

**Supplemental Materials and Methods:****The following yeast strains were used in this study:**

<u>Strain</u>	<u>Genotype</u>	<u>Source</u>
RDY 385	MAT a, <i>cdc4-1</i> , <i>his7</i> , <i>ura1</i>	(S1)
RDY 597	MAT a, <i>cdc34-2</i> , <i>his3Δ</i> , <i>ura3-52</i> , GAL+	(S2)
RDY 690	MAT a, <i>ura3</i> , <i>trp1</i> , <i>ade2</i> , <i>cdc53-1</i>	(S3)
RDY 1377	MAT a, <i>can1-100</i> , <i>ade2-1</i> , <i>leu2-3,-112</i> , <i>trp1-1</i> , <i>ura3-1</i> , <i>skp1-12</i>	(S4)
RDY 1716	MAT a, <i>ubc12::KanMX</i> , <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	(S5)
RDY 1721	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	(S5)
RDY 1735	MAT a, <i>rri1::KANMX</i> , <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	(S5)
RDY 1832	MAT α, <i>RRI1::KanMX</i> , <i>can1-100</i> , <i>leu2-3,-112</i> , <i>his3-11,-15</i> , <i>trp1-1</i> , <i>ura3-1</i> , <i>ade2-1</i>	This study
RDY 1835	MAT a, <i>rri1::KanMX</i> , <i>can1-100</i> , <i>leu2-3,-112</i> , <i>his3-11,-15</i> , <i>trp1-1</i> , <i>Ura3-1</i> , <i>ade2-1</i> , <i>pep4::TRP1</i> , <i>bar1::LEU2</i> , <i>skp1::HIS3</i> , <i>SKP1::Myc9</i>	This study
RDY 1914	MAT a, <i>rri1::KanMX</i> , <i>ura3</i> , <i>trp1</i> , <i>ade2</i> , <i>cdc53-1</i>	This study
RDY 1915	MAT a, <i>rri1::KanMX</i> , <i>cdc34-2</i> , <i>his3Δ</i> , <i>ura3-52</i> , GAL+	This study
RDY 1916	MAT a, <i>rri1::KanMX</i> , <i>cdc4-1</i> , <i>his7</i> , <i>ura1</i>	This study
RDY 1917	MAT a, <i>rri1::KanMX</i> , <i>can1-100</i> , <i>ade2-1</i> , <i>leu2-3,-112</i> , <i>trp1-1</i> , <i>ura3-1</i> , <i>skp1-12</i>	This study
RDY 2096	MAT a, <i>pci8::KanMX</i> , <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	(S5)
RDY 2097	MAT a, <i>YOL117W::KanMX</i> , <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	(S5)

The following *S. pombe* strains were used in this study: *csn5Δ* (RDY 1712)(S6); *Pcu1<sup>myc13</sup>* (RDY 1568)(S7); *csn5Δ*, *Csn1<sup>myc13</sup>* (RDY 2092)(S8); *csn5Δ*, *Csn2<sup>myc13</sup>* (RDY 2093)(S8).

**The following Plasmids were used in this study:**

<u>Plasmid</u>	<u>RDB number</u>
pREP41	1549
pREP41-FLAG-Csn5	1469
pREP41-FLAG-Csn5 (H118A)	1487
pREP41-FLAG-Csn5 (H120A)	1488
pREP41-FLAG-Csn5 (D131N)	1489
pEG(KT)	1572
pEG(KT)-Rri1	1471
pEG(KT)-Rri1 (H179A)	1478
pEG(KT)-Rri1 (H181A)	1491
pEG(KT)-Rri1 (D192A)	1494

**Fission Yeast Extracts:** Cells were grown to mid-log phase in complete media (YES), pelleted by centrifugation, and washed in ice cold STOP buffer (50mM NaF, 25mM TRIS pH7.2, 0.02% Sodium Azide). Cells were resuspended in an equal volume of lysis buffer (25mM TRIS pH 7.2, 150mM NaCl, 0.3% Triton X-100, 50mM NaF, 1mM EDTA, 1mM DTT, 1mM PMSF, and 1X protease inhibitor cocktail (5ug/mL of Aprotinin, Chymostatin, and Pepstatin A, 1ug/mL Leupeptin)). An equal volume of Glass Beads (average 500 microns, Sigma) was added and cells were vortexed for ten intervals of 40 seconds at max (cell suspension was incubated on ice for one minute between each vortex interval). Lysates were cleared by centrifugation (12Krpm).

