-self antigen(s), including, for example, 5 alloantigens, xenoantigens, viral antigens, bacterial antigens, unknown antigens, or allergens.

Further, for purposes of the present invention, the term "inflammatory disorder(s)" includes "autoimmune disease(s)." As used herein, the term "autoimmunity" is generally understood to encompass inflammatory immune-mediated processes involving 10 "self" antigens. In autoimmune diseases, self antigen(s) trigger host immune responses. The term "inflammatory disorder" also refers to inflammation associated with tissue transplant rejection, such as graft versus host disease. "Transplant rejection" or "graft rejection" refers to any host-mounted immune response against a graft including but not limited to HLA antigens, blood group antigens, and the like.

15 Non-limiting examples of inflammatory disorders that can be treated with the NEDD8 co-E3 inhibitors disclosed herein include systemic lupus erythematosus (SLE), CREST syndrome, inflammatory myositis, Sjogren's syndrome, mixed connective tissue disease, multiple sclerosis, inflammatory bowel disease, acute respiratory distress syndrome, pulmonary inflammation, idiopathic pulmonary fibrosis, osteoporosis, delayed 20 type hypersensitivity, asthma, primary biliary cirrhosis, and idiopathic thrombocytopenic purpura, discoid lupus, lupus nephritis, sarcoidosis, inflammatory arthritis, including juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, and gouty arthritis, rejection of an organ or tissue transplant, hyperacute, acute, or chronic rejection and/or graft versus host disease, multiple sclerosis, hyper IgE 25 syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), autoimmune hepatitis, pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia gravis, autoimmune thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease, Hashimoto's thyroiditis, immune complex disease, chronic fatigue immune dysfunction 30 syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis, cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, Type I and Type II diabetes mellitus, type 1, 2, 3, and 4 delayed-type hypersensitivity, allergy or allergic disorders, unwanted/unintended immune responses to therapeutic proteins (see for example, U.S. Patent Application No. US 2002/0119151 and Koren, *et al.* (2002) *Curr.*

Pharm. Biotechnol. 3:349-60), asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic dermatitis, allergic and irritant contact dermatitis, urticaria, IgE-mediated allergy, atherosclerosis, vasculitis, idiopathic inflammatory myopathies, hemolytic disease, Alzheimer's disease, chronic inflammatory demyelinating polyneuropathy, and the like.

5 Given the conservation of the NEDD8 signaling pathway in all eukaryotes, including fungal species, the presently disclosed NEDD8 co-E3 inhibitors inhibit the growth of pathogens and can be used to treat pathogenic infections, such as fungal infections. Further, viruses and bacterial pathogens routinely hijack the cullin-RING ligase machinery for ubiquitination and degradation of specific host factors. For example,
10 HIV-1 Vif targets a RNA-editing enzyme (APOBEC3G) for Cul5-mediated degradation in a manner that reportedly depends on the NEDD8 pathway (see, for example, Barry and Fruh (2006) *Sci STKE* 335:21; Ribert and Cossart (2010) *Cell* 143:694-702). Therefore, the growth of viruses and bacterial pathogens that target host proteins for cullin RING ligase-dependent degradation can also be inhibited by the presently disclosed NEDD8 co-E3
15 inhibitors and these inhibitors find further use in treating infections of these viral and bacterial pathogens. Non-limiting examples of pathogenic organisms whose growth can be attenuated by the NEDD8 co-E3 inhibitors disclosed herein include fungal species within the genus *Candida*, *Aspergillus*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*, *Stachybotrys*, and *Paracoccidioides*, including but not limited to, *Candida albicans*,
20 *Aspergillus fumigatus*, *Aspergillus flavus*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *Histoplasma capsulatum*, *Pneumocystis jirovecii*, *Stachybotrys chartarum*, and *Paracoccidioides brasiliensis* (see, for example, San-Blas and Calderone (2008) *Pathogenic Fungi: Insights in Molecular Biology*. Caister Academic Press. 264 pages; and Chen *et al.* (2010) *Curr Opin Pharmacol* 10(5):522-30, each of which is herein
25 incorporated by reference in its entirety); viral pathogens such as human immunodeficiency virus (HIV) and other lentiviruses; and bacterial pathogens that target host proteins for cullin RING ligase-dependent degradation.

 When administration is for the purpose of treatment, administration may be for either a prophylactic (i.e., preventative) or therapeutic purpose. When provided
30 prophylactically, the substance is provided in advance of any symptom. The prophylactic administration of the substance serves to prevent or attenuate any subsequent symptom. When provided therapeutically, the substance is provided at (or shortly after) the onset of a symptom. The therapeutic administration of the substance serves to attenuate any actual symptom.

It will be understood by one of skill in the art that the treatment modalities described herein may be used alone or in conjunction with other therapeutic modalities (i.e., as adjuvant therapy), including, but not limited to, surgical therapy, radiotherapy, chemotherapy (e.g., with any chemotherapeutic agent well known in the art) or

5 immunotherapy.

The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective
10 amount of a NEDD8 co-E3 inhibitor can include a single treatment or, preferably, can include a series of treatments. It will also be appreciated that the effective dosage of a NEDD8 co-E3 inhibitor used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

15 It is understood that appropriate doses of such active compounds depends upon a number of factors within the knowledge of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the active compounds will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable. Exemplary
20 doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of an active agent depend upon the potency of the active
25 agent with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these molecules is to be administered to an animal (e.g., a human) in order to reduce the activity of the NEDD8 co-E3 protein, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an
30 appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Therapeutically effective amounts of a NEDD8 co-E3 inhibitor can be determined by animal studies. When animal assays are used, a dosage is administered to provide a target tissue concentration similar to that which has been shown to be effective in the animal assays. It is recognized that the method of treatment may comprise a single
5 administration of a therapeutically effective amount or multiple administrations of a therapeutically effective amount of the NEDD8 co-E3 inhibitor.

The inhibitors of the E2 binding pocket of the co-E3 enzyme disclosed herein can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the compound (e.g., peptide, small molecule) and a
10 pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any
15 conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include
20 parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. In addition, it may be desirable to administer a therapeutically effective amount of the pharmaceutical composition locally to an area in need of treatment (e.g., to an area of the body where inhibiting a T_R cell function is desired). This can be achieved by, for example, local or regional infusion or
25 perfusion during surgery, topical application, injection, catheter, suppository, or implant (for example, implants formed from porous, non-porous, or gelatinous materials, including membranes, such as sialastic membranes or fibers), and the like. In one embodiment, administration can be by direct injection at the site (or former site) of a cancer that is to be treated. In another embodiment, the therapeutically effective amount of the
30 pharmaceutical composition is delivered in a vesicle, such as liposomes (see, e.g., Langer (1990) *Science* 249:1527-33; and Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez Berestein and Fidler (eds.), Liss, N.Y., pp. 353-65, 1989).

In another embodiment, the peptide NEDD8 co-E3 inhibitors can be formulated with a cell-penetrating peptide, including but not limited to the HIV tat protein, that is

either covalently bound to the peptide or otherwise associated therewith (see, for example, Okuyama *et al.* (2007) *Nature Methods* 4:153-159).

In yet another embodiment, the therapeutically effective amount of the pharmaceutical composition can be delivered in a controlled release system. In one
5 example, a pump can be used (see, *e.g.*, Langer (1990) *Science* 249:1527-33; Sefton (1987) *Crit. Rev. Biomed. Eng.* 14:201-40; Buchwald *et al.* (1980) *Surgery* 88:507-16; Saudek *et al.* (1989) *N. Engl. J. Med.* 321:574-79). In another example, polymeric materials can be used (see, *e.g.*, Levy *et al.* (1985) *Science* 228:190-92; During *et al.* (1989) *Ann. Neurol.* 25:351-56; Howard *et al.* (1989) *J. Neurosurg.* 71:105-12). Other
10 controlled release systems, such as those discussed by Langer (1990) *Science* 249:1527-33, can also be used.

Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or
15 other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral
20 preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration,
25 suitable carriers include physiological saline, bacteriostatic water, Cremophor[®] EL (BASF; Parsippany, NJ), or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a
30 solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can

be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth, or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser that contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art,

and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated with each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a peptide" is understood to represent one or more peptides. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

Throughout this specification and the claims, the words "comprise," "comprises," and "comprising" are used in a non-exclusive sense, except where the context requires otherwise.

As used herein, the term “about,” when referring to a value is meant to encompass variations of, in some embodiments $\pm 50\%$, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as
5 such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, when an amount, concentration, or other value or parameter is given as either a range, preferred range, or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper
10 range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the presently disclosed subject matter be limited to the specific values recited when defining a range.

15 The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

20 Example 1. Activation of ubiquitin-like protein ligation by acetylation-dependent E2-E3 interaction.

Exogenously expressed C-terminally tagged and/or untagged human or yeast Ubc12 were purified from 293T cells, yeast, and/or SF9 cells (hereafter human proteins denoted with “h”, and yeast with “y”). Endogenous hUbc12 was co-immunoprecipitated with TAPhNedd8 expressed in 293T cells. Tandem mass spectrometry (LC-MS/MS)
25 identified exogenous yeast and human, and endogenous human Ubc12 as retaining the amino-terminal (N-terminal) methionine (Met) and being N-terminally acetylated (data not shown). N-terminal acetylation has been well-characterized in budding yeast: N-terminal Acetyltransferase (Nat) enzyme specificities depend upon a combination of: (1) whether or not the N-terminal Met is removed by methionine-aminopeptidase; and (2)
30 the second residue in a protein’s sequence (Polevoda and Sherman (2003) *J Mol Biol* 325(4):595-622). yUbc12’s N-terminal sequence, Met-Leu, is predicted to retain the Met and be acetylated by NatC, a heterotrimeric enzyme consisting of Mak3p, Mak10p and Mak31p (Polevoda and Sherman (2001) *J Biol Chem* 276(23):20154-20159). Indeed, deletion of the yeast *Mak3* gene prevents yUbc12 N-terminal acetylation. Furthermore,

coexpression with NatC subunits is sufficient for yUbc12 N-terminal acetylation in bacteria (Fig. 1A). Thus, yeast NatC performs yUbc12 N-terminal acetylation.

To address the question of whether Ubc12 N-terminal acetylation influences Nedd8 ligation to cullins such as Cul1, levels of yNedd8~yCul1 (aka Rub1p~Cdc53p, but here human nomenclature used for simplification) complexes in yeast were examined with gene deletions for subunits of the major N-terminal Acetyltransferases, NatA, NatB, and NatC. Only yeast deleted for genes encoding NatC subunits displayed decreased levels of yNedd8~yCul1 (Fig. 1B), indicating a correlation between yUbc12 N-terminal acetylation and yNedd8 modification of yCul1 *in vivo*. Furthermore, loss of NatC activity was synthetically lethal in combination with the *cdc34-2* temperature-sensitive allele (data not shown) – a hallmark for Nedd8 pathway components due to roles in yCul1/SCF-regulated cell division (Kurz et al. (2008) *Mol Cell* 29:33; Lammer et al. (1998) *Genes Dev* 12:914).

In vitro, Ubc12 N-terminal acetylation strikingly and specifically dictates Dcn1^P-mediated Nedd8 transfer to Cul1, as revealed by assays with three forms of Ubc12: Ubc12^{AcMet} is N-terminally acetylated; Ubc12^{Met} is identical to Ubc12^{AcMet} sequence but lacks N-terminal acetylation; and yUbc12^{GSMet} is not acetylated and has Gly-Ser N-terminal of Met (Scott et al. (2010) *Mol Cell* 39(5):784-795). Although yDcn1^P enhances yNedd8 transfer to yCul1 even from yUbc12^{Met} or yUbc12^{GSMet}, consistent with the partial defect observed in NatC null yeast (Fig. 1B), yDcn1^P E3 activity is substantially magnified for yUbc12^{AcMet} (Fig 2A). For human proteins, hUbc12 N-terminal acetylation is absolutely required to observe any hDcn1^P mediated potentiation of neddylation (Fig 2B). With apparently saturating concentrations of hDcn1^P, hNedd8 transfer to hCul1 from hUbc12^{AcMet} is complete in less than 20 seconds under conditions where we estimate the reaction would take ~8 minutes either in the absence of hDcn1^P, or with hDcn1^P but without Ubc12 N-terminal acetylation (Fig. 2B and 3C). The effect of Ubc12 N-terminal acetylation is specific for Dcn1^P, because Rbx1-mediated transfer of Nedd8 to Cul1 is independent of the state of Ubc12's N-terminus (Fig 2A and 2B). Nonetheless, even in the presence of Dcn1^P and Ubc12 N-terminal acetylation, Cul1 neddylation still requires Rbx1's RING E3 activity and is blocked by an inhibitor, CAND1 (Fig. 3).

To gain insights into mechanistic roles of Ubc12 N-terminal acetylation, isothermal titration calorimetry (ITC) was used to quantify interactions with peptides corresponding to the N-termini of yeast or human Ubc12. Strikingly, N-terminal acetylation increases affinity for Dcn1^P by roughly two orders-of-magnitude (Table 23).

Ubc12's N-terminal Met is also required for Dcn1^P-binding. For comparison, N-terminal acetylation has little effect on Ubc12 peptide binding to E1, although the role of the Met differs between yeast and human, likely due to idiosyncratic Nedd8 E1-E2 interactions in yeast (Fig. 4) (Huang *et al.* (2009) *Mol Cell* 33(4):483-495).

5

Table 23. Thermodynamic parameters determined by isothermal titration calorimetry (ITC) for binding between the indicated Ubc12 peptides and Dcn1^P and E1.

Protein : Titrant	Kd (μ M)	Δ H (cal/mol)	Δ S (cal/mol/deg)	N
yDcn1 ^P : yUbc12 ¹⁻²⁴ (44) : (10)	>40	3,777	33.4	1.16
yDcn1 ^P : Acetyl-yUbc12 ¹⁻²⁴ (44) :	0.444	4,145	43.6	0.99
yDcn1 ^P : Acetyl-yUbc12 ²⁻²⁴	>37	3,761	33.4	0.92
hDcn1 ^P : hUbc12 ¹⁻²⁶	>130	1,259	22.2	0.75
hDcn1 ^P : Acetyl-hUbc12 ¹⁻²⁶	1.11	-6,107	5.84	0.92
hDcn1 ^P : Acetyl-hUbc12 ²⁻²⁶	>100	1,719	24.1	0.52
yE1 : yUbc12 ¹⁻²⁴	0.070	-8,571	2.65	1.05
yE1 : Acetyl-yUbc12 ¹⁻²⁴	0.016	-11,260	-3.8	0.99
yE1 : Acetyl-yUbc12 ²⁻²⁴	1.58	-9,625	-7.21	0.92
hE1 : hUbc12 ¹⁻²⁶	0.585	-8,881	-1.99	1.29
hE1 : Acetyl-hUbc12 ¹⁻²⁶	0.943	-9,425	-4.79	1.14
hE1 : Acetyl-hUbc12 ²⁻²⁶	0.390	-8,899	-1.24	1.04

Kd: dissociation constant; Δ H: change in enthalpy; Δ S: change in entropy; N: stoichiometry, number of molecules of titrant the protein binds

- 10 To understand how N-acetyl-methionine mediates interactions, the crystal structures of yeast and human Dcn1^P bound to Ubc12 peptides were determined (Table 24). The structure with human proteins is also in complex with a domain of the Nedd8 target, Cull1. hDcn1^P-hCull1 interactions superimpose with the prior structure of yeast counterparts (Scott *et al.* (2010) *Mol Cell* 39(5):784-796), and are not discussed in detail
- 15 here. Overall, Dcn1^P superimposes with prior structures, forming a helical domain containing 2 EFhand-like folds (Kurz *et al.* (2008) *Mol Cell* 29(1):23-25; Scott *et al.* (2010) *Mol Cell* 39(5):784-796; and Yang *et al.* (2007) *J Biol Chem* 282(34):24490-

24494). The Ubc12 N-terminal peptides are α -helical, as in full-length yUbc12^{GSMet} (Scott *et al.* (2010) *Mol Cell* 39(5):784-796). A Dcn1^P groove at the junction between the two EF-hand-like subdomains cradles Ubc12's helix, culminating in Ubc12's N-acetyl-methionine filling a conserved, hydrophobic crevasse in Dcn1^P (Fig. 5 and 6).

