

**Ubistatins Inhibit Proteasome-Dependent Degradation
by Binding the Ubiquitin Chain**

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Materials and Methods

Preparation of Proteins for Screening

To construct a cyclin-luciferase fusion protein (pSP cyc-luc), the N-terminal sequence of *Xenopus laevis* cyclin B1, including amino acids 2-97, was amplified by PCR, digested with BstEII, and ligated into the pSP-lucNF expression vector (Promega). The resulting vector was sequence verified. The fusion protein was expressed by coupled *in vitro* transcription and translation in reticulocyte lysate using the SP6-TNT Coupled Reticulocyte Lysate System (Promega) and flash frozen in liquid nitrogen until the time of use. The parental pSP-lucNF vector was used to express unmodified luciferase.

A vector for expression of cyclin-luciferase in *E. coli* (pET cyc-luc) was also constructed; this protein behaved identically in all assays to the protein expressed in reticulocyte lysate, but could be made in higher quantities necessary for screening. pSP cyc-luc was digested with HindIII and XhoI. The resulting 1949 bp fragment containing the cyclin B1-luciferase sequence was ligated into the pET 28b expression vector

(Novagen) containing an N-terminal hexahistidine tag for protein purification. To express this fusion protein, one liter of LB containing *E. Coli* strain BL21(DE3) was grown at 37 °C to an OD600 of 0.6. Expression was induced for 3 hrs with 1mM IPTG. The cells were pelleted and lysed, and protein purified by Ni-NTA batch purification under native protein conditions (Qiagen). Sea urchin cyclin BΔ90 was prepared as described (S1). Methylated ubiquitin was prepared by reductive methylation of bovine ubiquitin as described (S2).

Preparation of *Xenopus* Egg Extracts

Xenopus egg extracts were prepared from eggs laid overnight according to the protocol of Murray (S3) with the exception that eggs were activated with 2 µg/ml calcium ionophore (A23187, free acid form, Calbiochem) for forty minutes prior to the crushing spin. Extracts were frozen in liquid nitrogen and stored at -80 °C. Eggs laid from 40 frogs typically yielded a total 70 ml of cytoplasmic extract.

Assay Validation

Extracts were rapidly thawed and diluted to a final concentration of 75% in extract buffer (XB) just prior to assay (XB; 100 mM KCl, 0.1 mM CaCl₂, 1 mM MgCl₂, 10 mM Potassium HEPES, pH 7.7, 50 mM sucrose). Extracts were kept on ice and supplemented with 1 mg/ml bovine ubiquitin (Sigma) unless otherwise stated. Cyc-luc expressed in reticulocyte lysate was diluted 1/200 for most assays. High-throughput screening utilized the bacterially expressed and purified cyclin-luc fusion protein, which was added to extracts at a final concentration of 0.1 µg/ml. To induce entrance into

mitosis, cyclin B Δ 90 protein was added to a final concentration of 10 μ g/ml. For the dose response analysis (Fig. S2), inhibitors were purchased from Calbiochem (San Diego, CA) and dissolved in DMSO as 10 mM stocks. Cyclin-luciferase and cyclin B Δ 90 were mixed with interphase extract and placed on ice. Inhibitors were then added to extracts to yield a final DMSO concentration of 1%. 10 μ l aliquots of extract were distributed into 384-well white Cliniplates (Labsystems), and cell cycle progression was initiated by warming the samples to room temperature. After 60 minutes, 30 μ l of luciferin reagent (20 mM Tricine pH 7.8, 470 μ M D-Luciferin [Molecular Probes], 270 μ M Coenzyme A, 0.1 mM EDTA, 33 mM DTT, and 530 μ M ATP) was added using a multidrop dispenser (Labsystems). Luminescence was measured on an Analyst plate reader (LJL Biosystems). The values for 3 replicates were averaged. For the dose response analysis (Fig S2), percent inhibition was calculated as $100 \cdot (T-M)/(I-M)$ where T equals the test value for the inhibitor, M equals the value in a mitotic extract lacking inhibitor, and I equals the value in an interphase extract. For the experiment in Fig. S1, methylated ubiquitin was added to interphase extracts at a concentration of 1 mg/ml in the presence or absence of added unmodified ubiquitin (1 mg/ml) used as competitor. Cyclin-luciferase and cyclin B delta 90 were then added. After 60 minutes, the reactions were stopped and analyzed as described above.

Chemical Libraries and Chemical Characterization of Active Compounds

Compound collections screened included 16,320 compounds from Chembridge Corporation (San Diego, CA; Diverset E); 1991 compounds from the NCI Diversity set, and 90,802 compounds from the NCI open collection. Compounds that retained activity

after retesting were reobtained as dry powders and characterized by LC-MS; only compounds that were greater than 90% pure with an appropriate mass were evaluated in further experiments and presented in Figure 1.

For ubistatin A (C92), we performed additional structural characterization of a sample of dry powder provided by the NCI (NSC665534). NMR spectra were recorded at ambient temperature in D₂O with a 5 mm probe operating at 500 MHz (¹H) or 75 MHz (¹³C). For ¹H NMR the internal reference was TSP (δ 0.00). ¹H NMR (D₂O) δ 8.51 (d, J=3.0 Hz, 2H), 8.21 (d, J=2.1 Hz, 2H), 7.98(br, J=8.1 Hz, 2H), 7.80 (m, 6H), 7.60 (dd, J=8.4; 2.1 Hz, 2H) 7.35 (dd, J=8.4; 4.2 Hz, 2H), 6.82 (d, J=8.7 Hz, 2H, H-d). These measurements are identical to those reported in the literature (S4). Elemental analysis was also performed (M-H-W laboratories, Phoenix AZ): Anal. Calcd. for C₃₄H₂₀N₄Na₄O₁₆S₄; C, 42.50; H, 2.10; N, 5.83. Found: C, 42.65; H, 2.24; N, 5.88.

High-throughput screening

Chilled interphase *Xenopus* extracts containing cyclin B Δ90 and cyclin-luciferase were spread onto chilled custom-designed 1536-well plates (S5) that held 2 microliters of extract per well. 100 nl of compound (5-10 mM in DMSO) was then transferred into the assay plate using a custom-designed pin-transfer robot (S5). After a 60 minute incubation at room temperature, a PixSys 3200 dispenser (Cartesian Technologies, Irvine, CA) was used to dispense 200 nl of a 10x concentrated luciferin solution to each well of the assay plate. Immediately after filling, the plate was imaged using the Leadseeker system (Amersham Pharmacia Biotech, Amersham, U.K.). Exposure times were typically 2

minutes. Image analysis and quantitation was performed using the MCID Assayvision software (Imaging Research Incorporated, St. Catherines, Canada).

A total of 109,113 compounds were screened in duplicate. A low threshold was set for identification of initial hits, and included compounds that increased the luminescence reading by two-fold above the median value calculated for all wells on the plate. We included compounds that scored on either of the duplicate set of plates, and noted that about 65% of the compounds scored on both plates, whereas 35% were active only on a single plate. This rate of overlap may be a result of variability in the amount of compound transferred. The initial screen identified a total of 1017 hits (247 from the Chembridge Library and the NCI diversity set; 770 from the NCI open collection). In the next step, we cherry picked the DMSO stocks of active compounds to create a new master plate. All of the hits from the Chembridge Library and the NCI diversity set were replated, whereas only 444 compounds from the NCI open collection were replated, as high fraction of compounds were excluded due to chemical or structural considerations (compounds with very simple structures, reactive structures, or which contained metal complexes were excluded). The resulting 691 compounds were then retested in 384-well plates at 200 μ M concentration under the conditions described for validation of the assay. In this case, compounds were prediluted in XB prior to addition to extracts by pipetting, assuring more accurate compound concentration. A large number of compounds failed to retest as positive at this point, presumably due to the higher concentrations used in the primary screen. The 96 most active compounds were then reobtained as dry powders from either Chembridge or the NCI. These compounds were retested in the four assays described in Table 1, at 200 μ M concentration, with the exception of C10 and C92, which

were tested at 100 μM concentration. Compounds were tested at high concentration, as *Xenopus* extract generally require much higher concentration of compounds than corresponding experiments in mammalian cells or with purified proteins, due to the high concentration of lipid and protein in extracts. The data in Table 1 represent the 22 most active compounds (compounds that showed at least 30% inhibition) whose structures could also be confirmed by LC-MS analysis. Table S1 provides the relevant Chembridge ID or NCI identification numbers for each of these compounds. Structures of these compounds will also be accessible on the ChEMBL web site upon publication (<http://chembank.med.harvard.edu/>).

