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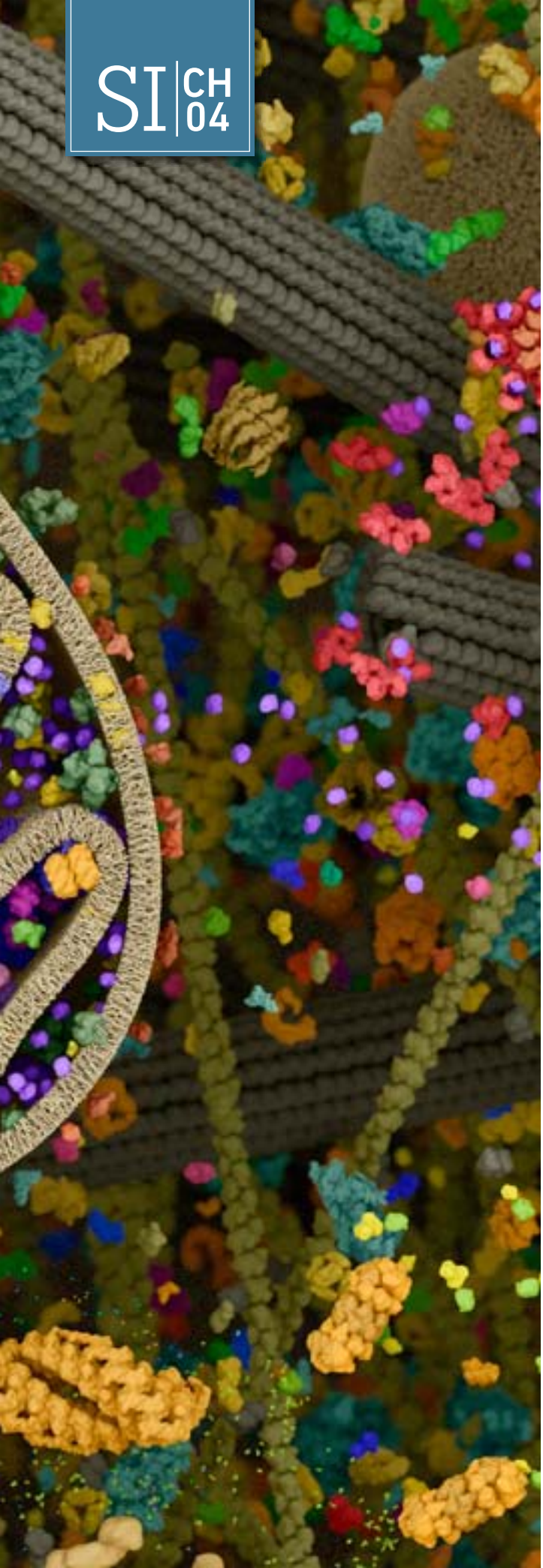


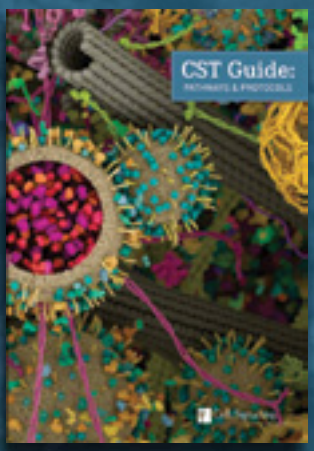
Cell Biology

An excerpt from the first edition of the
CST Guide: Pathways & Protocols

Cell Signaling TECHNOLOGY®

First Edition

CST Guide



GUIDE COVER PHOTO:

Cellular Landscape:

Vesicle Trafficking

Multiple levels shown of key pathways and structures involved in ER and Golgi-mediated trafficking and protein processing, including post-translational modifications.

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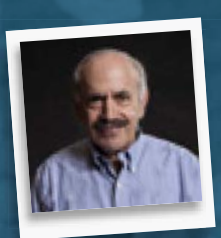

...the time has come for us... to puzzle out, one protein at a time, how signals are really processed inside cells to create the marvelously functioning apparatus the eukaryotic cell.

Dr. Robert A. Weinberg

Daniel K. Ludwig Professor for Cancer Research, MIT

Diagram & Table Keys

Pathway Diagram Key

The pathway diagrams found in this guide and on our website have been assembled by CST scientists and outside experts to provide succinct and current overviews of selected signaling pathways.

Direct Stimulatory Modification	Deacetylase
Direct Inhibitory Modification	Ribosomal subunit
Multistep Stimulatory Modification	TIM-3
Multistep Inhibitory Modification	Galectin-9
Tentative Stimulatory Modification	B7-H3
Tentative Inhibitory Modification	B7-H4
Separation of Subunits	CTLA-4
Joining of Subunits	CD80, 86
Translocation	PD-1
Transcriptional Stimulatory	PD-L1
Transcriptional Inhibitory	TCR
Kinase	MHC
Phosphatase	ICOS
Transcription Factor	ICOSL
Caspase	OX40
Receptor	OX40L
Enzyme	CD40
pro-apoptotic	CD40L
pro-survival	CD27
GAP/GEF	CD70
GTPase	CD137
G-protein	CD137L
Acetylase	CD28

Applications Key

While all of our antibodies are rigorously tested in a number of relevant applications, some products are more suitable for a specific application. This information is summarized in various lists and tables found throughout this guide.

WB	Western Blotting	**ChIP**	Chromatin Immunoprecipitation
IP	Immunoprecipitation	**-IC**	Immunocytochemistry
IHC	Immunohistochemistry	**-P**	Paraffin
IF	Immunofluorescence	**-F**	Frozen
F	Flow Cytometry	**E-P**	Peptide ELISA

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Cell Signaling
TECHNOLOGY®

04

Cell Biology

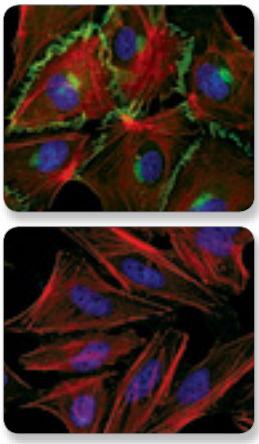
ADHESION AND EXTRACELLULAR MATRIX

Cells can form a number of connections with the cells and matrix in their surrounding environment, including adherens junctions (cell-cell), tight junctions (impermeable cell-cell), and focal adhesions (cell-matrix).

Adherens Junctions

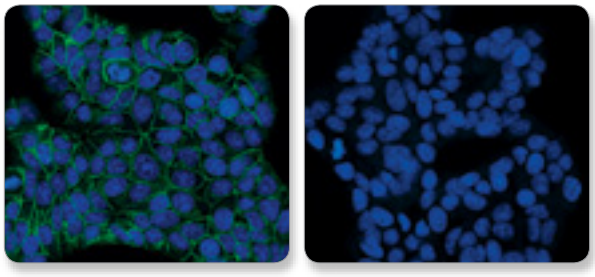
Adherens junctions are cell-cell connections mediated through a cadherin-catenin complex composed of cadherin, β-catenin, and α-catenin. The connection between cell junctions and the cytoskeleton is more dynamic than originally considered, relying on multiple, weak associations between the cadherin-catenin complex and the actin cytoskeleton or on other membrane-associated proteins (i.e. nectin and afadin). Monomeric α-catenin binds β-catenin at adherens junctions, and upon release, forms α-catenin dimers that promote actin bundle formation. The transition from branched actin networks to bundled actin filaments correlates with the creation of mature, strong adherens junctions, and a decrease in membrane lamellipodia. As with most dynamic cellular systems, a collection of kinases, phosphatases, and adaptor proteins regulate the activity and localization of a few key effector proteins. p120 catenin (δ-catenin) binds and stabilizes cadherin at the plasma membrane. Membrane-bound and cytosolic tyrosine kinases phosphorylate β-catenin at weak or nascent junctions, while phosphatases remove added phosphates from β-catenin and δ-catenin at established junctions. Rho family GTPases modulate the availability and activation state of catenins and other essential adherens proteins. Together, this collection of structural proteins, enzymes, and adaptor proteins creates dynamic cell-cell junctions necessary for temporary associations during morphogenesis and maintains the integrity of complex tissues and structures following development.

VE-cadherin is expressed in adherens junctions of endothelial cells.



VE-Cadherin (D87F2) XP® Rabbit mAb #2500: Confocal IF analysis of HUVE cells (top) and HeLa cells (bottom) using #2500. Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

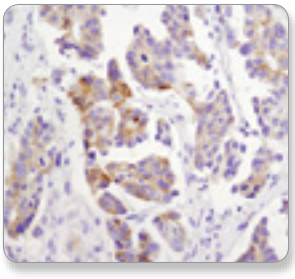
E-cadherin, an important component of adherens junctions, localizes to the plasma membrane.

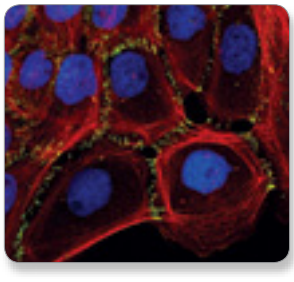


E-Cadherin (24E10) Rabbit mAb #3195: Confocal IF analysis of MCF7 cells using #3195 (green, left) compared to an isotype control (right). Blue pseudo-color = DRAQ5® (fluorescent DNA dye).

N-cadherin, a central component of adherens junctions, is up-regulated in many cancers.

N-Cadherin (D4R1H) XP® Rabbit mAb #13116: IHC analysis of paraffin-embedded human ovarian carcinoma using #13116.





P-cadherin, an adherens junction component expressed in epithelial cells and some cancers, localizes to the plasma membrane.

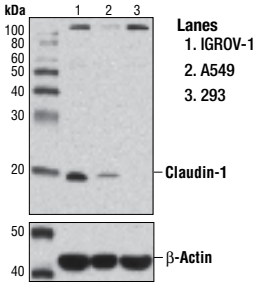
P-Cadherin (C13F9) Rabbit mAb #2189: Confocal IF analysis of A-431 cells using #2189 (green). Actin filaments were labeled with Alexa Fluor® 555 Phalloidin #8953 (red). Blue pseudocolor = DRAQ5® (fluorescent DNA dye).

Tight Junctions

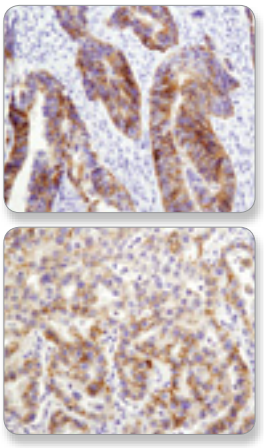
Tight junctions are impermeable cell-cell junctions that form a continuous barrier to fluids across the epithelium and endothelium. They function in regulation of paracellular permeability and in the maintenance of cell polarity, blocking the movement of transmembrane proteins between the apical and the basolateral cell surfaces. The primary protein families composing tight junctions are claudin, occludins, and junctional adhesion molecules (JAMs) transmembrane proteins, which join the junctions to the cytoskeleton. Occludin is thought to be important in the assembly and maintenance of tight junctions. Differential phosphorylation of occludin at various residues may regulate its interaction with other tight junction proteins such as ZO-1.

Differential expression of Claudin-1, a component of tight junctions, in IGROV-1 (high), A549 (moderate), and 293 (absent) cells

Claudin-1 (D3H7C) Rabbit mAb #13995: WB analysis of extracts from IGROV-1, A549, and 293 cells using #13995 (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).



Altered expression of Claudin-1 can be found in many types of cancer.

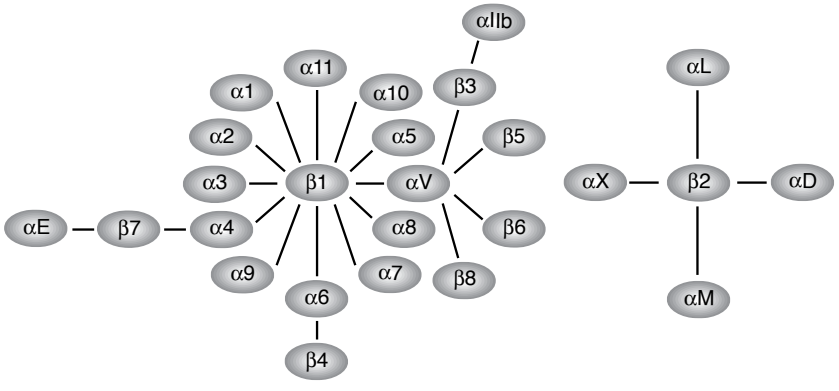


Claudin-1 (D5H1D) XP® Rabbit mAb #13255: IHC analysis of paraffin-embedded human colon carcinoma (top) and human lung carcinoma (bottom) using #13255.

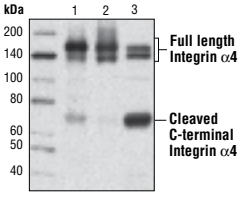
Focal Adhesions

Focal adhesions are the connections that form between a cell and the extracellular matrix (ECM) and are mediated primarily through integrins. Integrins are cell surface receptors that play a pivotal role in cell adhesion, migration, invasion, growth, and survival. Integrins are α/β heterodimeric proteins composed of one α and one β subunit. The integrin family contains at least 18 α and 8 β subunits that form 24 known integrin pairs with distinct tissue distribution and overlapping ligand specificities. The intracellular tail of integrins interacts with cytoskeletal proteins vinculin, talin, and α-actinin, as well as numerous signaling molecules such as focal adhesion kinase (FAK). Activation of FAK by integrin clustering leads to autophosphorylation at Tyr397, which is a binding site for the Src family kinases PI3K and PLCγ.

α/β Integrin Pairs



On SDS-PAGE, Integrin α4 can migrate as a 150 kDa mature protein, a 140 kDa precursor protein, or as an 80 kDa or 70 kDa cleavage fragment.

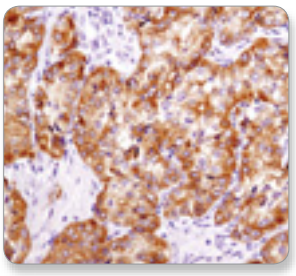


Integrin α4 (D2E1) XP® Rabbit mAb #8440: WB analysis of extracts from various cell lines using #8440.

Lanes
1. Jurkat
2. MOLT-4
3. C6

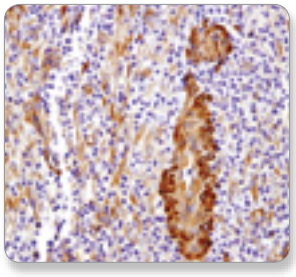
Example of Integrin β3 expression in cancer

Integrin β3 (D7X3P) XP® Rabbit mAb #13166: IHC analysis of paraffin-embedded human papillary renal cell carcinoma using #13166.



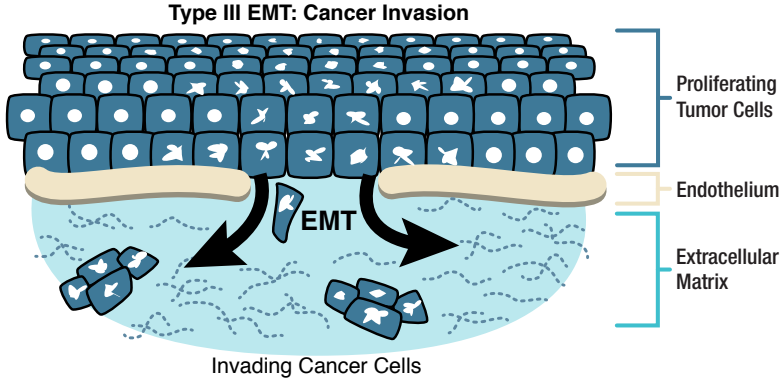
Vinculin is expressed in human breast ductal carcinoma *in situ*.

Vinculin (E1E9V) XP® Rabbit mAb #13901: IHC analysis of paraffin-embedded human breast ductal carcinoma *in situ* using #13901.



Epithelial-Mesenchymal Transition (EMT)

EMT is an essential process during development whereby epithelial cells acquire mesenchymal, fibroblast-like properties and display reduced intracellular adhesion and increased motility. This is a critical feature of normal embryonic development (type I) and wound healing (type II), but it is also utilized by malignant epithelial tumors to spread beyond their origin (type III). This tightly regulated process is associated with a number of cellular and molecular events. EMT depends on a reduction in expression of several cell adhesion molecules. For example, E-cadherin is a critical component of adherens junctions and is considered an active suppressor of invasion and growth for many epithelial cancers. Research studies have shown that cancer cells typically downregulate expression of E-cadherin and upregulate expression of N-cadherin. This is referred to as the cadherin switch and is one of the hallmarks of EMT. Downregulation of E-cadherin expression occurs by binding of transcriptional repressor proteins such as Slug, Snail, and ZEB to the E-cadherin promoter region.



Select Reviews

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Commonly Studied Adhesion Targets

Target	M	P
ADAMTS1	●	
ADAM9	●	●
ADAM10		●
Afadin	●	●
Phospho-Afadin (Ser1718)		●
Ajuba		●
BSP II		●
Pan-Cadherin	●	●
E-Cadherin	●	
N-Cadherin	●	●
OB-Cadherin	●	●
P-Cadherin	●	●
VE-Cadherin	●	●
α-E-Catenin	●	●
Phospho-α-E-Catenin (Ser652)		●
Phospho-α-E-Catenin (Ser655/Thr658)		●
α-N-Catenin	●	●
γ-Catenin		●
Phospho-Catenin δ-1 (Tyr228)		●
Phospho-Catenin δ-1 (Ser252)		●
Phospho-Catenin δ-1 (Ser320)		●
Phospho-Catenin δ-1 (Tyr904)		●
Catenin δ-1		●
CD54/ICAM-1		●

Target	M	P
CD102/ICAM-2	●	
CEA/CD66e	●	
Claudin-1	●	●
Connexin 43		●
Phospho-Connexin 43 (Ser368)		●
CYR61		●
Phospho-Desmoplakin (Ser165/166)		●
EpCAM	●	●
FAK	●	●
Phospho-FAK (Tyr397)	●	●
Phospho-FAK (Tyr576/577)		●
GIT-1		●
GIT2	●	●
Phospho-GIT2 (Tyr392)	●	
Phospho-GIT2 (Tyr592)	●	
Hic-5		●
ILK1	●	●
βIG-H3	●	●
Integrin α2b	●	
Integrin α4	●	●
Integrin α5		●
Integrin αV		●
Integrin α6		●
Integrin β1	●	●
Integrin β3	●	●
Integrin β4		●
Integrin β5	●	●

Target	M	P
LPP	●	
Lyric/Metadherin	●	
Maspin		●
Mesothelin		●
MMP-2	●	●
MMP-7	●	
MMP-9	●	●
MUC1	●	
NCAM (CD56)	●	●
α-Parvin	●	●
Paxillin	●	●
Phospho-Paxillin (Tyr118)		●
PSA/KLK3	●	
RECK	●	
Renin		●
Talin-1	●	
Phospho-Talin (Ser425)	●	●
TIMP1	●	
TIMP2	●	
TIMP3	●	
uPAR	●	●
Vinculin	●	●
ZO-1	●	●
ZO-2		●
ZO-3	●	
Zyxin		●
Phospho-Zyxin (Ser142/143)	●	●

These protein targets represent key nodes within adhesion signaling pathways and are commonly studied in adhesion research. Primary antibodies, antibody conjugates, and antibody sampler kits containing these targets are available from CST.

Listing as of September 2014. See our website for current product information.

