Supplementary Materials

for

Taylor & Radding, 2020

M$^{\text{pro}}$ site	2003 SARS-CoV	2019 SARS-CoV-2	# Non-identical
nsp4/5	SAVLQ/SGFRK	SAVLQ/SGFRK	0
nsp5/6	GVTFQ/GKFKK	GVTFQ/SAVKR	4
nsp6/7	VATVQ/SKMSD	VATVQ/SKMSD	0
nsp7/8	RATLQ/AIASE	RATLQ/AIASE	0
nsp8/9	AVKLQ/NNELS	AVKLQ/NNELS	0
nsp9/10	TVRLQ/AGNAT	TVRLQ/AGNAT	0
nsp10/11	EPLMQ/SADAS	EPMLQ/SADAQ	3
nsp12/13	HTVLQ/AVGAC	HTVLQ/AVGAC	0
nsp13/14	VATLQ/AENVT	VATLQ/AENVT	0
nsp14/15	FTRLQ/SLENV	FTRLQ/SLENV	0
nsp15/16	YPKLQ/ASQAW	YPKLQ/SSQAW	1

Figure S1. The 11 known M$^{\text{pro}}$ cleavage sites in the 2003 and 2019 SARS coronaviruses with a comparison of the 10-residue protein sequences spanning the cleavages sites in the two viruses. The alignments underneath each of the virus names (2003 SARS-CoV and 2019 SARS-CoV-2) were used to generate the logos shown in Fig. 1A and 1B. The cleavage sites and sequences for the SARS-CoV (Genbank NC_004718.3) are given in reference (10), and the equivalent sequences in SARS-CoV-2 were taken from its Genbank Reference Sequence, NC_045512.

S.2 Web server protease cleavage site prediction results cited in Figure 2.

Genbank source files for the protein sequences: **GCLC:** NP_001309424.1; **SELENOP:** NP_005401.3; **GLRX1:** NP_001112362.1; **TXNRD1:** NP_877393.1 **SELENOF:** NP_004252.2; **GPX1:** NP_000572.2

S.2.1. NetCorona results from cbs.dtu.dk/services/NetCorona

NetCorona 1.0 Server - prediction results
Technical University of Denmark

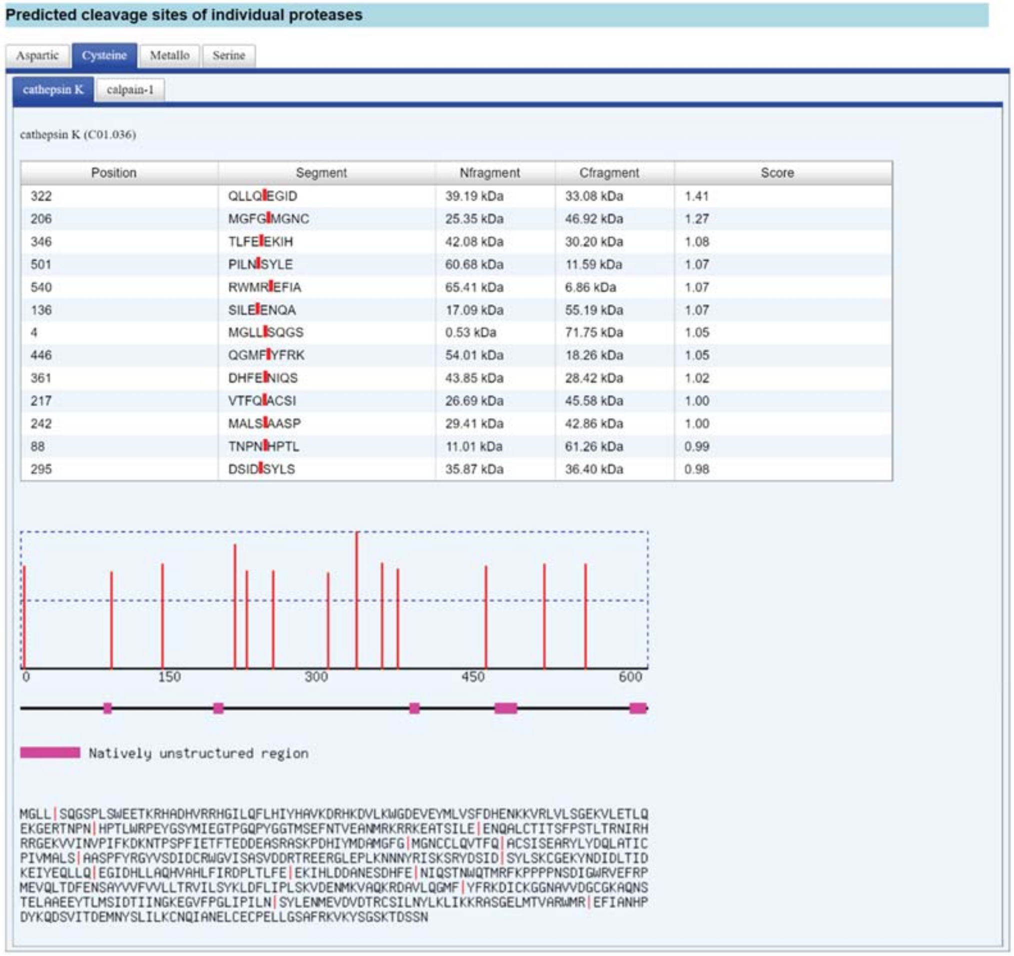
```
   165 SelenoF
MVAMAAGPSGCLVPAFGLRLLLATVLQAVSAFGAEFSSEACRELGFSSNLLCSSCDLLGQFNLLQLDPDCRGCCQEEAQF        80
ETKKLYAGAILEVCGCKLGRFPQVQAFVRSDKPKLFRGLQIKYVRGSDPVLKLLDDNGNIAEELSILKWNTDSVEEFLSE       160
KLERI   Selenocysteine (U) at #96 changed to C for search as NetCorona ignores U
..........................C.....................................................        80
................................................................................       160
.....

Pos     Score   Cleavage

27      0.846   ATVLQ^AVSAF     SelenoF
60      0.067   none    SelenoF
65      0.073   none    SelenoF
75      0.066   none    SelenoF
79      0.064   none    SelenoF
103     0.064   none    SelenoF
105     0.219   none    SelenoF
120     0.136   none    SelenoF
```

```
   499 TXNRD1
MNGPEDLPKSYDYDLIIIGGGSGGLAAAKEAAQYGKKVMVLDFVTPTPLGTRWGLGGTCVNVGCIPKKLMHQAALLGQAL        80
QDSRNYGWKVEETVKHDWDRMIEAVQNHIGSLNWGYRVALREKKVVYENAYGQFIGPHRIKATNNKGKEKIYSAERFLIA       160
TGERPRYLGIPGDKEYCISSDDLFSLPYCPGKTLVVGASYVALECAGFLAGIGLDVTVMVRSILLRGFDQDMANKIGEHM       240
EEHGIKFIRQFVPIKVEQIEAGTPGRLRVVAQSTNSEEIIEGEYNTVMLAIGRDACTRKIGLETVGVKINEKTGKIPVTD       320
EEQTNVPYIYAIGDILEDKVELTPVAIQAGRLLAQRLYAGSTVKCDYENVPTTVFTPLEYGACGLSEEKAVEKFGEENIE       400
VYHSYFWPLEWTIPSRDNNKCYAKIICNTKDNERVVGFHVLGPNAGEVTQGFAAALKCGLTKKQLDSTIGIHPVCAEVFT       480
TLSVTKRSGASILQAGCCG   (Penultimate U changed to C as NetCorona ignores U)
................................................................................        80
................................................................................       160
................................................................................       240
...............................C................................................       320
................................................................................       400
................................................................................       480
.............C.....

Pos     Score   Cleavage

33      0.068   none    TXNRD1
72      0.104   none    TXNRD1
78      0.071   none    TXNRD1
81      0.123   none    TXNRD1
106     0.073   none    TXNRD1
133     0.071   none    TXNRD1
230     0.075   none    TXNRD1
250     0.070   none    TXNRD1
258     0.065   none    TXNRD1
272     0.505   RVVAQ^STNSE     TXNRD1
323     0.061   none    TXNRD1
348     0.191   none    TXNRD1
355     0.064   none    TXNRD1
450     0.107   none    TXNRD1
464     0.074   none    TXNRD1
494     0.640   ASILQ^AGCCG     TXNRD1
```

