Cell Reports, Volume 37

Supplemental information

Mechanistic insights into COVID-19

by global analysis

of the SARS-CoV-2 3CL^{pro} substrate degradome

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Table S1 related to Figures 1 and 6, and Table 1. Candidate $3CL^{pro}$ substrates (n = 58) and their cleavage sites (n = 65) detected in human embryonic kidney (HEK-293) and human lung epithelial (BEAS-2B) cells that require further validation.

Gene	UniProt	TAILS neo-N-Terminal P' Peptide	Byonic	P4 – P4'*	HEK-	- BEAS-2B		
Name			Score		293	Control	IFN-α	IFN-β
PAIRB†	SERBP1	AAAQTNSNAAGKQLR	809	SAAQ↓ ⁵⁷ AAAQ	•••		•••	
PAIRB†	SERBP1	GEGKIIDR	549	QQLQ ¹¹⁹ GEGK	•••	000	000	000
K2C8	KRT8	AEIEGLKGQR	644	SRLQ1 ³¹⁹ AEIE	•••	•••	•••	•••
PTBP1	PTBP1	GALAPLAIPSAAAAAAAAGR	641	PNVH 306GALA	•••	•••	•••	•••
PININ†	PNN	SQPPSQPEDLSLAVLQPTPQVTQEQGHLLPER		PVLQ 496SQPP	•••		•••	
PININ+	PNN				•••	000	000	000
CHSP1	CARHSP1		503					
	ATL 2		502					
			102					
			403					
ALDUA	ALDUA		450	KRLQU ¹⁰ SIGT				
STMN1	STMN1	AFELILSPR	440	ASGQ ** AFEL	••••			
HSP/C	HSPA8	HGKVEIIANDQGNR	541	GVFQ ²³ HGKV	•••	•0•		
TCPZ	CCT6A	AALAVNISAAR	521	ARAQ º AALA	•••	0	•••	•••
FUS	FUS	SYNPPQGYGQQNQYNSSSGGGGGGGGGGGNYGQDQSSMSSGG GSGGGYGNQDQSGGGGSGGYGQQDR	265	GQQQ ¹⁴⁸ SYNP	•••	•••	•••	0
ZC3H13	ZC3H14	SSWVYETGR	271	LDMQ ²⁵¹ SSWV	•0•	0•0	•••	000
PRP4B	PRPF4B	GYESGSEEEGEIHEKAR	593	LILQ ¹³⁹ GYES	•••	000	000	000
HNRPD	HNRNPD	GAAAAAGSGAGTGGGTASGGTEGGSAESEGAKIDASKNEEDEG HSNSSPR	478	AATQ↓ ³⁶ GAAA	•••	000	000	000
ZFR	ZFR	SDVQPVGHDYVEEVRNDEGKVIR	469	AALQ↓ ⁵⁵⁹ SDVQ	•••	000	000	000
SFPQ	SFPQ	HHQGPPPGGPGGR	436	YHQQ↓ ²⁵⁵ HHQG	•••	000	000	000
GRAP1	GRIPAP1	AENTALQKNVAALQER	426	LRLQ↓ ¹⁴² AENT	•••			
CSTF2†	CSTF2	ASMQGGVPAPGQMPAAVTGPGPGSLAPGGGMQAQVGMPGSGP VSMER	414	PLMQ↓ ²⁴⁸ ASMQ	•••	000	000	000
CSTF2†	CSTF2	VGMPGSGPVSMER	342	MQAQ ²⁸² VGMP	000	•••	•••	•••
RBP56†	TAF15	SQSGYSQSYGGYENQKQSSYSQQPYNNQGQQQNMESSGSQGG	412	SYGQ↓ ⁵⁹ SQSG	•••	000	000	000
RBP56†	TAF15	SGYSQSYGGYENQKQSSYSQQPYNNQGQQQNMESSGSQGGR	390	GQSQ ⁶¹ SGYS	••0	000	000	000
ATX2	ATXN2	AGIIPTEAVAMPIPAASPTPASPASNR	386	ASPQ ⁷¹² AGII	•••	000	000	000
DAXX	DAXX	GTSSHSADTPEASLDSGEGPSGMASQGCPSASR	369	ARLQ1400GTSS	•••	000	000	000
PRRC1	PRRC1	GTGTTSAITEPEEOEDPR	338	SLAQ ¹⁸³ GTGT	•••	000	000	000
FPIPI	FPPK1	TSGII GPETI R	338	SELH 2724TSG	•••	000	000	000
TOX4	ΤΟΧ4		334	SVI OI ⁴²⁶ AAAA		000	000	000
EWS	EWSR1	SSSVGOOSSER	300	VS001258SSSV		000	000	000
			308			000	000	000
			304			000	000	000
	EIE4C1		204		000	000	000	
IF4G17	EIF4G1		322		000			
	EIF4G1	QAVPTESTONRR	263	SALQ Mar QAVP	000	-0-		0.00
ARPIN	ARPIN	SSYKVEAKGDIDR	286	GFLMJ ³⁰ SSYK		000	000	000
FLNA	FLNA	SGIINKPNKFIVEIR	273	PGIQ ¹³⁰⁷ SGTT	•••	000	000	000
ENOA	ENO1	VVGDDLTVTNPKR	273	AGIQ ³¹⁵ VVGD	•••	000	000	000
SF3B2	SF3B2	GVEVALAPEELELDPMAMTQKYEEHVR	268	PELQ ⁸²³ GVEV	•••	000	000	000
GOGA3	GOLGA3	AQVECSHSSQQR	256	TKLQ ⁴⁵¹ AQVE	•••	000	000	000
TAB1	TAB1	SEQQPSWTDDLPLCHLSGVGSASNR	240	SLLQ ¹¹ SEQQ	•••	000	000	000
SETD2	SETD2	AAPVPLPVDVAVR	237	TVLM ²²⁴ AAPV	•••	000	000	000
TMED8	TMED8	SEHTGAIDVLSADLESADLLGDHR	203	VMIQ↓ ¹²⁷ SEHT	•••	000	000	000
ROCK2	ROCK2	ALHIGLDSSSIGSGPGDAEADDGFPESR	406	SQLQ ¹¹²⁵ ALHI	• () •	000	000	000
CNN3	CNN3	MGTNKGASQAGMLAPGTR	386	ISLQ↓ ²⁰⁸ MGTN	0 • •	000	000	000
PUR9†	ATIC	SSESKDTSLETR	357	TEMQ ¹⁶⁰ SSES	• () •	000	000	000
PUR9†	ATIC	MDQSYKPDENEVR	272	CVLQ ³⁶⁷ MDQS	000	0 • •	•••	•••
PTBP3	PTBP3	SGSLALSGGPSNEGTVLPGQSPVLR	353	SAVQ1159SGSL	••0	000	000	000
NEB2	PPP1R9B	MGTTAGPSGEAGGGAGLAEAPR	312	MFLQ ¹⁶⁴ MGTT	0	000	000	000
ZKSC8	ZKSCAN8	SEATQHKSPVPQESQER	234	TQLQ 170SEAT	••0	000	000	000
PRUN2	PRUNE2	GTQLASFPDTCQPASLNER	378	TGLQ 2588GTOI	000	•••	•••	•••
TRIR	TRIR	GREAPGPAGGGGGGSR	350	AEPO 12 GREA	000			•••
ANIN	ANI N	AADTISDSVAVPASI I GMR	311		000			
FLYS	AHCTF1	AFEDMSAIPR	238		000			
	RARED1	SREOVSEELVR	2/0		<u></u>			
FAG8A	FAMORA		326		000	0.00		
		COMISAEDCEEIOR	250		000			
			208		000			
	BLOAD		201		000			
	DLGAP4		231		000			0
DKEB	DRIVI	AVVAGENIEEFFQAQAFFK	431	AArQ		$\cup = \cup$		

