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USP7: combining tools towards selectivity

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Experimental section

General:

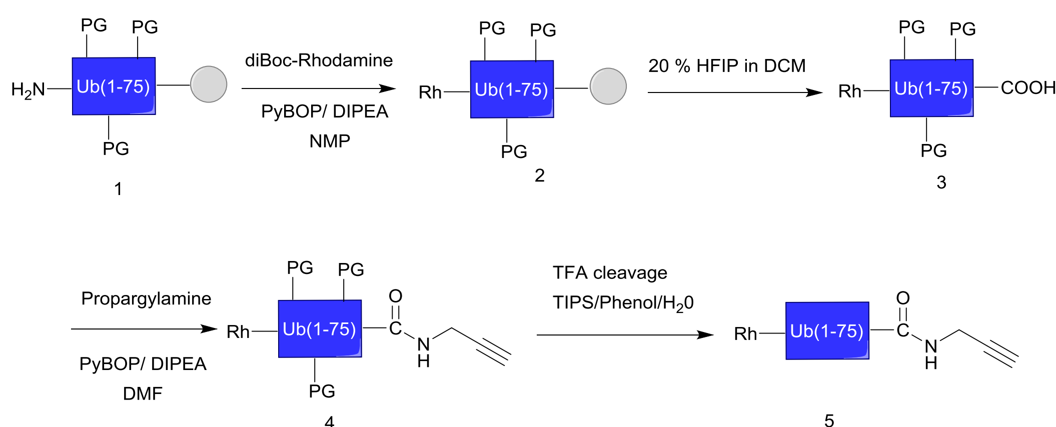
All chemicals (Aldrich, Fluka, Novabiochem) were used without further purification. All solvents were reagent grade or HPLC grade. LC-MS analysis was performed with a system containing a Waters 2795 separation module (Alliance HT), Waters 2996 Photodiode Array Detector (190-750 nm), Phenomenex Kinetex XBridge-C18 (2.1 x 50 mm) reversed phase column and a Micromass LCT-TOF mass spectrometer. Samples were run at 0.80 mL/min (Kinetex C18) with the use of a gradient of two mobile phases: A) aqueous formic acid (FA) (0.1 %), and B) formic acid in acetonitrile (CH_3CN) (0.1 %). Data processing was performed with Waters MassLynx 4.1 software (deconvolution with MaxEnt1 function). Preparative HPLC was performed with a Waters auto-purifier System connected to a 3100-mass detector. We used a purification method with Waters XBridge Prep C18 Column (19 x 150 mm, 5 µm OBD) column run with a flowrate of 30 mL/min for 23 min with the use of gradient elution [mobile phases: A) aqueous TFA (0.05 %) and B) TFA in CH_3CN (0.05 %)]. Gradient: 18 % B for 2.5 min; 18 % to 48 % B in 15 mins; washed at 95 % B for 2 mins; re-equilibrated back to 18 % B.

Softwares used in this study: FoldX: http://foldxsuite.crg.eu/ and UCSF Chimera https://www.cgl.ucsf.edu/chimera/

Computational predictions with FoldX

According to the protocol for FoldX, the structures were first adjusted using the repairpdb function to yield the most optimal structure with lowest energy. After, FoldX was run to allow for mutational analysis mutating single amino acids for all 20 proteogenic amino acids, as well as 4 post-translationally modified amino acids (PTR=phosphorylated threonine; TPO=phosphorylated tyrosine; SEP=phosphorylated serine; HYP=hydroxyproline). The newly calculated binding energies were subtracted from the WT reference yielding either a negative value (increased calculated binding) or a positive value (decreased calculated binding). Subsequently, we determined which residues caused an increased binding for Ub to USP7 over Ub for the other evaluated DUBs.

Synthesis of Rh-ubiquitin mutants-PA



Scheme S1. Synthesis of activity-based probe

Fmoc SPPS strategy:

SPPS was carried out on a Syro II MultiSyntech Automated Peptide synthesizer (Tip synthesis module, 96 positions reaction block), using standard 9-fluorenylmethoxycarbonyl (Fmoc) based solid phase peptide synthesis protocols in 2 µmol scales. All amino acids were used in excess and depending on the sequence, special dipeptides were used in specific positions, as previously reported[1]. All the procedures for the library synthesis were carried out in parallel following reported procedure[2].

To the protected ubiquitin (1) (positioned in the 96-reaction block) on trityl resin, 200 µL of a mixture in NMP of PyBOP (5.0 eq), diBoc-rhodamine (5.0 eq) or 5-carboxy-tetramethylrhodamine (TMR, in library I), DIPEA (10.0 eq) was added in parallel. After overnight coupling, the resins were washed with 2 x NMP and then 4 x DCM. The synthesis of diBoc-rhodamine was previously reported by our group[3].

Fully protected Ub (2) was cleaved from the resin by adding a solution of 200 µL of 20 % HFIP in DCM. After 30 min, the flow-through containing protected Ub with a free C-terminus was collected in a 96 deep well plate. The procedure was repeated twice. The flow-through was dried under N_2 flow and then freeze-dried using a mixture of CH_3CN/H_2O (1:1) overnight.

The crude product (3) obtained after freeze-drying was dissolved in 100 µL DMF. To the mixture we added 100 µL of a freshly made solution of PyBOP (5.0 eq), propargylamine (5.0 eq) and DIPEA (10.0 eq) and allowed to react overnight. Afterwards we added a mixture of CH_3CN/H_2O (1:1) to the reaction mix, prior to freeze-drying.

400 µL of freshly prepared cleaving mixture (TFA/TiPS/Phenol/H_2O; 92.5/2.5/2.5/2.5; v/v/v/v) was added to the crude material (4) present in the 96 deep well plate. After 2.5 h we added a cold mixture of Et_2O/Pentane (3/1), leading to the precipitation of the synthetic ubiquitin. After 2 rounds of washes with cold Et_2O, the precipitate was freeze-dried with CH_3CN/H_2O (1:1) overnight. The crude material (5) was dissolved in 500 µL DMSO and purified with the preparative HPLC. The pure fractions were analysed by LC-MS and combined and freeze-dried. All purified synthetic mutants were dissolved in DMSO to a final concentration of 2.0 mM. They were further diluted in MQ water until a final concentration of 20 µM.

Cell culture

HAP1 cells and USP7KO HAP1 (obtained from Horizon Genomics HAP1_USP7_00485-02) were cultured in IMDM (Gibco) supplemented with with 10% FCS and 1% Pen/Strep (FCS, Greiner). Cells were cultured at 37°C and 5 % CO_2.

Labelling experiments

Cell were lysed in 50 mM Tris, 150 mM NaCl, 0.5 % Triton X-100, pH=7.5. Final concentration of 2.0 mM DTT was used in the labeling experiments. HAP1 lysate (10 µL) was incubated for 30 min with the different synthetic mutants at a final concentration of 1.0 µM. Samples were separated by gel electrophoresis (4-12 % SDS-PAGE, precast gel from Invitrogen, in MOPS buffer, if not stated otherwise). In order to analyse the levels of the labelled DUBs, we used a Typhoon FLA 9500 (GE Healthcare LifeSciences using filters with λEx =473 and λEm =532) for fluorescence scanning.

Western-blotting

Proteins were transferred to a nitrocellulose membrane from Trans-Blot Turbo Mini Nitrocellulose Transfer Packs from Bio-Rad using a 7 min program for different molecular weight proteins (Trans-Blot Turbo Transfer System from Bio-Rad) The membranes were blocked in 5 % BSA in Tris buffer pH=7.5 (50 mM Tris, 150 mM NaCl). Subsequently they were incubated with a primary antibody diluted in 1 % BSA in 0.1 % Tris buffer -Tween 20 (TBST) for 1 h, washed three times for 5 min in 0.1 % PBST, then incubated with the secondary antibody diluted in 1 % BSA in 0.1 % TBST for 30 min and washed three times 5 min in 0.1 % TBST. β-actin antibody (Sigma-Aldrich, Cat# A5441) was used as a loading control in a 1:10000 dilution and USP7 antibody (Abcam, ab4080) was used at a dilution of 1:1000. As secondary antibodies we used IRDye 680LT goat anti-mouse IgG (H+L) (926-68020, Li-COR) and IRDye 800LT goat anti-rabbit IgG (H+L) 926-32211, Li-COR). The signal was detected using direct imaging by the Odyssey Classic imager (Li-Cor).

Pull-down experiment

$1x10^6$ cells were seeded in each 10 cm dish, in total 3 dishes per conditions. After 48 h, the cells were lysed in Tris buffer pH=7.5 (50 mM Tris, 150 mM NaCl) 0.5 % Triton X-100. The lysates were incubated with Rh-M6-PA or no probe for 30 min at 30°C, slowly shaking. The neutravidin beads (Thermo Fischer SCIENTIFIC) were washed twice with lysis buffer before addition. 50 µL of the 50 % aqueous slurry were added to the lysates and they were incubated at 4°C for 2.5 h. The beads were collected after being centrifuged at 1000 rpm at 4°C. The beads were washed multiple times in lysis buffer. 10 % of the final beads were boiled at 100°C in loading buffer and then analyzed by gel electrophoresis, followed by western blotting. The experiments were performed in triplicate for each of the following conditions.

1) HAP1 WT lysate with no probe (Control)
2) HAP1 WT lysate labelled with the probe
3) USP7KO HAP1 labelled with the probe

Mass spectrometry sample preparation.

Trypsin (V5111, Promega) digestions and peptide sample preparation were carried out according to the previously described protocols[4].

Mass spectrometry

LC-MS/MS analysis was performed on an EASY-nLC 1000 system (Proxeon, Odense, Denmark) connected to a Q-Exactive Orbitrap (Thermo Fisher Scientific, Germany) through a nano-electrospray ion source. The Q-Exactive was coupled to a 15 cm analytical column with an inner-diameter of 75 µm, in-house packed with 1.9 µm C18-AQ beads (Reprospher-DE, Pur, Dr. Maish, Ammerbuch-Entringen, Germany).

The chromatography gradient length was 60 minutes from 2% to 95% acetonitrile in 0.1% formic acid at a flow rate of 200 nL/minute. The mass spectrometer was operated in data-dependent acquisition (DDA) mode with a top-10 method. Full-scan MS spectra were acquired at a target value of 3×10^6 and

a resolution of 70,000, and the Higher-Collisional Dissociation (HCD) tandem mass spectra (MS/MS) were recorded at a target value of 1 x 10^5 and with a resolution of 35000 with a normalized collision energy (NCE) of 25%. The maximum MS1 and MS2 injection times were 50 ms and 120 ms, respectively. The precursor ion masses of scanned ions were dynamically excluded (DE) from MS/MS analysis for 60 sec. Ions with charge 1, and greater than 6 were excluded from triggering MS2 analysis.