***S. cerevisiae* native extracts and Cul1<sup>nedd8</sup>/Cdc53<sup>Nedd8</sup> conjugate purification:** Cells were grown to mid-log phase in complete media (YPD) and processed exactly as described above for *S. pombe* lysates. For deneddylation assays, clarified Skp1<sup>myc9</sup> *rrl1*Δ lysates were incubated 1 hour in the presence of protein A beads conjugated to α-myc (9E10) antibody. Beads were washed 3 times in buffer A (20mM TRIS (7.2), 150mM NaCl, and 0.3% Triton X-100) and 10uL of beads were used per reaction. psCSN was added to the beads and volume was brought to 10μL with Buffer A. Beads were incubated for 60 minutes at 30°C.

***S. cerevisiae* denatured extract:** Cells were manipulated as described in the text, pelleted by centrifugation, and washed in ice cold STOP buffer. Cell pellets were frozen in liquid nitrogen (LN<sub>2</sub>) and stored at -80°C. Pellets were thawed and an equal volume of SDS lysis buffer (25mM TRIS pH 7.5, 1% SDS, 1mM EDTA, 1mM PMSF, and 1mM DTT) and Glass beads (average 500 microns, Sigma) were added. Cells were briefly vortexed (2 seconds) and then boiled for three minutes. Cells were vortexed 3 minutes, boiled 2 minutes, than vortexed 2 minutes. Lysates were cleared by centrifugation (12 Krpm) in a microfuge.

***Drosophila* Larvae Extracts:** Third instar larvae were frozen in LN<sub>2</sub> and ground to a powder with a pestle. 20μL of lysis buffer (25mM TRIS (7.2), 150mM NaCl, 5mM DTT, 1mM PMSF, and 2mM MgCl<sub>2</sub>) was added and mixed briefly. Extracts were cleared by centrifugation and separated by SDS-PAGE followed by western blot analysis.

***Drosophila* Heat Shock Rescue:** Heat shock rescue of lethality was performed by mating *w;CSN5<sup>N</sup>/TM6C* to *w;P[w+,hs-CSN5] line 2/+; Df(3R)RK6-3/TM6C*. The transgene was followed by eye color. While the cross was maintained at 25°C, heat shocks were at 37°C for 30 min every 12 hr. Rescue of lethality was assessed by comparing white eye and red/orange eye progeny of each genotype raised with and without heat shock. The genotypes of adult progeny with red/orange eye color (with the transgene) were as follows: (with heat shock) *CSN5<sup>N</sup>/TM6C*, 35 (36.8%); *Df(3R)RK6-3/TM6C*, 31 (32.6%); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 29 (30.5%); and (without heat shock) *CSN5<sup>N</sup>/TM6C*, 42(51.2%); *Df(3R)RK6-3/TM6C*, 40 (48.8 %); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 0 (0%). The genotype of progeny with white eye color (without the transgene) were as follows: (with heat shock) *CSN5<sup>N</sup>/TM6C*, 27 (49.1%); *Df(3R)RK6-3/TM6C*, 28 (50.9%); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 0 (0%); and (without heat shock) *CSN5<sup>N</sup>/TM6C*, 43 (55.1%); *Df(3R)RK6-3/TM6C*, 35 (44.9%); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 0 (0%).

Similarly, heat shock rescue experiment was carried out with hs-*CSN5* (D148N).

w;*CSN5<sup>N</sup>/TM6C* was crossed to w;P[w+, hs-*CSN5* (D148N)] line 2/+; *Df(3R)RK6-*

*3/TM6C*. The transgene was followed by eye color as described above and the condition

of heat shocks was same as described above The genotypes of adult progeny with

red/orange eye color (with the transgene) were as follows: (with heat shock)

*CSN5<sup>N</sup>/TM6C*, 65 (53.7%); *Df(3R)RK6-3/TM6C*, 56(46.3%); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 0

(0%); and (without heat shock) *CSN5<sup>N</sup>/TM6C*, 39(52.0%); *Df(3R)RK6-3/TM6C*, 36

(48.0%); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 0 (0%). The genotype of progeny with white eye color

(without the transgene) were as follows: (with heat shock) *CSN5<sup>N</sup>/TM6C*, 61 (47.7%);

*Df(3R)RK6-3/TM6C*, 67 (52.3%); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 0 (0%); and (without heat shock)

*CSN5<sup>N</sup>/TM6C*, 37 (51.4%); *Df(3R)RK6-3/TM6C*, 35 (48.6%); *CSN5<sup>N</sup>/Df(3R)RK6-3*, 0

(0%).