Retesting of Active Compounds

Dry compounds were redissolved at 20 mM concentration in DMSO, or at 10 mM in a 50:50 water:DMSO mixture (for C10 and C92). These compounds were then diluted ten-fold in XB and mixed thoroughly. To retest compounds under conditions of the original screen, in which compounds were added prior to mitotic entry, chilled interphase extract was mixed with cyclin B $\Delta 90$ (10 $\mu\text{g}/\text{ml}$) and cyc-luc (0.1 $\mu\text{g}/\text{ml}$) in bulk and then 27 μl of extract was pipetted to each well of a chilled 384-well plate. Three microliters of each compound (diluted in XB) was then added to each well, and then compounds mixed thoroughly, yielding a final concentration of 200 μM (100 μM for C10 and C92). Plates were warmed to room temperature. After 70 minutes, 5 μl aliquots were transferred to another plate, and then 30 μl of luciferin reagent was added to each well and luminescence measured. Percent inhibition was calculated as described above. The reported values represent the average of three independent measurements. To determine

whether compounds lost inhibitory activity when added after mitotic entry, interphase extracts were treated with 10 μg /cyclin B $\Delta 90$ for 50 minutes at room temperature to drive entry into mitosis and then chilled on ice. Cyc-luc was then added, and then 27 μl of extract was pipetted to each well of a chilled 384-well plate. Three microliters of each compound (diluted in XB) was then added to each well, and then compounds mixed thoroughly. Plates were warmed to room temperature. After 60 minutes, 5 μl aliquots were transferred to another plate, and then 30 μl of luciferin reagent was added to each well and luminescence measured. To determine whether compounds inhibited Cdh1-induced proteolysis, recombinant Cdh1 (0.1 $\mu\text{g}/\text{ml}$) and cyc-luc were added to chilled interphase extracts. Extracts were then pipetted and compounds added as above. Plates were warmed to room temperature and incubated for three hours, following which luminescence was measured as above. To determine whether compounds inhibited degradation of a β -catenin reporter protein, chilled interphase extracts received recombinant Axin and beta-catenin-luciferase reporter as described (S6). Extracts were aliquoted to chilled plates and compounds distributed as described above. Plates were warmed to room temperature, incubated for three hours, and luminescence measured. In all assays, percent inhibition was calculated as $100 \times (\text{T}-\text{A})/(\text{I}-\text{A})$ where T equals the test value for the inhibitor, I equals the value for inactive interphase extracts lacking either cyclin B $\Delta 90$, Cdh1, or Axin protein, and A equals the value for an extract that lacks a chemical inhibitor but is stimulated to degrade the reporter protein by addition of cyclin B $\Delta 90$, Cdh1, or Axin protein. In the case of Cdh1 addition, we noted that many of the Class I compounds led to stimulation of proteolysis under these conditions (Table 1). As the same effect was observed with addition of the kinase inhibitor roscovitine to extracts,

we believe that these compounds stimulate Cdh1-dependent degradation by inhibiting an unidentified kinase in interphase *Xenopus* extracts.

Preparation of UbSic1

Sic1, expressed in *E. coli* as a Maltose-binding-protein chimera tagged at the C-terminus with the MycHis6 tag (MbpSic1^{mycHis6}) was purified as described (S7). It was phosphorylated and ubiquitinated utilizing insect expressed kinase and SCF^{Cdc4} complexes as described (S8). Ubiquitinated MbpSic1^{mycHis6} was designated UbSic1 in the text.

Purification of 26S proteasomes

26S proteasomes were purified from *S. cerevisiae* cells expressing a Flag-tagged proteasomal subunit (*PRE1*) essentially as described (S9). Briefly, lysates were immunoaffinity purified on anti-Flag resin in the presence of 2mM ATP and 5 mM MgCl₂, and eluted with Flag peptide. 26S proteasomes were purified from rat liver as described (S10).

Degradation of UbSic1

Ubiquitinated Sic1 (~300 nM) was incubated with purified 26S proteasomes (~100 nM) at 30°C for 5 min (S9). The reaction tubes were transferred to ice and quenched with 5X Laemmli SDS sample buffer. Aliquots were resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with polyclonal anti-Sic1 antibody.

Deubiquitination of UbSic1

Purified 26S proteasomes were preincubated with 100 μ M epoxomicin at 30°C for 45 min before addition of UbSic1 (*S11*). Reactions were processed as above.

Purification of Gst-Fusion Proteins

Recombinant proteins were purified as described (*S12*).

Binding of UbSic1 to 26S proteasomes

26S proteasomes were immunoprecipitated with anti-Flag resin from tagged and untagged control strains and incubated with 1 mM phenanthroline, 2.5 μ M Ub-aldehyde, and 100 μ M MG132 at 4°C for 45 min. UbSic1 was then added in binding buffer containing 25 mM Tris, pH 7.5, 1 mM ATP, 100 mM NaCl, 5 mM MgCl₂, 0.2 % Triton in the presence or absence of 5 μ M C92. Following binding, beads were washed twice with the same buffer, and once with 25mM Tris, 5mM MgCl₂, 1 mM ATP. Washed beads were resuspended in 2X SDS sample buffer and aliquots analyzed by immunoblotting with anti-Sic1 antibody.

Binding of UbSic1 to Gst-Fusion proteins

Recombinant Gst-Rpn10 and Gst-Rad23 were immobilized on glutathione sepharose beads. UbSic1 was added in binding buffer containing 25 mM Tris, pH 7.5, 150 mM NaCl and 0.2 % Triton in the presence or absence of C92 or C59. Following binding at 4°C for 90 minutes, the beads were centrifuged, and washed twice with the same buffer, and twice with buffer containing 25mM Tris, pH 7.5. They were then

resuspended in an equal volume of 2X SDS sample buffer. Aliquots were analyzed by immunoblotting.

Microinjection experiments

HEK-293 cells stably transfected with a plasmid that expresses AR-GFP were microinjected with 0.2 μ l (5-10% of cell volume) of a 200 mM KCl solution containing 10 μ M Protac and 50 mg/ml rhodamine dextran (MW 10,000 Da). For C92 and proteasome inhibition experiments, cells were co-injected with 1 μ M C92 or epoxomicin (yielding a final intracellular concentration of 50-100 nM) and Protac (10 μ M) as previously described (*S13*).

Ornithine Decarboxylase Degradation Experiments

Assays of the degradation of ^{35}S -mODC were performed as described (*S10*) in a volume of 20 μ l at 37 °C and contained: 50mM Tris-HCl pH7.5, 5 mM MgCl_2 , 1 mM ATP, 10 mM KCl, 10 % glycerol, an ATP regenerating buffer (2 mM DTT, 10 mM creatine phosphate, 1.6 mg/ml creatine kinase), 2 mg/ml BSA, 50 nM ^{35}S -mODC, and 50 nM rat proteasomes. Reactions were preincubated with inhibitors or cold ODC for 10 minutes, and degradation initiated by addition of proteasomes. Reactions were quenched after 30 minutes by adding 140 μ l of 20 % trichloroacetic acid. After microcentrifugation for 30 min at 14000g, 150 μ l of the supernatant was removed for scintillation counting to determine released counts. Total counts were obtained using water in place of trichloroacetic acid. Background of released counts without proteasome was about 0.5%

of total counts. Percentage of degradation of labeled ODC was determined by the formula: percent degradation= (released cam-background cpm)/total cpm.