S.2.2. PROSPER, the Protease Specificity Prediction Server, results from prosper.erc.monash.edu.au

S.2.2.B.

B. GCLC-a VTFQ/A **PROSPER** (1.00)
nsp5/6* VTFQ/G


Predicted cleavage sites of individual proteases

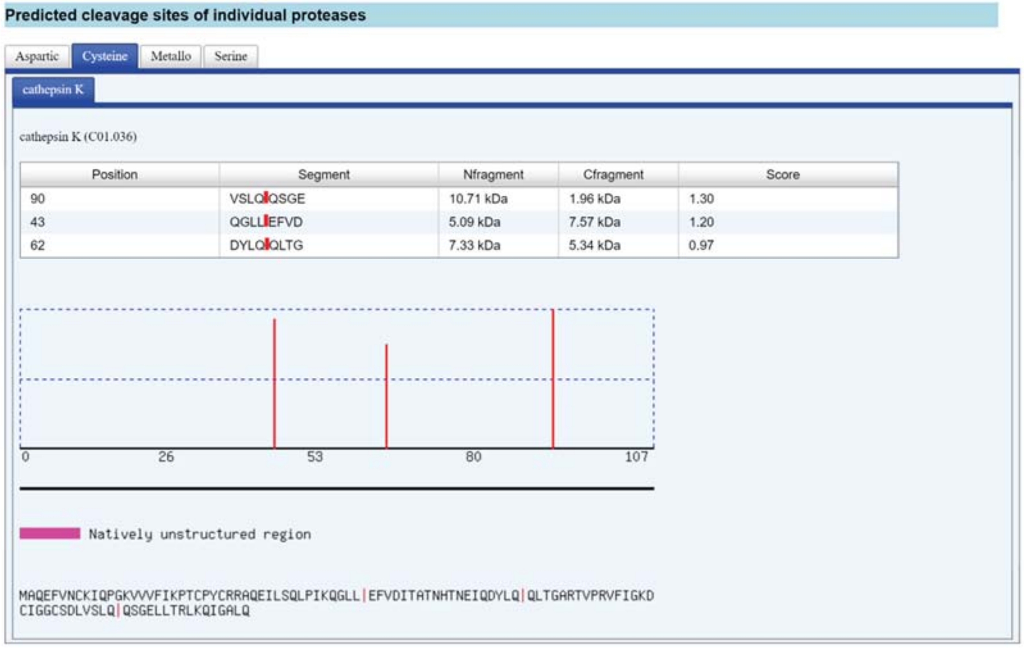
cathepsin K (C01.036)