12654695_Csn5a_Hsa	58-175	SALALLKMYMHARGG--GNLEVMGLMLGK-VDGE----TMIIMDSFAL-29-GHLENAIGWYHSHPGYGCWLSGIDVSTQMLNQOQFQEPFVAIVIDPTR
7300154_CH5_Dme	55-172	SALALLKMYMHARGG--GTLPEVMGLMLGK-VEDN----TMIVMDAFAL-29-GRMEHAVGWYHSHPGYGCWLSGIDVSTQMLNQTYQEPFVAIVVDPVR
17538322_CE06722_Cel	58-175	SAIALLKMTMHAKRG--GNLEIMGLLQGR-IDAN----SFIILDVFAL-29-GRKEKVGWYHSHPGYGCWLSGIDVSTQTLNQKFQEPFVAIVIDPLR
15219970_Atlg22920_Ath	62-179	SALALLKMYMHARGG--GTPEIMGLMQGK-TEGD----TIIVMDAFAL-29-GRLENVVGWYHSHPGYGCWLSGIDVSTQMLNQOQFQEPFVAIVIDPTR
6319985_Rr1p_Sce	89-216	SKLSCEKITHYAVRG--GNIEIMGILMGF-TLKD----NIVVMDCFNL-29-GAKLNVVGWYHSHPGYDCWLSNIDIQTQDLNQRFQDPYVAIVVDPLK
17483211_FLJ14981_Hsa	274-392	VSSNVFLFLDFHSHL--TRSEVVGYLGGRWDMNT--QMLTVLRAFPC-22-LRGLSLVGWYHSHPHSPALPSLQDDIQAQMD-9-NGFQPCIALLCSFPY
7297828_CG4751_Dme	286-405	VNSSALLLDFHCHL--TVREVCYLLGGTWMDS--HTLSITKTYPC-22-QDQLLVVGWYHSHPKFQAEPTRLRCDQAQD-10LTYTPCVSLIISPY
15620889_KIAA1915_Hsa	477-594	VSEALLINDFHCHL--SMAEIVGLLGGYSEVD--KVVEVCAAEPC-22-VRGFSVVGWYHSHPAFDPNPSLRDIDTQAK-6-RGGAKVLSVSPY
1168719_C6.1A_Hsa	15-140	ESDAFLVLCNHALST--EKEEVMGLCIGELNDDT-23-RIVHIHSVII-32-GRPMRVVGWYHSHPHITVWPSHVDVRTQAMYQMMDQGFVGLIFSCFI
15231308_At3g06820_Ath	2-131	SEDVWLTCTHALST--ETEEMIGLLLGDIEYSK----NGESATAMIW-39-GRTRVVGWYHSHPHITVLPSSHVDVRTQAMYQLLDSGFIGLIFSCFS
7243127_KIAA1373_Hsa	259-368	DLCHKFLQLAESNTV--RGIEETCGILCGKLTHNE-----FTITHVIV-24-QHLLTLGWYHSHPTQTAFLLSSVDLHTHCSYQLMLPEATAIVCSPKH
14043382_AMSH_Hsa	262-371	RLCPQFLQLASANTA--RGVETCGILCGKLMRNE-----FTITHVLI-24-QQGLITLGWYHSHPTQTAFLLSSVDLHTHCSYQMMLPESVAIVCSPKF
7301945_Amsh_Dme	255-364	DTMEVFLKALANTS--KNIETCGVLAGH-LSQN-----QLYITHIIT-24-QMQLITLGWYHSHPTQTAFLLSSVDLHTHCSYQIMPEALAVCAPKY
11499780_Af2198_Afu	4-101	SRGLLKTILEAAKSA--HPDEFIALLSGS-KDVMD----ELIFLFPVS-16-PIGMKVFGTVHSHSPSPCRPSSEEDLSLFTF---FGKYHIIIVCY-PYD
20093654_MK0214_Mka	10-110	DARLLDSLLEASDKN--HPDEFIALLSGS-IDAE----TITIDSLIV-15-VHTCDVIGTFSHSHPYGDPVPSSEEDLMLFKRLG---AVHATAAY-PYT
20090588_Mal1736_Mac	27-133	LLYMQIKGLARDTLD--FILEASKMSAPE-EFAGL-LQDGIITFEVLVI-15-MNCVAVV-15-MVTCVIGTFSHSHPGANRRRPSKADLRLFSKTG---NCHIIAGR-PYG
15678989_MTH971_Mth	16-126	FKPVRVVVDSEVMD--EVLLEIARRSHPH-EFAAL-LEVLVHVTGLIFL-16-PPFTGAVGSVHSHPGPVNLPSSAADLHFFSKNG---LFHLIIAH-PYT