M **Monoclonal Antibody**

P **Polyclonal Antibody**

Select Citations:

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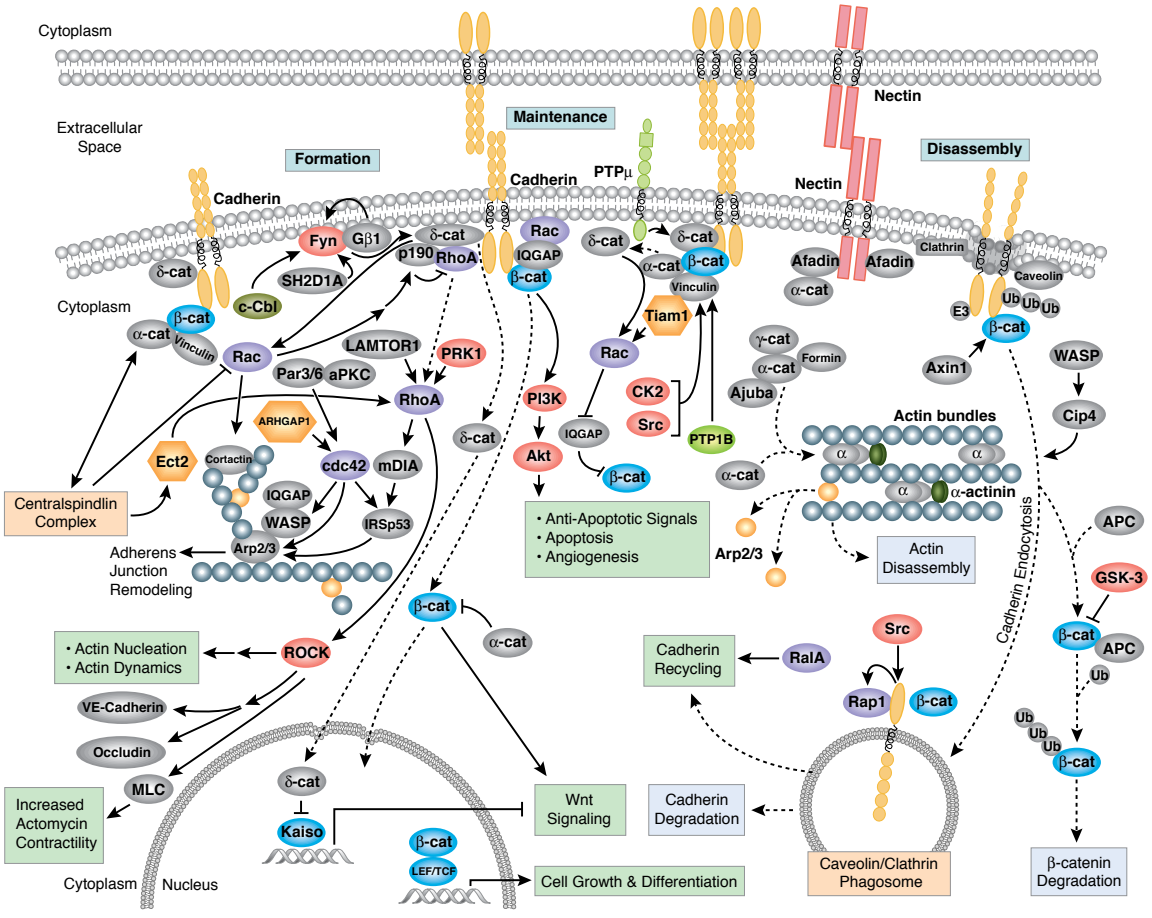
Gumireddy, K. et al. (2013) Identification of a long non-coding RNA-associated RNP complex regulating metastasis at the translational step. *EMBO J.* 32, 2672–2684.

163

2012–2014 CITATIONS

CST antibodies for E-Cadherin have been cited over 163 times in high-impact, peer-reviewed publications from the global research community.

Adherens Junction Dynamics



Adherens junctions are dynamic structures that form, strengthen and spread, degrade, and then re-form as their associated proteins create ephemeral connections with counterparts from adjacent cells. This view updates the traditional model of a stable complex composed of cadherin, β-catenin, and α-catenin bound to the actin cytoskeleton. Although cadherin does exist in a complex with β-catenin and α-catenin, this cadherin-catenin complex does not associate with the actin cytoskeleton. α-catenin does not directly anchor cell adhesion proteins to the actin cytoskeleton but acts as a regulatory protein to control actin filament dynamics.

Monomeric α-catenin binds β-catenin at adherens junctions and upon release forms α-catenin dimers that promote actin bundle formation. The transition from branched actin networks to bundled actin filaments correlates with the creation of mature, strong adherens junctions and a decrease in membrane lamellipodia. The connection between cell junctions and the cytoskeleton may be more dynamic than originally considered and may rely on multiple, weak associations between the cadherin-catenin complex and the actin cytoskeleton or rely on other membrane-associated proteins (i.e. nectin and afadin).

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Select Reviews:

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 • We would like to thank Prof. Kris DeMali of The University of Iowa Carver College of Medicine, Iowa City, IA for reviewing this diagram.

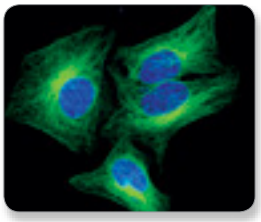
CYTOSKELETAL REGULATION

The cytoskeleton consists of three types of cytosolic fibers: microtubules, microfilaments (actin filaments), and intermediate filaments. Cytoskeletal signaling regulates several important cellular processes such as cell division, adhesion, polarity, migration, and movement through cilia and flagella.

Microtubules

Microtubules are composed of globular tubulin subunits, with α/β-tubulin heterodimers forming the tubulin subunit common to all eukaryotic cells. γ-tubulin is required to nucleate polymerization of tubulin subunits to form microtubule polymers. Many cell movements are mediated by microtubule action, including the beating of cilia and flagella, cytoplasmic transport of membrane vesicles, and nerve-cell axon migration. Microtubules also play a critical role in spindle assembly during mitosis/meiosis and are responsible for chromosome alignment during metaphase. Microtubules form the 9+2 structure of the centriole, a critical component of the centrosome that acts as a microtubule-organizing center (MTOC) and plays a role in cell polarity. Because of their role in mitosis, microtubules have been targets of chemotherapy in cancer. Microtubules continuously undergo a process of dynamic instability, whereby microtubule polymerization on the plus end competes with depolymerization at the minus end. This process is regulated by several signaling molecules including stathmin, diap1/2, tau, and the Rho family of small GTPases.

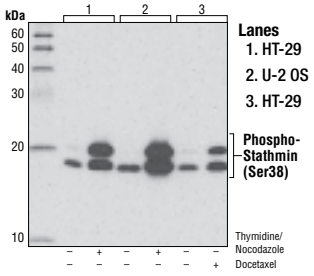
α/β tubulin heterodimers, the building blocks of microtubules, are found throughout the cytoplasm.



β-Tubulin (9F3) Rabbit mAb (Alexa Fluor® 488 Conjugate) #3623: Confocal IF analysis of HeLa cells using #3623 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Stathmin is a microtubule destabilizing protein phosphorylated at Ser38 in cells synchronized in mitosis.

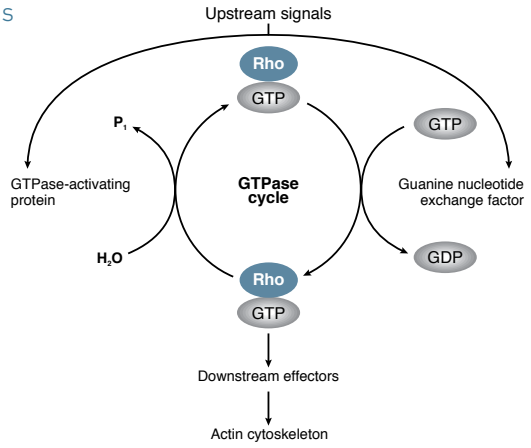
Phospho-Stathmin (Ser38) (D19H10) Rabbit mAb #4191: WB analysis of extracts from HT-29 and U-2 OS cells, untreated or synchronized in mitosis, using #4191. Mitotic synchrony was performed by using either a thymidine block followed by release into nocodazole (100 ng/ml, 24 hr) or using Docetaxel #9886 (100 ng/ml, 24 hr).



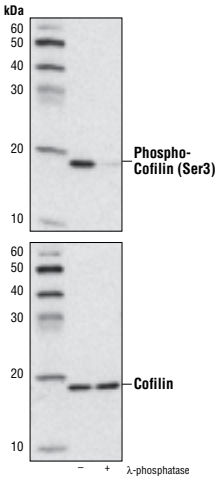
Microfilaments

Microfilaments are major structural components of the cytoskeleton and consist of fibrous polymers of actin, called F-actin. Microfilaments are important for changes in cell shape, migration, proliferation, and survival. Regulation of the actin cytoskeleton begins with signaling through G protein-coupled receptors (GPCRs), integrins, receptor tyrosine kinases (RTKs), and numerous other specialized receptors such as the semaphorin 1a receptor PlexinA. These receptors initiate a large number of signaling cascades that include the Rho family of small GTPases (Rho, Rac, and Cdc42) and their activators, guanine nucleotide exchange factors (GEFs) and their downstream protein kinase effectors (ROCK and PAK), as well as through direct binding of the GTPases to several actin regulatory proteins (cortactin, diap1/2, WAVE, and WASP). These cascades converge on proteins that directly regulate the behavior and organization of the actin cytoskeleton, including actin interacting regulatory proteins such as cofilin, ADF, Arp2/3 complex, Ena/VASP, profilin, and gelsolin.

Small GTPases



COS-7 cells express cofilin phosphorylated at Ser3, a modification known to inhibit cofilin activity.



Phospho-Cofilin (Ser3) (77G2) Rabbit mAb #3313: WB analysis of COS-7 cells, untreated or λ phosphatase-treated, using #3313 (upper) or Cofilin Antiobdy #3312 (lower).

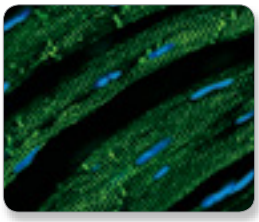
Intermediate Filaments

The major types of intermediate filaments are distinguished by their cell-specific expression.

Classes of Intermediate Filaments

Class	Protein	Expression
I	Acidic keratins	Epithelial cells
II	Basic keratins	Epithelial cells
III	Desmin, GFAP, vimentin	Muscle, Glial cells, Mesenchymal cells
IV	Neurofilaments (NFL, NFM and NFH)	Neurons
V	Lamins	Nucleus

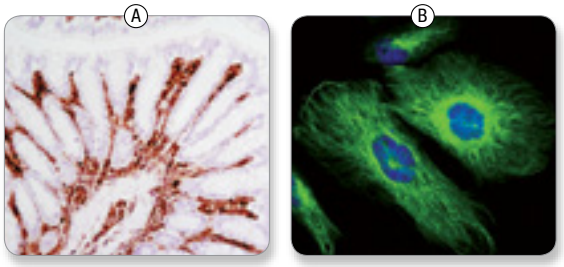
Intermediate filament protein desmin is expressed in muscle cells.



Desmin (D93F5) XP® Rabbit mAb #5332: Confocal IF analysis of mouse heart tissue using #5332 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Members of this group contain a globular N-terminal head domain, a central α–helical rod domain, and a variable C-terminal tail. Intermediate filaments provide structural support for the cell, act as anchorage points for organelles and molecular motors, and function as stress proteins that provide protection from intrinsic and environmental stresses. Intermediate filaments can be regulated through several mechanisms including phosphorylation, which affects their activity or ability to assemble with other intermediate filament-interacting proteins.

Vimentin is an intermediate filament protein expressed in connective tissue and other cells of mesenchymal origin.

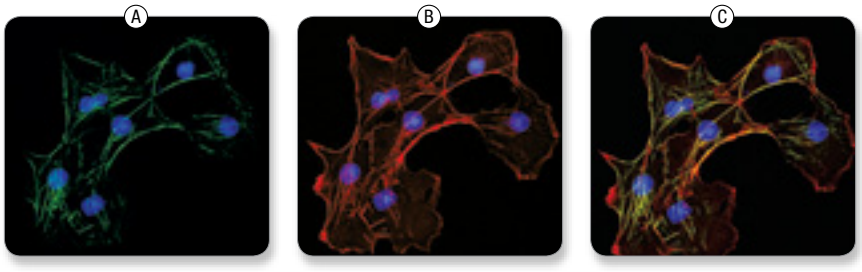


Vimentin (D21H3) XP® Rabbit mAb #5741: IHC analysis of paraffin-embedded mouse colon **(A)** using #5741. Confocal IF analysis of SNB19 cells **(B)** using #5741 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Myosins

Myosins are a large superfamily of actin-binding motor proteins that use ATP hydrolysis to generate motility. Myosins are categorized in over 25 classes. Of these, class II myosins, known as conventional myosins, comprise the largest class and contain myosin proteins specific to skeletal, cardiac, and smooth muscle as well as nonmuscle isoforms. Nonmuscle myosin is essential to cell motility, cell division, migration, adhesion, and polarity. The holoenzyme consists of two identical heavy chains and two sets of light chains. The light chains (MLCs) regulate myosin II activity and stability and exist in many isoforms with varying tissue distribution. The heavy chains (NMHCs) are encoded by three genes, MYH9, MYH10, and MYH14, which generate three different nonmuscle myosin II isoforms, IIa, IIb, and IIc, respectively. While all three isoforms perform the same enzymatic tasks, binding to and contracting actin filaments coupled to ATP hydrolysis, their cellular functions do not appear to be redundant and they have different subcellular distributions.

Nonmuscle myosin IIb heavy chain isoform colocalizes with actin.



Myosin IIb (D8H8) XP® Rabbit mAb #8824: Confocal IF analysis of COS-7 cells, showing Myosin **(A)**, Actin **(B)**, and merged **(C)**, using #8824 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Select Reviews

Blanchoin, L., Boujemaa-Paterski, R., Sykes, C., et al. (2014) *Physiol. Rev.* 94, 235–263. • Chircop, M. (2014) *Small GTPases* 5, e29770. • Chung, B.M., Rotty, J.D., and Coulombe, P.A. (2013) *Curr. Opin. Cell Biol.* 25, 600–612. • Gurel, P.S., Hatch, A.L., and Higgs, H.N. (2014) *Curr. Biol.* 24, 660–672. • Julian, L. and Olson, M.F. (2014) *Small GTPases* 5, e29846. • Kull, F.J. and Endow, S.A. (2013) *J. Cell Sci.* 126, 9–19. • Parker, A.L., Kavallaris, M., and McCarroll, J.A. (2014) *Front. Oncol.* 4, 153. • Snider, N.T. and Omary, M.B. (2014) *Nat. Rev. Mol. Cell Biol.* 15, 163–177. • Wojnacki, J., Quassollo, G., Marzolo, M.P., et al. (2014) *Small GTPases* 5, e28430.

Commonly Studied Cytoskeletal Regulation Targets

Target	M	P
14-3-3 Family		●
14-3-3 β/α		●
14-3-3 γ	●	
14-3-3 ε		●
14-3-3 ζ/δ	●	
14-3-3 η	●	●
14-3-3 τ		●
Phospho-Ack1 (Tyr284)		●
Pan-Actin	●	●
α-Actinin	●	●
Afadin	●	●
Annexin V		●
Phospho-AP2M1 (Thr156)	●	●
Phospho-ARHGAP42 (Tyr376)	●	
Cool2/αPix	●	
Cool1/βPix		●
ARP2	●	●
ARP3		●
Atlastin-1	●	
β-Actin	●	●
Phospho-Catenin δ-1 (Ser320)		●
β2-Chimerin	●	
Caldesmon-1	●	●
Caveolin-1	●	●
Phospho-Caveolin-1 (Tyr14)		●
Caveolin-2	●	
CD2AP		●
CDC37	●	●
Phospho-CDC37 (Ser13)	●	
Cdc42	●	●
CD71	●	
CdGAP	●	
Centrin-2		●
Chronophin/PDXP	●	
CIN85	●	
Claudin-1	●	●
CLIP1/CLIP170		●
Cofilin	●	●
Phospho-Cofilin (Ser3)	●	●
Cortactin		●
Phospho-Cortactin (Tyr421)		●
CrkII		●
Phospho-CrkII (Tyr221)		●
Dab2	●	●
Desmin	●	●

Target	M	P
Diap1		●
Diap2		●
DOCK180	●	
DRP1	●	
Phospho-DRP1 (Ser616)	●	●
Phospho-DRP1 (Ser637)	●	●
DSG2	●	
EB-1	●	
Emerin	●	●
EML4	●	●
EPAC1	●	
EPAC2	●	
EpCAM	●	●
EPLIN		●
Erlin-1		●
Erlin-2		●
EVL		●
Ezrin		●
Phospho-Ezrin (Tyr353)	●	●
Ezrin/Moesin/Radixin		●
Phospho-Ezrin (Thr567)/ Radixin (Thr564)/Moesin (Thr558)	●	●
Fascin	●	
Fer	●	
Fes		●
Fibrillarin	●	
Filamin A		●
Phospho-Filamin A (Ser2152)		●
Filamin B	●	●
Filamin C		●
Flotillin-1		●
Flotillin-2	●	●
FYVE-CENT		●
GEF-H1	●	●
Gelsolin	●	●
Phospho-GIT2 (Tyr392)	●	
GM130	●	●
Golgin-97	●	
HEF1/NEDD9	●	
Importin β1		●
IQGAP1		●
IQGAP2	●	
Integrin α2b	●	
Pan-Keratin	●	
Keratin 7	●	

Target	M	P
Keratin 8/18	●	
Keratin 17	●	
Phospho-Keratin 17 (Ser44)		●
Keratin 17/19	●	
Keratin 18	●	
Keratin 19	●	
Keratin 20	●	
Phospho-KIF1B (Ser1487)		●
KIFC1		●
KIF3A	●	
KIF3B		●
Kinectin 1	●	●
Lamin A/C	●	●
Phospho-Lamin A/C (Ser22)	●	●
Lamin B1	●	
Lamin B2	●	
LAMP1	●	
LASP1		●
LCP1	●	●
Phospho-LCP1 (Tyr28)		●
LIMK1		●
Phospho-LIMK1 (Thr508)/ LIMK2 (Thr505)		●
LIMK2	●	
LLGL1	●	
LMAN1	●	
Phospho-MARK Family (Activation Loop)		●
MARK1		●
MARK2		●
MARK3		●
MARK4		●
MCF2/Dbl		●
Moesin		●
M-RIP		●
MTSS1		●
Myosin IIa		●
Phospho-Myosin IIa (Ser1943)		●
Myosin IIb	●	●
Myosin IIc	●	●
Myosin Va		●
Myosin VI	●	
Myosin Light Chain 2	●	●
Phospho-Myosin Light Chain 2 (Ser19)	●	●
Phospho-Myosin Light Chain 2 (Thr18/Ser19)		●

These protein targets represent key nodes within cytoskeletal regulation signaling pathways and are commonly studied in cytoskeletal regulation research. Primary antibodies, antibody conjugates, and antibody sampler kits containing these targets are available from CST.

Listing as of September 2014. See our website for current product information.

M **Monoclonal Antibody**

P **Polyclonal Antibody**

104

2012–2014 CITATIONS

CST antibodies for β-tubulin have been cited over 104 times in high-impact, peer-reviewed publications from the global research community.

Select Citations:

Harikumar, K.B. et al. (2014) K63-linked polyubiquitination of transcription factor IRF1 is essential for IL-1-induced production of chemokines CXCL10 and CCL5. *Nat. Immunol.* 15, 231–238.

Kim, D.K. et al. (2014) Inverse agonist of estrogen-related receptor gamma controls Salmonella typhimurium infection by modulating host iron homeostasis. *Nat. Med.* 20, 419–424.

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Lucas, C.L. et al. (2014) Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat. Immunol.* 15, 88–97.

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Chowdhury, S. et al. (2013) Flavone-resistant Leishmania donovani Overexpresses LdMRP2 Transporter in the Parasite and Activates Host MRP2 on Macrophages to Circumvent the Flavone-mediated Cell Death. *J. Biol. Chem.* 289, 16129–16147.

Wang, D. et al. (2013) MicroRNA-205 controls neonatal expansion of skin stem cells by modulating the PI(3)K pathway. *Nat. Cell Biol.* 15, 1153–1163.

