Proteomic data and structure analysis combined reveal interplay of structural rigidity and flexibility on selectivity of cysteine cathepsins

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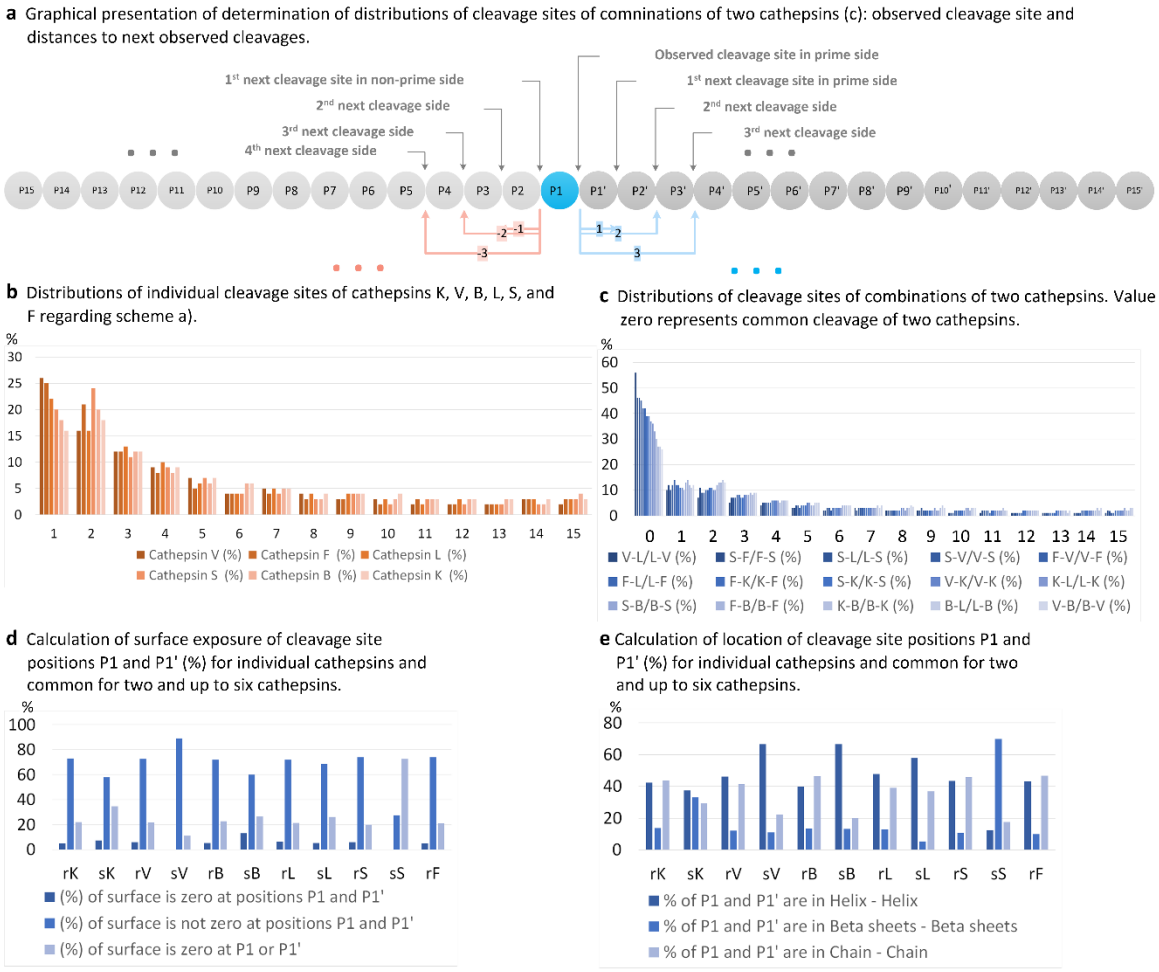
Supplementary Information

Supplementary figures and tables



Common (shared) cleavage sites of cathepsins K, V, B, L, S, and F are represented as rK, rV, rB, rL, rS, and rF, respectively. Specific (unique and single) cleavage sites of cathepsins K, V, B, L, S, and F are represented as sK, sV, sB, sL, sS, and sF, respectively.

Supplementary Fig. 1. Cleavage site separations and cleavage locations.

a. Graphical presentation of determination of neighboring cleavages of one selected cleavage.

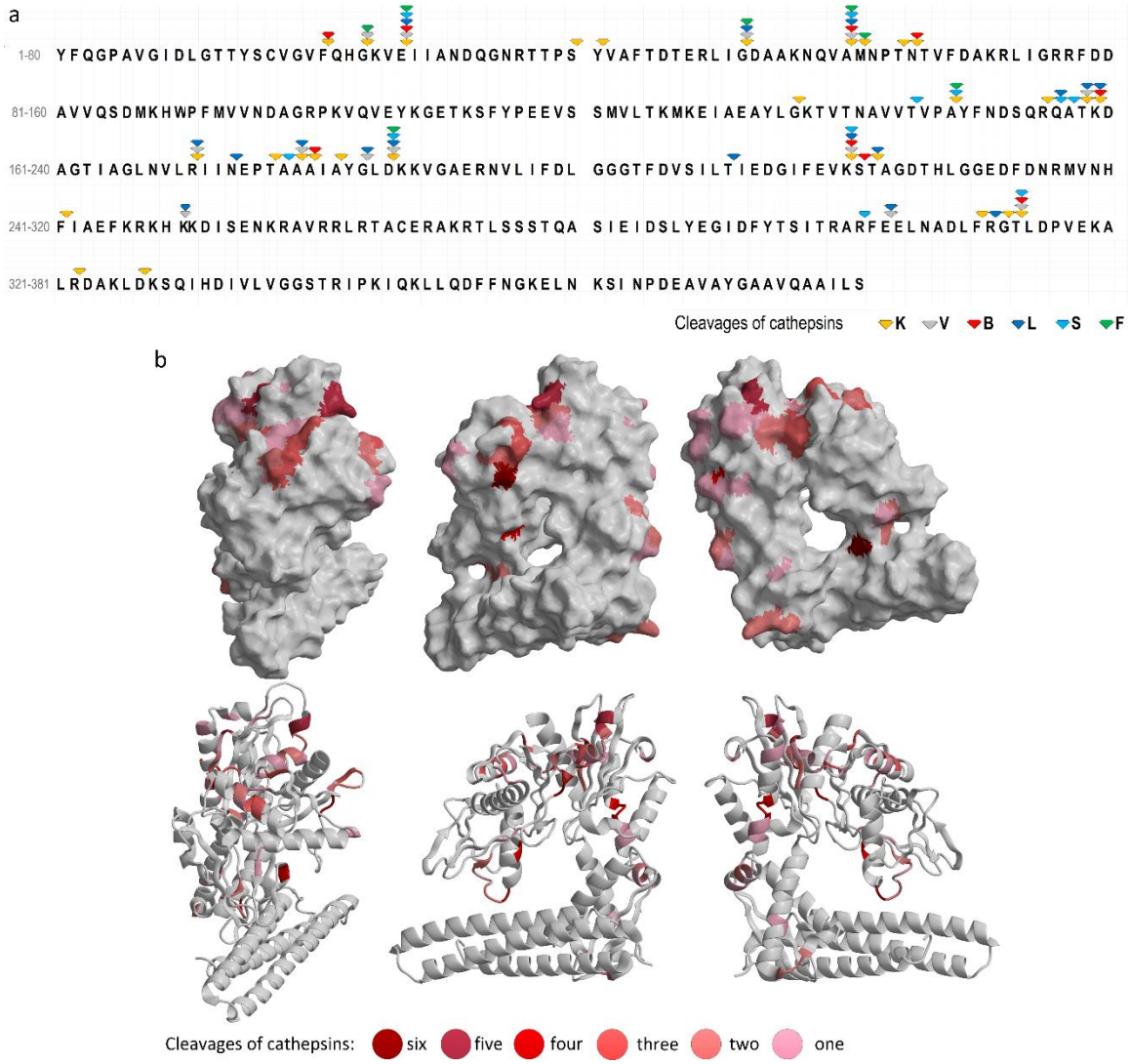
b. Cleavage site separations of individual cathepsins. The column diagram presents the share of neighborhood cleavage sites within the region of P15 to P15'.

c. Cleavage site separations of combinations of two cathepsins. The column diagram presents the shares of cleavages of the second cathepsin in the pair in the region of P15 to P15'.

d. The percentage of exposed surface area.

e. The percentage of cleavages with respect to the secondary structure.

These cleavage sites were analyzed for their solvent accessibility and secondary structure position (d and e) using MAIN[23]. The figures were generated with Excel.

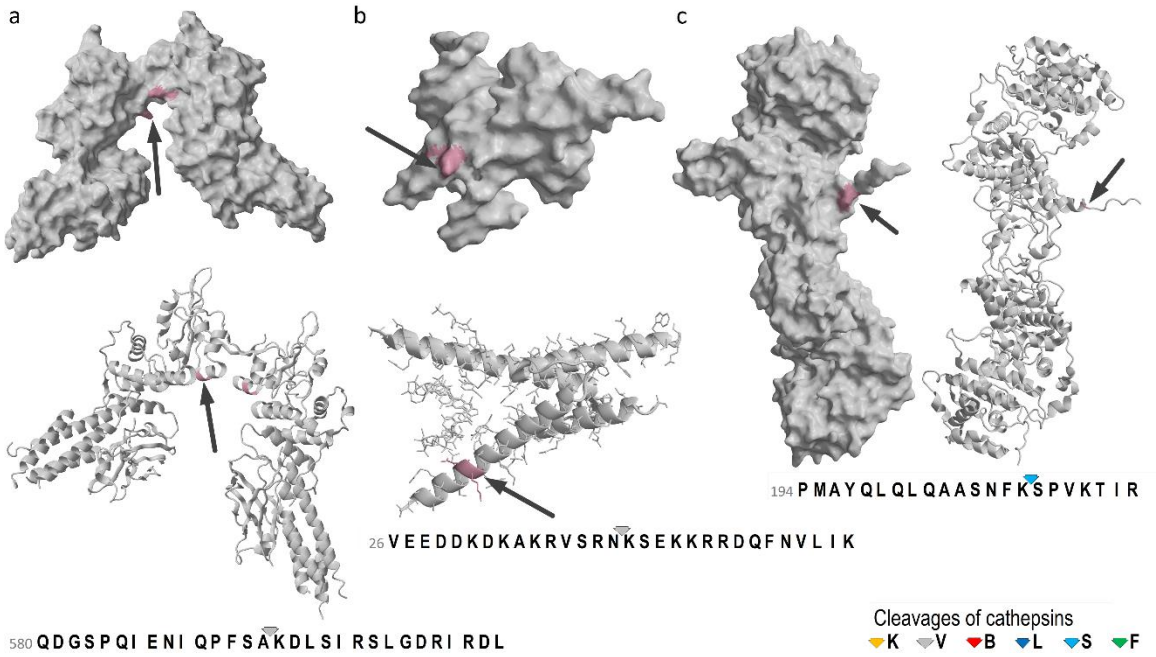


Supplementary Fig. 2. Protein with unique and shared cleavage sites.

a. Unique and shared cleavages mapped on the sequence. There were 41 cleavage sites in chain A and none in chain B in the 71 kDa heat shock "cognate" protein (PDB code 3LDQ and UniProt code P11142)[26].

b. Unique and shared cleavage cases mapped on the 3D structure. Three views of the structure of the nucleotide binding domain of the 72 kDa heat shock "cognate" are shown. Protein structures are shown in surface and ribbon presentations. Color coding shows the cleavage site residues P1 and P1', which are colored in 6 shades of red. The color shade corresponds to the number of cathepsins that performed the cleavage. Pale red corresponds to cleavage by one cathepsin, whereas the dark red areas were cleaved by all six cathepsins. The remainder of the structure is colored white.

The figures were generated with MAIN[23] and rendered with RASTER 3D[35].



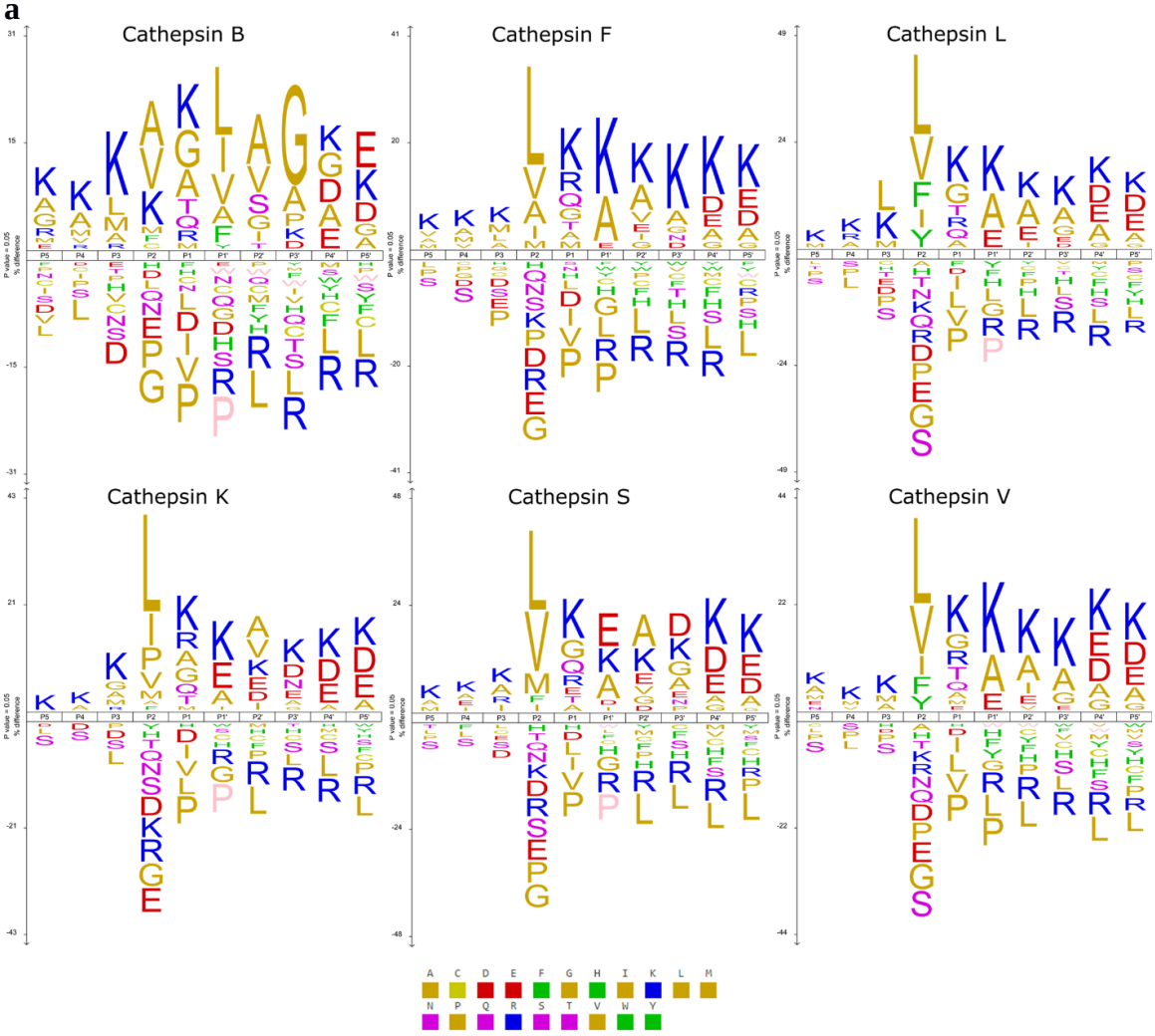
Supplementary Fig. 3. Single cleavage cases mapped on the 3D structures. The structure presentation and color coding are the same as in Fig. 1. The figures were generated with MAIN[23] and rendered with RASTER 3D[35].

a. Homodimer of the signal transducer and activator of the transcription 6 core fragment, namely, $STAT6^{CF}$ (PDB code 4Y5U and UniProt code P42226)[68], which was cleaved by cathepsin V between A594 and K595 within the sequence string PFSA↑KDLS of STAT6. The phosphorylated dimer of the STAT6 core fragment binds DNA within the cleaved region. This cleavage likely disrupted the dimeric structure of STAT6 and may have prevented the binding of DNA and, consequently, its transcription. The site is marked with a black arrow.

