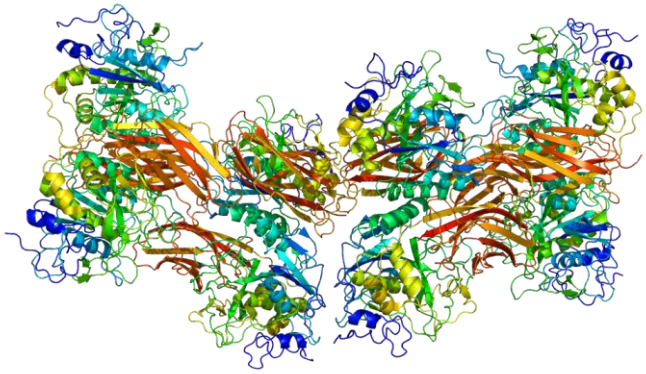
FURIN CLEAVAGE SITE IN SARS-COV-2

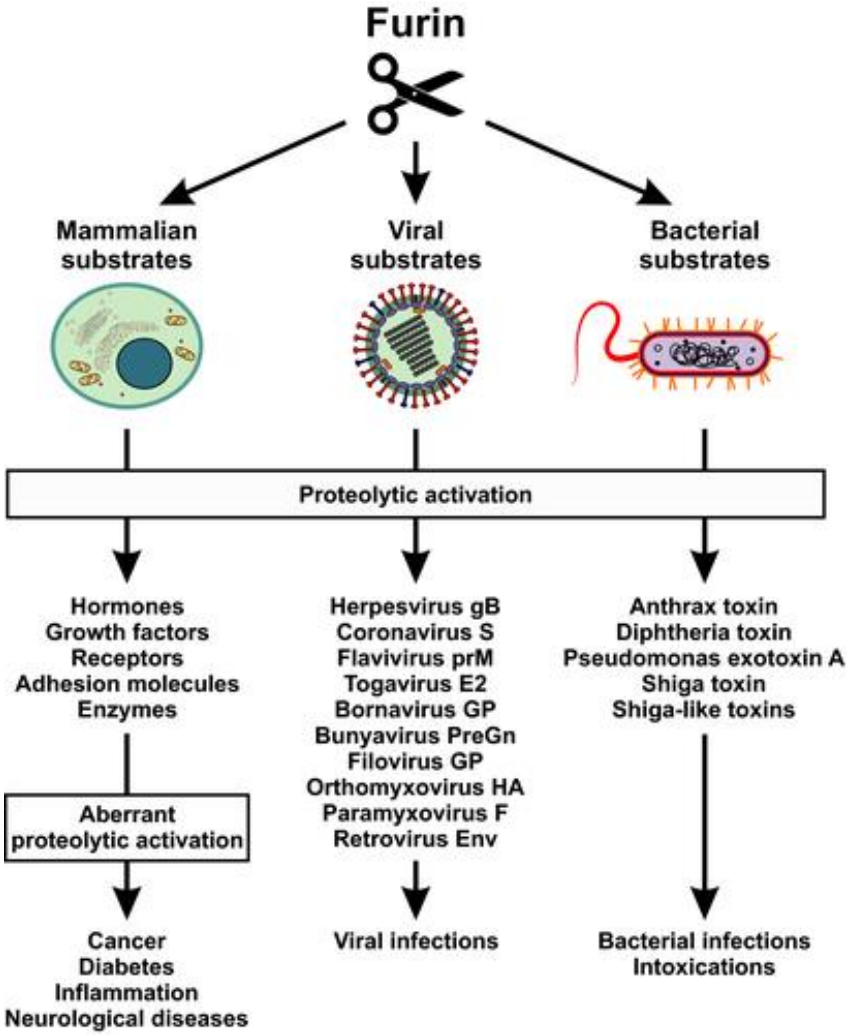
An evidence for laboratory leak or natural origin?

Anna Medgyes-Horváth

2024.