Target	M	P
Myosin Light Chain 2v (Cardiac Isoform)	●	
MYPT1	●	●
Phospho-MYPT1 (Ser507)		●
Phospho-MYPT1 (Ser668)		●
Phospho-MYPT1 (Thr696)		●
Phospho-MYPT1 (Thr853)		●
Na,K-ATPase		●
Phospho-Na,K-ATPase a1 (Tyr10)	●	●
Phospho-Na,K-ATPase a1 (Ser16)		●
Phospho-Na,K-ATPase a1 (Ser23)		●
N-WASP	●	●
NCK1	●	
NTF2	●	
NUP88	●	
NUP98	●	●
OSR1		●
PAK1/2/3		●
PAK1		●
Phospho-PAK1 (Ser144)/ PAK2 (Ser141)		●
Phospho-PAK1 (Ser199/204)/PAK2 (Ser192/197)		●
Phospho-PAK1 (Thr423)/ PAK2 (Thr402)		●
PAK2	●	●
Phospho-PAK2 (Ser20)		●
PAK3		●
PAK4		●
Phospho-PAK4 (Ser474)/ PAK5 (Ser602)/PAK6 (Ser560)		●
PAR2	●	
PCM-1		●
PDLIM2		●
Podoplanin	●	
PKG-1	●	
PKG-1α	●	
Plectin-1	●	●
PRC1		●

Target	M	P
PREX1	●	
Profilin-1	●	●
PTP4A3		●
PVR/CD155	●	
R-Ras	●	●
Rac1/Cdc42		●
Phospho-Rac1/cdc42 (Ser71)		●
Rac1/Rac2/Rac3		●
RACK1	●	●
Radixin	●	
RalA	●	●
RalB		●
RalBP1	●	●
Ran		●
RanBP1		●
Phospho-RanBP3 (Ser58)		●
Rap1A/Rap1B	●	●
Rap1B	●	
RasGRP3	●	
RCC1	●	●
Phospho-RCC1 (Ser11)	●	●
RCC2	●	●
Phospho-REPS1 (Ser709)	●	
RhoA	●	
RhoB		●
RhoC	●	
RhoE	●	
p190-A RhoGAP	●	●
p190-B RhoGAP		●
RhoGDI		●
p115 RhoGEF	●	
ROCK1	●	
ROCK2	●	●
SCAI	●	
Sec23A		●
Sec24A		●
Sec24B	●	●
Sec24C		●
Sec24D		●

Target	M	P
Sec31A	●	
SPAK		●
SSH1	●	
Stathmin		●
Phospho-Stathmin (Ser16)		●
Phospho-Stathmin (Ser38)	●	●
Talin-1	●	
Phospho-Talin (Ser425)	●	●
TCTP	●	●
Phospho-TCTP (Ser46)		●
Tensin 2		●
TESK1	●	
Troponin T (Cardiac)		●
Tropomyosin-1	●	
Tropomyosin-1/3	●	
Troponin I	●	●
Phospho-Troponin I (Cardiac) (Ser23/24)		●
α-Tubulin	●	●
Acetyl-α-Tubulin (Lys40)	●	●
α/β-Tubulin		●
β-Tubulin	●	●
γ-Tubulin		●
Twinfilin-1	●	●
VASP	●	●
Phospho-VASP (Ser157)		●
Phospho-VASP (Ser239)		●
Vav1	●	●
Vav2	●	
Vav3		●
Villin-1		●
Vimentin	●	●
Phospho-Vimentin (Ser39)		●
Phospho-Vimentin (Ser56)	●	●
Phospho-Vimentin (Ser83)	●	●
WASP	●	●
WAVE-2	●	
WAVE-3		●
ZO-1	●	●

Antibody Validation Principles

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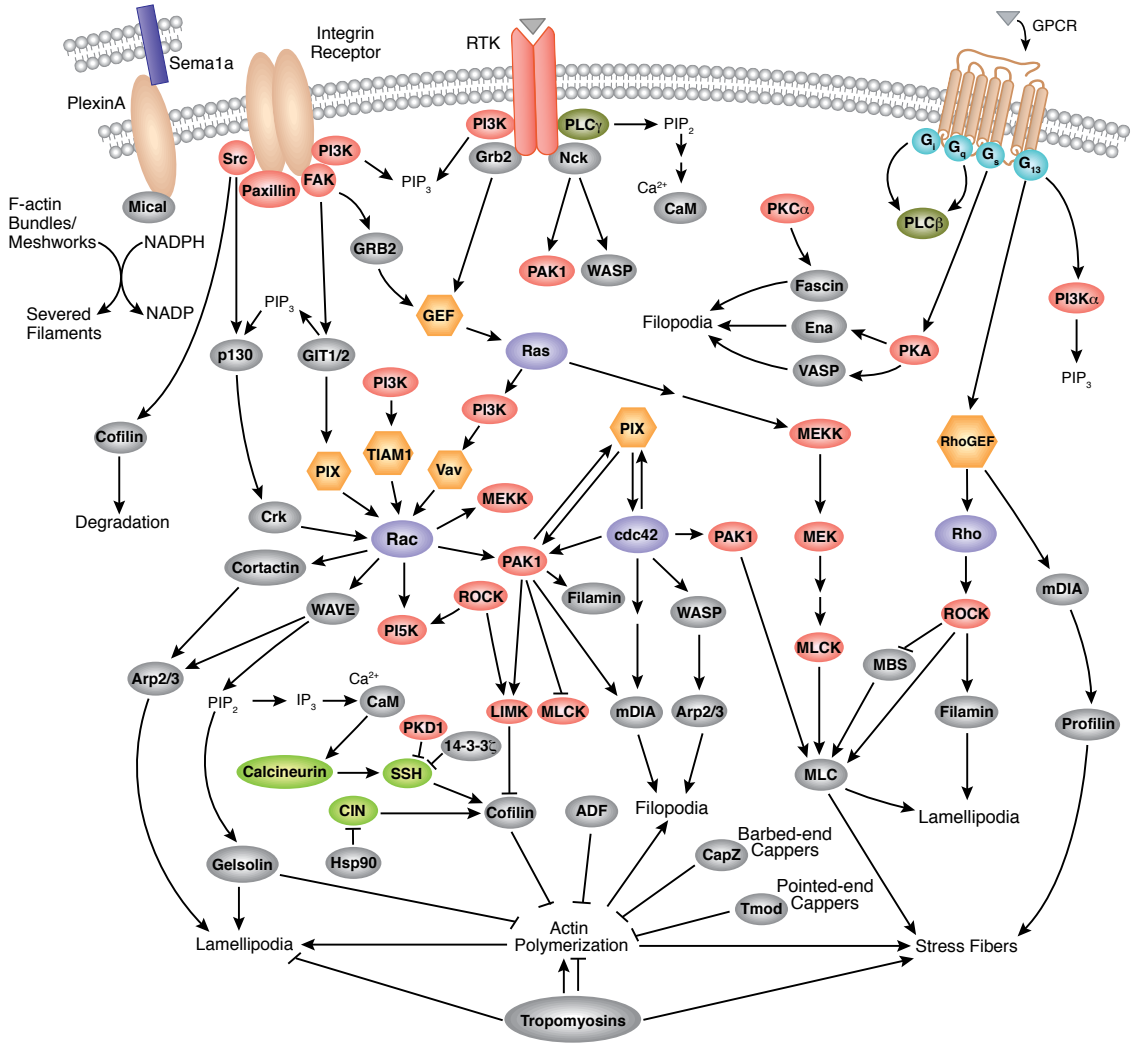
www.cellsignal.com/cstvalidation

Regulation of Actin Dynamics



Signaling to the cytoskeleton through G protein-coupled receptors (GPCRs), integrins, receptor tyrosine kinases (RTKs), and numerous other specialized receptors, such as the semaphorin 1a receptor PlexinA, can lead to diverse effects on cell activity, including changes in cell shape, migration, proliferation, and survival. Integrins, in conjuction with other components of focal adhesion complexes, serve as the link between the extracellular matrix and cytoskeleton in many cell types. Integrin activation leads to activation of focal adhesion kinase (FAK) and Src kinase, resulting in phosphorylation of other FA components such as paxillin and the Crk-associated substrate p130 Cas, as well as the recruitment of signaling adapter proteins.

Intracellular regulation of the cell's response to external cues occurs through a large number of signaling cascades that include the Rho family of small GTPases (Rho, Rac, and Cdc42) and their activators, guanine nucleotide exchange factors (GEFs), their downstream protein kinase effectors, including Rho-kinase/ROCK and p21 activated kinase (PAK), as well as through direct binding of the GTPases to several actin regulatory proteins, such as cortactin, mDia, WAVE, and WASP. These cascades converge on proteins that directly regulate the behavior and organization of the actin cytoskeleton, including actin interacting regulatory proteins such as cofilin, Arp2/3 complex, Ena/VASP, forminins, profilin, and gelsolin. Signaling through different pathways can lead to the formation of distinct actin-dependent structures whose coordinated assembly/disassembly is important for directed cell migration and other cellular behaviors. Migration is also regulated by signaling to myosin, which participates in leading edge actin dynamics and enables retraction of the rear of the cells. Tropomyosins stabilize F-actin by preventing binding of severing and dynamizing factors. Some tropomyosins may also enhance filament dynamics. Dynamic actin is required for most cellular actin-dependent processes; inhibiting actin assembly and preventing actin disassembly are equally inhibitory to most behaviors.

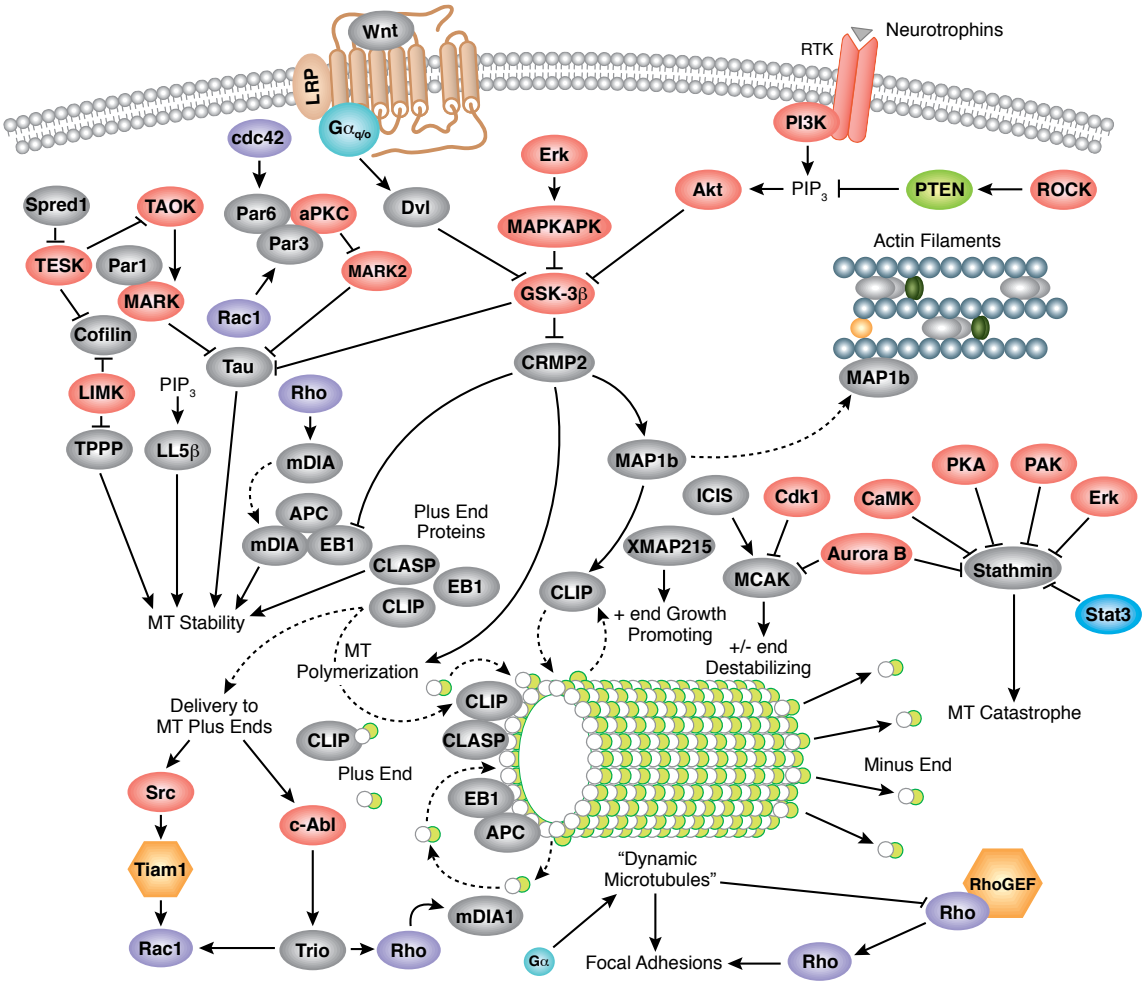
Aberrant control of cytoskeletal signaling, which can result in a disconnection between extracellular stimuli and cellular responses, is often seen in immune pathologies, developmental defects, and cancer.

Select Reviews:

Bernstein, B.W. and Bamburg, J.R. (2010) *Trends Cell Biol.* 20, 187–195. • Lee, S.H. and Dominguez, R. (2010) *Mol. Cells* 29, 311–325. • Levayer, R. and Lecuit, T. (2012) *Trends Cell Biol.* 22, 61–81. • Poukkula, M., Kremneva, E., Serlachius, M., and Lappalainen, P. (2011) *Cytoskeleton (Hoboken)* 68, 471–490. • Ridley, A.J. (2011) *Cell* 145, 1012–1022. • Rottner, K. and Stradal, T.E. (2011) *Curr. Opin. Cell Biol.* 23, 569–578.

 • We would like to thank Prof. James Bamburg, Colorado State University, Fort Collins, CO for reviewing this diagram.

Regulation of Microtubule Dynamics



Microtubules are required for the establishment of cell polarity, polarized migration of cells, intracellular vesicle transport, and chromosomal segregation in mitosis. Microtubules (MTs) are nonequilibrium polymers of α/β-tubulin heterodimers, in which GTP hydrolysis on the β-tubulin subunit occurs following assembly. Most microtubules are nucleated from organizing centers. The most prevalent microtubule behavior is dynamic instability, a process of slow plus end growth coupled with rapid depolymerization ("catastrophe") and subsequent rescue. Although microtubule minus ends show dynamic instability, albeit at a lower rate than the plus ends, the minus ends are usually capped and anchored at MT organizing centers and thus often do not participate in microtubule dynamics.

Maintaining a balance between dynamically unstable and stable microtubules is regulated in large part by proteins that bind either tubulin dimers or assembled microtubules. Proteins that bind tubulin dimers include stathmin, which sequesters tubulin and enhances MT dynamics by increasing catastrophe frequency, and collapsin response mediator protein (CRMP2), which increases MT growth rate by promoting addition of tubulin dimers onto microtubule plus ends. Other proteins that associate with assembled MTs include those that bundle MTs (e.g. MAP1c), those that stabilize MTs (e.g. tau), and those that maintain MTs in a dynamic state (MAP1b). A major signaling pathway that regulates MT dynamics involves GSK-3β, a kinase typically active under basal growth conditions but locally inactive in response to signals that enhance MT growth and dynamics.

In addition to the above factors, many MT motor proteins, and even non-motor proteins, aid in the dynamics of MTs. Proteins such as Xenopus microtubule associated protein 215 (XMAP215), promote MT assembly through binding to tubulin dimer to facilitate its incorporation in the growing plus end. XMAP215 also may compete with some of the MT plus end binding proteins (+TIPS), of which the end binding protein EB1 appears to be the master organizer. Complexes between the adenomateous polyposis coli (APC) protein and plus end binding proteins stabilize MTs by increasing the duration of the MT elongation phase. MT instability is promoted by several nonmotile kinesins from the kinesin-13 family. The mitotic centromere associated kinesin, MCAK, one of the most studied kinesin-13 family proteins, binds both plus and minus MT ends *in vitro*. The binding of MCAK to a MT end is thought to accelerate the transition to catastrophe by weakening the lateral interactions between the protofilaments.

Tubulin undergoes several post-translational modifications such as acetylation, poly-glutamylation, and poly-glycylation, which have been shown to alter the association with certain MT motors as well as other proteins that can affect MT stability and dynamics.

Select Reviews:

Anitei, M. and Hoflack, B. (2011) *Nat. Cell Biol.* 14, 11–19. • de Forges, H., Bouissou, A., and Perez, F. (2012) *Int. J. Biochem. Cell Biol.* 44, 266–274. • Etienne-Manneville, S. (2010) *Curr. Opin. Cell Biol.* 22, 104–111. • Mimori-Kiyosue, Y. (2011) *Cytoskeleton (Hoboken)* 68, 603–618. • van der Vaart, B., Akhmanova, A., and Straube, A. (2009) *Biochem. Soc. Trans.* 37, 1007–1013.

 • We would like to thank Prof. James Bamburg, Colorado State University, Fort Collins, CO for reviewing this diagram.

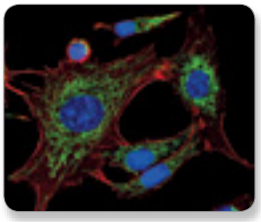
PROTEIN FOLDING AND VESICLE TRAFFICKING

Newly synthesized proteins must be properly folded and then directed to their correct subcellular locations in order to perform their biological functions. This highly regulated process includes molecular chaperone proteins that assist with proper folding and vesicle trafficking proteins that regulate delivery of cargo throughout the cell.

Protein Folding: Heat Shock Proteins

Heat Shock Proteins (HSPs) form seven families (small HSPs (sHSPs), HSP10, 40, 60, 70, 90, and 100) of molecular chaperone proteins that play a central role in the cellular resistance to stress and actin organization. They are involved in the proper folding of proteins and the recognition and refolding of misfolded proteins. HSP expression is induced by a variety of environmental stresses, including heat, hypoxia, nutrient deficiency, free radicals, toxins, ischemia, and UV radiation. HSP27 is a member of the sHSP family. It is phosphorylated at Ser15, Ser78, and Ser82 by MAPKAPK-2 as a result of the activation of the p38 MAP kinase pathway. Phosphorylation and increased concentration of HSP27 has been implicated in actin polymerization and reorganization. HSP70 and HSP90 interact with unfolded proteins to prevent irreversible aggregation and catalyze the refolding of their substrates in an ATP- and co-chaperone-dependent manner. HSP70 has a broad range of substrates including newly synthesized and denatured proteins, while HSP90 tends to have a more limited subset of substrates, most of which are signaling molecules. HSP70 and HSP90 are also essential for the maturation and inactivation of nuclear hormones and other signaling molecules.

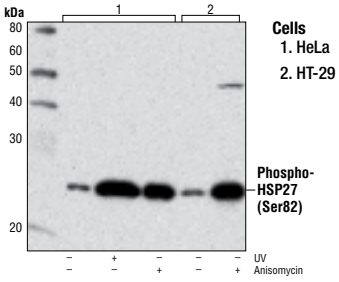
HSP60, an important chaperone for folding key mitochondrial proteins, is expressed in A-204 cells.



HSP60 (D6F1) XP® Rabbit mAb #12165: Confocal IF analysis of A-204 cells using #12165 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Activation of the p38 MAPK pathway by UV or anisomycin results in phosphorylation of HSP27 at Ser82.

Phospho-HSP27 (Ser82) (D1H2) XP® Rabbit mAb #9709: WB analysis of extracts from HeLa or HT-29 cells, untreated (-) or treated (+) with either UV (40 mJ/cm^2 with 30 min recovery) or anisomycin (25 µg/ml, 30 min), using #9709.



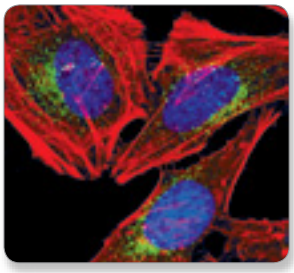
Vesicle Trafficking

Types of Endosomes

Endosomes are membrane-bound vesicles that transport molecules from the plasma membrane to the lysosomes for degradation and can be categorized as early, late, or recycling endosomes. Early endosomes fuse with clathrin-coated endocytic vesicles that contain extracellular particles, fluid, and membrane-bound receptors. EEA1 is an early endosome marker essential for membrane fusion and trafficking. Early endosomes increase in size and undergo a maturation process that results in their development into late endosomes, which can be identified using late endosome markers Rab7 and Rab9. Late endosomes fuse with lysosomes, which results in degradation of vesicle contents. Molecules contained within early endosomes can also be transported to the Golgi for sorting via recycling endosomes.

EEA1 is a marker for early endosomes.

EEA1 (C45B10) Rabbit mAb #3288: Confocal IF analysis of HeLa cells using #3288 (green). Actin filaments have been labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® (fluorescent DNA dye).

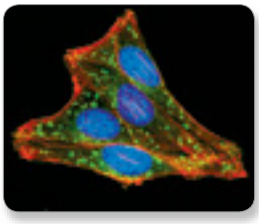


Rab Proteins

Rab proteins have been implicated in the regulation of intracellular protein trafficking in both the endocytic and biosynthetic pathways. GDP-bound Rabs are inactive and localize to the cytosol, while active GTP-bound Rabs localize to the cytoplasmic side of membrane compartments where they recruit downstream effector proteins that, along with Rabs, mediate vesicle formation, motility, docking, and fusion. Rab effector proteins include coat proteins, sorting adaptors, SNAREs, and microtubule- and actin-based motor proteins.

The Rab11 subfamily (Rab11a, Rab11b, and Rab25) localizes to the endosomal recycling compartment, as well as to the apical recycling endosomes of polarized epithelial cells. Endosomal trafficking events through these compartments are mediated by Rab11 family members and their effector molecules, such as the Rab11-family interacting proteins (FIPs), Rabphilin-11/Rab11BP, myosin Vb, and Sec15.

Rab7 localizes to late endosomes.

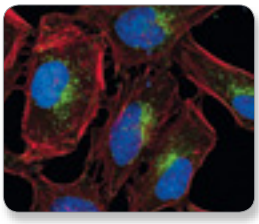


Rab7 (D95F2) XP® Rabbit mAb #9367: Confocal IF analysis of SK-MEL-28 cells using #9367 (green). Actin filaments were labeled using DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Rab proteins serve as markers for the various types of endosomes.

