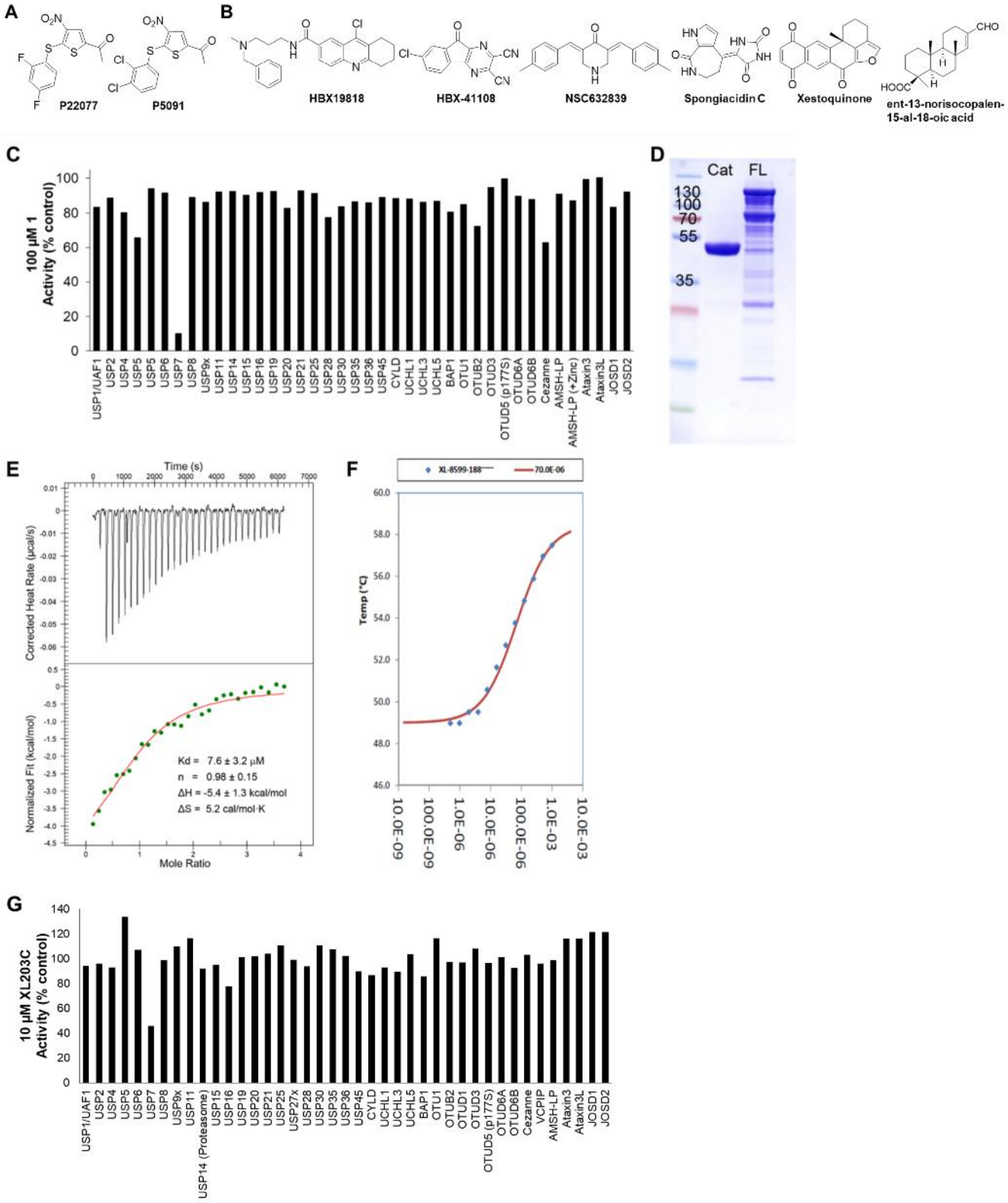


Figure S1. **Structure and characterization of USP7 inhibitors. Related to Figure 1.** A) USP7 inhibitors P22077 and P5091. B) Reported USP7 inhibitors. C) Inhibitory activity of **1**, at a concentration of 100 µM, across a panel of 38 purified DUBs using Ub-Rho as substrate. D) Assessment of **1** binding to USP7 using ITC. E) Measure of ability of XL188 to bind USP7 using differential scanning fluorimetry. F) Inhibitory activity of XL203C across a panel of 41 purified DUBs using Ub-Rho as substrate. G) Coomassie gel with USP7 catalytic domain (Cat) and full-length (FL) proteins.