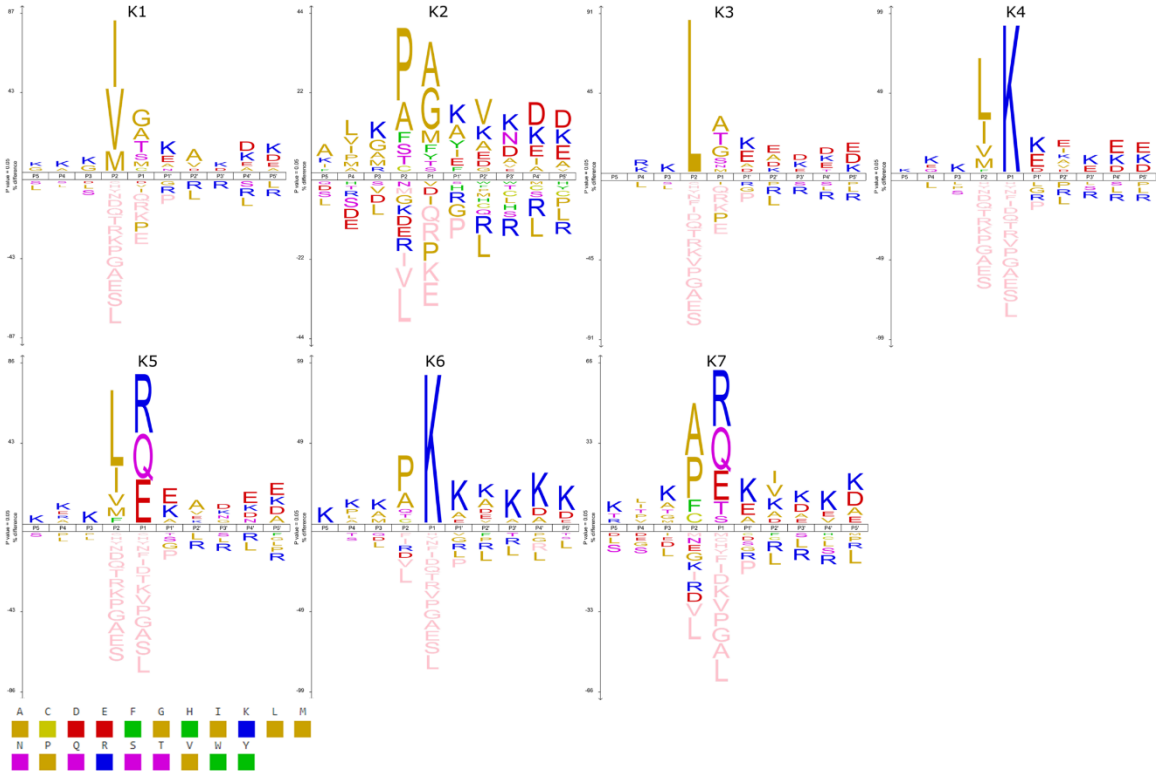
b. Human *c*ircadian locomotor output cycle kaput and brain and muscle ARNT-like 1 CLOCK-BMAL1 domain structures with E-box DNA (PDB code 4H10 and UniProt code O15516)[69], which was cleaved by cathepsin V between N40 and K41 within the sequence string VSRN↑KSEK in the CLOCK domain. This cleavage likely disrupted the structure of the DNA binding site and hints at either a regulative or disruptive role of cathepsin V in transcription. The site is marked with a black arrow.

c. β subunit of protein kinase CK2 (PDB code 4DGL and UniProt code P67870)[70], which was cleaved by cathepsin S between K208 and S209 in the middle of the SNFK↑SPVKT region, which contains three phosphorylation sites, among which S209 was shown to be related to enhancement of CK2 kinase activity in prostate cancer cells. The site is marked with a black arrow.

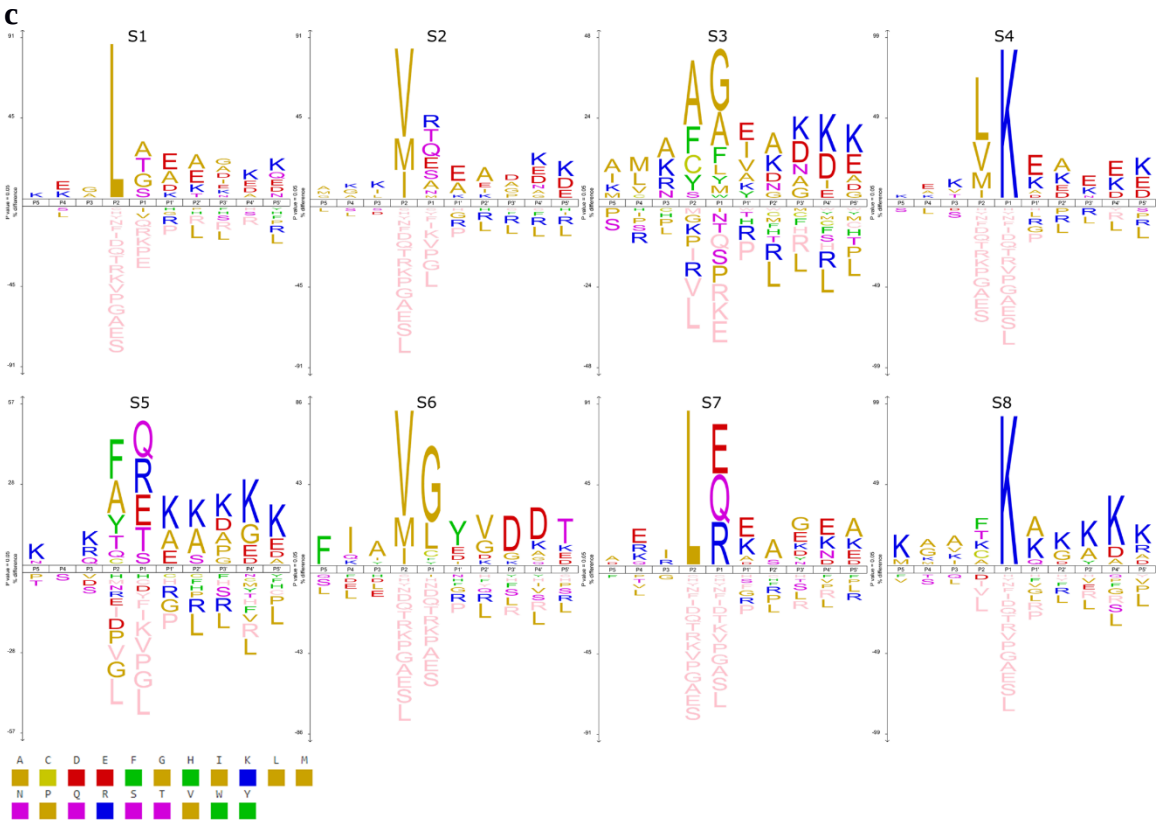
a


Cathepsin B
Cathepsin F
Cathepsin L
Cathepsin K
Cathepsin S
Cathepsin V
P5 P4 P3 P2 P1 P1' P2' P3' P4' P5'
P value = 0.05
% difference
A C D E F G H I K L M
N P Q R S T V W Y