The role of the furin protease

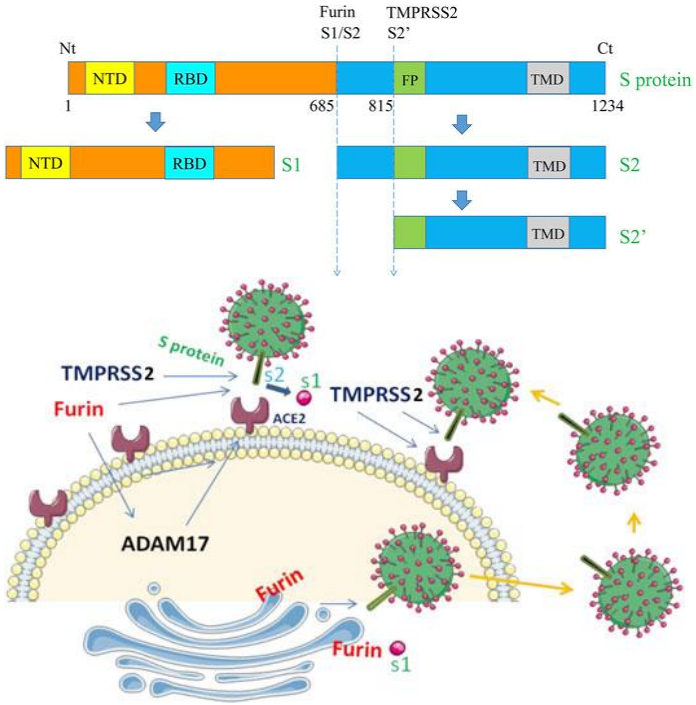




- Proprotein → active protein

Braun, E., & Sauter, D. (2019). Furin-mediated protein processing in infectious diseases and cancer. Clinical & Translational Immunology, 8(8). https://doi.org/10.1002/cti2.1073
https://en.wikipedia.org/wiki/Furin

The furin cleavage site (FCS) may increase the efficiency of virus infection in SARS-CoV-2



S protein – transmembrane protein is composed of two subunits:

S1 – receptor binding (to ACE2)	S2 – membrane fusion

The S1/S2 site (PRRAR) can be cleaved by the proprotein convertase furin that facilitates membrane fusion and viral spread.

(But may also happen in absense of furin by other proteases)

S2' site is cleaved by TMPRSS2 → S2 fusion peptide generation → initiates viral-host membrane fusion

FCS deletion				
Attenuated viral infection	Less efficient replication in lung and tracheal tissues	Less weigth loss and reduced lung damage in hamsters	Low titer virus shed - no transmission in ferrets	Augmented rate of replication in Vero E6 cells

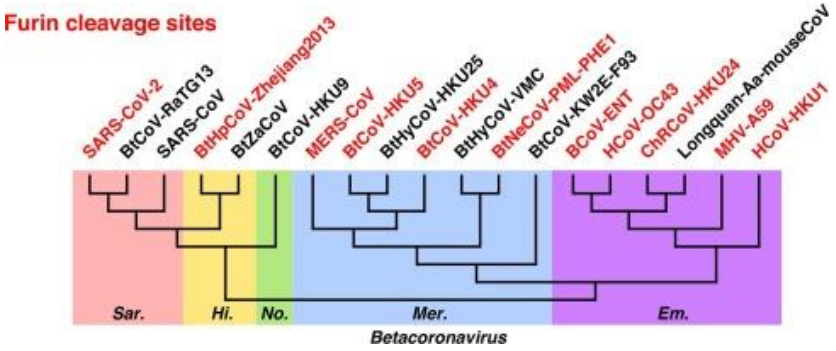
Villoutreix, B. O., et al. (2022). Furin and COVID-19: Structure, Function and Chemoinformatic Analysis of Representative Active Site Inhibitors. Frontiers in Drug Discovery, 2. https://doi.org/10.3389/fddsv.2022.899239

Peacock, T. P et al. (2021). The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. Nature Microbiology, 6(7), 899–909. https://doi.org/10.1038/s41564-021-00908-w

Johnson, B. A. et al. (2021). Loss of furin cleavage site attenuates SARS-CoV-2 pathogenesis. Nature, 591(7849), 293–299. https://doi.org/10.1038/s41586-021-03237-4

