

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 μ M, 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.





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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.

Δ		
~	SS-label~~~~	α1
	SS-stride~~~	HHHHHHHHHH -GGGGHHHHHHHHHH E-EEEEE TEEEEEE-EEEE-
	Numbering~~~	220230290
	USP07~~~~~	GLKNQGATCYMNSLLQTL ETLDSFMQHDVQELCRVLLDN YYDIQLSIKG VLHLQLMRFMYDPQTDQNIKINDR LHAVLVHSGDNHGGHYVVYLN CTNAYMLVYIRE
	XL188-site~~	CY
	Consens/100%	Gh.p.tshhss.Q.h]ttsth.h.h.paL.uH.Gttah
	Consensu/90%	Gl.NhGsTCahNuhlQs1QpOshhhhshl.nhplhRFtLhul.H.Gtutah.hhh.tt.hhh.h
	Consensu/80%	GL.NhGsTCahNuhlQsL htthtQpDspEhh.hhhshhslshhLhlpLpRFphth.phphhLhuVhsH.Gtutahhhhhh.ttthhhhh
	Consensu/70%	GLpNlGWTCahNullQsL .thpshpQpOupEhhthllstshhslslshlLhlpLKRFpasth.thphphhLhuVlsHpGthtsutahshhhhtppthhlhahpt.
	Segments~~~~	
	USP01~~~~~	GUNNLGNTCYLNSILQVLNPMYEGYLQHDAQEVLQCILGNDFQDISVPVQEEVITIHLKCFAASGLEFDCYGGGLSKLFAVVMHSGITISSGN/TASVKPTSTPYLLFYKKL
	USP02~~~~~	GLRNLGNTCFMNSILQCLAPRFVGYNQQDAQEFLRFLLDGPFWDLSLPIAKKILVLHLKRFSESRIRTSKLTTFVNFLYAVSNHSGTTMGGHYTAYCRRTSDAYLLFYELA
	USP03~~~~~	GLRNLGNTCFMNAILQSLMPNFRGYQQQDAHEFNALPFGPPFLDLSLDIPSKVLCLHLKRFHWTAYLRNKVDTYVEFLAAVVVHHGSGVGSGHYTAYATVKAKAYILFYVEH
	USP04~~~~~	GLGNLGNTCFMNSALQCLAPQFSGYQQQOSQELLAFLLDGPFCYLTLPLPLKILVVHLKRFSYNRYNROKLDTVVEFLIAVSNHYGAMGVGHYTAYAKVTKAAYVLFYQRR
	USP05~~~~~	GIRNLGNSCYLNSVVQVLHPEFSTNRQQDAQEFFLHLINMYIMQLPVPMDADYLVIQIXKFTFGLDNVPKKLDVSIELFAFISHMGTSTMCGHYVCHIXPKDLGYIYFYQRV
	USP06~~~~~	GLSNLGNTCHNNSSIQCVAPKFDGFQQQDSQELLAFLLDGPFNFLSLPLMPFLIIHLKRFQFVNDQNIKSQKIVRFLYAISCHSGILSGGHYITYAKDTDSAYILFYEQQ
	USP08~~~~~	GLRNLGNTCYMNSILQELNDQFAGYSQQSSQELLLFLMDGAFMYLSLPLASPVLLVHLXRFSYDGRNKQKLQTSVDFLFSVSNHYGGLDGGHYTAYCKXSSAAYILFYTSL
	USP9x~~~~~	GLKNAGATC/MNISVIQQLGEPVNLREQHDALEFFNSLVDSSFTTLNVDIRNPVLAIQL/RFDYDWERECAIKFNDYFLVGVLVH/GQASGGHY/SYIICFGGEYMGEVFDH
	USP9y~~~~~	GLKINAGA IC/IMINSVIQQLGEPVNLREQHDALEFFNSLVDSSFTTDIVDIRNRVLAIQLKRFDYDWRECAIKFNDYFLVGVLVHSGQASGGHYYSYIICFGGEYMGEVFDH
	USP10	GLINKGNACYINAI LQALSSLSEKGRQEDAEEYLGFILNGGIRSVVYQQSPVLVEHLXRPVYEKTGGCQKLIKNIEL-AVVYHHGNSATGGHYTTDVFAERTAVLLYYRV
