1. Supplemental Tables and Figures

1A His-UCHL1^{WT} + DMSO

m/z	Charge	Predicted Mass
1034	26	26859.2
1075.3	25	26858.4
1120.9	24	26878.8
1171.2	23	26915.3
1222.4	22	26869.9

Average Mass = 26,873.4 ± 17.2

1B His-UCHL1^{WT} + Compound **1**

m/z	Charge	Predicted Mass
1009.8	27	27237.6
1048.5	26	27236.2
1090.8	25	27245.2
1136.1	24	27243
1137.7	24	27281.6

Average Mass = 27,245.1 ± 12.7

1C His-UCHL1^{C90A} + DMSO

m/z	Charge	Predicted Mass
928.7	29	26902.7
994.7	27	26830.5
1037.5	26	26948.7
1075.6	25	26864.3
1124.3	24	26958.6
1167.5	23	26829.1
1169	23	26865
1220.4	22	26827.2
1278.8	21	26833.2
1343.1	20	26842.6

Average Mass = 26,874.5 ± 48.1

1D His-UCHL1^{C90A} + Compound **1**

m/z	Charge	Predicted Mass
1073.9	25	26822
1118.6	24	26822.6
1119.2	24	26835.9
1168.2	23	26845.2
1228.9	22	27012.8
1284.3	21	26949.7

Average Mass = 26,881.2 ± 76.6

1E

Compound 1 MW	Change in Detected Mass (His-UCHL1^{WT})	Change in Detected Mass (His-UCHL1^{C90A})
375.13	371.7	6.7

Table S1. **Compound 1 covalently modifies UCHL1 . Table 1A**) His-UCHL1^{WT} treated with DMSO, average deconvoluted mass ± S.D. shown below table; **Table 1B**) His-UCHL1^{WT} treated with compound **1** average deconvoluted mass ± S.D. shown below table. **Table 1C**) His-UCHL1^{C90A} treated with DMSO, average deconvoluted mass ± S.D. shown below table; **Table 1D**) His-UCHL1^{C90A} treated with compound **1** average deconvoluted mass ± S.D. shown below table. **Table 1E**) Molecular weight of compound **1** and detected mass shift from difference in samples treated with **1** and DMSO control.

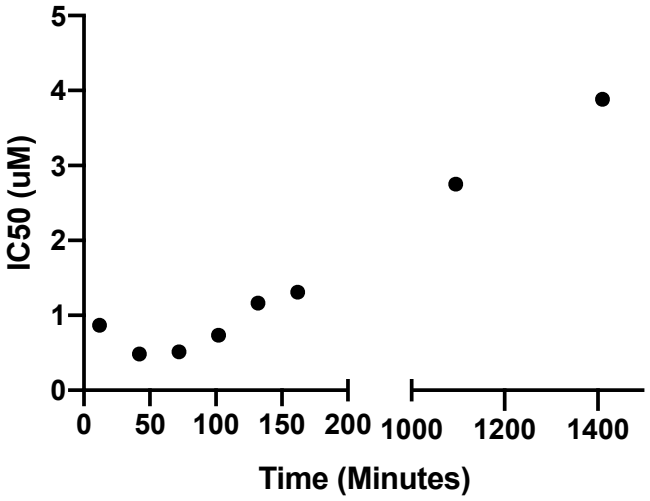


Figure S1. **Compound 1 is a slowly reversible inhibitor.** UCHL1 incubated with **1** regains activity over time.