Table 24. Data collection and refinement statistics for three crystal structures.

	yDcn1 ^P : Acetyl-yUbc12 ¹⁻²⁴	hCul1 ^{WHB} : hDcn1 ^P : Acetyl-hUbc12 ¹⁻¹⁶	hCul1 ^{WHB} : hDcn1 ^P : Acetyl-hUbc12 ¹⁻¹² (5:9 Staple)	hDcn3 ^P : Acetyl-hUbc2 ¹⁻²⁵
DATA COLLECTION				
Space group	C222 ₁	C2	C222 ₁	P2 ₁
Cell dimensions				
a, b, c (Å)	72.906, 98.421, 143.923	135.431, 65.454, 64.182	131.790, 190.210, 67.506	83.464, 44.579, 101.216
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 104.73, 90.0	90.0, 90.0, 90.0	90.000, 103.305, 90.000
Resolution (Å)	40.0-2.3 (2.38-2.3)	50.0-1.5 (1.55-1.5)	50.0-2.0	50.0-2.4
R _{merge}	0.123 (0.370)	0.090 (0.413)	0.078 (0.439)	0.110 (0.474)
I/σI	13.5 (2.6)	27.2 (2.6)	24.9 (2.5)	1.857
Completeness (%)	89.9 (65.8)	96.2 (88.0)	96.7 (77.2)	91.6 (70.0)
Redundancy	5.4 (3.4)	3.7 (3.3)	6.6 (3.5)	3.6 (3.2)
REFINEMENT				
Resolution (Å)	35-2.3	50.0-1.5	50.0-2.0	40.0-2.4
No. reflections	19967	78739	53080	27355
R _{work} /R _{free}	21.3/26.9	21.0/23.7	19.89/22.77	21.52/23.93
No. atoms	3788	5365	5131	
Protein	3651	4776	4646	3437
Water	137	589	485	135
B-factors				
Protein	27.06	19.56	17.2	34.381
Water	33.3	30.75	26.0	37.21
R.m.s deviations				
Bond lengths (Å)	0.009	0.006	0.008	0.009
Bond angles (°)	1.021	0.914	0.992	1.297
RAMACHANDRAN PLOT STATISTICS (%)				

Preferred regions	97.1	97.8	97.5	94.62
Allowed regions	2.9	2.2	2.5	4.1
Disallowed regions	0	0	0	1.28

Data for highest resolution shell is shown in parentheses. $R_{work} = \sum |F_o - F_c| / \sum F_o$, R_{free} is the cross-validation of R -factor, with 5-10% of the total reflections omitted in model refinement.

N-acetyl-methionine recognition consists of three major components (Fig 5). First, the methyl portion of the acetyl group fits snugly in a hydrophobic pocket consisting of the α -carbon from yDcn1's Glu105 (hDcn1's Ala98) and side-chains from yDcn1's Leu107, Leu110, and Leu193 (hDcn1's Ile100, Val102, Leu103, and Leu184).

5 Second, the amide makes a hydrogen bond with the carbonyl oxygen from yDcn1's Leu104 (hDcn1's Pro97). Third, the Met side-chain packs in an extensive hydrophobic channel formed by side-chains from yDcn1's Leu93, Ile97, Leu104, Thr109, Leu112, Ala113, Tyr118, Leu121, and Leu173 (hDcn1's Ile86, Cys90, Pro97, Val102, Ile105, Ala106, Cys115, Phe117, Leu121, and Phe164).

10 Four additional structural elements, two each from Dcn1^P and Ubc12, secure Ubc12's N-acetyl-methionine in place. First, yDcn1's Tyr190 (hDcn1's Tyr181) clamps between Ubc12's N-acetyl-Met1 and yDcn1-Leu2/hDcn1-Ile2 to press the N-acetyl-Met into the hydrophobic crevasse in Dcn1^P. Second, the loop between Dcn1^P's E- and F- α -helices acts as an additional clamp, closing down on top of Ubc12's N-acetyl-
15 methionine. Notably, in 7 prior structures of yDcn1^P (Kurz *et al.* (2008) *Mol Cell* 29(1):23-35; Scott *et al.* (2010) *Mol Cell* 39(5):784-796; and Yang *et al.* (2007) *J Biol Chem* 282(34):24490-24494), none of which are bound to Ubc12, both of these elements are repositioned and partially occupy and occlude access to the hydrophobic crevasse (data not shown). It seems likely that yDcn1^P is sufficiently flexible to initially engage Ubc12's
20 acetylated N-terminus, and subsequently clamps down around the hydrophobic yUbc12 N-acetyl-methionine. Such conformational flexibility may allow structural rearrangement accounting for yDcn1^P's ability to mediate low-level activation of yUbc12 even in the absence of N-terminal acetylation.

Ubc12's N-acetyl-methionine is further sealed into the hydrophobic crevasse by
25 interactions involving hydrophobic residues at positions 2 and 4 in Ubc12 (Fig 5C and 5D). This requires the helical structure, which places these side-chains on opposite sides of the N-acetyl-methionine. On one side, yUbc12's Leu2 (hUbc12's Ile2) buries the acetyl moiety via interacting with the aliphatic portion of yDcn1's Glu105 and Gln189 side-chains, Tyr190, and Leu193. The interaction is more extensive for the human proteins, with interactions with hDcn1's Ala98, Met177, Ala180, Tyr181, and Leu184. On the
30 other side, Ubc12's Leu4 side-chain seals the Met1 side-chain into place via interactions with yDcn1's Ile90, Val94, Leu104, and Leu121 (hDcn1's Ile83, Ile86, Gln87, Pro97, and Cys115). Furthermore, there are numerous additional, species-specific interactions between other downstream residues from both faces of the Ubc12 helix and surrounding

residues from the Dcn1^P groove. For example, yUbc12's Lys3 fits in an anionic pocket formed by side-chains from yDcn1^P's Glu122 and Asp176, and carbonyls from Ile174 and Leu175. Other interactions include the following: yUbc12's Arg5 forms a salt-bridge with yDcn1^P's Glu105; yUbc12's Leu7 makes hydrophobic contacts with yDcn1^P's Ile90 and Leu121; and yUbc12's Lys11 fits in an anionic pocket formed by side-chains from yDcn1^P's Asp89 and Asp91. Examples of interactions between hUbc12 and hDcn1 include the following: hUbc12's Lys3 makes a salt-bridge with hDcn1^P's Asn167; hUbc12's Leu7 makes hydrophobic contacts with hDcn1^P's Ile83 and Cys115; hUbc12's Lys8 forms a hydrogen bond with hDcn1^P's Gln87; and hUbc12's Lys11 forms a salt-bridge with hDcn1^P's Asp84.

Although yUbc12^{Met}'s N-terminal extension is helical in a prior structure (Scott *et al.* (2010) *Mol Cell* 39(5):784-796), experiments were performed to confirm a role for the helix with human proteins because hUbc12^{Met}'s N-terminal region is extended in complex with E1 (Huang *et al.* (2004) *Nat Struct Mol Biol* 11(10):927-935; Huang *et al.* (2007) *Nature* 445(7126):394-398). Specifically, Phe5 and Gln9 were replaced with (S)-2-(4'-pentenyl) alanine and these side-chains were covalently linked to test the effect of "stapling" an acetylated Ubc12 peptide into a helix (Bird *et al.* (2008) *Methods Enzymol* 446:369-386). The stapled helical peptide no longer binds E1 (data not shown), consistent with prior structures (Huang *et al.* (2004) *Nat Struct Mol Biol* 11(10):927-935; and Huang *et al.* (2007) *Nature* 445(7126):394-398). Moreover, a 2.0 Å resolution structure with the stapled peptide superimposes with the unstapled hUbc12^{AcMet} complex with hDcn1^P, confirming that the staple is completely solvent-exposed (data not shown). The helical staple improves binding to hDcn1^P 14-fold (Table 25). This is largely due to decreasing the entropic cost of binding, supporting the notion that locking the flexible hUbc12 N-terminal region into a helical conformation is important for the hDcn1^P interaction.

Table 25. Thermodynamic parameters determined by isothermal titration calorimetry (ITC) for binding between hDcn1^P or hE1 to control or hydrocarbon-stapled Ubc12 peptides.

Protein :	Titrant	K _d (μM)	ΔH (cal/mol)	ΔS (cal/mol/deg)	N
hDcn1P :	Acetyl-hUbc12 ¹⁻¹²	2.15	-4,992	8.79	0.99
hDcn1P :	Acetyl-hUbc12 ¹⁻¹² (5:9Staple)	0.15	-3,386	19.3	1.18
hE1 :	Acetyl-hUbc12 ¹⁻¹²	0.63	-11,490	-11.0	0.78
hE1 :	Acetyl-hUbc12 ¹⁻¹² (5:9Staple)	NB	NB	NB	NB

The structures suggest two primary mechanisms by which Ubc12's N-terminal acetylation dictates binding to Dcn1^P. First, the acetyl group interacts directly with Dcn1^P. Second, acetylation eliminates an N-terminal positive charge, which would impede burial in the Dcn1^P hydrophobic crevasse. To test these concepts, Dcn1^P binding to Ubc12 peptides that were N-terminally formylated was examined by ITC (Table 26). The formylated peptides lack the methyl portion of an acetyl, but retain the amide and are uncharged. Indeed, the formylated peptides bind better than those with a free N-terminus. Furthermore, the K_ds were decreased ~9- and ~17-fold compared with the acetylated human and yeast peptides, respectively, highlighting the importance of the acetyl methyl for mediating Ubc12-Dcn1^P interactions.

Table 26. Thermodynamic parameters determined by isothermal titration calorimetry (ITC) for binding between N-terminally formylated Ubc12 peptides and Dcn1^P.

Protein :	Titrant	K _d (μM)	ΔH (cal/mol)	ΔS (cal/mol/deg)	N
yDcn1P :	Formyl-yUbc12 ¹⁻²⁴	7.46	2,824	33.4	0.90
hDcn1P :	Formyl-hUbc12 ¹⁻²⁶	9.71	-4,893	5.79	1.00

The ultimate test of understanding the structural role of Ubc12 N-terminal acetylation would be to design Dcn1^P mutants that potentiate neddylation with unacetylated Ubc12. Given the structural malleability of yDcn1^P, it was reasoned that mutations alleviating repulsion of yUbc12's N-terminal charge might enhance the low-level yDcn1^P E3 activity toward yUbc12^{Met}. The structure indicated that aspartate substitutions for yDcn1^P Leu110 or Leu173 would approach yUbc12^{Met}'s N-terminus to

balance the positive charge. Also, an Ala replacement for the Tyr190 “clamp” would not force a charged yUbc12^{Met}’s N-terminus directly into the hydrophobic pocket. Indeed, the three Dcn1^P mutants showed enhanced activity specifically toward unacetylated yUbc12 in potentiating yNedd8 transfer to yCul1 (Fig 7A). To address whether a mutation could compensate for loss of NatC-mediated yUbc12 acetylation *in vivo*, HA-tagged versions of either wild-type or mutant yDcn1 were expressed in strains deleted for either *Dcn1*, or both *Dcn1* and the NatC subunit *Mak10*. Although Leu110Asp or Leu173Asp mutants were not expressed at levels comparable to the wild-type, strains expressing the Tyr190Ala mutant were obtained. Consistent with the *in vitro* results, relative to wild-type Dcn1, the Tyr190Ala mutant partially rescued the defect in yCul1~yNedd8 conjugate formation in the absence of NatC activity (Fig 7B).

Methods

Constructs, protein preparation, antibodies, and peptides

Constructs for expression and purification of hUbc12, yUbc12^{GSMet} (also referred to as Ubc12), yDcn1^P, hNedd8, yNedd8 (also referred to as Rub1), hCul1^{ctd}-hRbx1, yCul1^{C+}-yRbx1 (also referred to as Cdc53^{C+}-Hrt1), yCul1^{C+}-yRbx1^R (also referred to as Cdc53^{C+}-Hrt1^R), hE1 for Nedd8 (also referred to as APPBP1-UBA3 and NAE1-UBA3), yE1 for yNedd8 (also referred to as Ula1-Uba3), and hCAND1 were expressed in *E. coli* and purified as described previously (Walden *et al.* (2003) *Nature* 422(6929):330-334; Duda *et al.* (2008) *Cell* 134(6):995-1006; Scott *et al.* (2010) *Mol Cell* 39(5):784-796). hCul1-hRbx1 was expressed in insect cells and purified as described previously (Duda *et al.* (2008) *Cell* 134(6):995-1006).

hCul1^{ctd}-hRbx1^R contains a stop mutation introduced after residue 36 in hRbx1 to delete the RING domain, and was expressed and purified as previously described for hCul1^{ctd}-hRbx1 (Duda *et al.* (2008) *Cell* 134(6):995-1006). Expression constructs generated for this study were prepared by standard molecular biology techniques and coding sequences were entirely verified. Mutant versions used in this study were generated by QuickChange (Stratagene). hCul1^{WHB} corresponds to residues 702 to the C-terminal residue 776 of hCul1 (SEQ ID NO: 79). hDcn1^P corresponds to residues 62 to the C-terminal residue 259 of hDcn1 (aka SCCRO or DCUN1D1 (Sarkaria *et al.* (2006) *Cancer Res* 66(19):9437-9444); SEQ ID NO: 33).

hCul1^{WHB}, hDcn1^P, and hDcn1^{FL} were expressed as GST fusions in BL21(DE3)Gold *E. coli* and purified by glutathione affinity chromatography. After TEV protease treatment to release GST, proteins were further purified by ion exchange and gel filtration chromatography in 25 mM Tris, 125 mM NaCl, 5 mM DTT, pH 7.6 (Buffer A), concentrated (Amicon Ultra), aliquotted, flash-frozen and stored at -80°C.

For expression and purification of hUbc12^{Met} and yUbc12^{Met} proteins, His-SUMO-1 fusions were generated in pRSF-Duet, in which the Met start codon of hUbc12 or yUbc12 was directly fused to follow the GlyGly SUMO-1 C-terminus. His-SUMO-1 fusions were expressed in BL21(DE3)Gold *E. coli* and purified by Ni⁺² affinity chromatography. After treatment with the SUMO protease SENP2, hUbc12^{Met} and yUbc12^{Met} were further purified by gel filtration and passed back over a Ni⁺² affinity resin to remove any residual His-SUMO-1 that co-purified over sizing.

For purification of hUbc12^{AcMet}, hUbc12 was expressed untagged in insect cells. The Ubc12 coding sequence was cloned into pFastbac1, and baculoviruses were prepared using the Bac-to-Bac system (Invitrogen). Insect cell expression of hUbc12^{AcMet} was performed at 24°C for 1 day, followed by incubation at 18°C for 2 days, with lysates initially purified over gravity S-Sepharose with a step gradient elution. Fractions containing hUbc12^{AcMet} were diluted and reapplied to a Source-S column via FPLC, and eluted with a linear salt gradient. Fractions were pooled and concentrated (Amicon Ultra), and further purified by gel filtration chromatography in Buffer A.

For expression and purification of yUbc12^{AcMet}, the yUbc12 ORF was fused to a C-terminal hexahistidine tag and cloned into pRS426-Gal1 (Mumberg *et al.* (1994) *Nucleic Acids Res* 22(25):5767-5768). Proteins expressed from this vector are referred to as "yUbc12-His6", but subsequently the N-terminally acetylated version is referred to as "yUbc12^{AcMet}". BY4742, or *mak3* (i.e., strain Y15470) yeast cells harboring pRS426-Gal1-yUbc12-His6 under control of the galactose-inducible promoter were grown at 30°C in SD-Ura 2% Raffinose 0.1% Glucose media to mid-log phase (OD600 = 0.4-0.6). Cells were pelleted and resuspended at a density of 0.1 OD/ml in pre-warmed SD-Ura 2% Galactose media and induced at 30 °C for 14-16 hours. Cell pellets were resuspended in Phosphate-Buffer Saline containing Aprotinin, Leupeptin, PMSF, and protease inhibitor tablets (Sigma).