	USP11	GLTNLGNTCHMISALQCLASQFLGYQQHDSQELLSFLLDGPFCYLSVPLPIEILIIHDKRFSYTKFSREKLDILVEFLIAVSNHYGGMRDGHYTTFACESKAAYVLFYQRQ
	USP12~~~~~	GLUNFON CYCNSVUJALINELFONYMQQDAHEFENYLEN DFEDESVDVEQMILAEHEKREKMDQLHRYTRESYRVEVAVVVHGSGMRGHTALIV:NSESGYILFYQSR
	USP13~~~~~	GLKNLGNSCYLSSVMQALHPEFSSNRQQAQLEFFELLWILEAANRPELPEUPYLVVQLKKFTFGLDWPKKPDVSIDEFAFISHWGISINGGHYICHINPKDLGYMYYYRRI
	USPI4	GLI NLGWI CYMIA I WCLI PQCA BKGG QQ Y LQUDANE LWENW (UCLSC LI ALVI LI QWWEI PYKEKE SWIARW ELWY ELWY LI WGKSSSSG WYSWYRWHI A YVELY GYR
	USPISonoon	GLSNLGNTCHMISATQCLAPQFSGrQQQCQELLAPLEDGPFCYETEPLEMPUVHLIKEPSSRTMROKEDTEUDFELAVSIRTIGGMGGGMTTAFAKVSKAAVVEPYQRQ
	USP10~~~~~~	OLSINGNI CEPTIAVAWARKGYQQDSQEELKYLEDGSEDDDSEPYDPYZI ENDKRYQQGFREKKVIKALKE FYSYSGAI INKONTAVAKLISQAYLEFYEKI GONINGYTCAANACIOLAASEBUSED EEDISEPYDPYZI ENDKRYQQGFREKKVIKALKE FYSYSGAI INKONTAVAKLISQAYLEFYEKI
	USP1/202000	GLORINGN I CHVIRSEQUEAAGHRIGKQEDAHEF DIPI IVDAPYLUTADDIQAKVLLEVUKRESOVIGIRKTAKIWQYPETAVUKIABISCHIGHYTSYVKESQQAVUETTQK
	USP10000000	OURING CONSERVICE AND THE AND THE REPORT OF THE AND THE ADDRESS AND THE AND ADDRESS AND THE ADDRES
	02613000000	OLVINGHI CHINA YUQLASQI I GIAQIDAQEPHAFILDOPT CI UVVLQUKYI SIKSI JAKEVINGLI QUVINTIGINIGGITI ACAKVI KTATVUTIKKK GAVALI GICOMIA ALA DALI DALEGAADOT DELI DCI MOTTODI CI DI DOSTI CI U JEDIDO JAKEVINGLI OLVINGT GOVI ALAGVI KITATVUTIKKK
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	USP21aconder	SERVICE TERMINAL AND ANY
	USP24 approximation	GEDIGGATEWINAVEROLINE VIDEORIAVEETSI TIYAAMAI IN AVISSI VITII NEGENIKASTI VIDEORI VITII NEGENIKASTI VIDEORI VIDEORIAVEETVOVIMAVII EVIDIV
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	USP27~~~~~	GUTNI GUTCENNCTUDALABHI AGYRDODAHEFI TAALDVPCHDSLD DRVVACHEK/REFHS/AKORRKTTYTSELEAW/NHOGTI ESGHYTSETRI DSEGYL EYHKO
	USP28~~~~~	GLKINGKTCHESAVTOSLAFRSSEF0000VSETHKILDWECNNETEG0YPPVLTEELSBEERNOSLGOPEKTHINKILHAVLVHEGOANAGHWAYTYRNVSAYCLMYTND
	USP29~~~~~	GEPIN GVTC/MNAVLOSLAFTESGN/ONDAHEELGOCLDOPINY/STNLHORVLTTHUKRYSENNAM LVKINEOV/LTSW/SHTGSSPNSG//TSDWHSGYTEP/HN