Mass spectrometry data analysis

Proteomics data were analyzed using MaxQuant software (v1.5.3.30) according to, Tyanova *et al*[5]. using default settings with the following modifications: Label Free Quantification (LFQ) was employed with the Fast-LFQ algorithm disabled. We performed the search against an in silico digested UniProt reference proteome for Homo sapiens (18th June 2018). The match-between-runs feature was enabled with a 0.7 min match time window and a 20 min alignment time window.

Output files from MaxQuant were further processed in the Perseus (v 1.5.5.3) computational platform[6]. Proteins identified as common contaminants, only identified by site and reverse peptide were filtered out, and then all the LFQ intensities were log2 transformed. Different biological repeats of the experiment were grouped and only protein groups identified in all three biological replicates in at least one group were included for further analysis. Missing values were imputed using Perseus software by normally distributed values with a 1.8 downshift (log2) and a randomized 0.3 width (log2) considering whole matrix values. T-tests comparing the different groups were performed with a permutation based FDR of 0.05. Spreadsheets from the statistical analysis output were further processed in Microsoft Excel from comprehensive visualization and data analysis. Volcano plots were generated with a permutation based FDR of 0.05 and S0=0.1.

Mass spectrometry data availability.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[7] partner repository with the dataset identifier PXD012535.

Library screening:

The list of the mutants for each library and DUB activity-based probe assays are shown. The list of the special amino acids building blocks and their abbreviations used in these screening is as follows.

Pentafluoropheylalanine=5; Lys(Me_3Cl)=9 (K-trimethyl); Gln(Dmcp)=!; Lys(ivDde)=$; Glu(biotinyl-PEG)=#; Lys(biotin)=Z (K-bio).

Fmoc-pentafluoro-L-phenylalanine

Fmoc-Lys(Me_3Cl)-OH

Fmoc-Gln(DMCP)-OH

Fmoc-Lys(ivDde)-OH

Fmoc-Glu(biotinyl-PEG)-OH

Fmoc-Lys(biotin)-OH

Chart 1 Building blocks of the special amino acids

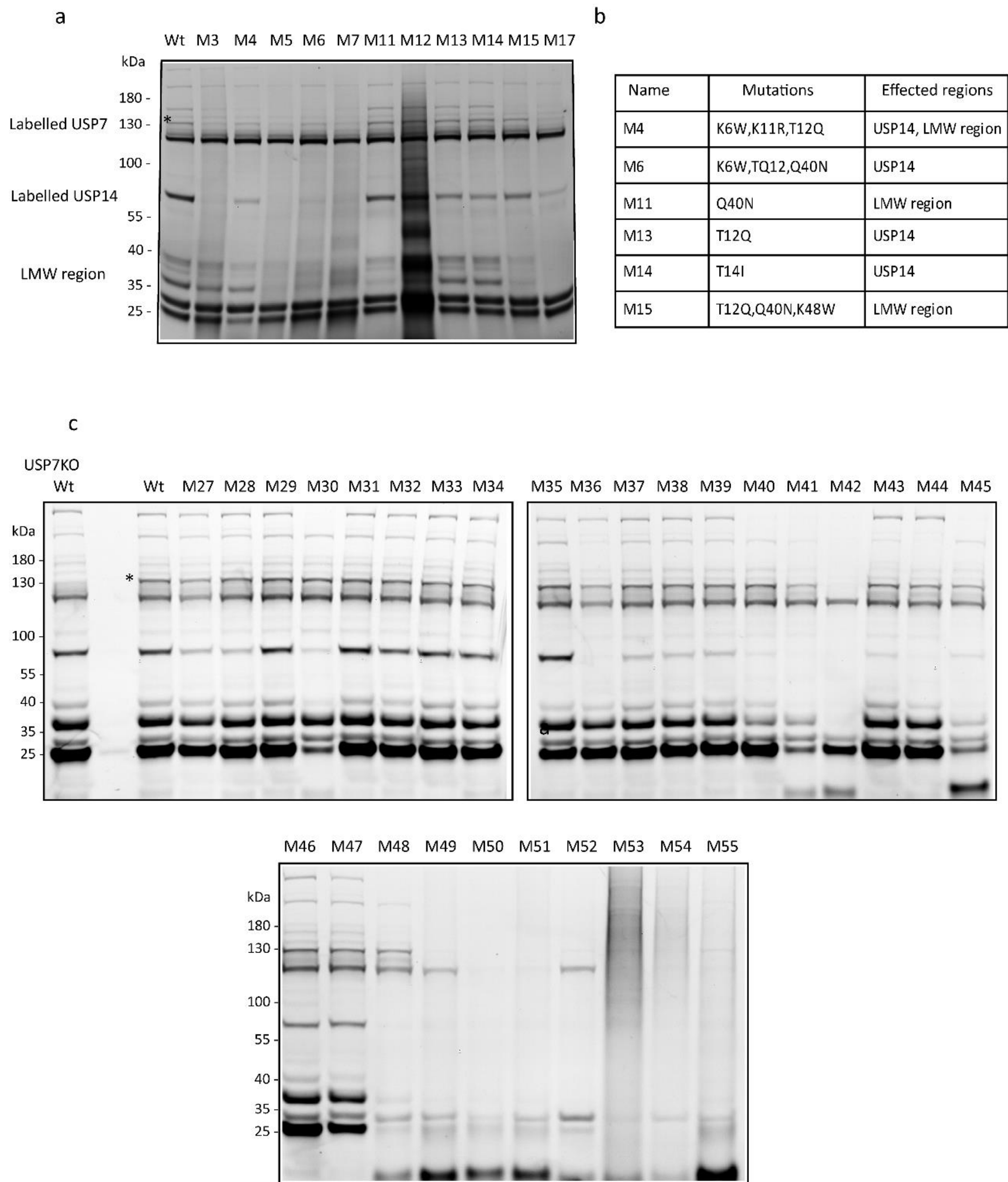
Table S1. List of the ubiquitin mutants from Library I (part I)

ID	Sequence	Mutation
Wt	TMR-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	
LIM1	TMR-MQIFVWTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W
LIM2	TMR-MQIFVWTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,T12Q
LIM3	TMR-MQIFVWTLTGKQIILEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,T12Q,T14I
LIM4	TMR-MQIFVWTLTGRQITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,K11R,T12Q
LIM5	TMR-MQIFVWTLTGKQIILEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,T12Q,T14I,Q40N
LIM6	TMR-MQIFVWTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,T12Q,Q40N
LIM7	TMR-MQIFVWTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFFGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,T12Q,Q40N,A46F
LIM8	TMR-MQIFVWTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDNQRLKFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,T12Q,Q40N,I44K
LIM9	TMR-MQIFVWTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDNQRLKFAGWQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K6W,T12Q,Q40N,I44K,K48W
LIM10	TMR-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGWQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	K48W
LIM11	TMR-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	Q40N
LIM12	TMR-MQIFVKTLTGKTDTLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	I13D
LIM13	TMR-MQIFVKTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T12Q
LIM14	TMR-MQIFVKTLTGKTIILEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T14I
LIM15	TMR-MQIFVKTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGWQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T12Q,Q40N,K48W
LIM16	TMR-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQWLIFAGKRLEDGRTLSDYNIQKESTLRLVLRLRG-PA	R42W,Q49R,H68R
LIM17	TMR-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDNQWLIFAGWRLEDGRTLSDYNIQKESTLRLVLRLRG-PA	Q40N,R42W,K48W,Q49R,H68R
LIM18	TMR-MQIFVKTLTGKQITLEVEPSDTIENVKAKIQDKEGIPPDNQWLIFAGWRLEDGRTLSDYNIQKESTLRLVLRLRG-PA	T12Q,Q40N,R42W,K48W,Q49R,H68R
LIM19	TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDNQWLEFAGWRLEDGRTLSDYNIQKLSTLRGVRRLRG-PA	U7Ub25.2540:Q40N,I44E,K48W,E64L
LIM20	TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQWLIFAGKRLEDGRTLSDYNIQKESTLRGVRRLRG-PA	U7Ub25.2540
LIM21	TMR-MQIFVKFRTGKTDTLEVEPSDTIENVKAKIQDKLGIPPDQQWLIFAGKRLEDGRTLSDYNIQKESTLRGVRRLRG-PA	U7Ub25.2540:Y13D
LIM22	TMR-MQIFVKFRTGKTDILEVEPSDTIENVKAKIQDKLGIPPDQQWLIFAGKRLEDGRTLSDYNIQKESTLRGVRRLRG-PA	U7Ub25.2540:Y13D,T14I
LIM23	TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQWLIFAGKRLEDGRTLSDYNIQKLSTLRGVRRLRG-PA	U7Ub25.2540:E64L
LIM24	TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQWLIFAGKRLEDGRTLSDYNIQKEYTLRGVRRLRG-PA	U7Ub25.2540:S65Y
LIM25	TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQWLEFAGKRLEDGRTLSDYNIQKESTLRGVRRLRG-PA	U7Ub25.2540:I44E
LIM26	TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQWLEFAGKRLEDGRTLSDYNIQKLSTLRGVRRLRG-PA	U7Ub25.2540:I44E,E64L

Table S2. List of the ubiquitin mutants from Library I (part II)