Cells were lysed by three passages through a cell disruptor at 15,000 psi and protein subsequently purified by Ni^{+2} affinity chromatography. Ni^{+2} column eluates were further purified over gel filtration chromatography in Buffer A.

N-terminal acetylation of bacterially expressed yUbc12 was achieved by co-expression of Mak3, Mak10, Mak31, and yUbc12-His6, followed by Ni^{+2} affinity and gel filtration chromatography. Briefly, untagged Mak10 and Mak3 were cloned into site 1 and 2, respectively, of pET-Duet (EMD Biosciences), and untagged Mak31 and yUbc12-His6 were cloned into site 1 and 2, respectively, of pRSF-Duet (EMD Biosciences).

For wild type and mutant rescue of *dcn1* yeast cells, yDcn1-HA was built by overlap PCR from yeast genomic DNA and cloned into the CEN/Leu vector pRS315. The resultant clone contains approximately 600 nucleotides 5' and 3' of the start and stop codon, respectively, in order to preserve the natural regulatory elements of genomic yDcn1.

Antibodies against Cdc53 (sc-50444) and HA tag (H6908) were obtained from Santa Cruz Biotechnology and Sigma, respectively, and used according to the manufacturer's instructions. Peptides were synthesized and purified by reversed-phase HPLC by the Hartwell Center for Bioinformatics and Biotechnology at St. Jude. All peptides were C-terminally amidated. For helical hydrocarbon stapling, residues 5 and 9 of Ac-hUbc12¹⁻¹² were substituted with (S)-N-Fmoc-2-(4-pentenyl)alanine, and the staple generated as described (Schafmeister *et al.* (2000) *J Am Chem Soc* 122:5891-5892). Sequences of peptides used in this study are provided in Table 27.

Table 27. Sequences of peptides used in Example 1.

SEQ ID NO:	Peptide name	Sequence
15	yUbc12 ¹⁻²⁴	MLKLRQLQKKKQKENENSSSIQPN-NH2
87	Acetyl-yUbc12 ¹⁻²⁴	Ac-MLKLRQLQKKKQKENENSSSIQPN-
89	Acetyl-yUbc12 ²⁻²⁴	Ac-LKLRQLQKKKQKENENSSSIQPN-
7	hUbc12 ¹⁻²⁶	MIKLFSLKQQKKEESAGGTKGSSKK-
8	Acetyl-hUbc12 ¹⁻²⁶	Ac-
9	Acetyl-hUbc12 ²⁻²⁶	Ac-IKLFSLKQQKKEESAGGTKGSSKK-
90	Acetyl-hUbc12 ¹⁻¹²	Ac-MIKLFSLKQQKK-NH2
20	Acetyl-hUbc12 ¹⁻¹² (5:9Staple)	Ac-MIKLXSLKXQKK-NH2 X=2-(4-pentenyl)alanine-
91	Formyl-yUbc12 ¹⁻²⁴	Formyl- MLKLRQLQKKKQKENENSSSIQPN-NH2

10	Formyl-hUbc12 ¹⁻²⁶	Formyl- MIKLFSLKQQKKEEESAGGTKGSSKK-
92	Acetyl-hUbc12 ¹⁻¹⁵	Ac-MIKLFSLKQQKKEEE-NH2

Cell line generation and sample preparation

293T cells with stable expression of C-terminally Flag-HA tagged hUbc12 were generated using lentiviral-mediated transduction. Puromycin-resistant cells were grown in DMEM supplemented with L-glutamine, 10% FBS, and pen/strep. TAP-Nedd8 cells were generated as described previously (Bennett *et al.* (2010) *Cell* 143(6):951-965). Cells were harvested and lysed in buffer containing 50 mM Tris pH 7.8, 150 mM NaCl, 0.5% NP40, and protease inhibitors (Roche). Cell lysates were then immunoprecipitated with HA conjugated resin. Complexes were washed, eluted with HA peptide, precipitated with TCA, and digested with GluC (NEB).

Yeast strains used in this study

Yeast strains for expression and purification of C-terminally His6-tagged yUbc12 ("yUbc12^{AcMet}") and for monitoring the yNedd8 modification status of yCul1 were obtained from the EUROScarf collection (Brachmann *et al.* (1998) *Yeast* 14(2):115-132). YBS001 was constructed by PCR-mediated gene disruption using pUG72 as a template for generation of a loxP-His3-loxP Dcn1 deletion cassette. Correct integration was confirmed by PCR screening of purified genomic DNA utilizing two primers that anneal internal to the His3 cassette and 5' and 3' primers flanking the intended disruption gene. Marker rescue was achieved as described with pSH47 (a kind gift from Randy Schekman's lab). After counter-selection on 5-FOA, YBS001 was subsequently used to generate YBS002 and correct integration verified as described above. Yeast strains used in this study are described in Table 28.

Table 28. Yeast strains used in Example 1.

Strain name or Accession Number	Genotype	Reference
BY4742	MAT his3- 1 leu2- 0 lys2- 0 ura3- 0	*
Y14085	Same as BY4742 but also dcn1::kanMX4	*
Y15214	Same as BY4742 but also ubc12::kanMX4	*
Y13914	Same as BY4742 but also csn5::kanMX4	*
Y10976	Same as BY4742 but also ard1::kanMX4	*

Y13736	Same as BY4742 but also nat1::kanMX4	*
Y15546	Same as BY4742 but also nat3::kanMX4	*
Y15470	Same as BY4742 but also mak3::kanMX4	*
Y10294	Same as BY4742 but also mak10::kanMX4	*
Y13501	Same as BY4742 but also mak31::kanMX4	*
YBS001	Same as BY4742 but also dcn1::loxP	This study
YBS002	Same as BY4742 but also mak10::loxP-His3-loxP	This study

* Brachmann *et al.* (1998) *Yeast* 14:115-132

Yeast extract preparation for western blotting

For Figure 1, cells were inoculated into YPD media and grown overnight at 30°C. After OD600 measurements, cells were subcultured into fresh YPD media at 0.2 OD/ml and grown at 30°C until OD600 = 0.9-1.0. Cells were pelleted from 2 ml of media and extracts made by the NaOH/BME/TCA method as described (Knop *et al.* (1999) *Yeast* 15(10B):963-972). For western blots from cell extracts in Figure 7, cells were treated as described above except they were grown in SD-Leu 2% glucose for plasmid maintenance. Cell extracts corresponding to approximately 0.3 OD's of cells were run on 10% NuPAGE gels (Invitrogen). After western transfer, nitrocellulose membranes were probed with anti-Cdc53 (referred to here as yCul1) or anti-HA antibodies as indicated and visualized by enhanced chemiluminescence.

Mass spectrometry

Peptides generated from GluC digestion of sample proteins were desalted offline using C18 stage tips. Peptides were eluted from the stage tip, dried down using a speed vac, and resuspended in 10 µl 5% formic acid, 5% acetonitrile. Peptide mixtures were separated by in line reverse phase using an 18 cm X 150 µm (ID) column packed with C18 (MAGIC C18 5µm particle, 200 angstrom pore size) using a 50-minute 8%-26% acetonitrile gradient. MS/MS data was generated using an LTQ-Velos mass spectrometer (Thermo), a data-dependent top10 instrument method, and zoom scan for MS1 assignments. Data was acquired using CID with the normalized collision energy set to 35% with activation times of 10ms. MS/MS triggering thresholds were set to 2000 and a 30s dynamic exclusion was used with an exclusion list size of 500. All of the mass spec data was validated using an LTQ-Orbitrap (Thermo) instrument to obtain high mass accuracy MS1 assignments for all Ubc12 peptides. Resultant MS/MS spectra were searched using Sequest against a concatenated forward and reverse human IPI database (v3.6), or a *Saccharomyces cerevisiae* database as needed. Methionine oxidation (+15.99),

acetylation (+42.01), and the combined modification (+58.09) were set as dynamic modifications.

For intact mass analyses, protein was desalted using a reverse phase C8 Zip Tip and eluted in 50% acetonitrile, 2% formic acid. The eluent was ionized by static nanospray on Waters LCT Premier XE mass spectrometer using positive mode to obtain MaxEnt LC-TOF spectra.

Isothermal Titration Calorimetry (ITC)

Protein samples were buffer matched by desalting over a NAP-5 column (GE Healthcare) into 50 mM Hepes, 125 mM NaCl, 1 mM BME, pH 7.0 (ITC buffer). Peptides were dissolved by weight to a final concentration of 10 mM in ITC buffer and diluted further in ITC buffer as required for the experiments. Measurements were performed using a MicroCal ITC200. hDcn1^P or yDcn1^P were placed into the sample cell at a final concentration of 400 μ M at 18°C. The peptide ligands (4 mM) were constantly injected (1.5 μ l). The interval time between each injection was 3 minutes and the duration of each injection was 3 seconds. For titrations involving hE1 and yE1, proteins were placed into the sample cell at a final concentration of 25 μ M at 22°C. Peptide ligands (250 μ M) were constantly injected (2.5 μ l). The interval time between each injection was three minutes and the duration of each injection was 5 seconds. For hCul1^{ctd}-Rbx1:hDcn1^P binding, proteins were buffer matched by desalting over a NAP-5 column in 25 mM Tris, 0.1 M NaCl, 1 mM BME, pH 7.6. hCul1^{ctd}-Rbx1 was placed into the sample cell at a final concentration of 55 μ M at 22°C. hDcn1^P (550 μ M) was constantly injected (2.5 μ l). The interval time between each injection was 3 minutes and the duration of each injection was 5 seconds. Obtained spectra were evaluated using Origin (V 7.0) to determine heats of binding and *K_d* values.

Crystallography

Crystals were grown by the hanging-drop vapor-diffusion method. Crystals of yDcn1^P-Acetyl-yUbc12¹⁻²⁴ grew as plate clusters at room temperature in 19-22% PEG3350, 0.1 M Bis-Tris Propane, 0.2 M sodium/potassium tartrate, pH 8.5. Single crystals were obtained by streak seeding into 16% PEG 3350, 0.1 M Bis-Tris Propane, 0.2 M sodium/potassium tartrate, pH 7.9. The crystals were harvested from mother liquor supplemented with 25% glycerol prior to flash-freezing in liquid nitrogen. Reflection data were collected at NECAT ID-24-E at

the Advanced Photon Source. The crystals belong to space group C2221 with two yDcn1^P-Acetyl-yUbc12¹⁻²⁴ complexes in the asymmetric unit.

Crystals of hCul1^{WHB}-hDcn1^P-Acetyl-hUbc12¹⁻¹⁵ were grown at 4°C in 27% PEG1500, 0.1 M MIB buffer (Qiagen), pH 4.0. The crystals grew as multiple
 5 clusters. Single crystals of the complex were obtained by streak-seeding into 17% PEG1500, 0.1 M MIB pH 4.0. The crystals were soaked in step gradients for 1-5 minutes, with sequential soaks containing well solution supplemented with 10%, 20%, and 30% ethylene glycol prior to flash-freezing in liquid nitrogen. Reflection data were collected at beamline 8.2.2 at the Advanced Light Source. Even after
 10 seeding, reflections from data for both the yDcn1^P-Acetyl-yUbc12¹⁻²⁴ and hCul1^{WHB}-hDcn1^P-Acetyl-hUbc12¹⁻¹⁵ complexes were streaky.

Crystals of hCul1^{WHB}-hDcn1^P-Acetyl-hUbc12^{Ac1-12}(5,9 Staple) contained a selenomethionine version of the peptide used for convenience in crystallography that binds hDcn1^P with essentially the same K_d as the methionine-containing peptide
 15 (not shown), and were grown at 4 °C in 21% PEG3350, 0.2 M KCl. The crystals were soaked in step gradients for 1-5 minutes, with sequential soaks containing well solution supplemented with 10%, 20%, and 30% of a 50:50 mixture of glycerol:ethylene glycol prior to flash-freezing in liquid nitrogen. Reflection data were collected at SERCAT 22-ID beamline at the Advanced Photon Source. All
 20 reflection data were processed with HKL2000 (Otwinowski and Minor (1997) *Methods in Enzymology, Macromolecular Crystallography, part A* 276:307-326).

Phases for all structures were obtained by molecular replacement using PHASER (Storoni *et al.* (2004) *Acta Crystallogr D Biol Crystallogr* 60(Pt 3):432-438) using the following search models: (1) for yDcn1^P-Acetyl-yUbc12¹⁻²⁴ structure, 2
 25 copies of yDcn1^P (3); (2) for hCul1^{WHB}-hDcn1^P-Acetyl-hUbc12¹⁻¹⁵ structure, 2 copies each of (a) a model of hDcn1^P generated by Modeller (Eswar *et al.* (2006) *Curr Protoc Bioinformatics* Chapter 5, Unit 5 6) and (b) residues 707-776 from a prior structure of hCul1 (SEQ ID NO: 79) (Zheng *et al.* (2002) *Nature* 416(6882):703-709); (3) for hCul1^{WHB}-hDcn1^P-Acetyl-hUbc12^{Ac1-12}(5,9 Staple) structure, 2 copies of hCul1^{WHB}-hDcn1^P
 30 from the hCul1^{WHB}-hDcn1^P-Acetyl-hUbc12¹⁻¹⁵ structure. In all cases, the peptides were built manually, and general manual rebuilding was performed with COOT (Emsley *et al.* (2004) *Acta Crystallogr D Biol Crystallogr* 60(Pt12Pt1):2126-2132). Refinement was performed using Phenix (Adams *et al.* (2010) *Acta Crystallogr D Biol*

Crystallogr 66(Pt2):213-221) and Refmac (Murshudov *et al.* (1997) *Acta Crystallogr D Biol Crystallogr* D53:240-255). Details of Refinement are provided in Table 24.

Biochemical assays

Dcn1^P-mediated co-E3 activity was monitored using pulse-chase assays to
 5 exclusively monitor effects of acetylation on Nedd8 transfer to Cul1 without
 sensing earlier steps in the reaction. For the “pulse”, 10 μM of the indicated
 versions of yUbc12 were charged with [³²P]-yNedd8 (also referred to as [³²P]-Rub1)
 as described previously (Scott *et al.* (2010) *Mol Cell* 39(5):784-796), resulting in
 formation of a thioester-linked yUbc12—[³²P]-yNedd8 intermediate. 10 μM of
 10 the indicated versions of hUbc12 were charged similarly with [³²P]-hNedd8, for
 15 minutes at room temperature using 0.1 μM hE1, 15 μM [³²P]-hNedd8, in 50
 mM Hepes, 100 mM NaCl, 1.25 mM ATP, 2.5 mM MgCl₂, pH 7.5. Formation of
 a yUbc12—[³²P]-yNedd8 or hUbc12—[³²P]-hNedd8 intermediate was quenched
 with 50 mM EDTA on ice for 5 minutes. Other than in Fig. 3, where additional
 15 details are provided, chase reactions with hUbc12 and yUbc12 variants involved
 dilution of the Ubc12—[³²P]-Nedd8 thioester conjugate to 40 nM in 50 mM BisTris,
 100 mM NaCl, 50 mM EDTA, 0.5mg/ml BSA, pH 6.7. Chase reactions were
 initiated at 0°C by the addition of hCul1^{ctd}-hRbx1, yCul1^{C+}-yRbx1, hDcn1^P-hCul1^{ctd}-
 hRbx1, or yDcn1^P-yCul1^{C+}-yRbx1 at a final concentration of 125 nM. Aliquots
 20 were removed at the indicated times and quenched with 2X SDS-PAGE sample
 buffer. Reaction products were heated at 70°C for 1.5 minutes and separated on
 4-12% NuPAGE gels (Invitrogen). Dried gels were exposed, as indicated in the
 figure legends, to a Storm (GE) Phosphorimager screen or film (Kodak).