DEN5B	DENND5B	SDVLATGPTSNNR	294	PKLQ↓660SDVL	000	•••	•••	0•0
SRRM2	SRRM2	SGSDSSPEPKAPAPR	222	PRAQ↓ ¹⁶¹⁶ SGSD	000	0•0	•••	•••
LRRF1	LRRFIP1	SREIDCLSPEAQKLAEAR	267	AAAQ ¹ 9SREI	000	•••	0 • •	•00
SPT6H	SUPT6H	SAQAQPQPSSSSR	357	TTPQ↓ ¹⁶⁴⁶ SAQA	000	00●	00 🔴	•0•
K1C18	KRT18	SVENDIHGLR	341	AMRQ ¹⁷⁷ SVEN	000	00●	000	•••
LRC59	LRRC59	HMKAVQADQER	243	KVLQ↓ ¹⁴⁷ HMKA	000	000	000	•••

Candidate substrates are those that did not meet high stringency manual inspection of the MS/MS spectra of the filtered neo-N-terminal P' peptides. Fields marked as " \bullet " or " \bullet " indicate in which of the *N* = 12 independent cell experiments the neo-N-terminal P' peptide was found by TAILS LC-MS/MS with an FDR \leq 0.01 at the peptide level. *, Amino acid sequence of the cleavage site and P1' amino acid position identified from the neo-N-terminal peptide. ↓, scissile bond. †, Proteins with multiple cut sites are shown with each other according to the highest-ranked peptide.

HEK-293 N-terminomics



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Figure S1 related to Figure 1. Quantitative TAILS and shotgun proteomics of HEK-293 and BEAS-2B cells.

(A) Distribution of free and N-terminal-modified peptides identified by TAILS and PreTAILS in N = 3 independent HEK-293 cell experiments.

(B) Venn diagrams of the total numbers of peptides and proteins identified, $FDR \le 0.01$.

(C) Classification of 1,085 quantified isotope-labelled, dimethylated N-termini by TopFinder 4.1 (N = 3).

(D) Log₂(fold change) distribution of all quantified neo-N-termini (n = 605) used to calculate the native protein P4–P4' cleavage site specificity of 3CL^{pro} (n = 198) versus other cell protease activity before cell harvesting and addition of protease inhibitors (n = 407).

(E) Distribution of free and N-terminal-modified peptides identified by TAILS and PreTAILS in N = 9 independent BEAS-2B cell experiments.

(F) Venn diagrams of the total numbers of peptides and proteins identified (left), and after 24-h treatment by IFN- α (N = 3), IFN- β (N = 3), and vehicle (N = 3), FDR ≤ 0.01 .

(G) Classification of 1,110 quantified isotope-labelled, dimethylated N-termini by TopFinder 4.1 (N = 9).

(H) Log₂(fold change) distribution of quantified neo-N-termini (n = 644) used to calculate the native protein P4–P4' cleavage site specificity of 3CL^{pro} (n = 170) versus other cell protease activity before cell harvesting and addition of protease inhibitors (n = 474).

(I) Volcano plot of log₂-fold change in expression of 2,767 proteins quantified in type I interferon-treated BEAS-2B cells (N = 6) relative to controls (N = 3). Red-filled circles, 3CL^{pro} substrates (n = 45). Yellow and blue-filled circles, IRG proteins with increased (n = 49) or decreased (n = 64) expression, respectively. A multiple sample t-test assessed statistical significance with Benjamini Hochberg correction. To focus on the 3CL^{pro} substrates, 113 IRG proteins with an adjusted *p*-value > 0.05 and log₂-fold change in expression ≥ 1.53 or ≤ -1.11 are not plotted and are listed in table S6.

(J) Immunoblot analysis of elevated phospho-STAT1-Tyr⁷⁰¹ protein, and unaltered FYCO1 and MAP4K5 protein levels in response to type I interferon signalling. b-actin loading controls. Protein levels of FYCO1 showed no apparent change, and although exhibiting a large relative fold-change at the peptide level by LC-MS/MS, this was not statistically significant. MAP4K5 peptides exhibited a small fold-change upon interferon treatment by LC-MS/MS that was significant. However, this small change was not readily apparent by immunoblotting and is unlikely to be biologically significant.



Figure S2 related to Figures 2, 3 and 4. 3CL^{pro} substrate validation by peptide and protein cleavage assays.

(A) MALDI-TOF-MS analyses of peptides assay incubated with $3CL^{pro}$ (1:20 molar ratio, E:S). Peptides from STMN1, CHSP1 and PAIRB, were not cut; thus, they were not included in our final substrate list (Table 1). The CREB1 cleavage site (TILQ²²³↓YAQT) was identified by Edman sequencing of recombinant human CREB1 cleavage products (Figure 4A) and is identical in ATF1. Product generation (red, site identified by TAILS; green, site identified by Edman sequencing) and substrate consumption (black) were calculated as the peak area normalized to the total peak area in the spectrum.