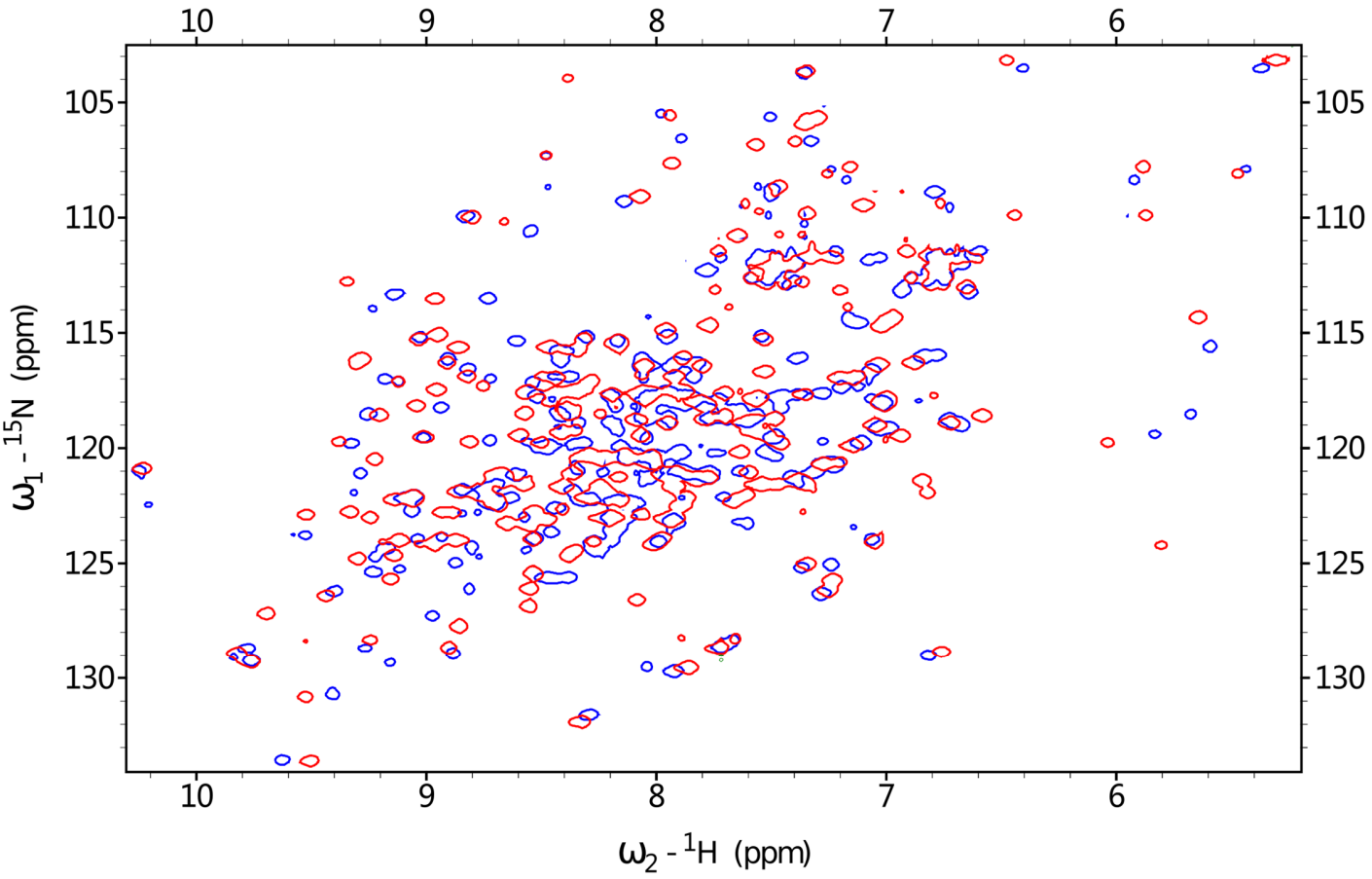


Figure S2. The overlaid ^{15}N-HSQC spectra for unligated (red) and UCHL1 bound (blue) with **1.**

Use of intermolecular NOE crosspeaks in computational docking

Intermolecular NOE crosspeaks were detected for 26 intermolecular contacts. Preliminary assignments were made for the NOE crosspeaks that matched in frequency with reported resonance assignments for aliphatic sidechains of UCHL1 (BMRB entry 17260). The BMRB assigned chemical shifts were first aligned with our measured spectra by minimizing the difference in ppm values between the BMRB entries and the peak positions in a ^{13}C-HSQC spectrum for unligated UCHL1. Eight intermolecular NOE crosspeaks overlaid peaks in both the unligated and bound ^{13}C-HSQC spectra and were given ambiguous assignments based on close matches in ppm values with the BMRB assignment list. This list of ambiguous assignments was inspected for residues near the active site pocket and these were selected for use as distance restraints in docking with CovDock (Schrödinger, LLC). Many of the docking computations failed to reach an acceptable complex structure. Docked poses were accepted for the case where distance-restrained docking was conducted with a single intermolecular NOE distance restraint between Ala 147 HB and H3 of **1**. Docking against the truncated form of UCHL1 without the N-terminal nine residues resulted in a pose with good interactions involving the oxyanion hole, and is the selected pose described in the main text.

