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Supplementary Materials for

Ubiquitination and degradation of SUMO1 by small-molecule degraders extends survival of mice with patient-derived tumors

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Materials and Methods

Real time PCR.

Total RNA was isolated using the RNeasy kit (Qiagen) and cDNA was synthesized using 1 μg RNA in a reverse transcription reaction (Quantitech Reverse Transcription kit). Quantitative PCR for SUMO1 using Quantitech Primer Assay (QT00014280), or β-actin (QT00095431) as an internal control gene, were performed on an Applied Biosystems 7500 FAST real time PCR system (Applied Biosystems). The expression concentrations of SUMO1 and were normalized to the β-actin mRNA concentrations and represented relative to the expression in control cells (means and standard deviations from PCR triplicates).

Cell lines and bacterial strains.

Cell lines: A549, H1299, H460, H292, H520, SW1573, H1975, H1944, CALU-1, DLD-1, HCT15, SW620, HCT116, HT29, COLO205, LN229, LN18, T98G, U138, MCF7, MD-MBA231, MD-MBA468 and BT549 were purchased from the American Type Culture Collection (ATCC). LN71, U343, LN215 were kindly provided by N. De Tribolet, Lausanne, Switzerland. Cells were maintained in a humidified incubator with 5% CO2 at 37°C and grown in RPMI 1640, DMEM or McCoy's supplemented with 10% FBS (Corning) and 50 IU ml-1 penicillin/streptomycin (Corning). Human normal cells Nuli-1, HIEC-6, MCF10A were purchased from ATCC and grown in the recommended media by ATCC. Normal Human Astrocyte (NHA) was purchased from Lonza and cultured as recommended. E.Coli cells (One Shot TOP10) for cloning were purchased from ThermoFisher Scientific.

Human primary tissues.

Human colorectal, lung, breast carcinomas and matched normal tissues from same patients were provided by the Ontario Tumor Bank (Ontario Institute for Cancer Research, Toronto, Ontario, Canada) and stored at -80°C until used in the study in accordance with protocols approved by the institute.

Constructs and reagents.

Human pCMV6-myc-DDK-FBXO42, pCMV6-myc-DDK-CDK6, pCMV6-myc-DDK-RBX1, pCMV6-myc-DDK-SKP1, pCMV6-myc-DDK-SENP1, pCMV6-myc-DDK-SENP3, pCMV6-myc-DDK-CUL3 were purchased from Origene. pCMV5-Flag-CUL1, pCMV5-Flag-CUL2 and pCMV5-Flag1-SUMO1 were obtained from MRC PPU Reagents and Services facility, University of Dundee. YFP-SUMO1ΔC4 (1–97), YFP-SUMO3ΔC11 were gifts from F. Melchior (Heidelberg University, Germany). pEF-IRES-P-HA-Ub-K0 and pEF-IRES-P-HA-Ub-WT were gifts from Z. J. Chen (UT Southwestern Medical Center, USA). Ctal-Myc-FBXO42 (EX-E3892-M09), Ntal-Myc-FBXO42 (EX-E3892-M43) were purchased from Genecopeia. pcDNA3 6xHis/SUMO1 was a gift from R. Hay (University of Dundee, UK). CAPRIN1 (Myc-Flag-tagged) plasmids was purchased from Origene (RC200300). The CAPRIN1 mutation DNA were generated by PCR amplification of DNA fragments encoding amino acid residue 112-709 , 112-260, 112-328, 112-352, 112-380, 328-709, 353-709, 381-709 and cloned into PCMV6-Entry plasmid (Origene). YFP-SUMO1-GV and YFP-SUMO3-GV mutants were generated using the Quickchange mutagenesis kit (Stratagene) at the Emory Custom Cloning Core Facility.

The chemicals and proteins used in the *in vitro* and *in vivo* assays include anti-Flag M2 agarose (Sigma-Aldrich), Flag and myc peptide (Sigma-Aldrich), recombinant CDK6 (Signal Chem), SUMO1, Sea1/Sea2 (Aos1/Uba2, Boston Biochem, Boston), ovalbumin (Sigma), ATP (Boston Biochem), protease inhibitors (Sigma), MLN4924 (Cayman Chemical), Cycloheximide (Sigma Aldrich), S-100 Fraction Degradation Kit (Boston Biochem), FBXO42 (Origene) and Promega RealTime-Glo™ MT

Cell Viability Assay (Promega). The following antibodies were used for western blotting: mouse SUMO1 (21C7) (33-2400 Thermo Fisher Scientific), rabbit SUMO2/3 (ab109005, Abcam), mouse Ubiquitin (sc-8017 AC, Santa Cruz Biotechnology), mouse FBXO42 (TA800210, Thermo Fisher Scientific), rabbit USP14 (11931, Cell Signaling Technology), rabbit CDK6 (13331, Cell Signaling Technology), mouse CDK6 (3136, Cell Signaling Technology), mouse Flag (P2983, Sigma-Aldrich), rabbit Flag (14793, Cell Signaling Technology), rabbit HA (3724,Cell Signaling Technology), mouse HA (MMS-101P, Covance), mouse Myc (2276, Cell Signaling Technology), rabbit UBC9 (4786, Cell Signaling Technology), rabbit SENP1 (11929, Cell Signaling Technology), rabbit SENP3 (5591, Cell Signaling Technology), rabbit SAE2 (IMG-5111A, IMGENEX), mouse GFP (2955, Cell Signaling Technology), rabbit GFP (2956, Cell Signaling Technology), mouse anti-Rb (pS780) (558385, BD Biosciences), rabbit SAE1 (13585, Cell Signaling Technology), mouse RB (9309, Cell Signaling Technology), rabbit anti-SKP1 (2156, Cell Signaling Technology), rabbit anti-G3BP1 (17798, Cell Signaling Technology), rabbit anti-CUL1 (4995, Cell Signaling Technology), rabbit anti-RBX1 (11922, Cell Signaling Technology), rabbit anti-CAPRIN1 (15112-1-AP, Proteintech), mouse anti-CAPRIN1 (66352-1-Ig, Proteintech), rabbit anti-CUL1 (ab75817, Abcam), rabbit anti- CUL2 (ab166917, Abcam).

Cell viability, proliferation and apoptosis assays.

Cell viability was measured by phosphatase assay and calculated based on the formula: 1 – (optical density of cells treated/optical density at 550 nm of cells untreated) × 100. Cell growth inhibition by HB007 at 2.5μM for CRISPR-CAS9 knockout clones was measured using Promega RealTime-Glo™ MT Cell Viability Assay at 24hrs according to protocol. IC50 were calculated using GraphPad Prism. BrdU cell proliferation assay was performed according to the manufacture's protocol (Biovision K306-

200). In brief, 3000 cells /well were seeded into 96-well plate and treated with HB007 at 3μM for 20h before addition of BrdU solution for 4h. The cells were then fixed, incubated with BrdU detection antibody for 1h followed by the addition of anti-mouse HRP-linked antibody for 1h. Cells were washed 3 times before addition of the HRP substrate for 10 minutes in room temperature. After the addition of the stop solution, BrdU incorporation was evaluated by measuring the absorbance at 450 nm. Apoptosis was evaluated by measuring the Casp3/7 activation using Caspase-Glo® 3/7 Assay System (Promega) that detect the cleavage of a luminogenic Casp3/7 substrate generating a luminescent signal proportional to the amount of casp3/7 activity.

Affinity Purification Mass Spectrometry.

Sample preparation methodology for the affinity purification mass spectrometry experiments were designed by adaptations from previous reports (5-8). The methodologies employed for the current study are detailed below.

Proteolytic digestion of the affinity captured proteins on solid support. This "on-bead" digestion procedure was carried using magnetic beads that were affinity captured with proteins were first mixed with 8 M urea in 100 mM Tris.HCl (30 μL) to induce denaturation of the proteins. These proteins were next subjected to reduction of the protein Cys-Cys bonds using 5 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and alkylation with 10 mM chloroacetaminde (CAM) to protect the reduced Cys residues from potential subsequent re-coupling. The resulting samples were subsequently diluted with 100 mM Tris.HCl to achieve a final 2 M Urea concentration. Proteins were then subjected to an overnight proteolytic digestion using 0.5 μg equivalent of Mass Spectrometry Grade Trypsin/Lys-C Mix (Promega Corporation, Madison, WI 53711-5399, U.S.A.) to derive peptides. The magnetic beads were next separated on a magnetic stand to separate the supernatant peptide solution which was acidified with formic acid (2 μL) to quench the digestion.

Nano-LC-MS/MS Analysis. This was on a Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer coupled to an UltiMate 3000 UHPLC RSLCnano System (Thermo Fisher Scientific). An 18 µL aliquot of the acidified peptide solution was loaded onto an Acclaim PepMapTM 100 trap column with nanoViper connections (inner diameter of 75 µm, and a length of 2 cm with C18 particles of 3 µm carrying 100 Å pore sizes) from Thermo Fisher Scientific at 4 µL/min for 5 mins using phase-A (FA/ H2O 0.1/99.9, v/v) to desalt and concentrate the peptides on the trap column. For the liquid chromatographic separation of the concentrated peptides, the trap column was then switched to align with the analytical column, PepMapTM RSLC C18 with nanoViper connections (inner diameter of 75 µm, and length of 15 cm with C18 particles of 3 µm carrying 100 Å pore sizes) that also carries an Easy-Spray tip at the outlet. After holding the MP composition at 97% phase A (FA/H2O 0.1/99.9, v/v) for 5 mins at 750 nL/min, the peptides were eluted using a varying mobile phase (MP) gradient by increasing phase B from the 3% to 35% for 75 mins.; next increasing the 35% phase B to 90% 0.1 mins; and then holding the 90% B composition for the next 4.9 mins to ensure elution of all the peptides. MP-composition was brought down to 3% phase B in 0.1 mins and kept at the same composition for the next 4.9 mins to ensure equilibration of the LC-column for the subsequent sample analyses.

Nano-LC mobile phase was introduced into the mass spectrometer using a Nanospray Flex Source apparatus (Proxeon Biosystems A/S). The nanospray was operated by applying an ion spray voltage of 2.75 kV onto the spray tip while keeping the ion transfer capillary at 275 oC. The mass spectrometer method was operated under data dependent mode, programmed to select top 15 most intense ions in a full MS scan with vendor defined parameters—Microscans 1; Resolution 35k; AGC target 3E6; Maximum IT 100 ms; Number of scan ranges 1; Scan range 200 to 2000 m/z; and Spectrum data type “profile”, and then to perform data dependent MS/MS scans with vendor defined parameters—Microscans 1; Resolution 17.5k; AGC target 1E5; Maximum IT 50 ms; Loop count 15;

MSX count 1; Isolation window 4.0 m/z; Fixed first mass 100 m/z; NCE 30.0; and Spectrum data type "profile". The respective data dependent settings were set with vendor defined default parameters.