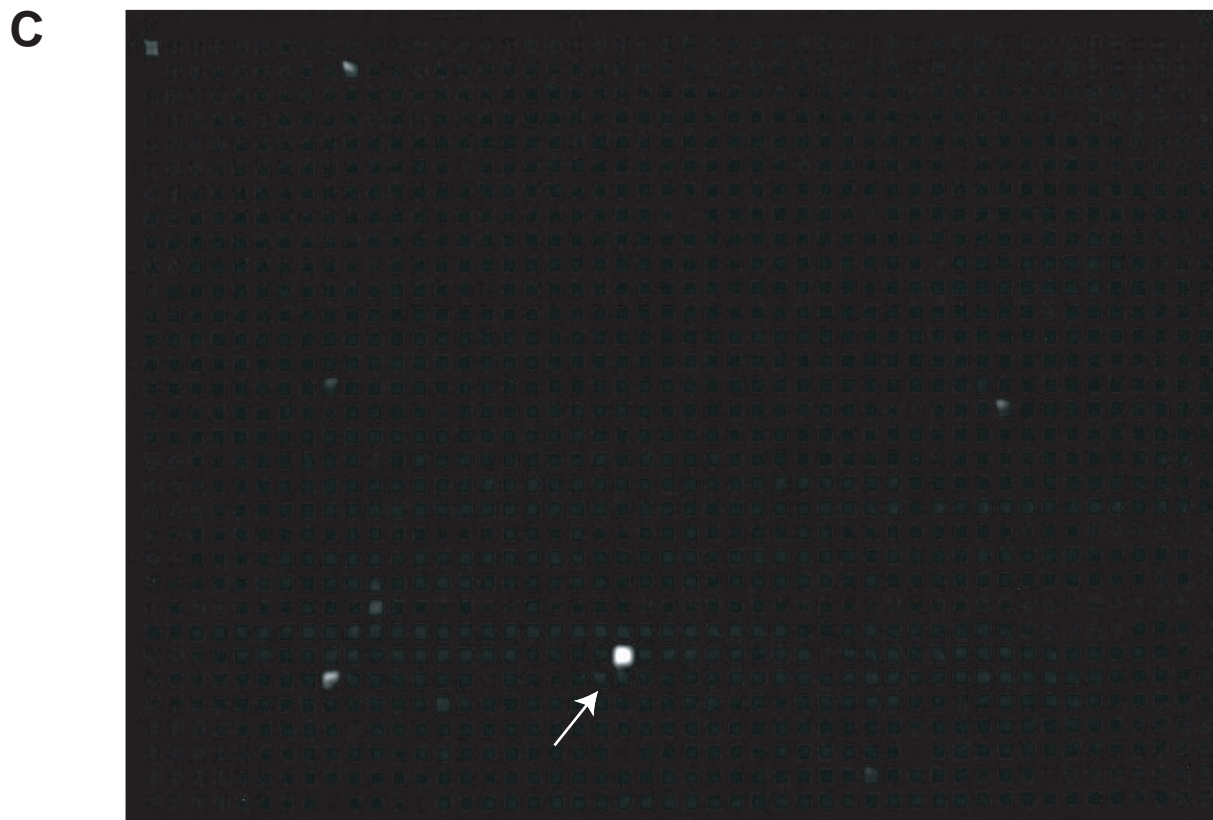
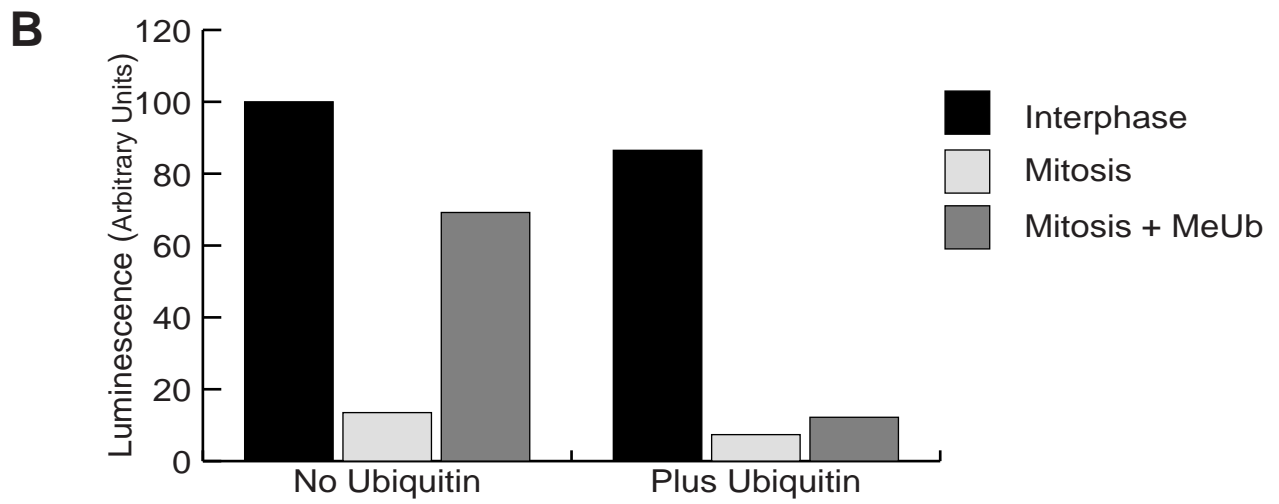
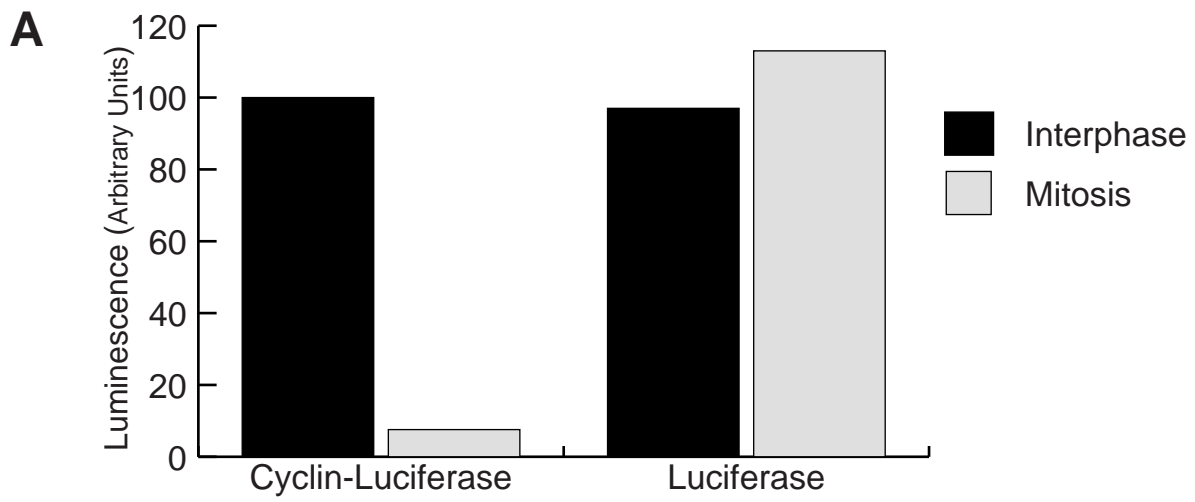
NMR studies

