# Supporting Information

## Guo et al. 10.1073/pnas.1113170108

### SI Materials and Methods

**UBLCP1 Protein Purification, Crystallization, and Structure Determination.** Full-length Drosophila ULBCP1 E112A was expressed using a modified pET28a vector as a $His_6$-SUMO (Small Ubiquitin-like MOdifier) fusion protein in *Escherichia coli* strain BL21-CodonPlus(DE3)-RIPL (Stratagene). Cultures were grown at 37 °C in LB medium to an $OD_{600}$ of 0.7 before induction with 0.4 mM IPTG at room temperature for 16 h. Cells were harvested by centrifugation and frozen at −80 °C.

Cell pastes of $His_6$-SUMO-UBLCP1 were resuspended in 50 mM Tris (pH 8.0), 300 mM NaCl, 20 mM imidazole, 0.5 mM TCEP [tris(2-carboxyethyl)phosphine], 1 mM Pefabloc SC, 1 mM benzamidine-HCl, 1 mM PMSF, and disrupted by sonication. The fusion protein was purified by Ni-NTA affinity chromatography then digested with the SUMO specific protease ULP1. $His_6$-SUMO and His-ULP1 protease were then removed by a second Ni-NTA chromatography step. Untagged UBLCP1 was recovered in the flow-through and buffer-exchanged into 20 mM Tris (pH 8.5), 100 mM NaCl, 2 mM $MgCl_2$, 2 mM DTT, applied to a Resource-Q anion exchange column (GE Healthcare), and eluted with a linear gradient of NaCl. Peak fractions were adjusted to 20 mM Tris (pH 8.5), 100 mM NaCl, 2 mM $MgCl_2$, 2 mM DTT, concentrated to 21.4 mg/mL, and flash-frozen with liquid nitrogen.

Se-Met substituted UBLCP1 was expressed in the methionine auxotroph *E. coli* strain B834(DE3)(Novagen) using SelenoMet minimal media plus Nutrient Mix (AthenaES) supplemented with 75 mg/L L(+)-Selenomethionine (Acros). Se-Met UBLCP1 was purified as described above except untagged Se-Met UBLCP1 was applied to a Superdex 200 size exclusion chromatography column (GE Healthcare) in place of anion exchange chromatography and run using 20 mM Tris (pH 8.5), 100 mM NaCl, 2 mM $MgCl_2$, and 2 mM DTT. Peak fractions were concentrated to 13.2 mg/mL and flash-frozen with liquid nitrogen.

Drosophila ULBCP1 crystals were grown at 16 °C via sitting-drop vapor diffusion method, using a 1:1 ratio of protein: reservoir solution containing 15–17% PEG 3350, and 0.2M sodium formate. Crystals grew to full size in several days and were transferred to 20% PEG 3350, 0.2M sodium formate, and 15% ethylene glycol before being flash-frozen under liquid nitrogen. The Se-Met derivative crystals used for phase determination were obtained using a 1:1 ratio of protein: reservoir solution containing 14–16% PEG 3350, 0.1M Hepes (pH 7.0), and 0.1M sodium formate and transferred to 20% PEG 3350, 0.1M Hepes (pH 7.0), 0.1M sodium formate, and 15% ethylene glycol for cryo protection before being flash-frozen under liquid nitrogen.

Diffraction data were collected at the Advanced Light Source beam line 8.2.1 and processed with HKL2000 (HKL Research) and CCP4 (1). The structure of UBLCP1 was determined by the multiple-wavelength anomalous diffraction method using the data collected from a Se-Met crystal. Heavy atom search, phase calculation and refinement, density modification, and initial model building were carried out with the AutoSol program (2) in the Phenix software suite (3). The rest of the molecule was traced manually with Coot (4) and refined using Phenix. Five percent randomly selected reflections were used for cross-validation (5), and the refinement strategy included simulated annealing, individual coordinates and *B*-factor refinements, and TLS (translation libration screw motion) refinement (6).

**Cell Culture, Retroviral Infection, and Transfection.** All cell lines were cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin (from Invitrogen and Cellgro). Stable populations were generated by retroviral infection as previously described (7) followed by selection with puromycin (1–2 μg/mL) and/or hygromycin (200 μg/mL) (Invitrogen). Transient transfection of plasmid DNAs was performed using FuGENE 6 Transfection Reagent (Roche), and transfection of siRNA oligonucleotides was achieved using HiPerFect (Qiagen) according to the manufacturer's protocol.