Data Analysis. Resulting RAW files were analyzed using Proteome DiscoverTM 2.2 (ThermoScientific). The MS/MS spectra were searched against in silico tryptic digest of a Homo sapiens (Human) proteins database (FASTA format) downloaded from the UniProt sequence database (v. June 2017) using the SEQUEST HT search engine. In order carry out the search, following specific search parameters were applied to vender provided basic "processing" and "consensus" workflow templates that correspond to Thermo Q-Exactive instruments: Trypsin as the proteolytic enzyme; searched for peptides with a maximum number of 2 missed cleavages; precursor mass tolerance of 10 ppm; and a fragment mass tolerance of 0.2 Da. The only static modifications used for the search was, carbamidomethylation on cysteine(C) residues. Dynamic modifications used for the search were oxidation of methionines and acetylation of N-termini. Percolator False Discovery Rate was set to a strict setting of 0.01 and a relaxed setting of 0.05. Resulting files from Proteome DiscoverTM 2.2 based search analyses were then uploaded to ScafoldTM (2.2) s/w to isolate and visualize the data with 90% peptide probability threshold carrying at least one peptide. Using the tools available in ScafoldTM (2.2) s/w and spectral counts assigned to each protein identified, Fisher's Exact Test (7) was performed with Benjamini-Hochberg correction (8) to identify/screen out statistically significant enriched proteins in experimental samples compared to the respective control samples.

Biolayer Interferometry (BLI) Assay.

BLI experiments were carried out on an Octet RED384 instrument at the Bindley Biophysical Analysis Facility at Purdue University. BLI Measurements were performed with High Precision Streptavidin (SAX) biosensors (Forte Bio, Part No. 18-5117) in an Octet RED384 instrument in a 384-well tilted bottom plate at 1000 rpm shaking and 30° C temperature. SAX biosensors were hydrated in 10 mM Tris, 1 mM EDTA, pH=7.6 and 2% DMSO (Buffer A) for 10 min prior to the assay run. During the

assay, the biosensors were equilibrated in Buffer A for 2 min, followed by loading of 1 μM biotinylated HB007 in Buffer A for 2.5 min. The biosensors were next quenched with 50 μg/mL biocytin (Sigma Aldrich, Catalog No. B4261-25MG) in Buffer A for 2 min. An equilibration step in 10 mM Tris, 1 mM EDTA, pH=7.6, 6% glycerol, 1 μM biotin (Sigma-Aldrich, Catalog No. 14400-100MG) (Buffer B) for 5 min followed the quenching step. The biosensors were then dipped into CAPRIN1 solution in Buffer B with varying concentration between 5 nM and 200 nM and the association step was run for 10 min. This was followed by a dissociation step in Buffer B for 10 min. A reference well without any analyte (CAPRIN1) was present for subtracting out the background signal from dissociation of captured HB007. For each concentration of CAPRIN1, a reference sensor without any loading of biotinylated HB007 was present.

Non-Specific Binding Analysis. To consider non-specific binding (NSB) of CAPRIN1 to the SAX biosensors, biocytin was used to quench the sensors after the loading step. Apart from that, double referencing method was utilized for the assay where for each biosensor, a corresponding reference biosensor was present with loading of Buffer A instead of biotinylated HB007. The signal from the reference sensors were subtracted from the assay biosensors during analysis.

Caprin1- Biotin Interaction analysis. To confirm whether CAPRIN1 also interacts with biotin, BLI assay with the same experimental setup as described above was performed with the exception of loading 1 μM biotin in Buffer A instead of biotinylated HB007. Buffer B for this assay did not contain any biotin.

The assay between CAPRIN1 and biotin revealed that CAPRIN1 shows a strong affinity of ~2 nM (±1 nM) to biotin (Fig. 2). To make sure that the binding we observed between CAPRIN1 and biotinylated HB007 is between the small molecule and protein, and not between biotin and protein, we added 1 μM biotin in Buffer B and pre-mixed the protein and buffer B at least 30 minutes before the assay was started. If binding was still observed, it had to be between HB007 and CAPRIN1. Even after

premixing CAPRIN1 with biotin, and then performing the BLI assay between biotinylated HB007 and CAPRIN1, we observed the binding phenomenon as shown in main Fig. 4h. This suggests that the biotin binding to CAPRIN1 has a different site than HB007 binding.

Before the final assays between CAPRIN1 and biotinylated HB007 were performed, multiple optimization steps were performed to accurately determine the concentration range of the protein, amount of biotinylated HB007 to be loaded on the sensor, amount of biocytin for blocking, and the amount of biotin to be premixed with the protein. The binding affinity between biotinylated HB007 and the protein has been consistently between 4 nM and 10 nM during all the optimization steps.

Western blot analysis and immunoprecipitation.

To assess SUMO-conjugated proteins concentration, tissues and cells were lysed in a denaturing buffer (50mM Tris-HCL pH7.4, 5% SDS, 30% glycerol, 1mM EDTA, 1mM PMSF, 50mM sodium fluoride, 5mM sodium pyrophosphate, 4mM sodium tartrate, 1mM sodium molybdate, 2mM imidazole, 10mM β-glycerophosphate, 0.5mM DTT, 1mM PMSF and protease inhibitors) supplemented with 20mM NEM and heated at 90^{o}C for 10 min. Lysates were subjected to a brief sonication after diluted three times in a RIPA buffer. For immunoprecipitation, transfected cells were lysed in 1% triton buffer (50mM Tris at ph 7.4, 150mM Nacl, 10% glycerol, 1% Triton x100) or RIPA buffer followed by immunoprecipitation overnight with anti-Flag M2 or Myc-agarose. For endogenous IP, cell lysate were mixed with the corresponding primary antibody (1.5μg) overnight, followed by 4 hrs rotation with protein G beads (Millipore). The immunoprecipitates were washed with corresponding buffer and eluted with 2x Laemmli buffer or 150 ng/μl 3x Flag peptide for Flag-elution and tested by western blotting.

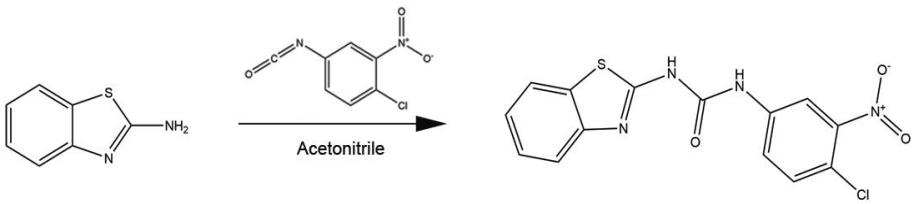
shRNA sequences and transduction. The lentiviral shRNA vectors included scrambled control (SHC002), SUMO1–589 (TRCN0000148589, 5′-CCTTCATATTACCCTCTCCTT-3′), SUMO1–613

(TRCN0000148613, 5′-CGACCAATGCAAGTGTTCATA-3′), shRNA vector were from Sigma MISSION shRNA library (Sigma). Each of the lentiviral shRNA vectors were transduced into cells based on the protocol as we reported29. Transduced cells were selected with puromycin.

Chemical synthesis procedures.

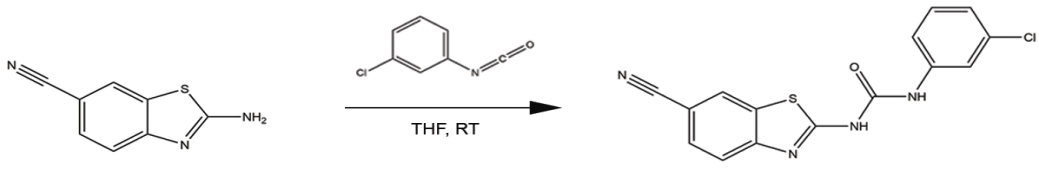
All reagents and solvents were purchased from commercial vendors and used as received. The chemical procedures are described below.

Preparation of 1-(1,3-Benzothiazol-2-yl)-3-(4-chloro-3-nitrophenyl) urea (CPD1).



A slurry of 4-chloro-3-nitrophenylisocyanate (5.05 g, 25.43mmol) in anhydrous acetonitrile (100 mL) was added to a solution of 2-Aminobenzothiazole (4.98 g, 33.16 mmol) in anhydrous acetonitrile (100 mL) and stirred under nitrogen overnight. After stirring overnight, the slurry was filtered and the solid was washed with acetonitrile (3X 30 mL), water (30 mL), and methanol (30 mL). The solid was then dried under vacuum affording a yellow solid. 1-(1,3-Benzothiazol-2-yl)-3-(4-chloro-3-nitrophenyl)urea was synthesized in one step. The final product was purified by filtration and washing. The percent yield for the overall synthesis was 79%. . ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 7.25 (t, *J*=7.55, 1 H), 7.40 (td, *J*=7.72, 1.37, 1 H), 7.59 (d, *J*=7.82 , 1 H), 7.70 (d, *J*=8.99, 1 H) 7.80 (d, *J*=9.05, 1 H) 7.88 (d, *J*=7.82, 1 H) 8.39 (d, *J*=2.34, 1 H) 9.81 (br. s., 1 H). ESI-MS (8.2 minutes, M^{+}+H: *m/z* = 349)

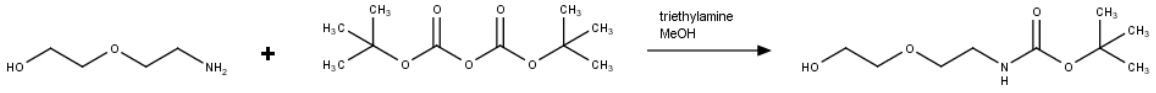
Preparation of 1-(3-chlorophenyl)-3-(6-cyano-1, 3-benzothiazol-2-yl) urea (HB007**)**



To a solution of 2-amino-1,3-benzothiazole-6-carbonitrile (200 mg, 1.14 mmol) in THF (8 ml) was added 3-chlorophenyl isocyanate (209 mg, 1.37 mmol) at room temperature. The solution was stirred at the same temperature for 24 hours. The solution was cooled under an ice-bath and the solid that precipitated out from the solution was collected by filtration. The solid was dried under vacuum to remove excess solvent. The solid was added to a boiling EtOH solution (10 ml) and was stirred at the same temperature for 30 minutes. The solution was cooled down to room temperature and the solid was collected by filtration. The resulting solid was washed with cold EtOH (10 ml) and was dried under vacuum. The yellow solid was confirmed to be the title product (220 mg, 60%). ^{1}H NMR (400 MHz, DMSO-D_6): δ 11.1 (brs, 1H), δ 9.50 (brs, 1H), δ 8.05 (d, *J*=40. 2H), δ 7.80 (d, *J*=4.0, 1H), δ 7.65 (m, 1H), δ 7.55 (m, 2H), δ 7.45 (dd, J=4.0, 1.2, 1H), ESI-MS: M^{+}+1: *m/z*= 329.03.

