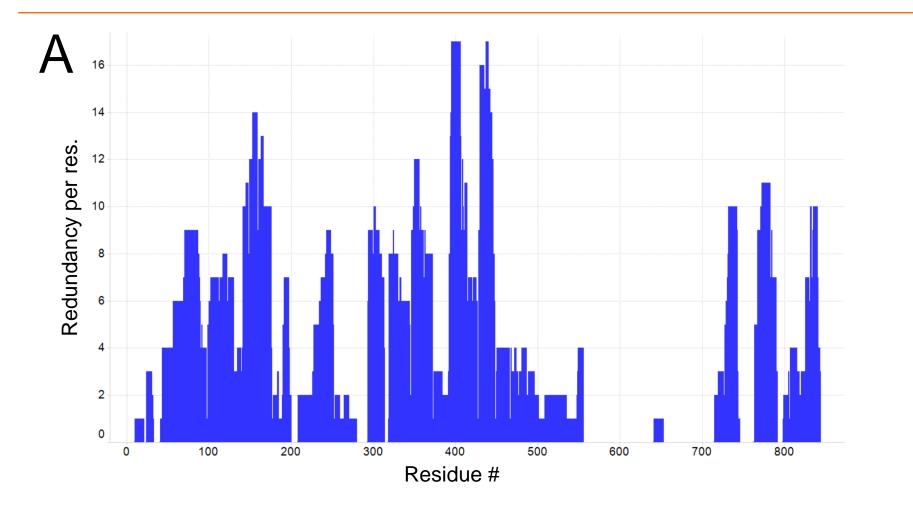


Figures: Figure S1: Sequence coverage and redundancy Figure S2: Differential fractional uptake and time course data vs. peptide ID Figure S3: Woods plot Figure S4: Volcano plot analysis

Tables: Table S1: HDX Experimental Data Summary Table S2: Table of peptides with statistically significant protection

Experimental details and parameters

Figure S1: Sequence coverage and redundancy



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Figure S2: Differential fractional uptake and time course data vs. peptide ID