**Plasmids and siRNAs.** The coding sequences of wild-type and truncated UBLCP1 were PCR-amplified from a human cDNA clone (Open Biosystems, Thermo, accession number NM_145049). Flag-, GFP-, His-, and GST-tagged UBLCP1 variants were made by inserting the PCR fragments into the pQCXIP-Flag retroviral vector (from Clontech, modified in our lab), the pEGFP-C1 vector (Clontech), the pSJ2-His vector (a gift from Zhaohui Xu, University of Michigan, Ann Arbor, MI) and the pGEX-KG vector (originally made in our lab), respectively. Site-directed mutagenesis was accomplished using the QuikChange method (Stratagene). N-terminally Flag-tagged 19S subunits were kindly provided by Shigeo Murata (University of Tokyo, Tokyo, Japan). pQCXIP-hRpn11-HTBH has been described previously (8). The nuclear localization signal (NLS) of SV40 Large T antigen (PKKKRKV) was introduced to the end of hRpn11-HTBH by PCR to make hRpn11-HTBH-NLS, which was then cloned into the pQCXIH vector (Clontech). The same NLS was also fused to the N terminus of GFPu, which was then used to replace paGFPu from the paGFPu-IRES-mCherry reporter (provided by Gentry Patrick, University of California, San Diego, CA). $Ub^{G76V}$-GFP was obtained from Addgene, and the same NLS was added to its C terminus to make $Ub^{G76V}$-GFP-NLS. All primer sequences are available upon request. The mammalian expression construct of GST-tagged C-terminal domain is a gift from Jean Wang (University of California, San Diego, CA). AllStars negative control siRNA (AF 488, catalog no. 1027284) and UBLCP1-specific siRNA (sense sequence: 5′-GCACCUAAAUCGUGAUAAATT-3′, antisense sequence: 5′-UUUAUCACGAUUUAGGUGCGC-3′, catalog no. SI02758105) were purchased from Qiagen. For stable UBLCP1 knockdown, the UBLCP1-targeting sequence GGACAGTAGTTACAAGTTA (sense) was used to generate the pSuperRetro-puro construct (Oligoengine) according to the supplier's protocol, and the pSR-GL2 control vector has been reported (7).

**Antibodies and Reagents.** The UBLCP1 polyclonal antibody was generated by immunizing rabbits with His-tagged full-length human UBLCP1 according to standard protocols, and affinity-purified through an antigen-bound *HiTrap™ NHS-activated HP* Column (GE Healthcare). The following antibodies were purchased from the indicated companies: Flag (M2, Sigma); GST, Rpn1, and Rpn2 (Santa Cruz Biotechnology); Rpt3, Rpt6, 20S core particle (CP), and α123567 (Enzo); Lamin A/C and β-tubulin (Cell Signaling); RNA Pol II, H5, and H14 (Covance). Proteasome substrates (Suc-LLVY-AMC, Ac-GPLD-AMC, and Ac-RLR-AMC) and proteasome inhibitors (MG-132 and epoxomicin) were all purchased from Enzo. Cycloheximide was from Sigma and calyculin A from EMD Chemicals.

**Western Blot and Immunoprecipitation.** Subconfluent cells were lysed with NETN lysis buffer (150 mM NaCl, 0.5 mM EDTA, 50 mM Tris, pH 7.5, 0.5% NP-40) supplemented with a protease inhibitor cocktail (1 mM Pefabloc, 1 mM benzamidine hydrochloride, 1 μM leupeptin, and 1 μM E64) and phosphatase inhi-

bitors (10 mM NaF, 20 mM β-glycerolphosphate, and 50 nM calyculin A). ATP (1 or 5 mM) was included whenever proteasome integrity was required. For immunoprecipitation, 2–3 μg of antibody was added to 0.5–1.0 mg of cell lysates and incubated for 2 h at 4 °C. Then protein A or G agarose beads (Invitrogen) were added and incubated for another hour. Beads were extensively washed with NETN buffer and boiled in Laemmli loading buffer. Proteins were separated on SDS-PAGE gels (Mini-PROTEAN TGX, BioRad), transferred to PVDF membranes, blocked in 5% milk, and probed with the desired antibodies.

**Flag Protein Purification.** To identify UBLCP1-interacting proteins by mass spectrometry, ten 10-cm plates of each 293T stable line were collected by centrifugation, washed once with PBS, and lysed in 5 mL of NETN lysis buffer with protease and phosphatase inhibitors as described above. After centrifugation, cell lysates were precleared with 100 μL of IgG Sepharose (GE Healthcare, NETN buffer-washed, 50% slurry) at 4 °C for 1 h. After centrifugation, the supernatant was incubated with 50 μL anti-Flag M2-Agarose slurry (Sigma, preblocked with 1 mg/mL BSA overnight and washed twice with NETN buffer) at 4 °C for 4 h. After extensive washes with NETN buffer, immunoprecipitated proteins were eluted with 100 μL of 3×Flag peptide (Sigma, 250 ng/μL in TBS—25 mM Tris, 125 mM NaCl, pH 8.0) at room temperature for 1 h. The supernatant was collected and the elution step was repeated. Eluted proteins were pooled, concentrated by SpeedVac, boiled in Laemmli loading buffer, and separated on SDS-PAGE. Protein bands were stained with Colloidal blue (Invitrogen), excised, and analyzed by tandem mass spectrometry.