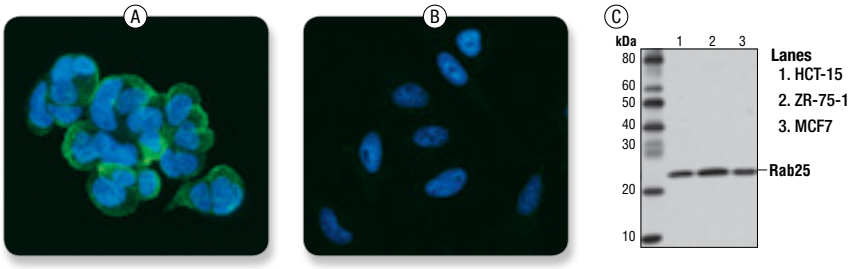
Compartment	Marker
Early Endosome	EEA1, Rab4, Rab5, Rab10
Late Endosome	Rab7, Rab9
Lysosome	LAMP1
Recycling Endosome	Rab4, Rab11, Rab25, Rab35
Perinuclear and Apical Recycling Endosome	Rab17
Golgi-to-Membrane Anterograde Endosome	Rab38
Exocytosis	Rab3A
Endoplasmic Reticulum	PDI, Calnexin
Golgi Apparatus	RCAS1
ER-to-Golgi Anterograde Endosome	Rab1A
Golgi-to-ER Retrograde Endosome	Rab6
Trans Golgi Network-to-Basolateral Membrane Endosome	Rab8

LAMP1 localizes to lysosomes.



LAMP1 (D2D11) XP® Rabbit mAb #9091: Confocal IF analysis of HeLa cells using #9091 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

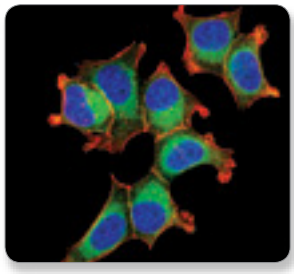
Rab25, which associates with apical recycling vesicles, is expressed in multiple cell lines.



Rab25 (D4P6P) XP® Rabbit mAb #13048: Confocal IF analysis of MCF7 (positive) **(A)** and HeLa (negative) **(B)** cells using #13048 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). WB analysis of extracts from various cell lines **(C)** using #13048.

Rab10, an early endosome marker, mediates protein transport between early endosomes and basolateral compartments.

Rab10 (D36C4) XP® Rabbit mAb #8127: Confocal IF analysis of MCF7 cells using #8127 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



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Commonly Studied Protein Folding and Vesicle Trafficking Targets

Target	M	P
ACE2		●
APPL1	●	
Arf6	●	●
Bag1	●	
BAG6		●
BiP	●	●
Calnexin	●	●
Calpastatin		●
Caveolin-1	●	●
Phospho-Caveolin-1 (Tyr14)		●
CCT2		●
CDC37	●	●
Phospho-CDC37 (Ser13)	●	
CHOP	●	
Clathrin Heavy Chain	●	●
CRYAB		●
Derlin-1		●
DNAJC2/MPP11	●	
Phospho-DNAJC2/MPP11 (Ser47)		●
Dynamin-I	●	
Dynamin I/II		●
EEA1	●	●
Eps15	●	●
Ero1-Lα		●
ERp44	●	●
ERp57		●
ERp72	●	●
FKBP4	●	●
GCN2		●
GM130	●	●
GOPC	●	

Target	M	P
Grp75	●	●
Grp94		●
Hip	●	●
Hop	●	●
HRS		●
HSF1	●	●
HSP27	●	●
Phospho-HSP27 (Ser15)		●
Phospho-HSP27 (Ser78)		●
Phospho-HSP27 (Ser82)	●	●
HSP40	●	●
HSP60	●	●
HSP70	●	●
TRAP1/HSP75		●
HSP90	●	●
Phospho-HSP90α (Thr5/7)		●
HSP90β	●	●
HSPA4/Apg-2		●
HSPA8	●	
HSPB8/HSP22		●
HYOU1		●
IGF-II Receptor/CI-M6PR	●	
IRE1α	●	
MBTPS2		●
NSF	●	●
OCRL1		●
OS-9	●	
p58IPK	●	
PDI	●	●
PERK	●	
Phospho-PERK (Thr980)	●	

Target	M	P
PKR	●	●
Prostate Specific Membrane Antigen	●	
Rab1A	●	
Rab3A	●	●
Rab4		●
Rab5	●	●
Rab6	●	●
Rab7	●	●
Rab8	●	
Rab9	●	
Rab10	●	●
Rab11	●	●
Rab11a		●
Rab11b		●
Rab11FIP1	●	
Rab17	●	
Rab25	●	●
Rab35		●
Rab38		●
Rabex-5	●	
RBX1	●	●
RCAS1	●	
REPS1	●	
SGTA		●
Phospho-SGTA (Ser305)	●	
SNIP/p140Cap		●
STAM1		●
Syntaxin 6	●	
Tid-1	●	
VAMP8		●

These protein targets are commonly studied in protein folding and vesicle trafficking research. Primary antibodies, antibody conjugates, and antibody sampler kits containing these targets are available from CST.

Listing as of September 2014. See our website for current product information.

M **Monoclonal Antibody**

P **Polyclonal Antibody**

Select Citations:

Kalwa, H. et al. (2014) Central role for hydrogen peroxide in P2Y1 ADP receptor-mediated cellular responses in vascular endothelium. *Proc. Natl. Acad. Sci. USA* 111, 3383–3388.

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Simone, L.C. et al. (2013) Role of phosphatidylinositol 4,5-bisphosphate in regulating EHD2 plasma membrane localization. *PLoS One* 8, e74519.

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32

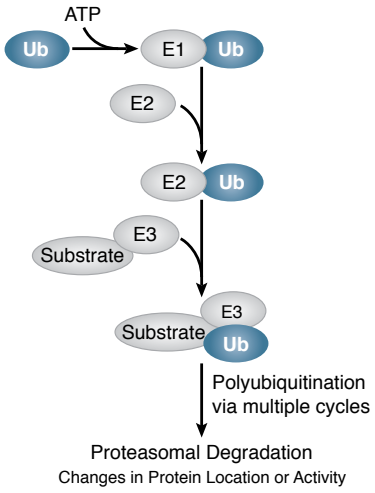
2012–2014 CITATIONS

CST antibodies for Caveolin have been cited over 32 times in high-impact, peer-reviewed publications from the global research community.

UBIQUITIN AND UBIQUITIN-LIKE PROTEINS

The ubiquitin-proteasome system (UPS) is the primary means by which cellular proteins are degraded. The UPS is a highly regulated system for elimination of misfolded or damaged proteins as well as proteins whose activity is acutely regulated by signaling pathways. This system plays a central role in cell proliferation, transcriptional regulation, apoptosis, immunity, development, and many other cellular processes (e.g., organelle biogenesis, cellular response to infection, etc.). Ubiquitin is a highly conserved 76-amino acid protein that can be covalently linked to many cellular proteins through an enzymatic cascade. Ubiquitination is an ATP-dependent process carried out by three classes of enzymes. A "ubiquitin activating enzyme" (E1), UBA1, forms a thio-ester bond with ubiquitin. This reaction allows subsequent binding of ubiquitin to "ubiquitin conjugating enzymes" (E2s), followed by the formation of an isopeptide bond between the C-terminus of ubiquitin and the ε-amino group of a lysine residue on the substrate protein. The latter reaction requires a "ubiquitin ligase" (E3). Several hundred E3 ligases exist within the eukaryotic cell; each ligase can only modify a subset of substrate proteins, thereby providing substrate specificity to the system. Ubiquitinated proteins are then targeted to the 26S proteasome for degradation or experience changes in protein location or activity.

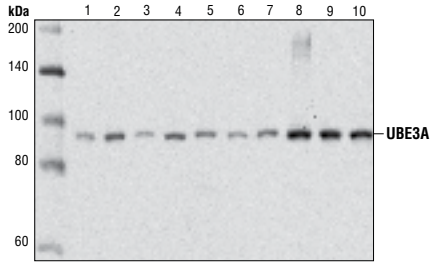
Ubiquitination



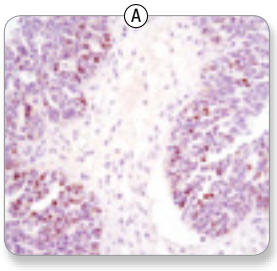
UBE3A, a widely expressed E3 ubiquitin ligase

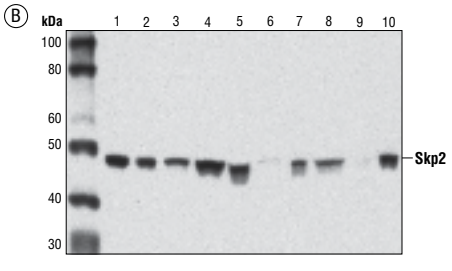
UBE3A (D10D3) Rabbit mAb #7526: WB analysis of extracts from various cell lines using #7526.

Lanes
1. K-562
2. SK-N-SH
3. SK-N-MC
4. A172
5. T-47D
6. HEK001
7. T24
8. KNRK
9. NIH/3T3
10. COS-7



Skp2, a substrate recognition subunit of the Skp-Cullin-F-box (SCF) ubiquitin ligase complex, is expressed in many cell lines and cancers.





Lanes
1. U-2 OS
2. LNCaP
3. MDA-MB-468
4. K-562
5. U-87 MG
6. WI-3B
7. SK-OV-3
8. MCF7
9. MCF-10A
10. COS-7

Skp2 (D3G5) XP® Rabbit mAb #2652: IHC analysis of paraffin-embedded human ovarian carcinoma **(A)** using #2652. WB analysis of extracts from various cell lines **(B)** using #2652.

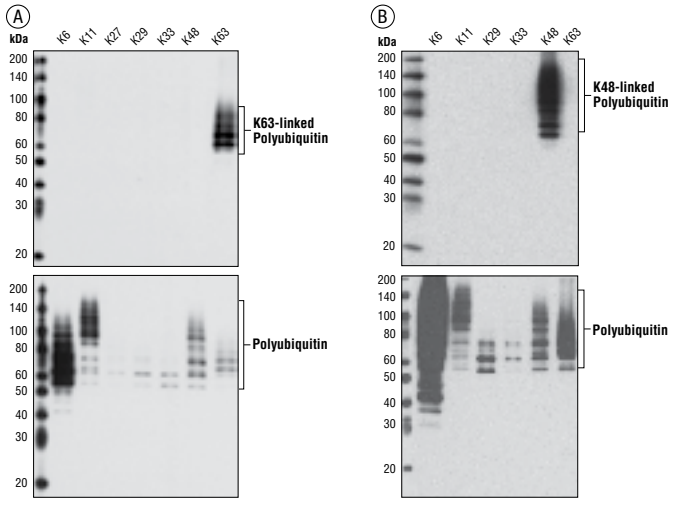
Ubiquitin Linkages

Ubiquitin can be linked to a substrate as a single unit, monoubiquitination, or as a branched chain, polyubiquitination. Substrate proteins are linked to ubiquitin using seven distinct ubiquitin lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63). Formation of a polyubiquitin chain occurs when a lysine residue of ubiquitin is linked to the C-terminal glycine of another ubiquitin. Polyubiquitinated proteins have distinct fates depending upon the nature of the ubiquitin linkage through which they are conjugated; K48-linked polyubiquitin chains mainly target proteins for proteasomal degradation while K63-linked polyubiquitin chains typically regulate protein function, subcellular localization, and protein-protein interactions. The K63 linkage sometimes results in proteasomal degradation as well. K11-linked polyubiquitin chains regulate cell cycle targets and progression through mitosis.In addition, ubiquitin also can be linked to a target protein through its N-terminal methionine residue. This linkage, termed linear ubiquitination, is catalyzed by the linear ubiquitin chain assembly complex (LUBAC) and plays a critical role in NF-κB signaling.

Distinct ubiquitin linkages can be detected using linkage-specific antibodies.

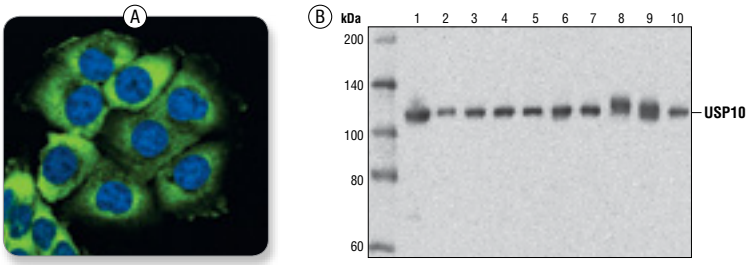
K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb #5621: WB analysis of seven distinct recombinant polyubiquitin chains **(A)** using #5621 (upper) and Ubiquitin Antibody #3933 (lower).

K48-linkage Specific Polyubiquitin (D9D5) Rabbit mAb #8081: WB analysis of six distinct recombinant polyubiquitin chains **(B)** using #8081 (upper) and Ubiquitin (P4D1) Mouse mAb #3936 (lower).



Deubiquitinating Enzymes

Deubiquitinating enzymes (DUBs) reverse the process of ubiquitination by removing ubiquitin from its substrate protein. DUB activity maintains ubiquitin recycling and ensures the cellular pool of ubiquitin molecules remains steady. DUBs are categorized into 5 subfamilies: USP, UCH, OTU, MJD, and JAMM, each with a specific tissue and ubiquitin-linkage specificity. Overexpression or misregulation of DUBs have been linked to cancer and other diseases. For example, overexpression of USP6 has been linked to bone and other mesenchymal tumors, mutations in CYLD are associated with skin tumors, and overexpression of USP33 to von Hippel-Lindau diease. In addition, other DUB mutations are associated with neurodegenerative disorders, including a role for UCHL1 in Parkinson's disease and ATX3 in spinocerebellar ataxia.



USP10, a DUB known to act on p53, Vps34, and CFTR, is found in the cytoplasm and is widely expressed in many cell lines.

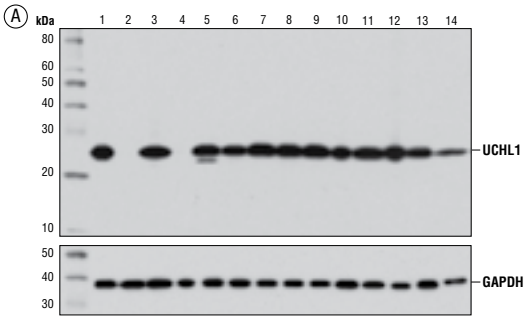
USP10 (D7A5) Rabbit mAb #8501: Confocal IF analysis of HCT 116 cells **(A)** using #8501 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). WB analysis of extracts from various cell lines **(B)** using #8501.

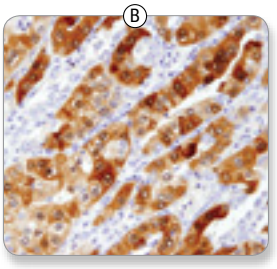
UCHL1 is expressed in many cell lines and human non-small cell lung carcinoma.

UCHL1 (D3T2E) XP® Rabbit mAb #13179: WB analysis of extracts from various cell lines **(A)** using #13179 (upper) and GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). IHC analysis of paraffin-embedded human non-small cell lung carcinoma **(B)** using #13179.

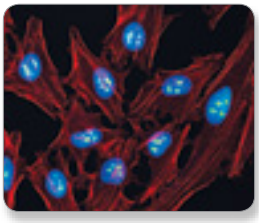
Lanes

1. DU 145	8. A172
2. LNCaP	9. SH-SY5Y
3. NCI-H1299	10. T98G
4. NCI-H358	11. SK-N-AS
5. WI-38	12. Neuro-2a
6. BT-549	13. C6
7. U266	14. COS-7



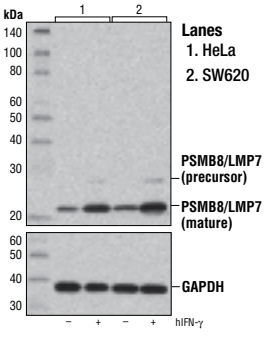


SENP3, a SUMO protease, localizes to the nucleolus.



SENP3 (D20A10) XP® Rabbit mAb #5591: Confocal IF analysis of HeLa cells using #5591 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Interferon-γ induces expression of PSMB8, a core particle subunit of the immunoproteasome.



PSMB8/LMP7 (1A5) Mouse mAb #13726: WB analysis of extracts from HeLa and SW620 cells, untreated or treated with Human Interferon-γ (hIFN-γ) #8901 (100 ng/ml, 72 hr), using #13726 (upper) and GAPDH (D16H11) XP® Rabbit mAb #5174 (lower).

Sumoylation and Neddylation

Small ubiquitin-related modifier 1, 2, and 3 (SUMO-1, -2, and -3) and NEDD8 are members of the ubiquitin-like protein family. SUMO and NEDD8 can be covalently attached to proteins (termed sumoylation and neddylation, respectively) in a manner analogous to ubiquitination using an E1, E2, E3 conjugation system. Unlike ubiquitination, however, sumoylation and neddylation of substrate proteins does not typically result in degradation. Instead, the SUMO and NEDD modifications affect subcellular localization, protein function, or protein-protein interactions.

Sumoylation substrates include RanGAP, PML, p53, IκB-α, topoisomerase II, and APP. Sumoylation has numerous cellular effects including nuclear trafficking, regulation of transcriptional activity, and protein stability. SUMO modifications can be removed (desumoylation) by proteases such as SENP3, which catalyzes the release of SUMO2 and SUMO3 monomers from sumoylated substrates.

Neddylation is the covalent attachment of NEDD8 via its C-terminal glycine residue to a lysine residue within the target protein. In contrast to the ubiquitin pathway, only a handful of neddylation substrates are known to date. NEDD8 and ubiquitin modifications are closely connected in that neddylation is an important regulator of E3 ubiquitin ligase activity. For example, neddylation of cullin proteins activates the SCF (Skp1-Cullin-F-box) E3 ubiquitin ligase complex by promoting complex formation and enhancing the recruitment of the E2-ubiquitin intermediate. Other neddylation substrates include p53, Mdm2, RPL11, and VHL.

Proteasome

The 26S proteasome is a highly abundant proteolytic complex involved in the degradation of ubiquitinated substrate proteins. It consists largely of two sub-complexes, the 20S catalytic core particle (CP) and the 19S/PA700 regulatory particle (RP) that can cap either end of the CP. The CP consists of two stacked heteroheptameric β-rings (β1-7) that contain three catalytic β-subunits and are flanked on either side by two heteroheptameric α-rings (α1-7). The RP includes a base and a lid, each having multiple subunits. The base, in part, is composed of a heterohexameric ring of ATPase subunits belonging to the AAA (ATPases Associated with diverse cellular Activities) family. The ATPase subunits function to unfold the substrate and open the gate formed by the α-subunits, thus exposing the unfolded substrate to the catalytic β-subunits. The lid consists of ubiquitin receptors and DUBs that function in recruitment of ubiquitinated substrates and modification of ubiquitin chain topology. Other modulators of proteasome activity, such as PA28/11S REG, can also bind to the end of the 20S CP and activate it. Proteasome activity can be inhibited by the chemical modulator bortezomib.

Constitutively expressed core particle subunits PSMB5, PSMB7, and PSMB6 provide chymotrypsin-like, trypsin-like, and caspase-like activities, respectively. In immune cells involved in antigen presentation, these subunits are replaced by highly homologous, induced β-subunits PSMB8, PSMB9, and PSMB10 to form the immunoproteasome. The immunoproteasome functions in degradation of proteins into fragments of the correct size for presentation on MHC class I molecules.