(B) PTBP1 isoforms 1, 2 and 3 sequence alignments by Clustal Omega. P and P' sites identified by TAILS (AALQ¹⁵² \downarrow AVNS) and Edman sequencing (AIPQ³²² \downarrow AAGL, isoform-3 numbering), blue/red and blue/green, respectively.

(C) Relative location of the $3CL^{pro}$ cleavage site in the importin beta binding domain (IBB) of IMA4 identified by the TAILS neo-N-terminal peptide (red). Representative MS/MS spectrum of neo-N-terminal peptides identifying the IMA4-cleavage site in every TAILS experiment (N = 12/12).

(D) Validation of $3CL^{pro}$ cleavage of recombinant human IMA4 by SDS-PAGE and immunoblotting for IMA4 and $3CL^{pro}$ (mouse anti-FLAG antibody). IMA4 was incubated (1:5 mol/mol, E:S) with $3CL^{pro}$ or inactive $3CL^{pro}$ -C145A, 18 h. Δ IMA4, no sequence of cleavage product was obtained. Figure S3C, Edman sequence data for IMA4 cleavage products 1 and 2. (E) IMA4 immunoblot of cell lysates from HAEC from five donors. Lysates were incubated with $3CL^{pro}$ -C145A (1:200 w/w, E:S) for 18 h, 37° C. β -tubulin, loading control.