**In Vitro Binding Assay.** $^{35}$S-Methionine labeled proteasome subunits were in vitro transcribed and translated using a TnT T7 coupled wheat germ extract system (Promega) in a reaction volume of 50 μL containing 0.6 $\mu$Ci/μL $^{35}$S-methionine (PerkinElmer) and 1 μg DNA template. All 19S regulatory particle (RP) subunits except Rpn11 were in the pcDNA3.1 vector and N-terminally Flag-tagged [from Shigeo Murata (University of Tokyo, Tokyo, Japan)]. Rpn11-HTBH was subcloned into the pBluescript KS vector. Both vectors contain the T7 promoter. Recombinant GST or GST-UBLCP1 (20 μg) was incubated with equilibrated GST-Bind Resin (Novagen) in 200 μL buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 0.5% Triton X-100). Upon completion of the in vitro trancription and translation reactions, 2% of the total input was removed for analysis, and the remainder was incubated with the above immobilized recombinant proteins for 2.5 h at 4 °C. Reactions were then washed 4 times with the above buffer, removed of all remaining liquid by needle and syringe and eluted in 2× SDS loading buffer. Samples were then subjected to SDS-PAGE and analyzed by Coomassie staining followed by autoradiography.

**Fluorescence Microscopy.** For UBLCP1 and Rpt6 immunostaining, HaCaT cells were grown on glass coverslips in a 12-well plate overnight. After PBS wash, cells were fixed with 4% paraformaldehyde and 4% sucrose in PBS, permeabilized with 0.2% Triton X-100, rehydrated, and washed with PBS. The cells were then blocked with 10% normal goat serum (NGS, Jackson Immunoresearch) for 1 h and incubated with mixed UBLCP1 (0.1 μg/mL) and Rpt6 (1:1,000) antibodies diluted in 3% NGS for another hour. Coverslips were washed with PBS and stained with premixed Alexa Fluor 568-conjugated goat-anti-rabbit antibody and Alexa Fluor 488-conjugated goat-anti-mouse antibody (Invitrogen, 1:2,000 each) for 45 min in the dark. After PBS wash, the coverslips were mounted on slides with Prolong Gold antifade reagent containing DAPI (Invitrogen). Cell images were taken with a Leica fluorescence microscope using a 63× oil lens, and processed using ImageJ (NIH). Images of HeLa cells expressing GFP fusion proteins were analyzed using Metamorph software (Universal Imaging Corporation). DAPI images of GFP-positive cells were thresholded and nuclear contour was traced automatically by Metamorph. The nuclear outlines were then transferred onto GFP images.

**pNPP and Malachite Green Assays.** The assays were essentially performed as reported (9). For pNPP assay, a 50-mM substrate was incubated with 1 μg of wild-type His-UBLCP1 or equimolar mutants at 37 °C for 15 min in UBLCP1 phosphatase buffer (100 mM NaOAc, 50 mM bis-Tris, 50 mM Tris, pH 5.0, 1 mM DTT, 10 mM $MgCl_2$). The phospho-peptide array was treated as in ref. 9 with His-UBLCP1 at pH 5.0 and 25 °C for 1 h, and inorganic phosphate release was determined by the Malachite green method.

**Cell Fractionation.** For nucleocytoplasmic fractionation of cells in bulk, the Lamond Lab protocol (http://www.lamondlab.com/f7nucleolarprotocol.htm) was used with the following adaptations: First, instead of Dounce homogenizing, 0.005% digitonin (Sigma) was added to the hypotonic buffer for gentle yet efficient cell permeabilization (10). This reduced sample preparation time to preserve proteasome modifications. PBS-washed cell pellets were resuspended in this buffer by pipetting and incubated on ice for 2 min. Second, the nuclear pellet was quickly washed twice with the above buffer with 0.005% digitonin before passing through the sucrose cushion. Third, nuclear lysis was achieved using the same hypotonic buffer above but with 10-fold more digitonin (0.05%). We chose digitonin over other detergents because it is more compatible with subsequent proteasome activity assays (11). All buffers included 1 mM DTT, 1 mM ATP, and phosphatase inhibitors as described above.

**Gel Overlay Assay and "in-Well" Proteasome Assay.** Gel overlay assays were performed as described (12). Equal amounts of cytosolic and nuclear extracts were separated on a 4.5% native gel. Proteasome activity was visualized after soaking the gel in proteasome assay buffer (50 mM Tris-HCl, pH 7.5, 40 mM KCl, 5 mM $MgCl_2$, 1.0 mM ATP, 1 mM DTT, 50 μg/mL BSA, and phosphatase inhibitors, ref. 11) containing Suc-LLVY-AMC and 0.02% SDS.