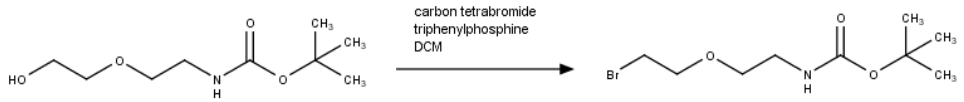
<u>Preparation of the HB007-immobilized FG beads and HB007-biotin.</u>

Preparation of the alcohol:



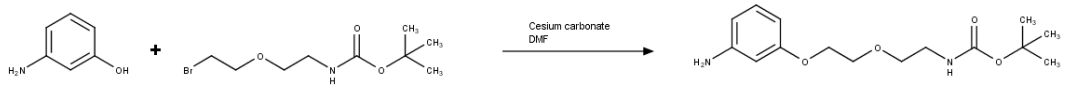
To the aminoalcohol (1 g, 9.51 mmol) in MeOH (10 mL) was added the anhydride (2.28 g, 10.47 mmol), followed by trimethylamine (1.06 g, 10.47 mmol). The mixture was stirred at room temperature (rt) overnight and concentrated under vacuum to give the product (1.97 g, 92%).

Preparation of the bromide:



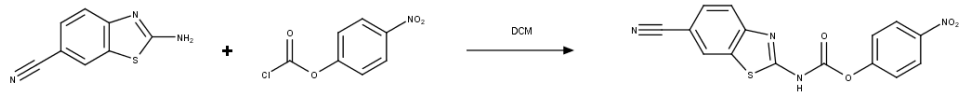
To the alcohol (0.5 g, 2.44 mmol) in DCM (20 mL) at 0 ○C was added PPh3 (963 mg, 3.67 mmol), followed by CBr4 (1.22 g, 3.67 mmol). The mixture was stirred at rt overnight and concentrated under vacuum. The residue was triturated with EtOAc/hexane (1:1). The solid was filtered. The filtrate was purified on silica gel using a gradient of 0-20% EtOAc/hexane to give the product (0.57 g, 87%).

Preparation of the aniline derivative:



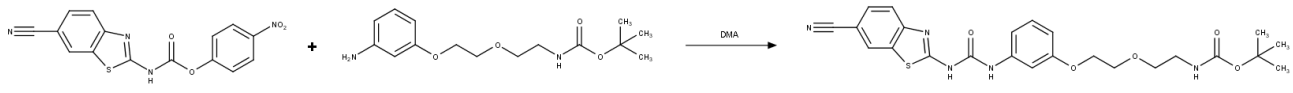
A mixture of 3-aminophenol (109 mg, 1 mmol), the bromide (268 mg, 1.1 mmol), cesium carbonate (652 mg, 2 mmol) and DMF (3 mL) was stirred at 50 ○C for 5 days. Water was added. The mixture was extracted with EtOAc. The organic layer was washed with water, followed by brine. It was then dried over MgSO4. The drying agent was filtered. The filtrate was concentrated under vacuum. The crude was purified on silica gel using a gradient of 0 – 60% EtOAc in hexane to give the product (276 mg, 93%).

Preparation of the nitrophenyl carbamate:



To 2-amino-6-cyanobenzothiazole (1 g, 5.7 mmol), pyridine (0.9 g, 11.4 mmol) and DCM (40 mL) was added 4-nitrophenyl chloroformate (2.3g, 11.4 mmol) in DCM (20 mL) at 0 ○C. The mixture was stirred at rt overnight. The solid was filtered to give the product (1.74g, 89%).

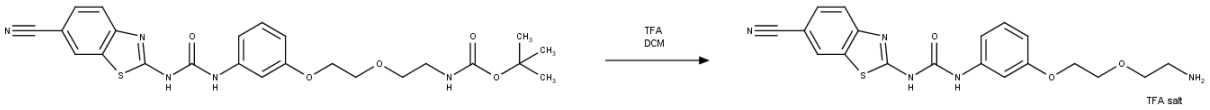
Preparation of the urea:



The mixture of the nitrophenyl carbamate (0.24 g, 0.706 mmol), the aniline derivative (0.21 g, 0.706 mmol) and DMA was stirred at rt overnight. Water was added and the product was taken up in DCM.

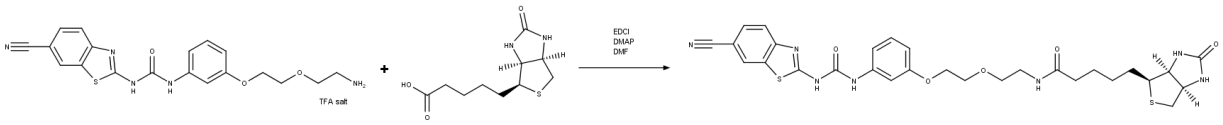
The organic layer was washed with water and brine sequentially, followed by drying over MgSO4. The drying agent was removed by filtration. The filtrate was concentrated under vacuum. The crude product was purified on silica gel using 0-100% EtOAc in hexane to give the product (310 mg, 88%).

Preparation of the trifluoracetic acid of the amine:



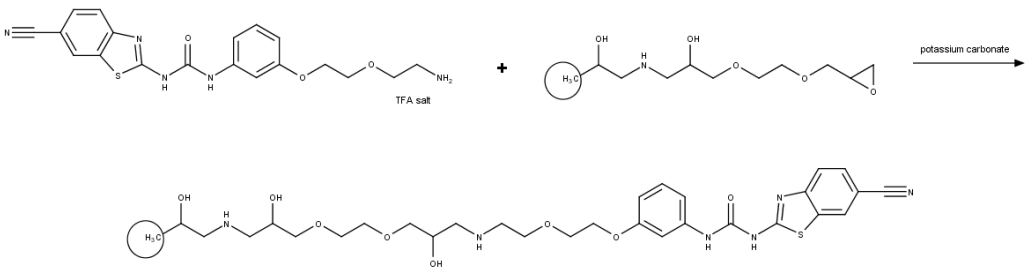
The carbamate (0.25 g, 0.50 mmol) in trifluoroacetic acid (1 mL) and DCM (5 mL) was stirred at rt for 1h. The mixture was concentrated down under vacuum. The crude was suspended in DCM and concentrated down again under vacuum to give the salt (0.35 g).

Preparation of the biotin probe:



A mixture of the trifluoroacetic acid salt of the amine (100 mg, 0.196 mmol), biotin (47.8 mg, 0.196 mmol), 4-dimethylaminopyridine (96 mg, 0.784 mmol), EDCI hydrochloride (38 mg, 0.196 mmol) and DMF (2 mL) was stirred at rt for 3 days. It was diluted with EtOAc. The mixture was washed with water and brine sequentially. The organic layer was dried over MgSO4. The solid was filtered and the filtrate was concentrated down under vacuum. The crude product was purified on silica gel (~ 40 g) using 0-10% MeOH in DCM to give the product as a white solid (39 mg, 32%).

Preparation of the bead probe:



Step 1: the trifluoroacetic salt of the amine (12.8 mg, 0.025 mmol) was dissolved in DMF (0.5 mL) to make a 50 mM solution.

Step 2: a bead suspension (0.125 mL or 2.5 mg of beads, ~ 100 nM/mg) was added to a 1.5 mL micro-tube.

Step 3: the microtube was centrifuged at 14,000 rpm for 5 minutes and the supernatant was discarded.

Step 4: DMF (0.5 mL) was added to the micro-tube and the beads were dispersed in a sonicator for 1 minute.

Step 5: the microtube was centrifuged at 14,000 rpm for 5 minutes and the supernatant was discarded.

Step 6: Step 4 and 5 were repeated two more times.

Step 7: the solution (0.5 mL) from step 1 was added to the beads. The mixture was mixed by sonication and transferred to a pear-shaped flask charged with potassium carbonate (210 mg).

Step 8: the flask was rotated in a 60 ○C water bath overnight. The mixture was transferred to a 1.5 mL micro-tube.

Step 9: the micro-tube was centrifuged at 14,000 rpm for 5 minutes and the supernatant was discarded.

Step 10: to the beads was added 50% DMF in water (0.5 mL). The mixture was sonicated for 1 min and centrifuged at 14,000 rpm for 5 minutes. The supernatant was discarded.

Step 11: step 9 and 10 were repeated one more time.

Step 12: water was added to the beads and the mixture was sonicated for 1 minute.

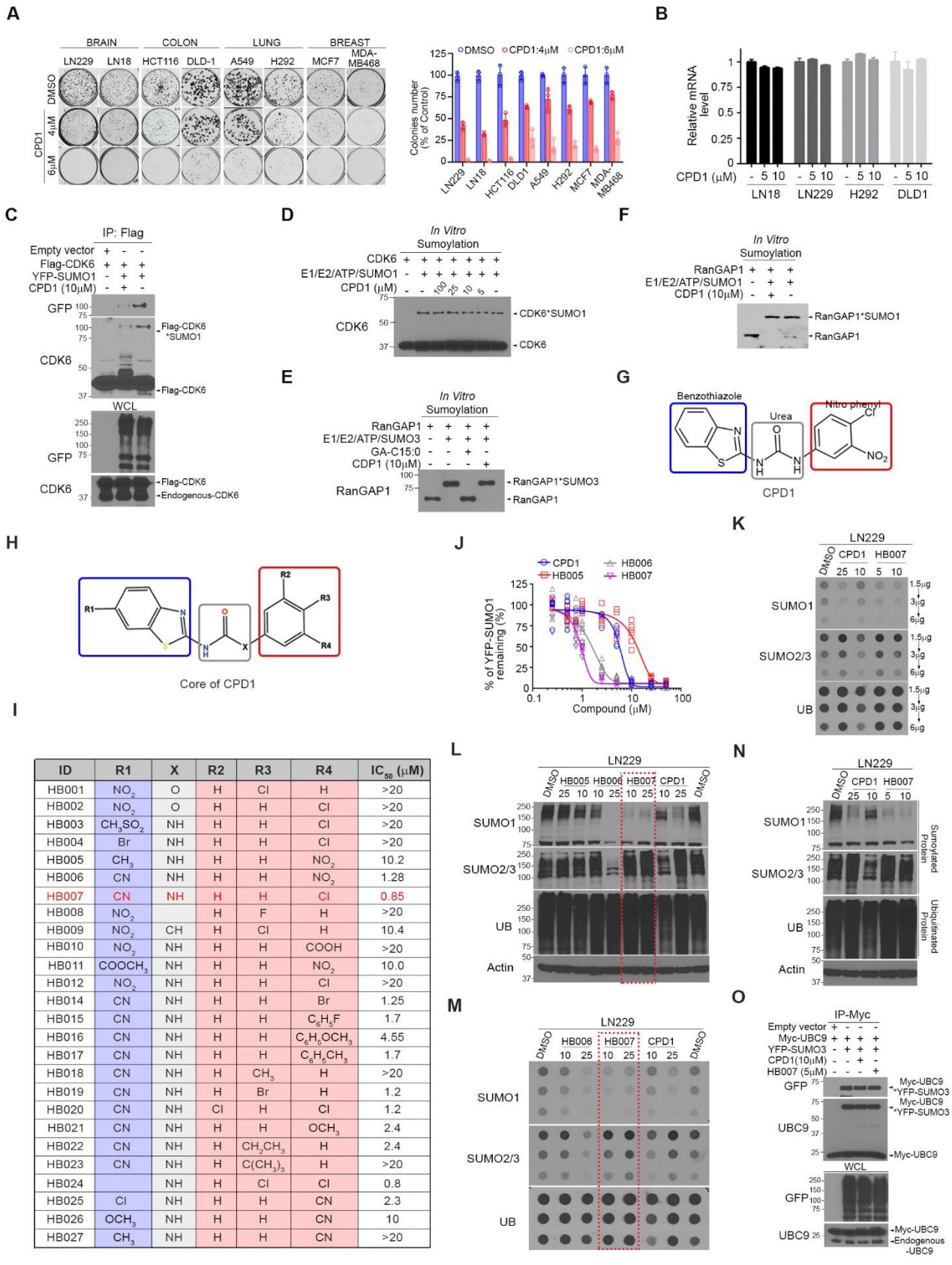
Step 13: the micro-tube was centrifuged at 14,000 rpm for 5 minutes and the supernatant was discarded.