Position	Segment	Nfragment	Cfragment	Score
322	QLLQ|EGID	39.19 kDa	33.08 kDa	1.41
206	MGFG|MGNC	25.35 kDa	46.92 kDa	1.27
346	TLFE|EKIH	42.08 kDa	30.20 kDa	1.08
501	PILN|SYLE	60.68 kDa	11.59 kDa	1.07
540	RWMR|EFIA	65.41 kDa	6.86 kDa	1.07
136	SILE|ENQA	17.09 kDa	55.19 kDa	1.07
4	MGLL|SQGS	0.53 kDa	71.75 kDa	1.05
446	QGMF|YFRK	54.01 kDa	18.26 kDa	1.05
361	DHFE|NIQS	43.85 kDa	28.42 kDa	1.02
217	VTFQ|ACSI	26.69 kDa	45.58 kDa	1.00
242	MALS|AASP	29.41 kDa	42.86 kDa	1.00
88	TNPN|HPTL	11.01 kDa	61.26 kDa	0.99
295	DSID|SYLS	35.87 kDa	36.40 kDa	0.98

```
MGLL|SQGSPLSWEETKRHADHVRRHGILQFLHIYHAVKDRHKDVLKWGDEVEYMLVSFDHENKKVRLVLSGEKVLETLQ
EKGERTNPN|HPTLWRPEYGSYMIEGTPGQPYGGTMSEFNTVEANMRKRRKEATSILE|ENQALCTITSFPSTLTRNIRH
RRGEKVVINVPIFKDKNTPSPFIETFTEDDEASRASKPDHIYMDAMGFG|MGNCCLQVTFQ|ACSISEARYLYDQLATIC
PIVMALS|AASPFYRGYVSDIDCRWGVISASVDDRTREERGLEPLKNNNYRISKSRYDSID|SYLSKCGEKYNDIDLTID
KEIYEQLLQ|EGIDHLLAQHVAHLFIRDPLTLFE|EKIHLDDANESDHFE|NIQSTNWQTMRFKPPPPNSDIGWRVEFRP
MEVQLTDFENSAYVVFVVLLTRVILSYKLDFLIPLSKVDENMKVAQKRDAVLQGMF|YFRKDICKGGNAVVDGCGKAQNS
TELAAEEYTLMSIDTIINGKEGVFPGLIPILN|SYLENMEVDVDTRCSILNYLKLIKKRASGELMTVARWMR|EFIANHP
DYKQDSVITDEMNYSLILKCNQIANELCECPELLGSAFRKVKYSGSKTDSSN
```

S.2.2.D.

D. GLRX-1 V*S*LQ/*Q* **PROSPER** (1.30)
nsp8/9 VKLQ/N


Predicted cleavage sites of individual proteases

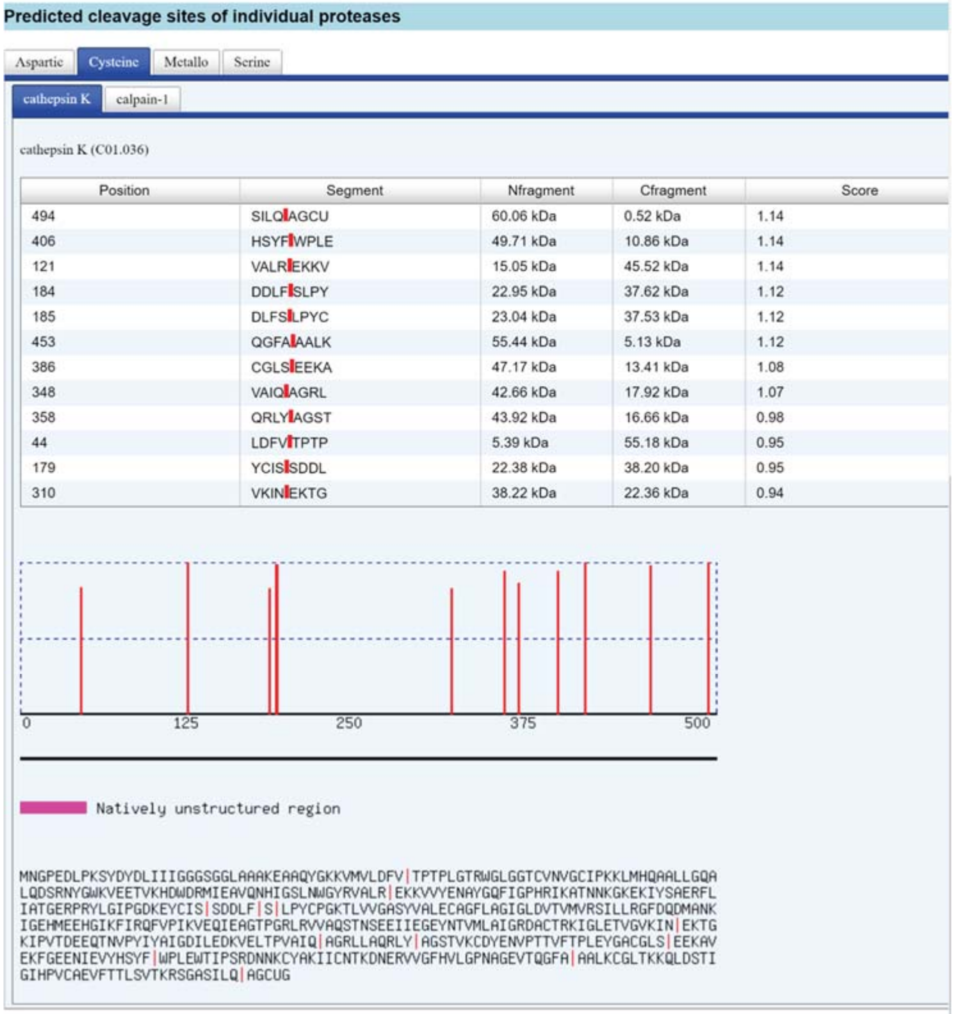
cathepsin K (C01.036)