Concerns about FCS

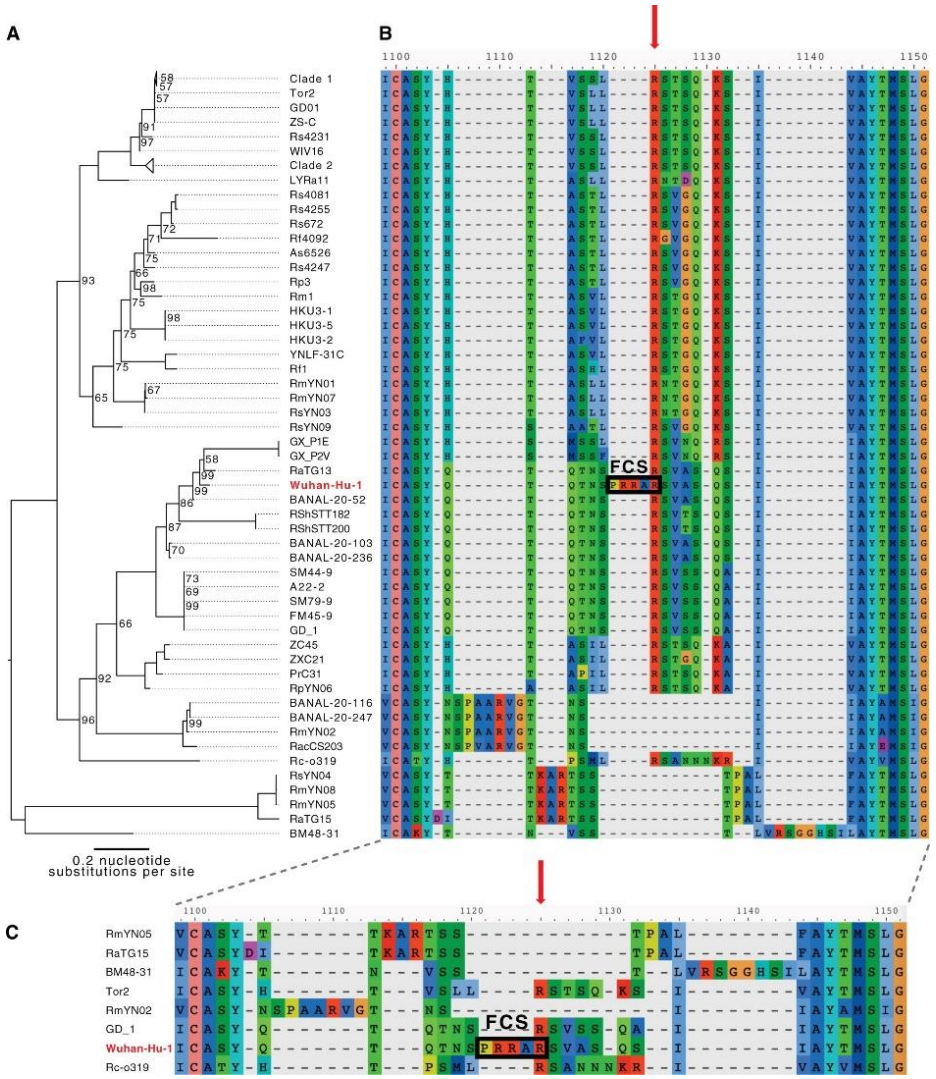
- FCS appears to destabilize the spike and the first known adaptive mutation in spike (D614G) is likely to have evolved to primarily compensate for this destabilization. Delta P/R (RRRA), Omicron P/histidine, H (HRRA).
- No known Sarbecovirus except SARS-CoV-2 has an FCS insertion at the S1/S2 junction. (But present in some Alphacoronaviruses, Betacoronaviruses, Gammaproteobacteria)
- Unique four-residue P-R-R-A (681–684) insertion at its spike S1/S2 junction, producing an FCS.
  - "non-canonical" -- not an R-R-X-R-R
  - highly functional
  - similar to FCSs found in other CoVs such as MERS
- Insertion is out of frame, arginine coding codon (CGG-CGG) is very rare (2/42)




```
Pangolin MP789      G  A  G  I  C  A  S  Y  Q  T  Q  T  N  S  -  -  -  -  R  S  V  S  S  X  A  I  I
   (nt 23527):      ggt gca gga ata tgt gcc agt tat cag act caa act aat tca --- --- --- --- cgt agt gtt tca agt cna gct att at

RaTG13              G  A  G  I  C  A  S  Y  Q  T  Q  T  N  S  -  -  -  -  R  S  V  A  S  Q  S  I  I
   (nt 23543):      ggt gca gga ata tgc gcc agt tat cag act caa act aat tca --- --- --- --- cgt agt gtg gcc agt caa tct att at

SARS-CoV-2          G  A  G  I  C  A  S  Y  Q  T  Q  T  N  S  P  R  R  A  R  S  V  A  S  Q  S  I  I
   (nt 23561):      ggt gca ggt ata tgc gct agt tat cag act cag act aat tct cct cgg cgg gca cgt agt gta gct agt caa tcc atc at

      Black  = common for all 3
      Red    = unique to SARS-CoV-2
      Green  = unique to RaTG13
      Blue   = common difference of RaTG13 and SARS-CoV-2 from MP789
```