	USP30~~~~~~	GLVNLGNTCENNSLLOGLRWOTSSFEEDOAHELEHVITSSDTEDSLSLSIPSSHGTPL/RHEHVOFNEFL/MDIYKYLMAWW/HGDMHSGHEVTYRRLSSSAVLLEYERV
	USP31~~~~~	GLRNHGVTCFMNATLOCLALOVRGNSOHDAGEFLLWLLDRPFLCISLPIPLDVLIIHLKRFROEGDRRMKLONMVKFLYAVCNHHGTMOGGHYTAYCKOTAYILFYORR
	USP32~~~~~~	GLSNLGNTCFMNSSIOCVAPRENGF000DS0ELLAFLLDGPENFLSLPLPMPILIIHLKRF0FVNGRWIKS0KIVKFLYAISCHSGILGGGHV/TYAKDTDSAYILFYE00
	USP33~~~~~	GLKNIGHTCYMNAALOALHPTFRGYSOCOAOEFLRCLMDLENGSRCFSEDNEILCIHLKRFRHELMFSTKISTHVSFLLSVICHHGTASSGHYIAYCRONAEAYVLFYRKS
	USP34~~~~~	GLTNLGATCYLASTIOQLKOPLNTGEOKOMTEFFTDLITKKNTVKSLFGGVRILSFNTMRYTFNMVTMMKEKVNTHFLIGVTVHTGTADGGHYYSFIRDKTHSAYMLFYKRM
	USP35~~~~~	GLINLGNTCVVNSILQALPWFSPGTQQDCSEYLKYLLDRLEAFTDLSLAFPCYLILTLLRFSFDLRTMRRRKILDDVLCSVVVHSGVSSESGHYYCYARDTAYVLFYRQR
	USP36~~~~~	GLHNLGWTCFLNATIQCLARHFRFGNQEDAHEFLRYTIDAPYLDVALEIRQNVLTLSLKRFANFSGGKITKDVGYPELYAVLVHSGYSCHAGHYYCYVKLNQQAYVLFYLRI
	USP37~~~~~	GFSKLGNTCYMNAILQSLAERFSGYMQNDAHEFLSQCLDQQENDLSIDLPRRVLILHLKRYSFNVALSLNNKIGQQVLISVVSHIGSTSSSDHYISDVYRSGYIFPYMHK
	USP38~~~~~~	GLINLGNTCYMNSVIQALPPWFTPRSQQDCSEYLRFLLDRAFTDLSLAFCPEYLILTLLRFSYDQKYHVRRKILDNVLSSWVHSGISSESGHYYSYARPKDTAYVLLYKKQ
	USP39~~~~~	GLWNIKANDYANAVLQALKKTFQITKQGDGVDFLSWFLNAEQLLHNDEYQEPYLIFCIKRFTKNNFPVEKNPTIVNFLIANIVHDGKPSEGSYRIHVLTLSEAYIQIWKRR
	USP40~~~~~	GIRNQGGTCYLNSLLQTLWTSNEBMRQHDVQELNRILFSADFLDLTVAVKNPFLTVSLLRFNFDFVKCERYKETSCYLFSVIIHKGGCYGGHYHVYIKGKESAYMLFYRKS
	USP41~~~~~	GLHNIGQTCCLNSLIQVFKYNVPLFVQHDAAQLYLKLWNLSMLTLRLSFFDQTLTIHLMRFSIRNSQTRKICHSLYFLFAVIAHVGMADSGHYCVYIRWLPSRAH
	USP42~~~~~~	GLQNLGNTCFANAALQCLARHFRFGNQEDAHEFLQYTVDAPYLDITLEIKANVLTLSLKRFANFTGGKIAKDVKYPELYAVLVHTGFNCHAGHYFCYIKLSQQAYVLFYIRS
	USP43~~~~~	GLKNHGNTCFMNAVVQCLGSQFQGNSQHDALEFLLWLLDRPFLCVSLPIPLDILIIHLKRFCQVGERRNKLSTLVKFLYAVCNHHGNLQGGHYTAYCRRGAYILFYQKR
	USP44~~~~~	GLRNLG/TC/MNSVLQVLIPAFRG/AQQDAQEFLCELLDKPFWDLSLEFPEQVLRLHLKRFRWSGRNNREKIGVHVGLSAVVMHHGKGFGSGH/TAYCYCKAQAYILFYTQR
	USP45~~~~~	GITNLGNTCFFNAVMQNLAPRFKDFQQQDSQELLHYLLDAPFIDISLPIIEAVLILHLKRFHKAGLSLRKVNRHVDFLYGIVEHSGSMREGHYTAYVKLSAQAYLLFYERV