A RPAP1 10	20	. 30	4.0	5.0	60		Recombinant partial RPA	AP1 analysed	in Fig. 3B		
GSSHHHHHĦÅ	LSRPKPGEŠĔ	VDL LHFQSŎŤ	LAAGAAPAVŎ	lvkkgnrgğğ	DANSDRPPLÖ		Bands	ID	Edman	MW _{Theor}	MW _{Exp}
DHRDVVMLDN	LPDI.PPAL.VP	SPPKRARPSP	100 GHCLPEDEDP	110 EEBLBBHDOH	TTAVI.TKITE		Intact RPAP1 (1 - 351)	RPAP1	GSSHH	39.4	~52.0
130	140	150	160	170	180		G1 – Q27 S28 – T360	-	- n/a	3.1	- ~11 9
RDTSSVAVŇĹ	PVPSGVAFPÁ	VFLRSRDTQG	KSATSGKRŠÍ	FAQEIAARRÍ	AEAKGPSVGÉ		G1 – Q245	2	n/a	26.4	~33.0
190 VVPNVGPPEG	200 AVTCETPTPR	210 NQGCQLPGSS	220 HSFQGPNLVT	230 GKGLRDQEAE	240 QEAQTIHEEN	RPAP1-I	N S28 - Q245	3	SQFLA	23.2	~29.0
	260 ETLOEOOBLL	270	280 LESHSHTOEO	290	300 PGGPSANVTK		Truncated RPAP1	5	GSSHH	unknown	~15.0
310	320	330	340	350	360		n/a: no amino acids iden	6 tified 225 RF	SQFLA 2AP1-N 270	356 RPAP1	~8.0
EEPLMSAFAS	EPRKRDKLEP	EAPALALPVT	POKEMTHWDI	VELEKTHM.LŐ	DT55AKKŐŐJ			RPAP1-	like N-terminal	RPAP1-like C	-terminal
B PTBP1-3 ₁₀	20	30	NLS 40	50	60		Recombinant PTBP1-3 a	analvsed in Fig	a. 3B		
HHHHHHMDG1	VPDIAV <u>GTKR</u>	GSDELFSTCV	TNGPFIMSSN	SASAANGNDS	KKFKGDSRSA		Bands	ID	Edman	MW _{Theor}	MW _{Exp}
GVPSRVIHIR	KLPIDVTEGE	VISLGLPFGK	VTNLLMLKGK	NQAFIEMNTE	EAANTMVNYY	RRM1	Intact PTBP1 (1 – 557)	PTBP1	sequencing n/a	(kDa) 60.5	(kDa) ~64.0
130 TSVTPVLRGO	140 PIYTOFSNHK	150 ELKTDSSPNO	ARAOAALOAV	170 NSVOSGNIAL	180 AASAAAVDAG		H1 - Q158	-	-	17.2	-
190	200	210	~ ~ 220	230	240		H1 – Q327	-	-	43.3 35.1	-
MAMAGQSPVL	RIIVENLFYP	VTLDVLHQIF	SKFGTVLKII	TFTKNNQFQA	LLQYADPVSA	RRM2	A328 – 1563 A159 – unknown	1	AAGLS AVNSV	25.3	~27.0 ~24.0
QHAKLSLDGQ	NIYNACCTLR	IDFSKLTSLN	VKYNNDKSRD	YTRPDLPSGD	SQPSLDQTMA		A159 – Q327	3	AVNSV	18.0	~23.0
310 AAFGAPGIIS	320 ASPYAGAGEP	BTEATPOAAG	340	350 APLATPSAAA	360 AAAAAGRTAT		n/a: no amino acids iden	tified 11 NLS	48 Nuclear loca	alization signa	I
370	380	390	400	410	420		60 RRM1 129				
PGLAGAGNSV	LLVSNLNPÉŘ	VTPQSLFİLF	GVYGDVQRVK	ILFNKKENAL	VQMADGNQAQ	RRM3	185 RRM2 254				
430 LAMSHLNGHK	440 LHGKPIRITL	450 SKHQNVQLPR	460 EGQEDQGLTK	470 DYGNSPLHRF	480 KKPGSKNFQN		RNA r	ecognition mot	if		
490	500	510	520	530	540	DDM4	338 RRM3 407				
IFPPSATLEL 550	SNIPPSVSEE 560	DLKVLFSSNG	GVVKGEKEEQ	KDRKMALIQM	GSVELAVQAL	RKM4	455 RRM4 525				
IDLHNHDLGE	NHHLRVSFSK	STI									
C IMA4 10	20	30	40	50	60		Recombinant partial IMA	4 analysed in	Fig. S2D	N/10/	N414/
GSSHHHHHHĖ	NPSLENHRIK	SFKNKGRDVĚ	TMRRHRNEVT	VELRKNKRDÉ	HLLKKRNVPÓ	IBB	Bands	ID	sequencing	(kDa)	(kDa)
70 EESLEDSDVD	80 ADFKAQNVTL	EAILQNATSD	100 NPVVQLSAVQ	AARKLLSSD <mark>R</mark>	120 NPPIDDLIKS	Armodill	Intact IMA4 (3 – 220)	IMA4	GSSHH	25.4	~29.0
130	140	150	160	170	180	repeats	G1 – Q85	1	n/a n/a	15.3	~16.0 ~15.0
GILPILVACL	200	210	SGISAQIQAV	VQSNAVPLFL	KLLKSPRQNV		n/a: no amino acids iden	tified 11 IBB	93 Importin bet	a binding don	nain
CEQAVWALGN	IIGDGPQCRD	YVISLGVVKP	LLSFISPSIP	ITFLRNV			103 Armadillo re	peats (1 to 8)	447 Arm-3 449	Atypical arma	adillo repeat
D MAP4K510	20	30	4.0	50	60		~				
нннннмзрі	LGYWKIKGLŸ	QPTRLLLEYL	EEKYEEHLYE	RDEGDKWRŇK	KFELGLEFPN	1	Recombinant MAP4K5 a	nalysed in Fig	9. 4C	N/10/	N414/
	0.0	0.0	100	110	120		Rande	ID	Edman	IVI VV Theor	IVI VV Exp
70 Lpyyidgdvk	LTOSMAIIRY	IADKHNMLGG	CPKERAEISM	LEGAVLDIRY	GVSRIAYSKD)	Danus	U	sequencing	(kDa)	(kDa)
70 LPYYIDGDVK 130	80 LTQSMAIIRY 140	IADKHNMLGG 150	CPKERAEISM	LEGAVLDIŘÝ 170	GVSRIAYŠŘĎ 180		Intact MAP4K5 (1 – 846)	MAP4K5	sequencing n/a	(kDa) 121.3	(kDa) ~119.0
70 LPYYIDGDVK 130 FETLKVDFLS	LTQSMAIIRY 140 KLPEMLKMFE 200	IADKHNMLGG 150 DRLCHKTYLN 210	CPKERAEISM 160 GDHVTHPDFM 220	LEGAVLDĪRŸ 170 LYDALDVVLY 220	GVSRIAYŠKD 180 MDPMCLDAFP 240		Intact MAP4K5 (1 – 846) H1 – M680 S681 – Y1070	MAP4K5 1 2	sequencing n/a n/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
70 LPYYIDGDVK 130 FETLKVDFLS 190 KLVCFKKRIE	LTQSMAIIRY 140 KLPEMLKMFE 200 AIPQIDKYLK	90 IADKHNMLGG 150 DRLCHKTYLN 210 SSKYIAWPLQ	CPKERAEISM 160 GDHVTHPDFM 220 GWQATFGGGD	LEGAVLDÍŘÝ 170 LYDALDVVLY 230 HPPKMEAPLR	GVSRIAYŠKĎ 180 MDPMCLDAFP 240 PAADILRRNP		Intact MAP4K5 (1 – 846) H1 – M680 <u>S681 – Y1070</u> n/a: no amino acids iden	MAP4K5 1 2 tified	sequencing n/a n/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