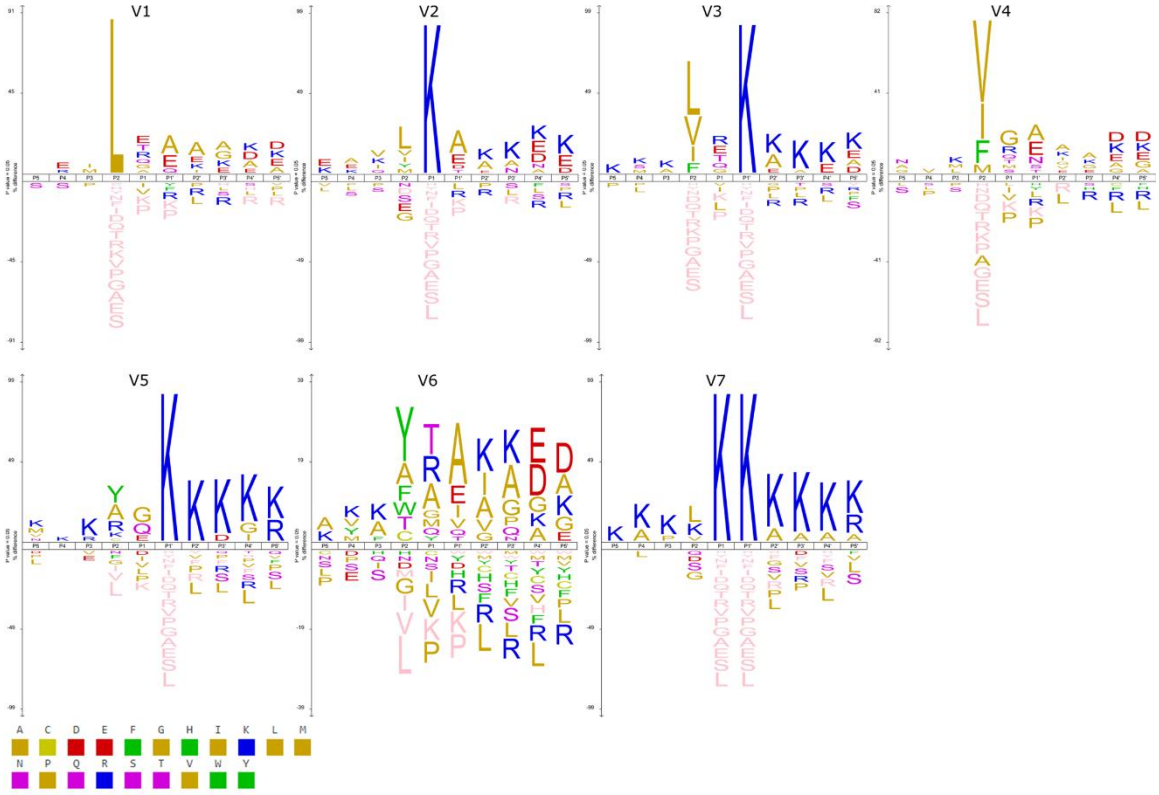
b


K1
K2
K3
K4
K5
K6
K7
A C D E F G H I K L M
N P Q R S T V W Y

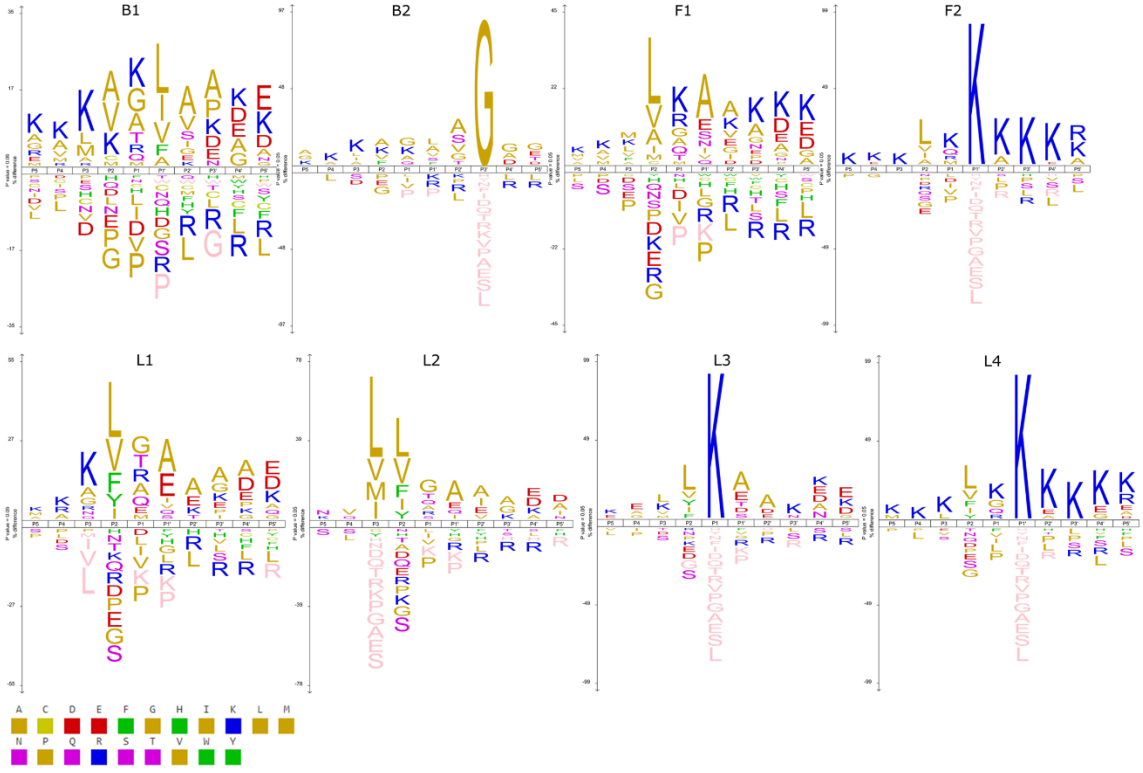
c


S1
S2
S3
S4
S5
S6
S7
S8
A C D E F G H I K L M
N P Q R S T V W Y

d

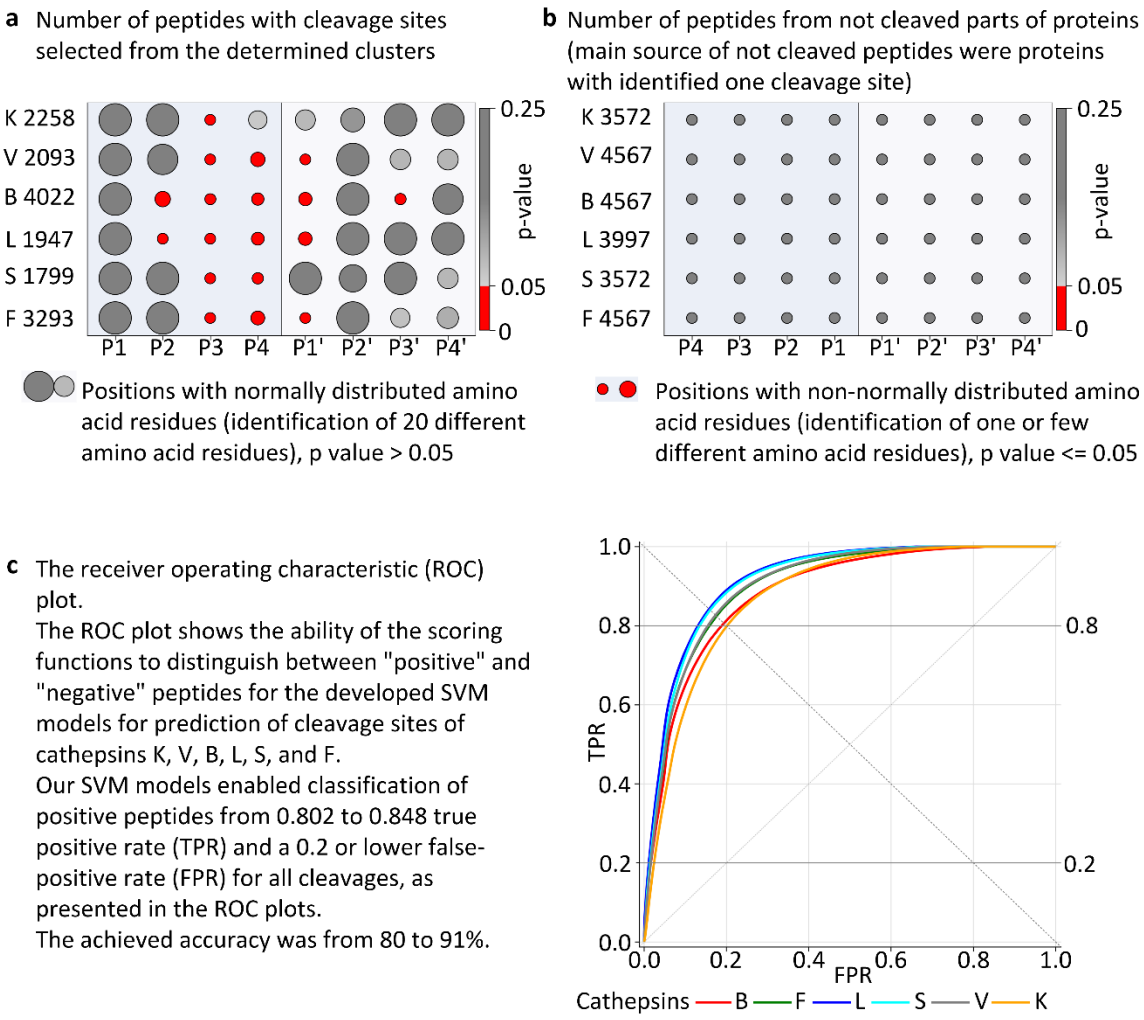

V1
V2
V3
V4
V5
V6
V7
A C D E F G H I K L M
N P Q R S T V W Y

e

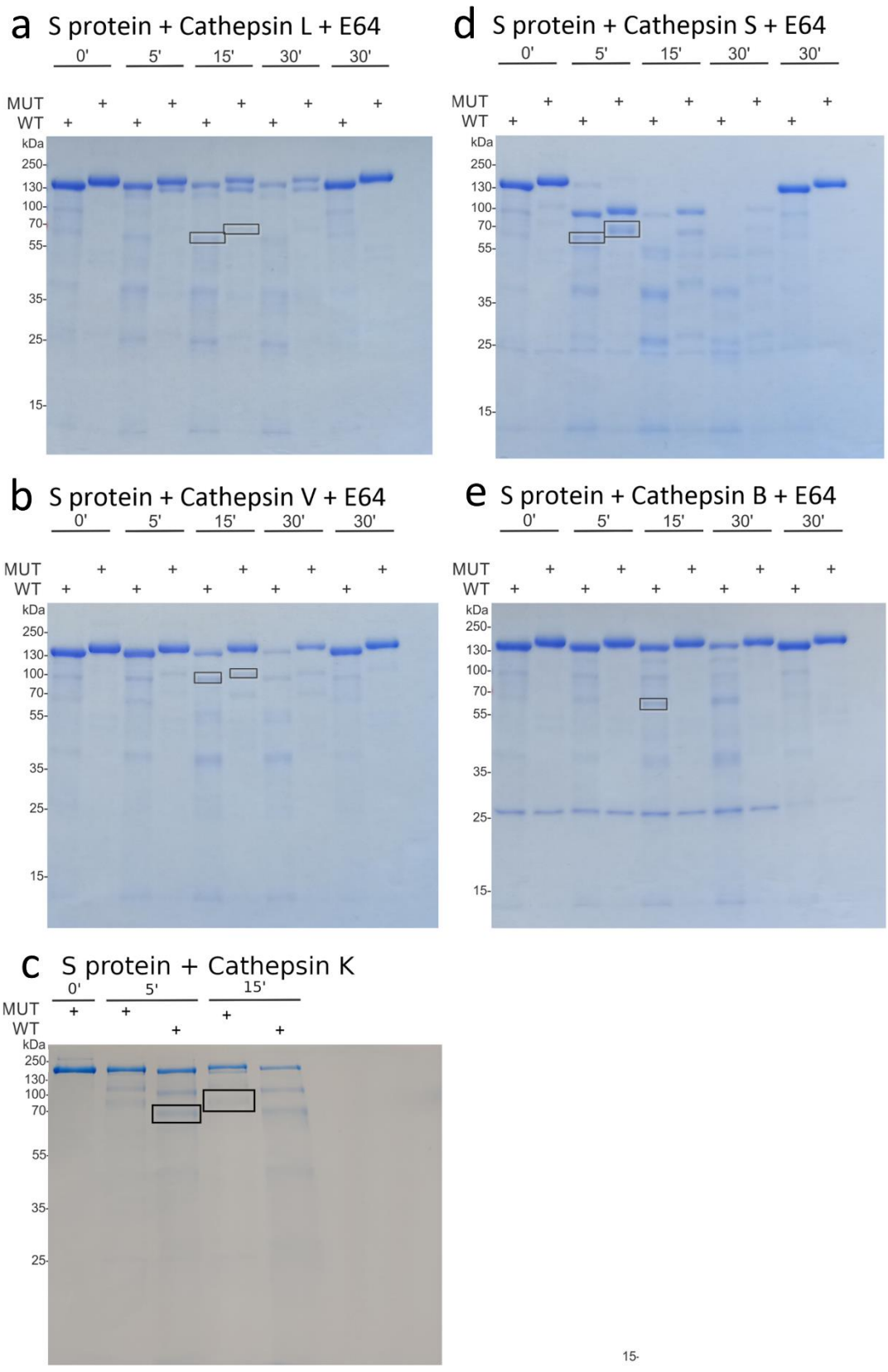

B1
B2
F1
F2
L1
L2
L3
L4
A C D E F G H I K L M
N P Q R S T V W Y

Supplementary Fig. 4. iceLogo plots of datasets of substrates of cathepsins K, V, B, L, S, and V (a) and its belonging 30 clusters (b, c, d, e). The clusters of substrates of cathepsins K are marked as K1, K2, K3, K4, K5, K6, and K7 (**b**); of cathepsin S as S1, S2, S3, S4, S5, S6, S7 and S8 (**c**); of cathepsin V as V1, V2, V3, V4, V5, V6, and V7 (**d**); of cathepsin B as B1 and B2, of cathepsin F as F1 and F2, and of cathepsin L as L1, L2, L3, and L4 (**e**)[14].

The plots visualize significantly different residue frequencies (above the abscissa) and significantly under represented residues (below the abscissa). The character size corresponds to the percentage of difference from the average amino acid occurrence. P value was set to 0.05.

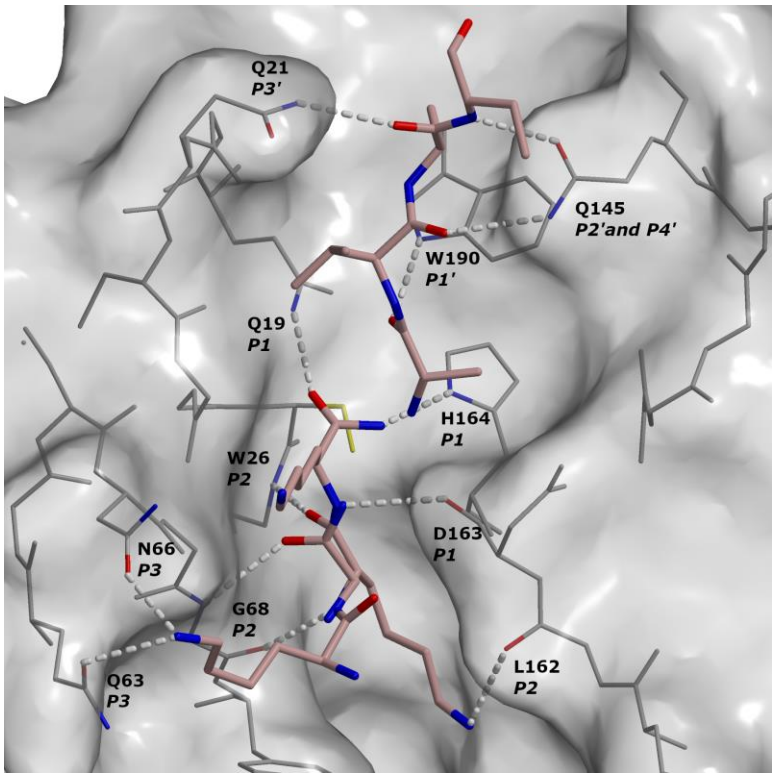


Supplementary Fig. 5. The selection of training sets for developed SVM models and achieved ability of prediction of cleavage sites for substrates of cathepsins K, V, B, L, S, and F on the basis of presented receiver operating characteristic (ROC) plot (the plot was calculated by using PCSS server[16]).

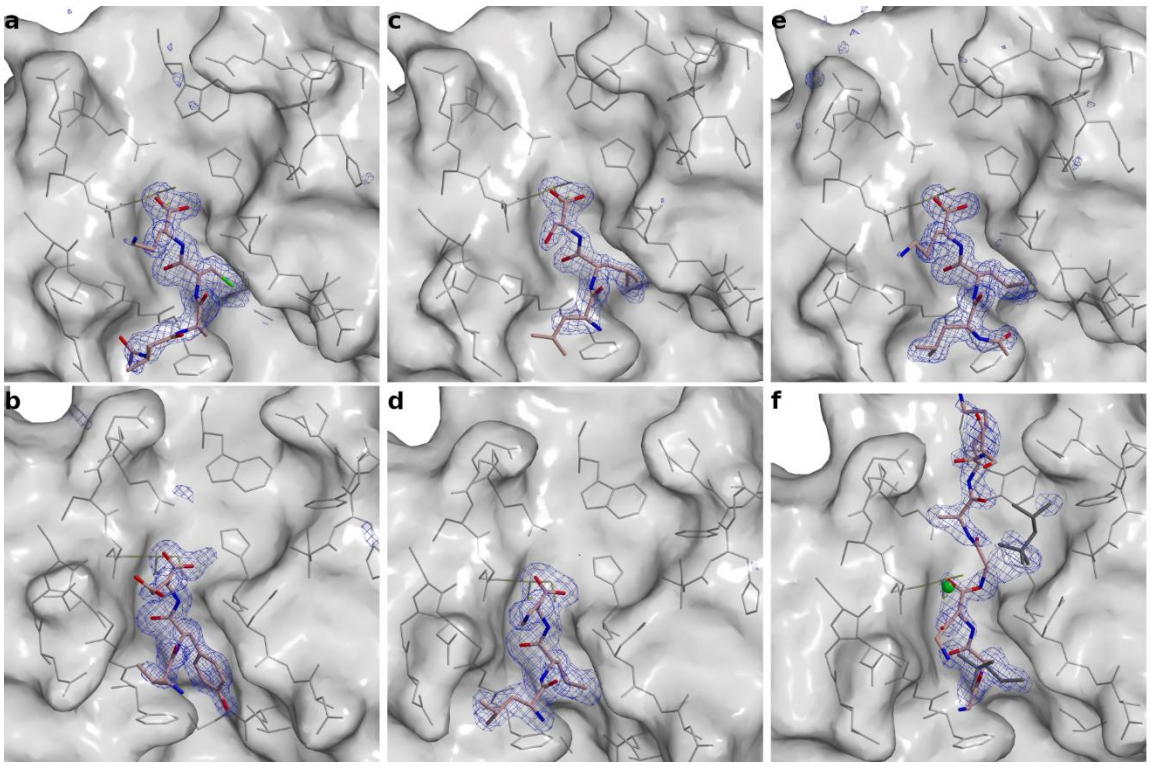


Selected samples for N terminal sequence analysis ☐

Supplementary Fig. 6. SDS page analysis of SARS-CoV-2 S protein degradation/processing; Cleavages of SARS-CoV-2 wild-type (WT) and mutated furin cleavage site (MUT) S proteins.
a. SDS–PAGE analysis of the processing of WT and MUT S proteins by cathepsins L,
b. Cathepsin V,
c. Cathepsin K,
d. Cathepsin S, and
e. Cathepsin B are presented.
WT and MUT were loaded in air on SDS–PAGE columns at 0, 5, 15 or 30 min after incubation with cathepsins. The control samples of S protein (WT and MUT) contain in addition cathepsin inhibitor E-64 (second number 30' in panels a, b, d, and e). Assuming that the first degrading product corresponds to the S protein N-terminal fraction, the second fragments were analyzed by N-terminal sequencing (analyzed samples are marked with black boxes).



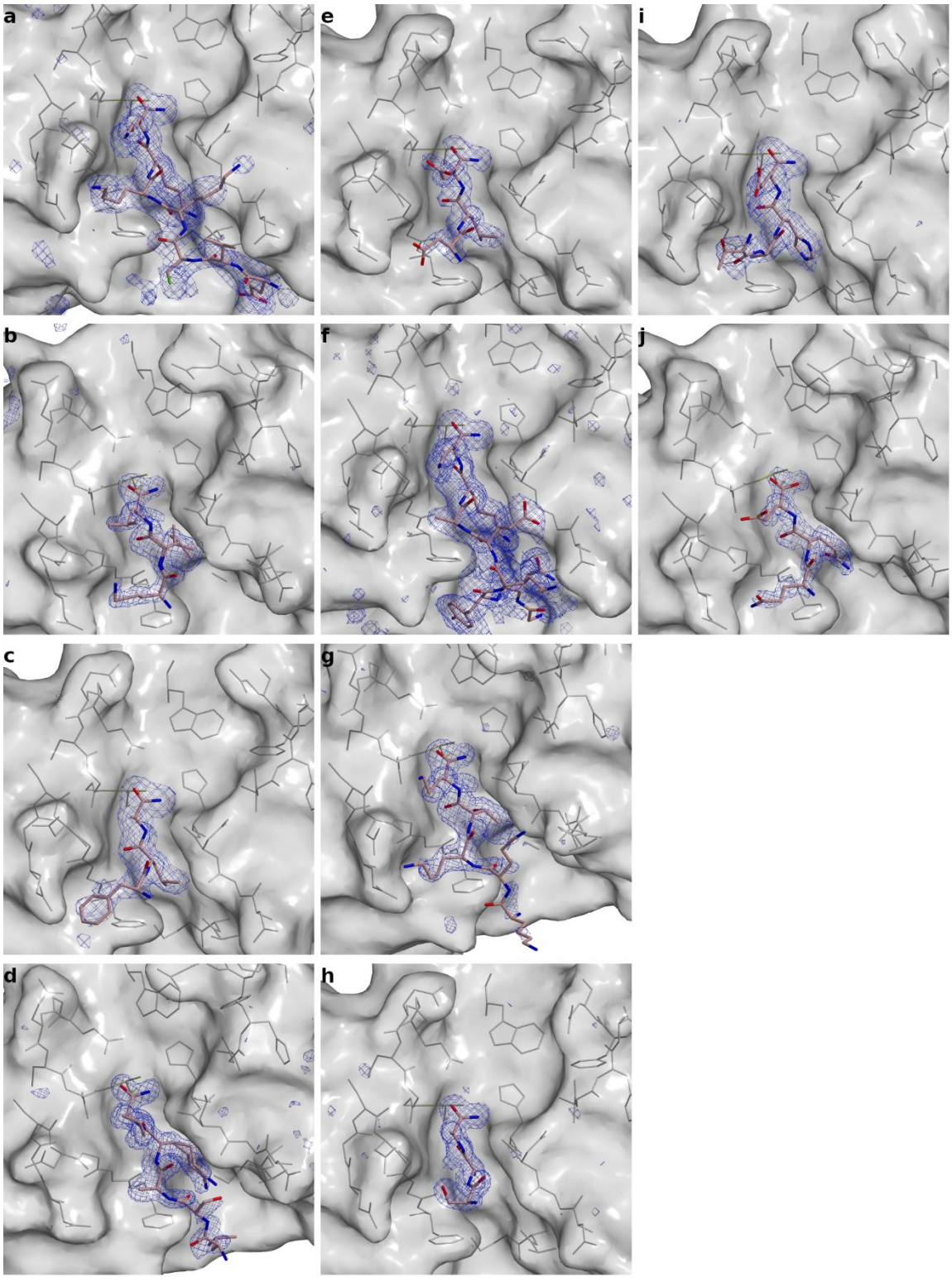
Supplementary Fig. 7. H-bonding pattern between cathepsin V and substrates. Peptide fragments KKK (P3–P1) and AVAE (P1'–P4') are shown on the surface of a semi-transparent cathepsin V structure. Interacting oxygen, nitrogen, and carbon atoms of peptides and cathepsin are shown in red, blue, rose and gray, respectively. H-bonds are presented as white dashed lines. At position P2, two main chain conformations are presented. In one conformation, the H-bond is formed between the O of P2 and the N of W26, and in the other, the O of P2 forms H-bond with the N of G68. Cathepsin residues that participated in peptide H-bonding are marked with sequence IDs and the peptide position. The mutant of catalytic residue S25 is highlighted in yellow. Figures in the panel were prepared using MAIN[23] and rendered using Raster 3D[35].



Supplementary Fig. 8. Electron density maps of peptides in the group of pattern I. Peptide nitrogen atoms are shown in blue, oxygen atoms in red, and carbon atoms in pale pink.

a. Fragment VACK of peptide VACKSSQP (structure 7QFF).
b. Fragment VYE of peptide VYEKKP (structure 7QNS).
c. Fragment LLS of peptide LLSGKE (structure 7Q8O).
d. Fragment LLK of peptide LLKVAL (structure 7Q8K).
e. Fragment LLK of peptide LLKAVAEKQ (structure 7Q9H).
f. Peptide GAK of peptide GAKSAA (structure 7QO2) is shown in the non-primed site. Primed site is occupied by peptide GAKSAA, belonging to a group of pattern IV.

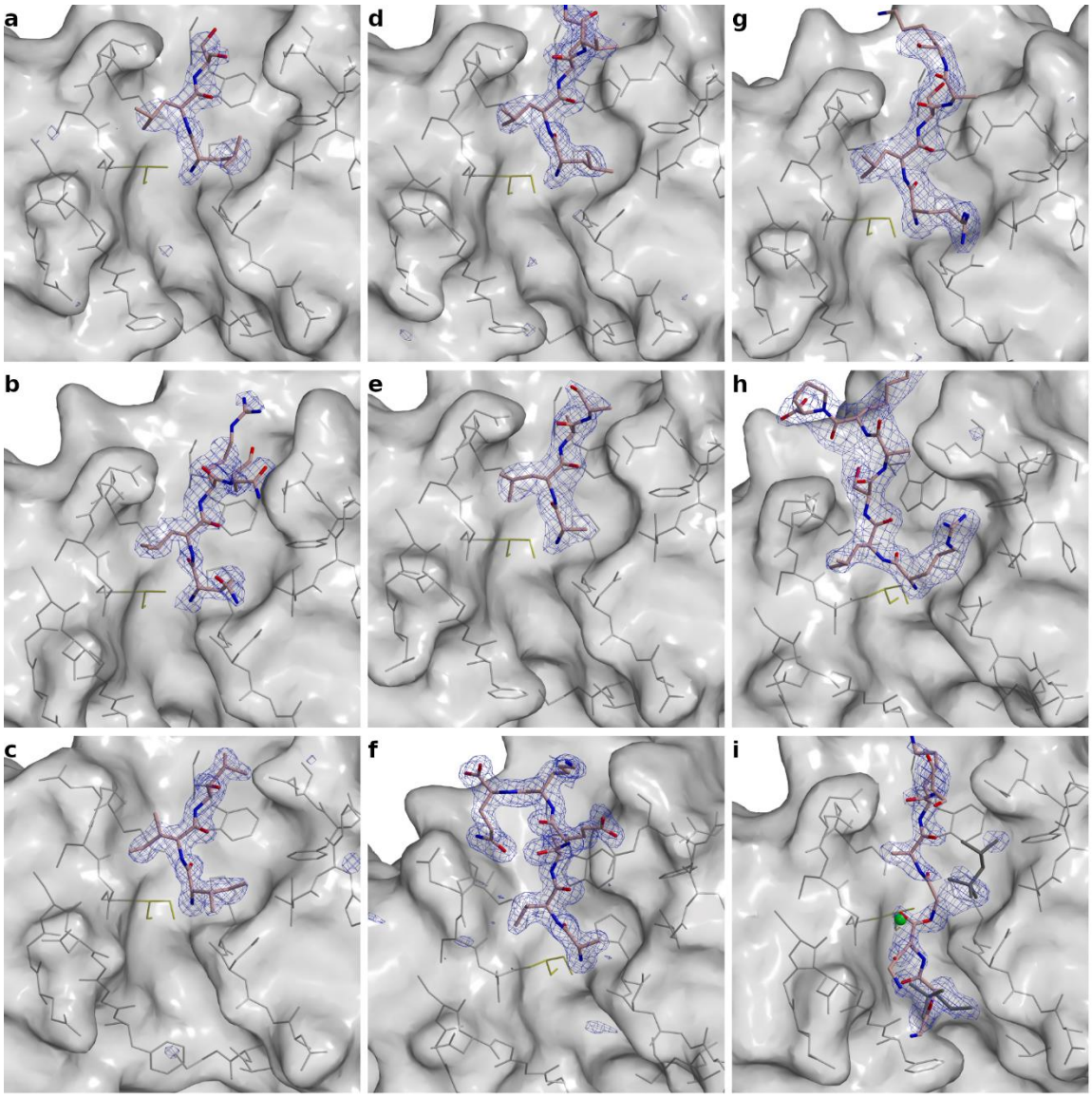
Electron densities were constructed using free kick omit Fo–Fc map[39]. Figures in the panel were prepared using MAIN[23] and rendered using Raster 3D[35].


a
e
i
b
f
j
c
g
d
h

Supplementary Fig. 9. Electron density maps of peptides in the group of pattern II. Peptide nitrogen atoms are shown in blue, oxygen atoms in red, and carbon atoms in pale pink.

a. Peptide EVCKKKK (structure 7Q8H).
b. Fragment KVL of peptide AYFKKVL (structure 7QFH).
c. Fragment FLA of peptide KKYDAFLA (structure 7Q8N).
d. Fragment LSAKP of peptide RLSAKP (protected) (structure 7Q9C).
e. Fragment DLE of peptide TRESEDLE (structure 7Q8D).
f. Peptide GNYKEAKK (structure 7Q8F).
g. Fragment KKKTK peptide KPKKKTK (structure 7Q8M).
h. Fragment SAA of peptide GAKSAA (structure 7QHJ).
i. Fragment TAHE of peptide VPCGTAHE (structure 7Q8L).
j. Fragment QQE of peptide QLRQQE (structure 7QHK).