Position	Segment	Nfragment	Cfragment	Score
90	VSLQ|QSGE	10.71 kDa	1.96 kDa	1.30
43	QGLL|EFVD	5.09 kDa	7.57 kDa	1.20
62	DYLQ|QLTG	7.33 kDa	5.34 kDa	0.97

```
MAQEFVNCKIQPGKVVVFIKPTCPYCRRAQEILSQLPIKQGLL|EFVDITATNHTNEIQDYLQ|QLTGARTVPRVFIGKD
CIGGCSDLVSLQ|QSGELLTRLKQIGALQ
```

S.2.2.E.

E. TXNRD1 *SI*LQ/A **PROSPER** (1.14)
nsp12/13 TVLQ/A



Predicted cleavage sites of individual proteases

cathepsin K (C01.036)

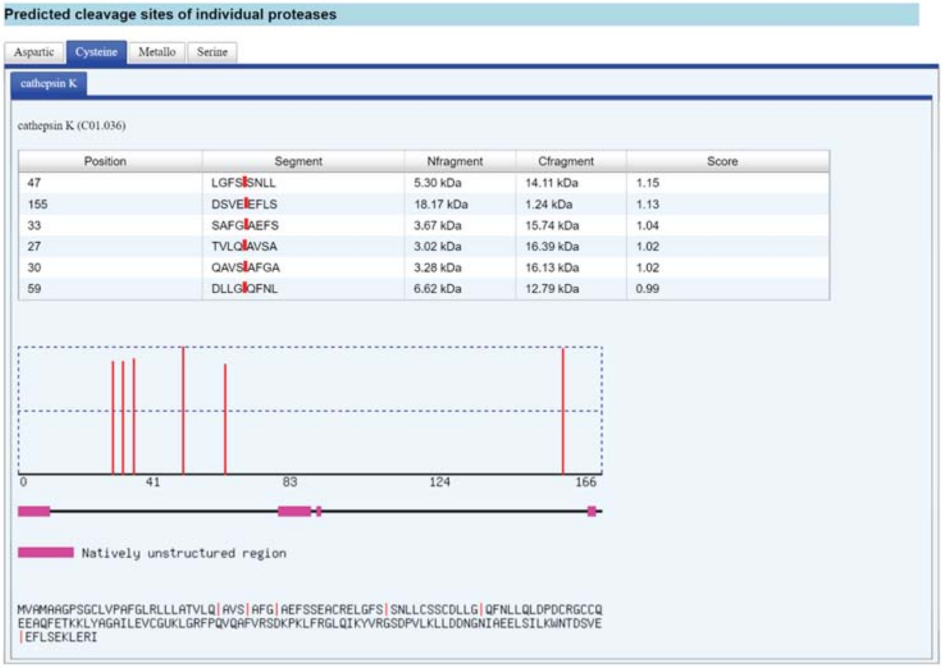
Position	Segment	Nfragment	Cfragment	Score
494	SILQ|AGCU	60.06 kDa	0.52 kDa	1.14
406	HSYF|WPLE	49.71 kDa	10.86 kDa	1.14
121	VALR|EKKV	15.05 kDa	45.52 kDa	1.14
184	DDLF|SLPY	22.95 kDa	37.62 kDa	1.12
185	DLFS|LPYC	23.04 kDa	37.53 kDa	1.12
453	QGFA|AALK	55.44 kDa	5.13 kDa	1.12
386	CGLS|EEKA	47.17 kDa	13.41 kDa	1.08
348	VAIQ|AGRL	42.66 kDa	17.92 kDa	1.07
358	QRLY|AGST	43.92 kDa	16.66 kDa	0.98
44	LDFV|TPTP	5.39 kDa	55.18 kDa	0.95
179	YCIS|SDDL	22.38 kDa	38.20 kDa	0.95
310	VKIN|EKTG	38.22 kDa	22.36 kDa	0.94

🟪 Natively unstructured region

```
MNGPEDLPKSYDYDLIIIGGGSGGLAAAKEAAQYGKKVMVLDFV|TPTPLGTRWGLGGTCVNVGCIPKKLMHQAALLGQA
LQDSRNYGWKVEETVKHDWDRMIEAVQNHIGSLNWGYRVALR|EKKVVYENAYGQFIGPHRIKATNNKGKEKIYSAERFL
IATGERPRYLGIPGDKEYCIS|SDDLF|S|LPYCPGKTLVVGASYVALECAGFLAGIGLDVTVMVRSILLRGFDQDMANK
IGEHMEEHGIKFIRQFVPIKVEQIEAGTPGRLRVVAQSTNSEEIIEGEYNTVMLAIGRDACTRKIGLETVGVKIN|EKTG
KIPVTDEEQTNVPYIYAIGDILEDKVELTPVAIQ|AGRLLAQRLY|AGSTVKCDYENVPTTVFTPLEYGACGLS|EEKAV
EKFGEENIEVYHSYF|WPLEWTIPSRDNNKCYAKIICNTKDNERVVGFHVLGPNAGEVTQGFA|AALKCGLTKKQLDSTI
GIHPVCAEVFTTLSVTKRSGASILQ|AGCUG
```

S.2.2.F.