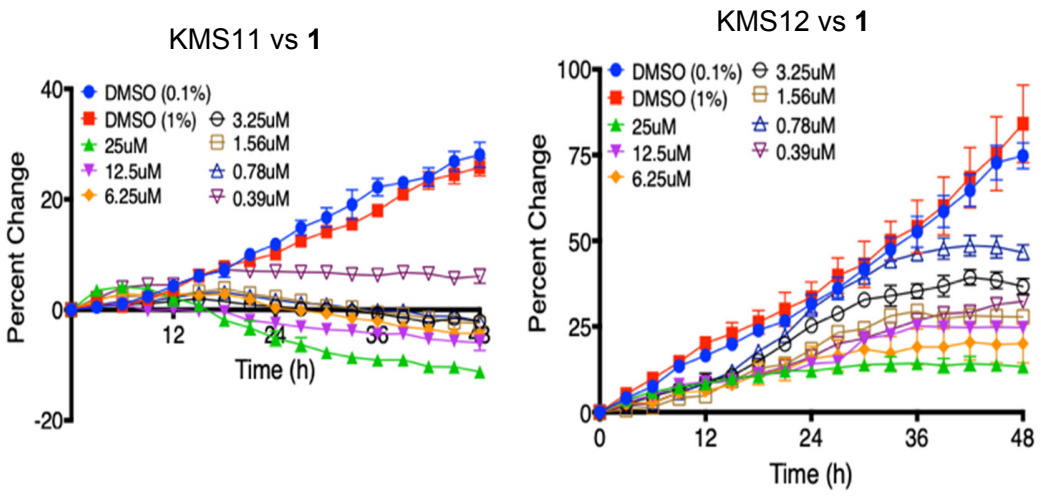


Figure S3. **Cell proliferation data for 1 versus KMS11 and KMS12 cells.** KMS11 and KMS12 cells incubated with various concentrations of **1** display a dose-dependent change in proliferation.

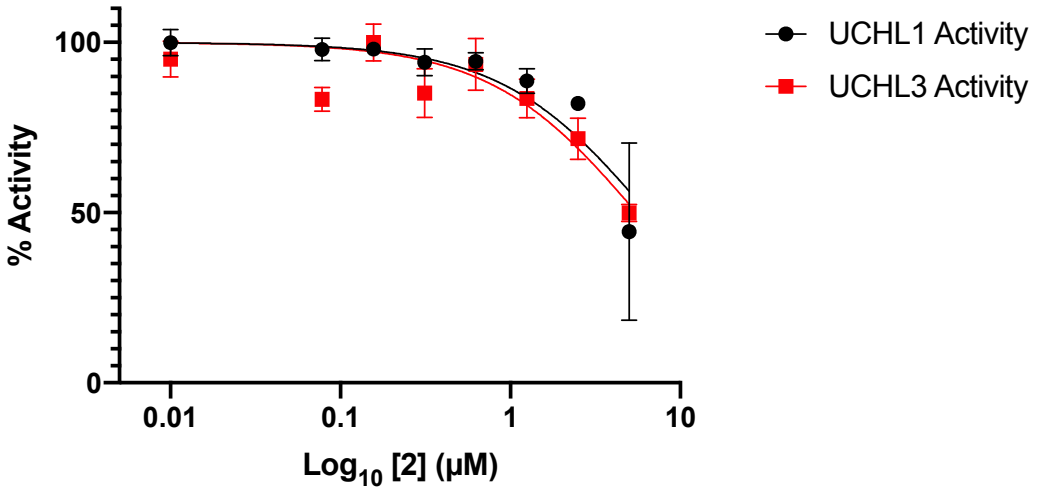


Figure S4. **Compound 2 dose-response inhibition assay.** IC_{50} curves for compound **2** against UCHL1 (black) and UCHL3 (red) after 30 minutes of preincubation.