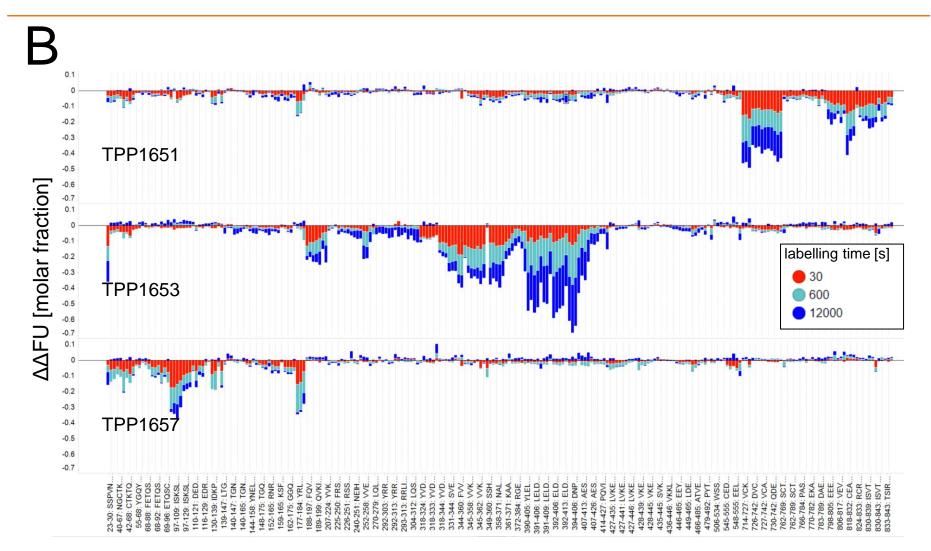


Figure S3: Woods Plot



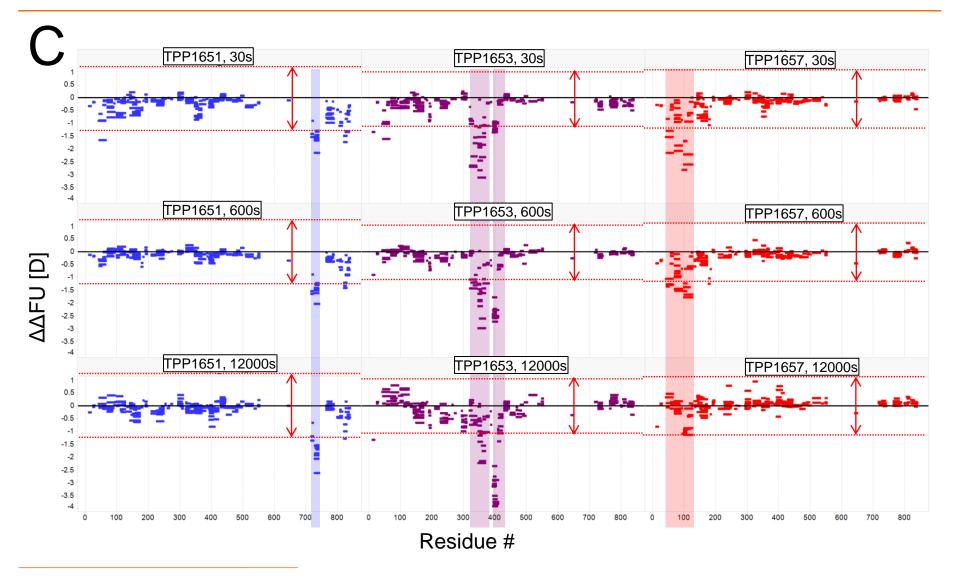
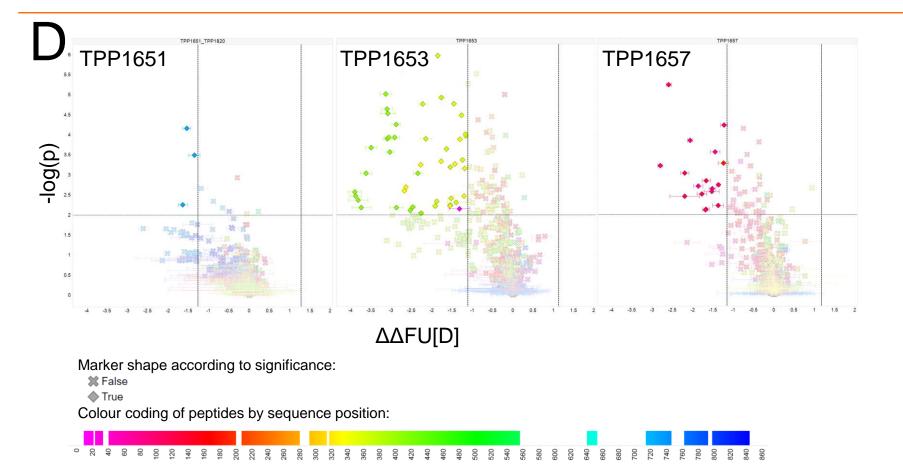


Figure S4: Volcano plot analysis, p=0.01





filename: ELN43007_1_HDX_DB_viewer.dxp



S1 Peptide map of C7: coverage and redundancy;

S2 Plot of differential fractional uptake values ($\Delta\Delta$ FU) of TPP1651 (top), TPP1653 (middle) and TPP1657 (bottom) in complex with C7, expressed as molar fraction of the maximum theoretical uptake for each peptide and normalised for the length of peptide;

S3 Associated Woods plots of the three complexes for separated by labelling time. The dotted lines indicate significance threshold at p<0.01. Regions that met the significance criteria (see Table HDX1) were highlighted.

S4 Volcano plot analysis of the three complexes. Peptides that represent significant hits at n=3, p<0.01 are highlighted and indicated by diamonds (see Table HDX2. Peptides below the threshold are indicated by crosses. The colour coding shows the position of the peptide in the sequence.

Table S1: Summary of Experimental Data



Protein States	Apo-C7, complexes of C7 with TPP1651, 1653 and 1657
Instrument Details	Liquid Handling: Leap2 Robot (Leap Trajan) UPLC-MS: Acquity HDX platform with SynaptG2Si mass spectrometer (Waters)
Sample details	Peptide mapping: 30pmol C7 in 5ul sample buffer; Labelling time course: 15pmol C7 in 5ul sample buffer as apo reference. Complexes were prepared by addition of 10pmol mAb (=0.7x molar excess).
Reaction details	H2O sample buffer: 50mM MOPS, 150mM NaCl in H2O, pH7.2; D2O labelling buffer: 50mM MOPS, 150mM NaCl in D2O, pD6.8 uncorrected (theoretical maximum incorporation= 91%D); labelling temperature 20degC; labelling times: 30, 600, 12000s quench buffer: 6M urea, 1M TCEP, 400mM formate, pH3.5 quench, trapping and LCMS temperature 0degC; quench time: 15min trapping time: 4min Pepsin digestion (Enzymate, Waters) at 15degC.
Data acquisition	solventA: 0.2% formic acid +0.03% TFA; solventB: 0.2% formic acid in acetonitrile analytical gradient: 11-28% solventB in 10min, then 28-40% in 2min. MS data were recorded with ion mobility, i.e. in HDMSE mode for peptide mapping and HDMS mode for labelled samples.
Coverage	249 peptides, 75.7% coverage, average length 15.4 aa.
Significance threshold for differential HDX	Volcano Plot analysis: Statistical significance of differential HDX was tested at p=0.01 confidence level. HDX protection was considered significant if \geq 3 peptides, or time points of the same peptide, in any contiguous region of the C7 sequence exceeded the significance threshold (see Woods plot). Significant thresholds and residue ranges with significant $\Delta\Delta$ FU were: TPP1651 complex: ±1.27D, res. 714-727; TPP1653 complex: ±1.12D, res. 318-371 and 390-413; TPP1657 complex: ±1.16D, res. 68-129. Assuming 13.4 exchangeable D for a peptide of average length this corresponded to a $\Delta\Delta$ FU Threshold of ca. 9%;
HDX data	A table of peptides and time points with statistically significant $\Delta\Delta FU$ is attached.