For tightly adherent cells such as HaCaT and ZR751, in-well fractionation and proteasome assay were performed in a black 96-well plate. Cells were seeded at a density of $4–8 \times 10^4$ cells per well in triplicate. Twenty-four hours later, cells were washed twice with PBS and permeabilized with 50 μL/well of hypotonic proteasome lysis buffer ("PLB", ref. 11) containing 0.005% digitonin. After gentle rocking at room temperature for 2 min, the released cytosolic proteins were transferred to new wells. The permeabilized cells were quickly washed twice with 50 μL of the same PLB, and the nuclei were lysed with 50 μL of PLB containing 0.05% digitonin at room temperature for 5 min with constant shaking. The activity assay was started by adding 50 μL of 200 μM (2×) substrate to each well (diluted in the proteasome assay buffer above, with or without proteasome inhibitors). All buffers included 1 mM DTT, 1 mM ATP, and phosphatase inhibitors.

**Size-Exclusion Chromatography.** Two 10-cm plates of 293T stable lines expressing control or UBLCP1 shRNA were lysed in 300 μL of buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 10% glycerol, 5 mM $MgCl_2$, 0.22 μm-filtered) supplemented with 0.5% NP-40, 1 mM DTT, 1 mM ATP, 50 nM calyculin A, and 10 mM NaF. Cell lysates were cleared twice by centrifugation, 0.22 μmfiltered, and quantified by Bradford protein assay. Equal amounts of each lysate (2.0 mg in 200 μL) were sequentially injected into a Superose 6 10/300 GL column (GE Healthcare) controlled by the ÄKTAFPLC™ system (GE Healthcare). The column was preequilibrated and run with the above buffer without NP-40

at a rate of 0.2 mL/min. Fractions (0.3 mL each) were collected from 0.12 to 0.82 column volume (CV = 24 mL), and 15 μL was withdrawn from each fraction for Western blot or proteasome activity assay.


1. Collaborative Computational Project, Number 4 (1994) The CCP4 suite: Programs for protein crystallography. *Acta Crystallogr D Biol Crystallogr* 50:760–763.
2. McCoy AJ, Storoni LC, Read RJ (2004) Simple algorithm for a maximum-likelihood SAD function. *Acta Crystallogr D Biol Crystallogr* 60:1220–1228.
3. Adams PD, et al. (2010) PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66:213–221.
4. Emsley P, Cowtan K (2004) Coot: Model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* 60:2126–2132.
5. Brunger AT (1992) Free *R* value: A novel statistical quantity for assessing the accuracy of crystal structures. *Nature* 355:472–475.
6. Afonine PV, Grosse-Kunstleve RW, Adams PD (2005) A robust bulk-solvent correction and anisotropic scaling procedure. *Acta Crystallogr D Biol Crystallogr* D61:850–855.


## Other Supporting Information Files

Dataset S1 (XLSX)

Peptides of proteasome subunits identified by mass spectrometry.


7. Guo X, et al. (2008). Axin and GSK3-β control Smad3 protein stability and modulate TGF-β signaling. *Genes Dev* 22:106–120.
8. Wang X, et al. (2007) Mass spectrometric characterization of the affinity-purified human 26S proteasome complex. *Biochemistry* 46:3553–3565.
9. Kim Y, et al. (2007) A conserved phosphatase cascade that regulates nuclear membrane biogenesis. *Proc Natl Acad Sci USA* 104:6596–6601.
10. Adam SA, Marr RS, Gerace L (1990) Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J Cell Biol* 111:807–816.
11. Kisselev AF, Goldberg AL (2005) Monitoring activity and inhibition of 26S proteasomes with fluorogenic peptide substrates. *Methods Enzymol* 398:364–378.
12. Leggett DS, Glickman MH, Finley D (2005) Purification of proteasomes, proteasome subcomplexes, and proteasome-associated proteins from budding yeast. *Methods Mol Biol* 301:57–70.