3257912_PH1488_Pho	26-140	LPKNIIEEITRSRE--SKIEICGFIFGT-KNG-----ERFIGKEVP-25-RKGLEVVITIEHSHLNCPPYPSKDDIKGMENWR---IPWLVSLKGD-
14590365_PH0451_Pho	6-104	RRELEYLELAKSF--YPREVAGFLRMK-DGVFE---EVLIVPKGFF-12-PHDESIGKTFHSHSPSPFPYPSSEGLMFFSKFG---GIHIIAFA-PYD
16554503_VNG1818a_Hsp	5-113	TREGYDSVLDAHAD--TPREACGVFVGE-RDGLD----RRVTAVRRV-26-AGVRELVGFYHSHHPVGFGRPSATDRREHAQ-----WPDVVVVASLA
15789943_VNG0778C_Hsp	4-120	GGRPSVLGAEDALED--FAEEAQQSDHPD-EYLLGL--DGYVVTDVVLVI-17-PNDMRNVGSVHSHPNVGLAPSDADRSMF-GKG---QLHIIILGH-PYG
15897071_SS00111_Sso	1-84	NRYFKINCWSRRFMD--NLKKEKCGIICNN-TFY-----ELKNISRTE-15-KCSDDIQAIVHSHHEES-CEPSYKDIKMSKIIWN---IPWIIISKKCIK
14600889_APE0681_Ape	1-97	ASIGPLRQLKLMAL--AHNEEAGLVIGA-RRGDT---VYAYILYRTD-24-KLGLVVGWYHSHHTTTPSPSGKDVVEGMKR-----WPGVWLIACGPE
18313041_PAE2024_Pae	1-102	MPKAFLEEARAKKCA--PEAECVGLIFGI-SDTAL--SWRWKNAF-20-ERNGEALLAIEHSHHPGP-PTPSWEDVRHMRL---WPTWIIIA-NVF
7514470_aq_1691_Aae	5-114	KKEVLEKMKQAERD--YPYETCGLLIGK-SEG-----GIRIAYEAF-28-SKGMEIVGVYHSHDPHPDRPSQFDLQRA-----FPDLSYIIFSVO
1652702_sl10864_Ssp	7-112	SQVHQDQIYRHGERC--YPEECCGLLIGK-ILIGE-HRHWQVVEVQPT-39-QKGLSIIIGIEHSHPHGQPIPSEEDRAIA-----WPEYIYLIASGE
17230399_al12907_Nsp	6-106	IANGVKTVEVPTANAWETEADNFQTEI-NKTNI---TSPTSSLKRR-16-DKSLNIIIGIYHSHDPHPAIPSECDRLYA-----WAGYSYIIVSVQ
15805429_DR0402_Dra	6-114	PAPLRRALWAQVRE--LPRCEVGLFGE--WVRGE---QVQAHALYPL-27-REGDLVLVYHSHHPGFAPASADRRLLAA-----YPVYLIADPAA
7479881_SCE19A.13c_Sco	5-110	TQALYDQIVAHARED--HPDEACGVVAGP-AGEGR---PERFIPLMNA-32-DRDEEPPVVIYHSHHTATEAHPASRTDVTYAN-----EPGAHYLVSTA
15608474_Rv1334_Mtu	14-123	RADLVNAMVAHARRD--HPDEACGVLAGP-EGS-----DRPERHIPM-25-DADEVVVIYHSHHTATEAHPASRTDVKLATEPDA---HYVLVSTRDPHR
16082790_YPMT1.08c_Ype	14-111	MQEIYLTAKR-----YPNDEACGFLVRT-TG-----EKYRFMEAR-20-EDAGDVVAIYHSHHTDESADADDRAGCEATE---VPWLIIAV-RKN
11347692_PA0639_Psa	4-104	SRSLQRAIAAHAARE--HPREACGFLVIRG-VRQ-----RRYVACRNA-18-EDQGEVLSVIYHSHDPVFAATPSMADRVSCELHG---LPWVILSW-PEG
15597298_PA2102_Psa	7-123	TEHALSVIYRHACRT--YPREECCGFVLAD-AKVKE---GTNIQDELHM-28-KTCSPVSAIYHSHDPVGAAYFASREDIDKALYAGEPMLPVYLVVDVAA