25 Example 2. DCUN1D(1-5) family members stimulate Ubc12^{AcMet} and Ubc2f^{AcMet} Nedd8 transfer to Cul1-5 family members.

Pulse-chase assays similar to those described in Example 1 were used to
 determine if the DCUN1D family members, DCUN1D1, DCUN1D2, DCUN1D3,
 DCUN1D4, and DCUN1D5 could stimulate Nedd8 transfer to cullin family members,
 30 Cul1, Cul2, Cul3, Cul4, and Cul5. Results are shown in Figures 8A and 8B.

Example 3. Peptide Binding Assays

The affinity of various peptides for hDcn1^P, hDcn2^P, hDcn3^P, hDcn4^P, and hDcn5^P was tested using isothermal titration calorimetry as described in Example 1 and is provided in Table 29.

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Table 29. Binding Kd (in μ M) of peptides for hDcn1^P, hDcn2^P, hDcn3^P, hDcn4^P, and hDcn5^P.

SEQ ID NO**:	Peptide	hDcn1 ^P	hDcn2 ^P	hDcn3 ^P	hDcn4 ^P	hDcn5 ^P
8	hUbc12 ^{Ac1-26}	1.5	0.735	10.8	10	8.6
7	hUbc12 ¹⁻²⁶	>200	-	-	-	-
9	hUbc12 ^{Ac2-26}	>150	-	-	-	-
10	hUbc12 ^{Formyl1-26}	9.7	-	-	-	-
11	hUbc12 ^{Ac1-Ac3-26}	4.6	-	-	-	-
12	hUbc12 ^{Ac1-12}	2.1	-	6.3	13.1	>30
17	hUbc12 ^{Ac1-12} (NorLeu)	5.6	-	-	-	-
93	hUbc12 ¹⁻¹² (NorLeu)	>400	-	-	-	-
18	hUbc12 ^{Ac1-12} (NorLeu) 5,9	0.440	-	-	-	-
19	hUbc12 ^{Ac1-12} (NorLeu) 6,10	0.8/1.2**	-	-	-	-
20	hUbc12 ^{Ac1-12} (Met) 5,9	0.153	-	-	-	-
21	hUbc12 ^{Ac1-12} (Met) SS 6,10	0.0018/0.187**	-	-	-	-
22	hUbc12 ^{Ac1-12}	0.34/1x10 ⁻⁶	-	-	-	-

SEQ ID NO*:	Peptide	hDen1 ^P	hDen2 ^P	hDen3 ^P	hDen4 ^P	hDen5 ^P
	(Met) RR 6,10	29**				
23	hUbc12 ^{Ac1-12} (SeMet) 5,9	0.182	-	-	-	-
24	hUbc12 ^{Ac1-12} (SeMet) 6,10	0.00093/0.361**	-	-	-	-
25	hUbc12 ^{Ac1-12} (Met) Oct:But 6,10	0.129	-	-	-	-
26	hUbc12 ^{Ac1-12} (Met) But:Oct 6,10	0.120	-	1.52	-	0.781
27	hUbc12 ^{Ac1-12} (Met) KE 5,9	1.06	-	-	-	-
94	hUbc12 ^{Ac1-12} (Met) KE 6,10	0.483	-	-	-	-
88	hUbe2f ^{Ac1-25}	>500	>400	2	>40	>30
14	hUbe2f ⁻²⁵	-	-	>400	-	-
87	yUbc12 ^{Ac1-24}	0.311	0.204	3.4	*1.2/13	2.4
88	yUbc12 ^{Ac1-12} (NorLeu)	0.628	0.793	-	-	-

SEQ ID NO*:	Peptide	hDen1 ^P	hDen2 ^P	hDen3 ^P	hDen4 ^P	hDen5 ^P
29	yUbc12 ^{AcI-12} (NorLeu) 5,9	0.418	0.515	-	-	-
30	yUbc12 ^{AcI-12} (NorLeu) 6,10	0.068	0.075	1.76	4.3	4.78
31	yUbc12 ^{AcI-12} (Met) 6,10	0.038/0.037**	-	-	-	-

* Each of the peptides had an amidated carboxy-terminus.

** The binding isotherm from the titration best fit a two-site model for binding, but the stoichiometry of binding is additive to approximately one.

Example 4. Peptide Inhibition.

Pulse chase assays were used to monitor the transfer of Nedd8 from Ubc12^{AcMet}~Nedd8 to Cull1^{CTD}-Rbx1 in the absence or presence of DCUN1D1^P and the indicated amounts of the peptide inhibitor Acetyl-yUbc12¹⁻¹²(6;10Staple). Results are
5 provided in Figures 9A and 9B.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same
10 extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. This application hereby incorporates by reference in its entirety U.S. Provisional Application No. 61/486,972, filed May 17, 2011.

Many modifications and other embodiments of the inventions set forth herein will
15 come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed
20 herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

THAT WHICH IS CLAIMED:

1. An isolated peptide selected from the group consisting of:
 - a) a peptide having the sequence set forth in SEQ ID NO: 7, 12, 13, 14, 15, or 16 or a fragment thereof; and
 - 5 b) a peptide having at least 75% sequence identity to SEQ ID NO: 7, 12, 13, 14, 15, or 16;wherein the nitrogen atom of the amino terminus of said isolated peptide is covalently bound to at least one -R group, wherein said -R group is uncharged under physiological conditions, provided that said -R group is not an acetyl group, and wherein
10 said isolated peptide binds to an E2-binding pocket of a NEDD8 co-E3 protein.
2. The isolated peptide of claim 1, wherein said E2-binding pocket comprises amino acid residues selected from the group consisting of:
 - a) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a
15 leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO:
20 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a
25 leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, and a leucine corresponding to position 193 of SEQ ID NO: 48;
 - b) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a
30 leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO:

48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, a leucine corresponding to position 193 of SEQ ID NO: 48, an asparagine corresponding to position 84 of SEQ ID NO: 48; an aspartic acid corresponding to position 89 of SEQ ID NO: 48, an aspartic acid corresponding to position 91 of SEQ ID NO: 48, a glycine corresponding to position 101 of SEQ ID NO: 48, a tyrosine corresponding to position 102 of SEQ ID NO: 48, an asparagine corresponding to position 103 of SEQ ID NO: 48, and a glutamic acid corresponding to position 186 of SEQ ID NO: 48;

15 c) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, and a leucine corresponding to position 184 of SEQ ID NO: 33;

 d) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID

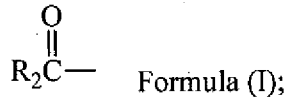
- NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, a leucine corresponding to position 184 of SEQ ID NO: 33, an aspartic acid corresponding to position 84 of SEQ ID NO: 33, an alanine corresponding to position 94 of SEQ ID NO: 33, a leucine corresponding to position 95 of SEQ ID NO: 33, and an aspartic acid corresponding to position 96 of SEQ ID NO: 33;
- 10 e) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, and a leucine corresponding to position 211 of SEQ ID NO: 39; and
- 20 f) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, a
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leucine corresponding to position 211 of SEQ ID NO: 39; a glutamic acid corresponding to position 109 of SEQ ID NO: 39; a valine corresponding to position 120 of SEQ ID NO: 39; and an aspartic acid at position 121 of SEQ ID NO: 39.

- 5 3. The isolated peptide of claim 2, wherein said E2-binding pocket is selected from the group consisting of:
- a) an E2-binding pocket having the atomic coordinates of Table 2, 3, 8, 9, 10, 11, 18, or 19;
 - b) a structural variant of the E2-binding pocket of part a), wherein said
10 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Table 2, 3, 8, 9, 10, 11, 18, or 19 of not more than 2 Å;
 - c) an E2-binding pocket having the atomic coordinates of Tables 2 and 5;
 - d) a structural variant of the E2-binding pocket of part c), wherein said
15 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 2 and 5 of not more than 2 Å;
 - e) an E2-binding pocket having the atomic coordinates of Tables 3 and 6;
 - f) a structural variant of the E2-binding pocket of part e), wherein said
20 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 3 and 6 of not more than 2 Å;
 - g) an E2-binding pocket having the atomic coordinates of Tables 8 and 13;
 - h) a structural variant of the E2-binding pocket of part g), wherein said
25 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 8 and 13 of not more than 2 Å;
 - i) an E2-binding pocket having the atomic coordinates of Tables 9 and 14;
 - j) a structural variant of the E2-binding pocket of part i), wherein said
30 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 9 and 14 of not more than 2 Å;

- k) an E2-binding pocket having the atomic coordinates of Tables 10 and 15;
- l) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 10 and 15 of not more than 2 Å;
- m) an E2-binding pocket having the atomic coordinates of Tables 11 and 16;
- n) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 11 and 16 of not more than 2 Å;
- o) an E2-binding pocket having the atomic coordinates of Tables 18 and 21;
- p) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 18 and 21 of not more than 2 Å;
- q) an E2-binding pocket having the atomic coordinates of Tables 19 and 22; and
- r) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 19 and 22 of not more than 2 Å.
4. The isolated peptide of any one of claims 1-3, wherein said -R group is a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.
5. The isolated peptide of any one of claims 1-3, wherein said -R group is a formyl group.

6. The isolated peptide of any one of claims 1-3, wherein said -R group has Formula (I):



wherein -R₂ group is a hydrophobic moiety.

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7. The isolated peptide of claim 6, wherein said -R₂ group is alkyl or aryl.

8. The isolated peptide of claim 1, wherein said peptide has the sequence set forth in SEQ ID NO: 10, 11, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 87, 88, 89, 90, 91, 92, 93, or 94.

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9. An isolated peptide selected from the group consisting of:

a) a peptide consisting of the sequence set forth in SEQ ID NO: 7, 12, 13, 14, 15, or 16 or a fragment thereof; and

b) a peptide having at least 75% sequence identity to SEQ ID NO: 7, 12, 13, 14, 15 or 16, wherein the peptide binds to an E2-binding pocket of a NEDD8 co-E3 protein;

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wherein the nitrogen atom of the amino terminus of said isolated peptide is covalently bound to at least one -R group, wherein said -R group is uncharged under physiological conditions, and wherein said isolated peptide binds to an E2-binding pocket of a NEDD8 co-E3 protein.

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10. The isolated peptide of claim 9, wherein said E2-binding pocket comprises amino acid residues selected from the group consisting of:

a) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID

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NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, and a leucine corresponding to position 193 of SEQ ID NO: 48;

b) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, a leucine corresponding to position 193 of SEQ ID NO: 48, an asparagine corresponding to position 84 of SEQ ID NO: 48; an aspartic acid corresponding to position 89 of SEQ ID NO: 48, an aspartic acid corresponding to position 91 of SEQ ID NO: 48, a glycine corresponding to position 101 of SEQ ID NO: 48, a tyrosine corresponding to position 102 of SEQ ID NO: 48, an asparagine corresponding to position 103 of SEQ ID NO: 48, and a glutamic acid corresponding to position 186 of SEQ ID NO: 48;

c) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID

- NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, and a leucine corresponding to position 184 of SEQ ID NO: 33;
- d) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, a leucine corresponding to position 184 of SEQ ID NO: 33, an aspartic acid corresponding to position 84 of SEQ ID NO: 33, an alanine corresponding to position 94 of SEQ ID NO: 33, a leucine corresponding to position 95 of SEQ ID NO: 33, and an aspartic acid corresponding to position 96 of SEQ ID NO: 33;
- e) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position

207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, and a leucine corresponding to position 211 of SEQ ID NO: 39; and

- f) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid
5 corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine
10 corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, a
15 leucine corresponding to position 211 of SEQ ID NO: 39; a glutamic acid corresponding to position 109 of SEQ ID NO: 39; a valine corresponding to position 120 of SEQ ID NO: 39; and an aspartic acid at position 121 of SEQ ID NO: 39.

11. The isolated peptide of claim 10, wherein said E2-binding pocket is
20 selected from the group consisting of:
- a) an E2-binding pocket having the atomic coordinates of Table 2, 3, 8, 9, 10, 11, 18, or 19;
- b) a structural variant of the E2-binding pocket of part a), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the
25 amino acids of Table 2, 3, 8, 9, 10, 11, 18, or 19 of not more than 2 Å;
- c) an E2-binding pocket having the atomic coordinates of Tables 2 and 5;
- d) a structural variant of the E2-binding pocket of part c), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the
30 amino acids of Tables 2 and 5 of not more than 2 Å;
- e) an E2-binding pocket having the atomic coordinates of Tables 3 and 6;

- f) a structural variant of the E2-binding pocket of part e), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 3 and 6 of not more than 2 Å;
- g) an E2-binding pocket having the atomic coordinates of Tables 8
5 and 13;
- h) a structural variant of the E2-binding pocket of part g), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 8 and 13 of not more than 2 Å;
- i) an E2-binding pocket having the atomic coordinates of Tables 9
10 and 14;
- j) a structural variant of the E2-binding pocket of part i), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 9 and 14 of not more than 2 Å;
- k) an E2-binding pocket having the atomic coordinates of Tables 10
15 and 15;
- l) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 10 and 15 of not more than 2 Å;
- m) an E2-binding pocket having the atomic coordinates of Tables 11
20 and 16;
- n) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 11 and 16 of not more than 2 Å;
- o) an E2-binding pocket having the atomic coordinates of Tables 18
25 and 21;
- p) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 18 and 21 of not more than 2 Å;
- q) an E2-binding pocket having the atomic coordinates of Tables 19
30 and 22; and

r) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 19 and 22 of not more than 2 Å.

5 12. The isolated peptide of any one of claims 9-11, wherein said -R group is a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

13. The isolated peptide of any one of claims 9-11, wherein said -R group is a formyl group or an acetyl group.

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14. The isolated peptide of any one of claims 9-11, wherein said -R group has Formula (I):



wherein -R₂ group is a hydrophobic moiety.

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15. The isolated peptide of claim 14, wherein said -R₂ group is alkyl or aryl.

16. The isolated peptide of any one of claims 1-7 and 9-15, wherein the methionine corresponding to position 1 in SEQ ID NO: 7, 12, 13, 14, 15, or 16 is substituted with another amino acid residue having an unbranched hydrophobic side chain.

20

17. The isolated peptide of claim 16, wherein said unbranched hydrophobic side chain is an alkyl, alkenyl, or alkynyl.

25 18. The isolated peptide of claim 16, wherein the methionine corresponding to position 1 in SEQ ID NO: 7, 12, 13, 14, 15, or 16 is substituted with a selenomethionine or a norleucine.

30 19. The isolated peptide of any one of claims 1-18, wherein said peptide has an alpha-helical structure.

20. The isolated peptide of any one of claims 1-7 and 9-18, wherein said peptide comprises a hydrophobic staple.

21. The isolated peptide of claim 20, wherein amino acid residues at positions
5 X and X+4 are substituted with hydrophobic staple-forming amino acid residues, wherein X is not the first amino acid residue of the peptide.

22. The isolated peptide of claim 21, wherein said hydrophobic staple-forming amino acid residues are selected from the group consisting of: (S)-2-(4-pentenyl)alanine,
10 (R)-2-(4-pentenyl)alanine, (S)-2-(3-butenyl)alanine, (S)-2-(7-octenyl)alanine, lysine, and glutamic acid.

23. The isolated peptide of any one of claims 1-22, wherein the carboxy terminus of said peptide is amidated.
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24. The isolated peptide of any one of claims 1-23, wherein said NEDD8 co-E3 protein is Dcn1, DCUN1D1, DCUN1D2, DCUN1D3, DCUN1D4, or DCUN1D5.

25. The isolated peptide of any one of claims 1-24, wherein said isolated
20 peptide inhibits co-E3 activity of said NEDD8 co-E3 protein.

26. The isolated peptide of claim 25, wherein said isolated peptide inhibits the neddylation of a cullin.

25 27. A pharmaceutical composition comprising a peptide and a pharmaceutically acceptable carrier, wherein the peptide is selected from the group consisting of:

- a) a peptide having the sequence set forth in SEQ ID NO: 7, 12, 13, 14, 15, or 16 or a fragment thereof; and
- 30 b) a peptide having at least 75% sequence identity to SEQ ID NO: 7, 12, 13, 14, 15, or 16;

wherein the nitrogen atom of the amino terminus of said isolated peptide is covalently bound to at least one -R group, provided that said -R group is uncharged under physiological conditions; and wherein said peptide binds to an E2-binding pocket of a NEDD8 co-E3 protein and inhibits co-E3 activity of said NEDD8 co-E3 protein.