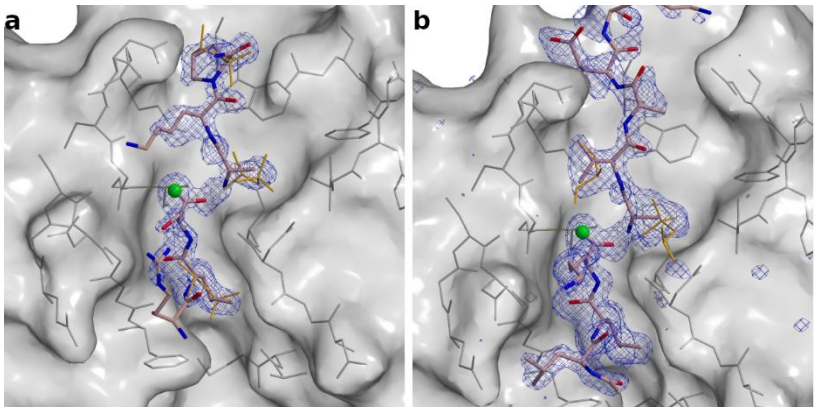
Electron densities were constructed using free kick omit Fo–Fc map[39]. Figures in the panel were prepared using MAIN[23] and rendered using Raster 3D[35].



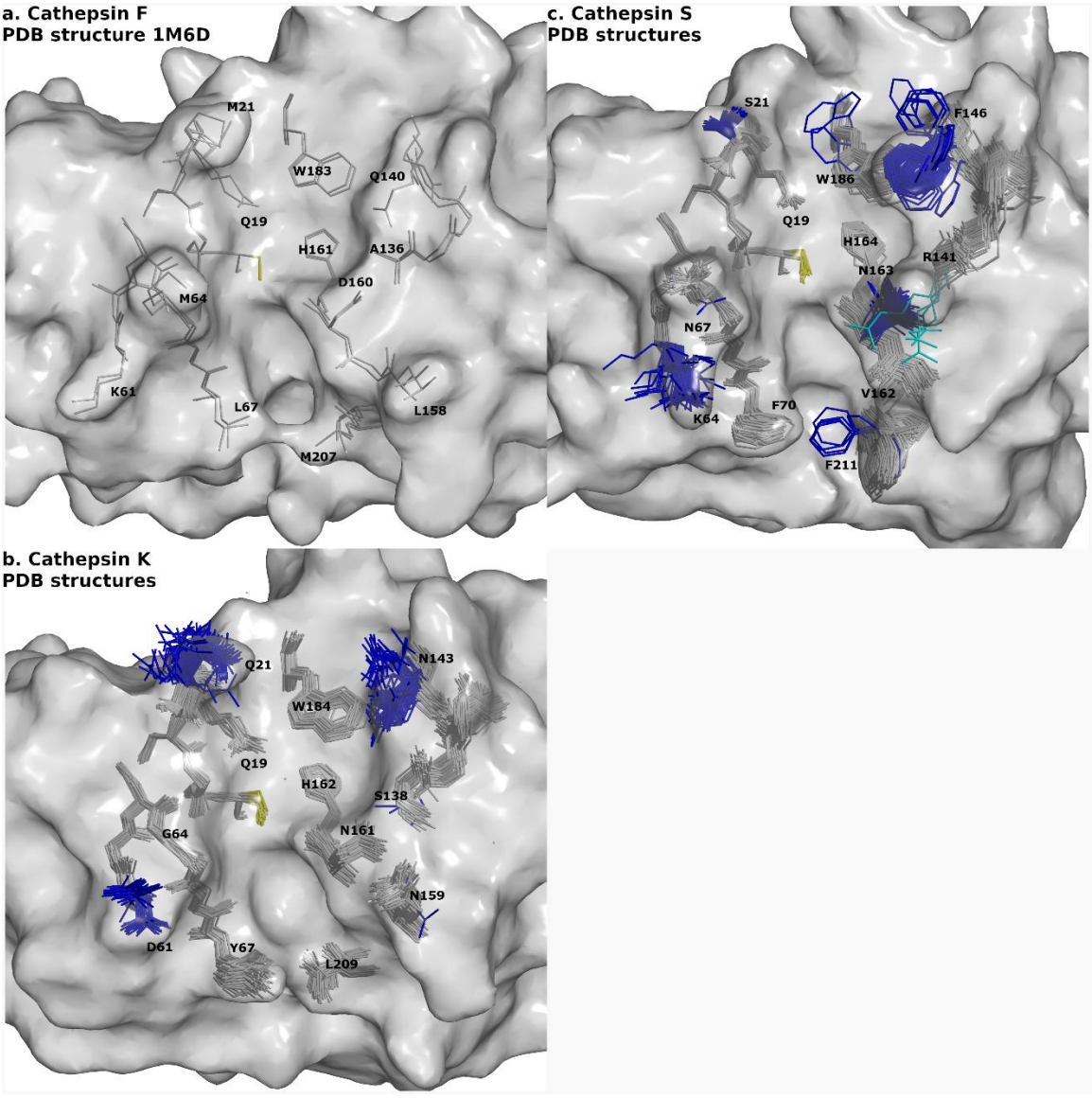
Supplementary Fig. 10. Electron density maps of peptides in the group of pattern III. Peptide nitrogen atoms are shown in blue, oxygen atoms in red, and carbon atoms in pale pink.

a. Fragment LLS of peptide LLSGKE (structure 7Q8O).
b. Fragment QLRQ of peptide QLRQQE (structure 7QHK).
c. Fragment IIL of peptide IILKEK (structure 7Q8J).
d. Fragment LLKV of peptide LLKVAL (structure 7Q8P).
e. Fragment ALAA of peptide ALAASS (structure 7Q8G).
f. Peptide AVAEKQ (structure 7Q8I).
g. Fragment RLSAK of peptide RLSAKP (non-protected; molecule A) (structure 7Q8Q).
h. Peptide RLSAKP (non-protected; molecule B) (structure 7Q8Q).
i. Fragment GAKS of peptide GAKSAA is shown in the primed site (structure 7QO2).

Non-primed site is occupied by peptide GAKSAA, belonging to group of pattern I. Electron densities were constructed using free kick omit Fo–Fc map[39]. Figures in the panel were prepared using MAIN[23] and rendered using Raster 3D[35].



Supplementary Fig. 11. Electron density maps of peptides in the group of pattern IV. Peptide nitrogen atoms are shown in blue, oxygen atoms in red, and carbon atoms in pale pink.
a. Fragments RLS and AKP of peptide RLSAKP (structure 7Q9C).
b. Fragments LLK and AVAEKQ of peptide LLKAVAEKQ (structure 7Q9H).
Electron densities were constructed using free kick omit Fo–Fc map[39]. Figures in the panel were prepared using MAIN[23] and rendered using Raster 3D[35].



Supplementary Fig. 12. Flexible and rigid residues of cathepsins K, S and F from PDB database. Surfaces of cathepsins are colored gray. Cathepsin residues are presented with bond models. Flexible residues around the active site are highlighted in blue. Catalytic residues at site 25 are shown in yellow.

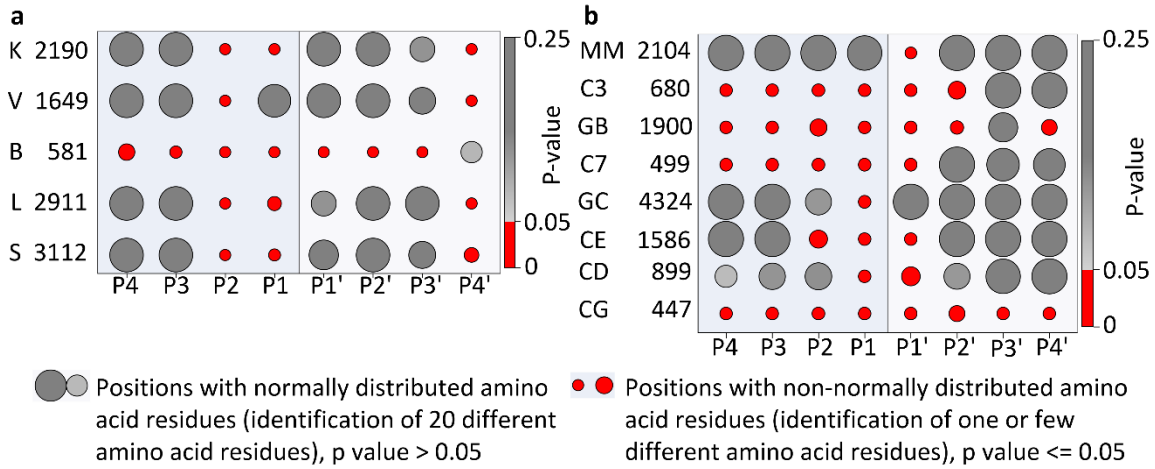
a. Cathepsin F (PDB entry *1M6D*).

b. Cathepsin K (*PDB entries 1BGO, 1NLJ, 1ATK, 1AU0, 1AU2, 1AU3, 1AU4, 1AYU, 1AYV, 1AYW, 1BY8, 1MEM, 1NL6, 1Q6K, 1SNK, 1TU6, 1U9V, 1U9W, 1U9X, 1YK7, 1YK8, 1YT7, 2ATO, 2AUX, 2AUZ, 2BDL, 2F7D, 2FTD, 2R6N, 3C9E, 3H7D, 3KW9, 3KWB, 3KWZ, 3KX1, 3O0U, 3O1G, 3OVZ, 4DMX, 4DMY, 4N79, 5N8W, 4X6H, 4X6I, 4X6J, 4YV8, 4YVA, 5J94, 5JA7, 5JH3, 5TDI, 5TUN, 5Z5O, 6ASH, 6HGY, 6PXF, 6QBS, 6QL8, 6QLM, 6QLW, 6QLX, 6QM0, 7NXL, 7NXM, 7PCK.*

c. Cathepsin S (*PDB entries 2HXZ, 2F1G, 2FT2, 3OVX, 2R9N, 2R9M, 1MS6, 2HHN, 4P6G, 2OP3, 4P6E, 6YYN, 2HH5, 2G7Y, 2FQ9, 6YYR, 6YYP, 2H7J, 2FRQ, 3N3G,*

2R9O, 2FRA, 6YYO, 3N4C, 2FUD, 1NPZ, 1NQC, 5QC0, 5QCH, 5QC4, 5QC2, 2C0Y, 5QCG, 5QCE, 5QCI, 5QCC, 5QCA, 5QC7, 5QBV, 5QC5, 5QBZ, 5QBX, 5QC9, 5QC3, 5QC1, 3IEJ, 5QCF, 5QCJ, 5QCD, 5QCB, 5QBW, 5QC8, 5QC6, 5QBU, 2G6D, 5QBY, 1GLO, 2FYE. Only three residues of flexible R141 are presented in cyan (entries *2FUD* and *5QCA*) for clarity of the figure.

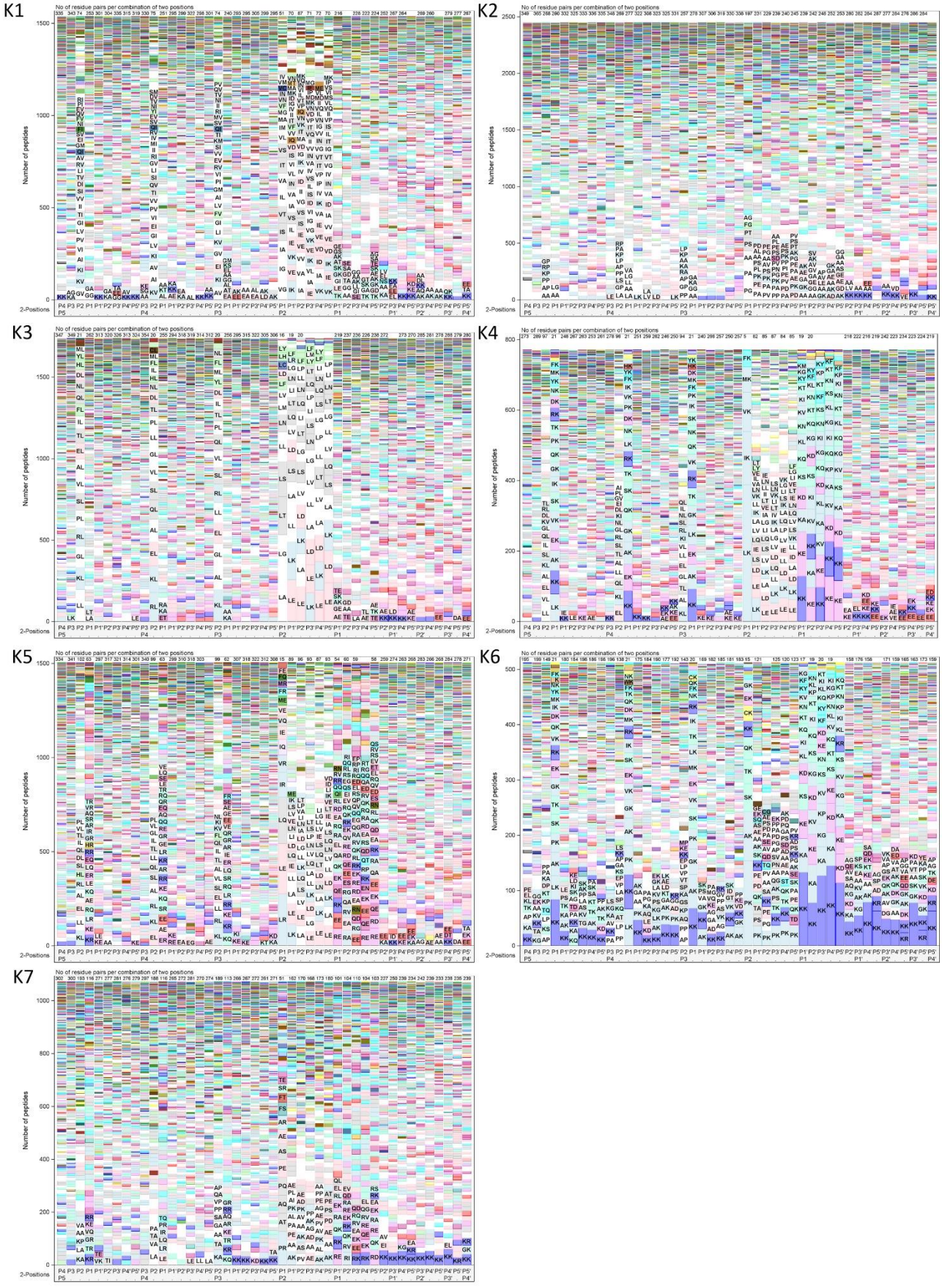
Figures in the panel were prepared using MAIN[23] and rendered using Raster 3D[35].