17546414_RSc1695_Rso	2-101	QETTLDAARRHAARE--HPREACGLVVVV-RGR-----ERYMACRNV-17-EDLGEVLAVYHSHPNASAEPSSEADRVACEASG---LPWHIIAW-PAD
15640211_VC0181_Vch	21-138	GHVVTRLLSYRQLHH--LTPESAAGVLIGE-RRGQ---HLVVCDISP-29-AGTHLYLGEWYHSHHPEDRPFPSATDRHSWRRNIVSDESMILLIVGRKD
16519909_Y4qB_Rsp	8-132	PESVVEAMLKDASRW--HDLTEGTFMGY-WSDAN---VAVITKMDIG-29-GRVDTYIGDWHSHHPNAQSEPSWTDRLRCLTIRSEPVMIILLCG-GPE
17546377_RSc1658_Rso	124-231	DLAFERLGAFFPMVR--AFIEAARKAAPN-EHA-----AVVVVDSFAL-24-EDHESYLVVDMYHSHGALAAFFSEQNRRDDAGE---VKISCVVGLDAD
17158696_alr7560_Nsp	58-166	LEPYFRLKVPKVPCC--AIAEIIINAASIN-PQQ-----EILFYLGVTN-25-KSYTDGLVEMHSHGTLAAYPSADNQEKGK---FRVFAIIGTLNN
16131509_RadC_Eco	105-205	SPEMTREFLOSQTLG--EEREIEMVIFLD-SQH-----RVITHRRLFS-18-KINASALILAHNHPSCAEPKADKLIITERI---IKSCQFMDLRVL
16079856_YsxA_Bsu	111-212	SPEDGANIYVMDMRF--LTQEHFVCLYLN-TKN-----QVIHKRTVFI-18-KRSASAIFICVHSHPSGDPPTPSREDIEVTRRL---FECGNLIGIELL
20090827_RadC_Mac	109-209	SPKDVYALYPRMRE--QTEKFTITLYLD-TKN-----QILKEEVSI-18-LESSASVIMVHSHPSGDPSPSREDIMVTEKL---VEGGKLLIGDIL
15965481_RadC_Sme	143-223	SWSAVIDYCHAAMAH--ETKEQFRILFLD-KRN-----TLIADEVQQQ-18-ELSATALILVHSHPSGDPPTPSRADIDMTKLI---AEAAPKPLGIALH
17547163_RadC_Rso	105-145	SPQSVKDFRLTLGH--RPQEVFACLFLD-VRH-----RLIAWEELFQ-18-HHNASALILSHNHPGHVVEPSESADLVLTREL---CRALALLDVRVL
17935503_RadC_Atu	113-213	SWSVIDYCHAAMAH--ETREQFRILFLD-KRN-----VLIADVQQQ-18-ELSGTALILVHSHPSGDPPTPSRADIEMTKTI---IDTAKPLGITVH
16331325_RadC_Ssp	116-216	SPEAAAIASQDLMW--QTEKFTITLYLD-VKN-----RLLATKVVIT-18-QGATRLIVAHNHPSGGLEPSPEDIRLTFEL---LQGAQYLGIPVL
15894524_RadC_Cac	110-210	SPKEAANLVMEQLRS--FNKEHLYVIMLN-TKN-----IVIKISDVS-18-LKHAASITLCHNHPSGDPKPSNEDLNITKRL---YECCKFIIGIELL
5606726_RadC_Aae	112-212	RNPQEAFAFLKDKFD--ERRSLIALYLD-LSN-----RLLDWEVVAI-18-KLSANGIIIAHNPQGEPSPSNEDLNFTERL---KKACCELLGFELL
15801143_YkfG_Eco	39-139	STRAAREWLIILNMAG--LEREHRVLYLN-NQN-----QLIAGETXFT-18-YHNAAAVLAHNPSPGSEVTPSKADRLITERL---VQALGLVDIRVP
17547339_YkfG_Rso	40-139	SPAAVKEYLRACKLAG--FEHEVFAVLFMD-TQH-----RLIEAYEMFT-18-RLNAAAVIVSHNHPGNEPSPGADRALTQRL---KEALGLVDIRVL
15641789_YkfG_Vch	40-139	RTENTTEYLIRCKLAG--YEHEVFAVLFMD-NQH-----RLIEFKELFR-18-NVNAAAVIFAHNPSPGPEPSEQADRRITQRL---KDALSLVDIRVL