F. SelenoF TVLQ/A **PROSPER** (1.02)
nsp12/13 TVLQ/A



Predicted cleavage sites of individual proteases

cathepsin K (C01.036)

Position	Segment	Nfragment	Cfragment	Score
47	LGFS|SNLL	5.30 kDa	14.11 kDa	1.15
155	DSVE|EFLS	18.17 kDa	1.24 kDa	1.13
33	SAFG|AEFS	3.67 kDa	15.74 kDa	1.04
27	TVLQ|AVSA	3.02 kDa	16.39 kDa	1.02
30	QAVS|AFGA	3.28 kDa	16.13 kDa	1.02
59	DLLG|QFNL	6.62 kDa	12.79 kDa	0.99

🟪 Natively unstructured region

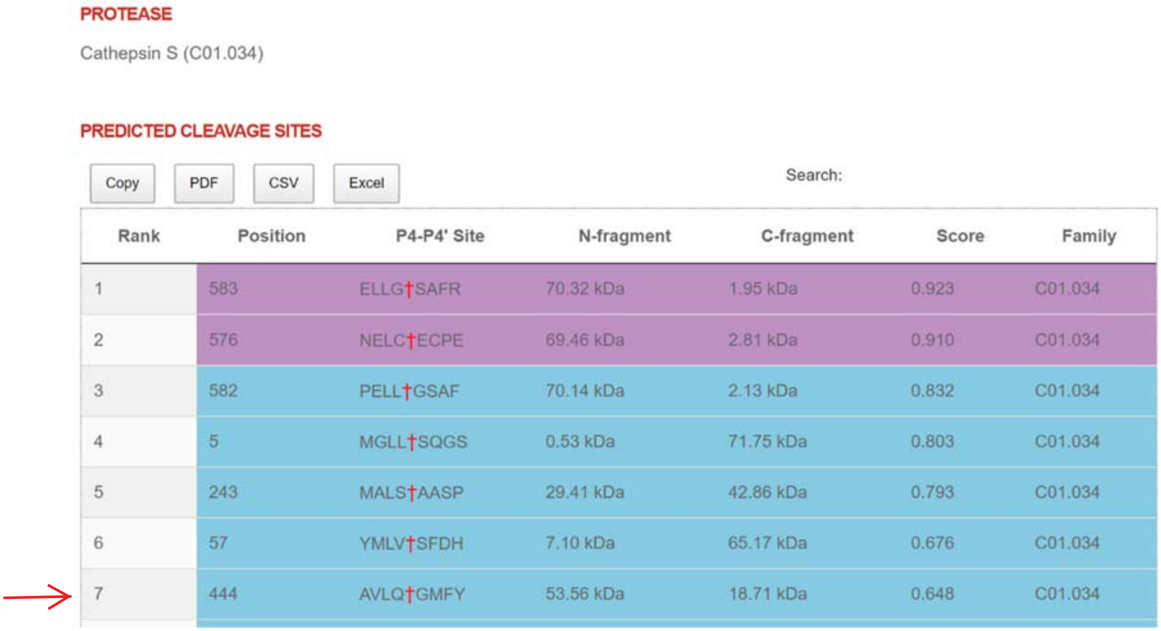
```
MVAMAAGPSGCLVPAFGLRLLLATVLQ|AVS|AFG|AEFSSEACRELGFS|SNLLCSSCDLLG|QFNLLQLDPDCRGCCQ
EEAQFETKKLYAGAILEVCGUKLGRFPQVQAFVRSDKPKLFRGLQIKYVRGSDPVLKLLDDNGNIAEELSILKWNTDSVE
|EFLSEKLERI
```

S.2.3. Procleave results from procleave.erc.monash.edu.au

S.2.3.A.

A. GCLC-b AVLQ/G
nsp4/5 AVLQ/S

ProCleave #7/592 for Cathepsin S



PROTEASE

Cathepsin S (C01.034)