5

28. The pharmaceutical composition of claim 27, wherein said E2-binding pocket comprises amino acid residues selected from the group consisting of:

a) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, and a leucine corresponding to position 193 of SEQ ID NO: 48;

b) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a

- leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, a leucine corresponding to position 193 of SEQ ID NO: 48, an asparagine corresponding to position 84 of SEQ ID NO: 48; an aspartic acid corresponding to position 89 of SEQ ID NO: 48, an aspartic acid corresponding to position 91 of SEQ ID NO: 48, a glycine corresponding to position 101 of SEQ ID NO: 48, a tyrosine corresponding to position 102 of SEQ ID NO: 48, an asparagine corresponding to position 103 of SEQ ID NO: 48, and a glutamic acid corresponding to position 186 of SEQ ID NO: 48;
- 10 c) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, 15 a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, and a leucine corresponding to position 184 of SEQ ID NO: 33;
- 20 d) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, 25 a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 30

of SEQ ID NO: 33, a leucine corresponding to position 184 of SEQ ID NO: 33, an aspartic acid corresponding to position 84 of SEQ ID NO: 33, an alanine corresponding to position 94 of SEQ ID NO: 33, a leucine corresponding to position 95 of SEQ ID NO: 33, and an aspartic acid corresponding to position 96 of SEQ ID NO: 33;

5 e) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, and a leucine corresponding to position 211 of SEQ ID NO: 39; and

 f) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, a leucine corresponding to position 211 of SEQ ID NO: 39; a glutamic acid corresponding to position 109 of SEQ ID NO: 39; a valine corresponding to position 120 of SEQ ID NO: 39; and an aspartic acid at position 121 of SEQ ID NO: 39.

29. The pharmaceutical composition of claim 28, wherein said E2-binding pocket is selected from the group consisting of:

- a) an E2-binding pocket having the atomic coordinates of Table 2, 3, 8, 9, 10, 11, 18, or 19;
- 5 b) a structural variant of the E2-binding pocket of part a), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Table 2, 3, 8, 9, 10, 11, 18, or 19 of not more than 2 Å;
- c) an E2-binding pocket having the atomic coordinates of Tables 2 and 5;
- 10 d) a structural variant of the E2-binding pocket of part c), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 2 and 5 of not more than 2 Å;
- e) an E2-binding pocket having the atomic coordinates of Tables 3 and 6;
- 15 f) a structural variant of the E2-binding pocket of part e), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 3 and 6 of not more than 2 Å;
- g) an E2-binding pocket having the atomic coordinates of Tables 8 and 13;
- 20 h) a structural variant of the E2-binding pocket of part g), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 8 and 13 of not more than 2 Å;
- i) an E2-binding pocket having the atomic coordinates of Tables 9 and 14;
- 25 j) a structural variant of the E2-binding pocket of part i), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 9 and 14 of not more than 2 Å;
- k) an E2-binding pocket having the atomic coordinates of Tables 10 and 15;

l) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 10 and 15 of not more than 2 Å;

5 m) an E2-binding pocket having the atomic coordinates of Tables 11 and 16;

n) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 11 and 16 of not more than 2 Å;

10 o) an E2-binding pocket having the atomic coordinates of Tables 18 and 21;

p) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 18 and 21 of not more than 2 Å;

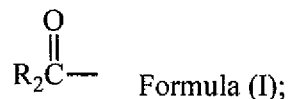
15 q) an E2-binding pocket having the atomic coordinates of Tables 19 and 22; and

r) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 19 and 22 of not more than 2 Å.

20 30. The pharmaceutical composition of any one of claims 27-29, wherein said -R group is a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

25 31. The pharmaceutical composition of any one of claims 27-29, wherein said -R group is a formyl group or an acetyl group.

32. The pharmaceutical composition of any one of claims 27-29, wherein said -R group has Formula (I):



wherein -R₂ group is a hydrophobic moiety.

30

33. The pharmaceutical composition of claim 27, wherein said peptide has the sequence set forth in SEQ ID NO: 10, 11, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 87, 88, 89, 90, 91, 92, 93, or 94.

5 34. The pharmaceutical composition of any one of claims 27-32, wherein the methionine corresponding to position 1 in SEQ ID NO: 7, 12, 13, 14, 15, or 16 is substituted with another amino acid residue having an unbranched hydrophobic side chain.

10 35. The pharmaceutical composition of claim 34, wherein said hydrophobic side chain is an alkyl, alkenyl, or alkynyl.

 36. The pharmaceutical composition of claim 35, wherein the methionine corresponding to position 1 in SEQ ID NO: 7, 12, 13, 14, 15, or 16 is substituted with a selenomethionine or a norleucine.

15 37. The pharmaceutical composition of any one of claims 27-36, wherein said peptide has an alpha-helical structure.

20 38. The pharmaceutical composition of any one of claims 27-32 and 34-36, wherein said peptide comprises a hydrophobic staple.

 39. The pharmaceutical composition of claim 38, wherein amino acid residues at positions X and X+4 are substituted with hydrophobic staple-forming amino acid residues, wherein X is not the first amino acid residue of the peptide.

25 40. The pharmaceutical composition of claim 39, wherein said staple-forming amino acid residues are selected from the group consisting of: (S)-2-(4-pentenyl)alanine, (R)-2(4-pentenyl)alanine, (S)-2-(3-butenyl)alanine, (S)-2-(7-octenyl)alanine, lysine, and glutamic acid.

30 41. The pharmaceutical composition of any one of claims 27-40, wherein the carboxy terminus of said peptide is amidated.

42. The pharmaceutical composition of any one of claims 27-41, wherein said isolated peptide inhibits the neddylation of a cullin.

43. The pharmaceutical composition of any one of claims 27-42, wherein said NEDD8 co-E3 protein is Dcn1, DCUN1D1, DCUN1D2, DCUN1D3, DCUN1D4, or DCUN1D5.

44. A small molecule that binds to an E2-binding pocket of a NEDD8 co-E3 protein.

10

45. The small molecule of claim 44, wherein said E2-binding pocket comprises amino acid residues selected from the group consisting of:

a) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, and a leucine corresponding to position 193 of SEQ ID NO: 48;

b) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding

to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a
5 leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, a leucine corresponding to position 193 of SEQ ID NO: 48, an asparagine corresponding to position 84 of SEQ ID NO: 48; an aspartic acid corresponding to position 89 of SEQ ID NO: 48, an
10 aspartic acid corresponding to position 91 of SEQ ID NO: 48, a glycine corresponding to position 101 of SEQ ID NO: 48, a tyrosine corresponding to position 102 of SEQ ID NO: 48, an asparagine corresponding to position 103 of SEQ ID NO: 48, and a glutamic acid corresponding to position 186 of SEQ ID NO: 48;

c) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an
15 isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to
20 position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine
25 corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, and a leucine corresponding to position 184 of SEQ ID NO: 33;

d) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an
isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33,
30 an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine

corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, a leucine corresponding to position 184 of SEQ ID NO: 33, an aspartic acid corresponding to position 84 of SEQ ID NO: 33, an alanine corresponding to position 94 of SEQ ID NO: 33, a leucine corresponding to position 95 of SEQ ID NO: 33, and an aspartic acid corresponding to position 96 of SEQ ID NO: 33;

e) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, and a leucine corresponding to position 211 of SEQ ID NO: 39; and

f) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, a

leucine corresponding to position 211 of SEQ ID NO: 39; a glutamic acid corresponding to position 109 of SEQ ID NO: 39; a valine corresponding to position 120 of SEQ ID NO: 39; and an aspartic acid at position 121 of SEQ ID NO: 39.

- 5 46. The small molecule of claim 45, wherein said E2-binding pocket is selected from the group consisting of:
- a) an E2-binding pocket having the atomic coordinates of Table 2, 3, 8, 9, 10, 11, 18, or 19;
 - b) a structural variant of the E2-binding pocket of part a), wherein said
10 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Table 2, 3, 8, 9, 10, 11, 18, or 19 of not more than 2 Å;
 - c) an E2-binding pocket having the atomic coordinates of Tables 2 and 5;
 - d) a structural variant of the E2-binding pocket of part c), wherein said
15 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 2 and 5 of not more than 2 Å;
 - e) an E2-binding pocket having the atomic coordinates of Tables 3 and 6;
 - f) a structural variant of the E2-binding pocket of part e), wherein said
20 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 3 and 6 of not more than 2 Å;
 - g) an E2-binding pocket having the atomic coordinates of Tables 8 and 13;
 - h) a structural variant of the E2-binding pocket of part g), wherein said
25 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 8 and 13 of not more than 2 Å;
 - i) an E2-binding pocket having the atomic coordinates of Tables 9 and 14;
 - j) a structural variant of the E2-binding pocket of part i), wherein said
30 structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 9 and 14 of not more than 2 Å;

- k) an E2-binding pocket having the atomic coordinates of Tables 10 and 15;
- l) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 10 and 15 of not more than 2 Å;
- m) an E2-binding pocket having the atomic coordinates of Tables 11 and 16;
- n) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 11 and 16 of not more than 2 Å;
- o) an E2-binding pocket having the atomic coordinates of Tables 18 and 21;
- p) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 18 and 21 of not more than 2 Å;
- q) an E2-binding pocket having the atomic coordinates of Tables 19 and 22; and
- r) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 19 and 22 of not more than 2 Å.

47. The small molecule of any one of claims 44-46, wherein said NEDD8 co-E3 protein is Dcn1, DCUN1D1, DCUN1D2, DCUN1D3, DCUN1D4, or DCUN1D5.

48. The small molecule of any one of claims 44-47, wherein said small molecule inhibits co-E3 activity of said NEDD8 co-E3 protein.

49. The small molecule of claim 48, wherein said isolated peptide inhibits the neddylation of a cullin.

50. A pharmaceutical composition comprising the small molecule of claim 48 or 49 and a pharmaceutically acceptable carrier.

51. A method for inhibiting co-E3 activity in a sample, said method comprising
5 contacting the sample with the peptide of claim 25 or 26, the small molecule of claim 48 or 49, or the pharmaceutical composition of any one of claims 27-43 and 50.

52. A method for inhibiting cell growth, said method comprising introducing
into said cell the peptide of claim 25 or 26, the small molecule of claim 48 or 49, or the
10 pharmaceutical composition of any one of claims 27-43 and 50.

53. A method for treating a cancer or an inflammatory disorder, said method
comprising administering a therapeutically effective amount of the peptide of claim 25 or
26, the small molecule of claim 48 or 49, or the pharmaceutical composition of any one of
15 claims 27-43 and 50 to a subject in need thereof.

54. The method of claim 53, wherein said cancer is a squamous cell carcinoma.

55. The method of claim 53, wherein said cancer is selected from the group
20 consisting of lung cancer, head and neck cancer, esophageal cancer, tongue cancer,
cancers of the urinary tract, cervical cancer, vulval cancer, vaginal cancer, ovarian cancer,
and oral tongue squamous cell carcinoma

56. A method for treating a pathogenic infection, said method comprising
25 administering a therapeutically effective amount of the peptide of claim 25 or 26, the small
molecule of claim 48 or 49, or the pharmaceutical composition of any one of claims 27-43
and 50 to a subject in need thereof.

57. The method of claim 56, wherein said pathogenic infection is a fungal
30 infection.

58. A method for identifying compounds that bind to the E2-binding pocket in a NEDD8 co-E3 protein, said method comprising comparing the three-dimensional structure of a test compound and a three-dimensional structure of the E2-binding pocket to determine if at least one atom of said test compound will interact with at least one atom of said E2-binding pocket, wherein said three-dimensional structure of the E2-binding pocket is selected from the group consisting of:
- a) an E2-binding pocket having the atomic coordinates of Table 2, 3, 8, 9, 10, 11, 18, or 19;
 - b) a structural variant of the E2-binding pocket of part a), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Table 2, 3, 8, 9, 10, 11, 18, or 19 of not more than 2 Å;
 - c) an E2-binding pocket having the atomic coordinates of Tables 2 and 5;
 - d) a structural variant of the E2-binding pocket of part c), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 2 and 5 of not more than 2 Å;
 - e) an E2-binding pocket having the atomic coordinates of Tables 3 and 6;
 - f) a structural variant of the E2-binding pocket of part e), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 3 and 6 of not more than 2 Å;
 - g) an E2-binding pocket having the atomic coordinates of Tables 8 and 13;
 - h) a structural variant of the E2-binding pocket of part g), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 8 and 13 of not more than 2 Å;
 - i) an E2-binding pocket having the atomic coordinates of Tables 9 and 14;
 - j) a structural variant of the E2-binding pocket of part i), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 9 and 14 of not more than 2 Å;

- k) an E2-binding pocket having the atomic coordinates of Tables 10 and 15;
- l) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 10 and 15 of not more than 2 Å;
- m) an E2-binding pocket having the atomic coordinates of Tables 11 and 16;
- n) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 11 and 16 of not more than 2 Å;
- o) an E2-binding pocket having the atomic coordinates of Tables 18 and 21;
- p) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 18 and 21 of not more than 2 Å;
- q) an E2-binding pocket having the atomic coordinates of Tables 19 and 22; and
- r) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 19 and 22 of not more than 2 Å.

59. The method of claim 58, wherein said method further comprises producing said compound.

60. The method of claim 59, wherein said method further comprises assaying the ability of said compound to bind to a NEDD8 co-E3 protein.

61. The method of claim 59 or 60, wherein said method further comprises assaying the ability of said compound to inhibit the co-E3 activity of said NEDD8 co-E3 protein.