70 LPYYIDGDVK 130 FETLKVDFLS 190 KLVCFKKIE 250 OODYELVORV	LTQSMAIIRY 140 KLPEMLKMFE 200 AIPQIDKYLK CSGTYGDVYK	IADKHNMLGG 150 DRLCHKTYLN 210 SSKYIAWPLQ 270 ARNVHTGELA	CPKERAEISM 160 GDHVTHPDFM 220 GWQATFGGGD AVKIIKLEPG 280	LEGAVLDÍŘÝ 170 LYDALDVVLY APPKMEAPLR 290 DDESLIOOEI	GVSRIAYŠKŬ 180 MDPMCLDAFP 240 PAADILRRNP 300 FMVKECKHCN		Intact MAP4K5 (1 – 846) H1 – M680 <u>\$681</u> – Y1070 n/a: no amino acids iden	MAP4K5 1 2 tified	sequencing n/a n/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
70 LPYYIDGDVK 130 FETLKVDFLS KLVCFKKRIE QQDYELVQRV 310	LTQSMAIIRY 140 KLPEMLKMFE 200 AIPQIDKYLK 260 GSGTYGDVYK 320	IADKHNMLGG 150 DRLCHKTYLN 210 SSKYIAWPLQ 270 ARNVHTGELA 330	CPKERAEISM 160 GDHVTHPDFM 220 GWQATFGG80 AVKIIKLEPG 340	LEGAVLDÍŘÝ 170 LYDALDVVLY HPPKMEAPLR 290 DDFSLIQOEI 350	GVSRIAYŠKĎ 180 MDPMCLDAFP 240 PAADILRRNP 300 FMVKECKHCN 360		Intact MAP4K5 (1 – 846) H1 – M680 <u>\$681</u> – Y1070 n/a: no amino acids iden	MAP4K5 1 2 tified	sequencing n/a n/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
70 LPYYIDGDVK 130 FETLKVDFLS NLVCFKKRIE 00DYELVORV 1VAYFGSYLS	LTQSMAIL8Y 140 KLPEMLKMFE 200 AIPQIDKYLK GSGTYGDVYK 320 REKLWICMEY	IADKHNMLGG 150 DRLCHKTYLM 210 SSKYIAWPLO 270 ARNVHTGELA 330 CGGGSLODIY	CPKERAEISM 160 GDHVTHPDFM 220 GWQATFGGGD 280 AVKIIKLEPG HVTGPLSEL0	LEGAVLDÍŘÝ 170 LYDALDVVLY HPPKMEAPLR 290 DDFSLIQQEI 350 IAYVCRETLQ	GVSRIAYŠKĚ 180 MDPMCLDAFP PAADILRNP 300 FMVKECKHCN GLAYLHTKGK	S – TKc	Intact MAP4K5 (1 – 846) H – M680 <u>\$681 – Y1070</u> n/a: no amino acids iden 20 S – TKc) 277 Serinu	MAP4K5 1 2 tified	sequencing n/a n/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
700 PFYTIDGDVK 130 FETLKVDFLS 190 KLVCFKKRIE 000 YELVORV IVAYFGSYLS 370 MHRDIKGANI	LTQSMAIL8Y 140 KLPEMLKMFE 200 AIPQIDKYLK 260 GSGTYGDVYK 260 REKLWICME 320 REKLWICME 380 LLTDHGDVKL	IADKHNMLGG 150 DRLCHKTYLM 210 SSKYIAWPLQ 270 ARNVHTGELA 330 CGGGSLQDIY 390 ADFGVAAKIT	CPKERAEISM 160 GDHVTHPDFM 220 280 AVKIIKLEPG AVKIIKLEPG HVTGPLSELQ 400 ATIAKRKSFI	LEGAVLDÍŘÝ 170 LYDALDVVLY HPPKMEAPLR 290 DDFSLIQQEI 350 IAYVCRETLQ 410 GTPYWMAPEV	GVSRIAYŠKD 180 MDPMCLDAFP 240 PAADILRNP 300 FMVKECKHCN GLAYLHTKGK 420 AAVEKNGGYN	S – TKc	Intact MAP4K5 (1 – 846) H1 – M680 <u>\$681 – Y1070</u> n/a: no amino acids iden 20 <mark>S – TKc</mark> 277 Sering	MAP4K5 1 2 tified	sequencing n/a n/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
200 FETLKVDFLS KLVCFKKRIE QQDYELVQRV IVAYFGSYLS MHRDIKGANI 01CDIMAYGT	LTQSMAILRY 140 KLPEMLKMFE 200 AIPQIDKYLK 260 GSGTYGDVYK 320 REKLWICMEY 380 LLTDHGDVKL 440 TALELCELOP	IADKHNNLGG 150 DRLCHKTVIN 210 SSKYIAWPLO 270 ARNVHTGELA 330 CGGGSLODIY ADFGVAAKIT 450 PMEDLHDWBA	CPKERAEISM 160 GDHVTHPDFM 220 GWQATFGGGD AVKIIKLEPG AVKIIKLEPG 400 ATIAKRKSFI 460 LEIMSKSNEO	LEGAVLDÎRY 170 LYDALDVVIY 230 HPPKMEAPLR 290 DFSLIQQEI 350 IAYVCRETLO GTPYWMAPEY 470 PEKLENTKM	GVSRIAYŠKO 1800 MDPMCLDAFP 240 PAADILRRNP 300 FMVKECKHCN GLAYLHTKGK 420 AAVEKNGGYN SSTEHNEVKT	S – TKc	Intact MAP4K5 (1 – 846) H1 – M680 <u>\$681 – Y1070</u> n/a: no amino acids iden 20 <mark>S – TKc</mark> 277 Serino	MAP4K5 1 2 tified	sequencing n/a N/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
270 PEYYIDGOVK FETLKVDFLS KLVCFKKRIE QQDYELVQRV IVAYFGSYLS MHRDIKGANI QLCDIWAVGI 490	LTQSMAILAY ALPEMLKMFE 200 AIPQIDKYLK 260 GSGTYGDVYK 320 REKLWICMEY 110 LITDHGDVKL 440 TAIELGELQP 500	IADKHNMLGG 150 DRLCHKTVIN 210 SSKYIAWPLO ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 450 PMFDLHPMRA 510	CPKERAEISM 160 GDHVTHPDFM 220 GWOATFGGGD AVKIIKLEPG HVTGPLSELQ ATIAKRKSFI 460 LFLMSKSNFO 520	LEGAVLDÎRŸ 170 LYDALDVVIY 230 HPPKMEAPLR 290 DDFSLIQOEI IAYVCRETL0 GTPYWMAPEV 470 PPKLKDKTKW 530	GVSRIAYŠŘÍ 180 MDPMCLDAPP 240 PAADILRNNP 3000 FMVKECKHEN 360 GLAYLHTKGK 420 AAVEKNGGYN 485 SSTFHNFVKI 540	S – TKc	Intact MAP4K5 (1 – 846) H1 – M680 <u>\$681 – Y1070</u> n/a: no amino acids iden 20 <mark>S – TKC</mark> 277 Serind	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 77.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