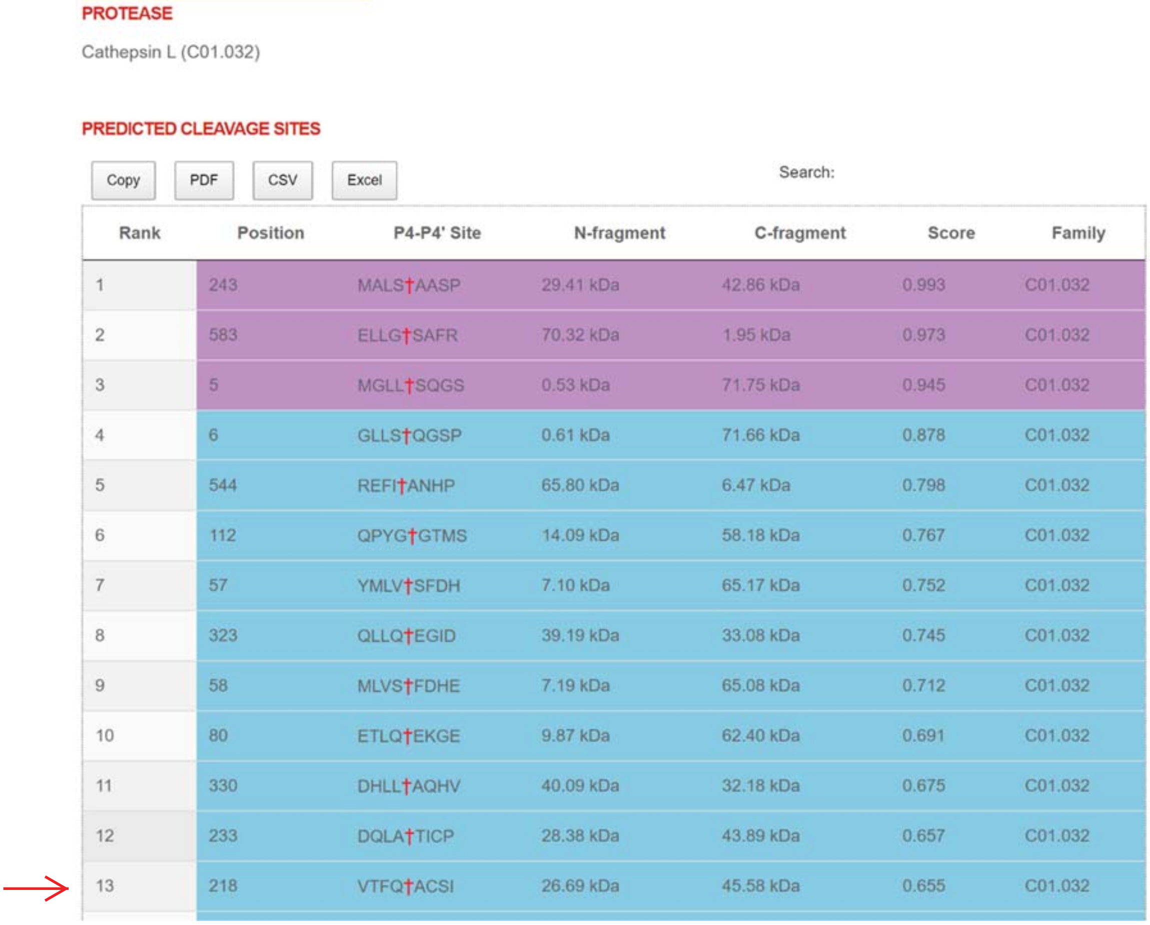
PREDICTED CLEAVAGE SITES

Rank	Position	P4-P4' Site	N-fragment	C-fragment	Score	Family
1	583	ELLG†SAFR	70.32 kDa	1.95 kDa	0.923	C01.034
2	576	NELC†ECPE	69.46 kDa	2.81 kDa	0.910	C01.034
3	582	PELL†GSAF	70.14 kDa	2.13 kDa	0.832	C01.034
4	5	MGLL†SQGS	0.53 kDa	71.75 kDa	0.803	C01.034
5	243	MALS†AASP	29.41 kDa	42.86 kDa	0.793	C01.034
6	57	YMLV†SFDH	7.10 kDa	65.17 kDa	0.676	C01.034
7	444	AVLQ†GMFY	53.56 kDa	18.71 kDa	0.648	C01.034

S.2.3.B.

B. GCLC-a VTFQ/A
nsp5/6* VTFQ/G

ProCleave #13 of 592 for Cathepsin L



PROTEASE

Cathepsin L (C01.032)

PREDICTED CLEAVAGE SITES

Rank	Position	P4-P4' Site	N-fragment	C-fragment	Score	Family
1	243	MALS†AASP	29.41 kDa	42.86 kDa	0.993	C01.032
2	583	ELLG†SAFR	70.32 kDa	1.95 kDa	0.973	C01.032
3	5	MGLL†SQGS	0.53 kDa	71.75 kDa	0.945	C01.032
4	6	GLLS†QGSP	0.61 kDa	71.66 kDa	0.878	C01.032
5	544	REFI†ANHP	65.80 kDa	6.47 kDa	0.798	C01.032
6	112	QPYG†GTMS	14.09 kDa	58.18 kDa	0.767	C01.032
7	57	YMLV†SFDH	7.10 kDa	65.17 kDa	0.752	C01.032
8	323	QLLQ†EGID	39.19 kDa	33.08 kDa	0.745	C01.032
9	58	MLVS†FDHE	7.19 kDa	65.08 kDa	0.712	C01.032
10	80	ETLQ†EKGE	9.87 kDa	62.40 kDa	0.691	C01.032
11	330	DHLL†AQHV	40.09 kDa	32.18 kDa	0.675	C01.032
12	233	DQLA†TICP	28.38 kDa	43.89 kDa	0.657	C01.032
13	218	VTFQ†ACSI	26.69 kDa	45.58 kDa	0.655	C01.032

S.2.3.C.