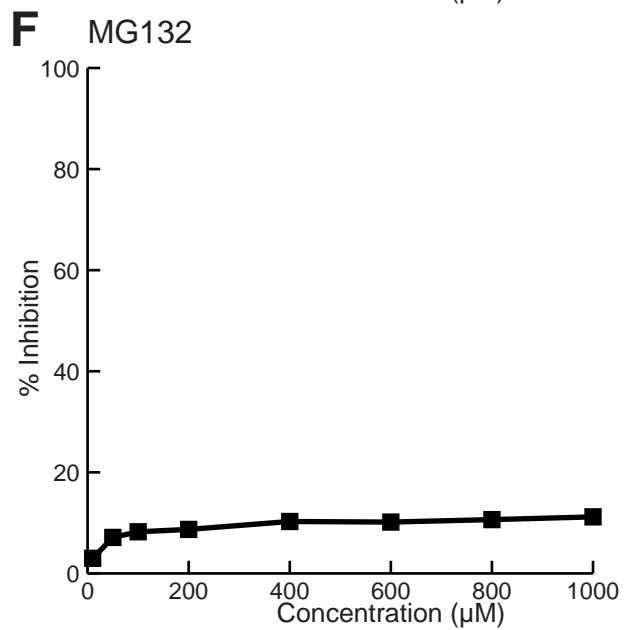
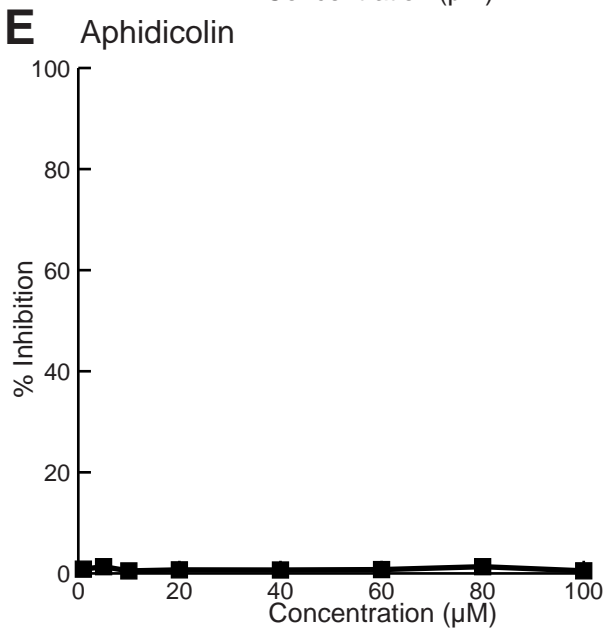
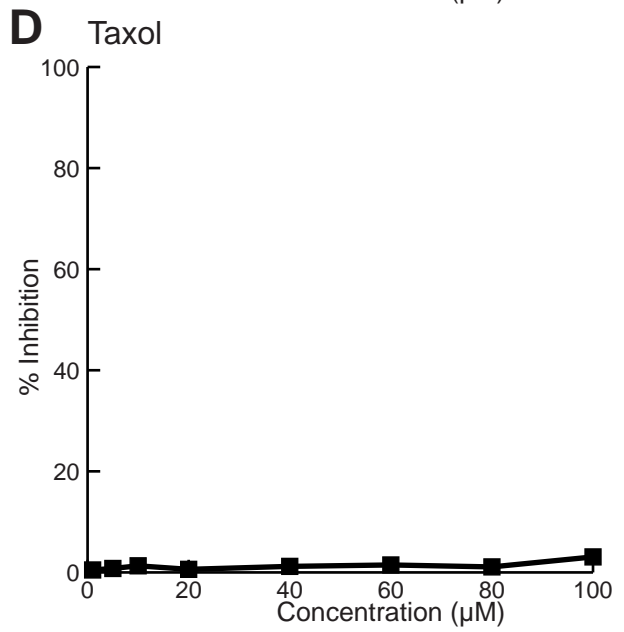
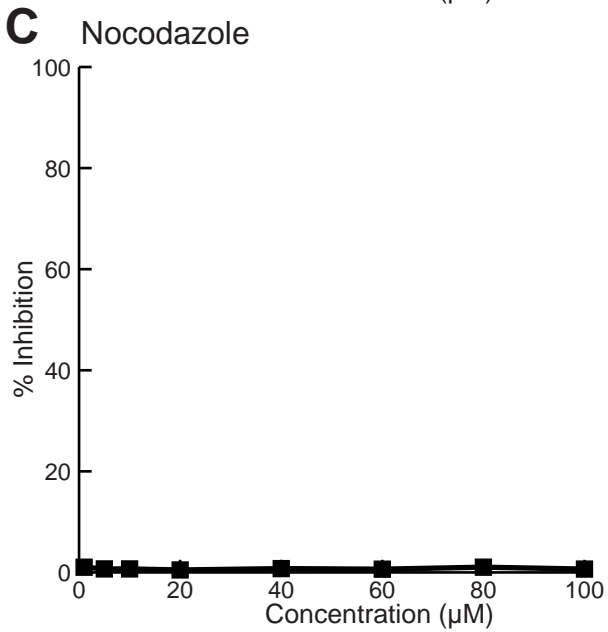
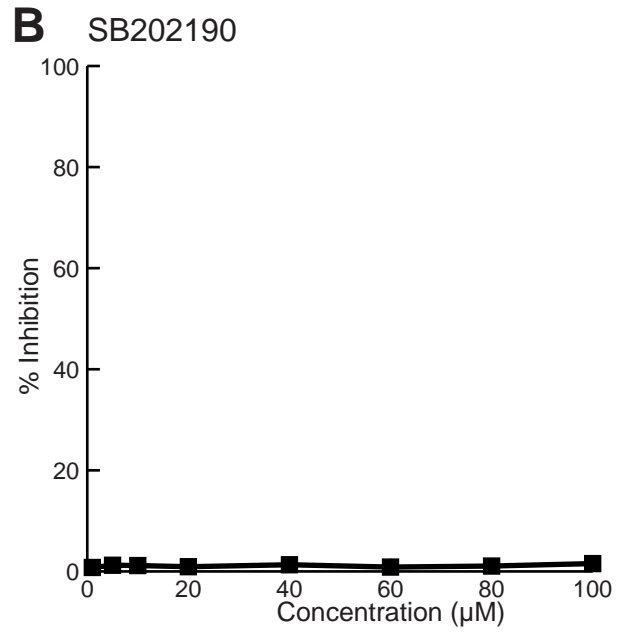
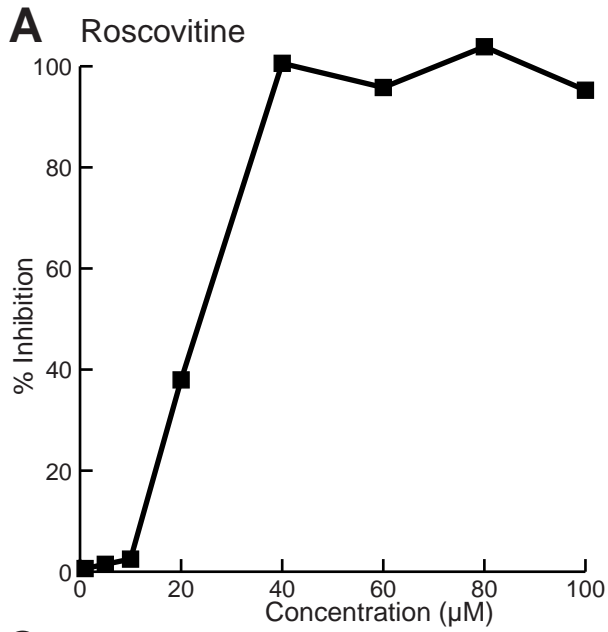
Synthesis of segmentally ^{15}N -labeled Ub₂ chains was performed as described in (S14). Ubiquitin monomers and C170S-E2_{25K} were expressed and purified as described in (S15). ^{15}N -labeled Ub (D77) and Ub (K48C) were expressed in *E. coli* cells grown in minimal media with $^{15}\text{NH}_4\text{Cl}$ as the sole source of nitrogen. E1 and Ub C-terminal hydrolase were from Boston Biochem Inc., and ethyleneimine was from Chemservice.