62. A method for identifying compounds that bind to an E2-binding pocket in a NEDD8 co-E3 protein, said method comprising contacting a NEDD8 co-E3 protein with a test compound and determining if said test compound binds to the E2-binding pocket, wherein said E2-binding pocket comprises amino acid residues selected from the group consisting of:

- 5 a) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid
10 corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid
15 corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, and a leucine
20 corresponding to position 193 of SEQ ID NO: 48;
- b) an isoleucine corresponding to position 90 of SEQ ID NO: 48, a leucine corresponding to position 93 of SEQ ID NO: 48, a valine corresponding to position 94 of SEQ ID NO: 48, an isoleucine corresponding to position 97 of SEQ ID NO: 48, a leucine corresponding to position 104 of SEQ ID NO: 48, a glutamic acid
25 corresponding to position 105 of SEQ ID NO: 48, an aspartic acid corresponding to position 106 of SEQ ID NO: 48, a leucine corresponding to position 107 of SEQ ID NO: 48, a threonine corresponding to position 109 of SEQ ID NO: 48, a leucine corresponding to position 110 of SEQ ID NO: 48, an alanine corresponding to position 113 of SEQ ID NO: 48, a leucine corresponding to position 121 of SEQ ID NO: 48, a glutamic acid
30 corresponding to position 122 of SEQ ID NO: 48, a leucine corresponding to position 173 of SEQ ID NO: 48, an isoleucine corresponding to position 174 of SEQ ID NO: 48, a leucine corresponding to position 175 of SEQ ID NO: 48, an aspartic acid corresponding to position 176 of SEQ ID NO: 48, a glutamine corresponding to position 189 of SEQ ID NO: 48, a tyrosine corresponding to position 190 of SEQ ID NO: 48, a leucine

corresponding to position 193 of SEQ ID NO: 48, an asparagine corresponding to position 84 of SEQ ID NO: 48; an aspartic acid corresponding to position 89 of SEQ ID NO: 48, an aspartic acid corresponding to position 91 of SEQ ID NO: 48, a glycine corresponding to position 101 of SEQ ID NO: 48, a tyrosine corresponding to position 102 of SEQ ID NO: 48, an asparagine corresponding to position 103 of SEQ ID NO: 48, and a glutamic acid corresponding to position 186 of SEQ ID NO: 48;

c) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, and a leucine corresponding to position 184 of SEQ ID NO: 33;

d) an isoleucine corresponding to position 83 of SEQ ID NO: 33, an isoleucine corresponding to position 86 of SEQ ID NO: 33, a glutamine corresponding to position 87 of SEQ ID NO: 33, a proline corresponding to position 97 of SEQ ID NO: 33, an alanine corresponding to position 98 of SEQ ID NO: 33, a serine corresponding to position 99 of SEQ ID NO: 33, a valine corresponding to position 102 of SEQ ID NO: 33, a leucine corresponding to position 103 of SEQ ID NO: 33, an alanine corresponding to position 106 of SEQ ID NO: 33, a glutamine corresponding to position 114 of SEQ ID NO: 33, a cysteine corresponding to position 115 of SEQ ID NO: 33, a phenylalanine corresponding to position 117 of SEQ ID NO: 33, a phenylalanine corresponding to position 164 of SEQ ID NO: 33, an asparagine corresponding to position 167 of SEQ ID NO: 33, a methionine corresponding to position 177 of SEQ ID NO: 33, an alanine corresponding to position 180 of SEQ ID NO: 33, a tyrosine corresponding to position 181 of SEQ ID NO: 33, a leucine corresponding to position 184 of SEQ ID NO: 33, an aspartic acid corresponding to position 84 of SEQ ID NO: 33, an alanine corresponding to position

94 of SEQ ID NO: 33, a leucine corresponding to position 95 of SEQ ID NO: 33, and an aspartic acid corresponding to position 96 of SEQ ID NO: 33;

- e) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, and a leucine corresponding to position 211 of SEQ ID NO: 39; and

- f) a glutamic acid corresponding to position 108 of SEQ ID NO: 39, a methionine corresponding to position 111 of SEQ ID NO: 39, a glutamic acid corresponding to position 112 of SEQ ID NO: 39, a proline corresponding to position 122 of SEQ ID NO: 39, a threonine corresponding to position 123 of SEQ ID NO: 39, a glutamic acid corresponding to position 124 of SEQ ID NO: 39, a valine corresponding to position 127 of SEQ ID NO: 39, a leucine corresponding to position 128 of SEQ ID NO: 39, an alanine corresponding to position 131 of SEQ ID NO: 39, a methionine corresponding to position 139 of SEQ ID NO: 39, a cysteine corresponding to position 140 of SEQ ID NO: 39, a phenylalanine corresponding to position 142 of SEQ ID NO: 39, a phenylalanine corresponding to position 189 of SEQ ID NO: 39, an isoleucine corresponding to position 204 of SEQ ID NO: 39, an alanine corresponding to position 207 of SEQ ID NO: 39, a leucine corresponding to position 208 of SEQ ID NO: 39, a leucine corresponding to position 211 of SEQ ID NO: 39; a glutamic acid corresponding to position 109 of SEQ ID NO: 39; a valine corresponding to position 120 of SEQ ID NO: 39; and an aspartic acid at position 121 of SEQ ID NO: 39.

63. The method of claim 62, wherein said E2-binding pocket is selected from the group consisting of:

- a) an E2-binding pocket having the atomic coordinates of Table 2, 3, 8, 9, 10, 11, 18, or 19;
- 5 b) a structural variant of the E2-binding pocket of part a), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Table 2, 3, 8, 9, 10, 11, 18, or 19 of not more than 2 Å;
- c) an E2-binding pocket having the atomic coordinates of Tables 2 and 5;
- 10 d) a structural variant of the E2-binding pocket of part c), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 2 and 5 of not more than 2 Å;
- e) an E2-binding pocket having the atomic coordinates of Tables 3 and 6;
- 15 f) a structural variant of the E2-binding pocket of part e), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 3 and 6 of not more than 2 Å;
- g) an E2-binding pocket having the atomic coordinates of Tables 8 and 13;
- 20 h) a structural variant of the E2-binding pocket of part g), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 8 and 13 of not more than 2 Å;
- i) an E2-binding pocket having the atomic coordinates of Tables 9 and 14;
- 25 j) a structural variant of the E2-binding pocket of part i), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 9 and 14 of not more than 2 Å;
- k) an E2-binding pocket having the atomic coordinates of Tables 10 and 15;

- l) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 10 and 15 of not more than 2 Å;
- 5 m) an E2-binding pocket having the atomic coordinates of Tables 11 and 16;
- n) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 11 and 16 of not more than 2 Å;
- 10 o) an E2-binding pocket having the atomic coordinates of Tables 18 and 21;
- p) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 18 and 21 of not more than 2 Å;
- 15 q) an E2-binding pocket having the atomic coordinates of Tables 19 and 22; and
- r) a structural variant of the E2-binding pocket of part k), wherein said structural variant comprises a root mean square deviation from the back-bone atoms of the amino acids of Tables 19 and 22 of not more than 2 Å.
- 20 64. The method of claim 62 or 63, wherein determining if said test compound binds to the E2-binding pocket comprises performing a competitive binding assay using a peptide according to any one of claims 1-26.

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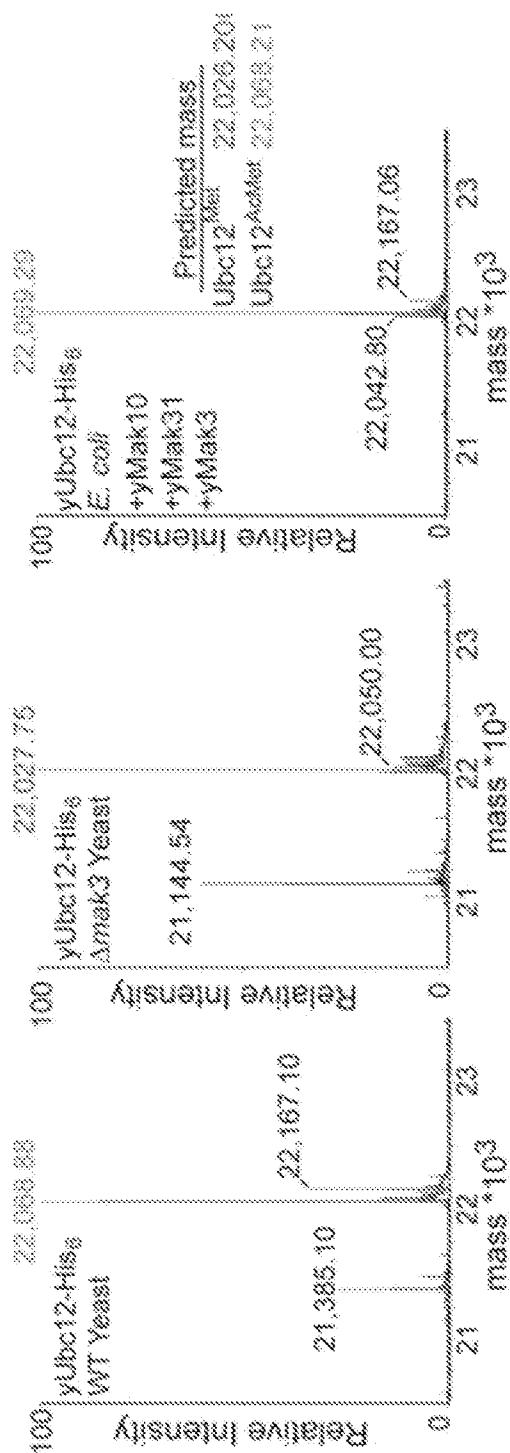


FIG. 1A

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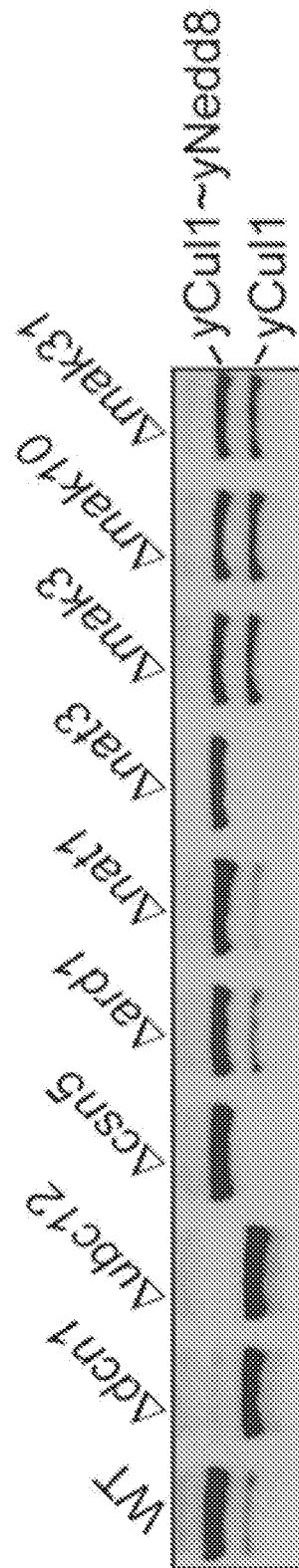


FIG. 1B

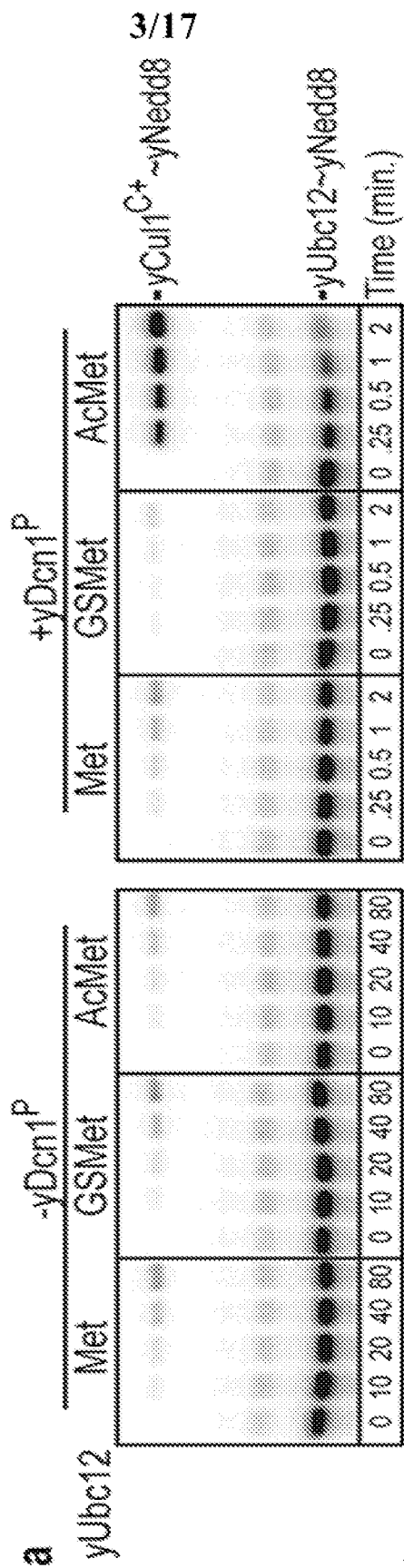


FIG. 2A

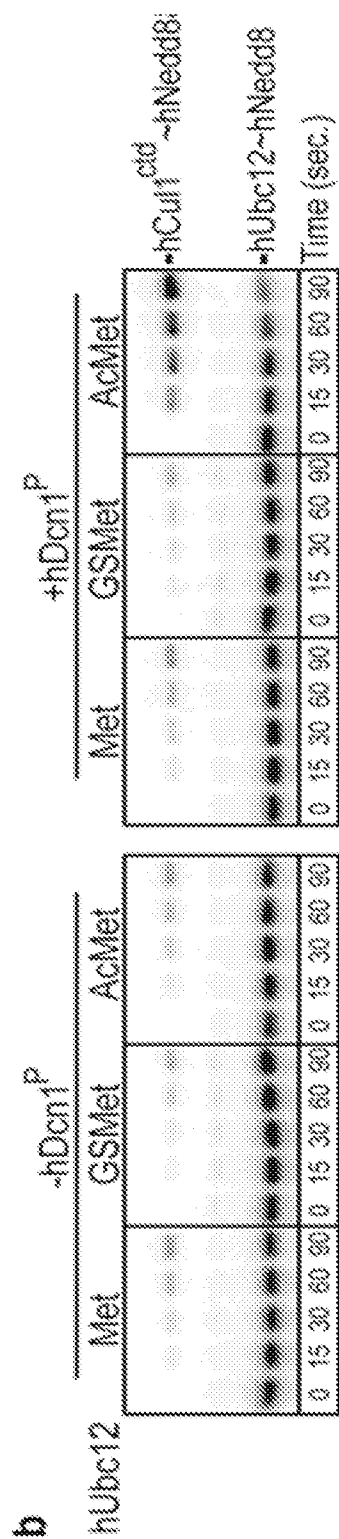


FIG. 2B

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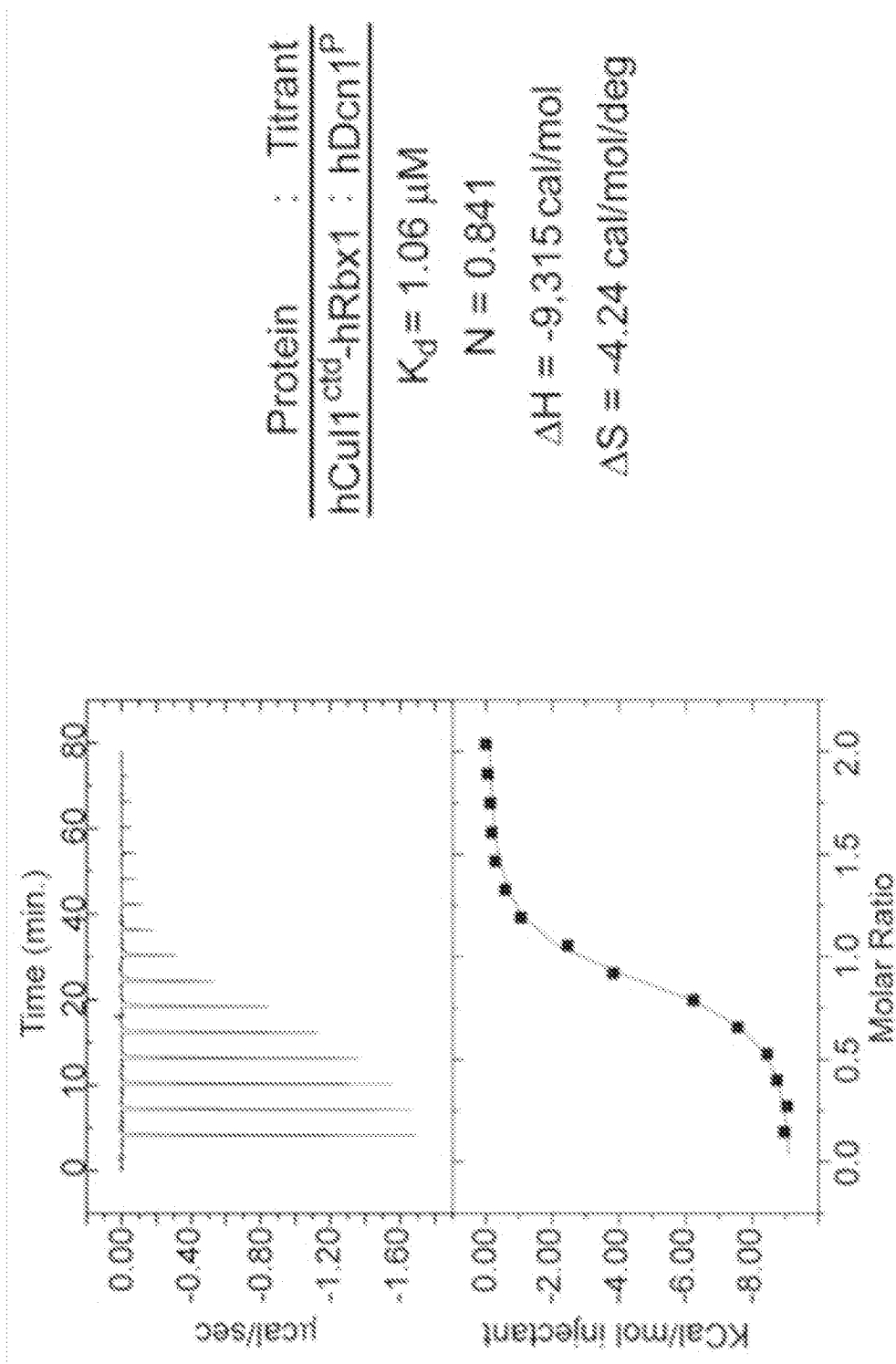