C. SelenoP A*L*LQ/A
nsp7/8 ATLQ/A

ProCleave #3/374 for Cathepsin L

PROTEASE

Cathepsin L (C01.032)

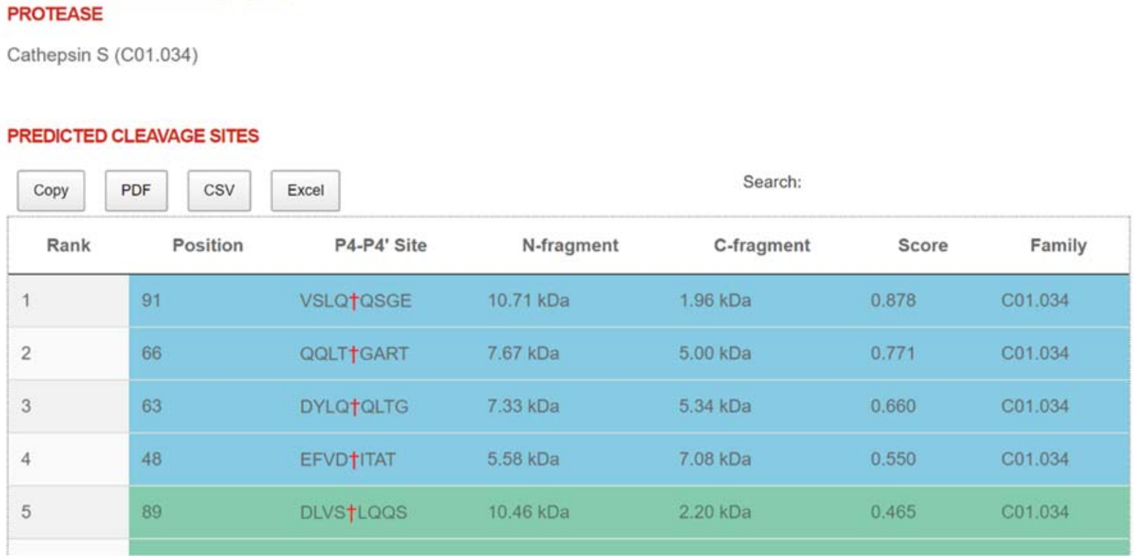
PREDICTED CLEAVAGE SITES

Rank	Position	P4-P4' Site	N-fragment	C-fragment	Score	Family
1	200	KTVE†TPSP	23.45 kDa	20.25 kDa	0.976	C01.032
2	16	CLLP†SGGT	1.75 kDa	41.96 kDa	0.900	C01.032
3	57	ALLQ†ASUY	6.42 kDa	37.29 kDa	0.892	C01.032

S.2.3.D.

D. GLRX-1 V*S*LQ/*Q*
nsp8/9 VKLQ/N

ProCleave #1/99 for Cathepsin S



PROTEASE

Cathepsin S (C01.034)

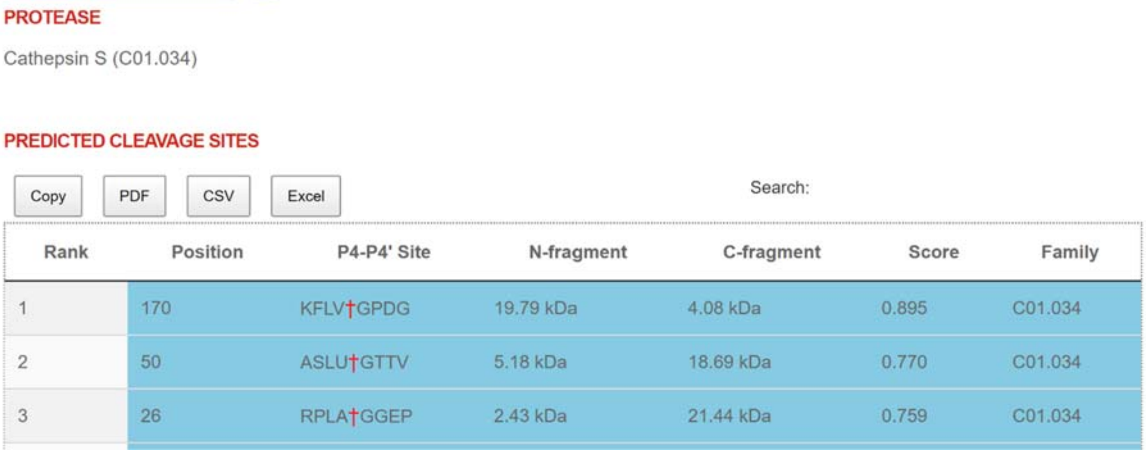
PREDICTED CLEAVAGE SITES

Rank	Position	P4-P4' Site	N-fragment	C-fragment	Score	Family
1	91	VSLQ†QSGE	10.71 kDa	1.96 kDa	0.878	C01.034
2	66	QQLT†GART	7.67 kDa	5.00 kDa	0.771	C01.034
3	63	DYLQ†QLTG	7.33 kDa	5.34 kDa	0.660	C01.034
4	48	EFVD†ITAT	5.58 kDa	7.08 kDa	0.550	C01.034
5	89	DLVS†LQQS	10.46 kDa	2.20 kDa	0.465	C01.034

S.2.3.G.