Step 14: to the beads was added 50% MeOH in water and the mixture was sonicated for 1 minute.

Step 15: the micro-tube was centrifuged at 14,000 rpm for 5 minutes and the supernatant was discarded.

Step 16: step 14 and 15 were repeated for two more times.

Step 17: the beads were resuspended in 50% MeOH in water (0.1 mL) and stored at 4 ○C.



ID	R1	X	R2	R3	R4	IC_{50} (μM)
HB001	NO_2	O	H	Cl	H	>20
HB002	NO_2	O	H	H	Cl	>20
HB003	CH_3SO_2	NH	H	H	Cl	>20
HB004	Br	NH	H	H	Cl	>20
HB005	CH_3	NH	H	H	NO_2	10.2
HB006	CN	NH	H	H	NO_2	1.28
HB007	CN	NH	H	H	Cl	0.85
HB008	NO_2		H	F	H	>20
HB009	NO_2	CH	H	Cl	H	10.4
HB010	NO_2	NH	H	H	COOH	>20
HB011	$COOCH_3$	NH	H	H	NO_2	10.0
HB012	NO_2	NH	H	H	Cl	>20
HB014	CN	NH	H	H	Br	1.25
HB015	CN	NH	H	H	C_6H_5F	1.7
HB016	CN	NH	H	H	$C_6H_5OCH_3$	4.55
HB017	CN	NH	H	H	$C_6H_5CH_3$	1.7
HB018	CN	NH	H	CH_3	H	>20
HB019	CN	NH	H	Br	H	1.2
HB020	CN	NH	Cl	H	Cl	1.2
HB021	CN	NH	H	H	OCH_3	2.4
HB022	CN	NH	H	CH_2CH_3	H	2.4
HB023	CN	NH	H	$C(CH_3)_3$	H	>20
HB024		NH	H	Cl	Cl	0.8
HB025	Cl	NH	H	H	CN	2.3
HB026	OCH_3	NH	H	H	CN	10
HB027	CH_3	NH	H	H	CN	>20

Fig. S1. Discovery of the hit CPD1 and lead HB007 of SUMO1 degraders. (**A**) Colony formation assays (*left panel*) and percentages (*right panel*) of indicated cancer cell lines treated with CPD1 for 10 to 15 days in the indicated doses (points: means ± SD; *n*=3). (**B**) The cancer cell lines as indicated were treated with CPD1 in indicated doses and analyzed by RT-PCR for *SUMO1* mRNA amounts (means ± SD; *n*=6). (**C**) Flag IP and western blotting of Flag-CDK6 and YFP-SUMO1 transfected LN229 cells treated with CPD1 for 24h for SUMO1-CDK6 conjugates as indicated (*right*). (**D-F**) In vitro sumoylation reaction assay with the indicated elements added with CDK6 as a SUMO1 substrate (D) and RanGAP as both SUMO3 (E) and SUMO1 substrate (F) in the presence or absence of CPD1 or ginkgolic acid (GA-C15:0). (**G**) The structure of CPD1 with the three moieties highlighted. (**H**) The core structure of CPD1 with each of three cores highlighted. (**I**) The compositions of matter generated through chemical modifications of the core structures. IC_{50} values of compounds was determined by cell viability assay. (**J-L**) One round of SAR studies of the compounds that was analyzed by cell viability assay for the activity (*n*=6 per compound) (J), dot blotting for the decrease of the total amounts of SUMO1 protein (K) and western blotting for the selective inhibition of SUMO1 conjugation (L) in LN229 cells treated for 72h with each of the four compounds. (**M**, **N**) Dot blot (M) and western blot analysis (N) of LN229 cells after treated for 72h with the indicated doses of CPD1 or HB007 for the total and conjugated amounts of SUMO1 with SUMO2/3, UB and actin as the selectivity and loading control. (**O**) Myc IP and western blot analysis of myc-UBC9 and YFP-SUMO3 transfected LN229 cells treated with CPD1 and HB007 for SUMO3-UBC9 conjugates (*right*). Whole cell lysate was used as a loading control.

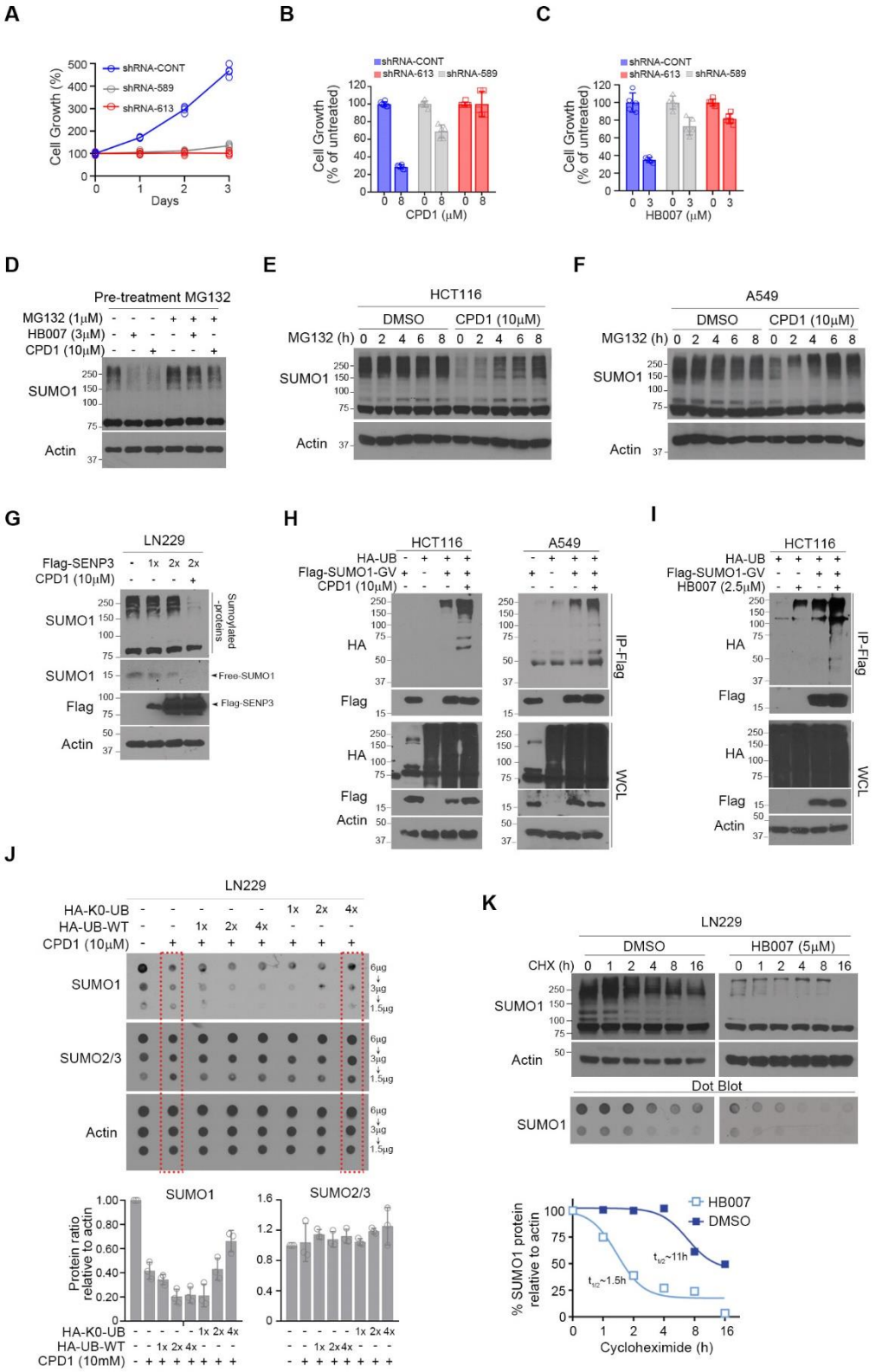
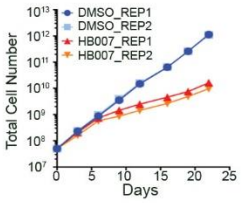

A
Cell Growth (%)
shRNA-CONT
shRNA-589
shRNA-613
Days
B
shRNA-CONT
shRNA-613
shRNA-589
Cell Growth (% of untreated)
CPD1 (μM)
C
shRNA-CONT
shRNA-613
shRNA-589
Cell Growth (% of untreated)
HB007 (μM)
D
Pre-treatment MG132
MG132 (1μM)
HB007 (3μM)
CPD1 (10μM)
SUMO1
Actin
E
HCT116
DMSO
CPD1 (10μM)
MG132 (h) 0 2 4 6 8 0 2 4 6 8
SUMO1
Actin
F
A549
DMSO
CPD1 (10μM)
MG132 (h) 0 2 4 6 8 0 2 4 6 8
SUMO1
Actin
G
LN229
Flag-SENP3 - 1x 2x 2x
CPD1 (10μM)
SUMO1
Sumoylated proteins
SUMO1
Free-SUMO1
Flag
Flag-SENP3
Actin
H
HCT116
A549
HA-UB
Flag-SUMO1-GV
CPD1 (10μM)
HA
Flag
IP-Flag
HA
Flag
Actin
WCL
I
HCT116
HA-UB
Flag-SUMO1-GV
HB007 (2.5μM)
HA
Flag
IP-Flag
HA
Flag
Actin
WCL
J
LN229
HA-K0-UB
HA-UB-WT
CPD1 (10μM)
SUMO1
SUMO2/3
Actin
6μg
3μg
1.5μg
SUMO1
SUMO2/3
Protein ratio relative to actin
HA-K0-UB
HA-UB-WT
CPD1 (10mM)
K
LN229
DMSO
HB007 (5μM)
CHX (h) 0 1 2 4 8 16 0 1 2 4 8 16
SUMO1
Actin
Dot Blot
SUMO1
HB007
DMSO
% SUMO1 protein relative to actin
t1/2~11h
t1/2~1.5h
Cycloheximide (h)