ID	Sequence	Mutation
LIM27	Rh-MQIWVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	F4W
LIM28	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T12W
LIM29	Rh-MQIFVKTLTGKTITLEVEPSDTIENAKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	V26A
LIM30	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGNPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	I36N
LIM31	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGATLSDYNIQKESTLHLVLRLRG-PA	R54A
LIM32	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTASDYNIQKESTLHLVLRLRG-PA	L56A
LIM33	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSAYNIQKESTLHLVLRLRG-PA	D58A
LIM34	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSWYNIQKESTLHLVLRLRG-PA	D58W
LIM35	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLALRLRG-PA	V70A
LIM36	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	F4W,T12W
LIM37	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSWYNIQKESTLHLVLRLRG-PA	T12W,D58W
LIM38	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSAYNIQKESTLHLVLRLRG-PA	T12W,D58A
LIM39	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGATLSDYNIQKESTLHLVLRLRG-PA	T12W,R54A
LIM40	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T12W,Q40N
LIM41	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T12W,I36N
LIM42	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLKFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T12W,Q40N,I44K
LIM43	Rh-MQI5VKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	F4(pentafluorophenylalanine)
LIM44	Rh-MQIFVKTLTGK5ITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T12(pentafluorophenylalanine)
LIM45	Rh-MQIFVKTLTGKTITLEVEPSDTIENAKAKIQDKEGIPPDQQRLIFAGKQLEDGRTASDYNIQKESTLHLVLRLRG-PA	V26A,L56A
LIM46	Rh-MQIFVKTLTGKTITLAVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTASDYNIQKESTLHLVLRLRG-PA	E16A,L56A
LIM47	Rh-MQIFVKTLTGKTITLAVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTASDYNIQKESTLHLALRLRG-PA	E16A,L56A,V70A
LIM48	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	F4W,T12W,I36N
LIM49	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDNQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	F4W,T12W,I36N,Q40N
LIM50	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDQQRLKFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	F4W,T12W,I36N,I44K
LIM51	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDNQRLKFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	F4W,T12W,I36N,Q40N,I44K
LIM52	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDNQRLIFAGKQLEDGRTLSWYNIQKESTLHLVLRLRG-PA	F4W,T12W,I36N,Q40N,D58W
LIM53	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDNQRLIFAGKQLEDGATLSWYNIQKESTLHLVLRLRG-PA	F4W,T12W,I36N,Q40N,R54A,D58W
LIM54	Rh-MQIWVKTLTGKWITLEVEPSDTIENVKAKIQDKEGNPPDNQRLKFAGKQLEDGATLSWYNIQKESTLHLVLRLRG-PA	F4W,T12W,I36N,Q40N,I44K,R54A,D58W
LIM55	Rh-MQIFVKTLTGKTITLEVEPSDTIENAKAKIQDKEGNPPDQQRLIFAGKQLEDGRTLSWYNIQKESTLHLVLRLRG-PA	V26A,I36N,D58W

Pentafluorophenylalanine=5



Name	Mutations	Effected regions
M4	K6W,K11R,T12Q	USP14, LMW region
M6	K6W,TQ12,Q40N	USP14
M11	Q40N	LMW region
M13	T12Q	USP14
M14	T14I	USP14
M15	T12Q,Q40N,K48W	LMW region

Fig. S1. DUB activity-based probe assay for Library I. Labelled DUBs were analysed using in-gel fluorescence scanning. Labelling of USP7KO HAP1 lysate with wild-type ubiquitin reagent was used as control. a) DUB activity-based probe assay for the active mutants from library I (Part I); b) A list of the main mutants (Library I, Part I) which have shown a decrease in labelling for USP14 and in the LMW region, still labelling USP7; c) DUB activity-based probe assay for mutants from library I (Part II). *= labelled USP7; LMW=Low Molecular Weight

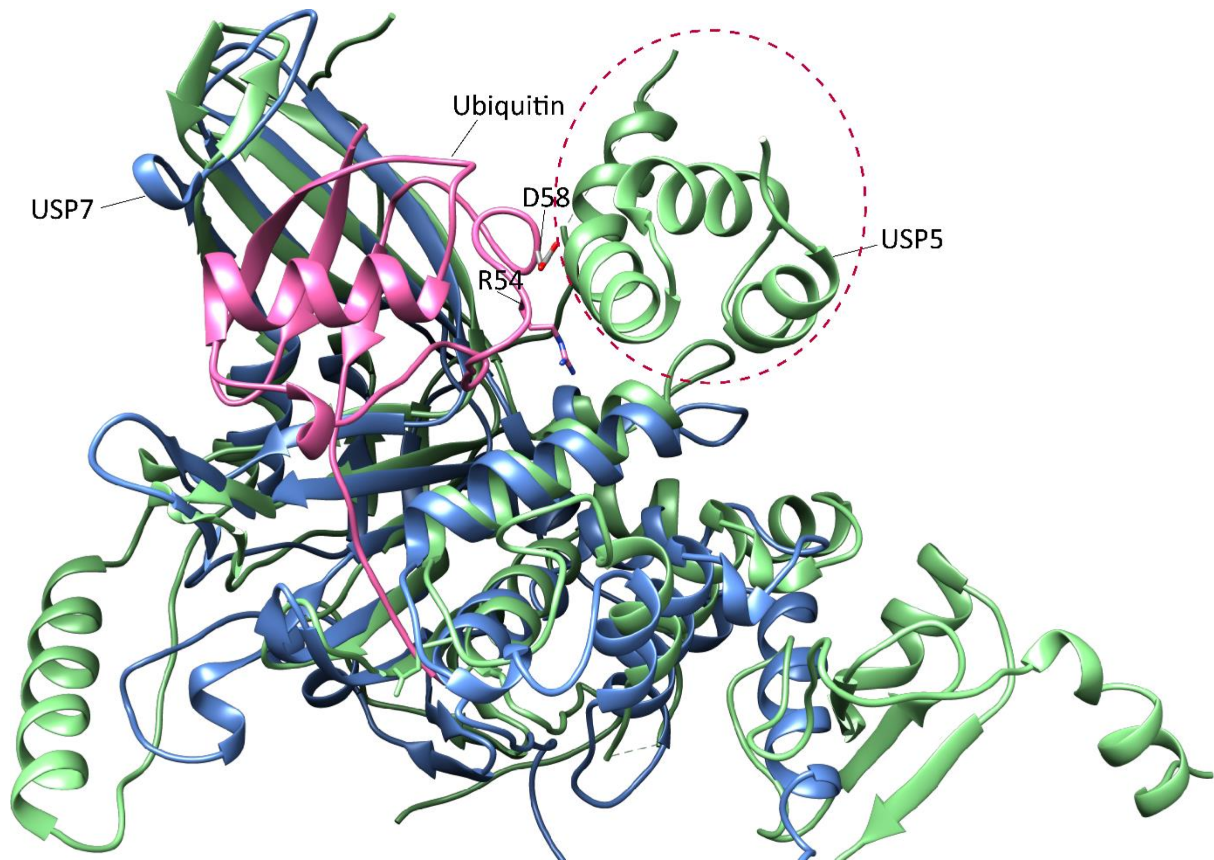


Fig. S2. Representation of the superimposition of the structure of USP7 catalytic domain (blu, PDB ID: 1NBF) to the crystal structure of USP5 in complex with ubiquitin (light green, PDB ID: 3IHP). Not all the motif of USP5 are present in this figure, only the ones interacting with ubiquitin. Residues R54 and D58 show close interaction with a motif of USP5 (red circle) which is absent in the case of USP7. The figure was generated using USCF Chimera.

Table S3. List of the ubiquitin mutants from Library II (LII)

ID	Sequence	Mutation
Wt	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	
LIIM1	Rh-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHGVRRLRG-PA	T7F,L8R,I13Y,E34L,L69G,L71R
LIIM2	Rh-MQIFVKFGTGKTYTLEVEPSDTIENVKAKIQDKIGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHWVRRLRG-PA	T7F,L8G,I13Y,E34I,L69W,L71R
LIIM3	Rh-MQIFVKFATGKTYTLEVEPSDTIENVKAKIQDKIGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHGVRRLRG-PA	T7F,L8A,I13Y,E34I,L69G,L71R
LIIM4	Rh-MQIFVKFGTGKTYTLEVEPSDTIENVKAKIQDKLGFPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHCVRRLRG-PA	T7F,L8G,I13Y,E34L,I36F,L69C,L71R
LIIM5	Rh-MQIFVKFATGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHGVRRLRG-PA	T7F,L8A,I13Y,E34L,L69G,L71R
LIIM6	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,L69A,L71A
LIIM7	Rh-MQIFVKSYTGKTYTLEVEPSDTIENVKAKIQDKIGNPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHIVGRLRG-PA	T7S,L8Y,I13Y,E34I,I36N,L69I,L71G
LIIM8	Rh-MQIFVKCLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHCVLRLRG-PA	T7C,L69C
LIIM9	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVRRLRG-PA	L71R
LIIM10	Rh-MQIFVKCLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHCVRRLRG-PA	T7C,L69C,L71R
LIIM11	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGYPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	I36Y
LIIM12	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGFPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	I36F
LIIM13	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSYYNIQKESTLHLVLRLRG-PA	D58Y
LIIM14	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGOTLSWYNIQKESTLHLVLRLRG-PA	R54TPO,D58W
LIIM15	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGOTLSYYNIQKESTLHLVLRLRG-PA	R54TPO,D58Y
LIIM16	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGOTLSWYNIQKESTLHLVLRLRG-PA	T12W,R54TPO,D58W
LIIM17	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGOTLSYYNIQKESTLHLVLRLRG-PA	T12W,R54TPO,D58Y
LIIM18	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGOTLSYYNIQKESTLHLVLRLRG-PA	T12W,Q40N,R54TPO,D58Y
LIIM19	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLSWYNIQKESTLHLVLRLRG-PA	T12W,Q40N,R54K-bio,D58W
LIIM20	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDG9TLSWYNIQKESTLHLVLRLRG-PA	T12W,Q40N,R54K-trimethyl,D58W
LIIM21	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLS9YNIQKESTLHLVLRLRG-PA	T12W,Q40N,R54K-bio,D58K-trimethyl
LIIM22	Rh-MQIFVKTL9GKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLS9YNIQKESTLHLVLRLRG-PA	T9K-trimethyl,T12W,Q40N,R54K-bio, D58K-trimethyl
LIIM23	Rh-MQIFVKTL9GKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLSWYNIQKESTLHLVLRLRG-PA	T9K-trimethyl,T12W,Q40N,R54K-bio,D58W
LIIM24	Rh-MQIFVKTLWGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLS9YNIQKESTLHLVLRLRG-PA	T9W,T12W,Q40N,R54K-bio,D58K-trimethyl
LIIM25	Rh-MQIFVKTL5GKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLS9YNIQKESTLHLVLRLRG-PA	T9(pentafluorphenylalanine),T12W,Q40N, R54K-bio,D58K-trimethyl
LIIM26	Rh-MQIFVKTL9GKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLS9YNIQKESTLHLVLRLRG-PA	T9K-trimethyl,T12W,Q40N,R54K-bio, D58K-trimethyl
LIIM27	Rh-MQIFVKTLZGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDG9TLSWYNIQKESTLHLVLRLRG-PA	T9K-bio,T12W,Q40N,R54K-trimethyl,D58W
LIIM28	Rh-MQIFVKTLWGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLSWYNIQKESTLHLVLRLRG-PA	T9W,T12W,Q40N,R54K-bio,D58W
LIIM29	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLS9YNIQKESTLHLVLRLRG-PA	D58K-trimethyl
LIIM30	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSZYNIQKESTLHLVLRLRG-PA	D58K-bio
LIIM31	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGZTLSDYNIQKESTLHLVLRLRG-PA	R54K-bio
LIIM32	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDG9TLSDYNIQKESTLHLVLRLRG-PA	R54K-trimethyl
LIIM33	Rh-MQIFVKTL5GKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T9(pentafluorphenylalanine)
LIIM34	Rh-MQIFVKTLWGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	T9W