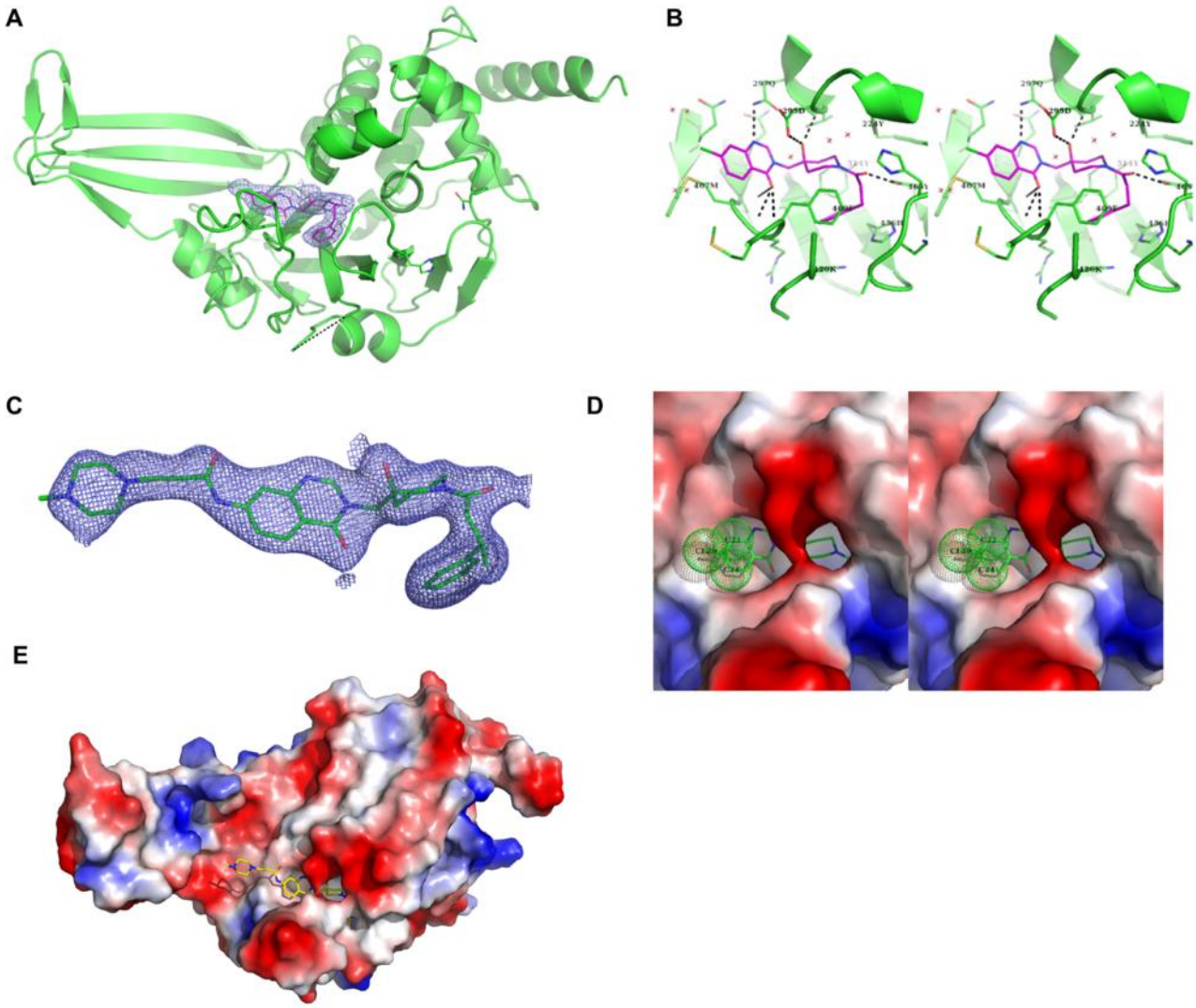
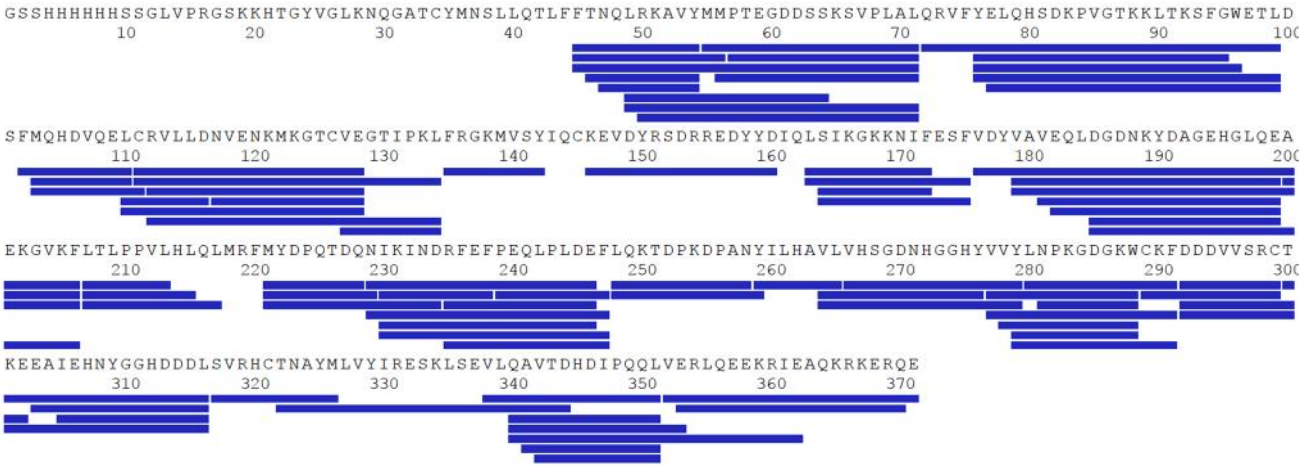


Figure S2. Crystallographic characterization of binding of 1 and XL188 to USP7. Related to Figure 2. A) Ribbon diagram of USP7 with **1**. B) Stereo view of the detailed interactions between **1** and USP7. C) Electron density map of XL188 bound to USP7. D) Molecular surface representation of **1** bound to USP7 zoomed in to the compound binding pocket. E) Molecular surface representation of XL188 bound to USP7.