Select Reviews

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Commonly Studied Ubiquitin Targets

Target	M	P	S
ADRM1	●	●	●
AMFR		●	
APC1	●		
APC2		●	●
APC3	●	●	●
APC11	●		●
BAP1	●		
Phospho-BAP1 (Ser592)		●	
β-Trcp	●		
CAND1	●	●	●
c-Cbl	●		●
Phospho-c-Cbl (Tyr700)	●		
Phospho-c-Cbl (Tyr731)		●	
Phospho-c-Cbl (Tyr774)		●	
Cbl-b	●	●	●
UBC3		●	
CHIP	●		
COPS5	●	●	
CUL1		●	
CUL3		●	
CUL4A		●	
CYLD	●	●	
Phospho-CYLD (Ser418)		●	
DDB-1	●	●	
DDB-2	●		
E2-25K/Hip2	●	●	
HAUSP	●	●	
HECTH9	●		
ISG15	●	●	●
ITCH	●		
KEAP1	●	●	●
KLHL12	●		●
MIB1		●	
NAE1/APPBP1	●		
NEDD4	●	●	
NEDD4L		●	
Phospho-NEDD4L (Ser342)	●		
Phospho-NEDD4L (Ser448)		●	
NEDD8	●	●	
NPL4		●	
OTUB1	●		
OTULIN		●	
PA28α	●	●	
PA28β		●	
PA28γ		●	
PSMA2	●	●	
PSMA3	●	●	
PSMA5		●	
PSMA6		●	
PSMB5	●	●	
PSMB6	●		
PSMB7	●	●	
PSMB8/LMP7	●		
PSMC3/TBP1		●	
PSMC5/TRIP1		●	
PSMD10/Gankyrin		●	
PSMD11	●		
PSMD14	●	●	
Rad23B	●		
RBX1	●	●	
RCHY1		●	
S5a/PSMD4	●	●	
SENP1	●		
SENP3	●		
Sharpin	●		
Skp1	●	●	●
Skp2	●	●	●
SPINK3		●	
STAMBP		●	
SUMO-1	●	●	
SUMO-2/3	●		
SYVN1		●	●
TRIAD1		●	●
TRIM25	●		
TRIM27		●	
UBA2	●	●	●
UBC3B		●	
Ubc9	●	●	
Ubc12	●	●	
Ubc13		●	
UbcH5C	●		
UBE1a		●	
UBE1a/b		●	
UBE1L2/UBA6		●	
UBE2C		●	
UBE2L3	●	●	
UBE2N	●		
UBE2S	●		●
UBE2T	●		●
UBE3A	●		
Ubiquitin	●	●	
K48-linkage Specific Polyubiquitin	●	●	
K63-linkage Specific Polyubiquitin	●		
UBLE1A/SAE1		●	
UBR5		●	
UCHL1	●	●	●
UCHL3	●	●	●
USP1	●		●
USP2		●	
USP4		●	
USP8	●	●	
USP9X		●	●
USP10	●	●	●
USP13	●		
USP14	●	●	●
USP18	●		
VCP	●	●	
VHL		●	
VPRBP		●	

These protein targets represent key nodes within ubiquitin signaling pathways and are commonly studied in ubiquitin research. Primary antibodies, antibody conjugates, and antibody sampler kits containing these targets are available from CST.

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P Polyclonal Antibody
S SignalSilence® siRNA

7

2012–2014 CITATIONS

CST antibodies for NEDD4 have been cited over 7 times in high-impact, peer-reviewed publications from the global research community.

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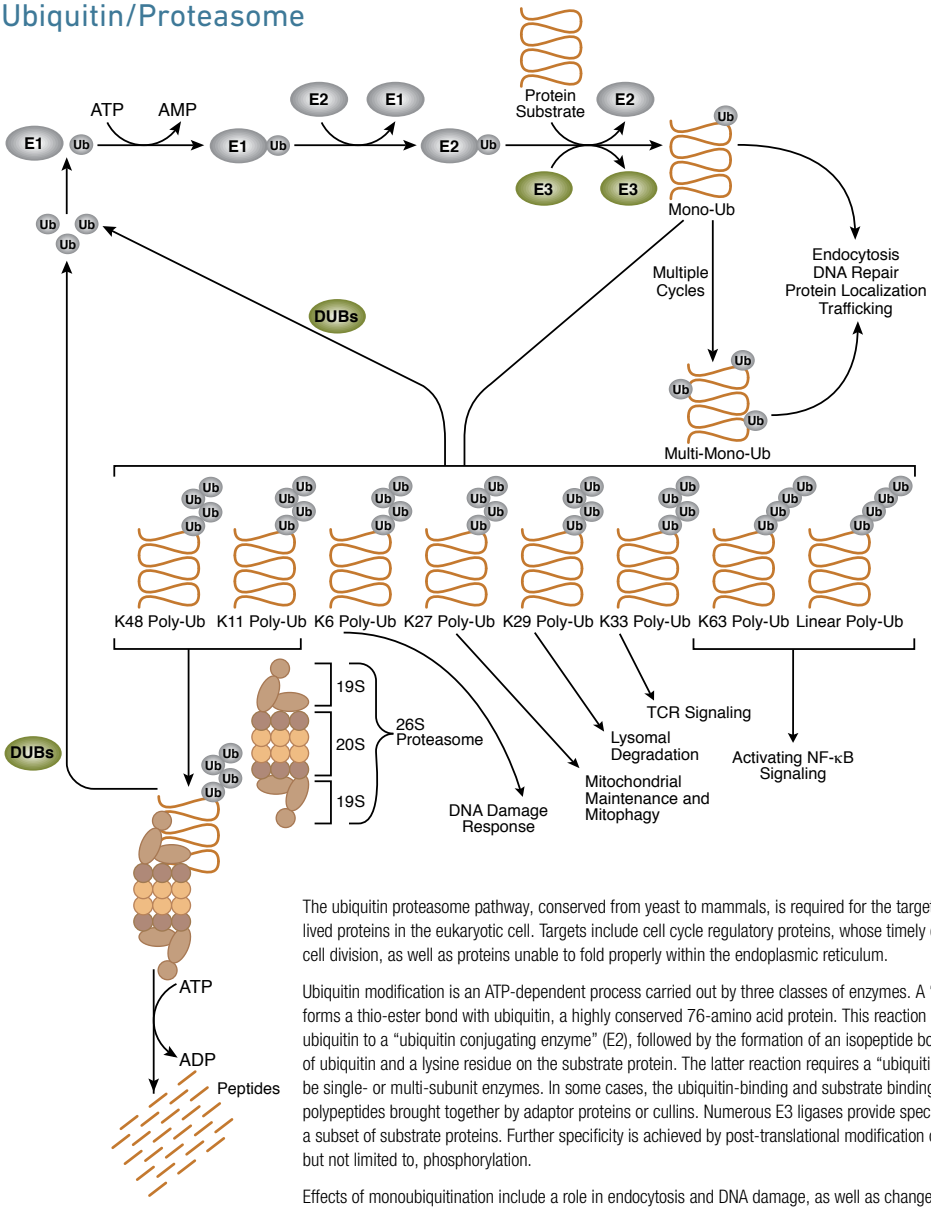
Ubiquitin/Proteasome



The ubiquitin proteasome pathway, conserved from yeast to mammals, is required for the targeted degradation of most short-lived proteins in the eukaryotic cell. Targets include cell cycle regulatory proteins, whose timely destruction is vital for controlled cell division, as well as proteins unable to fold properly within the endoplasmic reticulum.

Ubiquitin modification is an ATP-dependent process carried out by three classes of enzymes. A "ubiquitin activating enzyme" (E1) forms a thio-ester bond with ubiquitin, a highly conserved 76-amino acid protein. This reaction allows subsequent binding of ubiquitin to a "ubiquitin conjugating enzyme" (E2), followed by the formation of an isopeptide bond between the carboxy-terminus of ubiquitin and a lysine residue on the substrate protein. The latter reaction requires a "ubiquitin ligase" (E3). E3 ligases can be single- or multi-subunit enzymes. In some cases, the ubiquitin-binding and substrate binding domains reside on separate polypeptides brought together by adaptor proteins or cullins. Numerous E3 ligases provide specificity in that each can modify only a subset of substrate proteins. Further specificity is achieved by post-translational modification of substrate proteins, including, but not limited to, phosphorylation.

Effects of monoubiquitination include a role in endocytosis and DNA damage, as well as changes in subcellular protein localization and trafficking. However, multiple ubiquitination cycles resulting in a polyubiquitin chain are required for targeting a protein to the proteasome for degradation. The multisubunit 26S proteasome recognizes, unfolds, and degrades polyubiquitinated substrates into small peptides. The reaction occurs within the cylindrical core of the proteasome complex, and peptide bond hydrolysis employs a core threonine residue as the catalytic nucleophile. Polyubiquitin chains are also indicated in diverse cellular processes including DNA damage response, mitochondrial maintenance and mitophagy, lysosomal degradation, T Cell Receptor signaling, and NF-κB signaling.

Ubiquitinating enzymes (UBEs) catalyze protein ubiquitination, a reversible process countered by deubiquitinating enzyme (DUB) action. Five DUB subfamilies are recognized, including the USP, UCH, OTU, MJD, and JAMM enzymes. In humans, there are three proteasomal DUBs: PSMD14 (POH1/RPN11), UCH37 (UCH-L5), and Ubiquitin-Specific Protease 14, which is also known as the 60 kDa subunit of tRNA-guanine transglycosylase (USP14/TGT60 kDa).

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Ubiquitin Ligases

This table provides a list of E3 ubiquitin ligases, along with their substrates (when known), and corresponding references. This table was generated using PhosphoSitePlus®, Cell Signaling Technology's protein modification resource.

Ligase	Substrate	Function	PMID
AMFR	KAI1	AMFR is also known as gp78. AMFR is an integral ER membrane protein and functions in ER-associated degradation (ERAD). AMFR has been found to promote tumor metastasis through ubiquitination of the metastasis suppressor, KAI1.	18037895
APC/CDC20	Cyclin B, Securin	The anaphase promoting complex/cyclosome (APC/C) is a multiprotein complex with E3 ligase activity that regulates cell cycle progression through degradation of cyclins and other mitotic proteins. APC is found in a complex with CDC20, CDC27, SPATC1, and TUBG1.	17609108, 12070128
APC/Cdh1	CDC20, Cyclin B, Cyclin A, Aurora A, Skp2, Claspin	The anaphase promoting complex/cyclosome (APC/C) is a multiprotein complex with E3 ligase activity that regulates cell cycle progression through degradation of cyclins and other mitotic proteins. The APC/C-Cdh1 dimeric complex is activated during anaphase and telophase, and remains active until onset of the next S phase.	10548110, 11562349, 15014503, 19477924
ARIH1	4EHP	ARIH1 is an E3 ubiquitin ligase that may regulate protein translation by targeting eIF4E2 for ubiquitination and degradation by the proteasome.	14623119
BIRC2	Smac, TRAF2	BIRC2 is an apoptotic suppressor that prevents caspase activation by forming a complex with TNF receptor associated factors 1 and 2 (TRAF1 and TRAF2), which is then recruited to the tumor necrosis factor receptor 2 (TNFR2).	12525502, 18434593
BIRC3	Caspase 3 and 7, Smac, TRAF1	BIRC3 is an apoptotic suppressor that prevents caspase activation by forming a complex with TNF receptor associated factors 1 and 2 (TRAF1 and TRAF2), which is then recruited to the tumor necrosis factor receptor 2 (TNFR2).	10862606, 12525502, 15468071
BIRC4	Caspase 3, Smac, MEKK2	BIRC4 is an apoptotic suppressor that prevents caspase activation by forming a complex with TNF receptor associated factors 1 and 2 (TRAF1 and TRAF2), which is then recruited to the tumor necrosis factor receptor 2 (TNFR2). BIRC4 is also known as XIAP.	11447297, 12121969, 18761086
BIRC7	Smac	BIRC7 is an E3 ubiquitin ligase with anti-apoptotic activity. BIRC7 supports cell survival by targeting Smac for ubiquitination and degradation by the proteasome.	16729033
Bmi1	H2A K119	Bmi1 is a component of the polycomb group multiprotein PRC1- like (PcG PRC1) complex. Bmi1 is required for stimulating PcG PRC1 ubiquitin-protein ligase activity.	18650381
BRCA1	ER-a, Rpb8, CtIP, FANCD2	BRCA1 is an E3 ubiquitin ligase that maintains genomic stability by repairing DNA damage. Research studies have shown that mutations of this gene have been linked to breast cancer.	17392432, 17283126, 16818604, 11239454
C6orf157	Cyclin B	C6orf157 is also known as H10BH. C6orf157 is an E3 ubiquitin ligase that has been shown to ubiquitinate cyclin B.	15749827
Cbl		Cbl-b and c-Cbl are members of the Cbl family of adaptor proteins that are highly expressed in hematopoietic cells. Cbl proteins possess E3 ubiquitin ligase activity that downregulates numerous signaling proteins and RTKs in several pathways such as EGFR, T cell and B cell receptors, and integrin receptors. Cbl proteins play an important role in T cell receptor signaling pathways.	18759930, 9797470
CBLL1	CDH1	CBLL1 is also known as Hakai. CBLL1 is an E3 ubiquitin ligase that ubiquitinates the phosphorylated form of E-Cadherin, causing its degradation and loss of cell-cell adhesions.	11836526
CHFR	PLK1, Aurora A	CHFR is an E3 ubiquitin ligase that functions as a mitotic stress checkpoint protein that delays entry into mitosis in response to stress. CHFR has been shown to ubiquitinate and degrade the kinases PLK1 and Aurora A.	14562038, 19326084
CHIP	HSP70/90, iNOS, Runx1, LRRK2	CHIP is an E3 ubiquitin ligase that acts as a co-chaperone protein and interacts with several heat shock proteins, including HSP70 and HSP90, as well as the nonheat shock proteins iNOS, Runx1, and LRRK2.	19913553, 19362296, 19524548, 19536328
CUL3/ BACURD	RhoA	CUL3/BACURD is a ubiquitin ligase complex composed of CUL3 and the BTB domain adaptor BACURD. CUL3/BACURD controls actin cytoskeleton structure and cell movement by promoting ubiquitination and degradation of small GTPase RhoA.	19782033
CUL3/HIB/ SPOP	Ci/Gli	CUL3/HIB/SPOP is an E3 ubiquitin ligase complex composed of Cullin3, Hedgehog-induced MATH and BTB domain-containing protein (HIB), and SPOP. CUL3/HIB/SPOP targets the Hedgehog pathway transcription factor (Ci)/Gli for ubiquitination and degradation by the proteasome.	16740475
CUL3/KEAP1	Nrf2, IKKβ	CUL3/KEAP1 is part of an E3 ubiquitin ligase complex composed of RBX1, CULlin3 and the substrate recognition component, KEAP1. CUL3/KEAP1 targets Nrf2, a transcription factor that regulates antioxidant genes in response to oxidative stress for ubiquitination and degradation by the proteasome. In addition, CUL1/KEAP1 E3 ligase downregulates NF-κB signaling by targeting IKKβ ubiquitination.	12682069, 19818716
CUL3/ MEL-26	mei-1	CUL3/MEL-26 is an E3 ubiquitin ligase complex composed of Cullin3 and the substrate recognition component, MEL-26. MEL-26 targets mei-1 for ubiquitination and subsequent proteasomal degradation.	13679922

Ligase	Substrate	Function	PMID
CUL3/Ctb9/ KLHDC5	p60/katanin	CUL3/Ctb9/KLHDC5 is an E3 ubiquitin ligase complex required for efficient p60/katanin removal through promoting ubiquitination of p60/katanin to allow normal mitotic progression in mammalian cells.	19261606
CUL3/KLHL3	WNK1, WNK4	CUL3/KLHL3 is an E3 ubiquitin ligase complex composed of Cullin3 and kelch-like 3 (KLHL3) protein. WNK4 is a known target of CUL3/KLHL3-mediated ubiquitination and errors in that process are a common mechanism of human hereditary hypertension. Furthermore, mutations in KLHL3 and Cullin3 were identified to cause the human hypertensive disease pseudohypoaldosteronism type II (PHAII).	23387299, 23453970
CUL3/KLHL8	Rapsyn	CUL3/KLHL8, an E3 ubiquitin ligase complex, controls rapsyn stability through polyubiquitination, which is required for clustering of nicotinic acetylcholine receptors (nAChRs) at the neuromuscular junction.	19158078
CUL3/ KLHL12	Dsh	CUL3/KLHL12 E3 ubiquitin ligase targets Dishevelled for poly-ubiquitination and degradation, thus negatively regulating the Wnt/β-catenin pathway.	16547521
CUL3/ KLHL9/ KLHL13	Aurora B	CUL3/KLHL9/KLHL13 E3 ubiquitin ligase controls the dynamic behavior of mitotic chromosomes through ubiquitination of Aurora B, thereby coordinating mitotic progression and completion of cytokinesis.	17543862
CUL3/ KLHL20	PML, DAPK	CUL3/KLHL20 E3 ubiquitin ligase mediates hypoxia-induced PML proteasomal degradation via activation by HIF-1. The CUL3/KLHL20 complex also controls interferon responses by promoting DAPK polyubiquitination and proteasomal degradation.	21840486, 20389280
CUL3/ KLHL22	PLK1	CUL/KLHL22 regulates localization of PLK1 on the kinetochore thereby controlling spindle assembly checkpoint (SAC) activation. Ubiquitination of PLK1 signals degradation-independent removal from kinetochores and fulfillment of SAC leading to mitotic progression.	23455478
CUL3/ KLHL25	4E-BP1	CUL3/KLHL25 E3 ubiquitin ligase complex targets hypophosphorylated 4E-BP1 for degradation. Regulation of 4E-BP1 protein levels by CUL3/KLHL25 ubiquitination induces homeostatic control over the mRNA binding protein, eIF4E, for which 4E-BP1 acts as a repressor protein.	22578813
CUL3/SPOP	Gli2/Gli3, Daxx, SRC-3, AR, PTEN	The CUL3/SPOP E3 ubiquitin ligase displays both tumor suppressor and oncogenic functions in several types of human tissue. CUL3/SPOP regulates the proteolysis of the oncogene SRC-3, where underexpression of CUL3/SPOP leads to overexpression of SRC-3 in prostate cancer. Inversely, SPOP, a direct transcriptional target of HIFs in some tissues, is overexpressed in 85% of kidney cancers. The opposing roles of SPOP protein in prostate and kidney cancers may result from degradation of different substrates, such as AR in prostate cancers and PTEN in kidney cancer.	16524876, 19684112, 21577200, 24508459, 24656772
CUL4/CDT2	Cdt1, p21, Set8, CHK1	CUL4/CDT2 is an E3 ubiquitin ligase complex composed of DCX (DDB1-CUL4-X-box) and the substrate recognition component, CUL4/CDT2. CUL4/CDT2 regulates cell cycle progression into S phase by targeting CDT1 and SPD1 for ubiquitination and degradation by the proteasome.	16949367, 18794347, 20932471, 23109433
CUL4/DDB1/ Cereblon	IKZF1, IKZF3	Cereblon forms an E3 ubiquitin ligase complex with CUL4 and DDB1 to regulate limb development. Lenalidomide-bound Cereblon targets IKZF1 and IKZF3 for ubiquitination and degradation, helping to prevent B cell malignancies.	20223979, 24292623
CUL4/COP1	c-Jun, p53, ETV1, ETV4, ETV5	CUL4/COP1 is an E3 ubiquitin ligase that mediates ubiquitination and subsequent proteasomal degradation of target proteins. COP1 targets the oncoprotein c-Jun, transcription factor ETV1 and may target the tumor suppressor p53 for ubiquitination and degradation.	12615916, 16931761, 21572435, 20062082, 21572435
CUL4/DDB2	XPC, H3, H4	CUL4/DDB2 is an E3 ubiquitin ligase complex composed of DCX (DDB1-CUL4-ROC1) and the substrate recognition component, CUL4/DDB2. CUL4/DDB2 may target histone H2A, histone H3, and histone H4 at sites of UV-induced DNA damage to induce ubiquitination and degradation by the proteasome.	15882621, 16678110
CUL4/FBW5	TSC2	CUL4/FBW5 is an E3 ubiquitin ligase complex composed of DCX (DDB1-CUL4-ROC1) and the substrate recognition component, CUL4/FBW5. CUL4/FBW5 regulates TSC2 protein stability and TSC complex turnover.	18381890
CUL4/β-TrCP	REDD1	CUL4/β-TrCP E3 ligase complex contains DCX (DDB1-CUL4-ROC1) and β-TrCP. This complex targets REDD1 for ubiquitination and subsequent proteasomal degradation to re-activate the mTOR signaling pathway as cells recover from hypoxic stress.	19557001
CUL4/RBBP7	p150	CUL4/RBBP7 is an E3 ubiquitin ligase complex composed of DCX (DDB1-CUL4-ROC1) and the substrate recognition component, RBBP7. CUL4/RBBP7 targets p150 for ubiquitination and degradation.	21228219
CUL4/DDB1/ TRCP4A	N-Myc, C-Myc	CUL4/TRCP4A is an E3 ubiquitin ligase complex composed of DDB1-CUL4 and the substrate recognition component, TRCP4A. CUL4/DDB1/TRCP4A targets Myc for ubiquitination and degradation.	20551172
CUL4/VPRBP	TET	CUL4/VPRBP is an E3 complex consisting of CRL4 (DDB1-CUL4-RBX1), and VPRBP (DCAF1). CRL4/VPRBP is essential for regulating mammalian oocyte survival and reprogramming via activation of TET methylcytosine dioxygenases.	24357321