TPO=phosphorylated tyrosine; Pentafluoropheylalanine=5; Lys(Me_3Cl)=9 (K-trimethyl); Lys(biotin)=Z (K-bio)

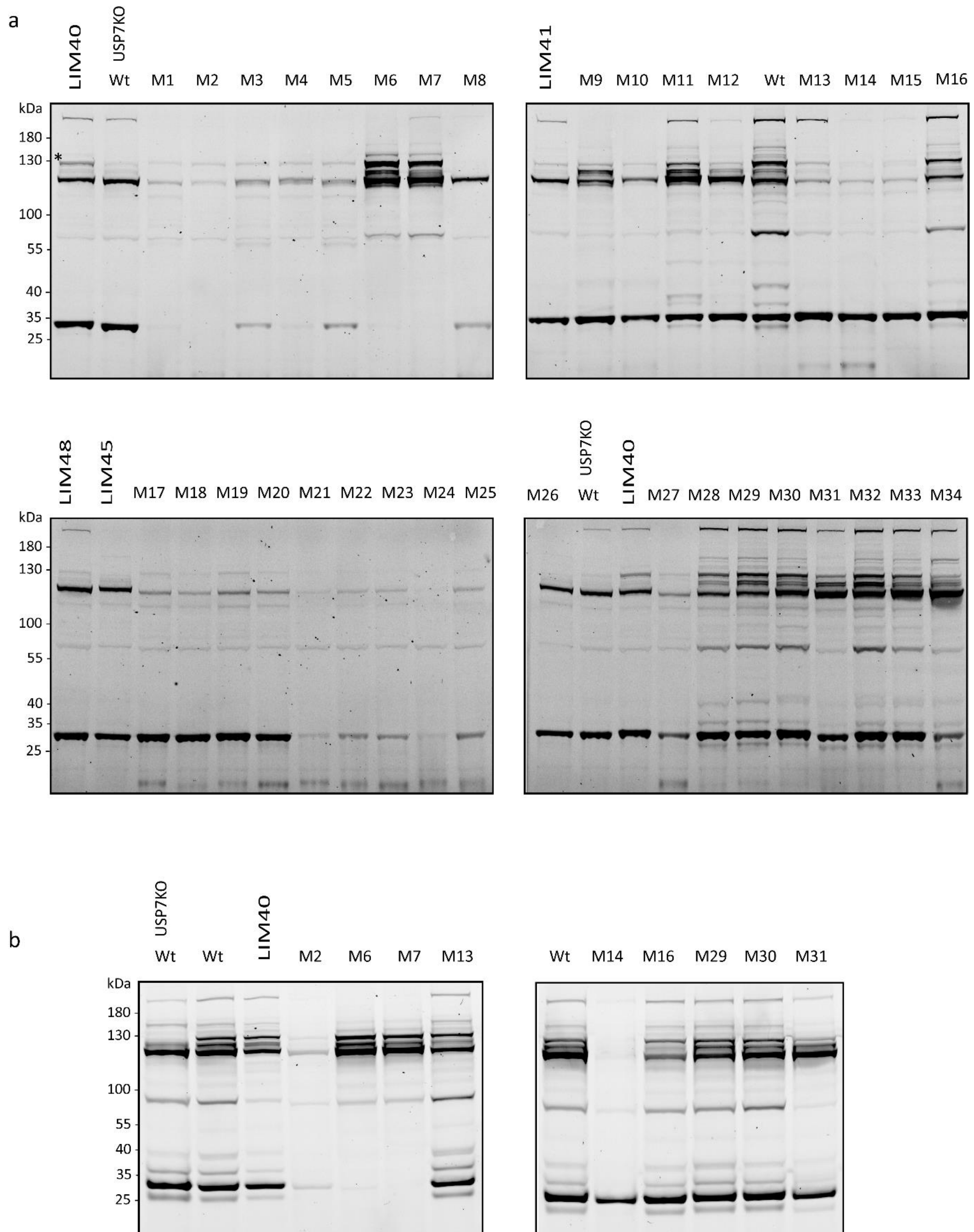


Fig. S3. DUB activity-based probe assay analysed using in-gel fluorescence scanning. a) DUB activity-based probe assay for all the mutants from Library II and a few mutants from Library I; b) another round of labelling experiments for a few hits from library II. Labelling of USP7KO HAP1 lysate with wild-type ubiquitin reagent was used as control. *=labelled USP7

Table S4. List of the ubiquitin mutants from Library III (LIII)

ID	Sequence	Mutation
LIIIM1	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDQQRLIFAGKQLEDGZTLSDYNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,R54K-bio,L69A,L71A,
LIIIM2	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDQQRLIFAGKQLEDGRTLSZYNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,D58K-bio,L69A,L71A
LIIIM3	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDQQRLIFAGKQLEDGRTLSYYNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,D58Y,L69A,L71A,
LIIIM4	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGZTLSDYNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N, R54K-bio,L69A,L71A,
LIIIM5	Rh-MQIFVKDYTGKWRTLEVEPSDTIENVKAKIQDKLGIPPDQQRLIFAGKQLEDGZTLSDYNIQKESTLHAVARLRG-PA	T7D,L8Y,T12W,I13R,E34L, R54K-bio,L69A,L71A,
LIIIM6	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N, D58K-bio,L69A,L71A,
LIIIM7	Rh-MQIFVKDYTGKWRTLEVEPSDTIENVKAKIQDKLGIPPNQQRLIFAGKQLEDGZTLSDYNIQKESTLHAVARLRG-PA	T7D,L8Y,T12W,I13R,E34L,Q40N, R54K-bio,L69A,L71A,
LIIIM8	Rh-MQIFVKSYTGKTYTLEVEPSDTIENVKAKIQDKIGNPPDQQRLIFAGKQLEDGZTLSDYNIQKESTLHIVGRLRG-PA	T7S,L8Y,I13Y,E34I,I36N,R54K-bio,L69I,L71G
LIIIM9	Rh-MQIFVKSYTGKTYTLEVEPSDTIENVKAKIQDKIGNPPDQQRLIFAGKQLEDGRTLSZYNIQKESTLHIVGRLRG-PA	T7S,L8Y,I13Y,E34I,I36N,D58K-bio,L69I,L71G
LIIIM10	Rh-MQIFVKSYTGKTYTLEVEPSDTIENVKAKIQDKIGNPPDNQRLIFAGKQLEDGZTLSDYNIQKESTLHIVGRLRG-PA	T7S,L8Y,I13Y,E34I,I36N,Q40N, R54K-bio,L69I,L71G
LIIIM11	Rh-MQIFVKSYTGKTYTLEVEPSDTIENVKAKIQDKIGNPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHIVGRLRG-PA	T7S,L8Y,I13Y,E34I,I36N,Q40N, D58K-bio,L69I,L71G
LIIIM12	Rh-MQIFVKSYTGKWYTLEVEPSDTIENVKAKIQDKIGNPPDQQRLIFAGKQLEDGZTLSDYNIQKESTLHIVGRLRG-PA	T7S,L8Y,T12W,I13Y,E34I,I36N, R54K-bio,L69I,L71G
LIIIM13	Rh-MQIFVKSYTGKWYTLEVEPSDTIENVKAKIQDKIGNPPDQQRLIFAGKQLEDGRTLSZYNIQKESTLHIVGRLRG-PA	T7S,L8Y,T12W,I13Y,E34I,I36N, D58K-bio,L69I,L71G
LIIIM14	Rh-MQIFVKSYTGKWYTLEVEPSDTIENVKAKIQDKIGNPPDNQRLIFAGKQLEDGZTLSDYNIQKESTLHIVGRLRG-PA	T7S,L8Y,T12W,I13Y,E34I,I36N,Q40N, R54K-bio,L69I,L71G
LIIIM15	Rh-MQIFVKSYTGKWYTLEVEPSDTIENVKAKIQDKIGNPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHIVGRLRG-PA	T7S,L8Y,T12W,I13Y,E34I,I36N,Q40N, D58K-bio,L69I,L71G
LIIIM16	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLSDYNIQKESTLHLVLRLRG-PA	T12W,Q40N,R54K-bio
LIIIM17	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLSYYNIQKESTLHLVLRLRG-PA	T12W,Q40N,R54K-bio,D58Y
LIIIM18	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGZTLSZYNIQKESTLHLVLRLRG-PA	T12W,Q40N,R54K-bio,D58K-bio
LIIIM19	Rh-MQIFVKTLTGKWITLEVEPSDTIENVKAKIQDKEGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHLVLRLRG-PA	T12W,Q40N,D58K-bio

Lys(biotin)=Z (K-bio)

