Twitter Thread by Billy Bostickson ■■&■ ■

Billy Bostickson ■■&■ ■
@BillyBostickson

Compilation Thread on ORF8 ORF8 or Fate?

1. ZLS (2018):

"Considering the variability of orf8 in bats, civets and humans, investigating the function of orf8 is a priority, particularly the contribution of these different variants to viral pathogenesis."

> 1/2
> Zheng Li Shi wrote this in 2018:
> "Considering the variability of orf8 in bats, civets and humans, investigating the function of orf8 is a priority, particularly the contribution of these different variants to viral pathogenesis." https://t.co/oNHpIs1aFP
>
> — Billy Bostickson \U0001f3f4\U0001f441&\U0001f441 \U0001f193 (@BillyBostickson) April 19, 2020

2. ORF8 or Fate?
"In addition to receptor binding, proteolytic cleavage of S & potentially other mutations that affect virion & trimer stability may be important for virus transmissibility in different hosts, & these factors need to be studied further."
https://t.co/oNHpIsiM4p

3. ORF8 or Fate?
Overwhelmingly these viruses had mutations or
29 deletions in ORF8 - associated with reduced replicative fitness of the virus.. may be associated with host adaptation
via @CarltheChippy
382-nt deletion during early evolution of SARS-CoV
https://t.co/W5IQwDunCN

4. ORF8 or Fate?
@franciscodeasis reviewed the list of read filenames according to dates and labels:
- Sat 2017-06-03 (7896)
- Sat 2017-06-17 to Tue 2017-06-20 (7896 and ORF8)
- Thu 2018-09-27 to Sun 2018-09-30 (RaTG13)
- Mon 2018-10-08 to Sun 2018-10-14 (no label and TSS)

orde	read	protein	sth	st	date unfo	sth_3	st	date	Day	main
30	SRR11806578.30	R-2-1	7896-2-F1		2017-06-03		A07	03-06-17	Saturday	7896
31	SRR11806578.31	R-2-1	7896-2-R1		2017-06-03		A08	03-06-17	Saturday	7896
26	SRR11806578.26	ORF8-1-1	ORF8-F		2017-06-17		A05	17-06-17	Saturday	ORF8
27	SRR11806578.27	ORF8-1-1	ORF8-R1		2017-06-17		A06	17-06-17	Saturday	ORF8
28	SRR11806578.28	R-1-1	7896-1-F1		2017-06-20		E03	20-06-17	Tuesday	7896
29	SRR11806578.29	R-1-1	7896-1-R1		2017-06-20		G03	20-06-17	Tuesday	7896
32	SRR11806578.32	R-4-1	7896-4-F		2017-06-20		F03	20-06-17	Tuesday	7896
33	SRR11806578.33	R-4-1	7896-4-R		2017-06-20		H03	20-06-17	Tuesday	7896
1	SRR11806578.1	1-2	RaTG13-1-F		2018-09-27		E05	27-09-18	Thursday	RaTG13
2	SRR11806578.2	1-2	RaTG13-1-R		2018-09-27		F06	27-09-18	Thursday	RaTG13
12	SRR11806578.12	3-2	RaTG13-3-F		2018-09-27		F05	27-09-18	Thursday	RaTG13
13	SRR11806578.13	4-2	RaTG13-4-F		2018-09-27		G05	27-09-18	Thursday	RaTG13
14	SRR11806578.14	4-2	RaTG13-4-R		2018-09-27		G06	27-09-18	Thursday	RaTG13
15	SRR11806578.15	5-2	RaTG13-5-F		2018-09-27		H05	27-09-18	Thursday	RaTG13
16	SRR11806578.16	6-2	RaTG13-6-F		2018-09-27		A06	27-09-18	Thursday	RaTG13
17	SRR11806578.17	6-2	RaTG13-6-R		2018-09-27		H06	27-09-18	Thursday	RaTG13
18	SRR11806578.18	7-2	RaTG13-7-F		2018-09-27		B06	27-09-18	Thursday	RaTG13
19	SRR11806578.19	8-2	RaTG13-8-F		2018-09-27		C06	27-09-18	Thursday	RaTG13
3	SRR11806578.3	10-3	RaTG13-10-F		2018-09-29		G04	29-09-18	Saturday	RaTG13
4	SRR11806578.4	10-3	RaTG13-10-R		2018-09-29		E05	29-09-18	Saturday	RaTG13
10	SRR11806578.10	2-3	RaTG13-2-R2		2018-09-29		D05	29-09-18	Saturday	RaTG13
11	SRR11806578.11	20-1	RaTG13-F		2018-09-29		H04	29-09-18	Saturday	RaTG13
8	SRR11806578.8	2-3	RaTG13-2-F		2018-09-30		A02	30-09-18	Sunday	RaTG13
9	SRR11806578.9	2-3	RaTG13-2-R1		2018-09-30		B11	30-09-18	Sunday	RaTG13
5	SRR11806578.5	11-2	18297-F	TSS	20181008	027-0303	G10	08-10-18	Monday	TSS
6	SRR11806578.6	12-2	22717-F	TSS	20181008	027-0303	H10	08-10-18	Monday	TSS
7	SRR11806578.7	12-2	24144-R	TSS	20181008	027-0303	C11	08-10-18	Monday	TSS
20	SRR11806578.20	9-5-1	21230-F		2018-10-11		A12	11-10-18	Thursday	-
21	SRR11806578.21	9-5-1	23258-R		2018-10-11		B12	11-10-18	Thursday	-
22	SRR11806578.22	9-5-4	9-5-f1		2018-10-14		A02	14-10-18	Sunday	-
23	SRR11806578.23	9-5-4	9-5-r1		2018-10-14		C02	14-10-18	Sunday	-
24	SRR11806578.24	9-5-5	9-5-f1		2018-10-14		B02	14-10-18	Sunday	-
25	SRR11806578.25	9-5-5	9-5-r1		2018-10-14		D02	14-10-18	Sunday	-

