

Table S1. Presentation of the non-canonical YXXXΦ [M/L/I/V/F] motifs, the reverse [M/L/I/V/F] ΦXXY motifs and the di-leucine motifs within the SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a		SARS-CoV-2-ORF3a	
Non-canonical YXXXΦ motifs			
peptide	position	peptide	position
YLYAI	107 - 111	YLYAV	107 - 111
YDYCI	154 - 158	YDYCI	154 - 158
YFTEV	206 - 210	-	-
-		YSTQL	215 - 219
Reverse ΦXXY motifs			
peptide	position	peptide	position
LALY	71-74	-	-
VTIY	88 - 91	VTVY	88 - 91
LYLY	106 - 109	LYLY	106 - 109
IGGY	186-189	IGGY	186-189
VKDY	197 – 200	-	-
VHGY	203 - 206	LHSY	203 - 206
-	-	FFIY	230 - 233
Di-leucine motifs			
peptide	position	peptide	position
SKNPLL	135-140	SKNPLL	135-140

Table S2. Predicted transmembrane (TM) helices topology within the SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-COV-ORF3a		SARS-COV-2-ORF3a	
topology	residues	location	residues
outside	1 - 42	outside	1 - 34
1 st TMhelix	43 - 64	1 st TMhelix	35 - 56
inside	65 - 75	inside	57 - 76
2 nd TMhelix	76 - 99	2 nd TMhelix	77 - 101
outside	100 - 104	outside	102 - 106
3 rd TMhelix	105 - 125	3 rd TMhelix	107 - 129
inside	126 - 274	inside	130 - 275

Table S3. Predicted α -helices, β -sheets and coil segments within the SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a		SARS-CoV-2-ORF3a	
structure	position	structure	position
1 st coil	1-3	1 st coil	1-3
1 st α -helix	4-5	-	-
1 st β -sheet	6-10	1 st β -sheet	4-15
2 nd coil	11-17	2 nd coil	16-28
2 nd β -sheet	18-20	-	-
3 ^d coil	21-28	-	-
3 rd β -sheet	29-35	2 nd β -sheet	29-38
4 th β -sheet	38	-	-
4 th coil	39-43	3 rd coil	39-43
2 nd α -helix	44-58	1 st α -helix	44-57
5 th coil	59	4 th coil	58-61
3 rd α -helix	60-61	-	-
5 th β -sheet	62-65	2 nd β -sheet	62-65
5 th coil	66-67	-	-
4 th α -helix	68-75	2 nd α -helix	66-74
6 th coil	76	5 th coil	75-76
5 th α -helix	77-98	3 rd α -helix	77-99
7 th coil	99-101	6 th coil	101-103
6 th α -helix	102-130	4 th α -helix	104-132
6 th β -sheet	131-132	-	-
8 th coil	133-139	6 th coil	133-139
7 th β -sheet	140-142	3 rd β -sheet	140-142
9 th coil	143-144	7 th coil	143-144
8 th β -sheet	145-150	4 th β -sheet	145-150
9 th β -sheet	156-158	5 th β -sheet	156-158
10 th coil	159-164	8 th coil	159-163
10 th β -sheet	165-170	6 th β -sheet	164-170
11 th coil	171-199	9 th coil	171-190
-	-	5 th α -helix	191 - 194
-	-	10 th coil	195-199
10 th β -sheet	200-205	7 th β -sheet	200-204
12 th coil	206	11 th coil	205-211
11 th β -sheet	207-215	8 th β -sheet	212-219
12 th coil	216	-	-
11 th β -sheet	217-220	-	-
13 th coil	221-228	12 th coil	220-225
12 th β -sheet	229 - 233	9 th β -sheet	226 - 234
7 th α -helix	234 - 236	-	-
14 th coil	237-243	12 th coil	235-243
13 th β -sheet	244 - 248	10 th β -sheet	244 - 249
15 th coil	249-274	13 th coil	250-275

Table S4. Predicted phosphorylated Ser, Tyr and Thr residues (in red) located within the YXX Φ motifs, the YXX Φ -like tetrapeptides or adjacently to them, for SARS-CoV-ORF3a and SARS-CoV-2-ORF3a. The kinase catalysing each phosphorylation is indicated.

SARS-CoV-ORF3a				
position	YXX Φ motif	sequence	score	kinase
-	-	-	-	-
S92	1 st	VTIYSHLLL	0.512	PKA
Y160	3 rd	YCIPYNSVT	0.525	unspecified
S162	3 rd	IPYNSVTD	0.971	unspecified
Y212	4 th	TEVYQLES	0.532	SRC
T217	-	QLESTQITT	0.518	DNAPK
SARS-CoV-2-ORF3a				
position	YXX Φ motif	sequence	score	kinase
S74	upstream	QLALSKGVH	0.706/ 0.535	PKC/ cdc2
S92	1 st	VTVYSHLLL	0.503	cdc2
Y160	3 rd	YCIPYNSVT	0.525	unspecified
T208	-	HSYFTSDYY	0.774	PKC
S209	-	SYFTSDYYQ	0.516/ 0.502	CKII/ PKA
Y211	4 th	FTSDYYQLY	0.698/ 0.518	unspecified NSR
Y212	4 th	TEVYQLES	0.512	SRC