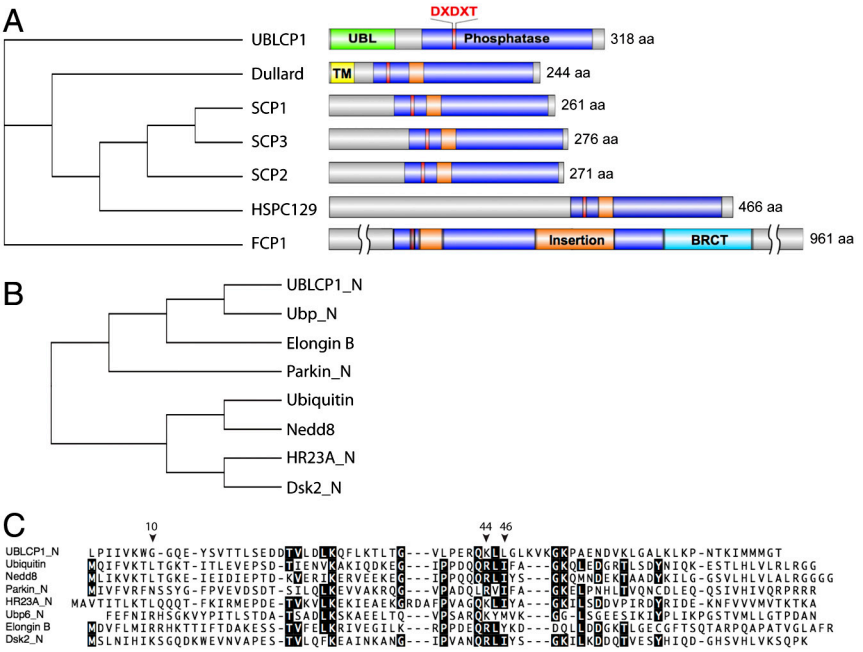


**Fig. S1.** Phylogenetic and sequence analysis of UBLCP1. (*A*) A phylogenetic tree of the seven haloacid dehalogenase-like phosphatase family members, based on ClustalW alignment of the human proteins. Schematics of the domain structures are shown (*Right*). The phosphatase domains are colored in dark blue, the DXDXT catalytic motif in red, insertion domains in orange, UBL domain in green, transmembrane (TM) region in yellow, and BRCT domain in light blue. The β-hairpin-like insertion domain is present in SCP1 and FCP1 and is predicted to exist in all other family members except for UBLCP1. FCP1 also contains an additional larger insertion (122 amino acids). (*B*) Phylogenetic analysis of ubiquitin and seven UBLs including UBLCP1_N. (*C*) Alignment of ubiquitin and UBL domain sequences from selected human proteins as in *B*. Residues of UBLCP1_N that were mutated for further analysis (Gly10, Lys44, and Leu46) are highlighted with arrowheads.