FIG. 3A

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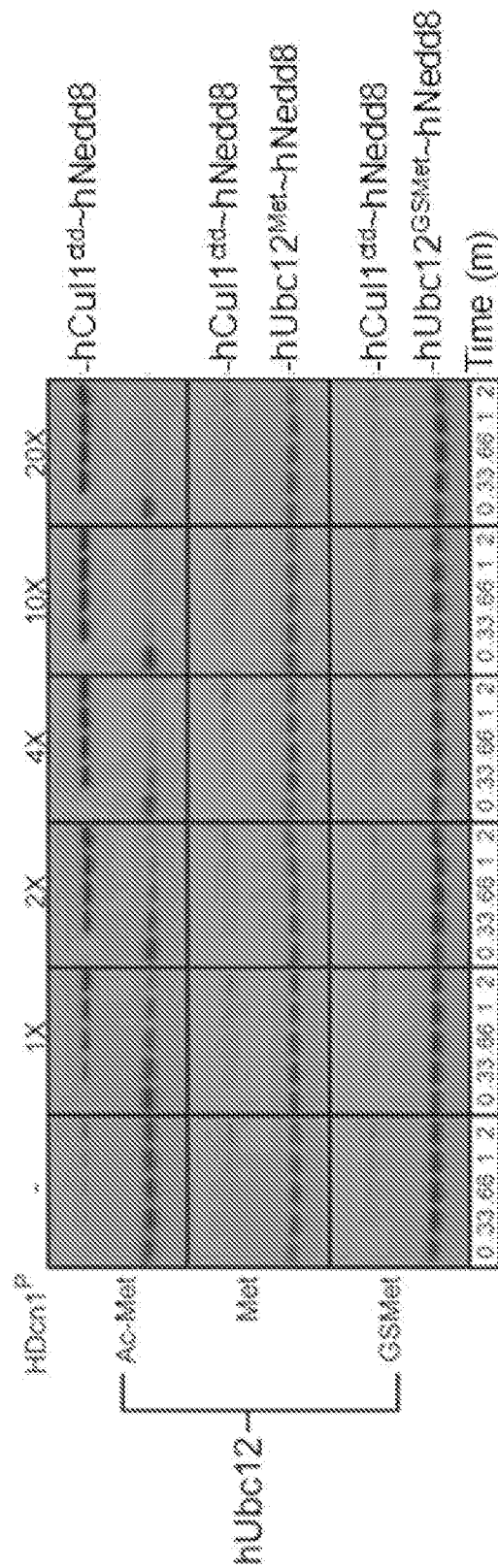


FIG. 3B



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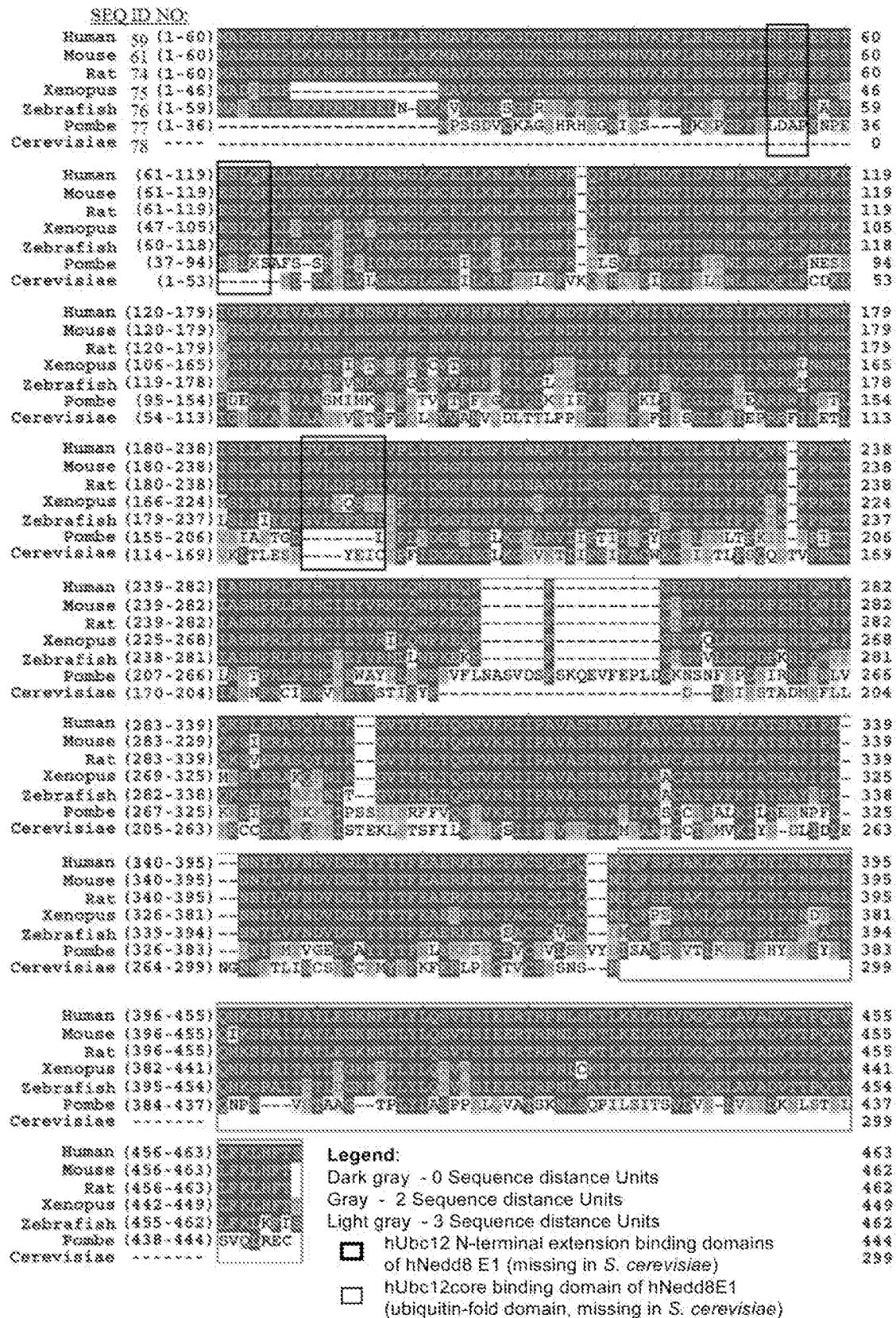


FIG. 4

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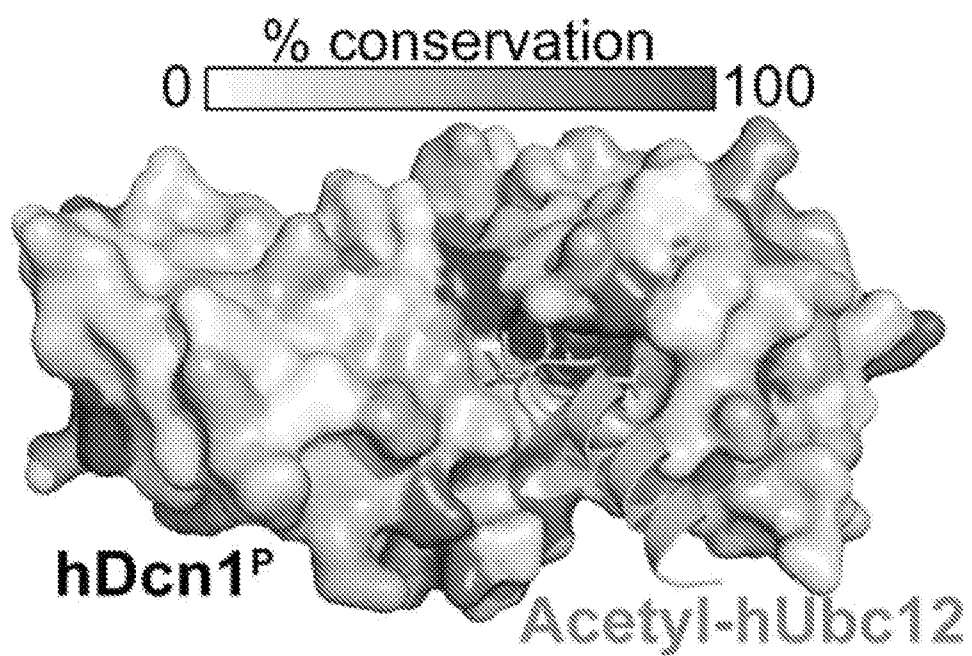


FIG. 5A

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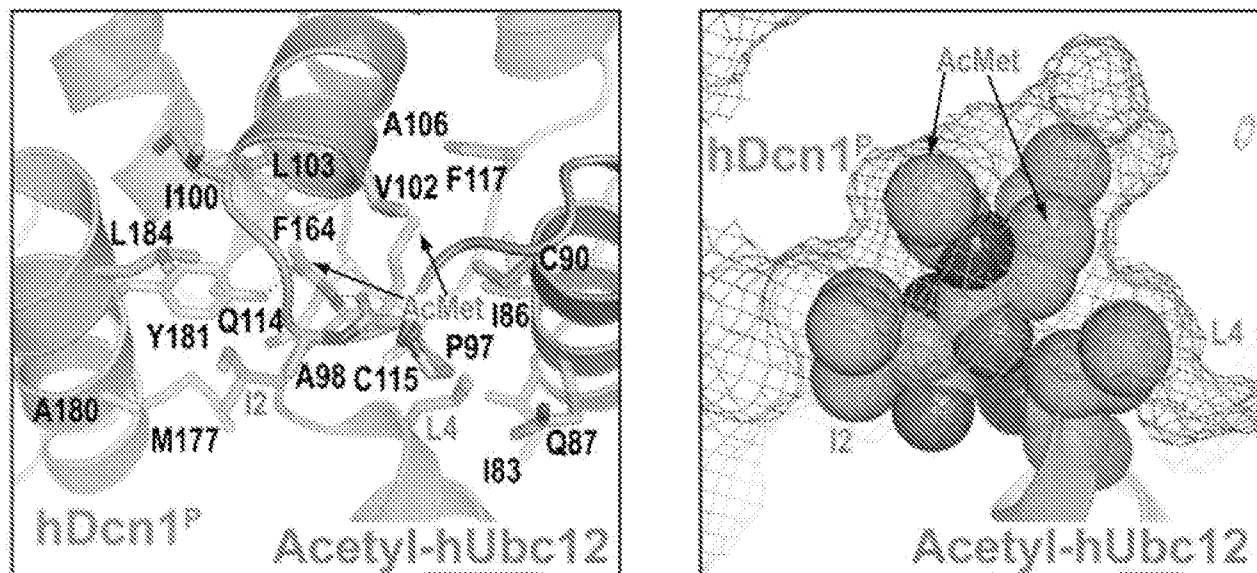


FIG. 5B

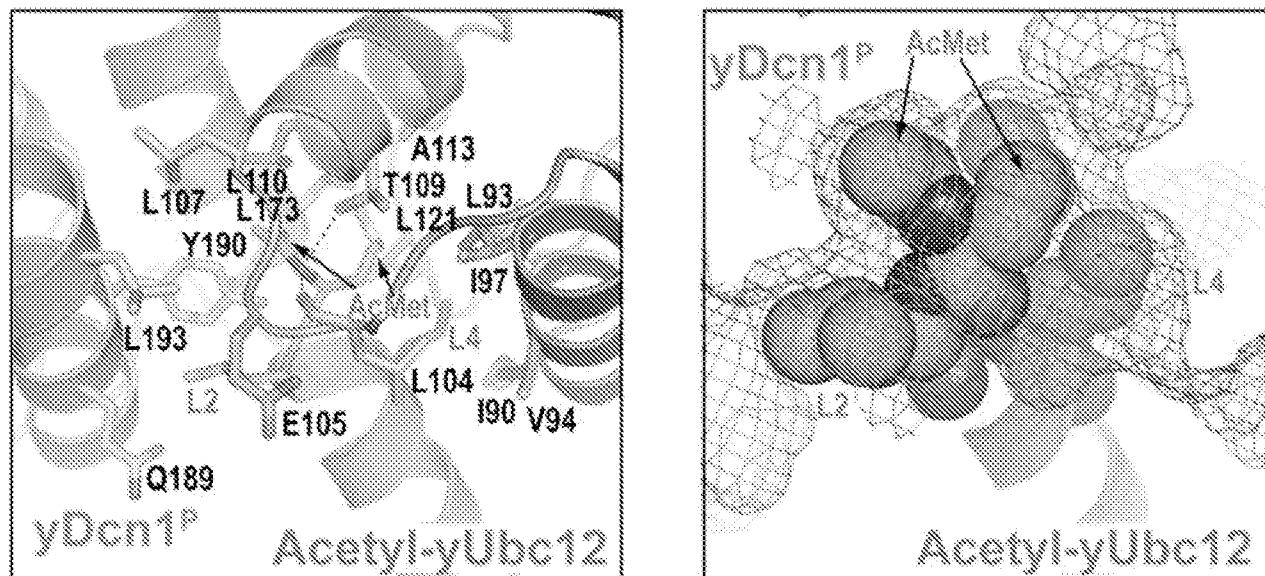
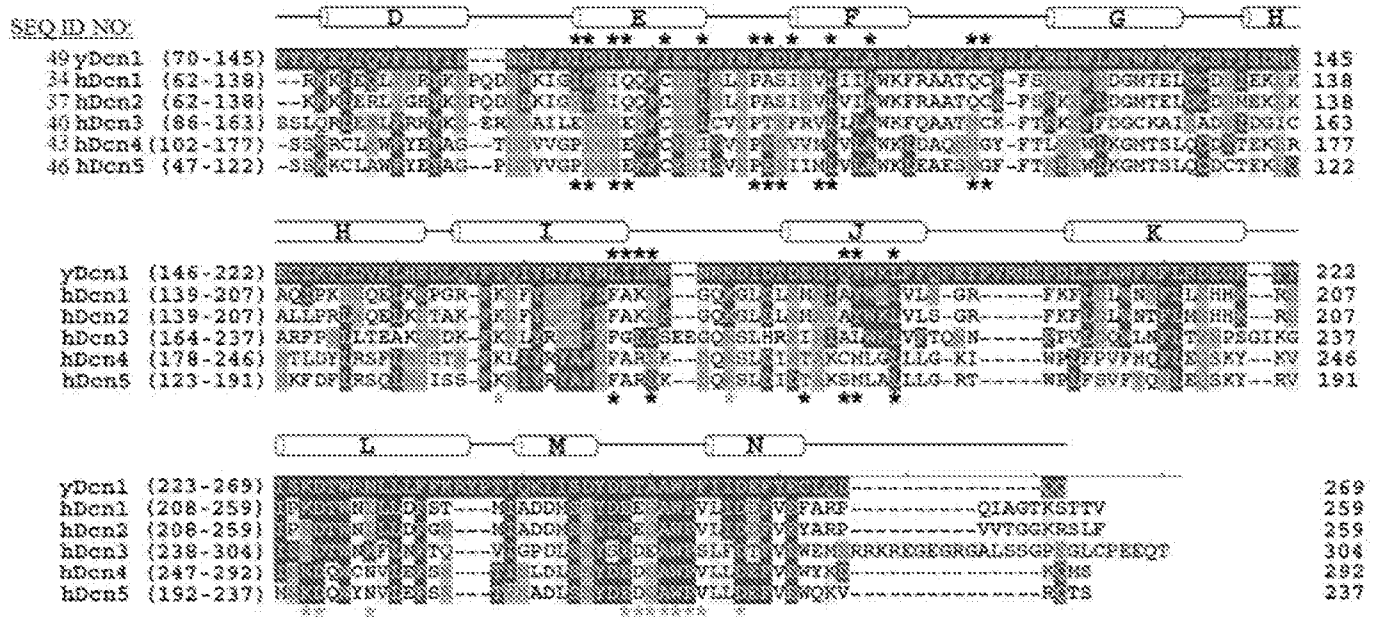