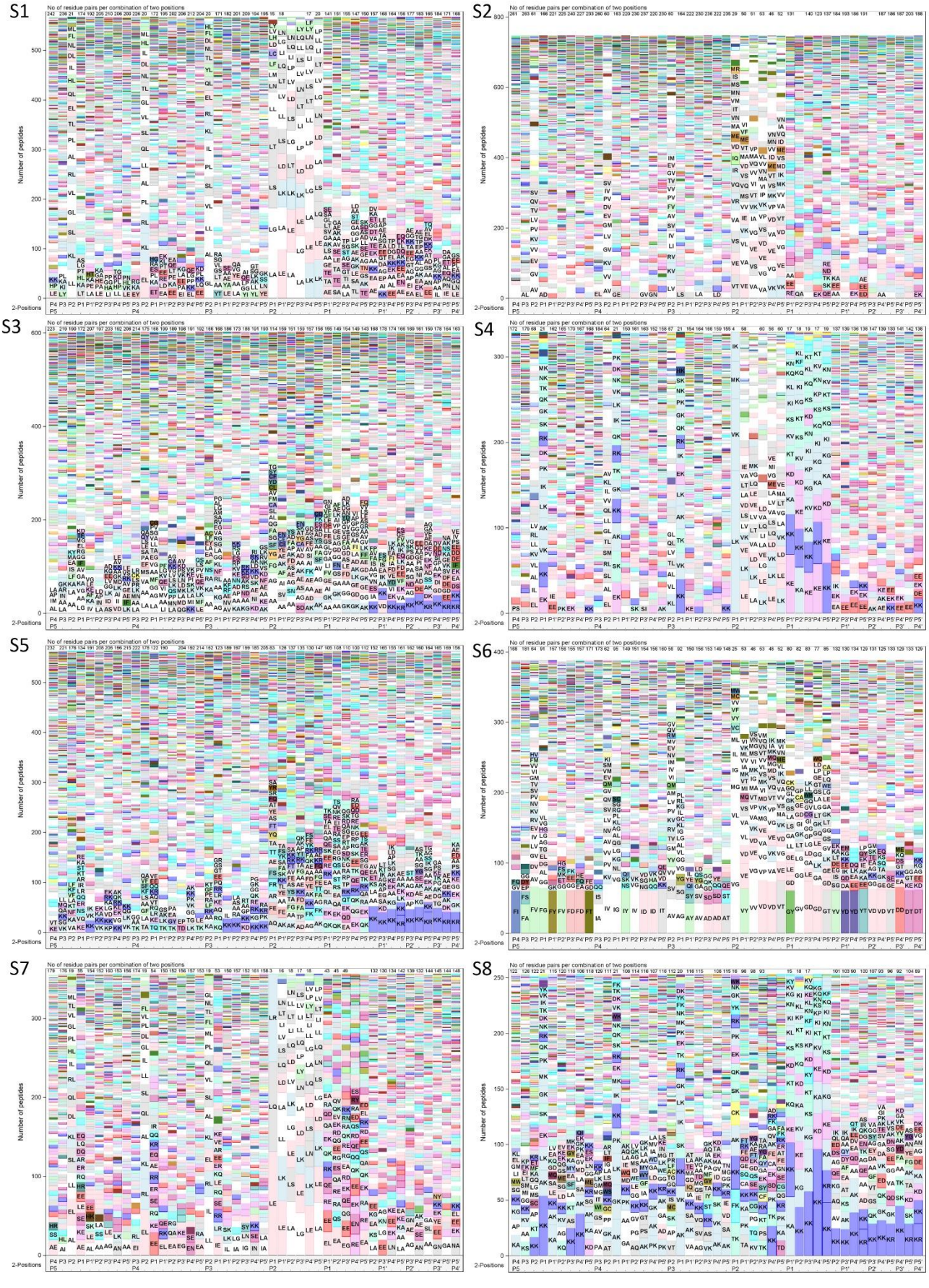


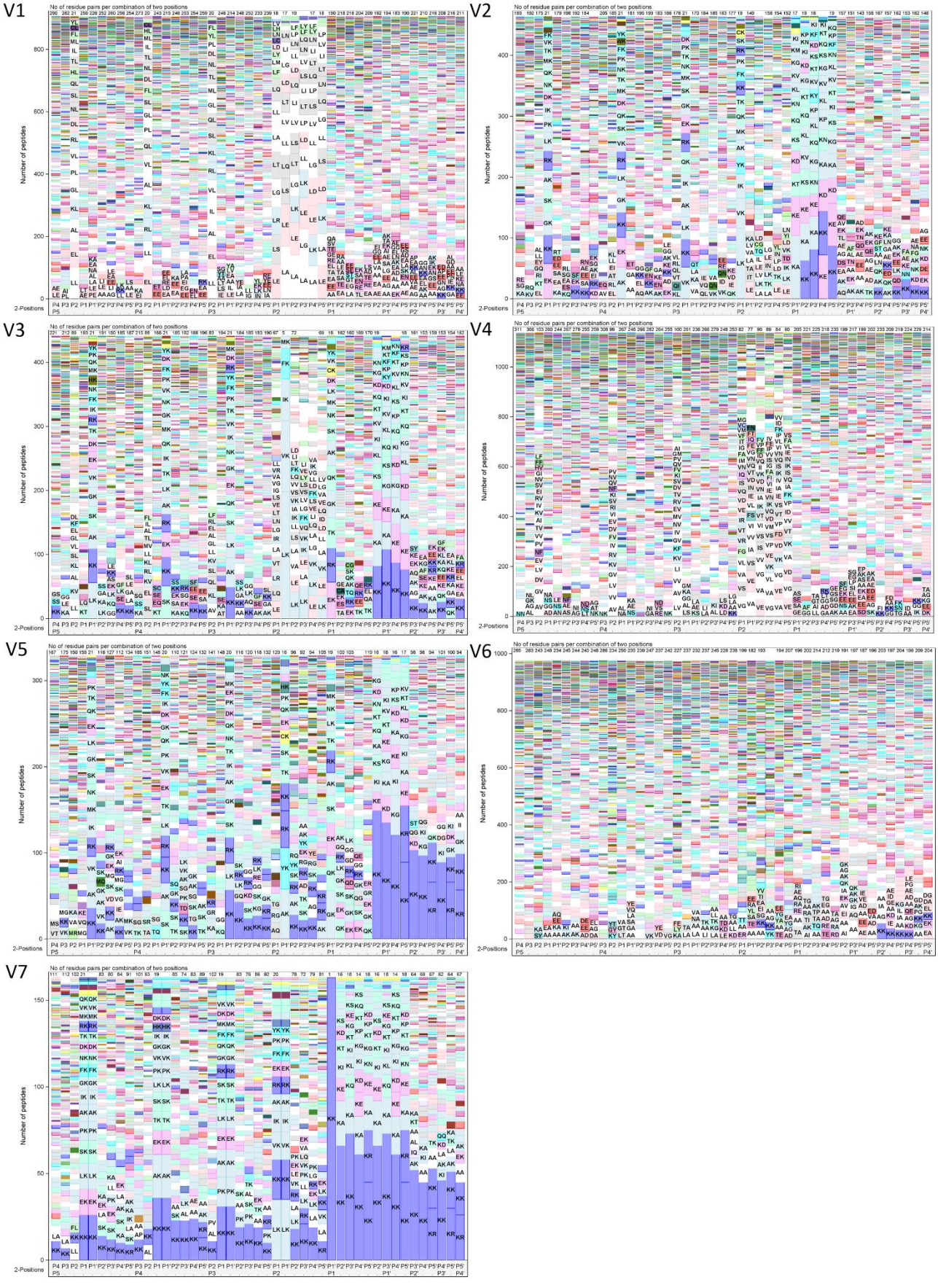
Supplementary Fig. 13. Calculated heterogeneous and homogeneous positions of substrates for selected enzymes downloaded from MEROPS database[40] **(https://www.ebi.ac.uk/merops/).** The substrate datasets for the following enzymes were used (the substrates without UniProt code were dropped out):

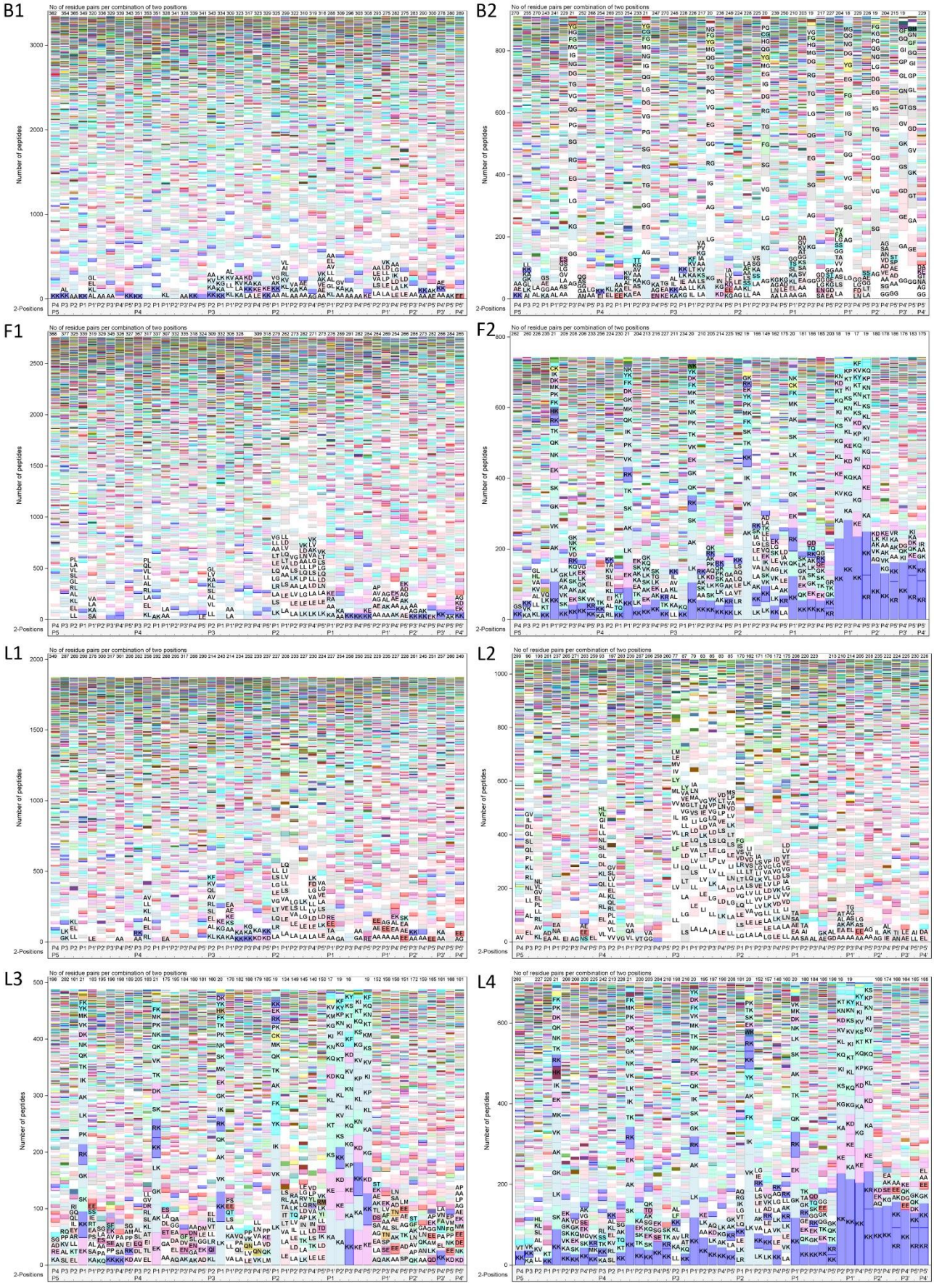
a. Cathepsins K (K), V (V), B (B), L (L), and S (S);

b. Peptidyl-Lys metallopeptidase (MM) which has on P1' only lysine, consequently p value was not calculated. To get a red spot we put p value equal to 0.0000000001; caspase-3 (C3); granzyme B (Homo sapiens-type) (GB); caspase-7 (C7); glutamyl endopeptidase I (GC); cathepsin E (CE); cathepsin D (CD); cathepsin G (CG).

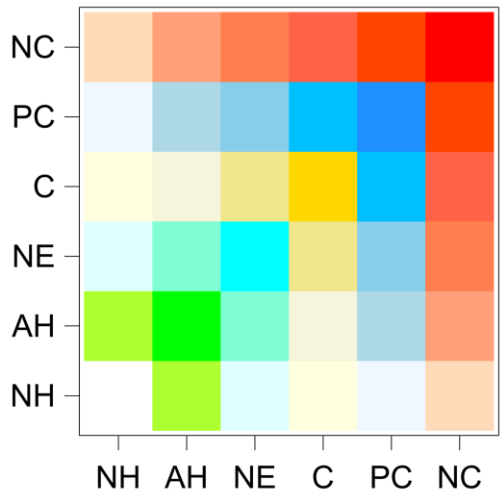

K1
K2
K3
K4
K5
K6
K7
No of residue pairs per combination of two positions
Number of peptides
2-Positions


S1
S2
S3
S4
S5
S6
S7
S8
No of residue pairs per combination of two positions
Number of peptides
2-Positions

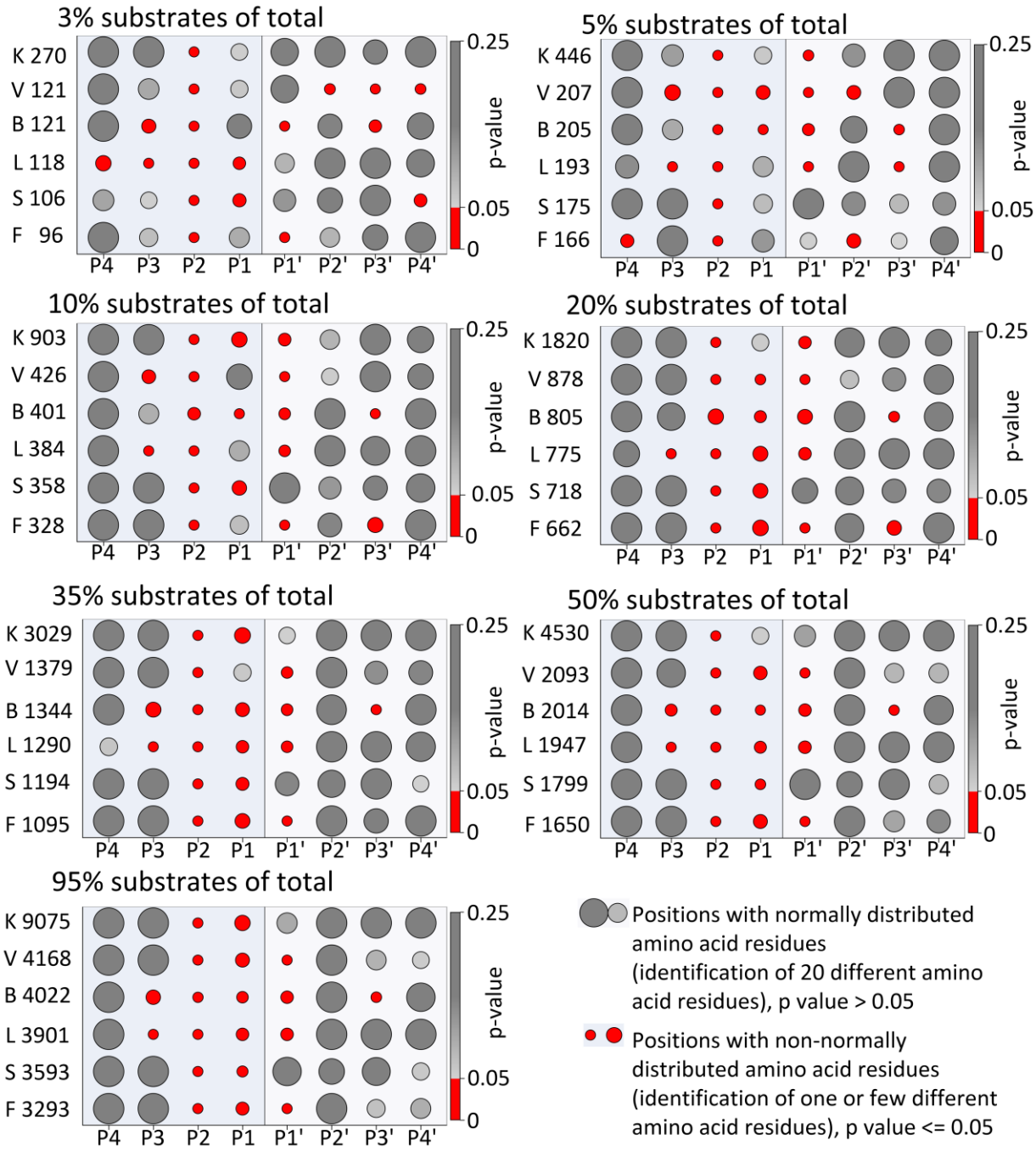

V1
V2
V3
V4
V5
V6
V7
No of residue pairs per combination of two positions
Number of peptides
2-Positions


B1
B2
F1
F2
L1
L2
L3
L4
No of residue pairs per combination of two positions
Number of peptides
2-Positions

Supplementary Fig. 14. Combinations of pair positions with pair residues for clusters of substrates of cathepsins K, S, V, B, F, and L. Each cluster presents bars of residue pairs at combinations of two sites in the region from P5 to P5'. The number of pairs is specified at the top of each bar, whereas combinations of positions are presented at the bottom of each bar. Each bar is composed of blocks, where each block represents a specified combination of amino acid residues. The size of the block corresponds to the occurrence of the pair. For pairs that occurred in blocks that are large enough to include characters of font size 12, the amino acid pair codes are shown. The colors of the pairs represent the chemical characteristics of the amino acid residues, namely, hydrophobic NH (white), hydrophilic NE (cyan), negatively charged NC (red), positively charged PC (blue), aromatic hydrophobic AH (green) and cysteine C (yellow), as presented in the small 6x6 color scheme.