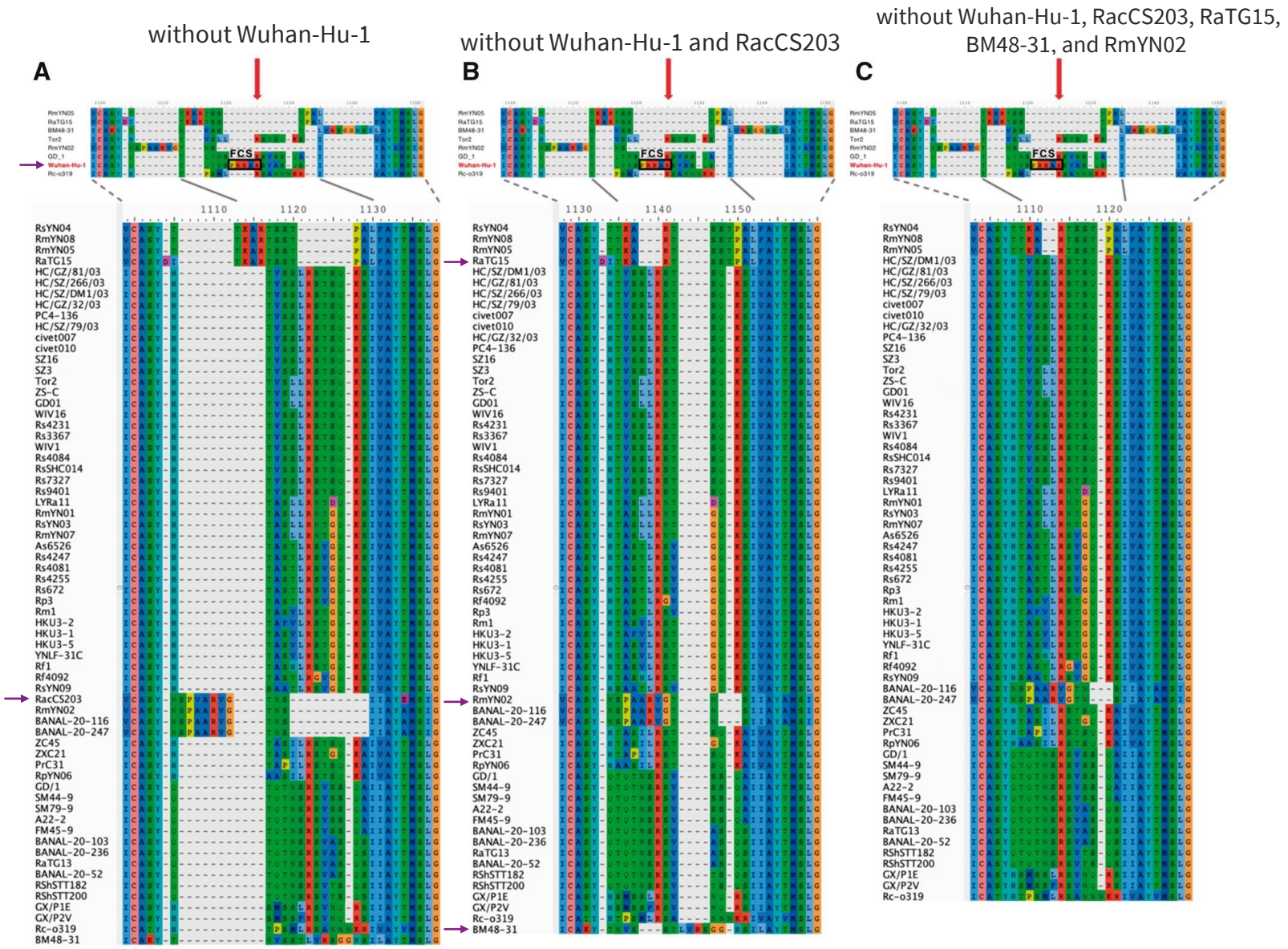

Chan, Y. A., & Zhan, S. H. (2022). The Emergence of the Spike Furin Cleavage Site in SARS-CoV-2. Molecular Biology and Evolution, 39(1). https://doi.org/10.1093/molbev/msab327
Whittaker, G. R. (2021). SARS-CoV-2 spike and its adaptable furin cleavage site. The Lancet Microbe, 2(10), e488–e489. https://doi.org/10.1016/S2666-5247(21)00174-9
Wu, Y., & Zhao, S. (2021). Furin cleavage sites naturally occur in coronaviruses. *Stem Cell Research*, *50*, 102115. https://doi.org/10.1016/j.scr.2020.102115
Zhang, L., et al. (2020). SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. *Nature Communications*, *11*(1), 6013. https://doi.org/10.1038/s41467-020-19808-4
Holmes, E. C., et al. (2021). The origins of SARS-CoV-2: A critical review. Cell, 184(19), 4848–4856. https://doi.org/10.1016/j.cell.2021.08.017
Segreto, R., & Deigin, Y. (2021). The genetic structure of SARS-CoV-2 does not rule out a laboratory origin. BioEssays, 43(3). https://doi.org/10.1002/bies.202000240

The close relatives of SARS-CoV-2 lack FCS



- S1/S2 region in unconserved and prone to substitutions, indels, and possibly recombinations.