FIG. 5C

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**Legend:**

Dark gray - 0 Sequence distance Units

Gray - 2 Sequence distance Units

Light gray - 3 Sequence distance Units

- * -Contacts between Acetyl-yUbc12¹⁻²⁴ and yDcn1^P (above alignment) or Acetyl-hUbc12¹⁻¹⁵ and hDcn1^P (below alignment)
- * -Contacts between hCul1^{WHB} and hDcn1^P

FIG. 6

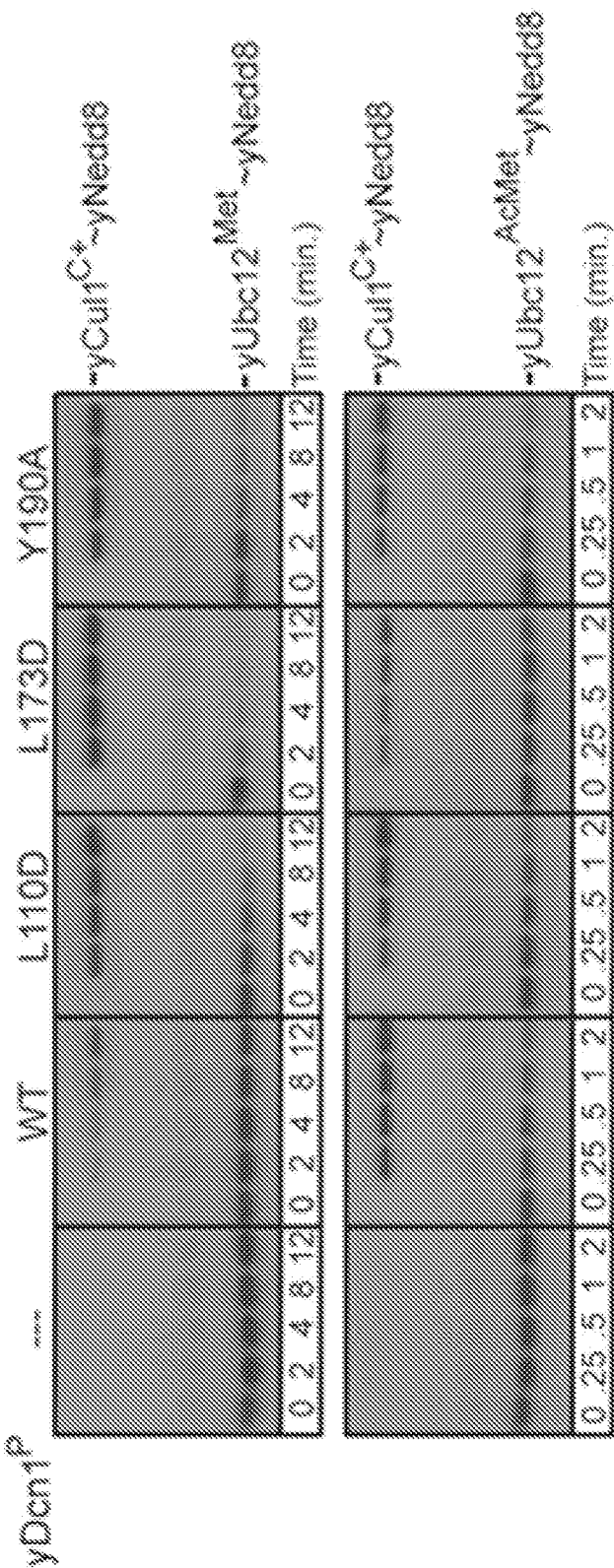


FIG. 7A

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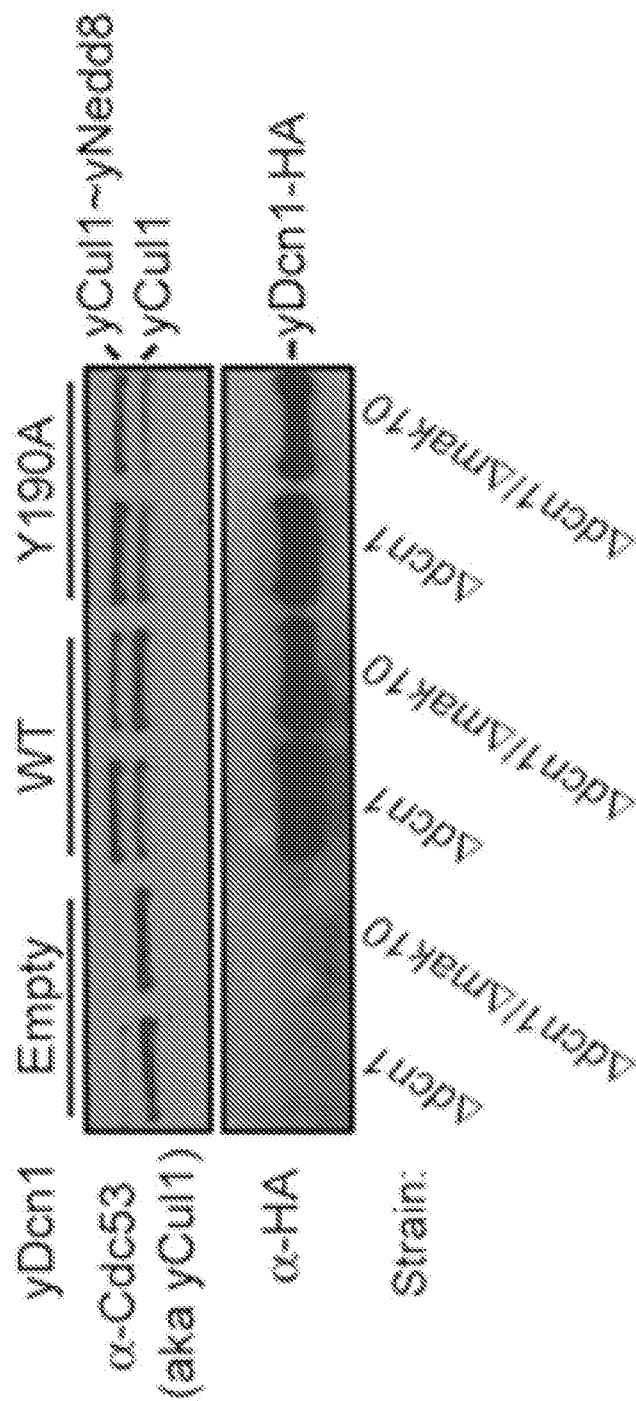


FIG. 7B

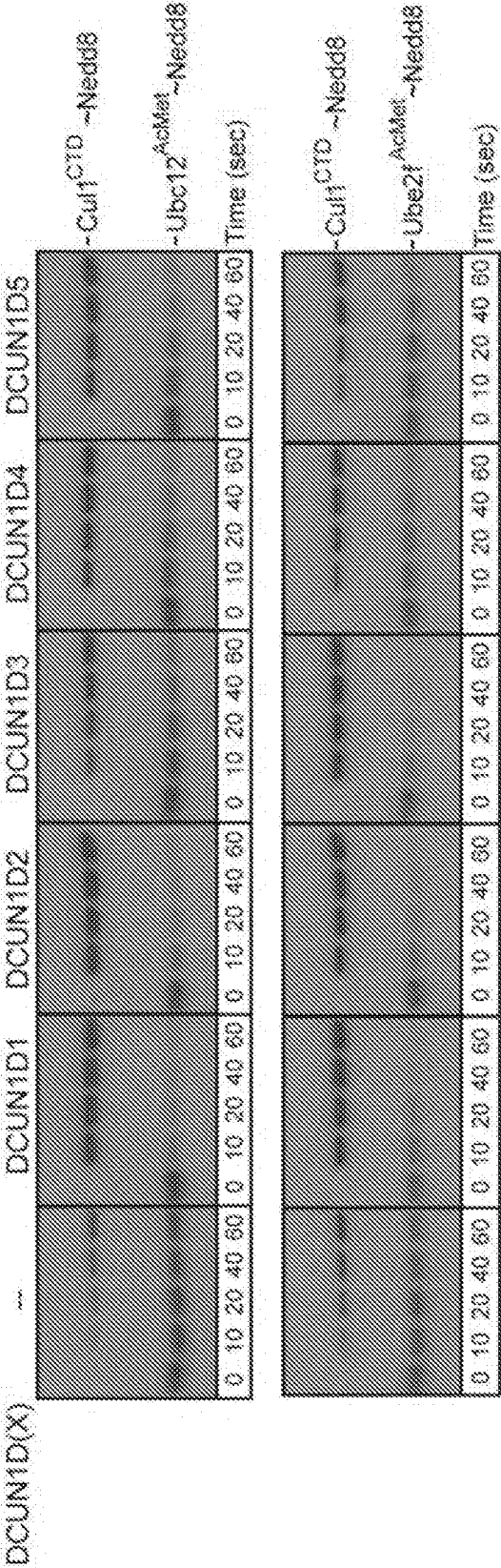


FIG. 8A

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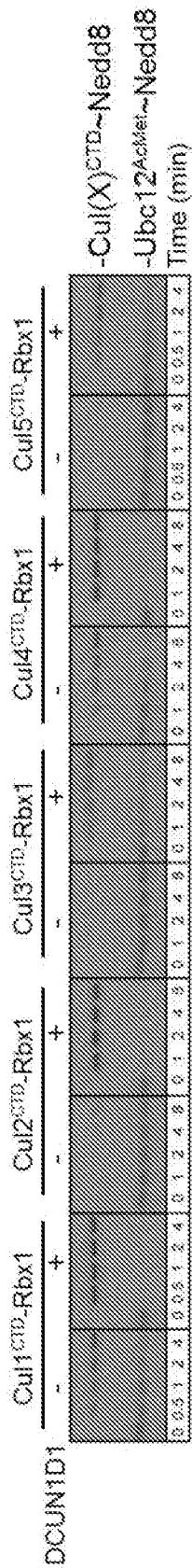


FIG. 8B

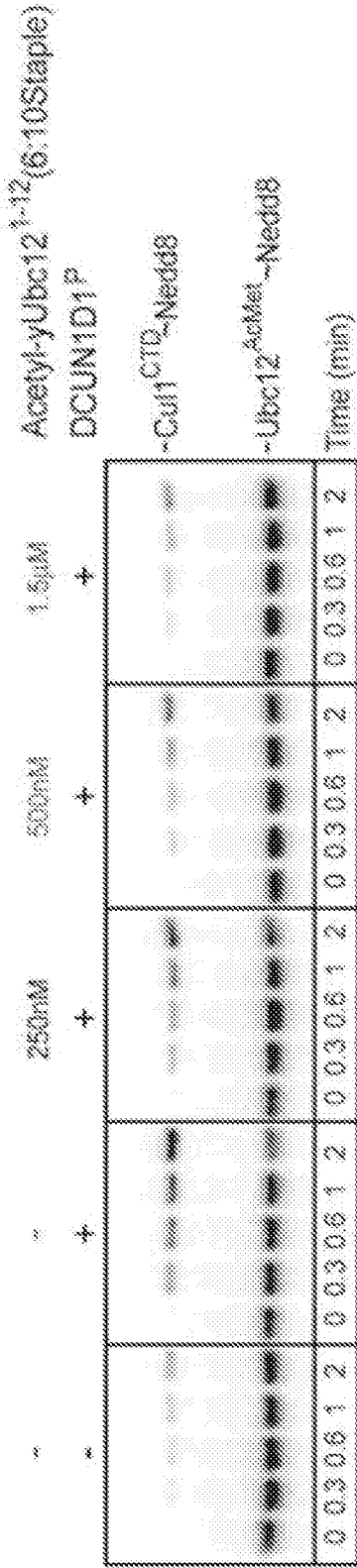


FIG. 9A

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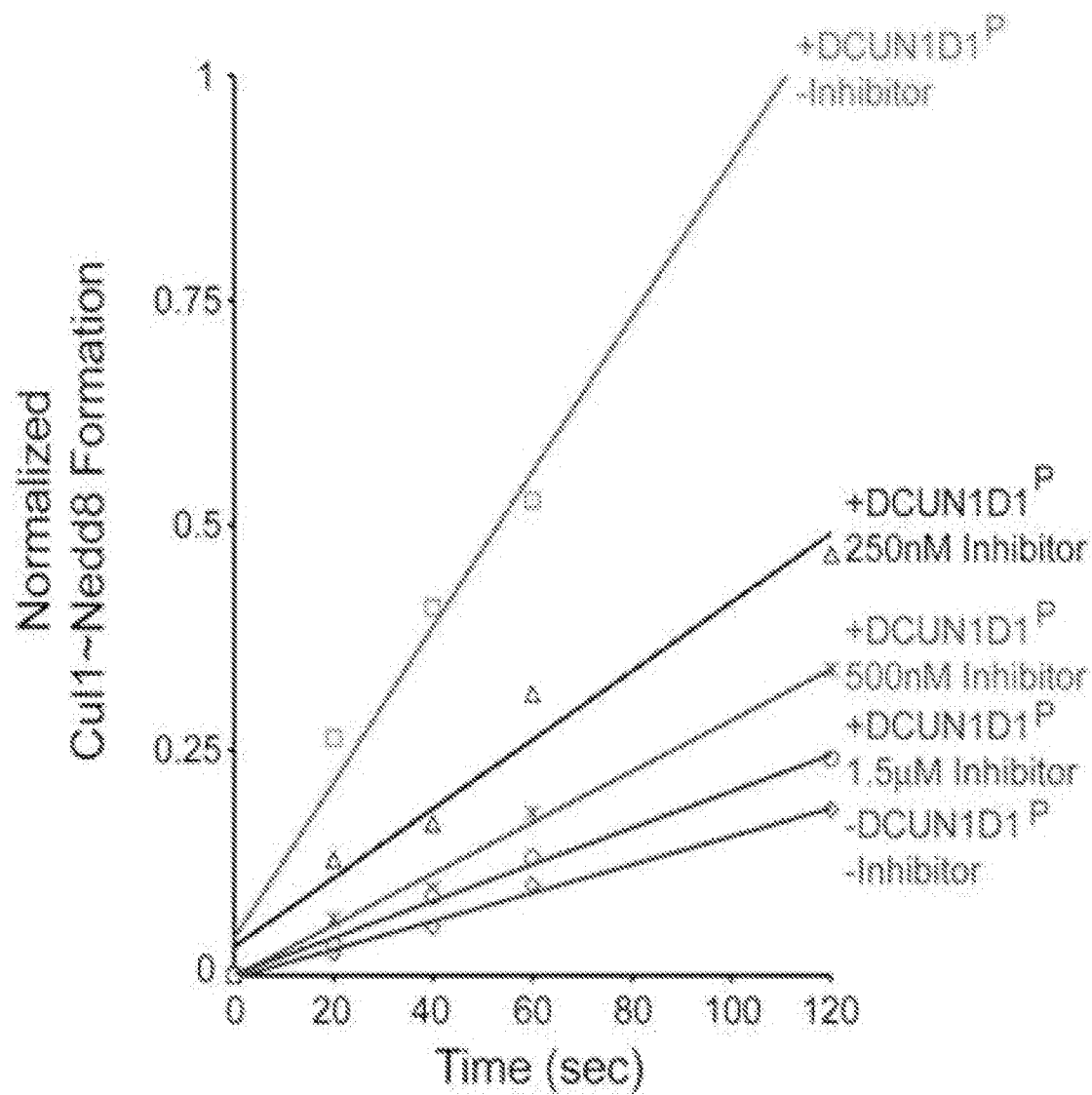


FIG. 9B