The clusters of substrates of cathepsins K are marked as K1, K2, K3, K4, K5, K6, and K7; of cathepsin S as S1, S2, S3, S4, S5, S6, S7 and S8; of cathepsin V as V1, V2, V3, V4, V5, V6, and V7; of cathepsin B as B1 and B2, of cathepsin F as F1 and F2, and of cathepsin L as L1, L2, L3, and L4.



Supplementary Fig. 15. **Identification of heterogeneous and homogeneous positions depend on the number and relevancy of selected peptides.** To show the importance of the selection of the adequate number of peptides for the investigation of the cathepsins' specificity the different shares of peptides from datasets divided into clusters to ensure the diversity of peptides were selected: 3%, 5%, 10%, 20%, 35%, 50% and 95%. The selection of 95% of peptides gave the same distributions of residues as it is presented in Fig. 2a.

Supplementary Table 1. Summary of the data sets of peptides for cathepsins K, V, B, L, S, and F.

a. Unique and shared cleavage sites in substrates that were cleaved by cathepsins (Cat) K, V, B, L, S, and F are presented. The columns present the number of peptides (A), number of originated proteins of peptides (B), number of unique cleavage sites (C) and its percentage share (C%), number of shared cleavages of 2 cathepsins (D) and its percentage share (D%), number of shared cleavages of 3 cathepsins (E) and its percentage share (E%), number of shared cleavages of 4 cathepsins (F) and its percentage share (F%), number of shared cleavages of 5 cathepsins (G) and its percentage share (G%), and number of shared cleavages of 6 cathepsins (H) and its percentage share (H%). The total number of identified cleavage sites was 29,674. Each cathepsin contributed between 3,500 and 9,583 cleavage sites in 3,167 proteins. A total of 1,592 of these proteins had at least a partial 3D structure in the protein structure database PDB (in January 2019, with 158,934 entries and 11,505 structures released annually[24]). In the case of multiple entries of the same protein, the entry with the highest sequence coverage and resolution was used in our analysis. The highest number of detected cleavages in one protein was 178 (cellular myosin with 1,960 residues and UniProt code P35579). Interestingly, among the shared cleavages, 243 were performed by all six cathepsins (Columns H and H (%)).

Cath	A	B	C	C%	D	D%	E	E%	F	F%	G	G%	H	H%
K	9583	2330	5182	54	1748	18	1174	12	695	7	541	6	243	3
V	4415	1501	756	17	1456	33	827	19	597	14	536	12	243	5
B	4254	1335	2192	52	829	19	480	11	313	7	197	5	243	6
L	4117	1454	780	19	1322	32	746	18	531	13	495	12	243	6
S	3805	1488	904	24	793	21	747	20	591	15	527	14	243	6
F	3500	1264	772	22	792	23	676	19	513	15	504	14	243	7
Total	29674		10586	36	6940	23	4650	16	3240	11	2800	9	1458	5

The frequencies (No) and shares (%) of combinations of shared cleavages of **b.** two, **c.** three, **d.** four, and **e.** five cathepsins are also presented. Interestingly, all possible combinations of shared cleavages of cathepsins appeared, but some were very rare.

b. Combinations of shared cleavages of two cathepsins.

Cat	Cat	No	%
V	L	961	28
K	B	459	13
K	F	395	11
K	S	363	10
K	V	318	9
K	L	213	6
S	F	156	5
B	F	145	4
B	S	135	4
V	F	74	2
V	S	73	2
L	S	66	2
B	L	60	2
V	B	30	1
L	F	22	1

c. Combinations of shared cleavages of three cathepsins.

Cat	Cat	Cat	No	%
K	V	L	359	23
K	S	F	244	16
K	B	F	153	10
V	L	S	122	8
K	B	S	120	8
K	V	S	75	5
K	V	F	73	5
V	L	F	57	4
V	B	L	54	3
K	L	S	53	3
B	S	F	52	3
K	B	L	34	2
V	S	F	32	2
K	L	F	32	2
K	V	B	31	2
L	S	F	23	1
V	B	S	16	1
B	L	S	10	1
V	B	F	8	1
B	L	F	2	0

d. Combinations of shared cleavages of four cathepsins.

Cat	Cat	Cat	Cat	No	%
K	V	L	S	166	21
K	B	S	F	126	16
K	V	L	F	119	15
K	V	S	F	97	12
K	V	B	L	62	8
V	L	S	F	59	7
K	L	S	F	56	7
V	B	L	S	30	4
K	V	B	S	27	3
K	V	B	F	20	2
K	B	L	S	12	1
K	B	L	F	10	1
V	B	S	F	9	1
B	L	S	F	9	1
V	B	L	F	8	1

e. Combinations of shared cleavages of five cathepsins.

Cat	Cat	Cat	Cat	Cat	No	%
K	V	L	S	F	363	65
K	V	B	S	F	65	12
K	V	B	L	S	56	10
K	V	B	L	F	33	6
K	B	L	S	F	24	4
V	B	L	S	F	19	3

Supplementary Table 2. Parameters of support vector machine SVM models and predictions of cathepsins' cleavage sites.

a. SVM models. The columns list the cathepsins (Cat), number of positive peptides (NoP), number of negative peptides (NoN), true positive rate critical point (TPR), false positive rate critical point (FPR), accuracy (Accuracy), and total number of testing peptides (NoT).

Cat	NoP	NoN	TPR	FPR	Accuracy (%)	NoT
K	2,253	3,526	0.802	0.198	80	10,466
V	2,081	4,508	0.833	0.167	85	2,002
B	4,006	4,508	0.809	0.192	89	1,326
L	1,938	3,948	0.848	0.152	88	1,978
S	1,792	3,526	0.84	0.160	87	1,773
F	3,277	4,508	0.828	0.173	91	993

b. **Predictions of cathepsins' cleavage sites.** The first column lists the virus and its protein with the UniProt code. The second column lists the predicted cleavage sites. Columns 3 to 8 contain the calculated false positive rates (FPRs), which are shown in bold when cleavages are predicted. In this case, the FPR values were less than or equal to 0.198 (the highest cathepsin critical value of FPR in Supplementary Table 2a). Identified cleavage sites of furin or cathepsin L or other proteases that were experimentally confirmed in the literature are marked with asterisks (*). The cleavage site that was confirmed in vitro in the JSI lab is marked with two asterisks (**). References are listed for the spike (S) protein of viruses SARS-CoV-2[9], SARS-CoV[8] and MERS-CoV[71].

		FPR of cathepsin cleavage sites					
Virus, its protein with UniProt code/cleaved by protease	Cleavage site	L	V	K	S	B	F
SARS-CoV-2, S protein, P0DTC2							
	Q675-T676	**0.118**	**0.092**	0.608	0.219	0.437	0.267
	Q677-T678	0.426	0.296	0.392	**0.160**	0.274	**0.176**
	N679-S680	0.966**	0.984	0.949	0.940	0.945	0.951
Furin[9]	R685-S686*	**0.194**	0.265	**0.107**	**0.091**	**0.167**	**0.109**
	T696-M697*	**0.162**	**0.132**	0.334	**0.172**	0.353	0.319
	M697-S698	0.406	0.425	0.677	0.576	**0.108****	0.240
	S698-L699	0.327	0.311	**0.164**	**0.068**	**0.050**	**0.063**
	G700-A701**	**0.018****	**0.018**	**0.032**	**0.021****	0.348	**0.001**
	E702-N703	0.516	0.265	**0.121**	0.220	0.352	0.208
	S816-F817	0.408	0.395	0.524	0.574	**0.096**	0.633
	F817-I818	0.684	0.868	0.441	0.345	**0.177**	0.445
SARS-CoV, S protein, P59594							
Trypsin[8]	R667-S668*	**0.080**	**0.095**	**0.139**	**0.079**	0.250	**0.060**
Cathepsin L[8]	T678-M679*	**0.111**	**0.149**	0.325	**0.177**	0.235	0.426
MERS-CoV, S protein, W6A028							
Furin[71]	R751-S752*	**0.062**	**0.043**	**0.119**	**0.013**	**0.040**	**0.019**
Furin[71]	R887-S888*	0.337	0.397	**0.163**	**0.069**	0.213	0.710

Supplementary Table 3. Selected peptide sequences for complexes with mutated cathepsin V.

The length of peptides is 6–11 residues, which are inserted in the columns at positions P5–P6'. The cleavage site is between P1–P1'. The cleavage site cluster for cathepsin V is presented under the cluster column (for example, V1 for cathepsin V cluster 1), as well as for cathepsins K, L, B, F, and S, when they had the same cleavage site. Residues that are shaded represent dominant residues in the corresponding cathepsin V cluster. If the sequence was found to have multiple cleavage sites, it is referred to as cleavage area, and if the sequence had only one cleavage site, it is referred to as positional cleavage, in the cleavage type column. Indices 1 and 2 refer to one or two separated cleavage areas in the originated protein, respectively, where cathepsin(s) acted. Indices 3 and 4 mark where there were only one or two cleavage sites in the originated protein, respectively. Termini of most peptides were protected with N-acetylation and C-amidation (marked "Y" in protection column). Some peptides were also synthesized without protection (marked "N") or with and without protection (marked "Y/N"). Peptides have UniProt codes (www.uniprot.org) of their corresponding proteins.

No	P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'	P5'	P6'	Cluster	Cleavage type	Prote-ction	UniProt
p1		T	C	L	C	Q	V	P	Q			**V1**, K3, F1, S1	Positional	Y	P49588
p2		I	L	L	T	E	A	P	L			**V1**, K3, L2, B1, F1, S1	Area	Y	Q6S8J3, P0CG38, P0CG39, Q9BYX7
p3		K	D	L	L	H	P	S	P			**V1**	Area[1]	Y	P42677
p4	E	I	D	L	R	N	P	K	G	N		**V1**, L1	Area	Y	P27695
p5		Q	L	L	V	A	C	K	V	K		**V1**, L2	Positional	Y	Q9Y490
p6		K	V	L	A	T	V	T	K			**V1**, F1, K3, S1	Area	Y	Q02878
p7			R	L	S	A	K	P				**V1**, K3, B1, L1, S1, F1, K3, L1	Area, Positional	Y/N	Q15651, O00479[*]
p8			L	L	S	G	K	E				**V1**, K3, L2	Area	Y/N	A6NHL2
p9			Q	L	R	Q	Q	E				**V1**, K5, L1, S7	Positional[4]	Y/N	O43818
p10		G	N	Y	K	E	A	K	K			**V2**	Positional	Y	P42704
p11		V	L	L	K	V	A	A	S			**V2**, L3	Positional[3]	Y	Q53FA7
p12		A	C	M	K	S	V	T	E			**V2**, F1, K4, S4	Area	Y	P63104
p13		V	A	C	K	S	S	Q	P			**V2**, B1, F1, K6, L3, S8	Positional	Y	P46013
p14			G	A	K	S	A	A				**V2**, K6, B1, L3, F1	Area	Y/N	Q8NC51
p15			G	V	T	K	A	A				**V3**, B1, F2, K1, L4, S2	Area	Y	P27797[**]
p16			G	M	C	K	A	G				**V3**, F2, K1, S6	Area	Y	Q6S8J3, P60709, P63261, A5A3E0, P0CG38, P0CG39
p17			K	I	A	K	T	H				**V3**	Positional	Y	O75533

p18			E	V	C	K	K	K	K			**V3**, F2, K1, L4, S6	Positional[3]	Y	Q92772
p19			I	I	L	K	E	K				**V3**, K1, L4	Positional[3]	Y/N	P07199
p20		R	G	I	R	E	A	A	K			**V4**, K5, B1, L1, S2, F1	Positional	Y	P25398
p21		K	R	F	Q	N	V	A	K			**V4**, F1, K5, S5	Area	Y	P14625
p22			A	Y	F	K	K	V	L			**V5**	Area	Y	P25205
p23			V	Y	E	K	K	P				**V5**, L4, S5, F2	Area	Y/N	P46777
p24		S	I	Y	E	V	D	K	Q			**V6**	Positional	Y	Q92747
p25		T	R	E	S	E	D	L	E			**V6**, B1, F1, K2, L1, S3	Positional[3]	Y	Q8N5V2
p26		V	P	C	G	T	A	H	E			**V6**	Positional	Y	O43823
p27		K	K	Y	D	A	F	L	A			**V6**, L1	Positional	Y	P62906
p28			A	W	K	K	E	A				**V7**, L4	Positional[4]	Y	Q9C0B0
p29			P	V	K	K	K	A	K			**V7**, F2, K4, S4	Area	Y	P16402
p30			K	P	K	K	K	T	K			**V7**, L4	Area	Y	Q6NWY9
p31			L	L	K	V	A	L				**V2**, L3, B1, F1, K4, S4		N	11,759***
p32			A	V	A	E	K	Q				**V4**, L1, B1, F1, K1, S2		N	6,261***
p33			A	L	A	A	S	S				**V1**, L1, B1, F1, K3, S1		N	40,482***
p34			A	V	R	A	R	L				**V4**, L1, B1, F1, K3, S2		N	33,891***
p35			L	L	K	A	V	A	E	K	Q	**V2**, L3, B1, F1, K4, S4		Y	1***

* Proteins O00479 and QI5652 were cleaved in the same location but with different cathepsins.

** Amount of peptide was sufficient to carry out structural analysis only.

*** Peptide was not part of our data sets (Supplementary Data 1). The number presents sequences found in the UniProt database.

Supplementary Table 4. Summary of peptide binding to crystals of cathepsin V C25S/A.

Peptide number and their corresponding sequences and clusters are shown in columns No, Sequence and Cluster, respectively. Peptide residues modeled to free–kick omit map (Fo-Fc) are marked in the columns that denote peptide positions P8–P6'. MPD is 2-methyl-2,4-pentanediol. Chlorine anion is marked as CL^-. Molecule column with "A" or "B" denotes 2 cathepsin V molecules in the asymmetric unit. Mutant column shows mutation at catalytic Cys residue: C25A or C25S. "Y" and "N" in the Protection column stand for peptide termini protection "yes" and "no". "s", "c", or "b" in the Method column stand for soaking, co-crystallization, or both techniques, respectively. PDB column contains the PDB codes of structures. Four patterns of peptide binding to cathepsin V were observed; table is divided into four parts: I, binding of cleaved peptide fragments to the non-primed site; II, binding shifted to the non-primed site; III, binding shifted to the primed site; and IV, binding of part cleaved peptides across the active site. Peptides exhibiting multiple binding patterns with respect to position in the asymmetric unit or crystallization method used are highlighted with colors in the sequence column. Peptides with the same sequence but different termini are not considered as equivalent peptides.

No.	Sequence	Cluster	P8	P7	P6	P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'	P5'	P6'	Molecule/ Mutant	Protection	Method	PDB code
Pattern I. Binding of cleaved peptide fragments at the non-primed site																				
p13	VACKSSQP	2					**V**	**A**	**C**	**K**	MPD						A, B / C25A	Y	s	7QFF
p23	VYEKKP	5						**V**	**Y**	**E**	MPD						A, B / C25S	N	s	7QNS
p14	GAKSAA	2						**G**	**A**	**K**	MPD						A / C25A	N	s	7QO2
p8	LLSGKE	1						**L**	**L**	**S**	MPD						B / C25A	N	s	7Q8O
p31	LLKVAL	2						**L**	**L**	**K**	MPD						A, B / C25S	N	c	7Q8K
p35	LLKAVAEKQ	2						**L**	**L**	**K**	MPD						B / C25A	Y	b	7Q9H
Pattern II. Binding shifted to the non-primed site																				
p18	EVCKKKK	3		**E**	**V**	**C**	**K**	**K**	**K**	**K**	MPD						A / C25A	Y	s	7Q8H
p22	AYFKKVL	5		A	Y	F	K	**K**	**V**	**L**	MPD						B / C25A	Y	s	7QFH
p7	RLSAKP	1			R	**L**	**S**	**A**	**K**	**P**	MPD						B / C25A	Y	b	7Q9C
p25	TRESEDLE	6	T	R	E	S	E	**D**	**L**	**E**	MPD						A, B / C25A	Y	s	7Q8D
p10	GNYKEAKK	2	**G**	**N**	**Y**	**K**	**E**	**A**	**K**	**K**	MPD						A / C25A	Y	s	7Q8F
p30	KPKKKTK	7		K	P	K	**K**	**K**	**T**	**K**	MPD						B / C25A	Y	s	7Q8M
p14	GAKSAA	2			G	A	K	**S**	**A**	**A**	MPD						A, B /	Y	b	7QHJ

																C25A			
p27	KKYDAFLA	6	K	K	Y	D	A	**F**	**L**	**A**	MPD				A, B / C25A	Y	s	7Q8N
p26	VPCGTAHE	6	V	P	C	G	**T**	**A**	**H**	**E**	MPD				A, B / C25A	Y	s	7Q8L
p9	QLRQQE	1			Q	L	R	**Q**	**Q**	**E**	MPD				B / C25A	N	s	7QHK
Pattern III. Binding shifted to the primed site																			
p8	LLSGKE	1						MPD	CL^-	**L**	**L**	**S**	G	K	E	A / C25A	N	s	7Q8O
p9	QLRQQE	1						MPD	CL^-	**Q**	**L**	**R**	**Q**	Q	E	A / C25A	N	s	7QHK
p7	RLSAKP	1						MPD	CL^-	**R**	**L**	**S**	**A**	**K**	**P**	B / C25S	N	s	7Q8Q
p14	GAKSAA	2						MPD	CL^-	**G**	**A**	**K**	**S**	A	A	A / C25A	N	s	7QO2
p19	IILKEK	3						MPD	CL^-	**I**	**I**	**L**	K	E	K	A / C25S	N	s	7Q8J
p31	LLKVAL	2						MPD	CL^-	**L**	**L**	**K**	**V**	A	L	A / C25S	N	s	7Q8P
p32	AVAEKQ	4						MPD	CL^-	**A**	**V**	**A**	**E**	**K**	**Q**	B / C25S	N	s	7Q8I
p33	ALAASS	1						MPD	CL^-	**A**	**L**	**A**	**A**	S	S	A / C25S	N	s	7Q8G
Pattern IV. Binding of cleaved peptide fragments across the active site																			
p7	RLSAKP	1						**R**	**L**	**S**	**A**	**K**	**P**				A / C25A	Y	b	7Q9C
p35	LLKAVAEKQ	2						**L**	**L**	**K**	**A**	**V**	**A**	**E**	**K**	Q	A / C25A	Y	b	7Q9H

Supplementary Table 5. Data collection and refinement statistics: PDB entries 7Q8H and 7Q8D.