Table S2: List of C7 peptides experiencing statistically significant protection P<0.01





VolcanoPlotP0.01

ELN43007 Claus Spitzfaden, SBS Stevenage, December 2021, January 2022



C6-C7 Residue Concordance of HDX Volcano Plot Hits



Clone (TPP)	SPR Bin	C7 HDX construct	C6 (crystal structure 3T5O)	ELN Reference
TPP1651&1820	1	714-727	765-779	N70158-1-06 & N70158-7-04
TPP1653	3	318-371, 390-413	359-417, 436-458	N70158-1-06
TPP1657	2	68-129	101-160	N70158-1-06

sequence alignment produced with DNAStar MegalignPro 17.1.1 filename: C6C7_alignments.msa

Experimental Parameters



- Experiment date: Oct 26, 2018
- Samples:

```
C7 (Complement Technologies #A124, lot 11b)
TPP-1651 (N61099-57-8; protection pattern identical to TPP1820, as shown in N70158-7-04)
TPP-1653 (N61099-57-2)
TPP-1657 (N61099-57-5)
```

Main Experimental Parameters:
 C7 concentration: 30pmol for peptide mapping, 15pmol for labelling;
 0.7eq mAb for complex samples;
 labelling buffer: MOPS pD6.8 uncorrected;
 labelling time: 4 time points, 0, 30, 600 and 12000s @20deg;
 replication: 3 replicates
 blanks: before and after blanks to assess background and carryover;
 quench buffer: 6M Urea +1.5M TCEP, pH3.5;
 quench time: 15min@0deg for labelling.
 Digestion column: Waters Enzymate (pepsin)

 Processing & analysis: PLGS &HDExaminer.
 Peptides with poor s/n, high background in blank and unidentified 100% signal were eliminated. Heatmap data were calculated with "very heavy" smoothing.



> Output:

Volcano plot, differential fractional uptake and average heatmap data.

File names:

Original HDExaminer file with all the mAbs: 70158_1_06_c02.hdx File with 1651=1820, 1653, 1657: ELN43007_1.hdx states file: ELN43007_1_statestable.csv HDX database entry #1722