Table S5. Predicted ubiquitinated Lys (in red) located within the YXX Φ -like tetrapeptides and proximal regions of SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a			
position	YXX Φ -motif	sequence	score
K19	-	SITAQPVKIDNASPA	0.64
K61	-	AVFQSATKIIALNKR	1.83
K132	-	MRCWLCWKCKSKNPL	1.74
K181	-	GISTPKLKEDYQIGG	0.52
K235	downstream-like	ATFFIFNKLVDPPN	0.31
SARS-CoV-2-ORF3a			
position	YXX Φ -motif	sequence	score
K16	-	TIGTVTLKQGEIKDA	0.63
K21	-	TLKQGEIKDATPSDF	1.13
K61	-	AVFQSASKIITLKKR	2.46
K66	-	ASKIITLKKRWQLAL	1.42
K67	-	SKIITLKKRWQLALS	0.46
K75	upstream-like	RWQLALSKGVHFVCN	0.98
K132	-	MRLWLCWKCRSKNPL	0.91

Threshold was set to 0.3

Table S6. *In silico* mutagenesis analysis for the adjacent residues (in red) to the Lys75 of the 1stYXXΦ-like tetrapeptide and ubiquitination prediction for these mutated sequences in SARS-CoV-2-ORF3a.

SARS-CoV-2-ORF3a		
	amino acids	score
consensus	SKGVHF	0.98
mutated	YKGVHF	<0.3
mutated	SKGFHF	0.48
mutated	SKGVQF	0.74

Threshold was set to 0.3

Table S7. Predicted methylated Lys residues (in red) of SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a		SARS-CoV-2-ORF3a	
position	YXXΦ-motif	position	YXXΦ-motif
-	-	K21	-
-	-	K66	-
K75	upstream	-	-
-	-	K136	-
-	-	K192	-
K235	-	K235	downstream
K238	-	-	-

Table S8. Predicted acetylated Lys residues (in red) of SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a			
position	YXXΦ motif	sequence	score
K61	-	VFQSATKIIALNK	1.32
K132	-	RCWLCWKCKSKNP	1.67
K134	-	WLCWKCKSKNPLL	0.64
K198	-	DRHSGVKDYVVVH	0.27
SARS-CoV-2-ORF3a			
position	YXXΦ motif	sequence	score
K16	-	IGTVTLKQGEIKD	0.92
K21	-	LKQGEIKDATPSD	1.46
K61	-	VFQSASKIITLKK	2.00
K66	-	SKIITLKKRWQLA	0.64
K75	upstream-like	WQLALS KGVHFVC	0.78
K132	-	RLWLCWKCRSKNP	0.73

Threshold was set to 0.3

Table S9. Predicted nitrated Tyr residues of SARS-CoV-ORF3a and SARS CoV-2-ORF3a.

SARS-CoV-ORF3a			SARS-CoV-2-ORF3a		
position	YXXΦ motif	score	position	YXXΦ motif	score
Y91	1 st	ΔG=0.111	-	1 st	-
Y141	-	ΔG=0.492	-	-	-
Y145	-	ΔG=0.025	Y145	-	ΔG=0.597
-	-	-	Y154	-	ΔG=2.703
Y263	-	ΔG=0.273	Y264	-	ΔG=0.606

ΔG: Free energy in kcal/mol.

Table S10. Predicted S-nitrosylated cysteines (in red) of SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a			SARS-CoV-2-ORF3a		
Position	FRP	sequence	Position	FRP	sequence
-	-	-	C200	3.77%	WESGVKDCVVLHSYF

FPR: false positive rate.

Table S11. Predicted S-glutathionylated Cys residues (in red) of SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a			SARS-CoV-2-ORF3a		
Position	FRP	sequence	Position	FRP	sequence
-	-	-	C130	4.67%	IIMRLWLCKWKCRSKN
-	-	-	C200	0.61%	WESGVKDCVVLHSYF

FPR: false positive rate.

Table S12. Predicted sulfated Tyr residues (in red) of SARS-CoV-ORF3a and SARS-CoV-2-ORF3a.

SARS-CoV-ORF3a			
Y position	YXXΦ motif	sequence	score
154	-	VCWHTHNYDYCIPYN	1.401
156	-	WHTHNYDYCIPYNSV	1.366
184	-	TPCLKEDYQIGGYSE	1.688
200	extra	RHSGVKDYVVVHGYF	0.738
211	4 th	HGYFTEVYQLESTQ	0.594
212	4 th	GYFTEVYQLESTQI	0.485
263	-	NPAMDPIYDEPTTTT	1.450
SARS-CoV-2-ORF3a			
Y position	YXXΦ motif	sequence	score
156	-	WHTNCYDYCIPYNSV	1.406
184	-	SPISEHDYQIGGYTE	2.025
211	-	HSYFTSDYQLYSTQ	1.767
212	-	SYFTSDYQLYSTQL	0.980
233	4 th	EHVTFFIYNKIVDEP	1.248
264	-	NPVMEPIYDEPTTTT	1.218

Threshold was set at 1.139