A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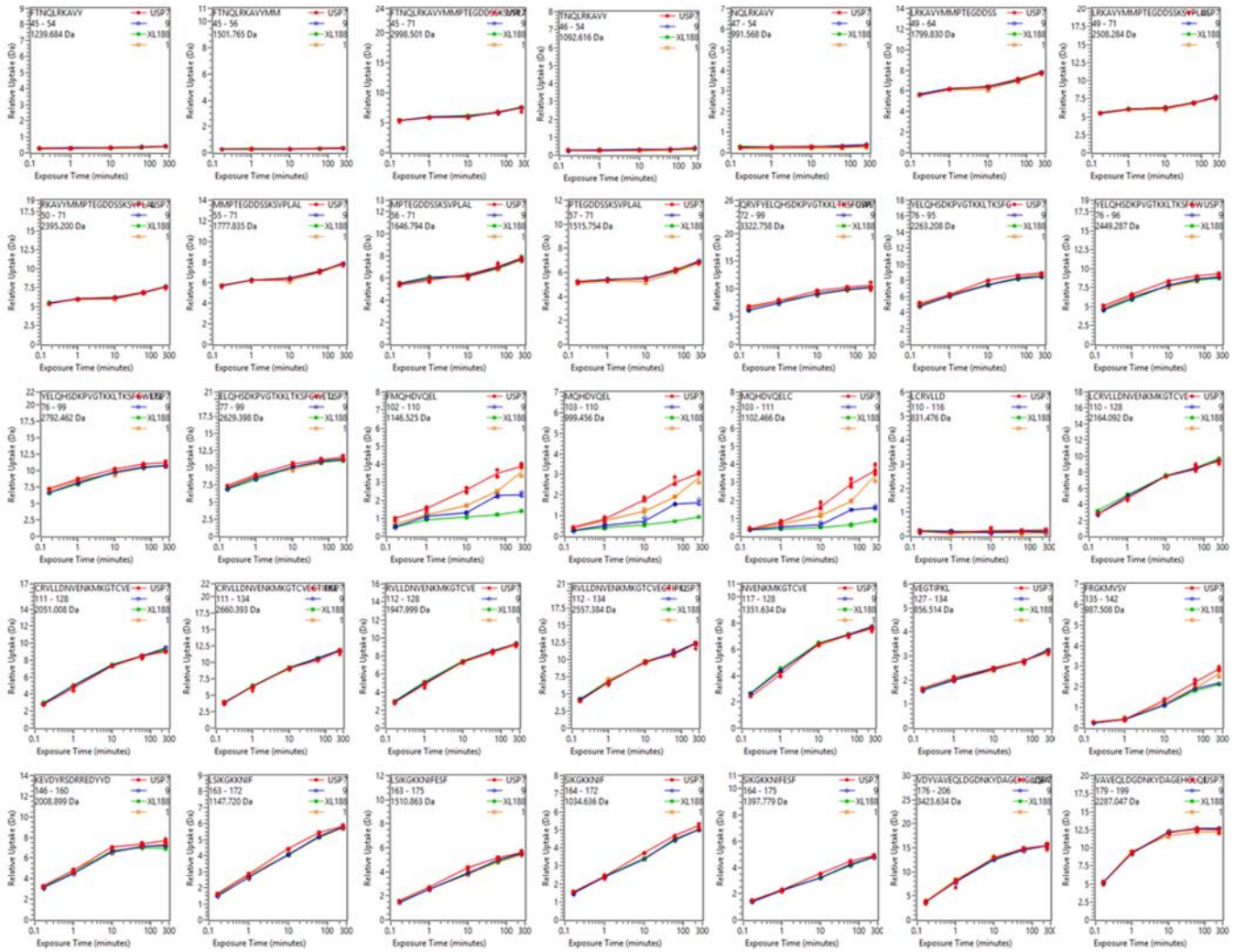
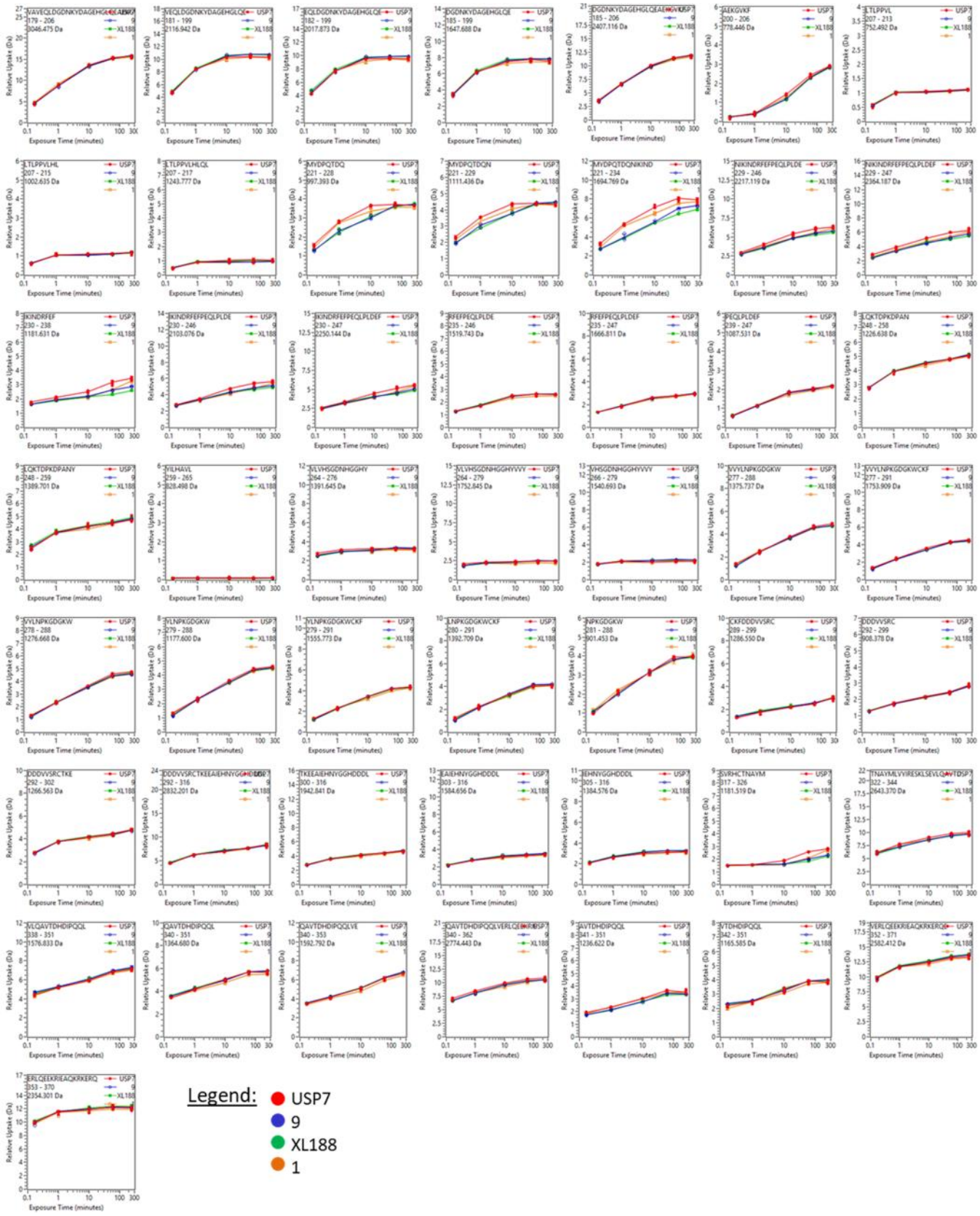
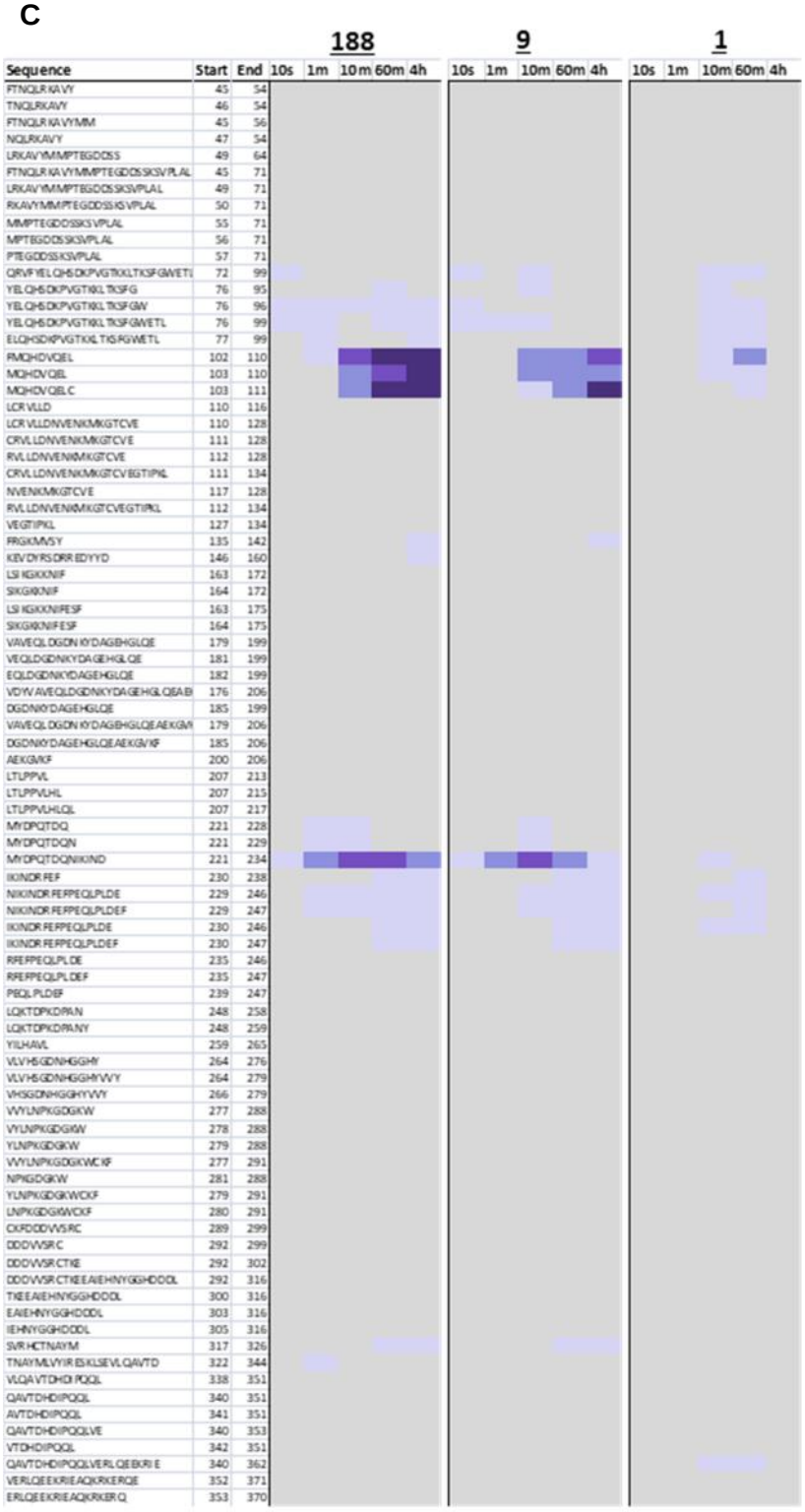
EKGVKFLTLPPVLHLQLMRFMYDPQTDQNIKINDRFEFPEQLPLDEFLQKTDPKDPANYILHAVLVHSGDNHGGHYVVYLNPKGDGKWCKFDDDVVSRCT
KEEAIEHNYGGHDDDLSVRHCTNAYMLVYIRESKLSEVLQAVTDHDIPQQLVERLQEEKRIEAQKRKERQE
Total: 85 Peptides, 85.4% Coverage, 3.95 Redundancy