	7Q8H	7Q8D
Data collection		
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	93.75, 93.75, 124.29	93.82, 93.82, 124.92
□□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	46.87 – 1.75 (1.86 – 1.75)	41.96 – 1.80 (1.91 – 1.80)
R_{sym} or R_{merge}	0.12 (1.19)	0.11 (1.51)
I / □I	10.17 (1.40)	13.42 (1.28)
Completeness (%)	99.8 (99.2)	99.6 (97.6)
Redundancy	10.1 (10.2)	13.7 (13.6)
Refinement		
Resolution (Å)	46.87 – 1.75	41.96 – 1.80
No. reflections	56397	52223
R_{work} / R_{free}	0.16 / 0.18	0.17 / 0.20
No. atoms		
Protein	3373	3373
Ligand	102	52
Water	420	392
B-factors		
Protein	23.35	30.50
Ligand	64.02	55.96
Water	48.13	52.05
R.m.s. deviations		
Bond lengths (Å)	0.018	0.015
Bond angles (□)	1.8	1.65

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 6. Data collection and refinement statistics: PDB entries **7Q8F and 7Q8L.**

	7Q8F	**7Q8L**
Data collection		
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	94.32, 94.32, 125.58	94.35, 94.35, 127.26
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	47.16 – 1.49 (1.58 – 1.49)	47.18 – 1.80 (1.91 – 1.80)
R_{sym} or R_{merge}	0.13 (2.19)	0.12 (1.45)
I / □I	16.64 (0.94)	11.36 (1.12)
Completeness (%)	99.9 (99.6)	99.9 (99.6)
Redundancy	24 (21.3)	12.8 (12.1)
Refinement		
Resolution (Å)	47.16 – 1.49	47.18 – 1.80
No. reflections	92736	53524
R_{work} / R_{free}	0.18 / 0.20	0.17 / 0.19
No. atoms		
Protein	3370	3372
Ligand	116	64
Water	428	492
B-factors		
Protein	24.14	41.75
Ligand	60.65	97.88
Water	45.53	66.01
R.m.s. deviations		
Bond lengths (Å)	0.018	0.014
Bond angles (□)	1.85	1.7

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 7. Data collection and refinement statistics: PDB entries **7Q8M and 7Q8N.**

Data collection	**7Q8M**	**7Q8N**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	94.23, 94.23, 125.61	94.15, 94.15, 126.10
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	47.11 – 1.57 (1.67 – 1.57)	45.78 – 2.00 (2.12 – 2.00)
R_{sym} or R_{merge}	0.06 / 1.15	0.12 (1.06)
I / □I	21.87 (1.99)	11.48 (1.86)
Completeness (%)	99.9 (99.5)	99.9 (99.9)
Redundancy	24.8 (21.9)	14.3 (13.5)
Refinement		
Resolution (Å)	47.11 – 1.57	45.78 – 2.00
No. reflections	79145	38867
R_{work} / R_{free}	0.19 / 0.21	0.17 / 0.20
No. atoms		
Protein	3367	3383
Ligand	60	50
Water	395	322
B-factors		
Protein	29.49	32.95
Ligand	57.78	86.79
Water	51.30	48.22
R.m.s. deviations		
Bond lengths (Å)	0.015	0.017
Bond angles (□)	1.7	1.7

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 8. Data collection and refinement statistics: PDB entries **7Q8I and 7Q9C.**

Data collection	**7Q8I**	**7Q9C**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	93.48, 93.48, 125.87	94.10, 94.10, 124.75
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	46.74 – 1.59 (1.69 – 1.59)	47.05 – 1.32 (1.40 – 1.32)
R_{sym} or R_{merge}	0.11 (0.89)	0.07 (1.89)
I / □I	16.99 (2.72)	16.05 (0.65)
Completeness (%)	99.9 (99.2)	99.6 (97.6)
Redundancy	13.5 (13.6)	11.9 (5.3)
Refinement		
Resolution (Å)	46.74 – 1.59	47.05 – 1.4
No. reflections	75384	110243
R_{work} / R_{free}	0.17 / 0.19	0.18 / 0.21
No. atoms		
Protein	3379	3372
Ligand	80	84
Water	402	489
B-factors		
Protein	20.87	20.32
Ligand	31.67	69.90
Water	37.12	55.31
R.m.s. deviations		
Bond lengths (Å)	0.017	0.014
Bond angles (□)	1.8	1.7

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 9. Data collection and refinement statistics: PDB entries **7Q9H and 7QHJ.**

Data collection	**7Q9H**	**7QHJ**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	94.51, 94.51, 124.78	94.31, 94.31, 125.37
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	47.26 – 1.29 (1.37 – 1.29)	47.16 – 1.35 (1.43 – 1.35)
R_{sym} or R_{merge}	0.09 (2.9)	0.08 (4.1)
I / □I	16.44 (0.76)	20.60 (0.57)
Completeness (%)	99.4 (97.4)	99.6 (97.6)
Redundancy	13.5 (13.4)	13.5 (12.7)
Refinement		
Resolution (Å)	47.26 – 1.4	45.67 – 1.40
No. reflections	141576	111254
R_{work} / R_{free}	0.18 / 0.20	0.19 / 0.21
No. atoms		
Protein	3374	3373
Ligand	93	34
Water	507	502
B-factors		
Protein	17.40	21.97
Ligand	41.30	36.01
Water	42.22	48.17
R.m.s. deviations		
Bond lengths (Å)	0.022	0.016
Bond angles (□)	2.1	1.7

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 10. Data collection and refinement statistics: PDB entries **7Q8K and 7Q8P.**

Data collection	**7Q8K**	**7Q8P**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	96.11, 96.11, 125.57	94.20, 94.20, 126.18
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	48.06 – 1.74 (1.85 – 1.74)	47.10 – 1.71 (1.81 – 1.71)
R_{sym} or R_{merge}	0.12 (0.93)	0.11 (1.01)
I / □I	14.89 (2.47)	15.19 (2.43)
Completeness (%)	99.5 (97.0)	99.9 (99.5)
Redundancy	13.3 (12.2)	13.4 (13.2)
Refinement		
Resolution (Å)	48.06 – 1.74	47.10 – 1.71
No. reflections	60130	62023
R_{work} / R_{free}	0.17 / 0.20	0.18 / 0.20
No. atoms		
Protein	3369	3381
Ligand	51	60
Water	379	353
B-factors		
Protein	25.81	28.11
Ligand	31.78	57.97
Water	39.80	43.02
R.m.s. deviations		
Bond lengths (Å)	0.018	0.012
Bond angles (□)	2.1	1.6

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 11. Data collection and refinement statistics: PDB entries **7QFF and 7QFH.**

Data collection	**7QFF**	**7QFH**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	94.40, 94.40, 126.83	94.09, 94.09, 126.42
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	47.20 – 1.50 (1.59 – 1.50)	45.83 – 1.52 (1.61 – 1.52)
R_{sym} or R_{merge}	0.05 (1.11)	0.09 (2.17)
I / □I	23.73 (1.69)	13.56 (0.76)
Completeness (%)	99.9 (99.6)	99.9 (99.8)
Redundancy	13.8 (13.3)	7.3 (7.4)
Refinement		
Resolution (Å)	47.20 – 1.50	45.83 – 1.52
No. reflections	91915	87563
R_{work} / R_{free}	0.17 / 0.19	0.18 / 0.21
No. atoms		
Protein	3378	3361
Ligand	62	46
Water	530	437
B-factors		
Protein	24.99	21.88
Ligand	60.86	56.12
Water	49.41	47.34
R.m.s. deviations		
Bond lengths (Å)	0.016	0.020
Bond angles (□)	1.7	1.8

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 12. Data collection and refinement statistics: PDB entries **7Q8G and 7Q8O.**

Data collection	**7Q8G**	**7Q8O**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	94.03, 94.03, 125.68	93.54, 93.54, 124.09
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	45.67 – 2.06 (2.18 – 2.06)	46.77 – 1.90 (2.02 – 1.90)
R_{sym} or R_{merge}	0.10 (0.71)	0.20 (1.15)
I / □I	12.23 (2.11)	9.83 (1.82)
Completeness (%)	99.6 (99.7)	100 (99.9)
Redundancy	13.3 (12.8)	7.8 (7.8)
Refinement		
Resolution (Å)	45.67 – 2.06	46.77 – 1.90
No. reflections	35511	43694
R_{work} / R_{free}	0.18 / 0.21	0.18 / 0.21
No. atoms		
Protein	3382	3371
Ligand	49	45
Water	335	330
B-factors		
Protein	35.95	21.46
Ligand	72.32	44.10
Water	52.05	35.07
R.m.s. deviations		
Bond lengths (Å)	0.017	0.017
Bond angles (□)	1.7	2.0

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 13. Data collection and refinement statistics: PDB entries **7Q8J and 7QHK.**

Data collection	**7Q8J**	**7QHK**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	93.82, 93.82, 125.63	93.69, 93.69, 124.75
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	46.91 – 1.64 (1.74 – 1.64)	45.42 – 1.83 (1.94 – 1.83)
R_{sym} or R_{merge}	0.08 (1.18)	0.08 (0.69)
I / □I	21.01 (1.94)	18.13 (1.96)
Completeness (%)	99.8 (99.1)	96.7 (83.1)
Redundancy	12.9 (11.3)	7 (3.9)
Refinement		
Resolution (Å)	46.91 – 1.64	45.42 – 1.83
No. reflections	68906	48185
R_{work} / R_{free}	0.19 / 0.22	0.17 / 0.20
No. atoms		
Protein	3375	3377
Ligand	52	65
Water	311	393
B-factors		
Protein	27.90	25.47
Ligand	42.60	84.54
Water	43.99	49.22
R.m.s. deviations		
Bond lengths (Å)	0.017	0.018
Bond angles (□)	1.8	1.8

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 14. Data collection and refinement statistics: PDB entries **7Q8Q and 7QNS.**

Data collection	**7Q8Q**	**7QNS**
Space group	P 43 21 2	P 43 21 2
Cell dimensions□□		
a, *b*, *c* (Å)	92.75, 92.75, 128.09	93.97, 93.97, 124.06
□□□□□□□□□□□□□ (□)	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	43.61 – 2.13 (2.26 – 2.13)	43.94 – 1.40 (1.48 – 1.40)
R_{sym} or R_{merge}	0.13 (1.09)	0.06 (1.05)
I / □I	14.20 (1.84)	25.16 (1.97)
Completeness (%)	99.3 (96.4)	99.6 (97.5)
Redundancy	8.6 (8.4)	12.5 (8.4)
Refinement		
Resolution (Å)	43.61 – 2.13	43.94 – 1.40
No. reflections	31531	108997
R_{work} / R_{free}	0.21 / 0.24	0.18 / 0.20
No. atoms		
Protein	3373	3388
Ligand	86	58
Water	284	341
B-factors		
Protein	40.99	20.53
Ligand	61.70	37.01
Water	53.11	43.17
R.m.s. deviations		
Bond lengths (Å)	0.015	0.012
Bond angles (□)	1.9	1.6

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 15. Data collection and refinement statistics: PDB entry **7QO2.**

Data collection	**7QO2**
Space group	P 43 21 2
Cell dimensions□□	
a, *b*, *c* (Å)	94.28, 94.28, 126.76
□□□□□□□□□□□□□□ (□)	90.00 90.00 90.00
Resolution (Å)	47.14 – 1.77 (1.88 – 1.77)
R_{sym} or R_{merge}	0.11 (1.03)
I / □*I*	15.87 (1.94)
Completeness (%)	99.8 (99.0)
Redundancy	7.7 (7.5)
Refinement	
Resolution (Å)	47.14 – 1.77
No. reflections	56230
R_{work} / R_{free}	0.18 / 0.20
No. atoms	
Protein	3385
Ligand	52
Water	401
B-factors	
Protein	25.13
Ligand	65.10
Water	45.28
R.m.s. deviations	
Bond lengths (Å)	0.018
Bond angles (□)	1.8

*Data was collected from one crystal. *Hydrogen atoms were excluded from calculations.

Supplementary Table 16. Peptide and protein cleavages.

a. Unique and shared cleavages of peptides. Unique cleavages were performed by only one cathepsin, whereas shared cleavages were performed by two or three cathepsins.

b. Peptide versus protein cleavages. Cleavage sites identical among peptides and proteins were separated from cleavages observed by one substrate type only.

a. Cleavages in numbers	**Cathepsin K**	**Cathepsin L**	**Cathepsin V**	**Cathepsins K, L, V (together)**
Total peptide cleavages	52	51	47	150
• Unique	11 (21%)	4 (8%)	3 (6%)	18 (12%)
• Shared	41 (79%)	47 (92%)	44 (94%)	132 (88%)
b. Comparison of peptide and protein cleavages	**Cathepsin K**	**Cathepsin L**	**Cathepsin V**	**Cathepsins K, L, V (together)**
Total cleavages	51, 29	49, 23	46, 38	146, 90
• Peptides only	34 (67%)	30 (61%)	20 (43%)	84 (58%)
• Proteins only	12 (41%)	4 (17%)	12 (32%)	28 (31%)
• Identical cleavages (peptides, proteins)	17 (33%, 59%)	19 (39%, 83%)	26 (57%, 68%)	62 (42%, 69%)

Supplementary Table 17. Peptide and protein cleavages and predictions.

The first column of the table (UniProt/Type) contains UniProt code of the protein origin (www.uniprot.org) and cleavage type. There are two types: positional (one cleavage in the sequence) and area (several cleavages in the sequence). The second column specifies whether the cleavages in the sequence in the next three columns (Cathepsin K, Cathepsin L and Cathepsin V) were obtained from protein or peptide analysis or from SVM based prediction. Cleavage sites are marked with arrows (↓). Asterisks (*) at the end of peptide sequences mark peptides with unique peptide cleavage. Protein sequences marked with (§) had no observed protein cleavage sites. The sequences marked with ($^{\&}$) were not selected from cleaved protein sequences. Indices 1 and 2 refer to one or two separated cleavage areas in the originated protein, respectively, where cathepsin(s) acted. Indices 3 and 4 mark where there were only one or two cleavage sites in the originated protein, respectively.