C6crystal/C7HDX Global Alignment



	A Pore forming
🗉 Overview	Cuttal Omega
Ruler	
G 3T5O_1 Chain	
© P10643 CO7_HUMAN	
Overview Sequences	Girstal Omena
- Sequences	TSP not present in C7: 1-58 TSP1 C7: 60-113 LDLRA: 117-154
Ruler 1	
©Consensus 3T5O_1 Chain	c f c dh y aw t gw t s c s k t c n s g t g s r h r x x y z x x x x x x F x x q i c s k g E x x x x x x x x Z x C x X x P x N C x x x x x x P x N C x x x x x x P x N C x x x x x Q x x x R S V x x x x x x Q x G C Q P x x x x x x Q x C x P x x x C x x Z E x C x x Z E x C x x Z E x C N G x D (x x x x x x P x N C x x x x x x P x N C x x x x x x P x N C x x x x x R X X X X X X X X X X X X X X
P10643 CO7_HUMAN	MKVISLFILVGFIG EFQSFSSASSPVNCQWDFYAPWSECNGCTKTQTRRRSVAVYGQYGGQPCVGNAFETQSCEPTRGCPTE EGCGERFRCFSGQCISKSLVCNGDSD(
	Poreforming: 155-501
Ruler 1	160 170 180 190 200 210 220 230 240 250 260 270 280 290
Consensus	D C C X X X M X X X X X X X P X P X X L X G X X X X X X G X X X X X X X X
3T5O_1 Chain P10643 CO7_HUMAN	D C GRTK AV CTRKYNP I PSVQLINGNGFH FLAGE PROEVIDNSFTOGICK TVKSSKTSNP KVPANLENVGFEVOTA EDDLKTDFYKDLTSIGHNENQGSFSSOGGSSFSVP I FYSSKRSENINHNSAK KVALI OA SHK KBE DRCEDSERPSCDIDKYPNIELTGRYNELTOGFNRVINTSFGOGCRKVFSGDGBORVESTORSONUSYTFOXITEN VEFYNDTWSTYKHTSTEN FTSSSRSSSSSRSYTSHTNEIHKAKS
	Poreforming: 155-501
Ruler 1	310 320 330 340 350 360 370 370 370 400 410 420 430 440
© Consensus	x x x ¥ x x F x x x x x x 1 x L x x F x X x 1 x H LP x Y x X x x Y x R x x 0 x x G T H Y x x SG S L G G x Y x x 1 x x x S E x L X x x x x x x 2 x X x x x x x x X X X X
3T5O_1 Chain P10643ICO7 HUMAN	VMK VLNFT - TKAK DLHLSDVFLKALNHLPLEVNSALVSR I FDDFGTHYFTSGSLGGVYDLLVQFSSEELKNSGLTEEEAKHCVR I ETKKRVLFAKKTKVEHRCTTNKLSEKHEGSFI QGAEKSI SLI RGGRSE YGAALAWE -) TVEVAQFI NNNPEFLQLAEPFWKELSHLPSLVDY SAV RRLI DQYGTHYLQSGSLGGEYRVLFYVDSEKLKQNDFNSVEEKKCKSSGWHFVVKFSS HGCKELENALKAASGTQNNVLRGEPFI RGGGAGFI SGLSYLEI
	Poreforming: 155-501 EGF:502-532 TSP2: 544-591
Ruler 1	460 470 480 490 500 510 520 530 540 550 560 570 580 590
© Consensus	x x x x x x x x x x x x x x x x x x x
3T5O_1 Chain P10643 CO7 HUMAN	K T F S EWLE S VK E N P A V I D F E L A P I V D L V R N I P C A V K R N N R K A LQ E Y A A K F D P CQCAP C P N G R P T L S G T E C L C V CQ S G T Y G E N C E K Q S P - D Y K S N A V D Q Q G C W S S W S T C D A T Y K R S R T R E C N P A P Q R G G K R C E G E K RQ I R R Y S AWA E S V T N L P Q V I K Q K L T P L Y E L V K E V P CA S V K K L Y L K WA L E F L D E F D P CH C R P CQ NG G L A T Y E G T H C L C H C K P Y T F G A A C E Q G V L Y G N Q A G G V D G G W S C W S S W S P C V Q G - K K T R S R E C N P P P S G G G R S C Y G E T E S
	CCP1: 621-680 CCP2: 681-742
Ruler 1	
Consensus	610 620 630 640 650 660 670 680 690 700 710 720 730 740 x x x x x x p c i n d d e e x x x x c x x x x x x x C x x G x x x x X X x X x x X x x X x X X x X X x X X x X X x X X X X X X X X X X
3T5O_1 Chain P10643 CO7_HUMAN	MENNGQ PCINDDE EMKEY-DLPEIEADSGCPQPVPPENGFIRNEKQLYLVGEDVEISCLTGFETVGYQYFRCLPDGTWRQGDVECQRTECIK PVVQEVLTITPFQRLYRIGESIELTCPKGFVVAGPSRYTCQGN-SWTPPIS
P10643[CO7_HUMAN	
	FIM1: 759-818 FIM2: 855-913
Ruler 1 © Consensus	†60 7 [†] 00 780 790 800 810 820 830 840 850 850 850 850 870 880 890 ■ x x L T x x x x x C Q x x x X x x x x x x x x x x x x x x x
3T5O_1 Chain	- DT LTKLKGHCQLGQKQSGSECTCMSPEEDCSHHSEDLCVFDTDSNDYFTSPACKFLAEKCLNNQQLHFLHTGSCQDGRQLEWGLERTRLSSNSTKKESCGYDTCYDWEKCSASTSKCVCLLPPQCFKGGNQLYCVKMGSST
	ENPLIDAVP KCORWEKLONSRCVCKMPYECG ·· PSLDVCAQDERSKRILPLTVCKMHVLHC ····································
P10643 CO7_HUMAN	FIM2
P10643]CO7_HUMAN Ruler 1	910 920 E G S X R C X X X X X X X A E L Q

C6 sequence and domain information from: <u>https://www.rcsb.org/sequence/3T5O</u>

C7 sequence: <u>https://www.uniprot.org/uniprot/P10643</u>

sequence alignment produced with DNAStar MegalignPro 17.1.1 filename: C6C7_alignments.msa