B


Relative Uptake (Da)
Exposure Time (minutes)

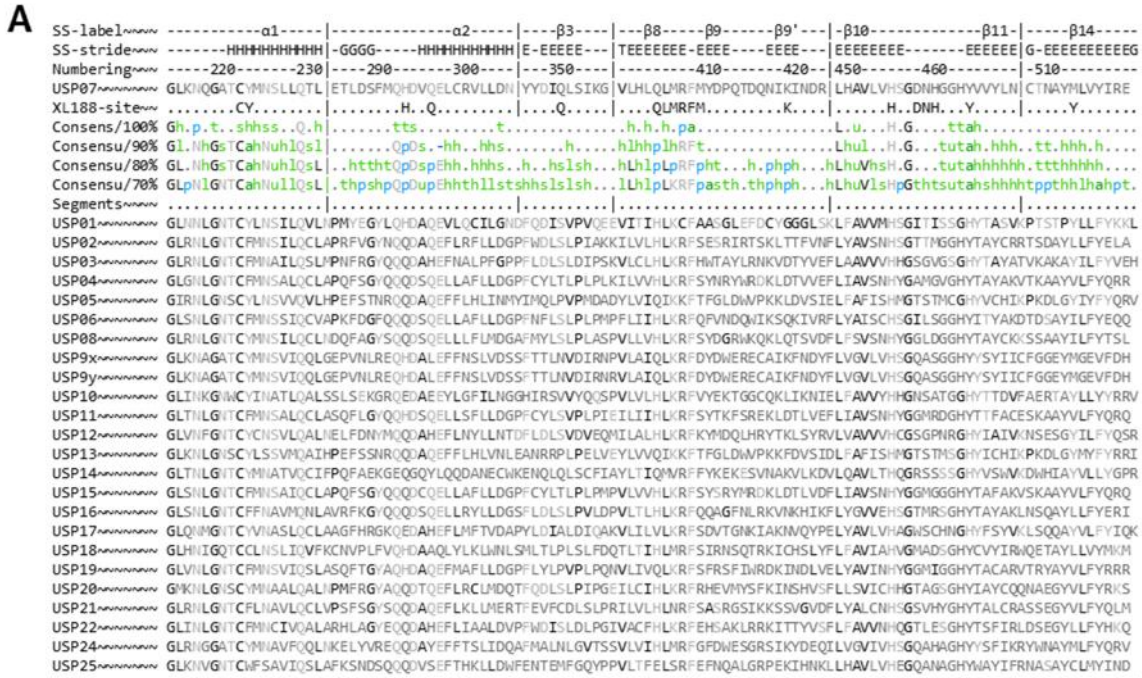

Legend:
USP7
9
XL188
1
Relative Uptake (Da)
Exposure Time (minutes)

C

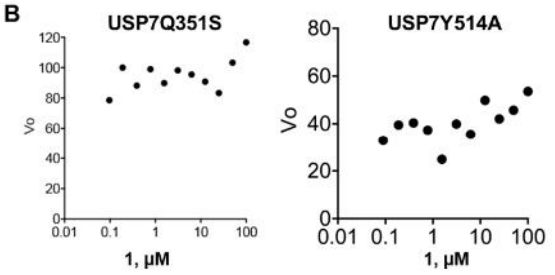


Maximal difference in deuteration (in Da): USP7-bound form vs. free USP7

Figure S3. Analysis of inhibitor binding by HDX MS. Related to Figure 2. A) The peptic map of USP7 catalytic domain showing all the peptic peptides (blue lines under the sequence) that were followed with HDX MS under all states investigated: free and individually bound to the compounds. B) Deuterium incorporation graphs for all the peptic peptides shown in A. The maximum of the *y* axis in each graph is the maximum amount of deuterium that could be incorporated in each peptide. Because these experiments were done as comparisons under identical experimental conditions, no corrections have been made for back-exchange thus the absolute value of each deuterium level is 20–30% higher than plotted based on totally deuterated standards.(Wales and Engen, 2006) C) Chiclet representation of the HDX MS data. The peptides from panel A and B are represented from N to C terminus (top to bottom) at each time point (left to right). The deuterium level for each bound state was subtracted from that of the protein alone and colored according to the legend shown.



Multiple sequence alignment of human catalytic USP domains, showing only the XL188-binding segments. Conserved residues are shaded according to MView.(Brown et al., 1998) Secondary structures are labeled and marked (H=helix, E=strand, G=H10 helix). Numbering corresponds to that of USP7. Residues that make direct contact with XL188 are shown (XL188-site). The 70-100% consensus lines according to MView are shown.



C

Compound	Protein	IC_{50} (μM)
1	WT USP7 full length (aa 1-1102)	8.8
	USP7Q351S full length (aa 1-1102)	> 100
XL188	WT USP7 full length (aa 1-1102)	0.08
	USP7Q351S full length (aa 1-1102)	9.4

Figure S4. **USP family sequence alignment and analysis of mutant USP7 enzymes. Related to Figure 3.** A) Alignment of 52 USP family DUBs with USP7 using MView. B) Dose-response inhibition of USP7Q351S and USP7Y514A catalytic domains (amino acids 208-560) by **1**. C) Summary of inhibition of full-length USP7 and USP7Q351S by **1** and XL188.