	USP46~~~~~	GLVNFGNTCYCNSVLQALIDLFDNYMQQDAHEFLNYLLNTDFLDLSVDVEQMILALHLKRFKYMEQLHRYTKLSYRVLVAVVVHCGSGPNRGHYITIVKNSESGYILFYQSR
	USP47~~~~~	GLVNQAMTCYLNSLLQTLNDSSEAWQQHDVQELCRVMFDATYLDIPLVIRPYLLTLQLKRFDFDYTTMHRIKLNDRMLFSVMVHSGSAAGGHYYACIKSSTNAYMLIYRLK
	USP48~~~~~	GLTNLGATCYVNTFLQVWALGLDTGQQQDAQEFSKLFMSLKFYELELNIQGCTLNLQLMRFVFDRQTGHKKKLNTYILSAVLIHRGVSAYSGHYIAHVKSRNAYMLVYRLQ
	USP49~~~~~	GLRNLGNTCYMNSILQVLIPAFRGYDQQDAQEFLCELLHKPFWDLSLEFPEQVLRLHLKRFRWSGRNHREKIGVHVVLSAVVMHHGKGFGSGHYTAYCYCKTQAYILFYTQR
	USP50~~~~~	GLWNLGNTCCVNAISQCLYPAFTKKMQQDAQEFLICVLNEVFTVFSLPIPSKIIIFHLKRFDIQGTTKRKLRTDIHYLCAVVNHFGDLDGGHYTAFCK
	USP51~~~~~	GLINLGKTCFMNCIVQALAEHLAGYRQQDAHEFLIAILDVGLQSDVTCQACIVACFHLKRFEHVGKQRRKINTFISFLFAVINHHGTLESGHYTSFIRLYSEGYLLFYHKQ

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.



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	93	98	92		
(Proteasome-					
VS@Kd)	00	05	05		
	90	90	90 70		
	92	00 107	101		
USPIS	92	107	101		
USP20	63	124	102		
03721	93	115	104		
05P25	91	132	111		
05P2/X	not tested	102	99		
USP28	78	111	94		
05P30	84	110	110		
05P35	86	107	108		
USP36	86	107	102		
05P45	89	113	90		
	89	96	87		
UCHL1	88	106	93		
UCHL3	86	101	89		
UCHL5	87	97	104		
BAP1	81	89	86		
0101	85	107	116		
OTUB2	12	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		

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

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

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	P21	P21	P21
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)
1/s/	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	1NB8	1NB8	1NB8
molecular replacement			
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	0.1M Tris pH 8.5, 0.2M	0.1M Hepes pH 7.8, 10mM DTT,	0.1M sodium citrate pH 5.5, 0.2M
condition	sodium acetate, 10mM DTT, 30% w/v PEG 4000	22.8% w/v PEG 8000	sodium formate, 10mM DTT, 36% w/v PEG 3350

Table S4. Primers used in generation of USP	7 proteins. Related to Figure 1, 3 and S4.
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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