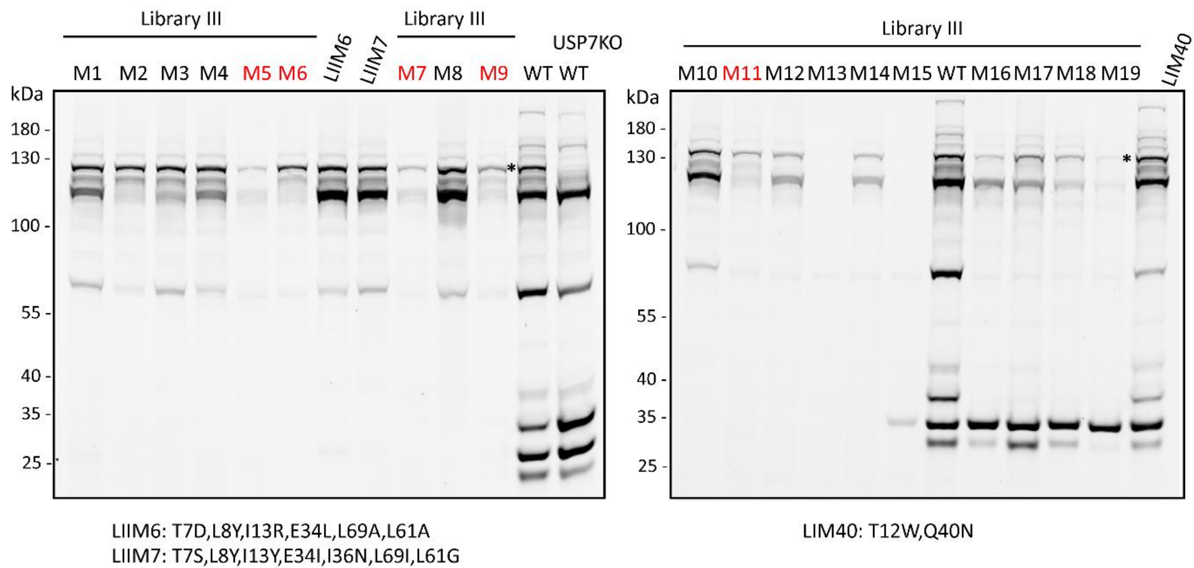
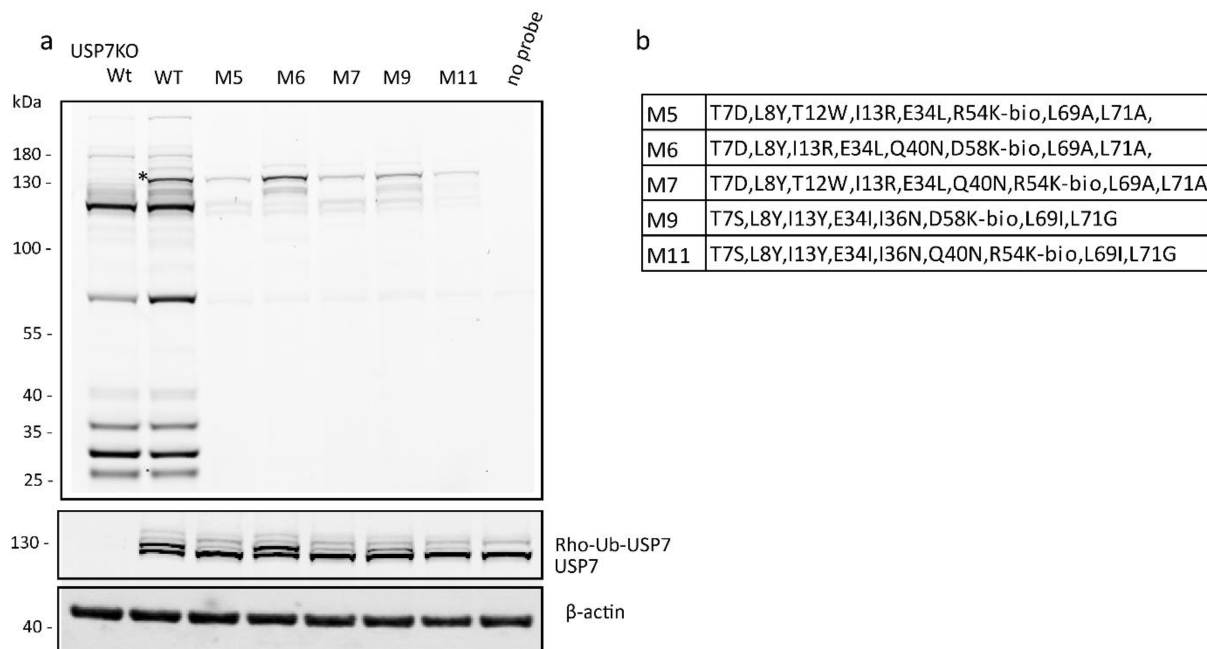


Fig. S4. DUB activity-based probe assay for Library III. Labelled DUBs were analysed using in-gel fluorescence scanning. Labelling of USP7KO HAP1 lysate with wild-type ubiquitin reagent (WT) was used as control. LIIM6 and LIIM7 were the best hits from Library II. *=labelled USP7



M5	T7D,L8Y,T12W,I13R,E34L,R54K-bio,L69A,L71A,
M6	T7D,L8Y,I13R,E34L,Q40N,D58K-bio,L69A,L71A,
M7	T7D,L8Y,T12W,I13R,E34L,Q40N,R54K-bio,L69A,L71A
M9	T7S,L8Y,I13Y,E34I,I36N,D58K-bio,L69I,L71G
M11	T7S,L8Y,I13Y,E34I,I36N,Q40N,R54K-bio,L69I,L71G

Fig. S5. a) DUB activity-based probe assay for the best hits from Library III. Labelled DUBs were analysed using in-gel fluorescence scanning (8 % precast gel, in MES buffer). In this case the labelling experiments were performed with 3x less final concentration of the probes. From all the mutants, Rh-M6-PA (LIIIM6) demonstrated higher reactivity towards USP7, confirmed by western blotting. β-actin was used as loading control; b) mutations present in the best hits. *=labelled USP7

Table 5. List of the ubiquitin mutants from Library IV (LIV).

ID	Sequence	Mutation
Wt	Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA	
LIVM1	Rh-MQIFVKTIKKPIITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKKLEDGRTLSDYNIRHASTLQLVIRRSG-PA	UbV.7.1: L8I,T9K,G10K,K11P,T12I,Q49K,Q62R, K63H,E64A,H68Q,L71I,L73R,R74S
LIVM2	Rh-MPIFVKTLTGKNITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFTGKKLEDGRTLSDYNIKFASTLHLVIRLRG-PA	UbV.7.2: Q2P,T12N,A46T,Q49K,Q62K,K63F,E64A,L71I
LIVM3	Rh-MQIFVKDYTGKWRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHAVARLRG-PA	T7D,L8Y,T12W,I13R,E34L,Q40N,D58K-bio,L69A,L71A
LIVM4	Rh-MQIFVKDIKKPIRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHAVARLRG-PA	T7D,L8I,T9K,G10K,K11P,T12I,I13R,E34L,Q40N, D58K-bio,L69A,L71,
LIVM5	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFTGKKLEDGRTLSZYNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N, A46T,Q49K D58K-bio,L69A,L71A
LIVM6	Rh-MPIFVKDYTGKNRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFTGKKLEDGRTLSZYNIQKESTLHAVARLRG-PA	Q2P,T7D,L8Y, T12N,I13R,E34L,Q40N,A46T, Q49K, D58K-bio,L69A,L71A,
LIVM7	Rh-MPIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHAVIRRSG-PA	Q2 ,T7D,L8Y,I13R,E34L,Q40N, D58K-bio,L69A,L71I;L73R,R74S
LIVM8	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHAVARRSG-PA	T7D,L8Y,I13R,E34L,Q40N, D58K-bio,L69A,L71,;L73R,R74S
LIVM9	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIKFASTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N, D58K-bio,Q62K,K63F;E64A, L69A,L71A
LIVM10	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIRHASTLQAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N, D58K-bio,Q62R,K63H,E64A,H68Q,L69A,L71A,
LIVM11	Rh-MPIFVKDITGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKKLEDGRTLSZYNIRKESTLHAVARLRG-PA	Q2P,L8I,I13R,E34L,Q40N,Q49K;D58K-bio,Q62R,L69A,L71A
LIVM12	Rh-MPIFVKDITGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKKLEDGRTLSZYNIRKESTLHAVIRLRG-PA	Q2P,L8I,I13R,E34L,Q40N,Q49K, D58K-bio,Q62R,L69A,L71I
LIVM13	Rh-MQIFVKDIKKPIRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHAVARLRG-PA	T7D;L8I;T9K;G10K;K11P,T12I;I13R,E34L,Q40N, D58K-bio,L69A,L71A
LIVM14	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLS!YNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N,D58varied,L69A,L71A
LIVM15	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLS#YNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N,D58varied,L69A,L71A
LIVM16	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLS$YNIQKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N,D58varied,L69A,L71A
LIVM17	Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIZKESTLHAVARLRG-PA	T7D,L8Y,I13R,E34L,Q40N,D58K-bio,Q62K-biotin, L69A,L71A

Gln(Dmcp)=!; Lys(ivDde)=$; Glu(biotinyl-PEG)=#; Lys(biotin)=Z (K-bio)

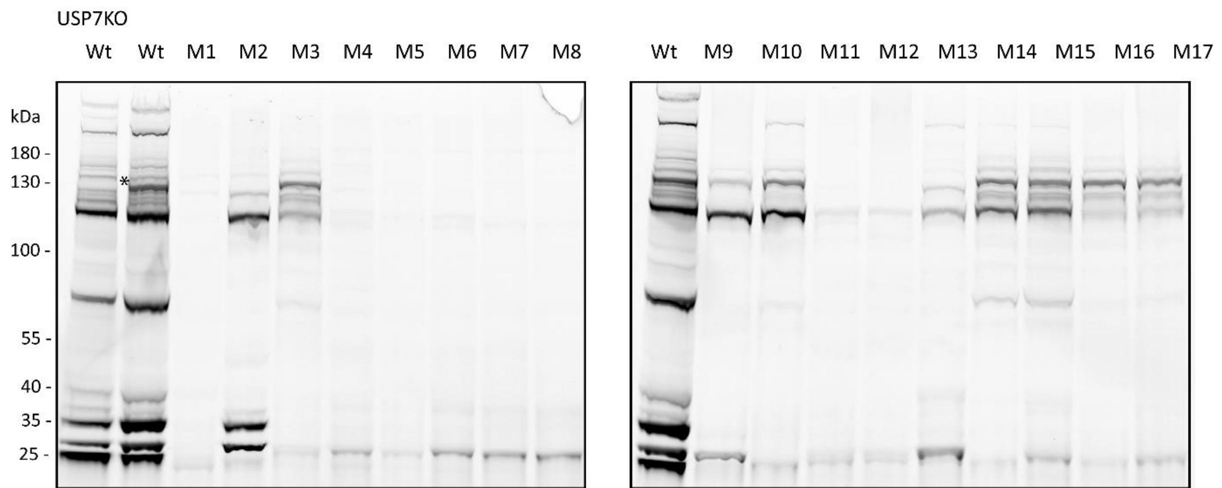


Fig. S6. DUB activity-based probe assay of Library IV. Labelled DUBs were analysed using in-gel fluorescence scanning. *=labelled USP7