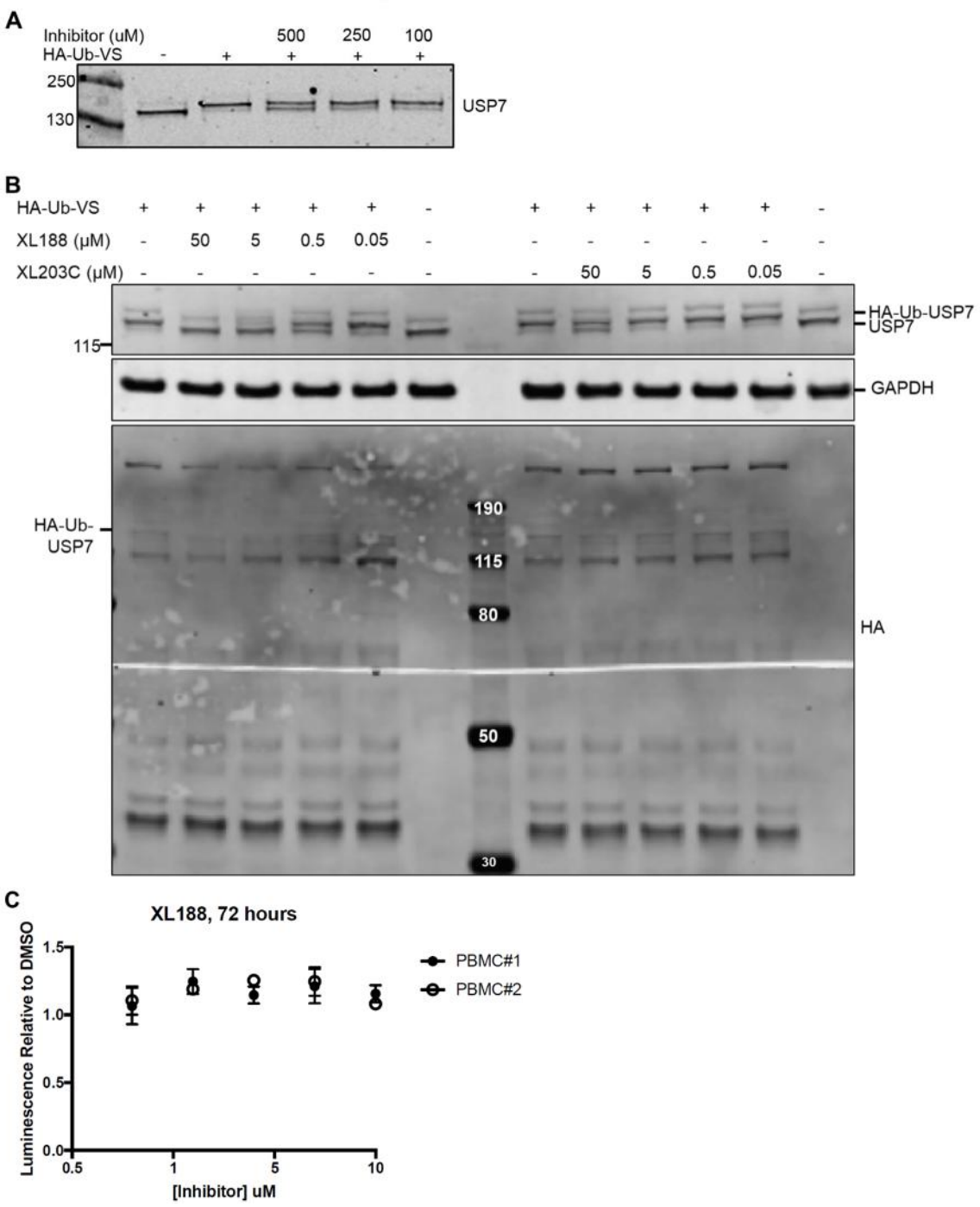


Figure S5. **Characterization of 1, XL188, and XL203C. Related to Figure 4.** A) Analysis of the ability of **1** to bind native USP7 in HEK293T lysates using competitive activity based protein profiling. B) Analysis of the ability of XL188 and XL203C to compete for labeling of DUBs by the DUB activity based probe HA-Ub-Vs. The USP7 blot is the same as appears in Figure 4C. A broad assessment of DUB inhibitory activity in lysates was conducted by blotting for HA. C) Analysis of the growth suppressive effects of XL188 on peripheral bone mononuclear cells (PBMCs).

Supplemental Tables

Table S1. **Analysis of the inhibitory activity of compounds versus a panel of purified DUBs. Related to Figure 1D, S1C and S1G.** *% Control = ((sample - mean no enzyme)/(mean plus enzyme - mean no enzyme))*100

	Activity (% control)*		
DUB	**100 μM 1**	**10 μM XL188**	**10 μM XL203C**
USP1/UAF1	83	94	94
USP2	89	93	96
USP4	80	89	93
USP5	66	126	134
USP6	92	111	107
USP7	10	1	46
USP8	89	99	99
USP9x	86	123	110
USP11	92	119	116
USP14 (Proteasome-VS@Kd)	93	98	92
USP15	90	95	95
USP16	92	88	78
USP19	92	107	101
USP20	83	124	102
USP21	93	115	104
USP25	91	132	111
USP27x	not tested	102	99
USP28	78	111	94
USP30	84	110	110
USP35	86	107	108
USP36	86	107	102
USP45	89	113	90
CYLD	89	96	87
UCHL1	88	106	93
UCHL3	86	101	89
UCHL5	87	97	104
BAP1	81	89	86
OTU1	85	107	116
OTUB2	72	87	97
OTUD1	not tested	96	97
OTUD3	95	97	108
OTUD5 (p177S)	100	101	97
OTUD6A	90	112	101
OTUD6B	88	81	92
Cezanne	63	100	103
VCPIP	not tested	100	96
AMSH-LP	91	97	99
Ataxin3	99	111	116
Ataxin3L	100	109	116
JOSD1	83	108	121
JOSD2	92	126	121

Table S2. ITC data for selected compounds. Related to Figure 1C, S1E.

Ligand	Kd (μM)			DH (kcal/mol)			n (stoichiometry)			DS (cal/mol·K)
XL188	0.104	±	0.015	-15.1	±	0.1	1.06	±	0.01	119.6
1	7.614	±	3.216	-5.4	±	1.3	0.98	±	0.15	5.3
7	No binding detected									
8	1.838	±	0.895	-3.9	±	0.2	2.46	±	0.09	13.1
9	0.797	±	-0.097	-13.5	±	0.3	0.96	±	0.01	-17.3

Table S3. Crystallization conditions and data collection and refinement statistics for crystal structures. Related to Figure 2A-B, S2.