- Numerous insertion at different positions in the S1/S2 region, but FCS is unique to SARS-CoV-2
- Conserved NSPXARVG motif in samples collected from different locations
  - RmYN02 (Yunnan, China)
  - RacCS203 (Thailand)
  - BANAL-20-116 and BANAL-20-247 (Laos)

Chan, Y. A., & Zhan, S. H. (2022). The Emergence of the Spike Furin Cleavage Site in SARS-CoV-2. Molecular Biology and Evolution, 39(1). https://doi.org/10.1093/molbev/msab327

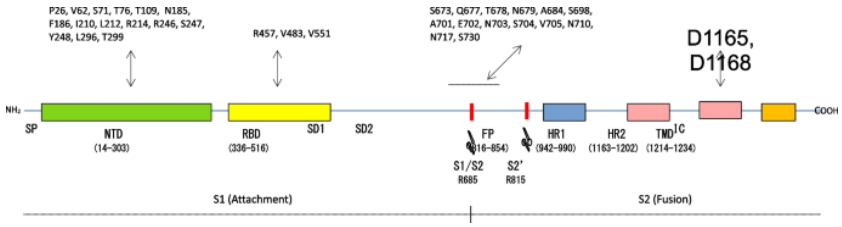


Chan, Y. A., & Zhan, S. H. (2022). The Emergence of the Spike Furin Cleavage Site in SARS-CoV-2. Molecular Biology and Evolution, 39(1). https://doi.org/10.1093/molbev/msab327

Possible human origin?

- Sequence analysis showed that the SARS-CoV-2 S gene 12-nucleotide fragments, potentially involved in the PRRA coding, 100% match to several NCBI human mRNA RefSeq transcripts
- These human transcripts could be good candidates as donors of mRNA template in a potential recombination link to a SARS-CoV-2 furin cleavage motif.
- Laos and Vietnam bat coronaviruses can infect human cells despite the absence of the furin cleavage motif - these may gain the FCS in human cells.
- Other PRRA-like insertions in the SARS-CoV-2 spike glycoprotein sequence