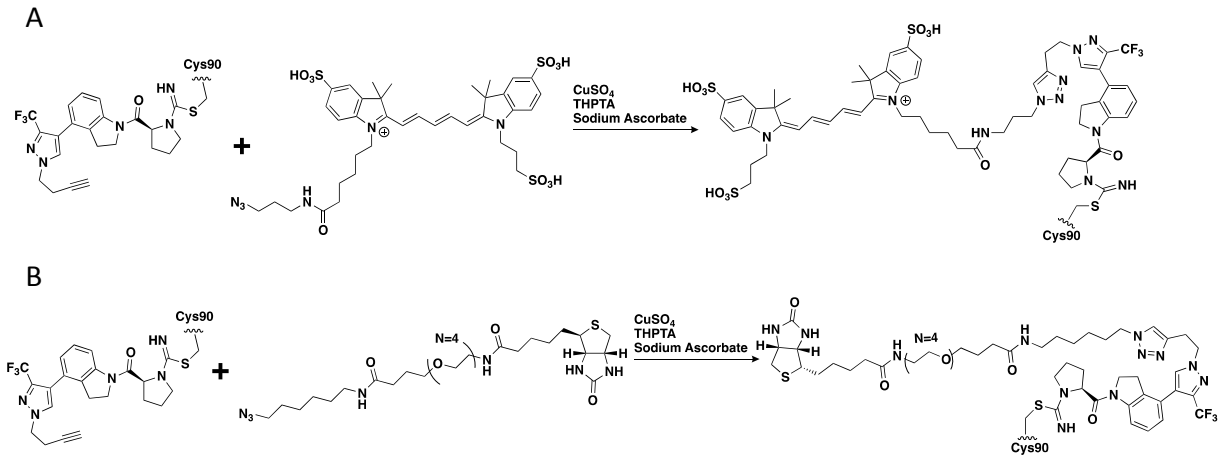


Figure S5. **CuAAC reaction of UCHL1 treated with 2.** General reaction scheme for the formation of UCHL1-Compound **2** complex, followed by the CuAAC ligation of (A) sulfo-Cyanine5 azide or (B) Biotin azide.

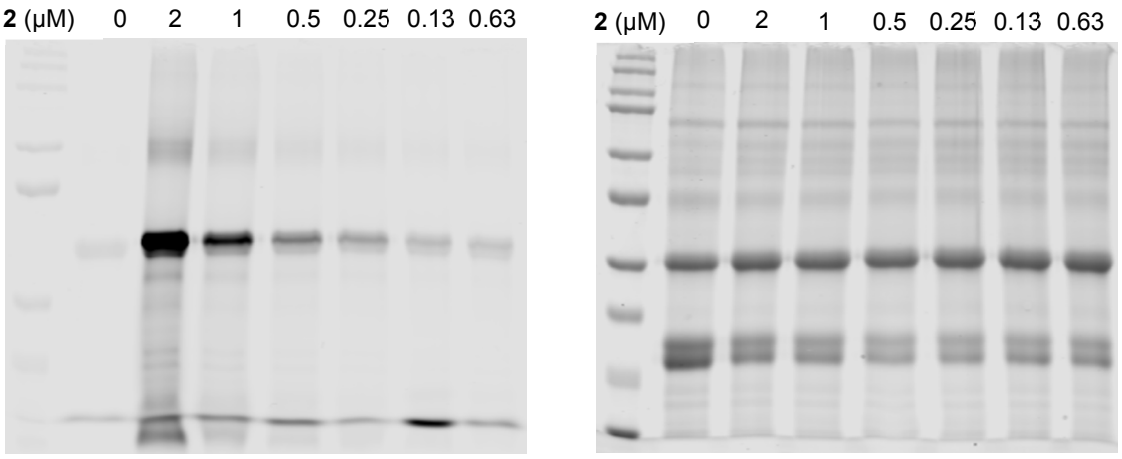


Figure S6. **Dose-dependent labelling of recombinant UCHL1 by 2.** Recombinantly expressed UCHL1^{WT} treated with decreasing concentrations of **2** results in a corresponding decrease in fluorescent band intensity (left). Coomassie stained gel (right) indicates equal loading of UCHL1.