G. GPX1 A*S*LU/G
nsp13/14 ATLQ/A

ProCleave #2/196 for Cathepsin S



PROTEASE

Cathepsin S (C01.034)

PREDICTED CLEAVAGE SITES

Rank	Position	P4-P4' Site	N-fragment	C-fragment	Score	Family
1	170	KFLV†GPDG	19.79 kDa	4.08 kDa	0.895	C01.034
2	50	ASLU†GTTV	5.18 kDa	18.69 kDa	0.770	C01.034
3	26	RPLA†GGEP	2.43 kDa	21.44 kDa	0.759	C01.034

Figure S3. Mapping of predicted M^{pro} cleavage sites to accessible surface regions of the target proteins

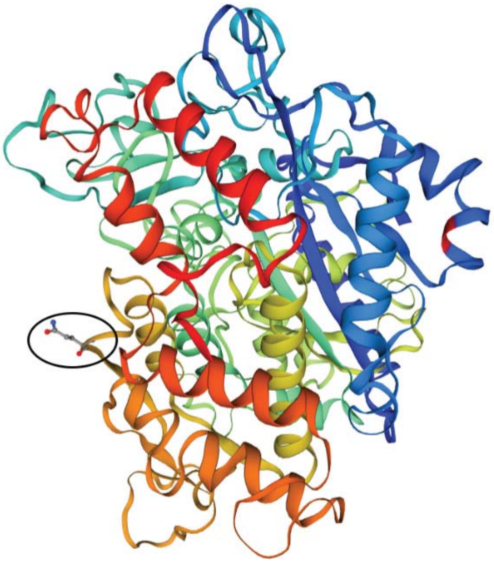


Fig. S3-A. Homology model of GCLC showing location of site GCLC-b, AVLQ/G (circled). The Gln in P1 is shown. The GCLC homology model used in lieu of an X-ray structure is based on the yeast enzyme PDB file 3ig5.1 and is found here: swissmodel.expasy.org/repository/uniprot/P48506

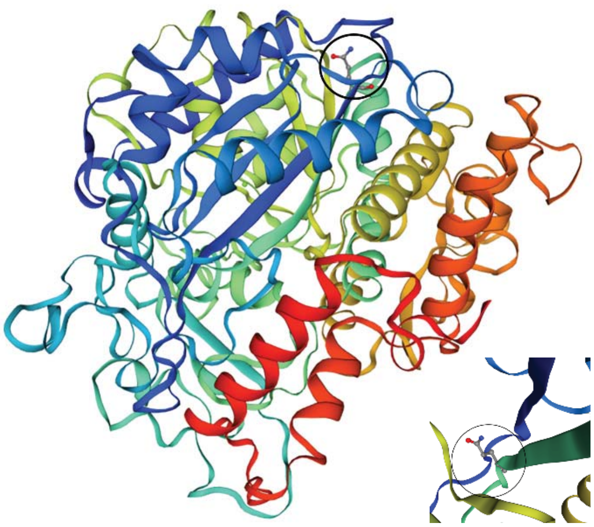


Fig. S3-B. The same homology model of GCLC showing location of site GCLC-a, VTFQ/A (circled). The Gln in P1 is shown. The site is at the protein surface but is recessed in a channel (inset). There is some uncertainty in the 3D structure, but an experimental structure was not available.

Fig. S3-D. NMR structure of human GLRX-1 (PDB file 1jhb). The predicted M^{pro} cleavage site region shown in Fig. 2D, VSLQ/Q, is circled and the Gln at P1 is displayed.

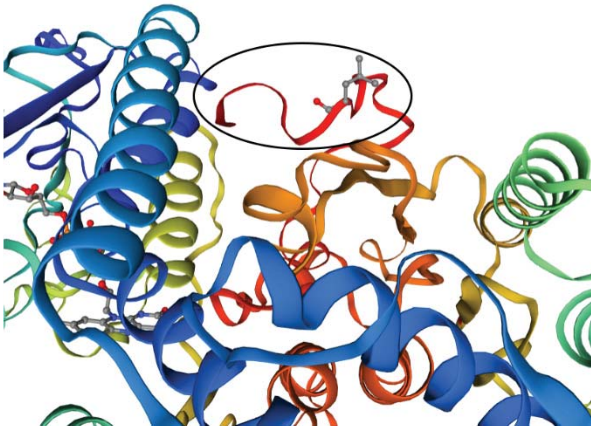


Fig. S3-E. X-ray structure of TXNRD1 (PDB file 2zzc). The C-terminal with the predicted M^{pro} cleavage site region shown in Fig. 2E, SILQ/A, is circled and the Leu at P2 is displayed. The AGCUG C-terminal redox center of TXNRD1 would be removed by M^{pro} cleavage at this site.

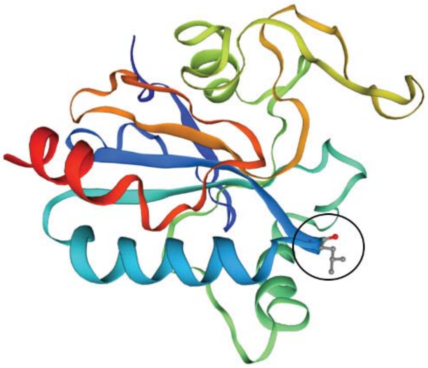


Fig. S3-G. X-ray structure of monomeric GPX1 (PDB file 2f8a). The predicted M^{pro} cleavage site region shown in Fig. 2G, ASLU/G, is circled and the Leu at P2 is displayed.

Fig. S3-C and S3-F: Structures were not available for SELENOF and SELENOP, but for these proteins, the predicted M^{pro} cleavage site was either near the N-terminal ER signal peptide cleavage site (position 27 of SELENOF) or right before a redox center active site (position 56 of SELENOP), so that a high probability of surface accessibility can be inferred in both cases.