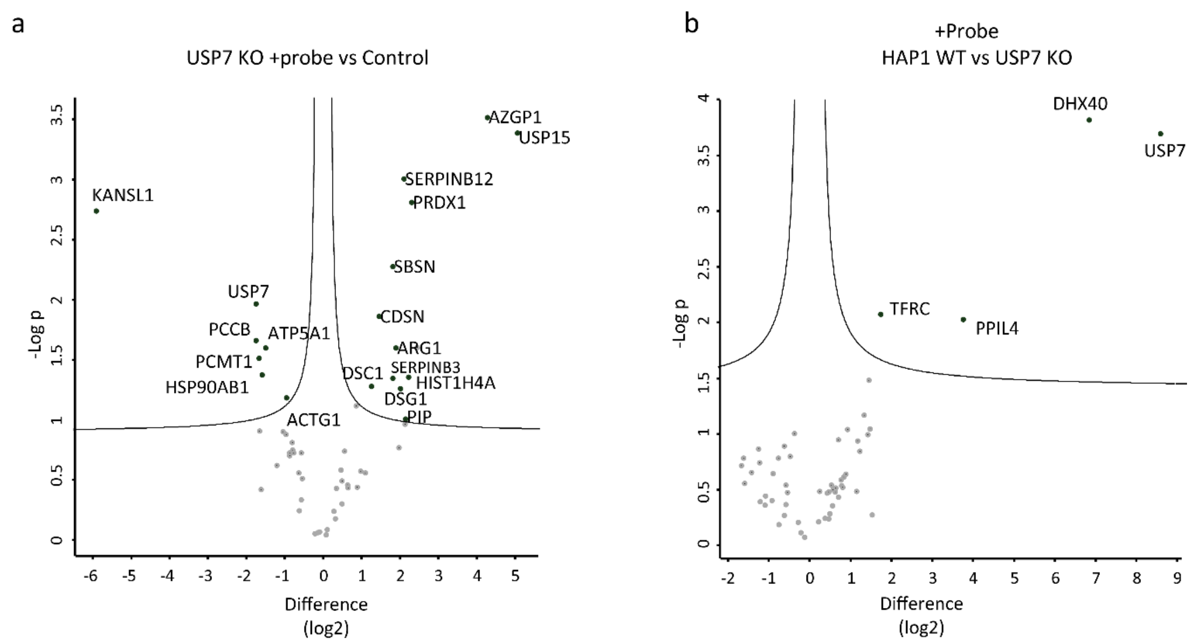


Fig. S7. Volcano plots depicting results from the Mass spectrometry data analysis. a) Difference in proteins identified in the pull-down from the USP7 KO HAP1 lysate versus Control. b) Difference in the proteins identified comparing the pull-down from the two lysate, HAP1 WT and USP7 KO HAP1. Control=HAP1 WT lysate incubated only with neutravidin beads.

Analytical data

For a few of the mutants the analytical data are reported: a) UV chromatogram (λ =190-600 nm); b) mass spectrum using LC-MS; c) mass spectrum of the main peak; d) deconvoluted mass of the spectra shown in c.

Rh-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-PA

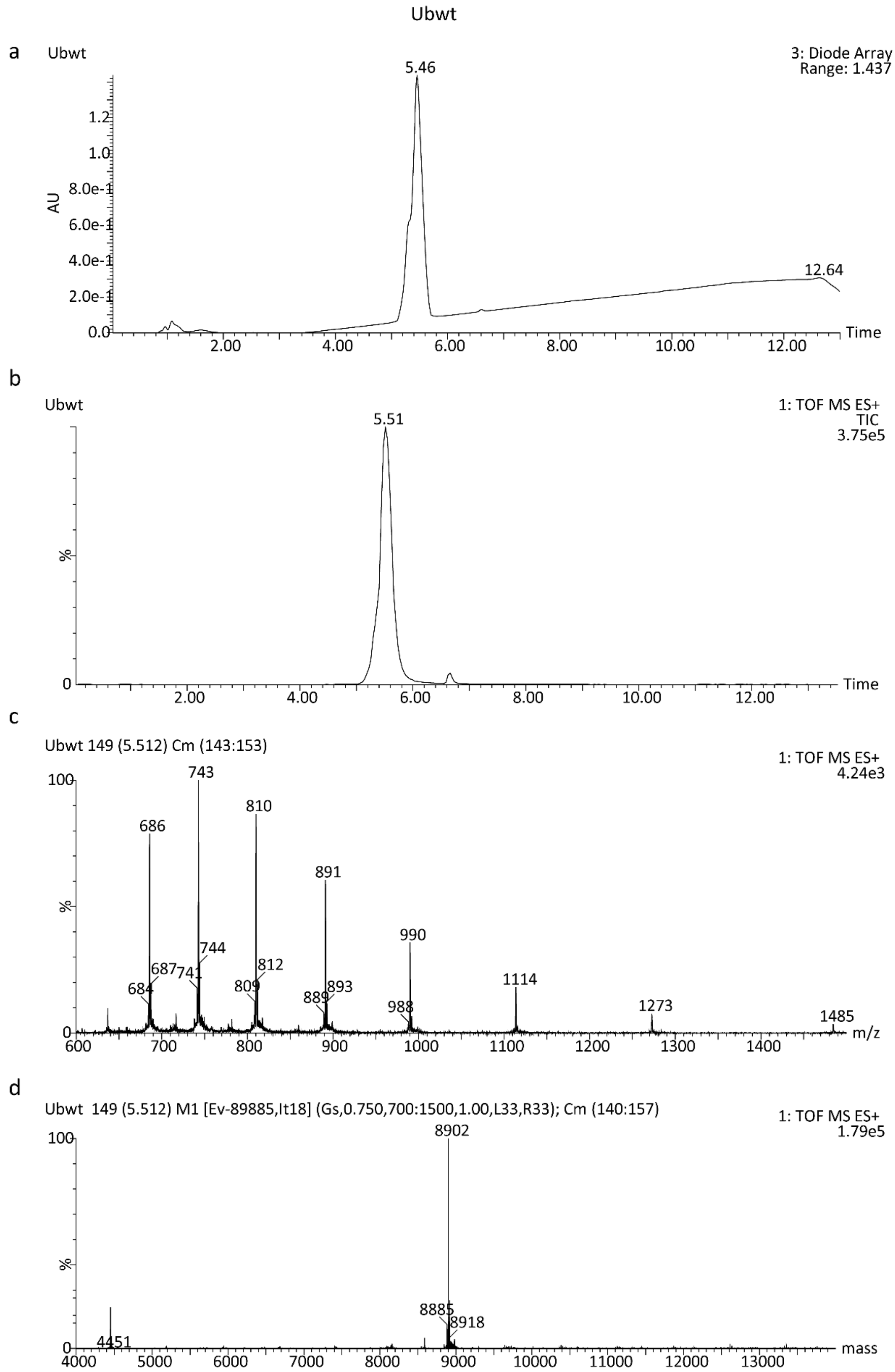


Fig. S8. Analytical data for Ubwt. ESI-Mass [M+H] Calculated: 8901 / Found: 8902.

Rh-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPPDNQRLIFAGKQLEDGRTLSZYNIQKESTLHAVARLRG-PA

LIIIM6

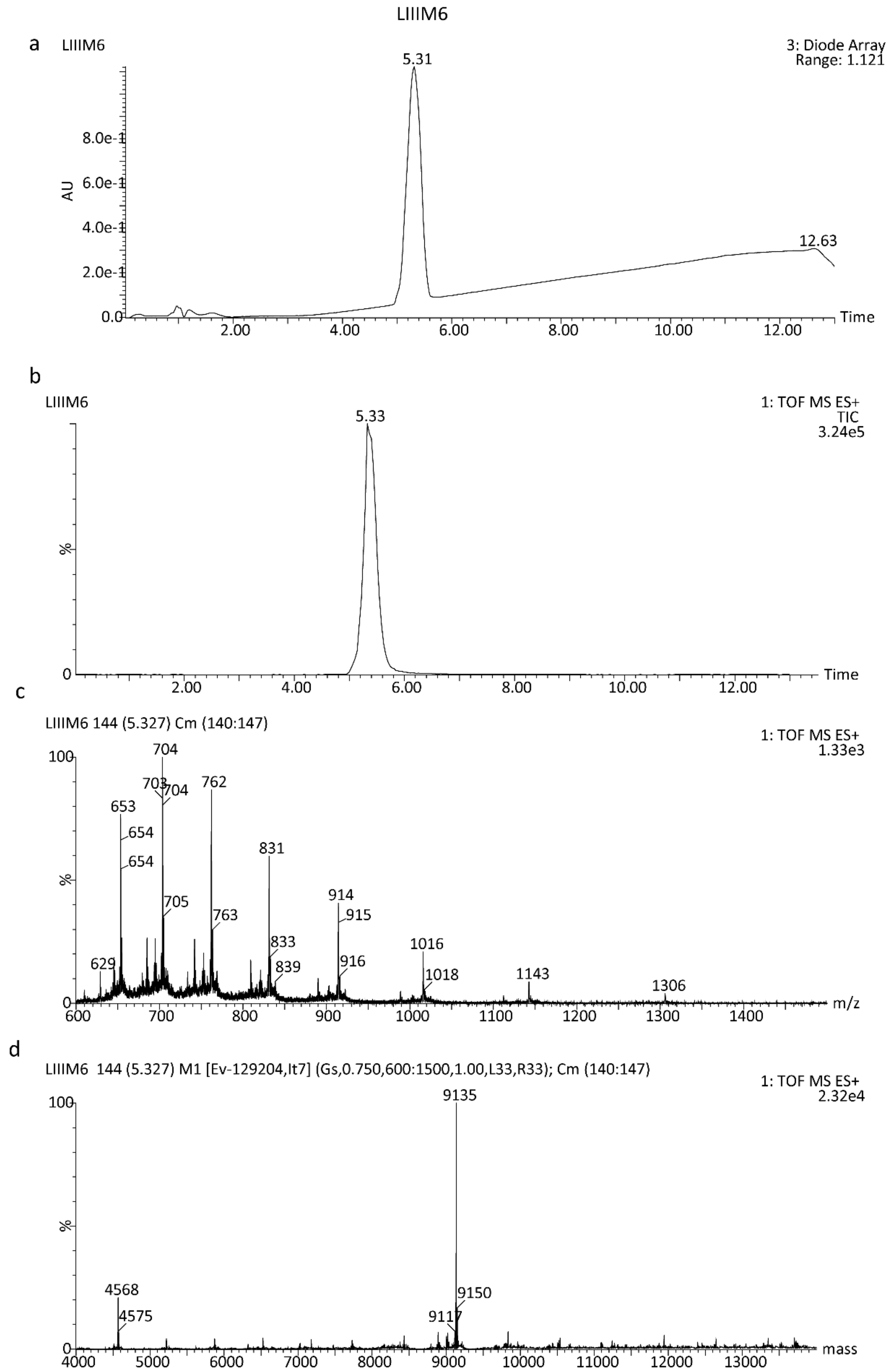


Fig. S9. Analytical data for LIIIM6. ESI-Mass [M+H] Calculated: 9133 / Found: 9135.

TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDNQWLEFAGWRLEDGRTLSDYNIQKLSTLRGVRRLRG-PA

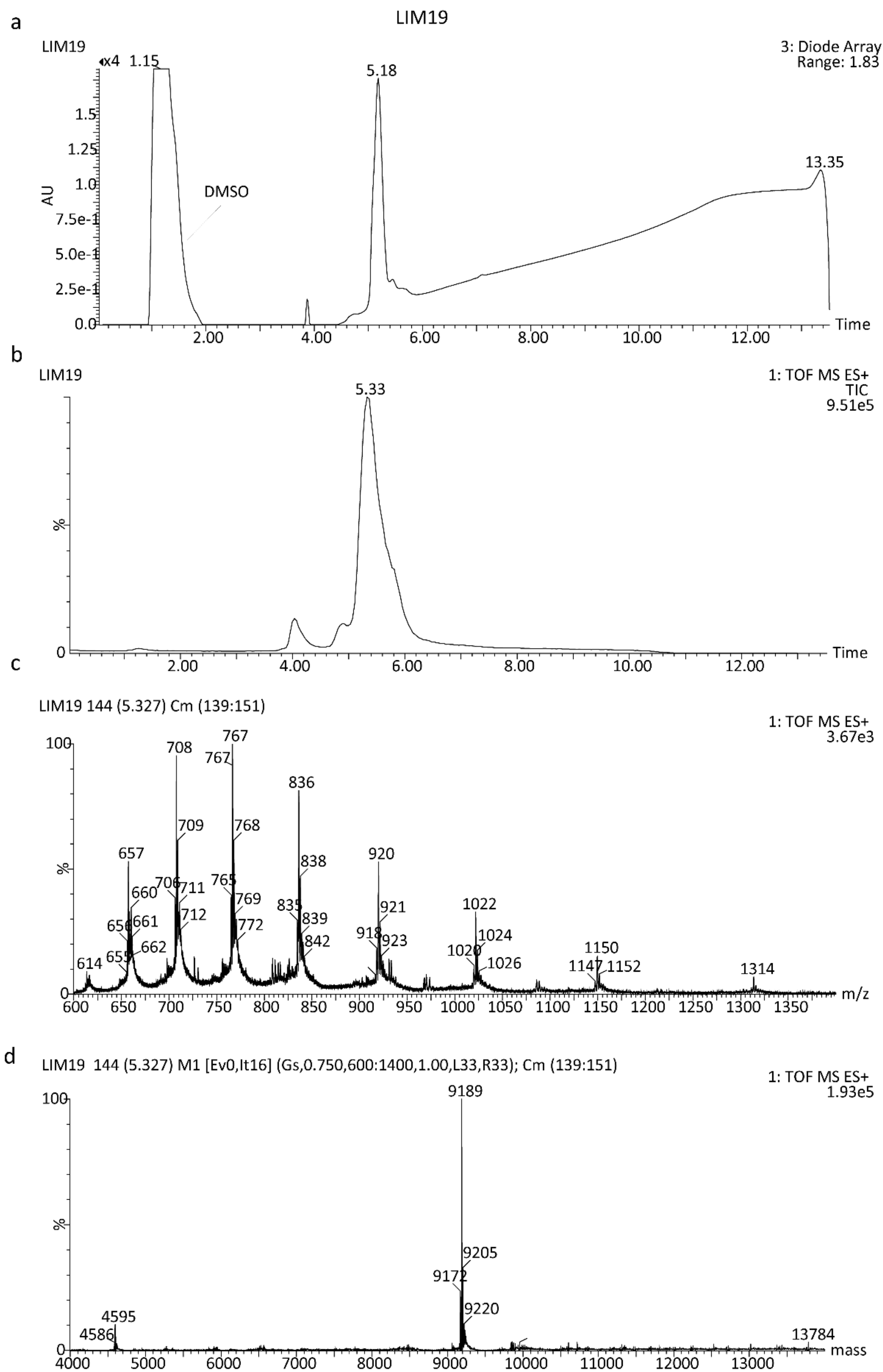


Fig. S10. Analytical data for LIM19. ESI-Mass [M+H] Calculated: 9188 / Found: 9189.

TMR-MQIFVKFRTGKTYTLEVEPSDTIENVKAKIQDKLGIPPDQQWLIFAGKRLEDGRTLSDYNIQKESTLRGVRRLRG-PA

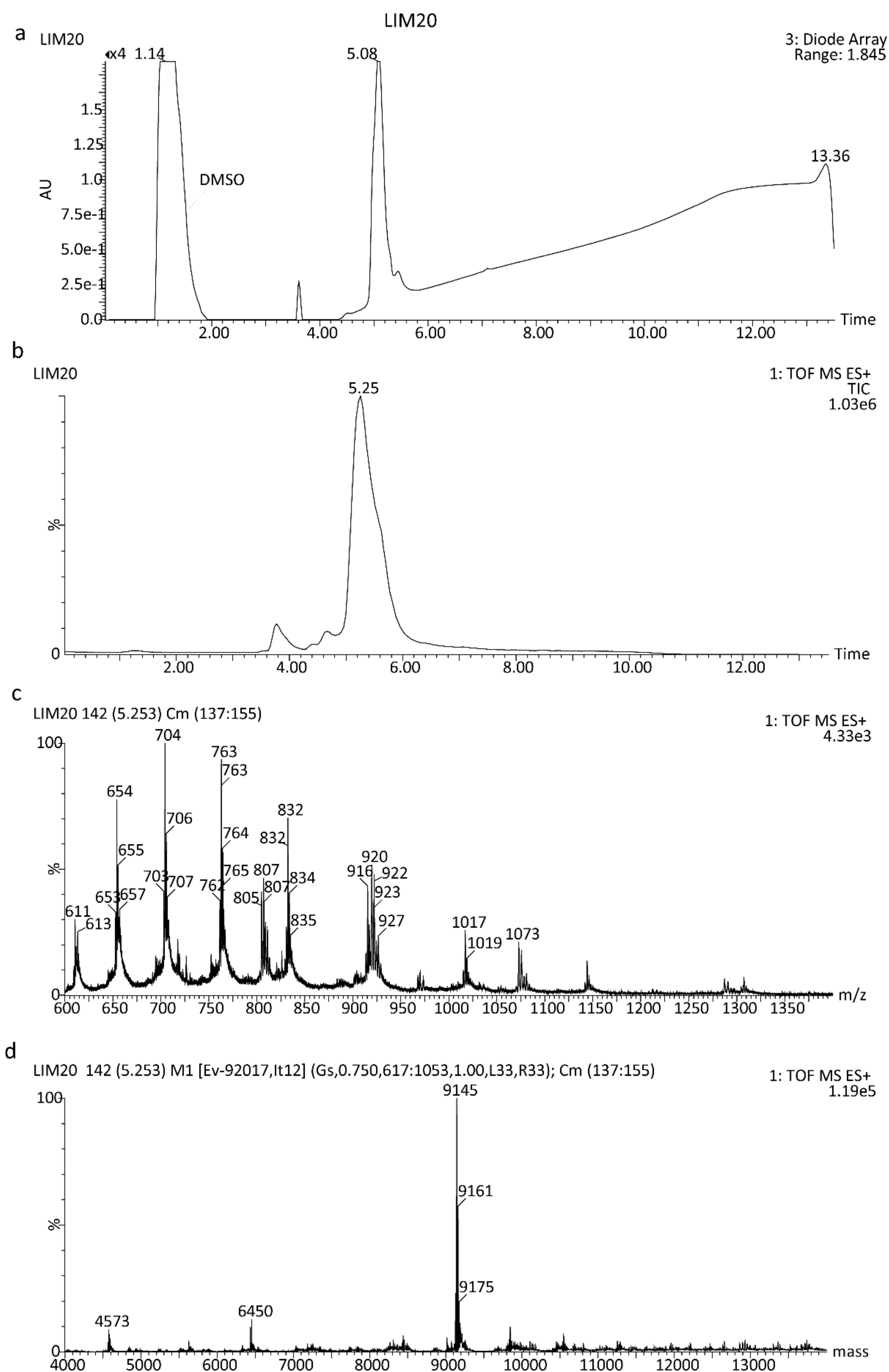


Fig. S11. Analytical data for LIM20. ESI-Mass [M+H] Calculated: 9144 / Found: 9145.