Ligase	Substrate	Function	PMID
CUL5/SOCS1	Dab1	CUL5/SOCS1 is part of an SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin ligase complex. CUL5/SOCS1 targets components of the Jak/Stat pathway as well as Dab1, a regulator of cortical development, for ubiquitination and degradation by the proteasome.	17974915
CUL5/SOCS4	EGFR	CUL5/SOCS4 is part of an SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin ligase complex. SOCS4 may target components of cytokine signal transduction pathways, such as EGF receptor (EGFR) for ubiquitination and degradation by the proteasome.	17997974
CUL5/Vif	APOBEC3G	CUL5/Vif is part of an SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin ligase complex. Vif targets APOBEC3G and APOBEC3F for ubiquitination and degradation by the proteasome. The interaction of Vif with APOBEC3G also blocks its cytidine deaminase activity in a proteasome-independent manner,	15574592
CUL7/ FBXW8	cyclin D1	CUL7/FBXW8 is an SCF-like E3 ubiquitin ligase complex composed of SKP1, CUL7, RBX1, GLMN isoform 1, and the substrate recognition component, FBXW8.	17205132
DZIP3	H2AK119	DZIP3 is an E3 ubiquitin ligase that blocks transcriptional elongation by ubiquinating H2A at lysine 119.	12538761
E6-AP	p53, Dlg	E6-AP is also known as UBE3A. E6-AP is a HECT domain E3 ubiquitin ligase that interacts with Hepatitis C virus (HCV) core protein and targets it for degradation. The HCV core protein is central to packaging viral DNA and other cellular processes. E6-AP also interacts with the E6 protein of the human papillomavirus types 16 and 18, and targets the p53 tumor-suppressor protein for degradation.	17108031
FANCL	FANC D2	FANCL is an ubiquitin ligase protein integral to the DNA repair pathway.	12973351
HACE1		HACE1 is an E3 ubiquitin ligase and tumor suppressor. Research has shown that aberrant methylation of HACE1 is frequently found in Wilms' tumors and colorectal cancer.	17694067
HECTD1		HECTD1 is an ubiquitin E3 ligase required for neural tube closure and normal development of the mesenchyme.	17442300
HECTD2		HECTD2 is a probable E3 ubiquitin ligase and may act as a susceptibility gene for neurodegeneration and prion disease.	19214206
HECTD3		HECTD3 is a probable E3 ubiquitin ligase and may play a role in cytoskeletal regulation, actin remodeling, and vesicle trafficking.	18194665
HECW1	DVL1, mutant SOD1, p53	HECW1 is also known as NEDL1. HECW1 interacts with p53 and the Wnt signaling protein DVL1, and may play a role in p53-mediated cell death in neurons.	14684739, 18223681
HECW2	p73	HECW2 is also known as NEDL2. HECW2 ubiquitinates p73, which is a p53 family member. Ubiquitination of p73 increases protein stability.	12890487
HERC2	RNF8	HERC2 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC2 plays a role in the DNA damage response through interaction with RNF8.	20023648
HERC3		HERC3 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC3 interacts with hPLIC-1 and hPLIC-2 and localizes to the late endosomes and lysosomes.	18535780
HERC4		HERC4 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC4 is highly expressed in testis and may play a role in spermatogenesis.	17967448
HERC5		HERC5 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC5 is induced by interferon and other pro-inflammatory cytokines and plays a role in interferon-induced ISG15 conjugation during the innate immune response.	16407192, 16815975
HLTF	PCNA	HLTF is both a helicase and an E3 ubiquitin ligase. HLTF participates in postreplication repair (PRR) of damaged DNA by polyubiquitination of chromatin-bound PCNA.	18316726
HOIP	PKC	HOIP is the E3 ubiquitin ligase of the LUBEC (linear ubiquitin chain assembly complex) which ubiquitinates signaling proteins, targeting them for proteasomal degradation.	17069764
HUWE1	N-Myc, C-Myc, p53, Mcl-1, TopBP1	HUWE1 is also known as Mule. HUWE1 is a HECT domain E3 ubiquitin ligase that regulates degradation of Mcl-1 and therefore regulates DNA damage-induced apoptosis. HUWE1 also controls neuronal differentiation by destabilizing N-Myc, and regulates p53-dependent and independent tumor suppression via ARF.	15989957
HYD	CHK2	HYD is also known as EDD or UBR5. HYD is a regulator of the DNA damage response and is overexpressed in many forms of cancer.	18073532
IBRDC2	p21, Bax	IBRDC2 is an E3 ubiquitin ligase involved in the regulation of apoptosis. IBRDC2 expression can be induced by p53 and may target apoptosis related proteins p21 and Bax.	12853982
IBRDC3	UCKL-1	IBRDC3 is an E3 ubiquitin ligase involved in the cytolytic activities of hematopoietic natural killer cells and T cells.	16709802

Ligase	Substrate	Function	PMID
ITCH	MKK4, RIP2, Foxp3	ITCH plays a role in T cell receptor activation and signaling through ubiquitination of multiple proteins including MKK4, RIP2, and Foxp3. Loss of ITCH function leads to an aberrant immune response and T helper cell differentiation.	19737936, 19592251, 20108139
LNX1	NUMB	LNX1 is an E3 ubiquitin ligase that plays a role in cell fate determination during embryogenesis through regulation of NUMB, the negative regulator of Notch signaling.	11782429
LRSAM1	Tsg101	LRSAM is an E3 ubiquitin ligase that mediates intracellular vesicular trafficking by monoubiquitination of TSG101.	15256501
Mahogunin		Mahogunin is an E3 ubiquitin ligase involved in melanocortin signaling. Loss of mahogunin function leads to neurodegeneration and loss of pigmentation, and may be the mechanism of action in prion disease.	19737927, 19524515
MALIN	laforin	Malin, also known as NHLRC1, is an E3 ubiquitin ligase that promotes the ubiquitination and proteasomal degradation of misfolded proteins.	15930137
MARCH-I	HLA-DRβ	MARCH1 is an E3 ubiquitin ligase found on antigen presenting cells (APCs). MARCH1 ubiquitinates MHC class II proteins and downregulates their cell surface expression.	19880452
MARCH-II		MARCH-II is a member of the MARCH family of E3 ubiquitin ligases. It associates with syntaxin6 in the endosomes and helps to regulate vesicle trafficking.	15689499
MARCH-III		MARCH-III is a member of the MARCH family of E3 ubiquitin ligases. MARCH-III associates with syntaxin6 in the endosomes and helps to regulate vesicle trafficking.	16428329
MARCH-IV	MHC class I	MARCH-IV is a member of the MARCH family of E3 ubiquitin ligases. MARCH-IV ubiquitinates MHC class I proteins and downregulates their cell surface expression.	14722266
MARCH-V	DRP1	MARCH-V is a member of the MARCH family of E3 ubiquitin ligases. March-V is located in the mitochondria and aids in the control of mitochondrial morphology.	16936636
MARCH-VI		MARCH-VI is also known as TEB4 and is a member of the MARCH family of E3 ubiquitin ligases. It localizes to the endoplasmic reticulum and participates in ER-associated protein degradation.	16373356
MARCH-VII	gp190	MARCH-VII is also known as axotrophin. MARCH-VII was originally identified as a neural stem cell gene, but has since been shown to play a role in LIF signaling in T lymphocytes through degradation of the LIF receptor subunit, gp190.	19901269
MARCH-VIII	B7-2, MHC class II	MARCH-VIII is also known as c-MIR. MARCH-VIII causes the ubiquitination/degradation of B7-2, which is a co-stimulatory molecule for antigen presentation. MARCH-VIII has also been shown to ubiquitinate MHC class II proteins.	16785530
MARCH-IX	ICAM-1, MHC-I	MARCH-IX is a member of the MARCH family of E3 ubiquitin ligases. MARCH-IX mediates ubiquitination of transmembrane proteins, marking them for endocytosis and sorting to lysosomes via multivesicular bodies.	17174307, 14722266
MARCH-X		MARCH-X is also known as RNF190. MARCH-X is a member of the MARCH family of E3 ubiquitin ligases. MARCH-X may be involved in spermiogenesis.	21937444
MARCH-XI	CD4	MARCH-XI is a member of the MARCH family of E3 ubiquitin ligases. MARCH-IX mediates ubiquitination of CD4, marking it for endocytosis and sorting to lysosomes via multivesicular bodies.	17604280
MDM2	p53	MDM2, an E3 ubiquitin ligase for p53, plays a central role in regulation of the stability of p53. Akt-mediated phosphorylation of MDM2 at Ser166 and Ser186 increases its interaction with p300, allowing MDM2-mediated ubiquitination and degradation of p53.	9153395
MEKK1	c-Jun, Erk	MEKK1 is a well known protein kinase of the STE11 family. MEKK1 phosphorylates and activates MKK4/7, which in turn activates JNK1/2/3. MEKK1 contains a RING finger domain and exhibits E3 ubiquitin ligase activity toward c-Jun and Erk.	12049732, 17101801
MGRN1	Tsg101	MGRN1 is an E3 ubiquitin ligase that mediates intracellular vesicular trafficking by monoubiquitination of TSG101.	17229889
MIB1	Delta, Jagged	Mindbomb homolog 1 (MIB1) is an E3 ligase that facilitates the ubiquitination and subsequent endocytosis of the Notch ligands, Delta and Jagged.	16000382
MIB2	Delta, Jagged	Mindbomb 2 (MIB2) is an E3 ligase that positively regulates Notch Signaling. MIB2 has been shown to play a role in myotube differentiation and muscle stability. MIB2 ubiquitinates NMDAR subunits to help regulate synaptic plasticity in neurons.	15824097, 18216171, 17962190
MID1	PP2A	Mid1, also known as Midline-1, is an E3 ubiquitin ligase that may target protein phosphatase 2 for ubiquitination and proteasomal degradation.	11685209
MKRN1	hTERT, p53, CDKN1A, FLIP1	MKRN1 is an E3 ubiquitin ligase that regulates both anti- and pro-apoptotic functions.	15805468
MycBP2	Fbxo45, TSC2	MycBP2 is an E3 ubiquitin ligase also known as PAM. MycBP2 associates with Fbxo45 to play a role in neuronal development. MycBP2 also regulates the mTOR pathway through ubiquitination of TSC2.	19398581, 18308511

Ligase	Substrate	Function	PMID
NEDD4		NEDD4 is an E3 ubiquitin ligase highly expressed in the early mouse embryonic central nervous system. NEDD4 downregulates both neuronal voltage-gated Na+ channels (NaVs) and epithelial Na+ channels (ENaCs) in response to increased intracellular Na+ concentrations.	9618557, 9792722
NEDD4L	Smad2, PTEN	NEDD4L is an E3 ubiquitin ligase highly expressed in the early mouse embryonic central nervous system. NEDD4L has been shown to negatively regulate TGF-β signaling by targeting Smad2 for degradation.	19917253, 17218260
NEURL	Jagged 1, Delta	NEURL is an E3 ubiquitin ligase involved in Notch signaling and neurological determination of cell fate.	17003037, 11696324
OSTM1	Gai3	OSTM1 is an E3 ubiquitin ligase localized to the cell membrane that regulates membrane associated G-proteins by ubiquitination and proteasomal degradation.	12826607
PARC		PARC is a cullin family member that acts as a p53-binding cytoplasmic anchor protein and is part of an atypical cullin-RING- based E3 ubiquitin ligase complex.	12526791
Parkin	Pael-R, CDC-rel, PLC-g1,MFN1	Parkin is an E3 ubiquitin ligase that has been shown to be a key regulator of the autophagy pathway. Mutations in Parkin can lead to Parkinson's Disease.	20074049, 18671761, 17553932, 16672220
PCGF1	H2A, K119	PCGF1 is a component of the polycomb group multiprotein PRC1- like (PcG PRC1) complex. PCGF1 is required for PcG PRC1 mediated monoubiquitination of H2A Lys119, which is central to the histone code and gene regulation.	18460542
PELI1	TRIP, IRAK	PELI1 is an E3 ubiquitin ligase that plays a role in Toll-like Receptor (TLR3 and TLR4) signaling to NF-κB via the TRIP adaptor protein. PELI1 has also been shown to ubiquitinate IRAK.	19734906, 17675297
PEX10	Pex5	PEX10 is localized to peroxisome membranes and has been associated with several peroxisomal biogenesis disorders.	15283676
PJA1	ELF	PJA1 is also known as PRAJA. PJA1 plays a role in downregulating TGF-β signaling in gastric cancer via ubiquitination of the Smad4 adaptor protein ELF.	16096365
PJA2		PJA2 is an E3 ubiquitin ligase found in neuronal synapses. The exact role and substrates of PJA2 are unclear.	12036302
RAD18	PCNA	RAD18 is an E3 ubiquitin ligase involved in post-replication repair of UV-damaged DNA.	17720710, 18245774
RBCK1	SOCS6, PKC, TAB2/3	RBCK1 is an E3 ligase that acts as an iron sensor by promoting the ubiquitination of oxidized IREB2 in the presence of high iron and oxygen. RBCK1 is a component of the LUBAC (linear ubiquitin chain assembly complex).	17449468, 16643902,
RCHY1	p27 Kip1, p53	RCHY1, also known as Pirh2, is an E3 ubiquitin ligase that contributes to the regulation of the cell cycle. RCHY1 is primarily associated with the ubiquitination and proteasomal degradation of tetrameric p53.	12654245, 18006823, 18344599
RFFL	p53	RFFL is also known as CARP2 and is an E3 ubiquitin ligase that inhibits endosome recycling. RFFL also degrades p53 through stabilization of MDM2.	15229288, 18382127
RFWD2	MTA1, p53, FoxO1	RFWD2 is also known as COP1. RFWD2 is an E3 ubiquitin ligase that ubiquitinates several proteins involved in the DNA damage response and apoptosis including MTA1, p53, and FoxO1.	19805145, 16931761, 18815134
Rictor	SGK1	Rictor interacts with CULlin1-Rbx1 to form an E3 ubiquitin ligase complex and promotes ubiquitination and degradation of SGK1.	20832730
RING1	H2A, K119	RING1, also known as RNF1, is an E3 ubiquitin ligase of the polycomb group multiprotein PRC1-like (PcG PRC1) complex. RING1 is required for PcG PRC1 mediated monoubiquitination of H2A Lys119, which is central to the histone code and gene regulation.	16359901
RNF2	H2A, K119, Geminin	RNF2, also known as Ring2, is an E3 ubiquitin ligase of the polycomb group multiprotein PRC1-like (PcG PRC1) complex. RNF2 is required for PcG PRC mediated monoubiquitination of H2A Lys119, which is central to the histone code and gene regulation.	17157253, 15509584
RNF5	JAMP, paxillin	RNF5 is also known as RMA5. RNF5 plays a role in ER-associated degradation of misfolded proteins and ER stress response through ubiquitination of JAMP. RNF5 also plays a role in cell motility and has been shown to ubiquitinate paxillin.	19269966, 12861019
RNF6	LIM1, Androgen receptor	RNF6 is an E3 ubiquitin ligase involved in the regulation of cell motility and differentiation. RNF6 targets LIMK for ubiquitination and degradation, inhibiting cytoskeleton stability.	16204183
RNF8	H2A, H2AX	RNF8 is a RING domain E3 ubiquitin ligase that plays a role in the repair of damaged chromosomes. RNF8 ubiquitinates Histone H2A and H2A.X at double-strand breaks (DSBs) which recruits 53BP1 and BRCA1 repair proteins.	18001824
RNF11	Smurf2	RNF11 is a required component of a ubiquitin-editing protein complex involved in modifying cellular inflammatory response to LPS and TNF signaling.	14562029
RNF12	CLIM, Ldb1, Ldb2	RNF12, also known as RLIM, is an E3 ubiquitin ligase. RNF12 is involved in telomere regulation and X chromosome inactivation.	12874135, 11882901

Ligase	Substrate	Function	PMID
RNF19	SOD1	RNF19 is also known as Dorfin. Accumulation and aggregation of mutant SOD1 leads to ALS disease. RNF19 ubiquitinates mutant SOD1 protein, causing a decrease in neurotoxicity.	19610091
RNF20	Histone H2B	RNF20 is also known as BRE1. RNF20 is an E3 ubiquitin ligase that mono-ubiquitinates Histone H2B. H2B ubiquitination is associated with areas of active transcription.	18832071
RNF34	Caspase-8, -10	RNF34 is also known as RFI. RNF34 inhibits death receptor mediated apoptosis through ubiquitination/degradation of caspase-8 and -10.	16596200
RNF40	Histone H2B	RNF40 is also known as BRE1-B. RNF40 forms a protein complex with RNF20 resulting in the ubiquitination of Histone H2B. H2B ubiquitination is associated with areas of active transcription.	16307923
RNF41	ErbB3, BIRC6, Parkin	RNF41 is an E3 ubiquitin ligase that has been implicated in the regulation of hematopoietic progenitor cell differentiation.	14765125, 12411582, 18541373
RNF111	Smad, SnoN, c-Ski	RNF111 is an E3 ubiquitin ligase that participates in mesoderm patterning by promoting the ubiquitination and proteasomal degradation of downstream Smads.	18451154, 14657019, 17591695
RNF123	CDKN1B	RNF123 is an E3 ubiquitin ligase that functions as part of the KPC complex. RNF123 aids in cell cycle regulation by targeting CDKN1B for ubiquitination and proteasomal degradation during G1.	15531880
RNF125		RNF125 is also known as TRAC-1. RNF125 has been shown to positively regulate T cell activation.	17990982
RNF128		RNF128 is also known as GRAIL. RNF128 promotes T cell anergy and may play a role in actin cytoskeletal organization in T cell/APC interactions.	19833735
RNF135	RIG-1	RNF135 is an E3 ubiquitin ligase involved in viral innate immunity. RNF135 targets the cytoplasmic viral nucleic acid receptor RIG-1 for ubiquitination and degradation by the proteasome.	19017631
RNF138	TCF/LEF	RNF138 is also known as NARF. RNF138 is associated with Nemo-like Kinase (NLK) and suppresses Wnt/β-Catenin signaling through ubiquitination/degradation of TCF/LEF.	16714285
RNF167	TSSC5 (SLC22A18)	RNF167 may act as an E3 ubiquitin ligase involved in the regulation of kidney transporter function.	16314844
RNF168	H2A, H2A.X	RNF168 is an E3 ubiquitin ligase that helps protect genome integrity by working together with RNF8 to ubiquitinate Histone H2A and H2A.X at DNA double-strand breaks (DSB).	19203579
RNF180	Zic2	RNF180 is an E3 ubiquitin ligase involved in neurological development. RNF180 targets the ZIC2 transcription factor for polyubiquitination and degradation by the proteasome.	18363970
RNF182	ATP6V0C	RNF 182 is an E3 ubiquitin ligase that targets ATP6V0C, a component of vacuolar ATPase, for polyubiquitination and degradation by the proteasome.	18298843
RNF190		see MARCH-X	
SCF/β-TrCP	IκBα, Wee1, Cdc25A, β-Catenin	SCF/β-TrCP is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, β-TrCP (also known as BTRC). SCF/β-TrCP mediates the ubiquitination of proteins involved in cell cycle progression, signal transduction, and transcription. SCF/β-TrCP also regulates the stability of β-catenin and participates in Wnt signaling.	10230406, 15070733, 14603323, 10339577
SCF/FBXW2	GCMa	SCF/FBXW2 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW2. SCF/FBXW2 promotes ubiquitination of GCMa which is important for trophoblast cell differentiation.	15640526
SCF/FBXW5	SASS6, Eps8	SCF/FBXW5 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW5. SCF/FBXW5 mediates the ubiquitination of SASS6, preventing centriole duplication.	18381890, 23314863
SCF/FBXW7	Cyclin-E, c-Myc, c-Jun	SCF/FBXW7 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW7. SCF/FBXW7 mediates the ubiquitination of proteins involved in cell cycle progression, signal transduction, and transcription. Target proteins for SCF/FBXW7 include the phosphorylated forms of c-Myc, Cyclin E, Notch intracellular domain (NICD), and c-Jun. Research has found that defects in FBXW7 may be a cause of many types of human cancers including T-ALL, colon cancer and breast cancer.	11533444, 15150404, 16023596
SCF/FBXW8	IRS-1, TBC1D3, Cyclin D1, IGFBP2, HPK1	SCF/FBXW8 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW8. SCF/FBXW8 plays critical roles in various cellular processes such as cell cycle progression, cell differentiation, development, and growth factor signaling pathway.	23142081, 23029530, 17205132, 24362026
SCF/FBXW10	CBX1, CBX5	SCF/FBXW10 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW10. SCF/FBXW10 contributes to gene expression by degradation of heterochromatin components CBX5 and CBX1.	20498703