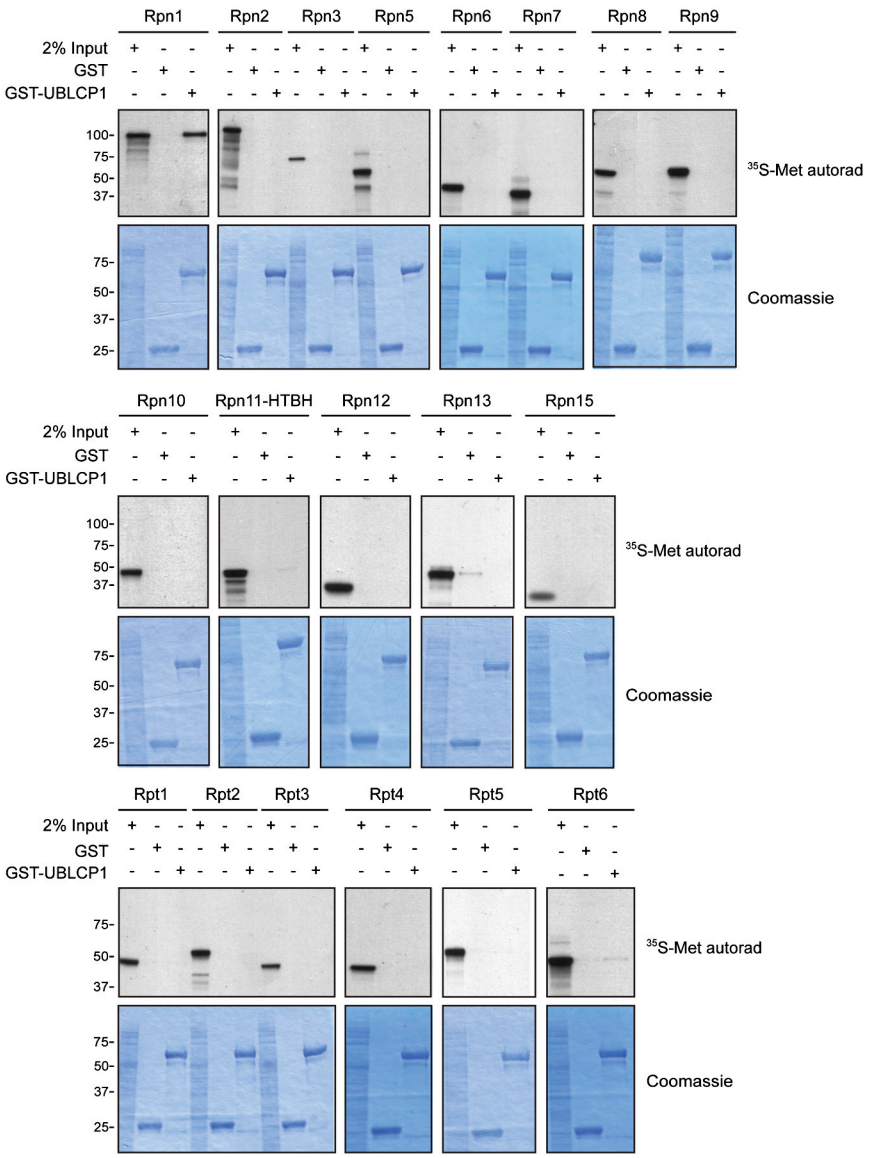


**Fig. S2.** In vitro binding assay between UBLCP1 and 19S subunits.

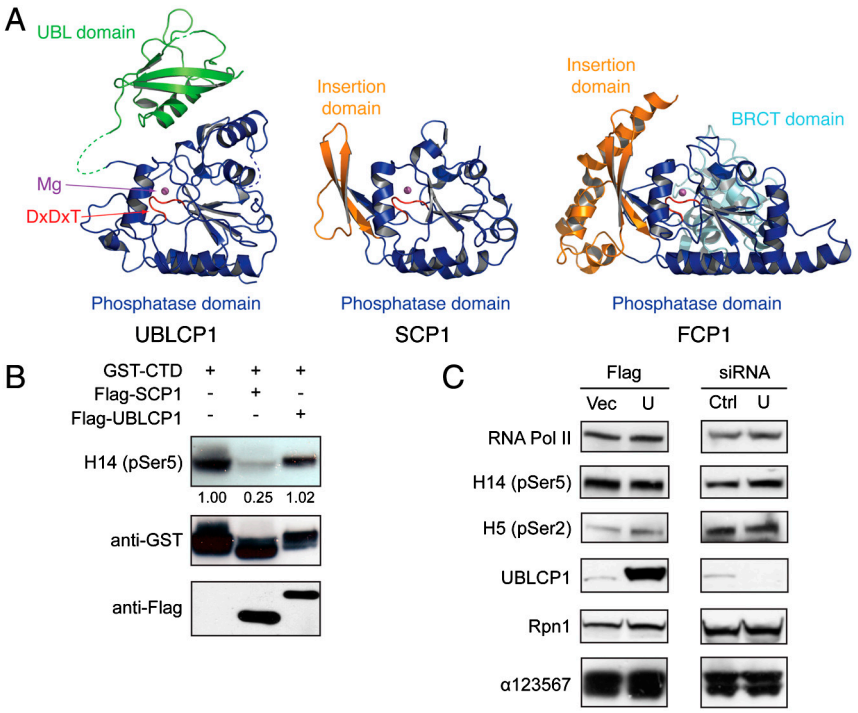


**Fig. S3.** Structural and biochemical comparison of UBLCP1, SCP1, and FCP1. (*A*) Crystal structures of fly UBLCP1 (PDB ID code 3SHQ), yeast SCP1 (PDB ID code 2GHQ), and human FCP1 (PDB ID code 3EF0). All functional domains are shown by the same color code as in Fig. S1*A*. The DXDXT motif (red) and the coordinated $Mg^{2+}$ ion (magenta) are also indicated. (*B*) GST-CTD dephosphorylation in 293T cells. The GST-CTD construct was cotransfected with either vector control, Flag-SCP1 or Flag-UBLCP1, and cell lysates were probed with the indicated antibodies. The pSer5/GST ratio of each sample was shown, with that of the control sample set to 1.00. (*C*) Ser2 and Ser5 phosphorylation of endogenous Pol II CTD in 293T cells transfected with control or UBLCP1 (U) plasmids/siRNAs. Proteasome proteins (Rpn1 and the α-subunits) remained at a constant level and are shown here as loading controls.