consensus/80% .....h.....E.h.hh.....l..hshPs..s..S..D.....h.hh.....  
Secondary structure HHHHHHHHHHHH HHHEEEEE E EEEEEEE EEEEEEE HHHHHHHHHH EEEEEEE

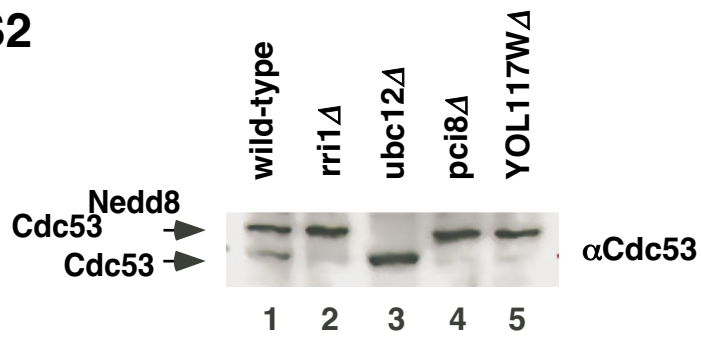
**Figure S1:** Alignment of predicted active JAMM domains.

The multiple alignment was constructed using the T-Coffee (S9) program and modified manually to ensure the correct superposition of the conserved motifs. The alignment includes all detected human JAMM proteins predicted to possess metal-dependent protease activity and their orthologs from the fruit fly *Drosophila melanogaster*, nematode *Caenorhabditis elegans*, and budding yeast *Saccharomyces cerevisiae*, whenever detectable. Additionally included are predicted active JAMM proteases from archaea and a representative set of bacteria, and a subset of bacterial RadC proteins. The sequences of Rpn11 and its orthologs are not included and neither are proteins containing apparently inactivated JAMM domains. For each protein, the position of the aligned region in the sequence is shown by numbers; poorly conserved spacers are not shown and are designated by numbers. The consensus includes amino acid residues conserved in 80% of the aligned sequences; l indicates aliphatic residues (A,I,L,V; yellow shading), h indicates hydrophobic residues (F,Y,W, A,I,L,V,M; yellow shading), and s indicates small residues (G,A,C,S,D,N,V,P; blue). The predicted metal-chelating and catalytic residues (see text) are shown in yellow against a dark-blue background. Secondary structure prediction was made using the PHD program with the multiple alignment submitted as the query (S10); E indicates extended conformation ( $\beta$ -strand) and H indicates  $\alpha$ -helix. Each protein is denoted by the GenBank identifier (GI) followed by the

gene name and an abbreviated species name. Species abbreviations: Hsa, *Homo sapiens*, Dme, *Drosophila melanogaster*, Cel, *Caenorhabditis elegans*, Ath, *Arabidopsis thaliana*, Sce, *Saccharomyces cerevisiae*; Afu, *Archaeoglobus fulgidus*, Mka, *Methanopyrus kandleri*, Mca, *Methanosarcina acetivorans*, Mth, *Methanothermobacter thermoautotrophicus*, Pho, *Pyrococcus horikoshii*, Hsp, *Halobacterium* sp., Sso, *Sulfolobus solfataricus*, Ape, *Aeropyrum pernix*, Pae, *Pyrobaculum aerophilum* (archaea); Aae, *Aquifex aeolicus*, Ssp, *Synechosystis* sp., Nsp, *Nostoc* sp., Dra, *Deinococcus radiodurans*, Sco, *Streptomyces coelicolor*, Mtu, *Mycobacterium tuberculosis*, Ype, *Yersinia pestis*, Psa, *Pseudomonas aeruginosa*, Rso, *Ralstonia solanaraceum*, Vch, *Vibrio cholerae*, Rsp, *Rhizobium* sp., Eco, *Escherichia coli*, Bsu, *Bacillus subtilis*, Sme, *Sinorhizobium meliloti*, Atu, *Agrobacterium tumefaciens*, Cac, *Clostridium acetobutylicum* (bacteria).



**S2**



**Figure S2:** Deletion of *PCI8* and *YOL117W* in *S. cerevisiae* alters Cdc53 neddylation pattern. Cell lysates (see supplemental methods) from the indicated strains were evaluated by western blot using antibodies specific for Cdc53.

**References and Notes:**

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