Ligase	Substrate	Function	PMID
SCF/ FBXW15	HBO1	SCF/FBXW15 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW15. SCF/FBXW15 controls DNA replication via degradation of the origin recognition complex protein HBO1.	23319590
SCF/Skp2/ FBXL1	p27, p21, FoxO1	SCF/Skp2 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, Skp2. SCF/Skp2 mediates the ubiquitination of proteins involved in cell cycle progression (specifically the G1/S transition), signal transduction and transcription. Target proteins for SCF/Skp2 include the phosphorylated forms of p27Kip1, p21Waf1/Cip1, and FoxO1.	15668399, 10559916
SCF/FBXL2	Aurora B, p85b, APP, Cyclin D2	SCF/FBXL2 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL2. SCF/FBXL2 is involved in cell cycle regulation, growth factor signaling and synapse formation.	23928698, 23604317, 22399757, 22323446
SCF/FBXL3	CRY1, CRY2	SCF/FBXL3 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL3. SCF/FBXL3 mediates circadian clock function by ubiquitination and subsequent degradation of CRY1 and CRY2.	17463251, 17463252, 17462724
SCF/FBXL4	KDM4A/JMJD2A	SCF/FBXL4 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL4. SCF/FBXL4 regulates chromatin structure through degradation of the lysine demethylase KDM4A/JMJD2A.	21757720
SCF/FBXL5	IRP2, p150(Glued)	SCF/FBXL5 is an ubiquitin ligase complex also known as SCF (SKP1-cullin-F-box). FBXL5 is an F-box protein that functions as an iron sensor by promoting the ubiquitination and subsequent degradation of IREB2/IRP2 under high iron and oxygen conditions.	19762597, 19762596
SCF/FBXL7	Aurora A	SCF/FBXL7 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL7. SCF/FBXL7 colocalizes with and promotes degradation of Aurora A during mitosis leading to impaired cell proliferation.	22306998
SCF/FBXL10	p15, c-Fos, Ink4a, RipK3	SCF/FBXL10 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL10. SCF/FBXL10 plays critical roles in cell proliferation, senescence and stem cell self-renewal.	18836456, 21252908, 21540074, 22825849
SCF/FBXL11	p65	FBXL11 is a lysine demethylase that regulates NF-κB signaling pathway through p65 demethylation. A substrate for ubiquitination has yet to be identified.	20080798
SCF/FBXL12	Ku80, Calmodulin kinase I	SCF/FBXL12 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL12. SCF/FBXL12 is involved in regulating cell cycle progression and DNA damage response.	23324393, 23707388
SCF/FBXL14	SNAIL1, SLUG, Mkp3	SCF/FBXL14 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component,FBXL14. SCF/FBXL14 is associated with metastasis and cancer development.	19955572, 16887825, 22410791
SCF/FBXL15	Timeless, SMURF1, SMURF2	SCF/FBXL15 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL15. FBXL15 targets negative regulators of the BMP signaling pathway, including SMURF1 and SMURF2, for ubiquitination and subsequent proteasomal degradation. FBXL15 is required for dorsal/ventral pattern formation and bone mass maintenance. SCF/FBXL15 also targets the *Drosophila* circadian clock protein timeless.	16794082, 21572392
SCF/FBXL19	ST2L, Rac1, RhoA	SCF/FBXL19 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL19. SCF/FBXL19 is an important regulator of cell proliferation and migration, cytoskeleton reorganization, and pulmonary inflammation.	22660580, 23512198, 23871831
SCF/FBXL20	RIM1	SCF/FBXL20 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL20. FBXL20 is localized to the synapse and its regulation of RIM1 by ubiquitination may play a role in neural transmission.	17803915
SCF/FBXL21	CRY	SCF/FBXL21 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL21. SCF/FBXL21 maintains circadian clock rhythms through controlling CRY turnover cooperatively with SCF/FBXL3.	23452856, 23452855, 18953409
SCF/FBXL22	ACTN/FLNC	SCF/FBXL22 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL22. FBXL22 is exclusively expressed in cardiomyocytes, and promotes ubiquitination and degradation of sarcomeric proteins, α-actinin-2 (ACTN) and Filamin C (FLNC).	22972877
SCF/FBXO1	CP110, RRM2	SCF/FBXO1 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO1. FBXO1 targets CP110 for ubiquitination and subsequent proteasomal degradation during cell cycle G2 phase, thereby inhibiting centrosome reduplication.	20596027, 22632967

Ligase	Substrate	Function	PMID
SCF/FBXO2	Pre-integrin β-1, CFTR, Connexin 26, NR1, SHPS-1, UL9	SCF/FBXO2 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO2 (NFB42). FBXO2 targets misfolded glycoproteins for ubiquitination and proteasomal degradation by recognition of sugar chains in the endoplasmic reticulum-associated degradation (ERAD) pathway.	12140560, 15809437, 14701835, 17494702
SCF/FBXO3	HIPK2, p300, Fbxl2	SCF/FBXO3 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO3. FBXO3 targets HIPK2 and p300 for ubiquitination and rapid degradation by the proteasome. The inclusion of PML in a complex with SCF/FBXO3, HIPK2, and p300 delays degradation of HIPK2 and allows synergistic activation of p53/TP53-dependent transactivation.	18809579, 23542741, 18809579
SCF/FBXO4	TERF1, Cyclin D1	SCF/FBXO4 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO4. FBXO4 may play a role in telomere homeostasis by recognition of TERF1 and promotion of its ubiquitination together with UBE2D1.	17081987, 19645770, 17081987
SCF/FBXO6	Chk1, ATRN, TCRa, TFRC	SCF/FBXO6 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO6. FBXO6 targets misfolded glycoproteins for ubiquitination and proteasomal degradation by recognition of sugar chains in the endoplasmic reticulum-associated degradation (ERAD) pathway. FBXO6 also targets the kinase Chk1, a cell cycle regulator involved in entry into mitosis.	19716789, 22268729, 12939278
SCF/FBXO7	BIRC2, DLGAP5, CD43	SCF/FBXO7 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO7. SCF/FBXO7 targets BIRC2 (cIAP1), an inhibitor of apoptosis, and DLGAP5, a cell cycle regulator, for ubiquitination and proteasomal degradation.	16510124, 21652635
SCF/FBXO8	Arf6	SCF/FBXO8 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO8. FBXO8 mediates the ubiquitination of Arf6. SCF/FBXO8 may function as a guanine nucleotide exchange factor (GEF) that activates ARF G proteins. Loss of FBXO8 correlates with poor survival in hepatocellular carcinoma.	18094045
SCF/FBXO9	Tel2, Tti1	SCF/FBXO9 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO9. Three isoforms of the human protein are produced by alternative splicing, and it is linked to promoting survival in multiple myeloma.	23263282
SCF/FBXO10	Bcl2	SCF/FBXO10 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO10. SCF/FBXO10 induces apoptosis in B-cells through degrading Bcl2.	23431138
SCF/FBXO11	Cdt2, Bcl6, p53	SCF/FBXO11 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO11. SCF/FBXO11 promotes the neddylation of p53 to suppress its transcriptional activity, and crosstalks with the cullin 4-RING ubiquitin ligase, CRL4-Cdt2, to control cell cycle progression. Mutations and deletions in FBXO11 lead to BCL6 stabilization and B-cell lymphoma. FBXO11 is also regarded as a haplo-insufficient tumor suppressor gene.	23478445, 23478441, 23892434, 22113614, 17098746
SCF/FBXO15	P-glycoprotein/ABCB1	SCF/FBXO15 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO15. SCF/FBXO15 activity may affect anticancer drug resistance through controlling the expression of P-glycoprotein (P-gp)/ABCB1 on cancer cell surfaces.	23465077
SCF/FBXO17	Arf1	SCF/FBXO17 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO17. In fission yeast, SCF/FBXO17 binds the Arf1 transcription factor and promotes Arf degradation to suppress stress-related gene expression. Stress-induced Arf phosphorylation dissociates Arf interaction with FBXO17.	19836238
SCF/FBXO22	KDM4A	SCF/FBXO22 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO22. SCF/FBXO22 is involved in transcriptional control through degradation of lysine demethylase KDM4A.	21768309
SCF/FBXO25	NKX2.55, Isl1, Hand1, Elk-1	SCF/FBXO25 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO25. FBXO25 is a cardiac-specific F-box protein. SCF/FBXO25 maintains cardiac protein homeostasis through degrading NKX2.5, Isl1, and Hand1, and suppresses mitogenic response by downregulating Elk-1.	21596019, 23940030
SCF/FBXO31	Cyclin D1, Par6c	SCF/FBXO31 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO31 (FBXO14). SCF/FBXO31 targets phosphorylated cyclin D1 for ubiquitination and degradation by the proteasome, resulting in G1 cell cycle arrest.	19412162
SCF/FBXO32	DUSP1, eIF3-f, MyoD, BK-β(1), FOXO1	SCF/FBXO32 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO32 (MAFbx). FBXO32 is a cardiac-specific F-box protein. SCF/FBXO32 maintains cardiac protein homeostasis. FBXO32 promoter is highly methylated in pediatric soft-tissue sarcoma.	22586590, 21454680, 19073596, 24213577

Ligase	Substrate	Function	PMID
SCF/FBXO33	YB-1	SCF/FBXO33 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO33. SCF/FBXO33 is involved in gene expression and protein translation through degradation of Y-box protein YB-1.	16797541
SCF/FBXO40	IRS-1	SCF/FBXO40 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO40. FBXO40 is a skeletal muscle-specific F-box protein. SCF/FBXO40 controls muscle size by regulating IRS-1 protein stability.	22033112
SCF/FBXO42	p53	SCF/FBXO42 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO42. FBXO42 targets p53/TP53 for ubiquitination and degradation by the proteasome.	19509332
SCF/FBXO44	BRCA1	SCF/FBXO44 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO44. SCF/FBXO44 activity may contribute to breast cancer development through targeting BRCA1 for proteasomal degradation.	23086937
SCF/FBXO45	UNC13A, p73	SCF/FBXO45 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXO45. SCF/FBXO45 aids in the regulation of neurotransmission at mature neurons by targeting UNC13A for ubiquitin dependent degradation by the proteasome. FBXO45 also targets the apoptotic protein p73 for ubiquitination and degradation.	19581926
SHPRH	PCNA	SHPRH is an E3 ubiquitin ligase that plays a role in DNA replication through ubiquitination of PCNA. PCNA ubiquitination prevents genomic instability from stalled replication forks after DNA damage.	18719106
SIAH1	β-catenin, Bim, TRB3	SIAH1 is an E3 ubiquitin ligase that plays a role in inhibition of Wnt signaling through ubiquitination of β-catenin. SIAH1 has also been shown to promote apoptosis through upregulation of Bim, and to ubiquitinate the signaling adaptor protein TRB3.	16413921, 19775288, 18276110
SIAH2	HIPK2, PHD1/3	SIAH2 is an E3 ubiquitin ligase that plays a role in hypoxia through ubiquitination and degradation of HIPK2. SIAH2 also ubiquitinates PHD1/3, which regulates levels of HIF-1α in response to hypoxia.	19043406, 15210114
SMURF1	Smad1/5, RhoA, MEKK2	SMURF1 is an E3 ubiquitin ligase that interacts with BMP pathway Smad effectors, leading to Smad protein ubiquitination and degradation. Smurf1 negatively regulates osteoblast differentiation and bone formation *in vivo*.	10458166, 15820682
SMURF2	Smads, Mad2	SMURF2 is an E3 ubiquitin ligase that interacts with Smads from both the BMP and TGF-β pathways. SMURF2 also regulates the mitotic spindle checkpoint through ubiquitination of Mad2.	11158580, 18852296
SYVN1	ERAD, Pael-R,p53, IRE-1	SYVN1 is an E3 ubiquitin ligase involved in the ER-associated degradation (ERAD) pathway. SYVN1 targets misfolded proteins and appropriately folded short-lived proteins for ubiquitination and degradation by the proteasome.	14593114, 17059562, 17170702, 18369366
TOPORS	p53, NKX3.1	TOPORS is an E3 ubiquitin ligase and a SUMO ligase. TOPORS ubiquitinates and sumoylates p53, which regulates p53 stability. TOPORS has also been shown to ubiquitinate the tumor suppressor NKX3.1.	19473992, 18077445
TRAF2	Rip1, other TRAFs	TRAF2 is a weak E3 ubiquitin ligase that acts as a component of several ubiquitination complexes. TRAF2 ligase activity is activated in the presence of cytoplasmic sphingosine-1-phosphate. TRAF2 is a major regulator of the apoptosis and cell survival machinery.	11909853, 15175328
TRAF6	NEMO, Akt1	TRAF6 is an E3 ubiquitin ligase that functions as an adaptor protein in IL-1R, CD40, and TLR signaling. TRAF6 promotes NF-κB signaling through K63 polyubiquitination of IKK, resulting in IKK activation. TRAF6 has also been shown to ubiquitinate Akt1, causing its translocation to the cell membrane.	19713527, 11057907
TRAF7		TRAF7 is an E3 ubiquitin ligase and SUMO ligase that functions as an adaptor protein in TNF Receptor and TLR signaling. TRAF7 has been shown to be capable of self-ubiquitination and plays a role in apoptosis via MEKK3-mediated activation of NF-κB.	15001576
TRIAD3	TLRs, RIP1	Triad 3 is an E3 ubiquitin ligase found in peripheral blood leukocytes of the immune system that regulates antiviral and cytokine induced cellular responses.	15107846, 16968706
TRIM8	SOCS-1	TRIM8 is an E3 ubiquitin ligase that regulates cytokine induced signal transduction by targeting SOCS1 for ubiquitination and degradation by the proteasome.	12163497
TRIM11	Humanin, ARC105, Pax6	TRIM11 is an E3 ubiquitin ligase that may promote the degradation of insoluble ubiquitinated proteins. TRIMM11 may also aid in anti-viral cellular functions.	12670303, 16904669, 18628401
TRIM13		TRIM13 is an E3 ubiquitin ligase that targets membrane and secretory proteins for ubiquitination and proteasomal degradation in the endoplasmic reticulum-associated degradation (ERAD) pathway.	17314412
TRIM21	IgG1 HC, IRF3	TRIM21 is an E3 ubiquitin ligase involved in intracellular antibody-mediated degradation of viral components by the proteasome.	18022694, 18641315
TRIM25	RIG-1	TRIM25 is an E3 ubiquitin ligase involved in viral innate immunity. TRIM25 targets the cytoplasmic viral nucleic acid receptor RIG-1 for ubiquitination and degradation by the proteasome.	12075357

Ligase	Substrate	Function	PMID
TRIM32	actin, piasy	TRIM32 is an E3 ubiquitin ligase involved in viral lysosome related vesicle trafficking. TRIM32 targets DTNBP1 for ubiquitination and degradation by the proteasome.TRIM32 may also mediate the activity of HIV Tat proteins.	14578165, 16243356
TRIM33	Smad4	TRIM33 is an E3 ubiquitin ligase involved in the regulation of the TGF-b/ BMP signaling pathway. TRIM33 targets SMAD4 for ubiquitination, nuclear exclusion, and proteasomal degradation.	15820681
TRIM41	PKC	TRIM41 is an E3 ubiquitin ligase that targets protein kinase C for ubiquitination and proteasomal degradation.	17893151
TRIM54		TRIM54 is an E3 ubiquitin ligase that may target and stabilize microtubules.	15967462
TRIM55		TRIM55 is an E3 ubiquitin ligase that may regulate gene expression and protein turnover in muscle cells	15967462
TRIM63	Troponin I, MyBP-C,	TRIM63 is also known as Murf-1. TRIM63 is a muscle-specific E3 ubiquitin ligase whose expression is upregulated during muscle atrophy. TRIM63 has been shown to ubiquitinate several important muscle proteins including troponin I, MyBP-C, and MyLC1/2.	19506036
MyLC1/2		TRIM63 is also known as Murf-1. TRIM63 is a muscle-specific E3 ubiquitin ligase whose expression is upregulated during muscle atrophy. TRIM63 has been shown to ubiquitinate several important muscle proteins including troponin I, MyBP-C, and MyLC1/2.	19506036
UBE3B		UBE3B is an E3 ubiquitin ligase identified through sequence analysis. The specific substrates and cellular function of UBE3B is currently unknown.	12837265
UBE3C		UBE3C is an E3 ubiquitin ligase also known as KIAA10. UBE3C is highly expressed in muscle and may interact with the transcriptional regulator TIP120B.	12692129
UBR1		UBR1 is an E3 ubiquitin ligase responsible for proteasomal degradation of misfolded cytoplasmic proteins. UBR1 has also been shown to be a ubiquitin ligase of the N-end rule proteolytic pathway, which regulates degradation of short-lived proteins.	19041308, 17962019
UBR2	Histone H2A	UBR2 is an E3 ubiquitin ligase that has been shown to ubiquitinate histone H2A, resulting in transcriptional silencing. UBR2 is also part of the N-end rule proteolytic pathway.	20080676, 19008229
UHRF1	Histone H3	UHRF1 is an epigenetic regulator that is also a putative E3 ubiquitin ligase.	14993289
UHRF2	PCNP	UHRF2 is also known as NIRF. UHRF2 is a nuclear protein that may regulate cell cycle progression through association with Chk2. UHRF2 also ubiquitinates PCNP and has been shown to play a role in degradation of nuclear aggregates containing polyglutamine repeats.	15178429, 14741369, 19218238
VHL	HIF-1α	VHL is the substrate recognition component of the ECV (Elongin B/C, CUL-len-2, VHL) E3 ubiquitin ligase complex responsible for degradation of the transcription factor HIF-1α. Ubiquitination and degradation of HIF-1α takes place only during periods of normoxia, but not during hypoxia, thereby playing a central role in the regulation of gene expression by oxygen.	11292862
VPS18	SNK	VPS18 is an E3 ubiquitin ligase that regulates intracellular vesicle trafficking. VPS18 may also regulate the POLO-like kinase SNK during the cell cycle.	16203730
WWP1	ErbB4	WWP1 is an E3 ubiquitin ligase commonly found to be overexpressed in breast cancer. WWP1 has been shown to ubiquitinate and degrade ErbB4. Interestingly, the WWP1 homolog in C. elegans was found to increase life expectancy in response to dietary restriction.	19561640, 19553937
WWP2	oct-4, PTEN	WWP2 is an E3 ubiquitin ligase that has been shown to ubiquitinate/degrade the stem cell pluripotency factor Oct-4. WWP2 also ubiquitinates the transcription factor EGR2 to inhibit activation-induced T cell death.	19274063, 19651900, 21532586
ZNRF1		ZNRF1 is an E3 ubiquitin ligase highly expressed in neuronal cells. ZNRF1 is found in synaptic vesicle membranes and may regulate neuronal transmissions and plasticity.	14561866

Deubiquitinase Table

This table provides a list of deubiquitinases (DUBs), along with their substrates (when known) and corresponding references.