270 PFYYIDGDVK FETLKVDFLS KLVCFKKRIE QQDYELVQRV IVAYFGSYLS MHRDIKGANI QLCDIWAVGI ALTKNPKKRP	LTQSMAILAY ALPEMLKMFE 200 AIPQIDKYLK GSGTYGDVYK REKLWICMEY LLTDHGDVKL 4400 TAIELGELQP TAERLLTHTF	IADKHNNLGG 1500 DRLCHKTVIN 210 SSKYIAWPLO 2700 ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 9MFDLHPMRA 510 VAQPGLSRAL	CPKERAEISM GDHVTHPDFM 220 GWOATFGGGD AVKIIKLEPG HVTGPLSELQ ATIAKRKSFI 460 LFLMSKSNFO AVELLDKVNN	LEGAVLDÎRŸ 170 LYDALDVVIY 230 HPPKMEAPLR 290 DFSLIQOEI IAYVCRETLO GTPYWMAPEV 470 PPKLKDKTKW 530 PDNHAHYTEA	GVSRIAYŠŘÍ 180 MDPMCLDAPP 240 PAADILRNNP 3000 FMVKECKHON GLAYLHTKGK AAVEKNGGYN 420 AAVEKNGGYN 5540 DDDDFEPHAI	S – TKc	Intact MAP4K5 (1 – 846) H1 – M680 <u>\$681 – Y1070</u> n/a: no amino acids iden 20 <mark>S – TKC</mark> 277 Serind	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 777.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
200 FETLKVDFLS KLVCFKKRIE QQDYELVQRV IVAYFGSYLS MHRDIKGANI 0LCDIWAVGI ALTKNPKKRP IRHTIRSTNR	LTQSMAILAY ALPONTRACTOR ALPOIDKYLK 200 ALPOIDKYLK 260 CSGTYGDVYK 320 REKLWICMEY ALTDHGDVKL 440 TAIELGELOP TAERLLTHTF 500 TAERLLTHTF 0 NARAERS	IADKHNNLGG 150 DRLCHKTYLN 210 SSKYIAWPLO 270 ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 9MFDLHPMRA VAQPGLSRAL 510 VAQPGLSRAL 0 INFDKLQFFP	CPKERAEISM CPKERAEISM CGHVTHPDFM 220 GWOATFGGGD AVKIIKLEPG AVKIIKLEPG 400 ATIAKRKSFI 460 LFLMSKSNFO AVELLDKVNN 520 AVELLDKVNN 520 AVELLDKVNN	LEGAVLDÍŘÝ 170 LYDALDVVIY 230 HPPKMEAPLR 290 DJFSLIQOEI IAYVCRETLO GTPYWMAPEV 470 PPKLKDKTKW PDNHAHYTEA 590 EMGLSSDPNF	GVSRIAYŠŘÍ 180 MDPMCLDAPP PAADILRRNP 3000 FMVKECKHON GLAYLHTKGK 420 AAVEKNGGYN 85TFHNFVKI DDDDFEPHAI DDDDFEPHAI 6500 MLQWNPFVDG	S – TKc	Intact MAP4K5 (1 – 846) H1 – M680 <u>\$681 – Y1070</u> n/a: no amino acids iden 20 <mark>S – TKC</mark> 277 Serind	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 777.2 44.1	(kDa) ~119.0 ~75.0 ~43.0
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270 PPYYIDGVK 130 FETLKVDFLS QQDYELVOFK 190 QQDYELVORV 310 IVAYFGSYLS MHRDIKGANI 0LCDIWAVGI ALTKNPKKRP IRHTIRSTNR ANTGKSTSKR 610 670	LTQSMAILAY ALPEMLKMFE 200 AIPQIDKYLK 250 GSGTYGDVYK 320 REKLWICMEY ALTDHGDVKL 440 TAIELGELOP TAERLLTHTF NARAERTASE AIPPPLPPLP	IADKHNNLGG 150 DRLCHKTYLN 210 SSKYIAWPLQ 270 ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 9MFDLHPMRA 510 VAQPGLSRAL INFDKLQFFP RISSYPEDN 630 690	CPKERAEISM GDHVTHPDFM 220 GWOATFGGGD AVKIIKLEPG 400 ATIAKRKSFI 400 ATIAKRKSFI 400 ATIAKRSFI 400 AU AU AU AU AU AU AU AU AU AU	LEGAVLDÍŘÝ 170 LYDALDVVIY 230 HPPKMEAPLR 290 DDFSLIQOET 1AYVCRETLO 6TPYMAPEV 410 GTPYMAPEV 470 PPKLKDKTKW PDNHAHYTEA 530 PDNHAHYTEA 530 MCPDSESRAP HCPDSESRAP	GVSRIAYŠŘÍ 180 MDPMCLDAPP PAADILRRNP 3000 FMVKECKHON GLAYLHTIGK 420 AAVEKNGGYN SSTFHNFVKI DDDDFEPHAI MLQWNPFVDG QILRRQSSPS 720	S – TKc	20 S – TKc 277 Serind	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 777.2 44.1	(KDa) ~119.0 ~75.0 ~43.0
2PYYIDGDVK 130 FETLKVDFLS QQDYELVQFK IVAYFGSYLS MHRDIKGANI 0LCDIWAVGI ALTKNPKKRP IRHTIRSTNR ANTGKSTSKR CGPVAETSSI	LTQSMAILAY ALPENLKMFE 200 AIPQIDKYLK 200 CSGTYGDVYK 320 REKLWICMEY 380 LLTDHGDVKL 440 TAIELGELOP TAERLLTHTF NARAERTASE AIPPPLPFKF GNGDGISKLM	IADKHNNLGG 150 DRLCHKTYIN 210 SSKYIAWPLQ 270 ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 9MFDLHPMRA VAQPGLSRAL INFDKLQFFP RISSYPEDNF SENTEGSAQA	CPKERAEISM GDHVTHPDFM 220 GWQATFGGGD AVKIIKLEPG 400 ATIAKRKSFI 400 ATIAKRKSFI 400 ATIAKRSFI 400 ATIAKRSFI 400 AUXIIKLEPG AUXIIKLEPG 400 AUXIIKLEPG AUXIIKLEP	LEGAVLDÍŘÝ 170 LYDALDVVLY 230 HPPKMEAPLR 290 DDFSLIQOEI 1AYVCRETLO GTPYWMAPEV 410 GTPYWMAPEV 410 GTPYWMAPEV 420 PPKLKDKTKW PDNHAHYTEA EMGLSSDPNF HCPDSESRAP DFPKPAINGL	GVSRIAYŠŘÍ 180 MDPMCLDAPP PAADILRRNP GLAVILRRNP GLAVILHTKGK 420 AAVEKNGGYN SSTFHNFVKI DDDDFEPHAI MLQWNPFVDG QILRRQSŠPS QILRRQSŠPS 720 PPTPKVLMGA	S – TKc	20 S – TKc 277 Serind	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 777.2 44.1	(KDa) ~119.0 ~75.0 ~43.0