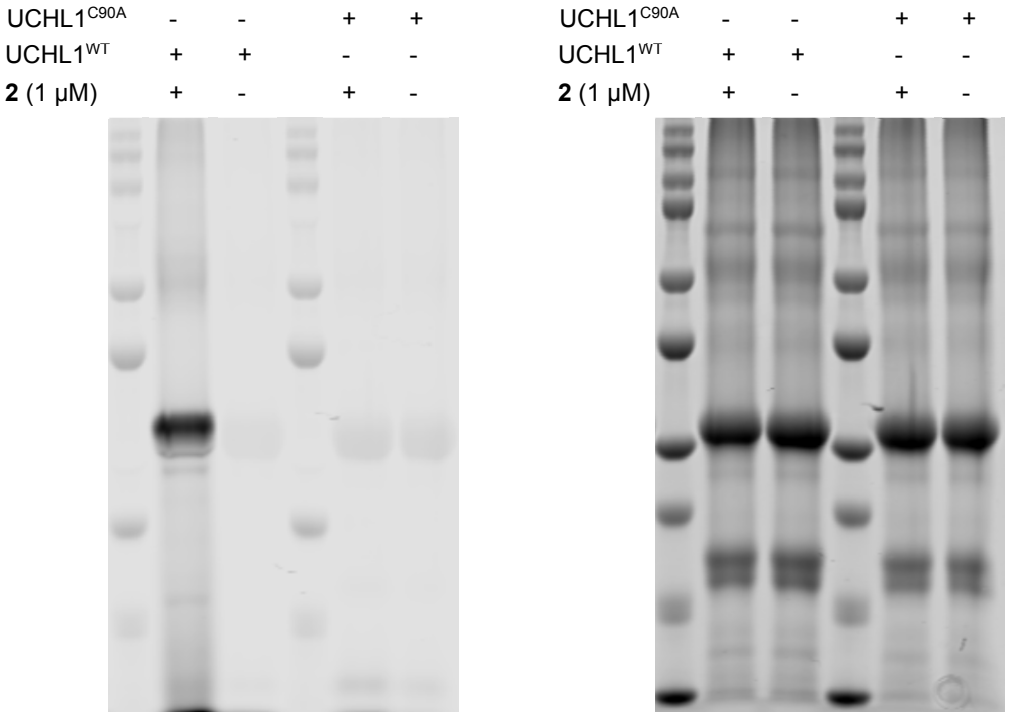


Figure S7. **Specific labelling of UCHL1s catalytic cysteine by 2.** Recombinantly expressed UCHL1^{WT} or catalytically inactive mutant UCHL1^{C90A} treated with 1 μM **2** results in selective labelling of UCHL1^{WT} at the catalytic cysteine (residue 90) as visualized by fluorescent bands (left). Coomassie stained gel (right) indicates equal loading of all proteins.