Fig. S2. CPD1 and HB007 induce SUMO1 ubiquitination and degradation. (A) LN229 cells were transduced with the *SUMO1* targeted shRNAs (shRNA-589, shRAN-613) and control shRNA (shRNA-CONT) and analyzed by cell viability assay, indicating that the SUMO1 knockdown drastically arrested the cell growth (n = 3). (**B**, **C**) LN229 cells were transduced with *SUMO1* targeted shRNAs and control shRNA, then treated with CPD1 (B) and HB007 (C) in indicated doses for 72h and finally analyzed by cell viability assay. (**D**) HCT116 cells were pre-treated with MG132 for 4 hrs, then treated with CPD1 or HB007 for 24 hrs and submitted to western blotting for SUMO1 conjugation amounts. (**E**, **F**) HCT116 (E) and A549 (F) were treated with CPD1 or DMSO for 24hrs, then treated with MG132 for indicated times and subjected to western blotting for SUMO1 conjugation amounts. (**G**) LN229 cells were overexpressed with Flag-SENP3 and, after treated or untreated with CDP1 for 48h, the cells were analyzed by western blotting for conjugated and unconjugated/free SUMO1 as indicated (*right*). (**H**, **I**) HCT116 and A549 cells were co-transfected with HA-UB and Flag-SUMO1-GV, then treated with CPD1 (H) or HB007 (I) for 48h and subjected to Flag IP and western blotting for SUMO1 poly-ubiquitination. (**J**) The conjugated HA-UB and non-conjugated HA-K0-UB mutant were overexpressed in LN229 cells and after 48h CPD1 treatment, the cells were examined by dot blots for total SUMO1 amounts (*upper panel*) with the densitometry analysis of SUMO1 and SUMO2/3 dots relative to actin (*bottom panel*). (**K**) LN229 cells were treated with HB007 for a total of 48h in the presence or absence of CHX and analyzed by western (*top panel*) and dot blotting (*middle panel*). The total concentrations of SUMO1 protein were normalized with actin using the Image J and plotted for a half-life indicating the HB007 treatment reduced the half-life of SUMO1 protein (*bottom panel*).

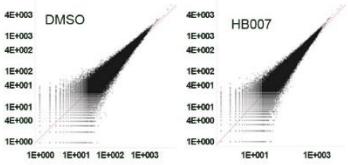
A



Dose range for screen

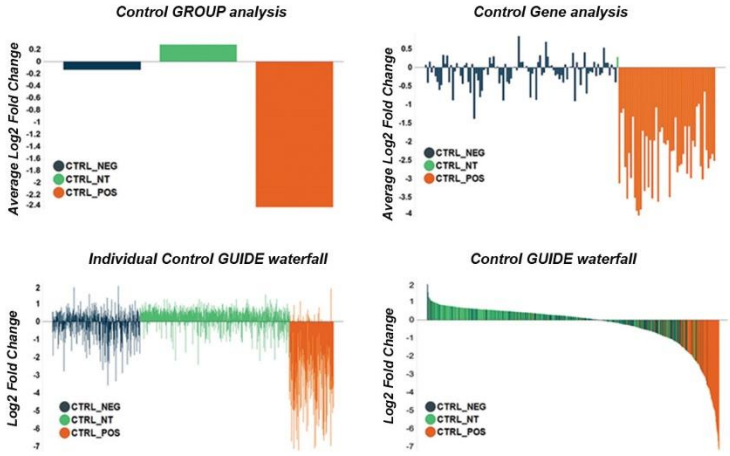
Condition	Screen dose (μM)
HB007	1-2

B



Sample Comparison	R^2
Library R1 and R2	0.99
DMSO R1 and R2	0.98
HB007 R1 and R2	0.98

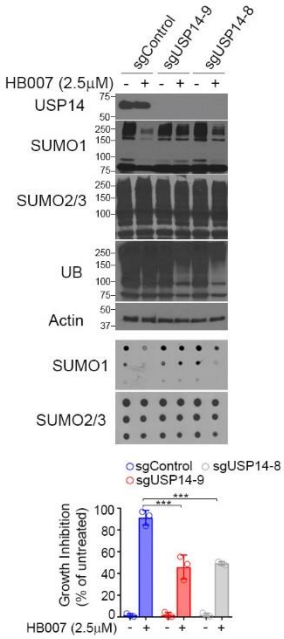
C



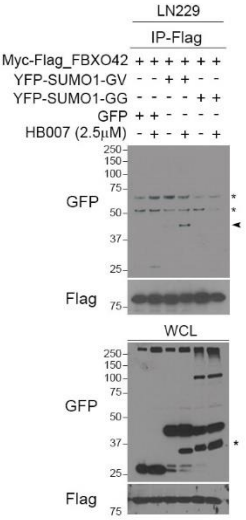
D

RANK	GENE	RRA Score	p-value	FDR	LogFC
1	STARD7	7.96E-14	2.40E-07	0.00165	1.149
2	G6PD	2.15E-12	2.40E-07	0.00165	1.3093
3	SLC7A1	2.58E-08	2.40E-07	0.00165	1.3068
4	KAT2A	7.98E-08	7.19E-07	0.002475	0.69899
5	CAB39	1.03E-07	7.19E-07	0.002475	1.0342
6	NOC4L	1.15E-07	7.19E-07	0.002475	0.65382
7	METTL23	1.58E-07	1.68E-06	0.00495	0.6462
8	CHD8	5.26E-07	3.59E-06	0.008416	0.46031
9	USP14	6.47E-07	4.07E-06	0.008416	1.0572
10	PAXIP1	6.56E-07	4.07E-06	0.008416	1.3785
11	CAD	8.23E-07	5.03E-06	0.009451	0.78405
12	TAF5L	1.23E-06	7.67E-06	0.013201	0.90181
13	MED23	1.30E-06	8.39E-06	0.013328	0.67004
14	EED	1.62E-06	9.34E-06	0.013791	0.88963
15	PDCD10	2.21E-06	1.27E-05	0.017492	0.43421
16	H2AFZ	2.77E-06	1.70E-05	0.021968	0.87797
17	DNAJC24	2.94E-06	1.89E-05	0.022277	0.75602
18	MED23	3.23E-06	1.94E-05	0.022277	0.96411
19	OLA1	3.59E-06	2.18E-05	0.02371	0.7516
20	TEN1	4.44E-06	2.47E-05	0.025495	0.86702
21	CBX4	6.41E-06	3.62E-05	0.035596	0.4693
22	NPRL2	9.02E-06	4.91E-05	0.045998	0.2503
23	G3BP1	9.74E-06	5.25E-05	0.045998	0.51804
24	MAPK1	9.92E-06	5.34E-05	0.045998	0.80863
25	CAPRIN1	1.03E-05	5.58E-05	0.046139	0.81886
26	EIF3H	1.13E-05	6.16E-05	0.047488	0.70721
27	FBXO42	1.14E-05	6.20E-05	0.047488	0.58362
28	WDR83	1.29E-05	7.16E-05	0.052864	0.54223

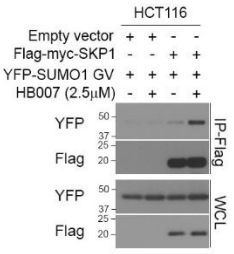
E



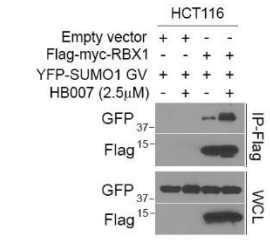
F



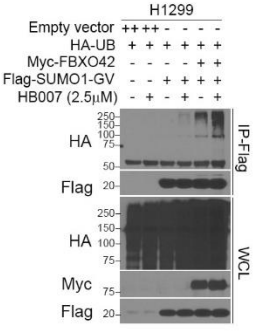
G



H



I



J

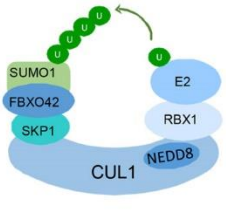
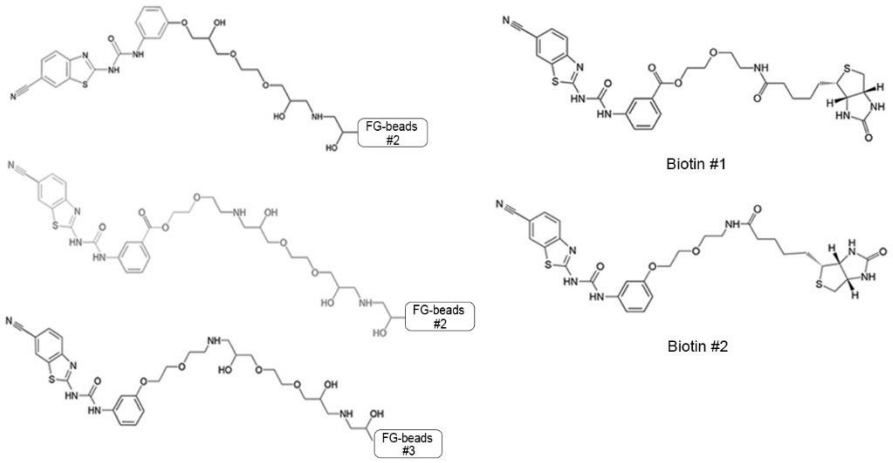
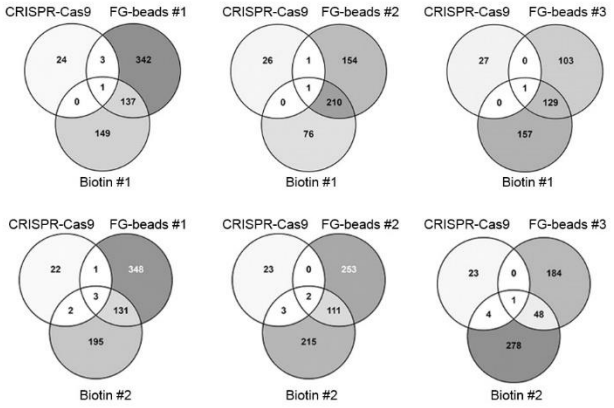