2PYYIDGVK FETLKVDFLS KLVCFKKRIE QQDYELVQRV IVAYFGSYLS MHRDIKGANI QLCDIWAVGI ALTKNPKKRP IRHTIRSTNR ANTGKSTSKR CGPVAETSSI CFSKVFDGCP	LTQSMAILRY 140 KLPEMLKMFE 200 AIPQIDKYLK 260 GSGTYGDVYK 320 REKLWICMEY 380 LLTDHGDVKL 440 TAIELGELOP TAERLLTHTF NARAERTASSE AIPPPLPFKF GNGDGISKLM 2740 LKINCATSWI	IADKHNNLGG 150 DRLCHKTYIN 210 SSKYIAWPLQ 270 ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 9MFDLHPMRA VAQPGLSRAL INFDKLQFFP RISSYPEDNF SENTEGSAQA HPDTKDQYII	CPKERAEISM GDHVTHPDFM 220 GWQATFGGGD 280 AVKIIKLEPG 400 ATIAKRKSFI 400 ATIAKRKSFI 400 ATIAKRKSFI 400 ATIAKRSFI 520 AVELLDKVNN PLRKETEARD PDEEKASTIK PDEEKASTIK 700 PQLPRKKDKR 700 FGTEDGIYTL	LEGAVLDÎRY 170 LYDALDVVLY 230 HPPKMEAPLR 290 DDFSLIQQEI 3500 IAYVCRETLQ 670 PPKLKDKTKW PDNHAHYTEA PDNHAHYTEA EMGLSSDPNF HCPDSESRAP DFPKPAINGL 770 NLNELHEATM	GVSRIAYŠŘÍ 180 MDPMCLDAPP PAADILRRNP GLAVILRRNP GLAVILHTKGK 420 AAVEKNGGYN 420 AAVEKNGGYN 0 SSTFHNFVKI MLQWNPFDOG QILRRQSŠPS QILRRQSŠPS QILRRQSŠPS 200 PPTPKVLMGA 200 200 200 200 200 200 200 20	S – TKc	20 S – TKc 277 Serind	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 777.2 44.1	(KDa) ~119.0 ~75.0 ~43.0
2PYYIDGVK FETLKVDFLS KLVCFKKRIE QQDYELVQRV IVAYFGSYLS MHRDIKGANI OLCDIWAVGI ALTKNPKKRP IRHTIRSTNR ANTGKSTŠKR CGPVAETŠSI CFSKVFDGCP 790	LTQSMAILRY 140 KLPEMLKMFE 200 AIPQIDKYLK 260 GSGTYGDVYK 320 REKLWICMEY 140 LITDHGDVKL 440 TAIELGELQP TAERLLTHTF NARAERTASE AIPPPLPER GNGDGISKLM LKINCATSWI	IADKHNNLGG 150 DRLCHKTYIN 210 SSKYIAWPLQ 270 ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 9MFDLHPMRA VAQPGLSRAL INFDKLQFFP RISSYPEDNF SENTEGSÁQA HPDTKDQYII 00000000000000000000000000000000000	CPKERAEISM GDHVTHPDFM 220 GWQATFGGGD 280 AVKIIKLEPG 400 ATIAKRKSFI 400 ATIAKRKSFI 400 ATIAKRKSFI 520 AVELLDKVNN PLRKETEARD PDEEKASTIK PDEEKASTIK 700 PQLPRKKDKR FGTEDGIYTL 820	LEGAVLDÎRY 170 LYDALDVVLY 230 HPPKMEAPLR 290 DDFSLIQQEI 1AYVCRETLQ 6TPYWMAPEV 470 PPKLKDKTKW PDNHAHYTEA EMGLSSDPNF HCPDSESRAP DFPKPAINGL 770 NLNELFEATM 830	GVSRIAYŠŘÍ 180 MDPMCLDAPP PAADILRRNP GLAVILRRNP GLAVILHTKGK 420 AAVEKNGGYN 0 SSTFHNFVKI MLQWNPFVDG QILRRQSŠPS QILRRQSŠPS PPTPKVLMGA 202 PPTPKVLMGA 202 202 202 202 202 202 202 20	S – TKc	20 S – TKc 277 Serind	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 777.2 44.1	(KDa) ~119.0 ~75.0 ~43.0
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1991100 FETLKVDFLS RETLKVDFLS QQDYELVORV 190 QQDYELVORV 310 IVAYFGSYLS MHRDIKGANI 0LCDIWAVGI ALTKNPKKRP IRHTIRSTNR ANTGKSTSKR ANTGKSTSKR CGPVAETSSI CFSKVFDGCP LYVINNTLMS PDTKGCHRCC MLVIPEQEYP DTVLVCLDRA	LTQSMAILRY 140 KLPEMLKMFE 200 AIFQIDKYLK 260 GSGTYGDVYK 320 REKLWICMEY 140 CSGTYGDVYK 320 REKLWICMEY 380 LITDHGDVKL 440 TATELGELQP TAERLLTHTF NARAERTASE AIPPPLPER 0 NARAERTASE AIPPPLPER 0 NARAERTASE 4 0 NARAERTASE 0 N	IADKHNNLGG 1500 DRLCHKTVIN 210 SSKYIAWPLQ 270 ARNVHTGELA 330 CGGGSLQDIY ADFGVAAKIT 900 800 900 800 900 800 900 800 900 9	CPKERAEISM CPKERAEISM GDHVTHPDFM 220 GWQATFGGGD AVKIIKLEPG AVKIIKLEPG AUTIAKRKSFI 400 ATIAKRKSFI 400 ATIAKRKSFI 520 AVELLDKVNN PLRKETEARD PDEEKASTIK FGTEDGIYTL AKKPGLAAHI VLLQWYEPMQ INLNSASSWF 1000 SFDFRIESVV	LEGAVLDÎRY 170 LYDALDVVLY 230 HPPKMEAPLR 290 DDFSLIQQEI 1AYVCRETLQ 6TPYWMAPEV 410 GTPYWMAPEV 470 PKLKDKTKW PDNHAHYTEA 650 EMGLSSDPNF HCPDSESRAP DFPKPAINGL 770 0FPKPAINGL 7710 0FPKPAINGL 770 0CHRFPDRIL 830 0THRFPDRIL 1010 CLQDSVLAFW	GVSRIAYŠŘÍ 180 MDPMCLDAPP PAADILRNP 240 PAADILRNP 360 GLAYLHTKGK 420 AAVEKNGGYN 420 AAVEKNGYN 420 420 420 420 420 420 420 420	S – TKc	Intact MAP4K5 (1 – 846) H1 – M680 S681 – Y1070 n/a: no amino acids iden 20 S – TKc 20 S – TKc 21 S – TKc 22 S – TKc 23 S – TKc 20 S –	MAP4K5 1 2 tified	sequencing n/a SENTE	(kDa) 121.3 777.2 44.1	(KDa) ~119.0 ~75.0 ~43.0
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fusion tag J3CL^{pro} cleavage site identified by TAILS J3CL^{pro} cleavage site identified by Edman sequencing