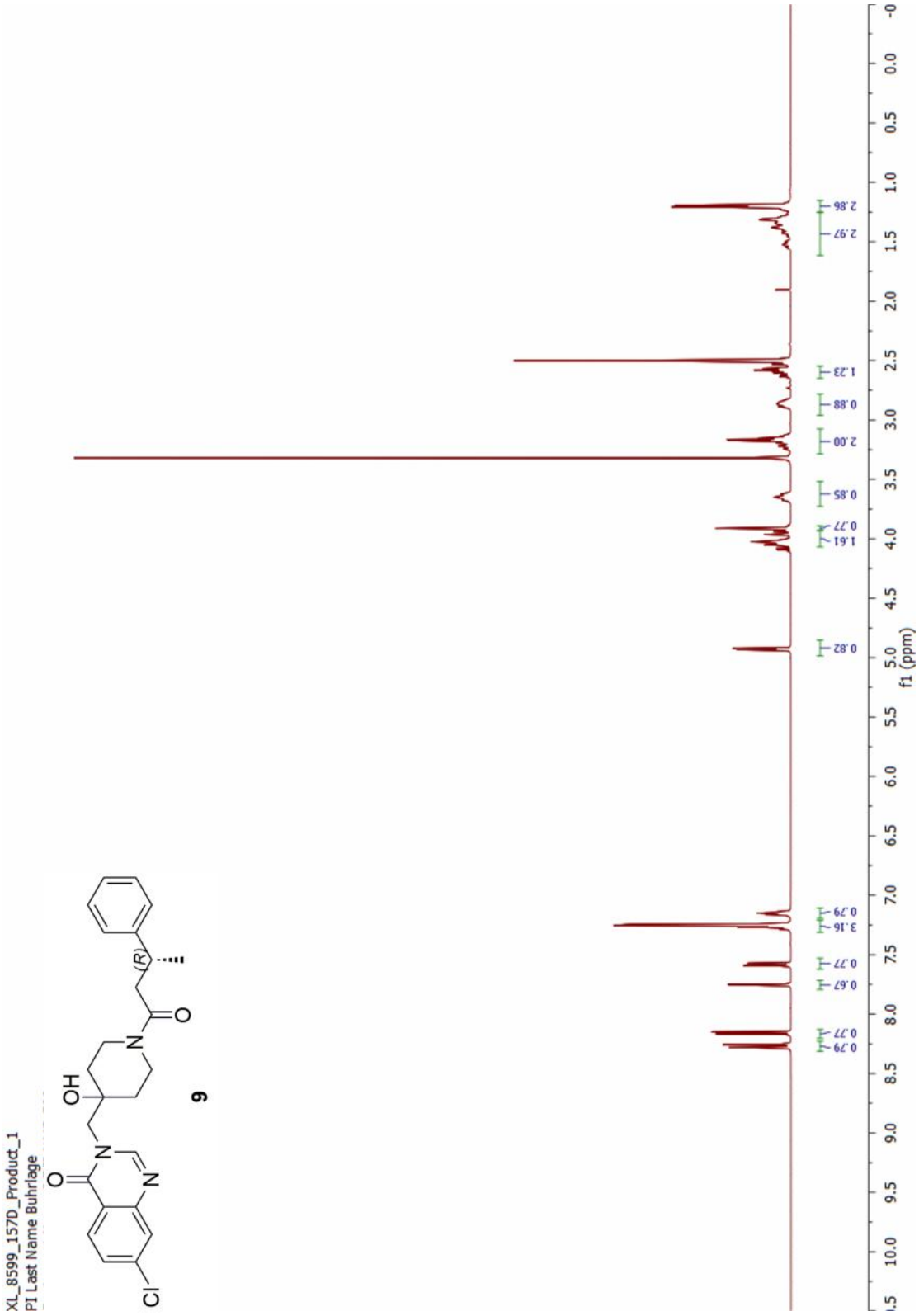
Structure Name	XL188	8	1
Ligand	XL188	8	1
RCSB accession code	5VS6	5VSK	5VSB
Data collection [a]			
Space group	$P2_1$	$P2_1$	$P2_1$
Cell dimensions			
a, *b*, *c* (Å)	75.8 68.52 80.31	72.39 69.63 77.97	61.97 73.74 84.77
a, b, g (°)	90.00 96.17 90.00	90.00 99.16 90.00	90.00 91.43 90.00
Resolution (Å)	52.00-2.27 (2.35-2.27) [b]	44.15-3.33 (3.45-3.33)	73.74-1.85 (1.90-1.85)
R_{pim}	0.078 (0.584)	0.038 (0.449)	0.058 (0.824)
I / s*I*	11.00 (1.59)	13.57 (1.94)	8.90 (1.10)
Completeness (%)	99.4 (99.3)	98.4 (98.2)	99.7 (99.3)
Redundancy	3.7 (3.7)	3.4 (3.4)	3.6 (3.2)
Structure solution			
PDB entries used for molecular replacement	1NB8	1NB8	1NB8
Refinement			
Resolution (Å)	52.00-2.27	44.15-3.33	73.74-1.85
No. reflections	37813	11217	64979
R_{work} / R_{free}	0.1817/0.2321	0.2183/0.2734	0.2282/0.2484
No. atoms	5740	4630	5726
Protein	5356	4563	5294
Ligand/ion	98	66	60
Water	286	1	372
B-factors			
Protein	46.1	121.07	38.3
Ligand/ion	50.2	142.76	26.2
Water	45.7	118.39	42.9
R.m.s. deviations			
Bond lengths (Å)	0.007	0.002	0.133
Bond angles (°)	0.86	0.49	2.71
Ramachandran Plot[e]			
Preferred	96.1%	94.0%	93.8%
Allowed	3.6%	5.5%	5.5%
Not Allowed	0.3%	0.5%	0.8%
Crystallization condition	0.1M Tris pH 8.5, 0.2M sodium acetate, 10mM DTT, 30% w/v PEG 4000	0.1M Hepes pH 7.8, 10mM DTT, 22.8% w/v PEG 8000	0.1M sodium citrate pH 5.5, 0.2M sodium formate, 10mM DTT, 36% w/v PEG 3350

Table S4. Primers used in generation of USP7 proteins. Related to Figure 1, 3 and S4.

USP7 protein	primer	
USP7 208-560	GTTCCGCGTGGTAGTAAGAAGCACACAGGCTACGTC	forward
	CAAGCTTCGTCATCATTCCTGCCGCTCCTTCCGC	reverse
USP7 1-1102	AGGAGATATACCATGAACCACCAGCAGCAGCAG	forward
	GGTGGTGGTGCTCGAGGTTATGGATTTTAATGGCCTT	reverse

USP7 mutant	primer	
Q351S	GAAGATTATTATGATATCTCGCTAAGTATCAAAGG	forward
	CCTTTGATACTTAGCGAGATATCATAATAATCTTC	reverse
M407K	CCAGTGTTACATCTACAACTGAAGAGATTTATGTATGACCC	forward
	GGGTCATACATAAATCTCTTCAGTTGTAGATGTAACACTGG	reverse
M410S	CTACAACTGATGAGATTTAGTTATGACCCTCAGACGGACC	forward
	GGTCCGTCTGAGGGTCATAACTAAATCTCATCAGTTGTAG	reverse
M407K/M410S	CCAGTGTTACATCTACAACTGAAGAGATTTAGTTATGACCCTCAGACGGACC	forward
	GGTCCGTCTGAGGGTCATAACTAAATCTCTTCAGTTGTAGATGTAACACTGG	reverse
K420A	CCCTCAGACGGACCAAAATATCGCGATCAATGATAGGTTTGAATTCC	forward
	GGAATTCAAACCTATCATTGATCGCGATATTTTGGTCCGTCTGAGGG	reverse
H456A	CTTCATGCAGTCCTGGTTGCTAGTGGAGATAATCATGGTGG	forward
	CCACCATGATTATCTCCACTAGCAACCAGGACTGCATGAAG	reverse
H461A	CTGGTTCATAGTGGAGATAATGCTGGTGGACATTATGTGG	forward
	CCACATAATGTCCACCAGCATTATCTCCACTATGAACCAG	reverse
Y514A	CGACACTGCACTAATGCTGCCATGTTAGTCTACATCAGGG	forward
	CCCTGATGTAGACTAACATGGCAGCATTAGTGCAGTGTCG	reverse