Fig. S3. Discovery of HB007-targeted E3 ligase pathway though genome-scale CRIPR-CAS9 knockout screen. (**A**) The growth of HCT116 cells treated with HB007 or DMSO for 21 days. (**B**) Scatter plots of guide-RNA abundance between replicates of DMSO and HB007 screen. Pearson's R values for each sample are shown in the Table. (**C**) Control group behavior: the *top left* shows the average Log2 Fold Change between the input sample (plasmid baseline) and the end point control (DMSO sample) for the group of controls. *Top right* shows the gene concentration average Log2 Fold change for each of the control genes. Bottom panels shows the individual control guide RNA concentration data either segregated by group (*left*) or in waterfall plot by value *(right*), indicating the partitioning of the positive control in the highly active group. (**D**) The top 28 genes were ranked based on the MAGeCK algorithm with the robust ranking aggregation (RRA) score, p-value, FDR and LogFC. (**E**) CRISPR-CAS9 *USP14* knockout and sgRNA control (sgControl) HCT116 clones were treated with HB007 for 72h and analyzed by western blots for conjugate SUMO1 (*upper panel*), dot blots for SUMO1 total amounts (*middle panel*) and cell viability assay for growth inhibition (*bottom panel*) (mean +/- S.D., n=3; ***P <0.001). (**F**) LN229 cells were co-transfected with myc-Flag-FBXO42, YFP-SUMO1-GV and/or YFP-SUMO1-GG, then treated with HB007 and subjected to Flag IP and western blot analysis for the interaction of FBXO42 and the unconjugated SUMO1-GV but not conjugated SUMO1-GG (* indicates a non-specific band). (**G**, **H**) HCT116 cells were transfected with YFP-SUMO1-GV together with Flag-myc-SKP1 (G) or Flag-myc-RBX1 (H), treated with HB007 for 48h and subjected to Flag IP and western blot analysis for SUMO1 interaction with SKP1 and RBX1. (**I**) HCT1299 cells were transfected with Flag-SUMO1-GV, Myc-FBXO42 and HA-UB, treated with HB007 for 48h and subjected to Flag IP and western blotting for SUMO1 poly-ubiquitination. (**J**), The protein-protein interaction studies suggested the molecule model of CUL1-FBXO42 E3 ligase complex.

A



B



	FG-beads			Biotin	
	#1	#2	#3	#1	#2
CONT-Beads	13	1	2	0	12
HB007-linked	60	9	6	6	21

C

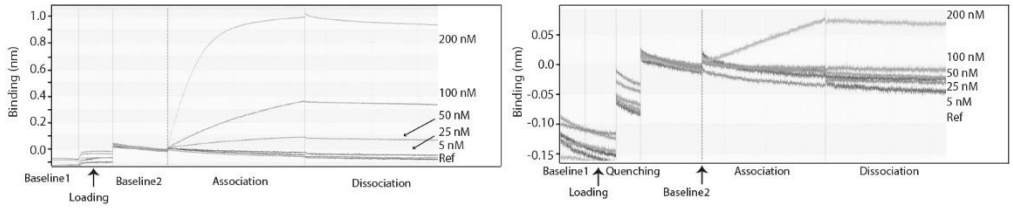


Fig. S4. Identification of the HB007 binding protein CAPRIN1. (**A**) Schemes of HB007 linked to FG-beads with three different linkers (*left*) and to biotin by two linkers (*right*), respectively. (**B**) Venn diagrams representing six combinations of five HB007-FG and HB007-biotin pull-downs with genome-scale CRISPPR-CAS9 knockout screen (*left panel*) and total peptides counts of CAPRIN1 from LC-MS/MS analysis of control and HB007-FG beads and HB007-biotin pull-downs (*right panel*). (**C**) Sensorgrams representing the raw data captured during the BLI assay and demonstrating the success of biocytin quenching in removing non-specific binding between CAPRIN1 protein and the SAX biosensors. The l*eft* panel shows the snapshot of assay between biotin loaded sensors and varying concentrations of CAPRIN1 in solution. The maximum binding response of biotin loaded sensors at 200 nM CAPRIN1 concentration is 1. The *right* panel shows the snapshot of assay between biocytin quenched reference sensors (with no small molecule loading) and varying concentrations of CAPRIN1-biotin mixture in solution. The maximum binding response of the quenched reference sensors at 200 nM CAPRIN1 concentration is 0.07.

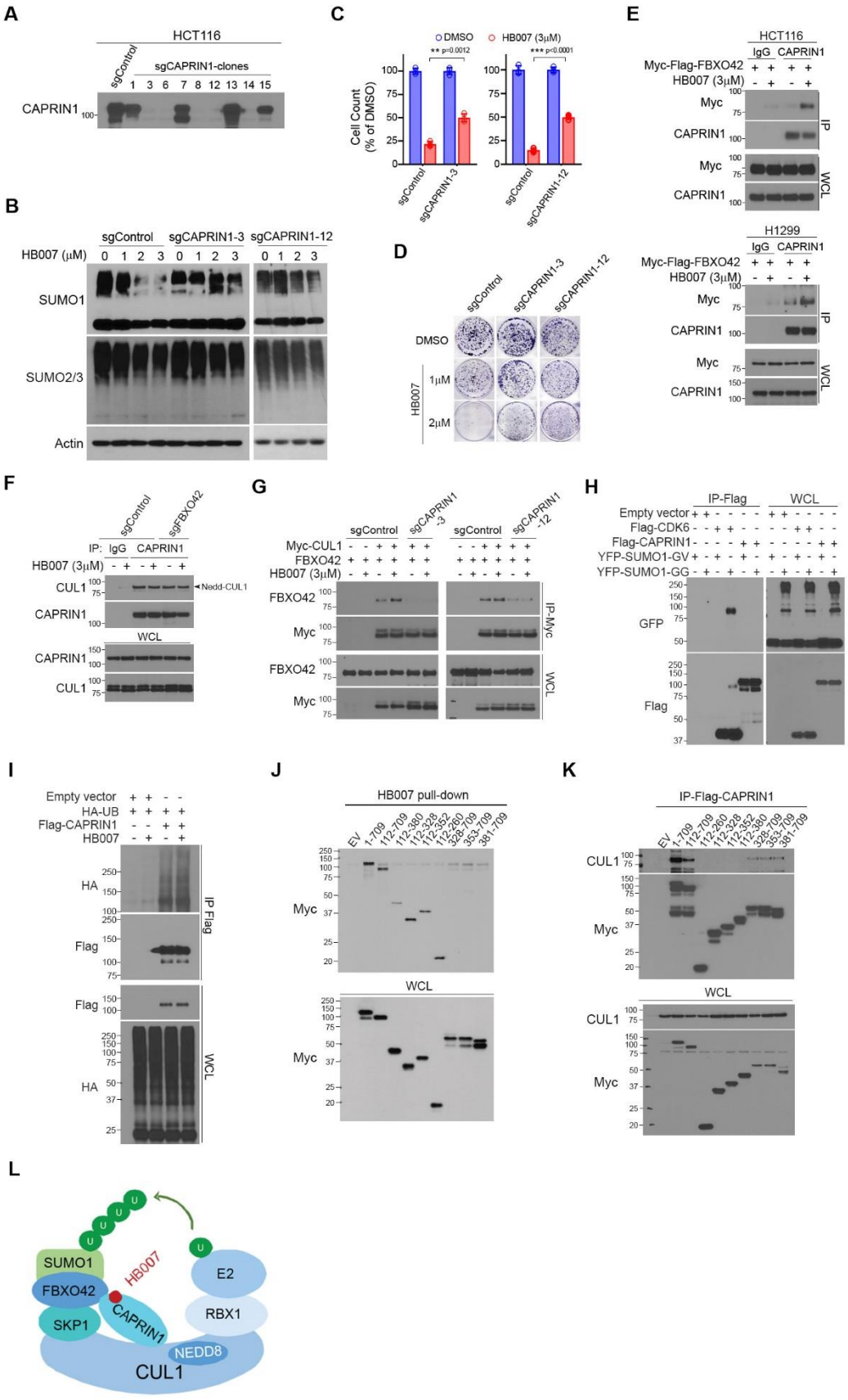
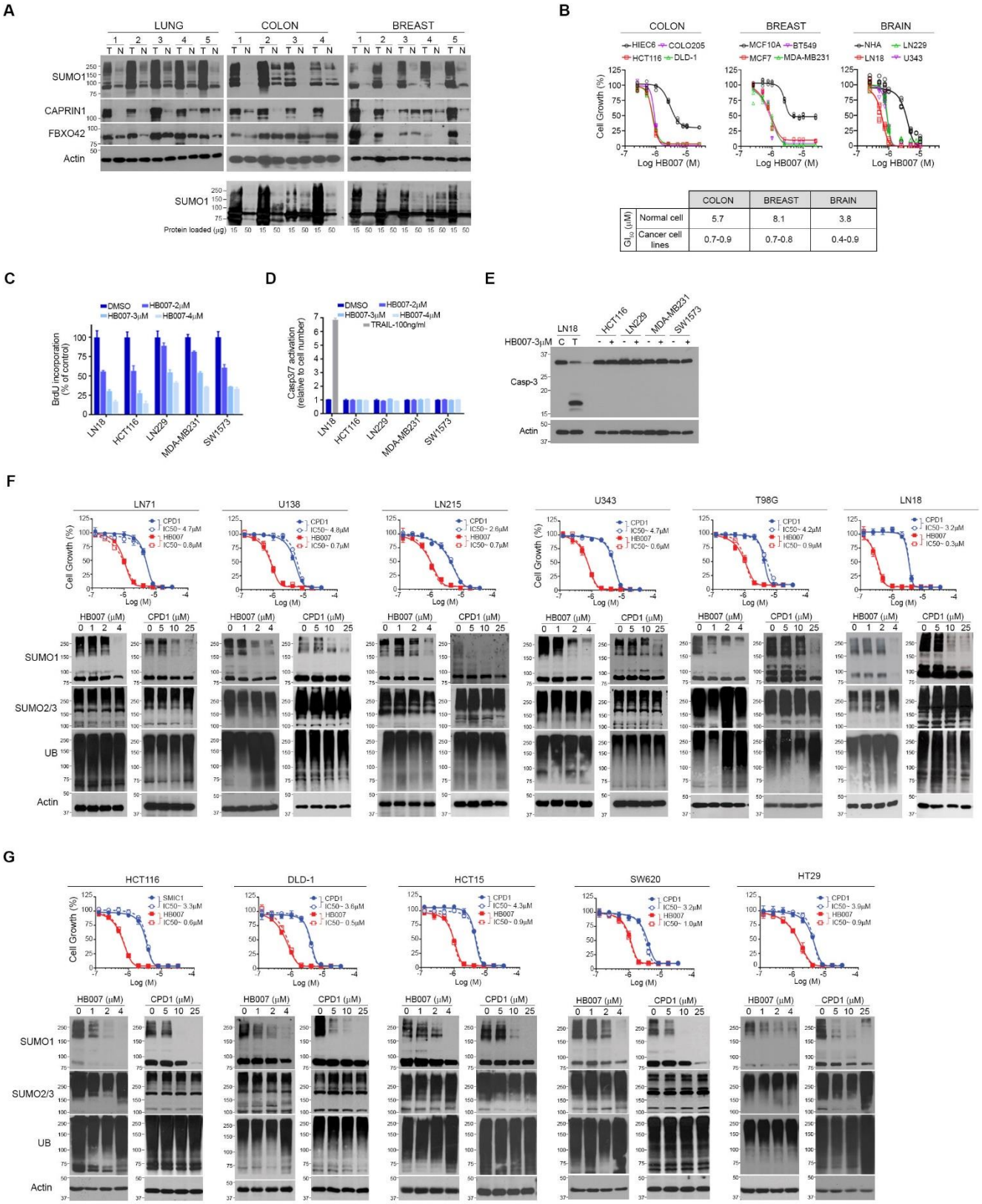