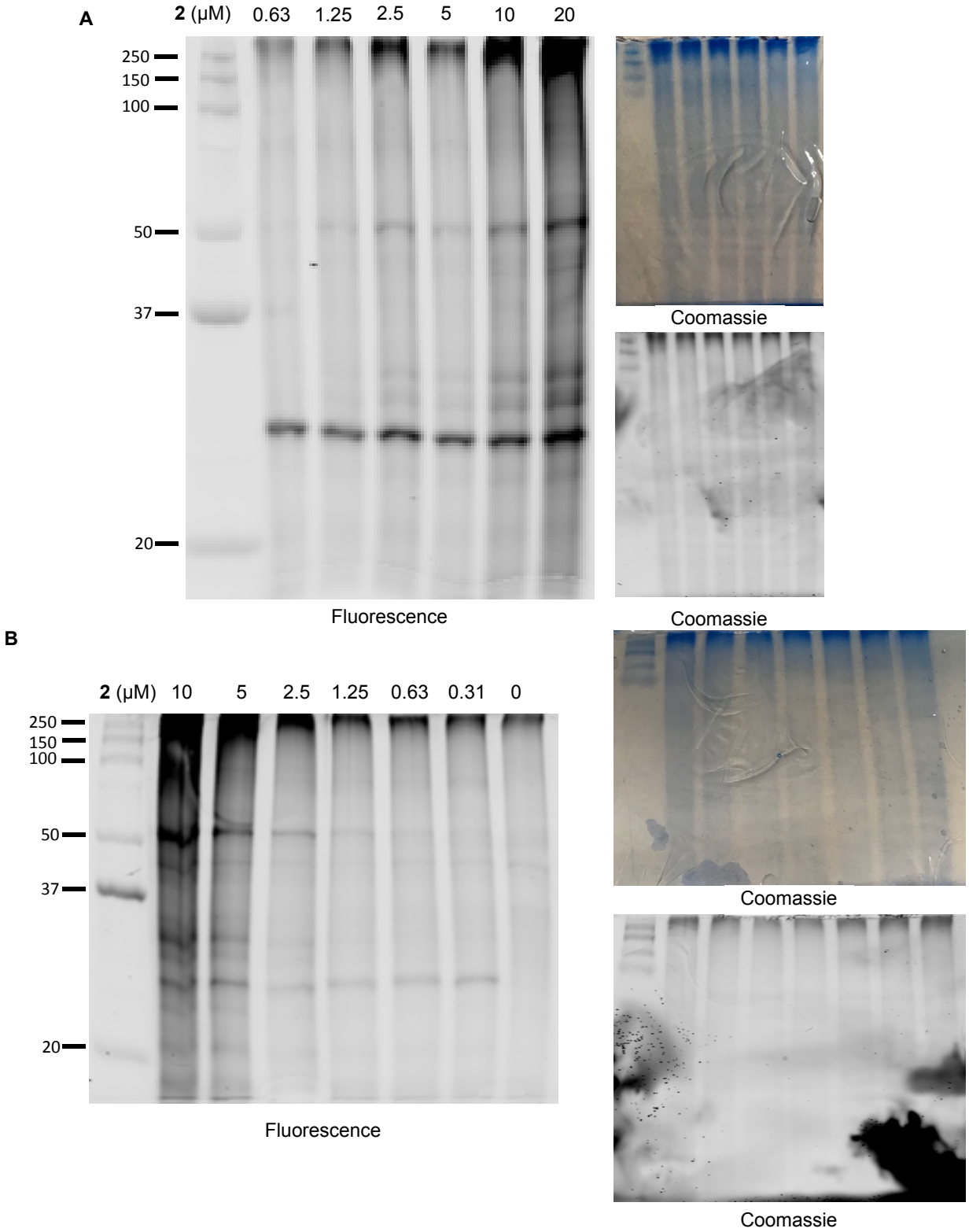


Figure S8. **Labelling of proteins in intact cells by 2**. KMS11 (A) and SW1271 (B) cells treated with various **2** results in a dose-dependent change in fluorescent band intensity. Bands were undetectable by Coomassie staining.

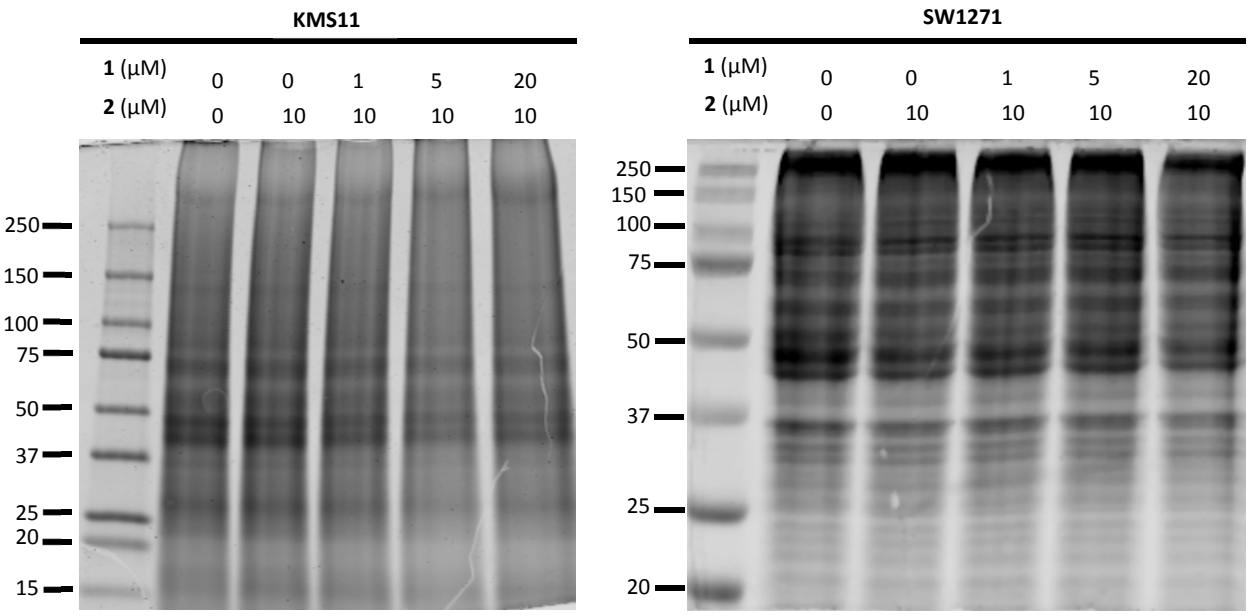


Figure S9. **Loading control of fluorescence gels in Figure 8.** KMS11 and SW1271 cells treated simultaneously with a 10 μM of compound **2** and increasing concentrations of compound **1** show equal loading in each lane as visualized by Coomassie stain.