All NMR measurements were performed at 24 °C on a Bruker DRX spectrometer operating at ^1H resonance frequency of 600.13 MHz. The protein samples (concentration range from 0.1-0.6 mM) were prepared in 20 mM phosphate buffer, pH 6.8, containing 7% D₂O and 0.02% NaN₃. ^1H - ^{15}N HSQC spectra were acquired with spectral widths of 7.2 kHz and 2 kHz in the ^1H and ^{15}N dimensions, respectively. For each 2D plane, 128 t₁ increments were collected, each consisting of 1024 complex points.

For NMR titration studies ubistatin A was added to the di-ubiquitin sample in small steps from a 1 mM stock solution in the same buffer as the protein. The titration continued up to a molar ratio, ubistatin:Ub₂, of 4:1 (distal-domain-labeled Ub₂), 5:1 (proximal-domain-labeled Ub₂). Binding of ubistatin A was monitored by signal attenuation and shifts in the resonance peak positions of the backbone amides in each Ub domain. Site-specific perturbations saturated at a molar ratio of ubistatin A:Ub₂ of 3:1. The combined amide chemical shift perturbations were computed as $\Delta\delta = [(\Delta\delta_{\text{H}})^2 + (\Delta\delta_{\text{N}}/5)^2]^{1/2}$, where $\Delta\delta_{\text{H}}$ and $\Delta\delta_{\text{N}}$ are the chemical shift differences (for ^1H and ^{15}N ,

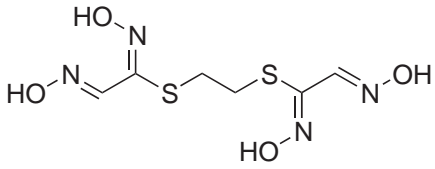
respectively) between the free and ubistatin A-bound di-ubiquitin for a given amide group. The signal attenuation for each residue was calculated as $(1-I/I_0)*100\%$, where I_0 and I are peak intensities in HSQC spectra of the free and C92-bound protein; the latter values were uniformly scaled to account for higher molecular weight of the complex and for the differences in the protein concentration and the experimental settings between the experiments. The atom coordinates used for Fig. 3 are from the crystal structure 1AAR.pdb (S16).



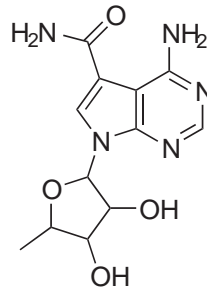


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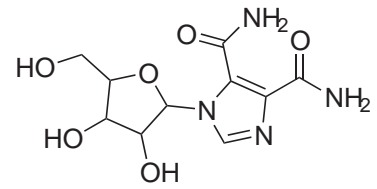
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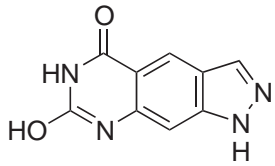
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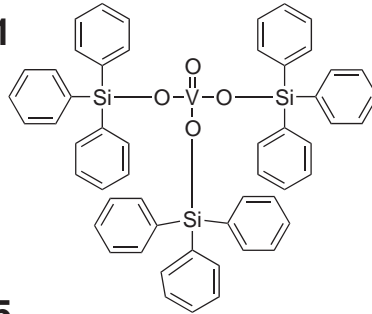
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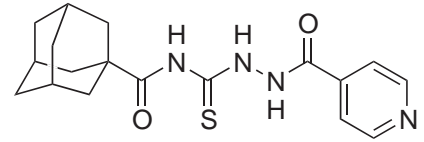
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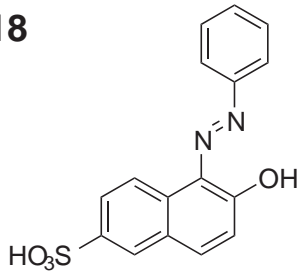
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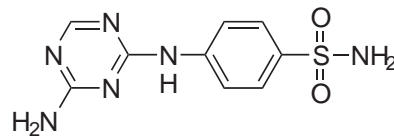
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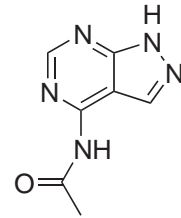
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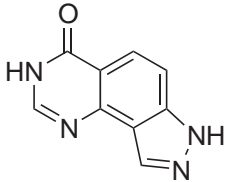
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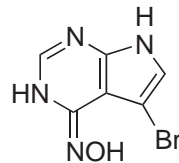
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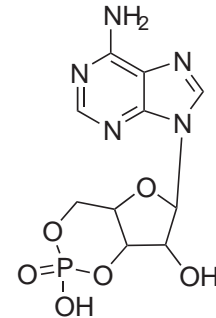
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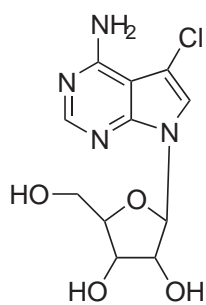


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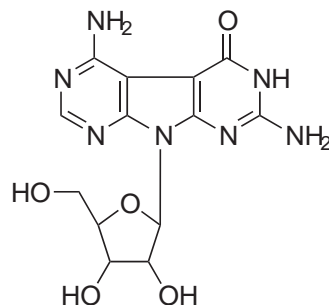


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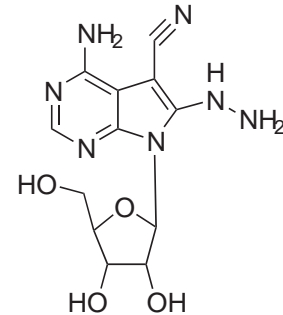
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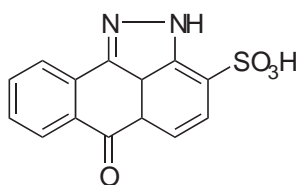
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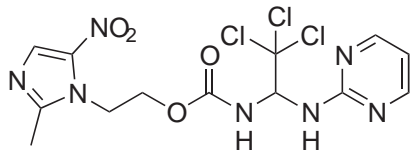


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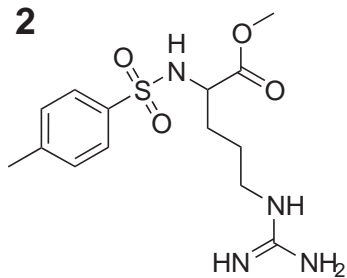


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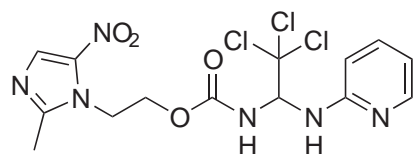
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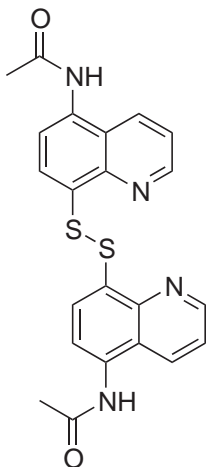


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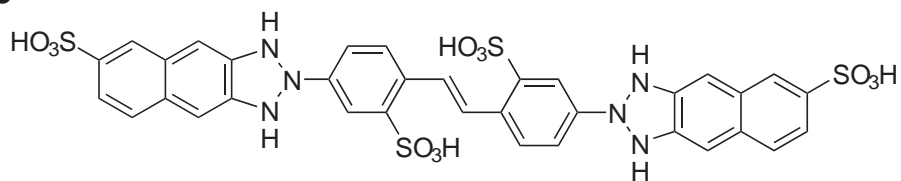