A
HCT116
sgControl
sgCAPRIN1-clones
1 3 6 7 8 12 13 14 15
CAPRIN1
100
B
sgControl
sgCAPRIN1-3
sgCAPRIN1-12
HB007 (μM)
0 1 2 3 0 1 2 3 0 1 2 3
SUMO1
SUMO2/3
Actin
C
DMSO
HB007 (3μM)
** p=0.0012
*** p<0.0001
Cell Count (% of DMSO)
100
75
50
25
0
sgControl
sgCAPRIN1-3
sgControl
sgCAPRIN1-12
D
sgControl
sgCAPRIN1-3
sgCAPRIN1-12
DMSO
HB007
1μM
2μM
E
HCT116
IgG
CAPRIN1
Myc-Flag-FBXO42
HB007 (3μM)
Myc
CAPRIN1
IP
Myc
CAPRIN1
WCL
H1299
IgG
CAPRIN1
Myc-Flag-FBXO42
HB007 (3μM)
Myc
CAPRIN1
IP
Myc
CAPRIN1
WCL
F
sgControl
sgFBXO42
IP: IgG CAPRIN1
HB007 (3μM)
CUL1
Nedd-CUL1
CAPRIN1
WCL
CAPRIN1
CUL1
G
sgControl
sgCAPRIN1-3
sgControl
sgCAPRIN1-12
Myc-CUL1
FBXO42
HB007 (3μM)
FBXO42
Myc
IP-Myc
FBXO42
Myc
WCL
H
IP-Flag
WCL
Empty vector
Flag-CDK6
Flag-CAPRIN1
YFP-SUMO1-GV
YFP-SUMO1-GG
GFP
Flag
I
Empty vector
HA-UB
Flag-CAPRIN1
HB007
HA
Flag
IP Flag
Flag
WCL
HA
J
HB007 pull-down
EV
1-709
112-709
112-380
112-328
112-352
112-260
328-709
353-709
381-709
Myc
WCL
Myc
K
IP-Flag-CAPRIN1
EV
1-709
112-709
112-260
112-328
112-352
112-380
328-709
353-709
381-709
CUL1
Myc
WCL
CUL1
Myc
L
U
SUMO1
FBXO42
SKP1
HB007
CAPRIN1
E2
RBX1
NEDD8
CUL1

Fig. S5. HB007-induced CAPRIN1-FBXO42 Interaction and SUMO1 recruitment to CUL1 E3 Ligase. (**A**) Western blots of sgRNA control (sgControl) and *CAPRIN1* kncokout (sgCAPRIN1) HCT116 clones for CAPRIN1 protein concentrations. (**B**) The sgCAPRIN1-3 and -12 knockout and sgRNA control HCT116 clones were treated with HB007 for 48h in indicated doses and examined by western blots for conjugated SUMO1 with SUMO2/3 and actin used as controls. (**C**) The sgCAPRIN1-3 and -12 knockout and sgRNA control HCT116 clones were treated with HB007 and DMSO for 48h and analyzed by cell viability assay with the cell number presented as % of DMSO control (means $\pm$ SD; n=3; $^{**}P<0.01$, $^{***}P$ <0.001 by unpaired t-test). (**D**) Colony formation assay of sgControl, sgCAPRIN1-3 and sgCAPRIN1-12 knockout HCT116 clones treated with HB007 for 10 days in indicated doses. The data was included in Fig.5B. (**E**), HCT116 and H1299 cells were transfected with myc-Flag-FBXO42, treated with HB007 for 24h and submitted to IP with a CAPRIN1 antibody, followed by western blot for the interaction of CAPRIN1 and FBXO42. (**F**) sgControl and sgFBXO42 knockout HCT116 clones were treated with HB007 for 24h and subjected to IP and western blots for CAPRIN1 and CUL1 interaction. (**G**) sgControl and sgCAPRIN1-3/-12 HCT116 clones were co-transfected with myc-CUL1 and FBXO42, treated with HB007 for 24h and submitted to myc-IP and western blots for CUL1 and FBXO42 interaction. (**H**) Flag-CAPRIN1, Flag-CDK6 and YFP-SUMO1-GG and/or YFP-SUMO1–GV were co-transfected in HCT116 cells and subjected to Flag-IP, followed by western blotting using GFP/YFP and Flag antibodies. Flag-CDK6 was used as a positive control. (**I**) Flag-CAPRIN1 and HA-UB were co-transfected in HCT116 cells, treated with HB007 for 24h, lysed in denaturing condition and submitted to Flag IP, followed by western blotting using a HA antibody detecting CAPRIN1 poly-ubiquitination. (**J**) Myc-Flag-CAPRIN1 domains were expressed in HCT116 cells and HB007-biotin/streptavidin-coated bead pull-downs of the cell lysates were examined by immunoblotting using myc antibodies for the binding of CAPRIN1 domains to HB007-biotin. The input (WCL) was used as the loading control. (**K**) HCT116 cells were transfected with Myc-Flag-CAPRIN1 domains and submitted to Flag IP and western blot using CUL1 and myc antibodies for the

binding of CAPRIN1 domains to CUL1.



GI_{50} (μM)	COLON	BREAST	BRAIN
Normal cell	5.7	8.1	3.8
Cancer cell lines	0.7-0.9	0.7-0.8	0.4-0.9

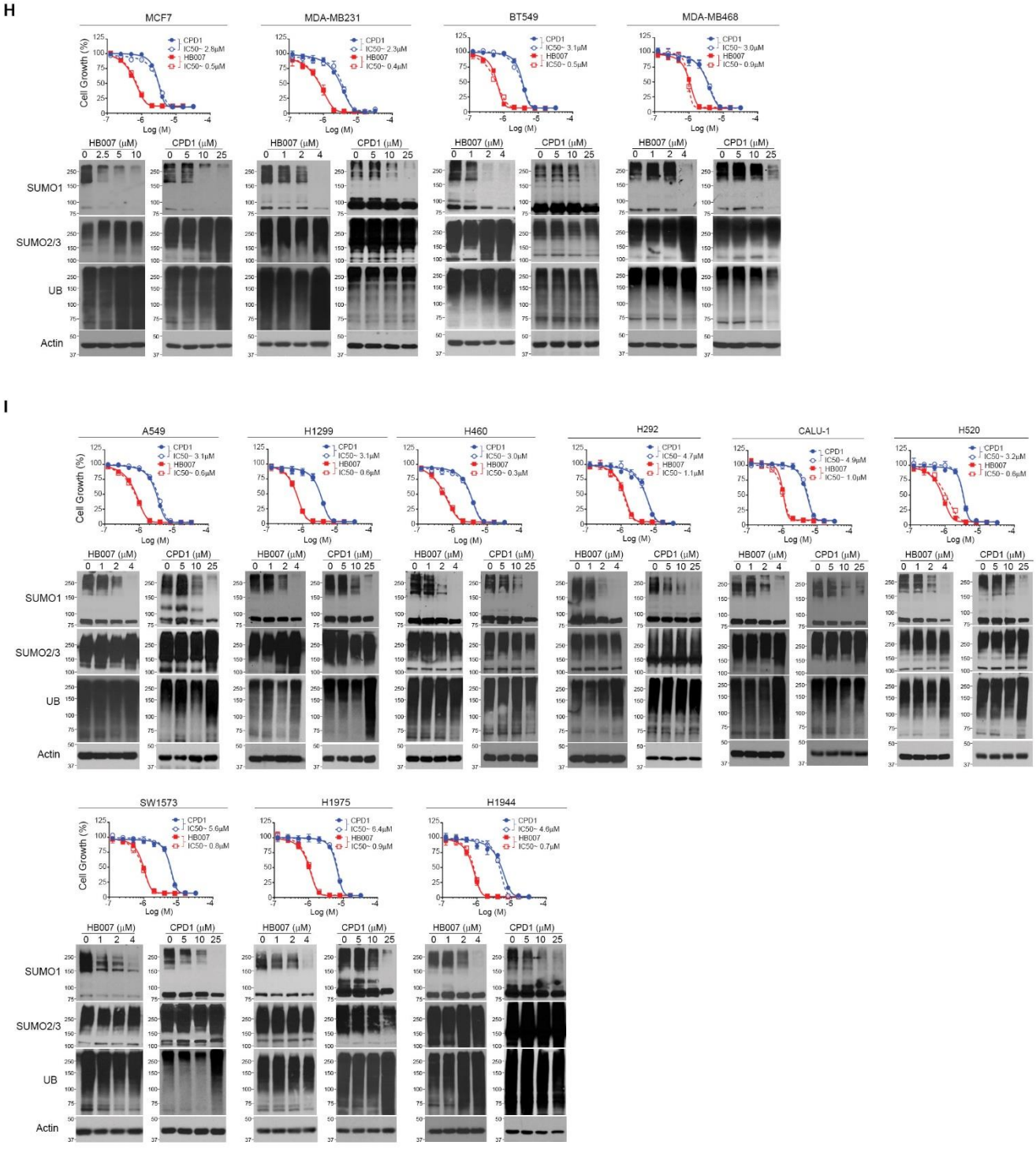
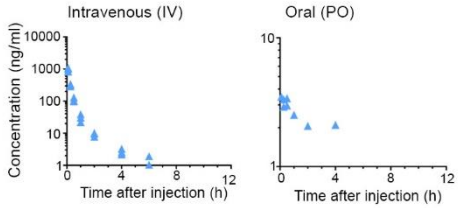

H
MCF7
MDA-MB231
BT549
MDA-MB468
Cell Growth (%)
Log (M)
CPD1
IC50~ 2.8μM
HB007
IC50~ 0.5μM
IC50~ 2.3μM
IC50~ 0.4μM
IC50~ 3.1μM
IC50~ 0.5μM
IC50~ 3.0μM
IC50~ 0.9μM
HB007 (μM)
CPD1 (μM)
SUMO1
SUMO2/3
UB
Actin
I
A549
H1299
H460
H292
CALU-1
H520
IC50~ 3.1μM
IC50~ 0.6μM
IC50~ 3.0μM
IC50~ 0.3μM
IC50~ 4.7μM
IC50~ 1.1μM
IC50~ 4.9μM
IC50~ 1.0μM
IC50~ 3.2μM
IC50~ 0.8μM
SW1573
H1975
H1944
IC50~ 5.6μM
IC50~ 0.8μM
IC50~ 8.4μM
IC50~ 0.9μM
IC50~ 4.6μM
IC50~ 0.7μM