UniProt /Type	**Substrate form**	**Cathepsin K**	**Cathepsin L**	**Cathepsin V**
P42677 Area[1]	Peptide	K D L L↓H P S P	K D L↓L↓H P S P*	K D L L↓H P S P
	Protein	K D L L H P S P§	K D L L H P S P§	K D L L↓H P S P
	Prediction	K D L L H P S P	K D L L H P S P	K D L L H P S P
Q6NWY9 Area[2]	Peptide	K P K↓K K T K	K P K↓K↓K T K	K P K↓K↓K T K
	Protein	K P↓K K K T K	K P K↓K K T K	K P K↓K K T K
	Prediction	K P K↓K↓K T K	K P↓K↓K↓K T K	K P K↓K↓K T K
P46777 Area	Peptide	V Y↓E K K P	V Y↓E↓K K P	V Y↓E↓K K P
	Protein	V Y↓E K K P	V Y E↓K K P	V Y E↓K K P
	Prediction	V Y↓E K K P	V Y↓E↓K K P	V Y↓E↓K K P
Q8NC51 Area	Peptide	G A K↓S A A	G A K↓S A A	G A K↓S A A
	Protein	G↓A K↓S A↓A	G A K↓S A A	G A K↓S A A
	Prediction	G↓A K↓S A↓A	G↓A K S A↓A	G↓A K S A↓A
P27695 Area	Peptide	E I D L R↓N P K↓G N	E I D L↓R↓N P K↓G N*	E I D↓L R↓N P K G N*
	Protein	E I D L R N P K↓G N	E I D L R↓N P K↓G N	E I D L R↓N P K G↓N
	Prediction	E I D L R↓N P K↓G N	E I D L R↓N P K G N	E I D L R↓N P K↓G↓N
P16402 Area	Peptide	P V K↓K K A K	P V K↓K K A K	P V K↓K K A K
	Protein	P V K↓K K A K	P V K K K A K§	P V K↓K↓K↓A K
	Prediction	P V K↓K↓K↓A K	P V K↓K↓K↓A K	P V K↓K↓K↓A K
Q15651 Area	Peptide	R L S↓A K P	R L S↓A K P	R L↓S↓A K P*
	Protein	R L S↓A K P	R L S↓A K P	R L S↓A↓K P
	Prediction	R↓L S↓A K P	R↓L S↓A↓K P	R↓L↓S↓A K P
A6NHL2 Area	Peptide	L L↓S↓G K E	L L↓S↓G K E	L L↓S↓G K E
	Protein	L L S↓G↓K↓E	L L S↓G↓K E	L L S↓G↓K E
	Prediction	L L↓S↓G↓K↓E	L L↓S↓G↓K↓E	L L↓S↓G↓K E
Q6S8J3, P60709, P63261, A5A3E0, P0CG38, P0CG39 Area	Peptide	G M C↓K A G	G M C↓K↓A G*	G M C↓K A G
	Protein	G M C↓K↓A G	G M C K↓A G	G M C↓K↓A G
	Prediction	G M C↓K↓A G	G M C K↓A G	G M C↓K↓A G
P14625	Peptide	K↓R↓F Q↓N↓V A↓K*	K R↓F↓Q↓N V A↓K	K↓R↓F↓Q↓N V A↓K

Area	Protein	K R F Q↓N V A↓K	K R F Q N V A↓K	K R F Q↓N V A↓K
	Prediction	K R F Q↓N↓V A↓K	K R F Q↓N V A↓K	K R F↓Q↓N↓V A↓K
P63104 Area	Peptide	A C M↓K S V T↓E	A C M↓K S V T↓E	A C M↓K S V T↓E
	Protein	A C M K↓S V↓T↓E	A C M↓K S V T↓E	A C M↓K↓S V T↓E
	Prediction	A C M↓K↓S V T↓E	A C M↓K↓S V T↓E	A C M↓K↓S V T↓E
P25205 Area	Peptide	A Y F K↓K V L	A Y F↓K↓K V L	A Y F↓K K V L
	Protein	A↓Y F K↓K V L	A Y F K↓K V L	A Y F↓K↓K V L
	Prediction	A↓Y F K↓K V L	A↓Y F↓K↓K V L	A Y F↓K↓K V L
Q02878 Area	Peptide	K V L↓A↓T V T↓K	K V L↓A T V T↓K	K V L↓A T V T↓K
	Protein	K V L A↓T↓V T↓K	K V L A T V T K§	K V L A↓T V T↓K
	Prediction	K V L↓A↓T V T↓K	K V L↓A↓T↓V T↓K	K V L↓A↓T↓V T↓K
Q92772 Positional[3]	Peptide	E V C↓K↓K K K	E V C↓K↓K K K	E V C↓K K K K
	Protein	E V C↓K K K K	E V C↓K K K K	E V C↓K K K K
	Prediction	E V C↓K K K K	E V C↓K↓K K K	E V C↓K↓K K K
P07199 Positional[3]	Peptide	I I L↓K↓E K	I I L↓K↓E K	I I L↓K↓E K
	Protein	I I L↓K E K	I I L↓K E K	I I L↓K E K
	Prediction	I I L↓K↓E K	I I L↓K↓E K	I I L↓K↓E K
Q8N5V2 Positional[3]	Peptide	T R↓E S E D L E	T R↓E↓S↓E D L E	T R↓E↓S↓E D L E
	Protein	T R E S↓E D L E	T R E S↓E D L E	T R E S↓E D L E
	Prediction	T R↓E S E D L E	T R E S E D L E	T R E S E D L E
Q53FA7 Positional[3]	Peptide	V L ↓L K↓V A A S	V L↓L K V A A S	V L↓L K V A A S
	Protein	V L L K V A A S§	V L L K↓V A A S	V L L K↓V A A S
	Prediction	V L↓L K↓V A↓A S	V L↓L K↓V A↓A S	V L L K↓V A↓A S
Q9C0B0 Positional[4]	Peptide	A W↓K↓K↓E A*	A W K↓K E A	A W K↓K E A
	Protein	A W K K E A§	A W K↓K E A	A W K↓K E A
	Prediction	A W K↓K↓E A	A W K↓K E A	A W↓K↓K E A
O43818 Positional[4]	Peptide	Q L↓R↓Q Q E	Q L↓R↓Q Q E	Q L↓R↓Q Q E
	Protein	Q L R↓Q Q E	Q L R↓Q Q E	Q L R↓Q Q E
	Prediction	Q L R↓Q Q E	Q L R↓Q Q E	Q L R↓Q Q E
O43823 positional	Peptide	V P C↓G T A H E*	V P C G↓T A H E	V P C G↓T A H E
	Protein	V P C G T A H E§	V P C G T A H E§	V P C G↓T A H E
	Prediction	V P C G↓T A H E	V P C G T A H E	V P C G T A H E
O75533 Positional	Peptide	K I A↓K T H	K I A↓K T H	K I A↓K T H
	Protein	K I A K T H§	K I A K T H§	K I A↓K T H
	Prediction	K I A↓K T H	K I A↓K T H	K I A↓K T H
P46013 Positional	Peptide	V A↓C K S S Q P	V A↓C K S S Q P	V A↓C K S S Q P
	Protein	V A C K↓S S Q P	V A C K↓S S Q P	V A C K↓S S Q P
	Prediction	V A↓C K↓S S Q P	V A↓C K↓S S Q P	V A↓C K↓S S Q P
P42704 Positional	Peptide	G N Y K↓E↓A↓K K*	G N Y K↓E A K K	G N Y K↓E A K K
	Protein	G N Y K E A K K§	G N Y K E A K K§	G N Y K↓E A K K
	Prediction	G N Y K↓E A K↓K	G N Y K↓E A K K	G N Y K↓E↓A K↓K
Q9Y490	Peptide	Q L L↓V↓A↓C↓K V K*	Q L L↓V A C↓K V K	Q L L V A C↓K V K

Positional	Protein	Q L L V A C K V K§	Q L L V↓A C K V K	Q L L V↓A C K V K
	Prediction	Q L L↓V A C↓K↓V K	Q L L↓V↓A↓C↓K↓V K	Q L L↓V A↓C↓K↓V K
P25398 Positional	Peptide	R G↓I R↓E A A K	R G↓I R↓E A A K	R G↓I R↓E A A K
	Protein	R G I R↓E A A K	R G I R↓E A A K	R G I R↓E A A K
	Prediction	R G I R↓E A A K	R G I R↓E↓A A K	R G I R↓E↓A A K
Q92747 Positional	Peptide	S I Y↓E V D↓K Q*	S I Y↓E↓V D K Q	S I Y↓E↓V D K Q
	Protein	S I Y E V D K Q§	S I Y E V D K Q§	S I Y E↓V D K Q
	Prediction	S I Y↓E V D K Q	S I Y↓E↓V D K Q	S I Y↓E↓V D↓K Q
P62906 Positional	Peptide	K K Y D↓A F↓L↓A*	K K Y D↓A F L↓A	K K Y↓D↓A F L↓A*
	Protein	K K Y D A F L A§	K K Y D↓A F L A	K K Y D↓A F L A
	Prediction	K K Y D A F↓L↓A	K K Y D↓A F L↓A	K K Y D↓A F L↓A
	Peptide	L L↓K A V A E K Q	L L↓K↓A V A E K Q	L L↓K A V A E K Q
	Protein&	No data	No data	No data
	Prediction	L L↓K↓A V A E K Q§	L L↓K↓A V A E K Q§	L L↓K↓A V A E K Q§

Supplementary Data 1. Input data – individual data sets of peptides for cathepsins K, V, B, L, S, and F.

Tables are provided in a separate excel file (SuppData1_Cat_KVBLSF.xlsx).

Supplementary Data 2. Specific 941 cleavages – only one cleavage in the whole protein.

Table is provided as separate excel file (SuppData2_941proteins.xlsx).

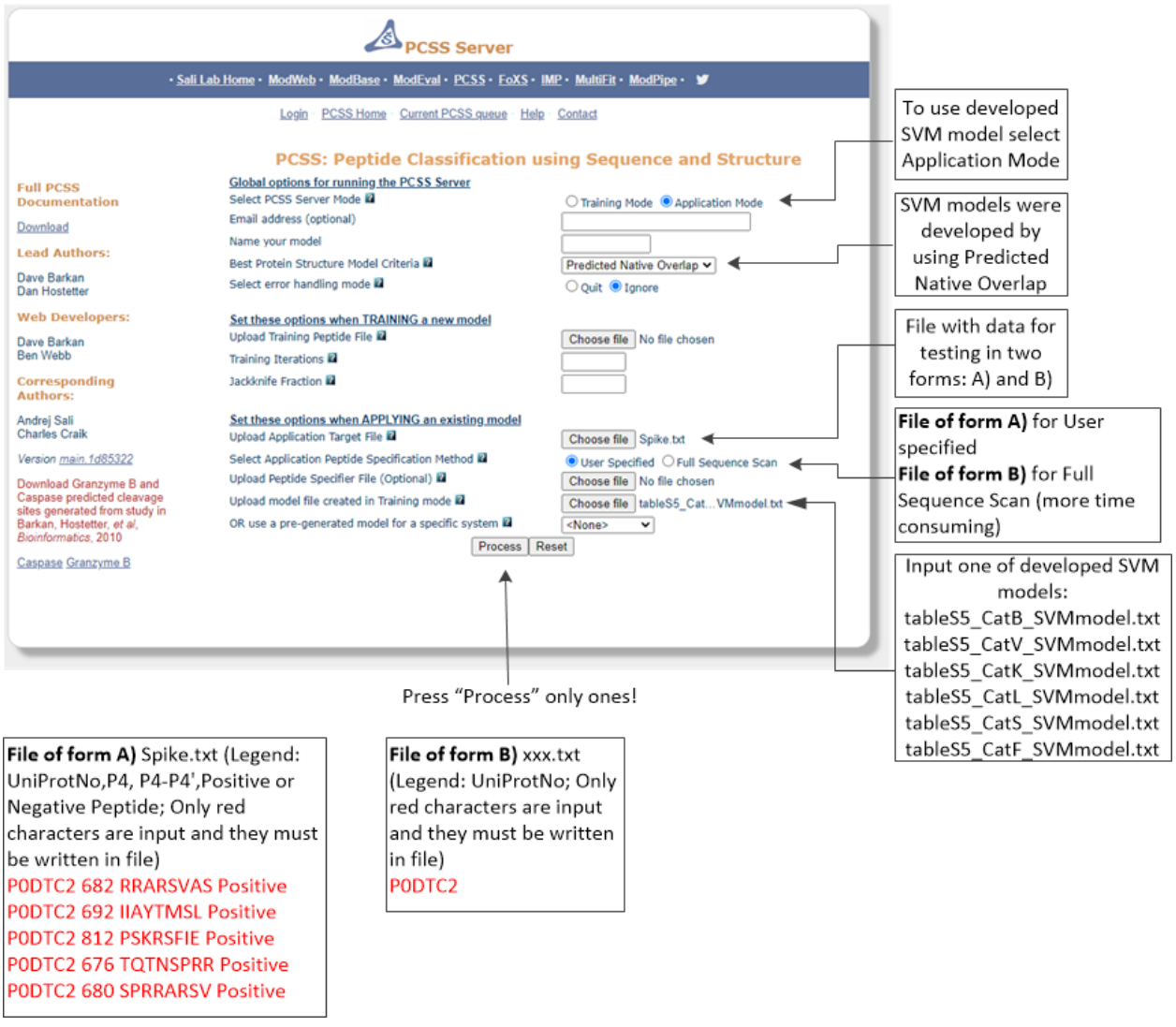
Supplementary Data 3. SVM models for predictions which can be used as input for PCSS server (https://salilab.org).

SVM models are provided in a separate ascii files for cathepsins K, V, B, L, S, and F:

SuppData3_CatK_SVMmodel.txt;
SuppData3_CatV_SVMmodel.txt;
SuppData3_CatB_SVMmodel.txt;
SuppData3_CatL_SVMmodel.txt;
SuppData3_CatS_SVMmodel.txt;
SuppData3_CatF_SVMmodel.txt;

in one "zip" file called "SuppData3_Cat_BFKLSV_SVMmodels.zip".

Short guidelines for using developed SVM models:



File of form A) Spike.txt (Legend: UniProtNo,P4, P4-P4',Positive or Negative Peptide; Only red characters are input and they must be written in file)
P0DTC2 682 RRARSVAS Positive
P0DTC2 692 IIAYTMSL Positive
P0DTC2 812 PSKRSFIE Positive
P0DTC2 676 TQTNSPRR Positive
P0DTC2 680 SPRRARSV Positive

File of form B) xxx.txt (Legend: UniProtNo; Only red characters are input and they must be written in file)
P0DTC2

Supplementary Data 4. Peptide fragment identification.

a. **Peptides treated with cathepsins were analyzed using reverse phase HPLC**. Peaks representing the peptide fragments were captured. Separation was monitored at 214 nm. The y-axis shows normalized signal of absorbance at A214 and the x-axis shows separation time in minutes. Peptide sequences as identified using MALDI-TOF are written on top or next to their corresponding HPLC signals. Characteristic signals at approximately 8 and 12 min correspond to buffer component dithiothreitol (DTT) and cathepsin, respectively.
b. **Processing of peptides AYFKKVL and KVLATVTK from 5 s–60 min.** Aliquots were taken after 5 s, 30 s, 2 min, 6 min, 20 min, and 60 min of incubation with cathepsins V and L (both peptides) or K (peptide KVLATVTK). Response at Y-axis is not normalized to quantitatively compare fragment signals at different time points.

HPLC spectra are provided as separate file (SuppData4.pdf).

Supplementary Note 1. Comparison of peptide and protein cleavages by cathepsins V, L and K.

After treatment with native cathepsins V, L, and K, we determined the cleavage sites of 28 peptides with N- and C-terminal protections (peptide p15 was used in the structural assay). In total, 150 cleavages were observed. Supplementary Table 16 shows that 42% of all peptide cleavages and 69% of all protein cleavages were identical, whereas the remaining cleavages were observed only with one type of substrate (58% of total peptide and 31% of total protein cleavages). Most cleavages were shared (performed by more than one cathepsin), whereas a few were unique to only one cathepsin (Supplementary Table 16a). Statistical comparison of the patterns of cleaved peptides and their protein counterparts showed that there was no significant difference between their cleavage patterns, demonstrating that the selected sequences indeed represented a variety of protein cleavage samples of all seven cathepsin V clusters, despite those peptides were cleaved in more places than their protein counterparts (146 peptide and 90 protein cleavages among the selected sequences; Supplementary Table 16b).

Of all 28 sequences treated with three different cathepsins, only 11 were cleaved into peptides and proteins at the same position by at least one cathepsin. Other sequences contained additional cleavages that was observed with only one substrate type. In contrast, sequences EVC↓KKKK, IIL↓KEK, and TRES↓EDLE had only one observed protein cleavage site, indicating very restrictive processing, whereas in peptides they were cleaved at two sites by all three cathepsins (IIL↓K↓EK), at two sites by cathepsins K and L (EVC↓K↓KKK), and at three sites by cathepsins V and L (TR↓E↓S↓EDLE). In addition, several sequences were not cleaved in proteins by cathepsin K, L, or both, whereas they cleaved each sequence at least at one site in the peptidyl form. Four of these sequences (AWKKEA, SIYEVDKQ, KKYDAFLA, and GNYKEAKK) appeared as weak substrates of cathepsin K in the peptidyl form and were only partially processed by cathepsin K during the incubation period. We also observed multiple fragments that had in their sequences embedded protein cleavage sites, which were evidently not cleaved when present in peptides. These were ESEDLE, ATVT, and KPK fragments with intact protein cleavage sites TRES↓EDLE, KVLAT↓VTK, KP↓KKKTK (cathepsin K), NPKGN, and AKP with cleavage sites EIDLRNPKG↓N and RLSA↓KP (cathepsin V), and KSVT with cleavage sites ACMK↓SVTE (cathepsins V and K) and ACMKSV↓TE (cathepsin K) (Supplementary Table 17, Supplementary Data 4a). These data indicated that the recognition of several sequences in proteins and peptides is not the same.

Interestingly, we discovered that cathepsins cleaved peptides along the entire length of peptide sequences, including the terminal residues and their protective groups, four of which had their C-terminal residues removed by all cathepsins, whereas cathepsins K and V cleaved the amino-terminal residue of one peptide each. This discovery suggests the exopeptidase activity of cathepsins toward peptides. To gain further insight, we followed the cleavage of peptides AYFKKVL and KVLATVTK from 5 s to 60 min. The analysis confirmed the carboxypeptidase activity of cathepsins V and L, but not that of K, which is evident from the processing of fragments AYFK and KVLA to AYF and KVL, respectively (Supplementary Data 4b).

Supplementary References

68. Li, J. *et al.* Structural basis for DNA recognition by STAT6. *Proc. Natl. Acad. Sci. U. S. A.* (2016) doi:10.1073/pnas.1611228113.
69. Wang, Z., Wu, Y., Li, L. & Su, X. D. Intermolecular recognition revealed by the complex structure of human CLOCK-BMAL1 basic helix-loop-helix domains with E-box DNA. *Cell Res.* (2013) doi:10.1038/cr.2012.170.
70. Lolli, G., Pinna, L. A. & Battistutta, R. Structural determinants of protein kinase CK2 regulation by autoinhibitory polymerization. *ACS Chem. Biol.* (2012) doi:10.1021/cb300054n.
71. Millet, J. K. & Whittaker, G. R. Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. *Virus Res.* (2015) doi:10.1016/j.virusres.2014.11.021.