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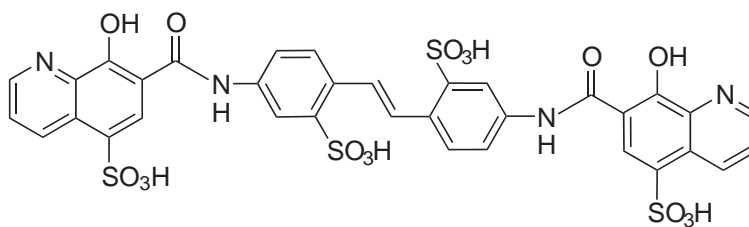
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92



Verma et al., Fig. S4

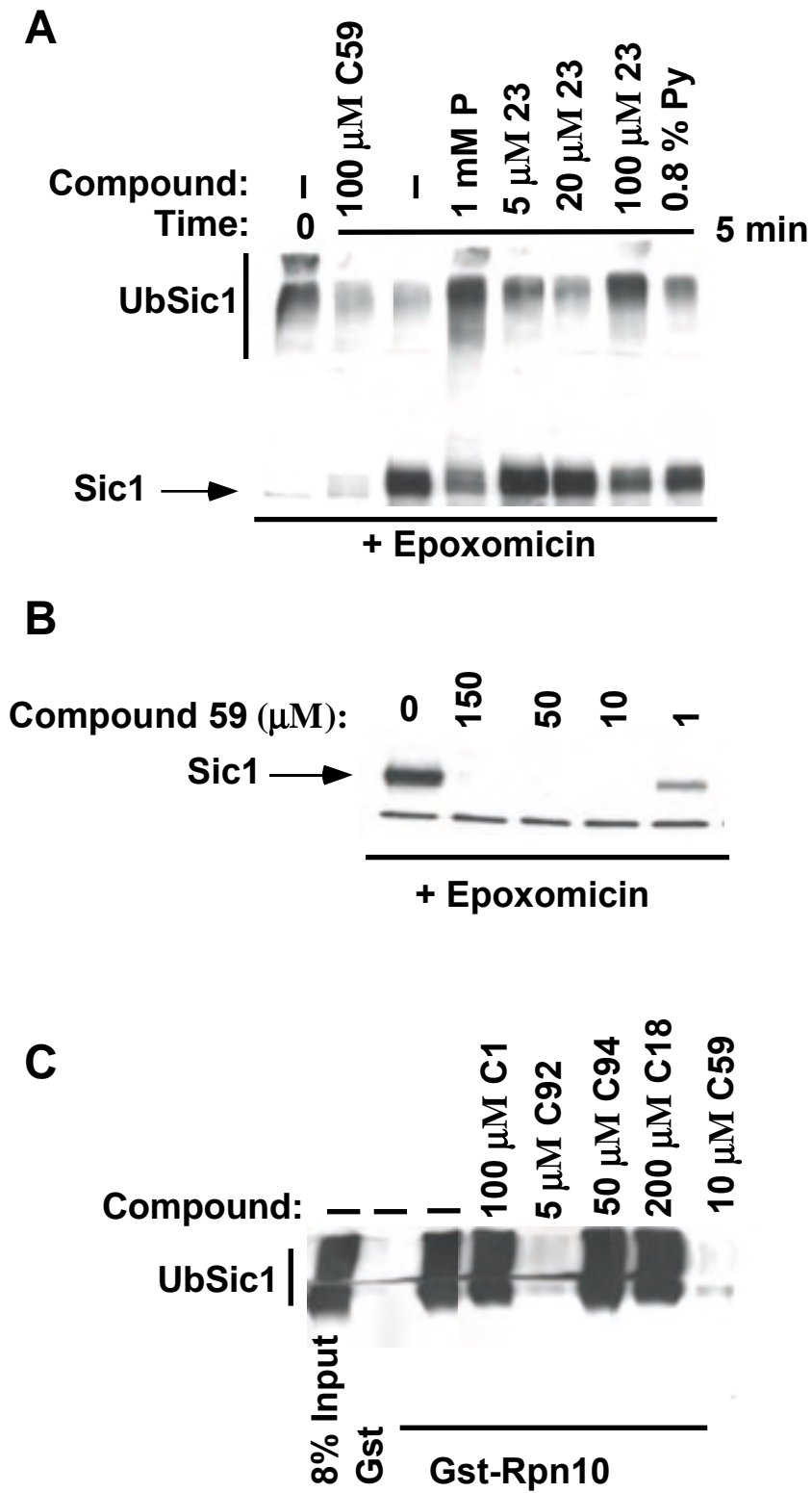


Fig. S5