Fig. S6. The selective activity of CPD1 and HB007 against various cancer cell lines. (**A**) Frozen tumor (T) and matched normal tissues (N) of NSCLC (lung), colon and breast carcinoma were analyzed by western blotting for conjugated SUMO1, CAPRIN1 and FBXO42 expression (*upper panel*). Western blots were repeated with normal tissues loaded in higher concentrations for visualization of RanGAP-SUMO1 conjugates (*lower panel*). (**B**) Colon, breast carcinoma and brain glioblastoma cell lines and their normal counterparts were treated with HB007 and analyzed by cell viability assay for growth inhibition after 72hrs treatment (*upper panel*) with the IC_{50} values calculated (*lower panel*). (**C**) Cell proliferation inhibition of colon, brain, breast and lung cancer cell lines following 24hrs treatment with various concentrations of HB007 was detected by BrdU incorporation. (**D**) Apoptotic cell death was evaluated through measurement of caspase-3/7 activity in cell lines treated with HB007 for 24hrs. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) was used as a positive control. (**E**) The cancer cell lines were treated with HB007 for 24hrs and subjected to western blots for Caspase 3 cleavage for apoptotic cell death. (**F -I**) Brain glioblastoma (F), colon carcinoma (G), breast carcinoma (H) and NSCLC cell lines (I) were treated with a series of concentrations of CPD1 and HB007 for 5 days and analyzed by cell viability assay (*upper panels*) and the IC_{50} values were calculated and presented in Fig. 6B. The treated cell lines were also examined by western blotting for the amounts of conjugated SUMO1 with SUMO2/3, UB and actin as controls following 72hrs treatment with CPD1 and HB007 (*lower panels*).

A

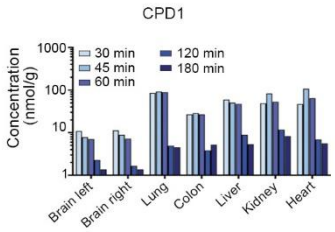
PK parameters	CPD1 i.p. 20mg/kg		HB007 i.p. 20mg/kg	
	Serum	Brain	Serum	Brain
No. points used for $T_{1/2}$	9	9	5	5
Cmax (ng/ml)	5266.37	5956.30	7080.19	4490.04
Tmax (hr)	0.50	1.00	0.50	0.50
T1/2 (hr)	1.42	0.74	1.28	0.60
$AUC_{0\text{-}inf}$ (ng.hr/ml)	10567.4	10092.43	16158.89	9200.31
Vz/F (L/kg)	3.87	n.a.	2.28	n.a.
CL/F (L/h/kg)	1.89	n.a.	1.23	n.a.
Kp		0.96		0.57

C



HB007 in Mice		
PK parameters	IV	PO
No. points used	9	8
Cmax (ng/ml)	1727.17	NR
T1/2 (hr)	1.37	NR
$AUC_{0\text{-}inf}$ (ng.hr/ml)	322.48	NR
Vz/F (L/kg)	6.03	NR
CL/F (L/h/kg)	52.15	NR

B



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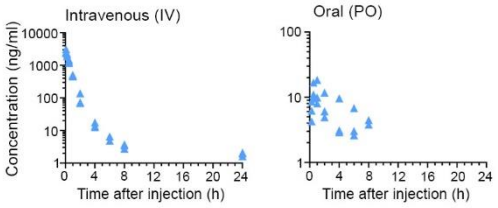
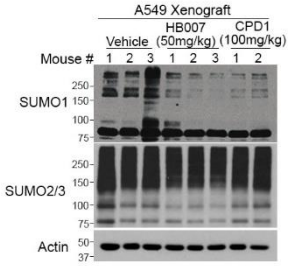


HB007 in Rat		
PK parameters	IV	PO
No. points used	9	8
Cmax (ng/ml)	3126.19	13.01
T1/2 (hr)	1.30	2.57
$AUC_{0\text{-}inf}$ (ng.hr/ml)	1850.13	60.76
Vz/F (L/kg)	1.01	
CL/F (L/h/kg)	9.05	
Bioavaibility (%F)		0.84

E



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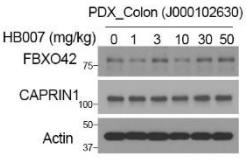

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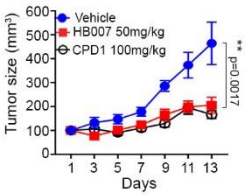

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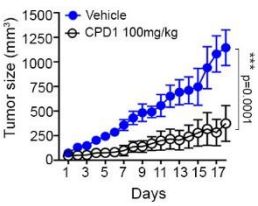

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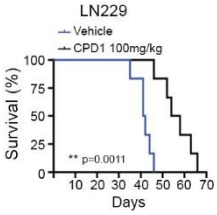

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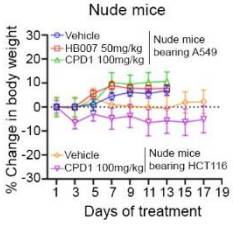

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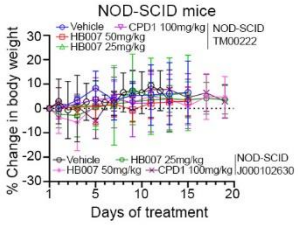

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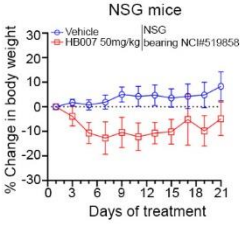

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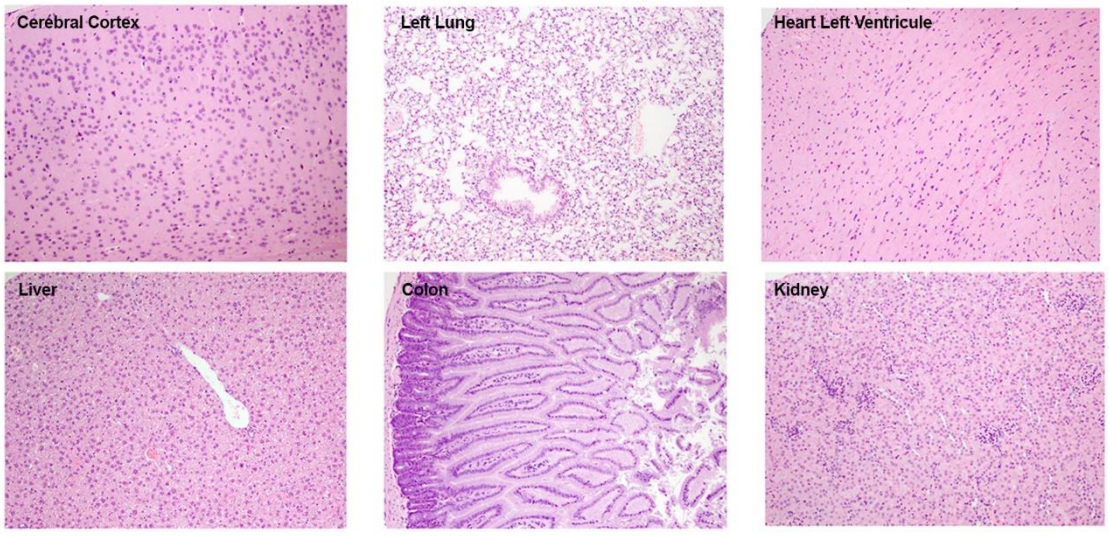

Fig. S7. The pharmacokinetics and in vivo anticancer activity of CPD1 and HB007. (**A**) The PK parameters of CPD1 and HB007in non-tumor bearing NOD/SCID mice, as determined by the PK analysis (Fig. 7b,c) including the maximum concentration (C_{max}), time to reach the C_{max} (T_{max}), and area under the concentration-time curve to the last sampling time ($AUC_{0\text{-}last}$), elimination half-time (T1/2), apparent volume of distribution at terminal phase (V_Z/F), and clearance (CL/F) were estimated using WinNonlin, (**B**) CPD1 distribution in organs as determined by the PK analysis of CPD1 (Fig. 7B) in non-tumor bearing NOD/SCID mice. (**C**, **D**) PK analysis of HB007 measured in plasma samples of non-tumor bearing ICR-CD1 mice (C) and Sprague Dawley rats (D) after the compound was administered intravenously (IV) (*left panel*) or orally (PO) (*middle panel*) with the summary of the PK parameters (*right panel*). (**E**) A549-derived xenograft mice were treated with HB007 and CDP1 through intraperitoneal injection once per day for 3 days, 10 days after inoculation; western blot analysis of the xenograft tissues showed that the treatment reduced the abundance of SUMO1 conjugation. (**F**) Colon cancer PDX mice were treated with HB007, a week after inoculation, at the indicated doses by intraperitoneal injection once a day for 3 days; xenograft tissue were examined by western blot for CAPRIN1 and FBXO42 expression. (**G**, **H**) Mice bearing A549 (G) or HCT116 xenografts (H) were treated with CPD1 (100mg/kg) or HB007 (50 mg/kg), 10 days after inoculation, xenograft sizes indicated that the treatment suppressed xenografts (Data represent means +/- s.e.m. For A549: n=7/group, $^{**}P<0.001$ by Friedman test; for HCT116: n=5/group, $^{***}P<0.0001$ by Wilcoxon test). (**I**) Kaplan Meier survival analysis of *NOD/SCID* mice bearing LN229 intracranial brain glioblastoma xenografts under the treatment of CPD1 (n=6 mice per group, $^{**}P<0.01$ by Log-rank test). (**J**-**L**) Percentage of change in body weights of the nude mice bearing A549 and HCT116 (J, as G, H), the NOD-SCID mice bearing colon PDX J000102630 and NSCLC/lung PDX TM00222 and (K, as Fig. 7I, J) and the NSG mice bearing colon PDX NCI#519858 (L, as Fig. 7M). (**M**) Representative hematoxylin and eosin staining sections of brain cerebral cortex, left lung, heart left ventricle, liver, colon and left kidney from A549 xenograft-bearing mice at the end of treatment with the 50mg/kg of

Tissue origin	Provenance	Reference Number

HB007 (the data presented in G). Magnifications of the images: 10 x 10. No histologic abnormalities are observed in these organs.

BREAST	Jackson Laboratory	TM00091
LUNG	Jackson Laboratory	TM00222
COLON	Jackson Laboratory	J000102630
COLON	NCI	519858

Table S1. Information regarding patient' derived xenografts.