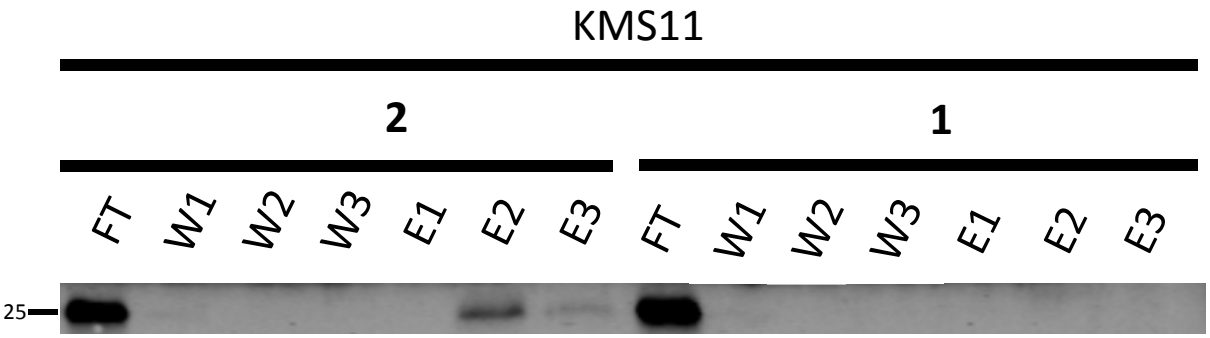
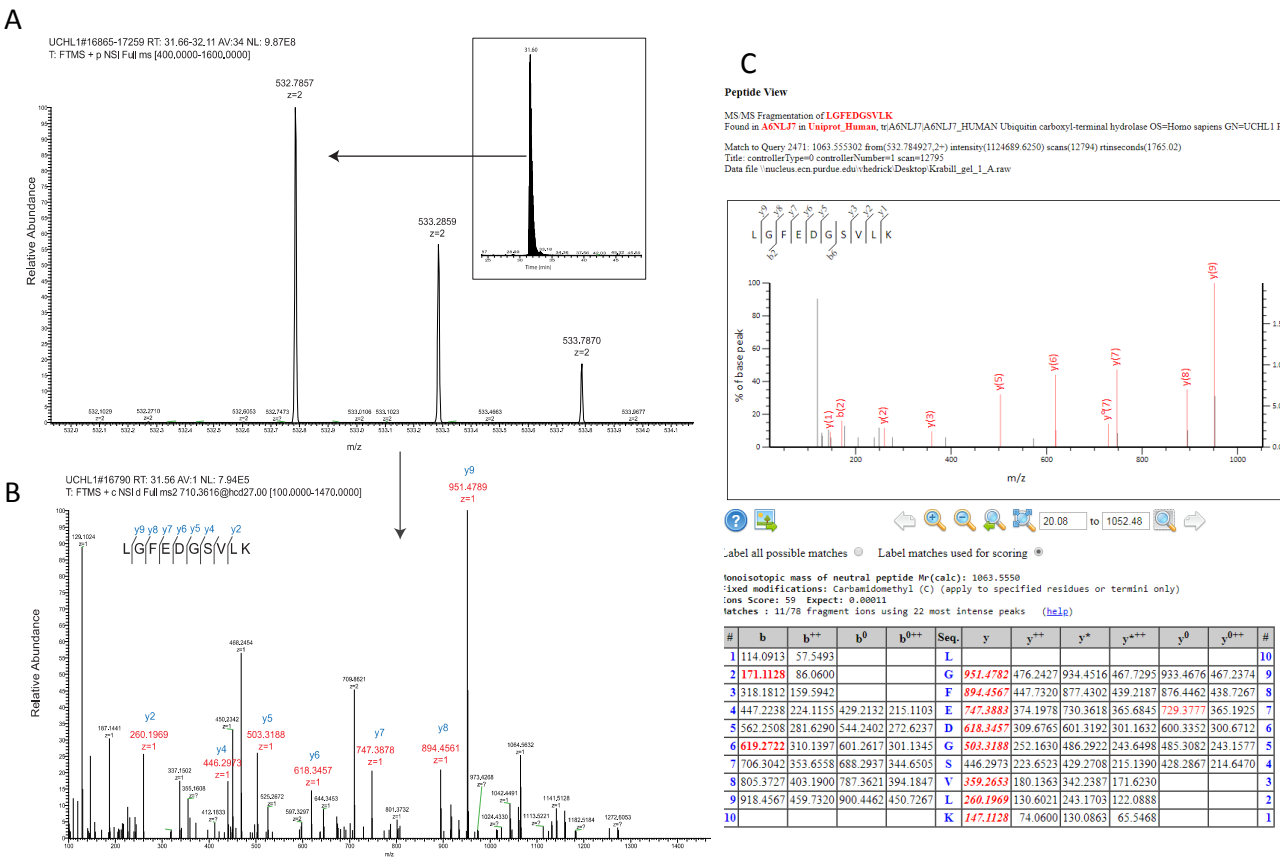