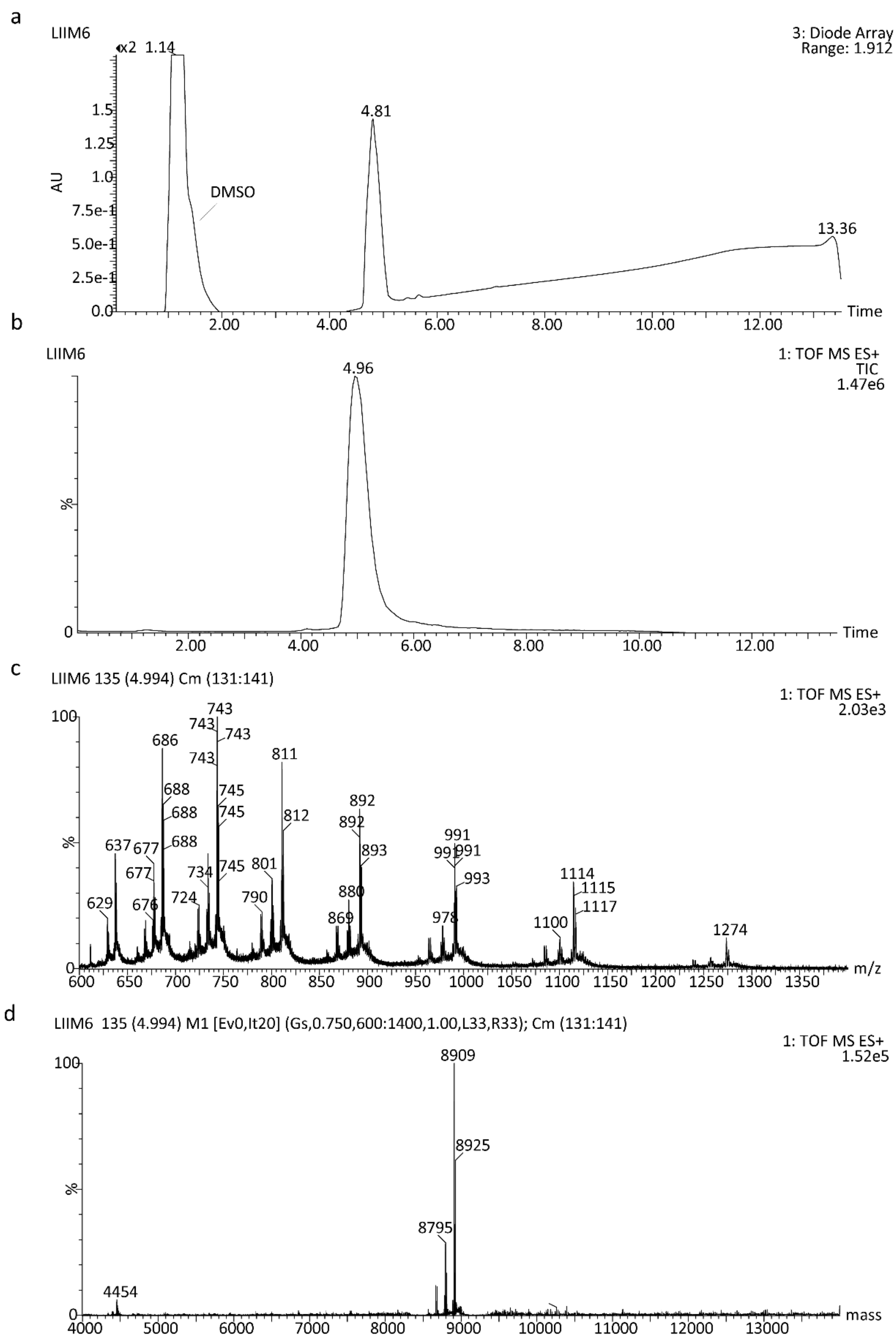


Fig. S12. Analytical data for LIIM6. ESI-Mass [M+H] Calculated: 8908 / Found: 8909.

Rh-MQIFVKSYTGKTYTLEVEPSDTIENVKAKIQDKIGNPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHIVGRLRG-PA

LIIM7

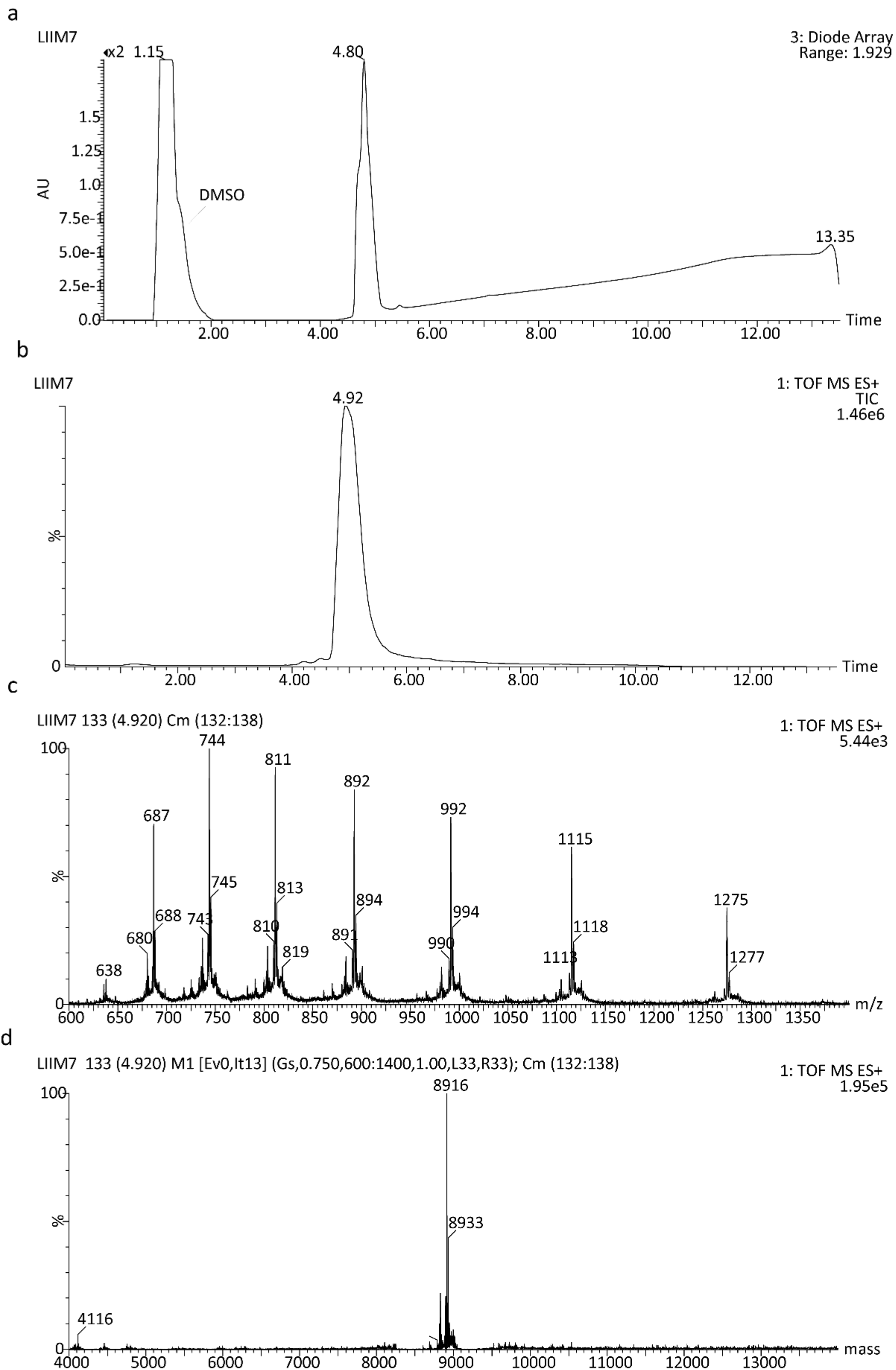


Fig. S13. Analytical data for LIIM7. ESI-Mass [M+H] Calculated: 8916/ Found: 8916.

Rh-MQIFVKTIKKPIITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKKLEDGRTLSDYNIRHASTLQLVIRRSG-PA

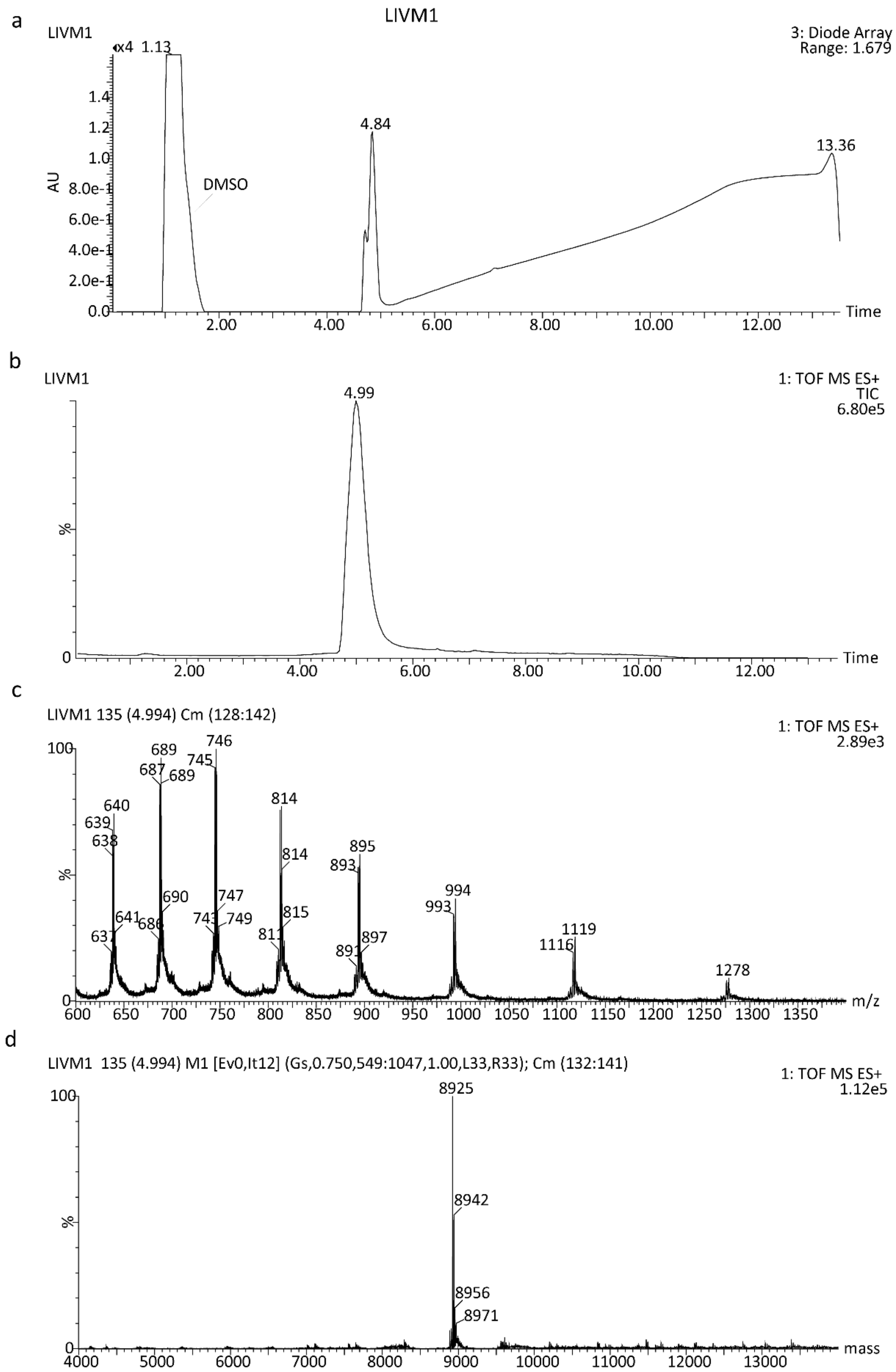


Fig. S14. Analytical data for LIVM1. ESI-Mass [M+H] Calculated: 8924 / Found: 8925.

References:

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