NMR spectra



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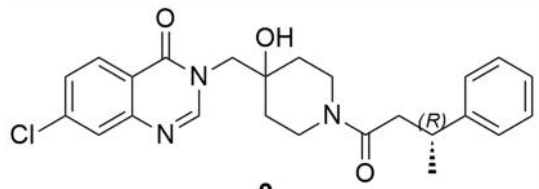

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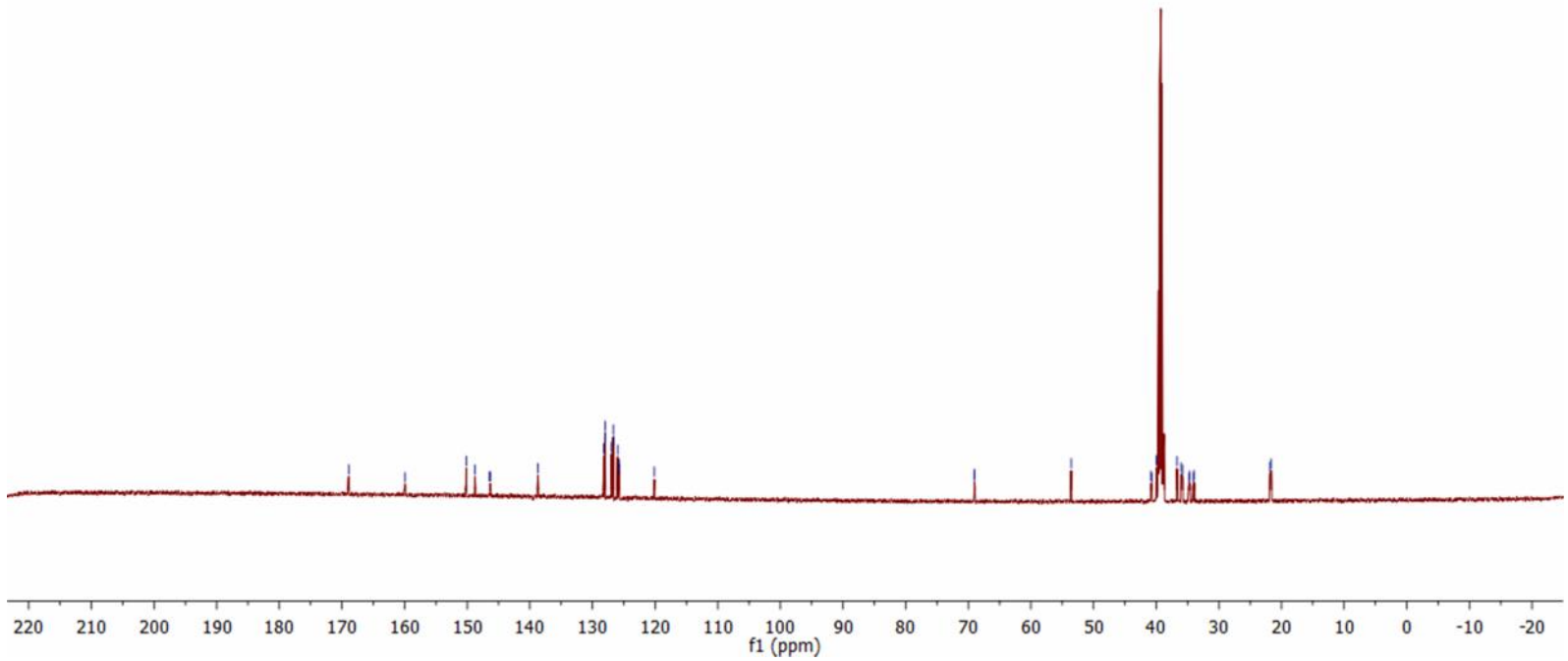

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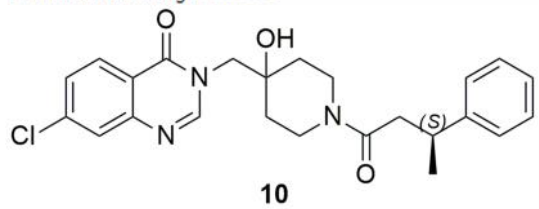

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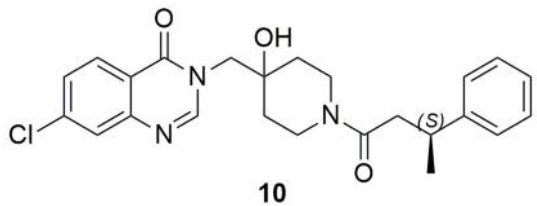

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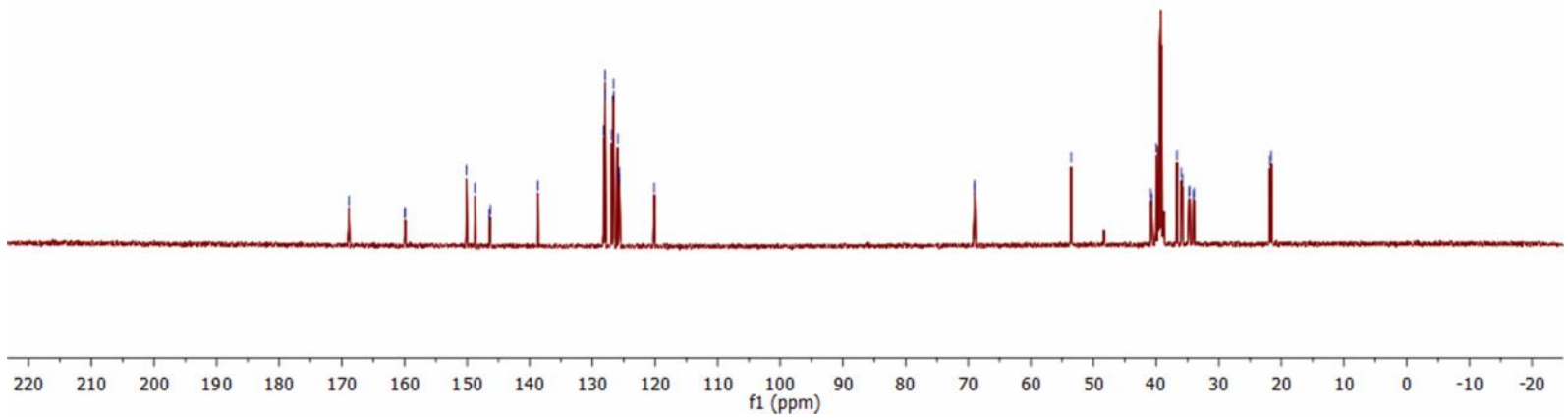