Figure S10. **Cellular engagement of UCHL1 by compound 2.** KMS11 cells were treated with **1** or **2** before lysis. Cell lysates were subjected to pulldown using magnetic streptavidin beads and analyzed by immunoblot (Developmental Studies Hybridoma Bank – antibody 15C7). UCHL1 can be seen eluting from the beads using elution condition 2 (E2). Compound **1,** which lacks an alkyne for click reactions, was not able to pull down UCHL1 from cell lysate. FT = flow-through, W = wash, E = elution



#	b	b^{++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	114.0913	57.5493			L							10
2	171.1128	86.0600			G	*951.4782*	476.2427	934.4516	467.7295	933.4676	467.2374	9
3	318.1812	159.5942			F	*894.4567*	447.7320	877.4302	439.2187	876.4462	438.7267	8
4	447.2238	224.1155	429.2132	215.1103	E	*747.3883*	374.1978	730.3618	365.6845	729.3777	365.1925	7
5	562.2508	281.6290	544.2402	272.6237	D	*618.3457*	309.6765	601.3192	301.1632	600.3352	300.6712	6
6	619.2722	310.1397	601.2617	301.1345	G	*503.3188*	252.1630	486.2922	243.6498	485.3082	243.1577	5
7	706.3042	353.6558	688.2937	344.6505	S	446.2973	223.6523	429.2708	215.1390	428.2867	214.6470	4
8	805.3727	403.1900	787.3621	394.1847	V	*359.2653*	180.1363	342.2387	171.6230			3
9	918.4567	459.7320	900.4462	450.7267	L	*260.1969*	130.6021	243.1703	122.0888			2
10					K	*147.1128*	74.0600	130.0863	65.5468			1

Figure S11. Identification of UCHL1 by tandem mass spectrometry analysis. (A) The monoisotopic peak (m/z = 532.7857) and isotopomers distribution of the peptide mapped to UCHL1. The inset shows the extracted ion current (XIC) chromatogram and its retention time in the LC column. (B) MS/MS spectra showing the y-ion fragments that were used to identify the LGFEDGSVLK peptide sequence. Data were obtained from recombinant UCHL1 tryptic peptides. The same peptide was also identified in the probe purified biological sample with the same y-ion fragments as shown in supplementary figure 9. (C) Predicted y-ions for for UCHL1 peptide sequence LGFEDGSVLK.

Protein Name	Gene Name	Molecular Weight (kDa)	LFQ Intensity DMSO	LFQ Intensity Compound 1	MS/MS Count DMSO	MS/MS Count Compound 1
Forkhead box protein N4	FOXN4	55.277	0	95486000	0	2
40S ribosomal protein S14	RPS14	16.273	0	44447000	0	2
Retina-specific copper amine oxidase	AOC2	83.672	0	9107700	0	3
Protein-glutamine gamma-glutamyltransferase K	TGM1	82.355	0	25026000	0	3
Membrane-spanning 4-domains subfamily A member 10	MS4A10	29.747	0	74649000	0	5
Alpha-amylase	AMY1A	56.21	0	4854700	0	5
Catalase	CAT	59.755	0	46581000	0	5
Cystatin-SN	CST1	16.387	0	13196000	0	6
Transcription factor HES-1	HES1	14.429	0	113100000	0	6
Fibronectin	FN1	246.7	0	178110000	0	31

Table S2. Identification of putative off-targets by tandem mass spectrometry analysis. Puative off-target interactions of compound **2** were identified using Mascot Daemon. Proteins were filtered and accepted if signal (LFQ Intensity) was present in only the probe-treated samples, and if the MS/MS count was greater than 2 in at least two of three samples tested.