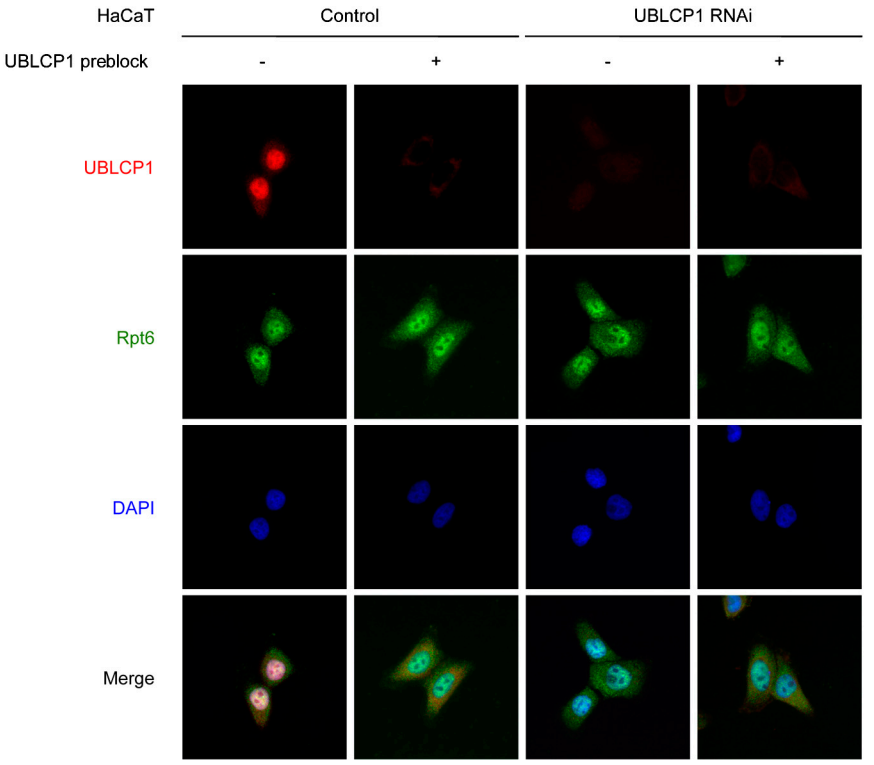


**Fig. S4.** Controls for UBLCP1 immunostaining. HaCaT pSuperRetro stable lines were immunostained as described in *SI Materials and Methods*. For antibody preblocking, 5 μg of purified His-UBLCP1 (WT) was added to 250 μL of working solution of anti-UBLCP1 antibody (0.1 μg/mL). The mixture was incubated at room temperature for 1 h with constant shaking and then used for staining.

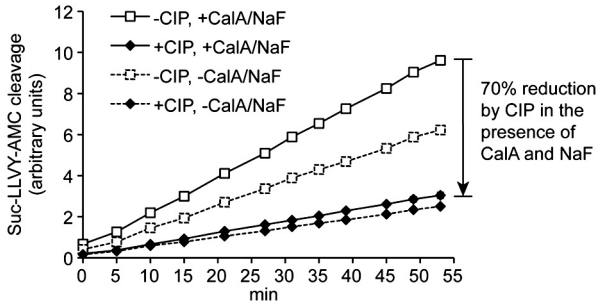


**Fig. S5.** Alkaline phosphatase (CIP) inhibits proteasome activity. Purified nuclear proteasomes (0.2 μg) from 293T pSuperRetro-UBLCP1/Rpn11-HTBH-NLS cells were mixed with or without CIP (NEB, 5 U/reaction) in NEB Buffer 2 (20 μL reaction volume) in the presence or absence of calyculin A (50 nM)/NaF (10 mM). The reactions were incubated at 37 °C for 45 min before the Suc-LLVY-AMC substrate solution was added. Proteasome activity was measured in triplicate for each condition, and the data are shown as average ± SD. CIP is not inhibited by calyculin A or NaF, whereas there was calyculin A/NaF-sensitive phosphatase(s) copurified with the proteasome (compare solid open squares with dashed open squares).