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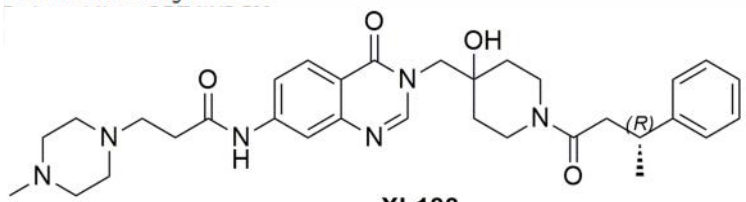

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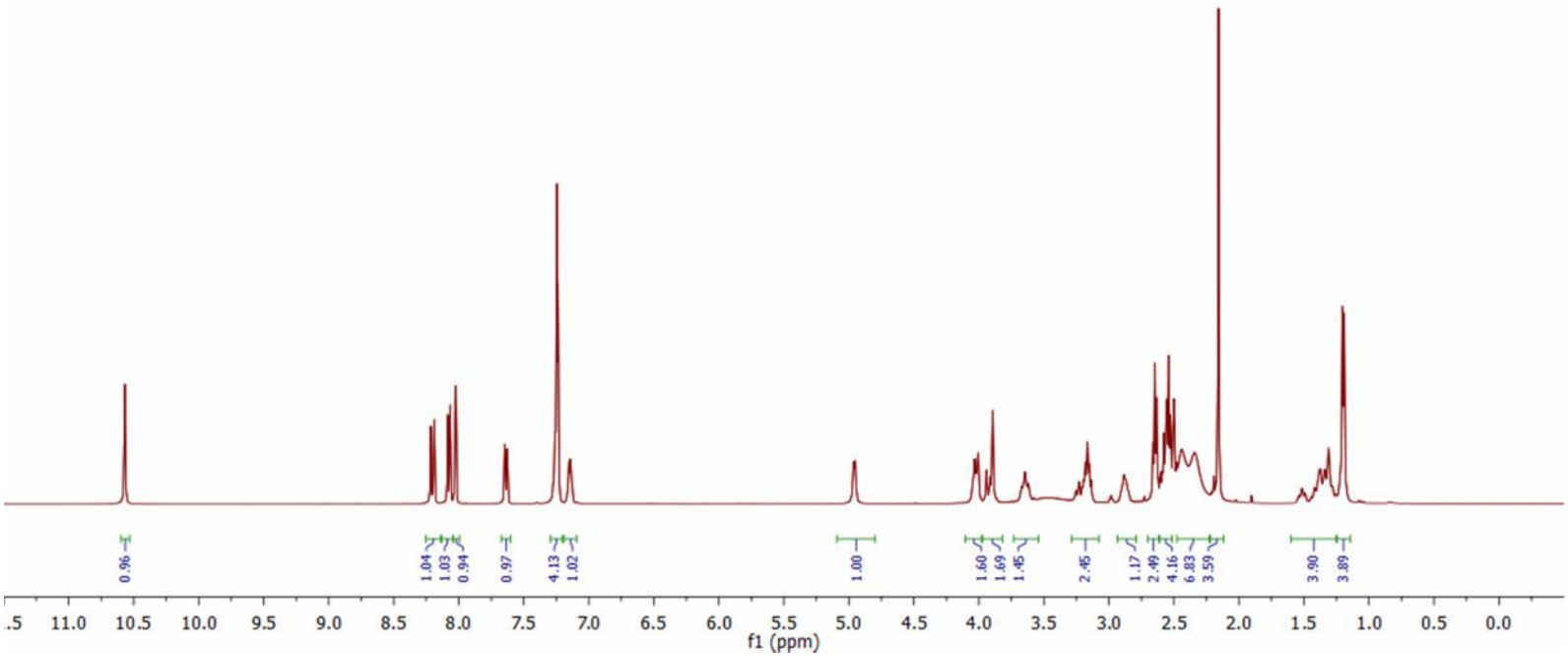

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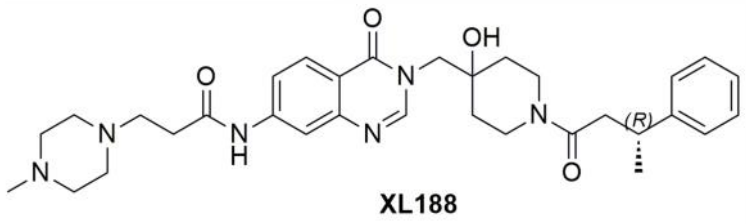

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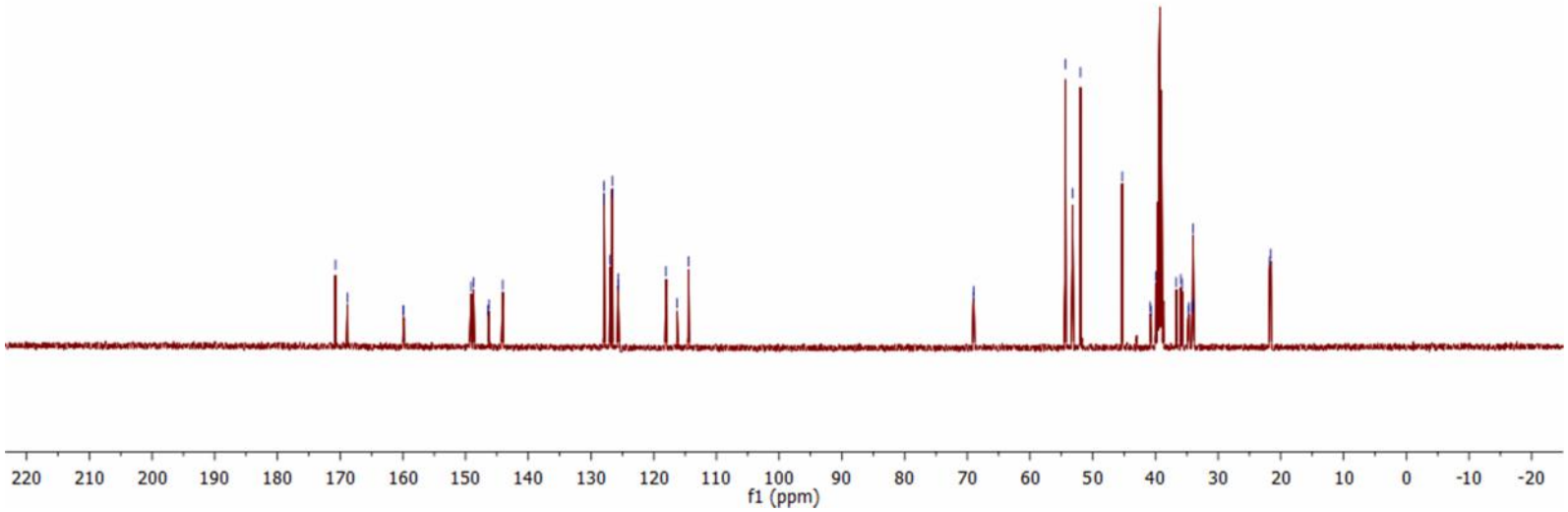

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XL203C