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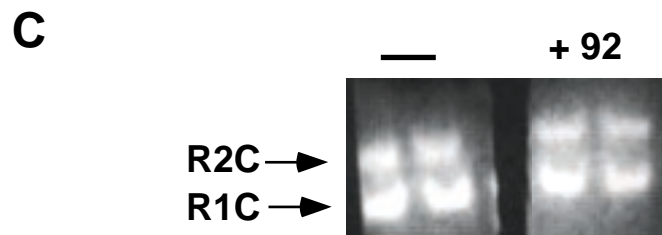
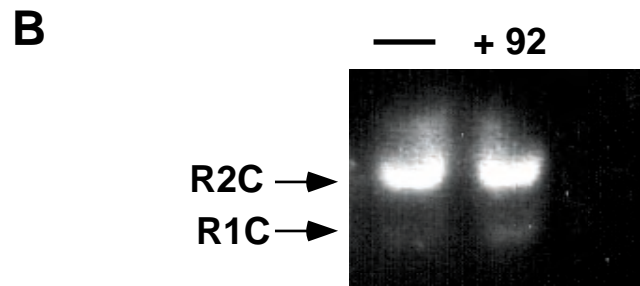
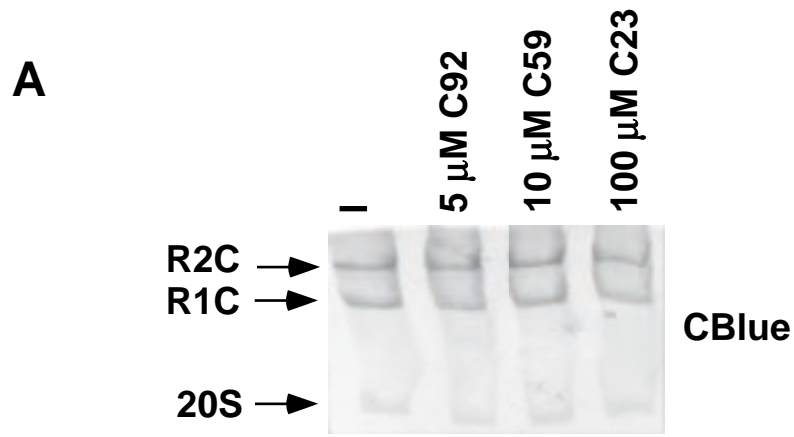
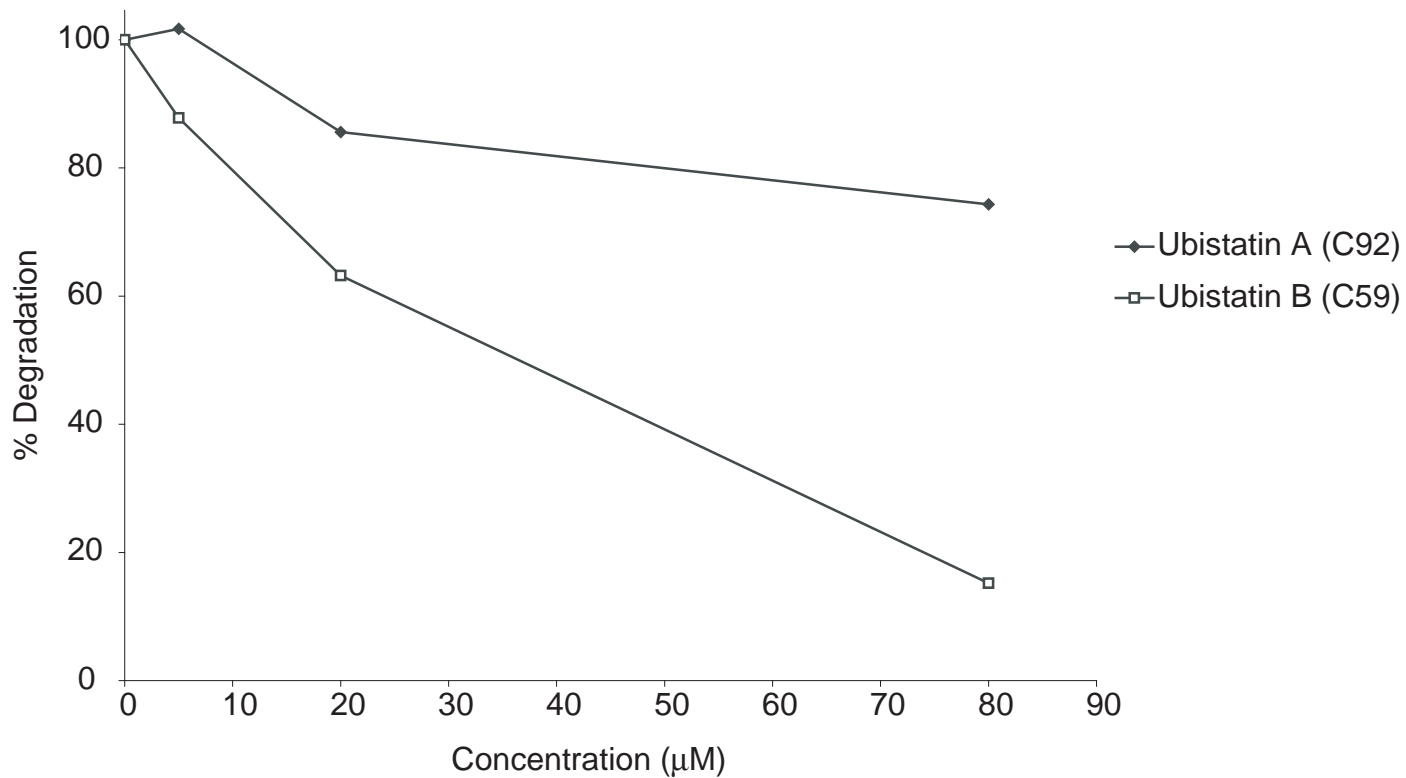
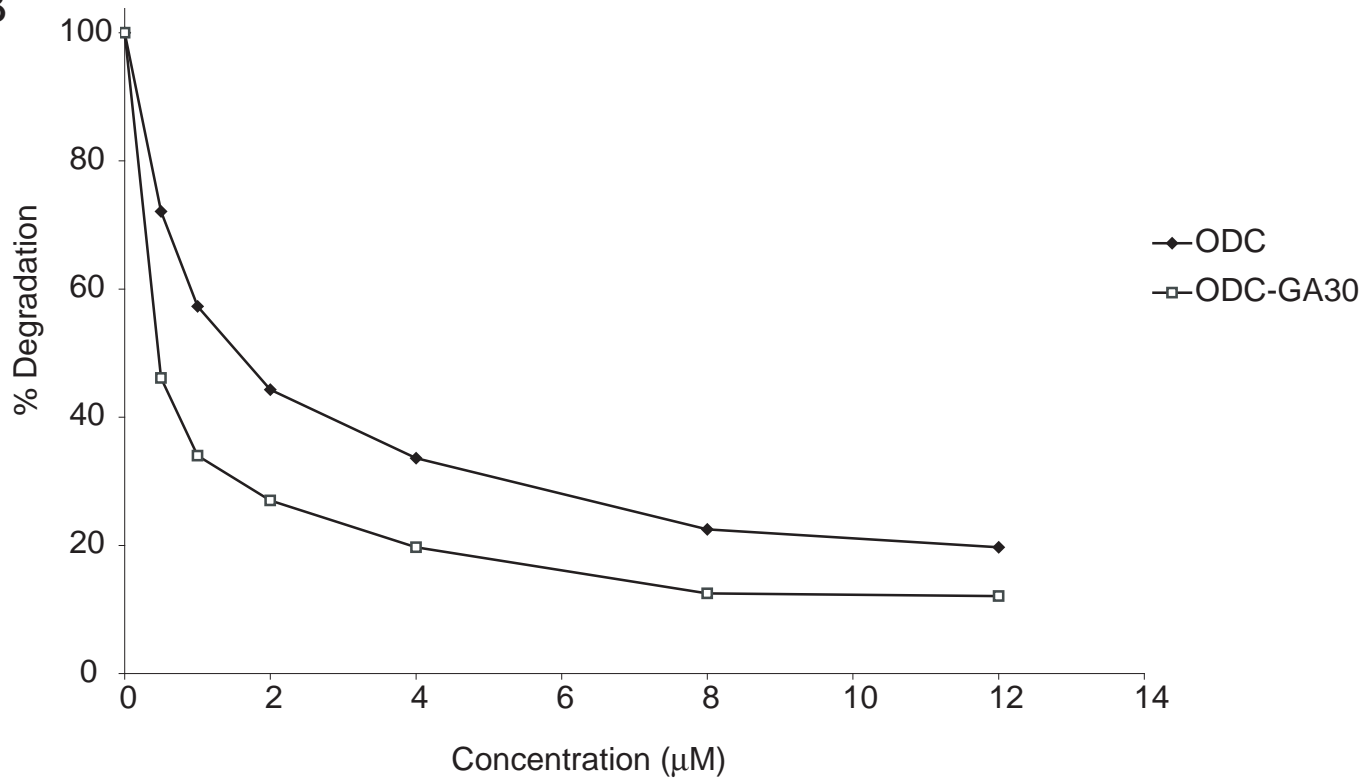
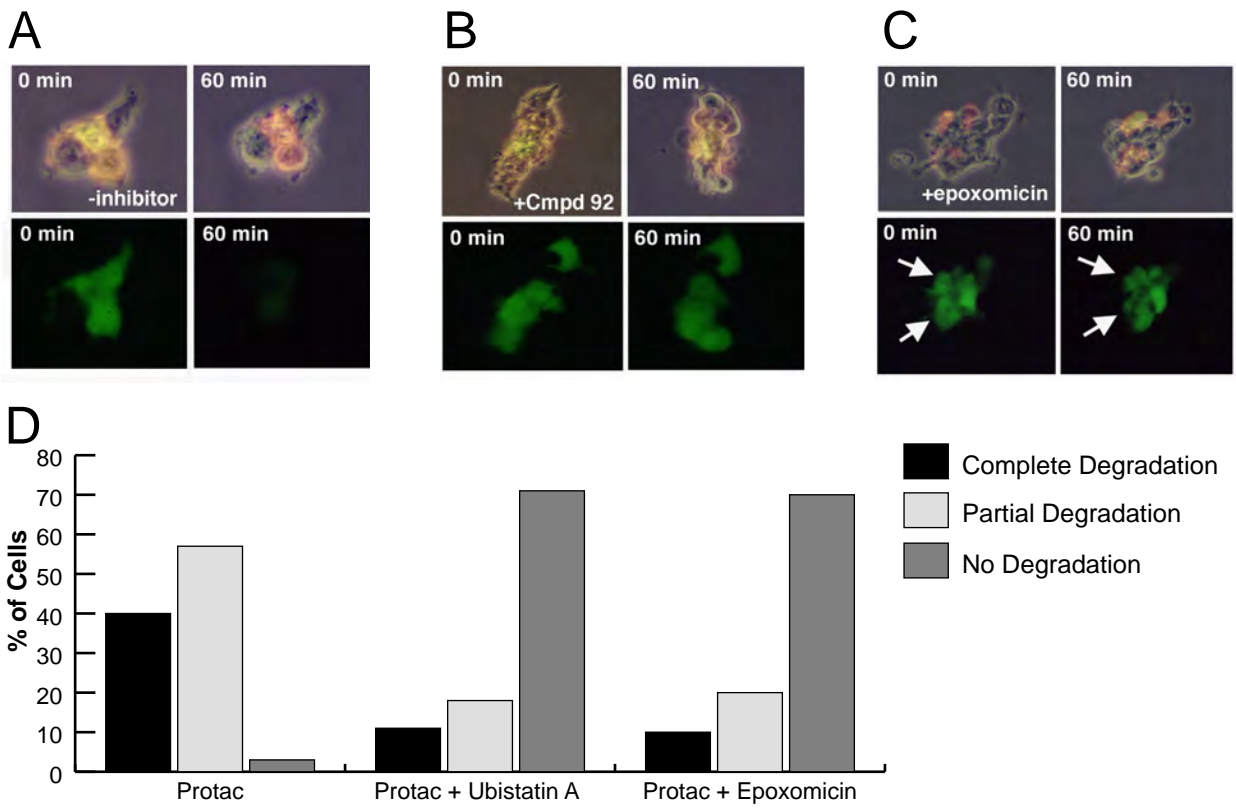