Query and tranacript position	RefSeq GenBank title	Chr	Exon range
CTCCTCGGCGGG-2921	NM_000466.3 Homo sapiens peroxisomal biogenesis factor 1 (PEX1), transcript variant 1, mRNA	7	Exon, 2869..3011
CTCCTCGGCGGG-2750	NM_001282677.2 Homo sapiens peroxisomal biogenesis factor 1 (PEX1), transcript variant 2, mRNA	7	Exon, 2669..2803
CTCCTCGGCGGG-2956	NM_001282678.2 Homo sapiens peroxisomal biogenesis factor 1 (PEX1), transcript variant 3, mRNA	7	Exon, 2869..3011
CTCCTCGGCGGG-1643	NM_001099289.3 Homo sapiens SH3 domain containing ring finger 3 (SH3RF3), mRNA	2	Exon, 1636..1739
CTCCTCGGCGGG-869	NM_001145873.1 Homo sapiens CD8a molecule (CD8A), transcript variant 3, mRNA	2	Exon, 762..1080
CTCCTCGGCGGG-851	NM_001382698.1 Homo sapiens CD8a molecule (CD8A), transcript variant 5, mRNA	2	Exon, 744..1062
CTCCTCGGCGGG-3430	NM_020910.3 Homo sapiens KIAA1549 (KIAA1549), transcript variant 1, mRNA	7	Exon, 3790..3967
CTCCTCGGCGGG-3430	NM_001164665.2 Homo sapiens KIAA1549 (KIAA1549), transcript variant 2, mRNA	7	Exon, 3790..3967
CTCCTCGGCGGG-853	NM_001291291.2 Homo sapiens MISP family member 3 (MISP3), transcript variant 1, mRNA	19	Exon, 1..1092
CTCCTCGGCGGG-853	NM_001393577.1 Homo sapiens MISP family member 3 (MISP3), transcript variant 3, mRNA	19	Exon, 1..1092
CTCCTCGGCGGG-169	NM_004717.3 Homo sapiens diacylglycerol kinase iota (DGKI), transcript variant 1, mRNA	7	Exon, 1..403
CTCCTCGGCGGG-279	NM_001321708.2 Homo sapiens diacylglycerol kinase iota (DGKI), transcript variant 2, mRNA	7	Exon, 1..513
CTCCTCGGCGGG-145	NM_001321710.2 Homo sapiens diacylglycerol kinase iota (DGKI), transcript variant 4, mRNA	7	Exon, 1..379
CTCCTCGGCGGG-279	NM_001388092.1 Homo sapiens diacylglycerol kinase iota (DGKI), transcript variant 5, mRNA	7	Exon, 1..513
CTCCTCGGCGGG-7	NM_004093.4 Homo sapiens ephrin B2 (EFNB2), transcript variant 1, mRNA	13	Exon, 1..820
CTCCTCGGCGGG-7	NM_001372056.1 Homo sapiens ephrin B2 (EFNB2), transcript variant 2, mRNA	13	Exon, 1..820
CTCCTCGGCGGG-7	NM_001372057.1 Homo sapiens ephrin B2 (EFNB2), transcript variant 3, mRNA	13	Exon, 1..820
CTCCTCGGCGGG-7	NM_001372058.1 Homo sapiens ephrin B2 (EFNB2), transcript variant 4, mRNA	13	Exon, 1..820
CTCCTCGGCGGG-118	NM_004637.6 Homo sapiens RAB7A, member RAS oncogene family (RAB7A), mRNA	3	Exon, 1..177
CTCCTCGGCGGG-307	NM_006843.3 Homo sapiens serine dehydratase (SDS), mRNA	12	Exon, 276..315
CTCCTCGGCGGG-187	NM_016085.5 Homo sapiens all-trans retinoic acid induced differentiation factor (ATRAID), transcript variant 1, mRNA	2	Exon, 1..248
CTCCTCGGCGGG-1032	NM_021620.4 Homo sapiens PR/SET domain 13 (PRDM13), mRNA	6	Exon, 600..3129
CTCCTCGGCGGG-36	NM_022831.4 Homo sapiens axin interactor, dorsalization associated (AIDA), mRNA	1	Exon, 1..284
CTCCTCGGCGGG-2215	NM_171999.4 Homo sapiens spalt like transcription factor 3 (SALL3), mRNA	18	CDS*, 458..4360



Romeu, A. R. (2023). Probable human origin of the SARS-CoV-2 polybasic furin cleavage motif. *BMC Genomic Data*, *24*(1), 71. https://doi.org/10.1186/s12863-023-01169-8

The presence of the FCS is not a direct evidence of the 'lab leak' hipothesis

- Loss of FCS in cell cultures → SARS-CoV-2 is not replicating efficiently in this form
- Poor replication in traditional animal models
- No direct evidence of genetic engineering
- The pathogenesis of SARS-CoV-2 may be dependent on the cleavage site loop length and not only the presence of the furin cleavage site.

Alwine, J. C., Casadevall, A., Enquist, L. W., Goodrum, F. D., & Imperiale, M. J. (2023). A Critical Analysis of the Evidence for the SARS-CoV-2 Origin Hypotheses. *Journal of Virology*, *97*(4). https://doi.org/10.1128/jvi.00365-23