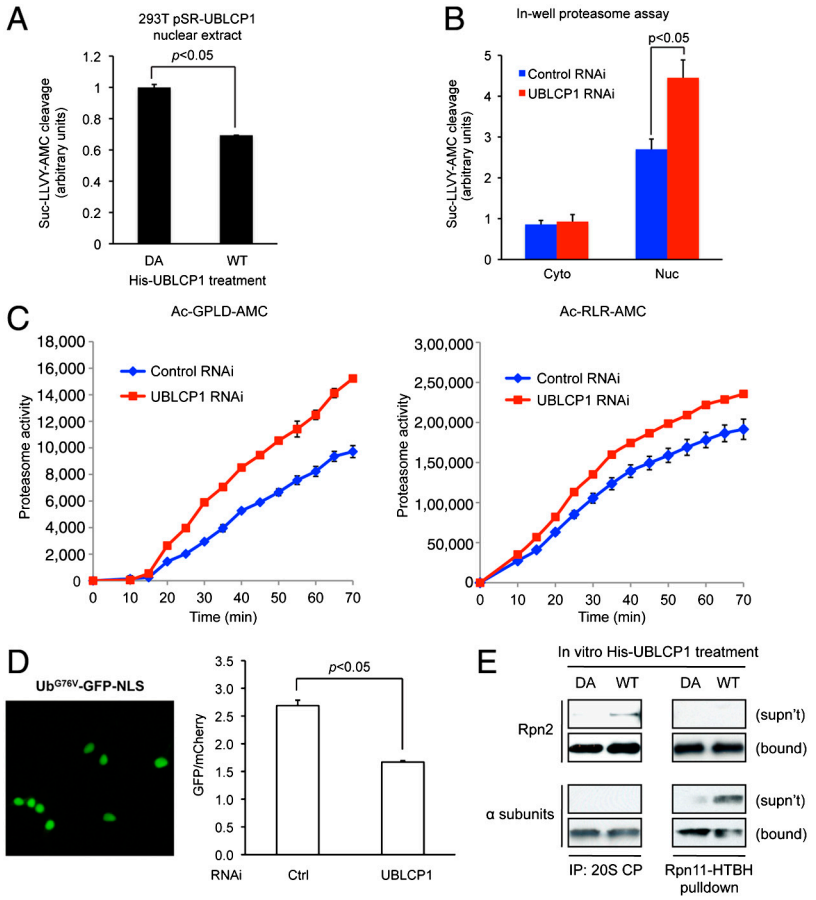


**Fig. S6.** UBLCP1 inhibits nuclear proteasome activity. (*A*) One 10-cm plate of 293T pSuperRetro-UBLCP1 cells were fractionated, and the nuclear extract (10 μg, containing DTT, ATP, phosphatase inhibitors but not protease inhibitors) was treated in vitro with purified His-UBLCP1 (WT or DA, 1 μg) at 37 °C for 45 min. Nuclear proteasome activity was then measured after incubation with the substrate at 37 °C for 15 min. (*B*) In-well fractionation and proteasome activity assay using HaCaT pSuperRetro stable lines. (*C*) In-well proteasome assay on nuclear lysates from ZR751 pSuperRetro stable lines with Ac-GPLD-AMC (*Left*) and Ac-RLR-AMC (*Right*) as substrates. UBLCP1 knockdown also enhances the caspase-like and trypsin-like activities of nuclear proteasomes. (*D*) 293T cells (control and UBLCP1 knockdown) were cotransfected with $Ub^{G76V}$-GFP-NLS and mCherry. Fluorescence was read from cell lysates at 24 h posttransfection. $Ub^{G76V}$-GFP-NLS was nuclear localized (*Left*) and expressed at a lower level in UBLCP1 knockdown cells, suggesting reduced protein stability (*Right*). (*E*) In vitro dissociation of RP and CP by UBLCP1 treatment. The 26S proteasomes were isolated by anti-20S IP (*Left*) or Rpn11-HTBH pulldown (*Right*) from calyculin A treated cell lysates. After washes, immobilized proteasomes were treated with UBLCP1 (WT or DA) at 37 °C for 45 min with constant rocking. Samples were then collected by brief centrifugation. The supernatants containing dissociated proteasome complexes were transferred to new tubes, whereas bound proteasomes were washed once with 1× UBLCP1 buffer, and both fractions were boiled in sample buffer for Western blot analysis.

**Table S1. Proteasome subunits identified by mass spectrometry following Flag-UBLCP1 immunoprecipitation**

| Band # | Protein name | Swissprot | RefSeq | Size, aa/kD | aa coverage, % |
|---|---|---|---|---|---|
| 1 | 26S proteasome non-ATPase regulatory subunit 1; RPN2; PSMD1; | Q99460 | NP_002798.2 | 953/106 | 36 |
| 2 | 26S proteasome non-ATPase regulatory subunit 2; RPN1; PSMD2; TRAP2 | Q13200 | NP_002799.3 | 908/100 | 43 |
| 3 | 26S proteasome non-ATPase regulatory subunit 3; PSMD3; RPN3 | O43242 | NP_002800.2 | 534/61 | 47 |
| 4 | 26S protease regulatory subunit 4; Proteasome 26S subunit ATPase 1; PSMC1; RPT2 | P62191 | NP_002793.2 | 440/49 | 51 |
| | 26S proteasome non-ATPase regulatory subunit 12; PSMD12; RPN5 | O00232 | NP_002807.1 | 456/53 | 34 |
| 5–7 | 26S protease regulatory subunit 6A; Proteasome 26S subunit ATPase 3; PSMC3; TBP1; RPT5 | P17980 | NP_002795.2 | 439/49 | 34 |
| | 26S protease regulatory subunit 7; Proteasome 26S subunit ATPase 2; PSMC2; MSS1; RPT1 | P35998 | NP_002794.1 | 433/48 | 33 |
| | 26S protease regulatory subunit 6B; Proteasome 26S subunit ATPase 4; PSMC4; TBP7; RPT3 | P43686 | NP_006494.1, | 418/47 | 40 |
| | 26S proteasome non-ATPase regulatory subunit 11; PSMD11; RPN6 | O00231 | NP_002806.2 | 422/47 | 30 |
| | 26S protease regulatory subunit 8; Thyroid hormone receptor-interacting protein 1; PSMC5; RPT6 | P62195 | NP_002796.4 | 406/46 | 22 |
| 8 | 26S proteasome non-ATPase regulatory subunit 14; PSMD14; POH1; RPN11 | O00487 | NP_005796.1 | 310/35 | 46 |