DUB	Substrate	Function	PMID
ATXN3	RAD23A, RAD23B, STUB1/CHIP	ATXN3 is a transcriptional regulation deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward long, four or greater, ubiquitin chains.	17696782
ATXN3L		ATXN3-like is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains.	21118805
BRCC36	FAM175A/ Abraxas	BRCC36 (BRCC3) is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains. BRCC36 targets K63-linked ubiquitin chains on H2A and H2X at the site of DNA double strand breaks as a component of the BRCA complex.	14636569, 16707425
COPS5	TP53, MIF, JUN, UCHL1, ESR1, RanBP9	COPS5 (CSN5) is the protease subunit of the COP9 signalosome complex (CSN), a key regulator of the ubiquitin conjugation pathway. COPS5 is essential for the CSN isopeptidase activity responsible for deneddylation of cullin-RING E3 ubiquitin ligase complexes.	9535219, 11285227, 23926111
COPS6	TP53, MIF, c-Jun, UCHL1	COPS6 is a component of the COP9 signalosome complex (CSN) which is involved in several cellular and developmental processes. The CSN complex regulates the ubiquitin (Ubl) conjugation pathway through the deneddylation of the cullin subunits of SCF-type E3 ligase complexes. This decreases the Ubl ligase activity of SCF-type complexes such as SCF, CSA, or DDB2.	11337588, 12732143, 12628923
USP17L2	CDC25A	USP17L2 is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward CDC25A, preventing CDC25A degradation and allowing cell cycle progression.	14699124, 20228808
eIF3F	Notch	eIF3F deubiquitinates activated Notch1 promoting its nuclear import, thereby acting as a positive regulator of Notch signaling.	21124883
eIF3H		eIF3H is involved in various stps of the initiation of protein synthesis as a component of the eukaryotic translation initiation factor 3 (eIF-3) complex.	16766523
JOSD1		JOSD1 is a deubiquitination enzyme that displays low ubiquitin isopeptidase activity *in vitro*.	21118805
JOSD2		JOSD2 is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K63-linked chains, and to a lesser extent K48-linked chains.	17696782, 21118805
MPND		MPND is an MPN domain and JAMM motif-containing protein with predicted ubiquitin isopeptidase activity.	
MYSM1	H2A, E4BP4, GFI1	MYSM1 is a deubiquitinating enzyme that acts as a transcriptional co-activator by directing preferential ubiquitin isopeptidase activity toward monoubiquinated H2A in hyperacetylated nucleosomes.	17707232, 24062447, 24014243
OTU1	VCP	OTU1, also known as YOD1, is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked polyubiquitin or di-ubiquitin chains. OTU1 is a part of the endoplasmic reticulum-associated degradation (ERAD) pathway for misfolded lumenal proteins.	19818707
OTUB1	RNF128, UbcH5, Smad2/3, c-IAP	OTUB1 is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward polyubiquitinated K48-linked chains. OTUB1 regulates protein turnover by preventing degradation and also plays a unique role in the regulation of T cell anergy. Furthermore, OTUB1 regulates p53 stability and activity via non-canonical inhibition of the MDM2 cognate Ub-conjugating enzyme (E2) UbcH5. OTUB1 also inhibits the ubiquitination of phospho-SMAD2/3 by binding to and inhibiting the E2 ubiquitin-conjugating enzymes independent of its catalytic activity. OTUB1 regulates NF-κB and MAPK signaling pathways as well as TNF-dependent cell death by modulating c-IAP1 stability.	12704427, 14661020, 24403071, 24071738, 23524849, 22124327
OTUB2	RNF-8	OTUB2 is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains. OTUB2 regulates protein turnover by preventing degradation. OTUB2 fine-tunes the speed of DSB-induced ubiquitination.	12704427, 18954305, 24560272
OTUD1		OTUD1 is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD3		OTUD3 is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD4	XPC	OTUD4 is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829, 24366067
OTUD5	TRAF3, p53	OTUD5 is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains. OTUD5 negatively regulates type I interferon (IFN) production by deubiquitination of TRAF3.	17991829, 24143256
OTUD6A		OTUD6A is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD6B		OTUD6B is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD7A/ Cezanne 2		OTUD7A is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains.	12682062
OTUD7B/ Cezanne	TRAF6, TRAF3	OTUD7B is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains. OTUD7B negatively regulates NF-κB.	11463333, 23334419
OTUD7C/ A20	NAF1, TAX1BP1, TRAF2	OTUD7C is a ubiquitination-editing enzyme that displays ubiquitin isopeptidase activity toward K63-linked chains and ubiquitination of K48-linked chains. OTUD7C is an essential regulator of inflammatory signaling pathways in the lymphoid system.	9882303, 14748687

DUB	Substrate	Function	PMID
OTULIN	Met-1 polyubiquitin	OTULIN disassembles Met1-Ub which is important for reducing Met1-Ub accumulation after stimulation of nucleotide-oligomerization domain-containing protein 2 (NOD2). Depletion of OTULIN alters signaling downstream of NOD2.	23806334
POH1		POH1 is the metalloprotease deubiquitination enzyme component of the 26S proteasome that displays ubiquitin isopeptidase activity toward K63-linked chains.	9374539, 19214193
PRPF8	SNRP116, WDR57/ SPF38	PRPF8 is a member of the deubiquitinating enzyme metalloprotease JAMM domain superfamily. PRPF8 is known to be a central component of the spliceosome, while PRPF8 ubiquitin isopeptidase activity is controversial.	2139226, 8702566
PSMD7	TRIM5	PSMD7 is a regulatory subunit of the 26S proteasome, and is involved in the ATP-dependent degradation of ubiquitinated proteins.	22078707, 7755639
STAMBP	CXCR4, EGFR, ErbB2	STAM-binding protein (STAMBP or AMSH) is an endosomal deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains.	18758443, 20159979, 22800866
STAMB-PL1		STAM-binding protein-like 1 (STAMBPL1 or AMSHLP) is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains.	18758443
TRABID	TRAF6, APC	TRABID is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains. TRABID acts as a positive regulator of the Wnt signaling pathway by deubiquitinating APC protein, a Wnt signaling pathway negative regulator.	18281465, 21834987
UCHL1	COPS5, CHT, NCAM, β-Catenin	UCHL1 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL1 functions as a ubiquitin hydrolase involved in the processing of both ubiquitin precursors and ubiquitinated substrates, generating free monomeric Ub. UCHL1 may play a role in regulating cholinergic function through CHT ubiquitination and degradation.	9790970, 24525247, 23061666, 22641175
UCHL2/ BAP1	BRCA1, HCFC1	UCHL1/Bap1 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL1/ Bap1 is a BRCA1-associated, nuclear localized ubiquitin hydrolase that suppresses cell growth.	9528852
UCHL3	ENAC	UCHL3 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL3 functions as a ubiquitin hydrolase involved in the processing of both ubiquitin precursors and ubiquitinated substrates, generating free monomeric Ub. UCHL3 shows dual specificity toward both ubiquitin (Ub) and NEDD8, a Ub-like molecule.	2530630
UCHL5	TGF-β Receptor I	UCHL5 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL5 is the deubiquitination enzyme component of the 19S regulatory subunit of the 26S proteasome that displays ubiquitin isopeptidase activity toward K48-linked chains.	16906146, 18922472, 23500140
USP1	FANCD2, PCNA, PHLPP1	USP1 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP1 is a negative regulator of DNA repair machinery.	15694335, 16531995, 22426999
USP2	CCND1, PER1	USP2 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP2 is characterized by its C19 peptidase activity, which is involved in ubiquitin recycling and in the disassembly of various forms of polymeric ubiquitin and ubiquitin-like protein complexes. USP2 is also a core component of circadian rhythm machinery.	17290220, 19917254, 19838211, 23213472
USP3	H2A, Rig-I , H2A, γH2A.X	USP3 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP3 deubiquitinates monoubiquitinated histone H2A and H2B. USP3 is required for proper progression through S phase and subsequent mitotic entry.	17980597, 24366338, 24196443
USP4	ADORA2A, RB1, Rig-I, RIP1, TRAF2, TRAF6, PDK1	USP4 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP4 is a proto-oncogene that deubiquitinates target proteins such as the receptor ADORA2A and TRIM21 and plays a role in the regulation of quality control in the ER.	7784062, 16316627, 23388719, 23313255, 22029577, 22347420
USP5/ ISOT	p53, TRIML1	USP5 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP5 preferentially cleaves branched and K48-linked polymers. USP5 binds linear and K63-linked polyubiquitin with a lower affinity. Knock-down of USP5 causes the accumulation of p53/TP53 and an increase in p53/TP53 transcriptional activity.	19098288
USP6	NF-κB	USP6 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP6 exhibits an ATP-dependent C-terminal isopeptidase activity.	20418905, 22081069
USP7/ HAUSP	FOXO4, PTEN p53, MDM2, Tip60, MCM, FoxP3 , UBE2E1, NF-κB, Aurora A	USP7, also known as herpes virus-associated ubiquitin-specific protease (Hausp), is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP7 deubiquitinates target proteins such as FoxO4, p53/TP53, MDM2, PTEN and DAXX. USP7 is Involved in cell proliferation during early embryonic development and also plays a role in the regulation of early adipogenesis.	11923872, 14506283, 15053880, 23775119, 24190967, 23973222, 23603909, 23267096, 23348568

DUB	Substrate	Function	PMID
USP8/ UBPY	EPS15, CLOCK, HIF-1α, Smo	USP8 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP8 is an essential growth-regulated enzyme that is indispensible for cell proliferation and survival. USP8 regulates endosomal ubiquitin dynamics, cargo sorting, membrane traffic at early endosomes, and maintenance of EGFR stability. In normoxia, USP8 maintains HIF1α and HIF transcriptional output which is essential for endosome-mediated ciliogenesis.	9628861, 16520378, 17711858, 23154984, 24378640, 22253573
USP9X	SMAD4, MARK4, NUAK1, BIRC5/ survivin, Smurf1, Mcl-1, ERG, Bcl10, PEX5	USP9X is an X-linked member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP9X hydrolyzes both 'Lys-29'- and 'Lys-33'-linked polyubiquitin chains. USP9X functions to regulate cell-cell contact interactions, TGF-β/BMP signaling, chromosome alignment and segregation, and specifically deubiquitinates monoubiquitinated Smad4. Moreover, USP9X is a mTORC1 and mTORC2 binding partner that negatively regulates mTOR activity and skeletal muscle differentiation.	16322459, 18254724, 19135894, 23184937, 23097624, 24591637, 23690623, 22371489
USP9Y	SMAD4	USP9Y is a Y-linked member of the ubiquitin-specific processing protease (USP/ USB) deubiquitinase superfamily required for sperm production. USP9Y functions to regulate TGF-β/BMP signaling, and specifically deubiquitinates monoubiquitinated Smad4.	19246359
USP10	G3BP, p53/ TP53, SNX3, NEMO, SirT6	USP10 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP10 functions as an essential regulator of p53/TP53 stability following DNA damage.	11439350, 18632802, 19398555, 24270572, 24332849
USP11	BRCA2, CHUK/IKKA, RANBP9/ RANBPM , PML, ALK5	USP11 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP11 aids in the regulation of pathways leading to NF-κB activation and also DNA repair after double-stranded DNA breaks. Depletion of USP11 causes inhibition of TGFβ-induced epithelial to mesenchymal transition.	15314155, 17897950, 18408009, 24487962, 22773947
USP12	WDR48, PHLPP1, Notch	USP12 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP12 requires interaction with WDR48 for high deubiquitinase activity. WDR48, in complex with deubiquitinase USP12, suppresses Akt-dependent cell survival signaling by stabilizing PHLPP1. USP12 and its activator UAF1 deubiquitinate nonactivated Notch.	19075014, 24145035, 22778262
USP13/ ISOT3	PTEN, Stat1, Ubl4A	USP13 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP13 is part of an autophagy regulatory loop involving the deubiquitination of USP10 that leads to regulation of p53 stability. USP13 is a tumor suppressing protein that functions through deubiquitylation and stabilization of PTEN. USP13 also modulates STAT1 and plays a role in host defense against viral infection.	9841226, 24270891, 23940278, 24424410
USP14	FANCC, CXCR4, ERN1, REST, I-κB	USP14 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP14 is one of three proteasome-associated deubiquitinases, along with POH and UCHL5. USP14 is thought to antagonize substrate degradation as a part of the proteasome.	18162577, 19135427, 19106094, 23754622, 23615914
USP15	E6, ubH2B, TRIM25, KEAP1, REST, BRAP, TβR-I	USP15 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP15 preferentially cleaves K48-linked polymers. USP15 deubiquitination protects APC and human papillomavirus type 16 protein E6 target proteins against proteasomal degradation. USP15 is a critical regulator of the TRIM25- and RIG-I-mediated antiviral immune response. USP15 also regulates the TGF-β pathway and is a key factor in glioblastoma pathogenesis.	16005295, 19576224, 24526689, 24399297, 23727018, 23708518, 23105109, 22344298
USP16	H2A	USP16 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP16 acts as a transcriptional co-activator by specifically targeting H2A for deubiquitination. USP16 deubiquitination of H2A is also required for entry into mitosis.	10077596, 17914355
USP17	RIG-I, MDA-5, SDS3	USP17 regulates virus-induced type I IFN signaling through deubiquitination of RIG-I and MDA5. USP17 specifically deubiquitinates Lys-63-linked ubiquitin chains from SDS3.	20368735, 21239494
USP18	TAK1, TAB1	USP18 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP18 catalyzes the removal of ISG15, an interferon-regulated ubiquitin-like protein, which maintains the critical cellular balance of ISG15-conjugated proteins important for normal development and brain function. USP18 deubiquitinates the TAK1/TAB1 complex thus inhibiting NF-κB and NFAT activation during Th17 differentiation.	10777664, 23825189
USP19	RNF123	USP19 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP19 deubiquitinates target proteins involved in cell proliferation, myogenesis, regulation of hypoxia, and modulation of the ERAD protein degradation pathway.	19465887
USP20	VHL, DIO2, HIF1	USP20 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP20 cleaves K48- and K63-linked chains. USP20 deubiquitinates β2-adrenergic receptor (ADRB2) as well as target proteins involved in thyroid hormone regulation and regulation of hypoxia.	12056827, 12865408, 15776016

DUB	Substrate	Function	PMID
USP21	H2A, RIG-I, GATA-3	USP21 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP21 is also known as USP23. USP21 acts as a transcriptional co-activator by specifically targeting H2A for deubiquitination. USP21 is capable of removing the ubiquitin-like NEDD8 from NEDD8 conjugates. USP21 acts as a negative regulator in antiviral responses by binding and deubiquitinating RIG-I. USP21 also stabilizes the transcription factor GATA-3 by mediating its deubiquitination.	10799498, 24493797, 23395819
USP22	ATXN7L3, NFATc2	USP22 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP22 deubiquitinates histones H2A and H2B as a component of the histone acetylation (HAT) complex SAGA. USP22 deubiquitinates specific targets required for transcription, nuclear receptor-mediated transactivation, and cell cycle progression. USP22 positively regulates NFATc2 through its deubiquitinase activity and promotes IL2 expression.	18206972, 18206973, 18469533, 24561192
USP23	H2A	See USP21	10799498
USP24	DDB-2	USP24 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. Mutations of the USP24 gene may correlate with risk of Parkinson's disease. USP24 interacts with and regulates stability of the DNA damage specific protein, DDB-2.	16917932, 23159851
USP25	ACTA1, MYBPC1, TRAF3, TRAF5, TRAF6	USP25 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP25 cleaves both K48- and K63-linked chains. The USP25 muscle-specific isoform may have a role in the regulation of muscular differentiation and function. USP25 targets TRAF5 and TRAF6 for deubiquitination and thus negatively regulates IL-17-mediated signaling and inflammation.	10612803, 11597335, 16501887, 23674823, 23042150
USP26	AR	USP26 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP26 regulates the androgen receptor signaling pathway by targeting the androgen receptor for deubiquitination.	20501646
USP27X		USP27X is an X-linked member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	12838346
USP28	P53bp1, Chk2, LSD1	USP28 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP28 can bind to and deubiquitinate several target proteins in the DNA damage pathway, resulting in their stability, including p53BP1 and Chk2. USP28 also plays an important role in Myc related signaling by binding through FBW7α to Myc. USP28 stabilizes LSD1 via deubiquitination, and USP28 overexpression has been linked to the upregulation of LSD1 upregulation in multiple cancer cell lines and breast tumor samples.	17558397, 16901780, 24075993
USP29	Claspin	USP29 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP29 helps regulate the ATR-Chk1 pathway and the control of DNA replication via Claspin deubiquitination.	10958632, 24632611
USP30		USP30 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP30 may participate in the maintenance of mitochondrial morphology.	18287522
USP31		USP31 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP32		USP32 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP32 is highly expressed in breast cancer cell lines and may be involved in tumorigenesis.	12604796, 20549504
USP33	ADRB2, CP110, RalB	USP33 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP33 is involved in cellular migration, β2-adrenergic receptor/ADRB2 recycling, and G protein-coupled receptor (GPCR) signaling. In addition, USP33 regulates centrosome biogenesis by deubiquitinating CP110. USP33 also regulates the autophagy and innate immune response of RalB through deubiquitination of RalB.	12865408, 23486064, 24056301
USP34	AXIN1, AXIN2	USP34 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP34 acts as an activator of the Wnt signaling pathway downstream of the β-catenin destruction complex by deubiquitinating and stabilizing AXIN1 and AXIN2.	21383061
USP35		USP35 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP36	RNA Polymerase I	USP36 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP36 may play a role in the maintenance of stem cells and regulation of cellular differentiation. Furthermore, USP36 regulates rRNA production through control of RNA Polymerase I stability.	22622177, 22902402
USP37	FZR1/CDH1, PLZF/RARα	USP37 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP37 antagonizes the anaphase-promoting complex (APC/C) during G1/S transition by mediating deubiquitination of cyclin A (CCNA1 and CCNA2), thereby promoting S phase entry. In addition, USP37 is involved in acute promyelocytic leukemia (APL) through regulating the stability of the oncogenic fusion protein PLZF/RARα.	21596315, 23208507
USP38		USP38 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP38 is expressed in skeletal muscle and adrenal gland.	19615732

DUB	Substrate	Function	PMID
USP39		USP39 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP39 may play a role in mRNA splicing as a competitor of ubiquitin C-terminal hydrolases (UCHs).	11350945
USP40		USP40 may be a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	16917932
USP41		USP41 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP42		USP42 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP42 may play a role in spermatogenesis.	14715245
USP43		USP43 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP44	Cdc20, Histone H2B	USP44 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP44 regulates the cell cycle by deubiquitination of CDC20, leading to stabilization of the MAD2L1-CDC20-APC/C ternary complex and avoidance of premature anaphase entry. USP44 modulates H2B ubiquitylation thus regulating stem cell differentiation.	17443180, 22681888
USP45		USP45 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP46	GAD1/ GAD67, PHLPP	USP46 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP46 requires interaction with WDR48 for high deubiquitinase activity. USP46 may act by mediating the deubiquitination of GAD1/GAD67. USP46 also regulates Akt signaling in colon cancer through control of PHLPP activation.	19075014, 22391563
USP47	POLB, CDC25A, katanin-p60	USP47 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP47 regulates base excision repair by deubiquitinating monoubiquitinated DNA polymerase β (POLB). USP47 may also regulate cell growth and survival by targeting CDC25A. USP47 promotes axonal growth of cultured rat hippocampal neurons through specifically deubiquitinating and stabilizing katanin-p60.	19966869, 23904609
USP48	TRAF2, RELA, NHE3	USP48 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP48 may be involved in the regulation of NF-κB activation by the TNF receptor superfamily via its interactions with RelA and TRAF2. USP48 can also prevent NHE3 degradation by deubiquitination and thus helps regulate blood pressure and sodium balance.	16214042, 24308971
USP49	Histone H2B	USP49 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP49 specifically regulates Histone H2B by deubiquitination and is required for efficient cotranscriptional splicing of exons.	14715245, 23824326
USP50		USP50 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	14715245
USP51		USP51 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP52	PAN3	USP52 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP52 is a member of the Pan nuclease complex, which regulates mRNA stability.	14583602, 16284618
USP53		USP53 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	14715245
USP54		USP54 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	14715245
USPL1		USPL1 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	
USPL2/ CYLD	NF-κB, HDAC6, RIP1	CYLD deubiquitinase regulates inflammation and cell proliferation by down regulating NF-κB signaling through removal of ubiquitin chains from several NF-κB pathway proteins. CYLD is a negative regulator of proximal events in Wnt/β-catenin signaling and is a critical regulator of natural killer T cell development. In selenite-treated colorectal cancer cells, CYLD regulates RIP1 deubiquitination and triggers apoptosis.	12917689, 12917690, 24577083
VCPIP1	VCP	VCPIP1 (valosin containing protein p97/p47 complex-interacting protein) is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily. VCPIP1 is necessary for VCP-mediated reassembly of Golgi stacks after mitosis.	15037600


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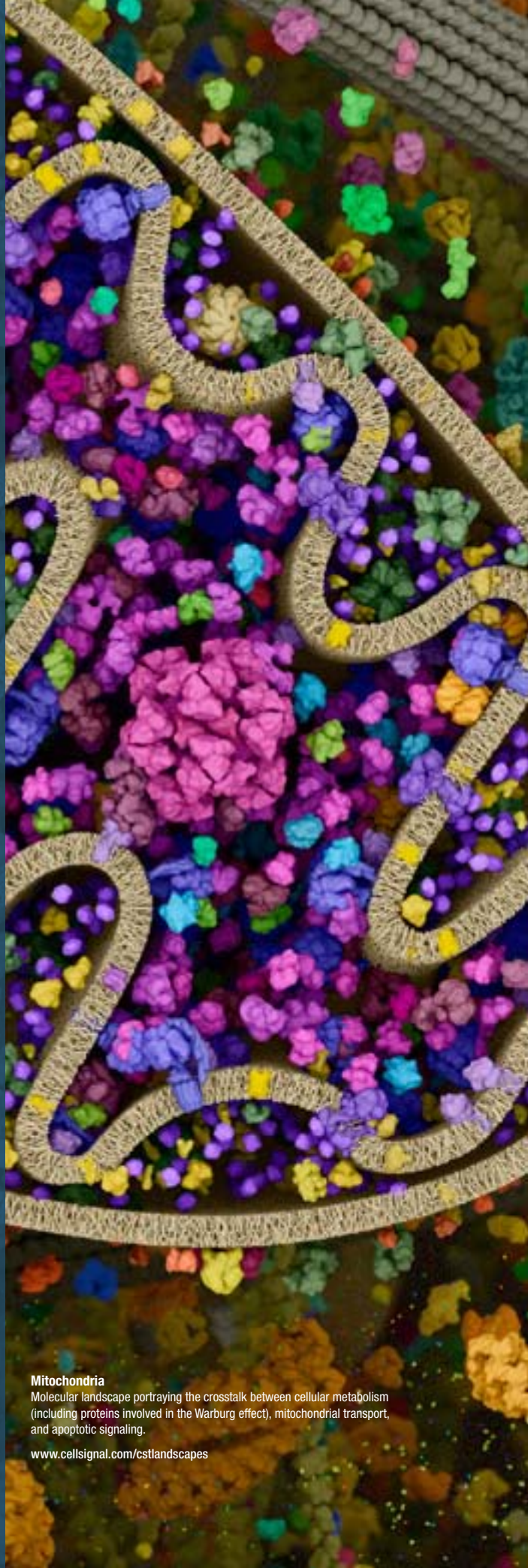

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