Fig. S6
Verma et al.

A**B**



Verma et al. Fig. S8

Figure Legends

Fig. S1. Mitosis-specific degradation of a cyclin-luciferase fusion protein. Luciferase or cyclin-luciferase was added to an interphase extract. Recombinant cyclin B $\Delta 90$ was added to one sample to induce entrance into mitosis. After 60 minutes, luciferin was added and luminescence measured. (B) Cyclin-luciferase is stabilized by methylated ubiquitin. The experiment in part (A) was repeated as described above, except that methylated ubiquitin was added to a concentration of 1 mg/ml. Extracts were supplemented with no additional ubiquitin ("no ubiquitin") or 1 mg/ml ubiquitin ("plus ubiquitin"), to compete with methylated ubiquitin. (C) An image of a sample assay plate from the screen. The arrow indicates a well that was considered a "hit" in the screen.

Fig. S2. Determination of sensitivity of the assay to a panel of inhibitors. Interphase extracts containing cyclin-luciferase were treated with compound and then induced to enter mitosis with cyclin $\Delta 90$ protein. Inhibitors were dissolved in DMSO and diluted to 1% final volume in extract. After 60 minutes, luciferin was added and luminescence measured. Percent inhibition was calculated as $100 \times (T-M)/(I-M)$ where T equals the test value for the inhibitor, M equals the value in a mitotic extract lacking inhibitor, and I equals the value in an interphase extract. In testing known inhibitors, we noted that even potent inhibitors, such as roscovitine, required much higher concentrations in *Xenopus* extracts than in purified enzyme systems. For example, roscovitine exhibited an IC_{50} of 30 μM in *Xenopus* extracts, whereas its reported IC_{50} against purified cyclin B1/cdc2 is 650 nM (S17). We believe this is due to the high concentration of lipid and protein (>30 mg/ml) in *Xenopus* extracts which may sequester compound. We also noted that MG132

and epoxomicin did not fully prevent loss of cyc-luc activity, even at high concentration (F). Assessment of proteins by western blotting, however, indicated that proteins are fully stabilized by higher concentrations of these inhibitors. We believe this discrepancy is due to proteasome-dependent unfolding and inactivation of the luciferase enzyme in the presence of 20S inhibitors.

Fig. S3. Structures of Class I inhibitors.

Fig. S4. Structures of Class II inhibitors.

Fig. S5. (A) Complete inhibition of deubiquitination by 100 μ M C59, and marginal inhibition by 100 μ M C23. Assays were performed and evaluated as summarized in the legend to Fig. 1B. The metal chelating reagent phenanthroline is designated as “P”, and “Py” refers to the pyridine solvent that is used as the solvent for C23. (B) Titration curve for C59. (C) Inhibition of binding of ubiquitin conjugates to Gst-Rpn10 by C92 and C59, but not other compounds. The experiment was performed as described for Fig. 2B.

Fig. S6. Class IIB compounds do not compromise 26S proteasome integrity at concentrations that inhibit ubiquitin-dependent proteolysis. (A) Purified 26S proteasomes were preincubated with test compounds before being resolved on a native gel. Following electrophoresis, the gel was stained with Coomassie Blue. (B) C92 does not affect 20S core activity. Same as (A), except that following electrophoresis, the gel was incubated with fluorescent substrate to determine peptidase activity, which was visualized using a

UV transilluminator (*S9*). C23 was also without effect (not shown), whereas C59 was too fluorescent by itself for this assay. (C) Untreated 26S proteasomes were resolved on a native gel. Following electrophoresis, the gel was incubated with the peptidase substrate in the presence of ubistatin. No inhibition could be observed, whereas epoxomicin inhibited peptidase activity under identical conditions (not shown).

Fig. S7. Effect of ubistatins on degradation of ornithine decarboxylase (ODC) by purified rat proteasomes. (A) Ubistatins A and B were titrated into a reaction containing 50 nM ³⁵S-labeled ODC and 50 nM proteasomes and incubated for 30 minutes. Data are normalized to ODC degradation observed in the absence of competing inhibitors and are expressed as residual degradation. (B) Inhibition of ³⁵S-labeled ODC turnover by cold ODC or ODC-GA30, a variant that can bind to the proteasome but not be degraded (*S18*).

Fig. S8. Ubistatin A blocks protein turnover in animal cells. We previously demonstrated that Protacs, comprised of a ubiquitin ligase-binding peptide from IκBα linked to a target-binding molecule, induce degradation of the target in a proteasome-dependent manner by recruiting the target to the SCF ubiquitin ligase (*S13, S19*). (A) Protac, which contains dihydroxytestosterone as the target-binding molecule, induces rapid degradation of androgen receptor-GFP (AR-GFP) upon introduction into cells. HEK293 cells expressing AR-GFP were injected with Protac alone or Protac plus ubistatin A or epoxomicin, and monitored for presence or absence of GFP by fluorescence microscopy. Protac induced rapid loss of AR-GFP without compromising cell integrity (as monitored by retention of rhodamine dye), but this effect was blocked by simultaneous introduction

of either 100 nM ubistatin A (B) or 100 nM epoxomicin (C). (D) Quantitation of results from one experiment in which the following number of cells were injected: Protac alone (n= 37); Protac + ubistatin A (n=28); Protac + epoxomicin (n=26). The results are representative of 3 experiments performed on 3 separate days.

Table S1

Cmpd	Class	ID #	CAS
C77	IA	NSC383123	NA
C58	IA	NSC298884	65562-56-3
C82	IA	NSC519257	66678-51-1
C62	IA	NSC350006	73908-01-7
C61	IA	NSC349960	18822-50-9
C13	IA	C5144324	301860-02-6
C18	IA	NSC7831	1934-20-9
C25	IA	NSC19742	92474-98-1
C54	IA	NSC205359	68341-64-0
C67	IA	NSC350138	81115-64-2
C40	IA	NSC124151	22276-98-8
C34	IA	NSC94017	60-92-4
C39	IB	NSC124149	24386-95-6
C57	IB	NSC279846	73024-72-3
C51	IB	NSC172599	22256-94-6
C10	IB	C5255908	NA
C1	IIA	C5271852	NA
C2	IIA	C5117023	901-47-3
C8	IIA	C145663	NA
C23	IIB	NSC14226	5429-79-8
C59	IIB	NSC306455	NA
C92	IIB	NSC665534	NA

Table S1: Identification numbers for compounds reported in Table 1. "ID#" refers to the identification number of the inhibitor obtained from the NCI (prefaced by NSC) or Chembridge (prefaced by C). CAS refers to the chemical abstract services number and is included where available; NA indicates that a CAS number was not available.

References and Notes

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