Figure S3 related to Figures 3, 4, and S2. Recombinant protein substrate details.

Protein sequence and Edman sequencing of 3CL^{pro}-cut sites in the recombinant proteins shown in Figures 3, 4 and S2. Blue, P-side. Red, P'-side (identified by TAILS). Green, P'-side (identified by Edman sequencing). Theoretical molecular weight (ProtParam, ExPASy) and experimental molecular weight (GelAnalyzer version 19.1) of intact proteins and the proteolytic fragments generated by 3CL^{pro}. Protein domain information was accessed from Smart (https://smart.embl.de).

(A) Partial recombinant RPAP1 (1 – 351 aa, BC000246).

(B) PTBP1 (1 – 557 aa, NP_002810.1).

(C) Partial recombinant IMA4 (3 – 220 aa, NP_002258.2).

(D) MAP4K5 (1 – 846 aa, NP_006566.2).

(E) CREB1 (1 – 327 aa, NM_004379).



Figure S4 related to Figures 3 and 4. 3CL^{pro} substrates in bronchial or SARS-CoV-2 infected human lung cells.

(A) Immunoblots for the proteins indicated of lysates from HAECs (N = 5 donors) incubated with 3CL^{pro} or 3CL^{pro}-C145A (1:200 w/w, E:S) for 18 h, 37°C. Anti-FLAG antibody verified that equivalent amounts of 3CL^{pro} and inactive 3CL^{pro}-C145A were incubated.

(B) Schematics of all substrates validated in Figures 3 and 4. 3CL^{pro} cleavage sites identified by TAILS (red arrow) and Edman sequencing (green arrow).

(C) Uncropped immunoblots of substrates in Calu-3 lung epithelial cells infected with SARS-CoV-2 for 24 and 48 h (MOI 0.1 and 1.0, n = 4, n = 2 mock). Proteolytic fragments indicated by apparent molecular weights or Δ .

(D) Immunoblots of $3CL^{pro}$ and nucleocapsid protein (N) in Calu-3 cells infected with SARS-CoV-2 at a MOI 0.1 and 1.0 for 24 (n = 4) and 48 (n = 4) hpi, or mock-treated (n = 3). Positive control, recombinant (r) $3CL^{pro}$ in control cell lysate. b-actin and b-tubulin loading controls.





Secondary antibody control

в

Goat α-mouse Alexa 546	DAPI	Goat α-rabbit Alexa 488	Uninfected (mock)-Vero E6 cells
Goat α-mouse Alexa 546	DAPI	Goat α-rabbit Alexa 488	SARS-CoV-2 infected Vero E6 ce

S-CoV-2 infected Vero E6 cells

Uninfected (mock)-Vero E6 cells

D

C Exp 1	Uninfected (mock)-Vero E6 cells	SARS-CoV-2 infected Vero E6 cells					
PTBP1	DAPI SARS-CoV-2 Spike	Merge	PTBP1	DAPI	SARS-CoV-2 Spike	Merge	PTBP1 DAPI
Exp. 2	2-8-9-P-8-4-1		Exp. 2				
					10 - 1 O		
Exp. 3			Exp. 3				
			and a				
Exp. 4			Exp. 4				

SARS-CoV-2 infected Vero E6 cells



Figure S5 related to Figure 3. Subcellular localization of PTBP1 in Vero E6 cells infected with SARS-CoV-2.

(A) Immunoblot of PTBP1 and cleaved (Δ) PTBP1 in the SARS-CoV-2-infected Vero E6 cells
(MOI 0.1, 48 hpi) used for subcellular localization in B–D. b-actin loading controls.
(B) Secondary antibody controls.

(C) Subcellular localization of PTBP1 in uninfected (N = 4, mock) and SARS-CoV-2-infected (N = 4) Vero E6 cells. Confocal images of immunofluorescent localization of PTBP1 and SARS-CoV-2 Spike S1 glycoprotein. DAPI, nuclear stain. Scale bar, 50 μ m. Far-right panels, merge of

PTBP1 and DAPI channels to visualize cytoplasmic versus nuclear PTBP1.

(D) Enlarged and uncropped confocal imaging of Figure 3G, Spike-positive (S+) and Spikenegative (S-) (scale bar, 50 μ m). Cyan boxes, S- uninfected cells. Green boxes, S+ infected cells. Dark blue-box, cytoplasmic PTBP1 in anaphase cells.



Figure S6 related to Figure 5. Loss of galectin-8 biological function and binding to SARS-CoV-2 Spike S1 glycoprotein after cleavage by 3CL^{pro}.

(A) MS/MS spectrum of TAILS neo-N-terminal peptide (red) identifying $3CL^{pro}$ cleavage site in galectin-8 (Gal8) in N = 9/9 BEAS-2B cell experiments.

(B) Reference sequence of recombinant Gal8 (short linker) (1 - 317) #AAF 19370.1. Edman sequencing of 3CL^{pro}-cleaved Gal8 in Figure 5C.

(C) Structural model of Gal8 P5–P1 (blue sticks) and P1'–P4' (red sticks) amino acid residues interacting with the 3CL^{pro} substrate-binding S and S'-subsites, respectively (grey surface).

(D) Cleaved recombinant human Gal8 (Δ Gal8) prepared for agglutination (S6E), adhesion (S6F), and binding (Figures 5G and 5J) assays.

(E) Human erythrocyte agglutination assay with intact or Δ Gal8, or 3CL^{pro} (control).

(F) Adherent *versus* non-adherent Jurkat T cells in serum-free RPMI containing intact or Δ Gal8, or 3CL^{pro} (control) were counted after DAPI/phalloidin staining. Scale bars, 20x: 50 µm; 100x: 20 µm. Mean ± SD, n = 2, *** P ≤ 0.001 , ** P ≤ 0.01 , ns P > 0.05, one-way ANOVA with Tukey's multiple comparisons test.

(G) Gal8 immunoblots of HAECs lysates from N = 5 donors incubated with 3CL^{pro} or 3CL^{pro}-C145A (1:200 w/w, E:S) for 18 h, 37°C. b-tubulin loading control.

(H) ELISA of SARS-CoV-2 Spike S1 glycoprotein binding to immobilized Gal8 (mean \pm SD, $n = 2, N = 2, * P \le 0.05$, Student's t-test).

(I) Anti-Gal8 antibody ELISA of Gal8 binding to immobilized SARS-CoV-2 Spike S1 glycoprotein in the presence of thiodigalactoside (TDG) (mean \pm SD, n = 2, N = 2, **** $P \le 0.0001$, Student's t-test).

(J) Anti-C-Gal8 antibody ELISA of intact Gal8, cleaved Δ Gal8, or intact Gal8 in the presence of TDG binding to immobilized Spike S1.

(K) Anti-C-Gal8 antibody ELISA of intact Gal8, cleaved Δ Gal8, or TDG-inhibited Gal8 binding to immobilized NDP52. One-way ANOVA with Tukey's multiple comparisons test (mean \pm SD, n = 3, ns P > 0.05).

(L) Transfected GFP-NDP52, WT-Gal8-FLAG or C-Gal8 (159-317)-FLAG 3CL^{pro}-cleavage analogue detected in the Hela-cell lysates before immunoprecipitation experiments shown in Figure 5H. Immunoblots were performed with specific anti-FLAG antibody, anti-NDP52 antibody, and with β -actin used as the loading control.

(M) SDS-PAGE of NDP52 and SARS-CoV-2 Spike S1 incubated with recombinant 3CL^{pro}.