5. ORF8 or Fate?

What is ORF8? This is Orf8!

https://t.co/21ugtiinhL

This is Orf8 pic.twitter.com/Vnkml5zyHu

— Billy Bostickson \U0001f3f4\U0001f441&\U0001f441 \U0001f193 (@BillyBostickson) July 16, 2020

6. ORF8 or Fate? keep on saying it!
orf8 NSP12 deletion found in India
https://t.co/1TQ9ftuToV
https://t.co/ysNXp88dWf

Position	Protein Annotation	Reference Nucleotide	Sample 1 ♂ 66 (Acc. No. MT435081)		Sample 2 ♀ 66 (Acc. No. MT435082)	
			Allele Nucleotide	Amino Acid Mutation	Allele Nucleotide	Amino Acid Mutation
241	5' UTR: 5' UTR	C	T	-	T	-
1059	ORF1ab	C	T	Thr > Ile	T	Thr > Ile
3037	ORF1ab-nsp1	C	T	-	T	-
14408	ORF1ab-nsp12	C	T	Pro > Leu	T	Pro > Leu
15371	ORF1ab-nsp12	C	T	Thr > Met	C	-
17747	ORF1ab-nsp13	C	C	-	T	Pro > Leu
23403	S	A	G	Asp > Gly	G	Asp > Gly
25563	ORF3a	G	T	Gln > His	T	Gln > His
28221	ORF8	G	T	Glu > *	T	Glu > *
28254	ORF8	A	A	-	Del	Ile > FS
28371	Gene: N	G	T	Ser > Ile	T	Ser > Ile
28549	Gene: N	A	Del	Arg > FS	A	-

7. ORF8 or Fate?

Via @ConsciousEvolu1

What is critically important is that Orf8 and surface Glycoproteins create Porphyrin penetration of the Cell. Resulting in more Severe Infections, Bio-chemic Processes interuption, Heme cleavage and Hypoxia.

https://t.co/XyiU1879Rq

8. ORF8 or Fate?

via @Rossana38510044

"The ORF8 Protein of SARS-CoV-2 Mediates Immune Evasion through Potently Downregulating MHC-I"

https://t.co/m9KY0Fkxkx

9. ORF8 or Fate?

"Immune evasion via SARS-CoV-2 ORF8 protein?"

https://t.co/oEncCgDDvb

Immune evasion via SARS-CoV-2 ORF8 protein?

In this preprint, Zhang et al. elucidate a potential immune evasion strategy involving the SARS-CoV-2 ORF8 protein. They show that expression of ORF8, which directly binds to MHC class I molecules, downregulates their surface expression on HEK293T cells. ORF8 co-localizes with MHC class I molecules in lysosomes, thereby disrupting antigen presentation. When healthy human donor-derived cytotoxic T lymphocytes (CTLs) sensitized to the SARS-CoV-2 epitope SSp-1 were exposed to autologous dendritic cells pre-pulsed with SSp-1, there was reduced killing of ORF8-expressing HEK293T cells compared with ORF8 non-expressing cells. These results were replicated using CTLs isolated from a patient recovering from COVID-19 that responded to a mixture of SARS-CoV-2 N and S proteins. This potential mechanism for SARS-CoV-2 evasion of host immune surveillance warrants further investigation.

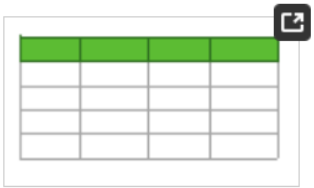
10. ORF8 or Fate? Stress Trigger!

"However, 2 proteins (orf8 & orf10) in SARS-CoV-2 have no homologous proteins in SARS-CoV. The amino acid sequence of orf8 in SARS-CoV-2 is different from sequences of conserved orf8 or orf8b derived from human SARS-CoV" https://t.co/Y76d3b3sH3

3.9. Proteomic Comparison

To further explore whether all encoded proteins of SARS-CoV-2 are homologous to that of SARS-CoV, we performed a protein sequence alignment analysis using Blastp. The results showed that most of SARS-CoV-2 proteins are highly homologous (95%–100%) to the proteins of SARS-CoV virus, indicating the evolutionary similarity between SARS-CoV and SARS-CoV-2 (**Table 2**). However, two proteins (orf8 and orf10) in SARS-CoV-2 have no homologous proteins in SARS-CoV. The amino acid sequence of orf8 in SARS-CoV-2 is different from sequences of conserved orf8 or orf8b derived from human SARS-CoV [**11**]. Orf8 protein of SARS-CoV-2 does not contain known functional domain or motif. An aggregation motif VLVVL (amino acid 75–79) has been found in SARS-CoV orf8b which was shown to trigger intracellular stress pathways and activate NOD-like receptor family pyrin domain-containing-3 (NLRP3) inflammasomes [**90**]. Therefore, it will be clinically meaningful to analyze the biological function of these two specific proteins (orf8 and orf10) in SARS-CoV-2.

Table 2. Comparison of protein sequences SARS-CoV-2 and SARS-CoV by Blastp.

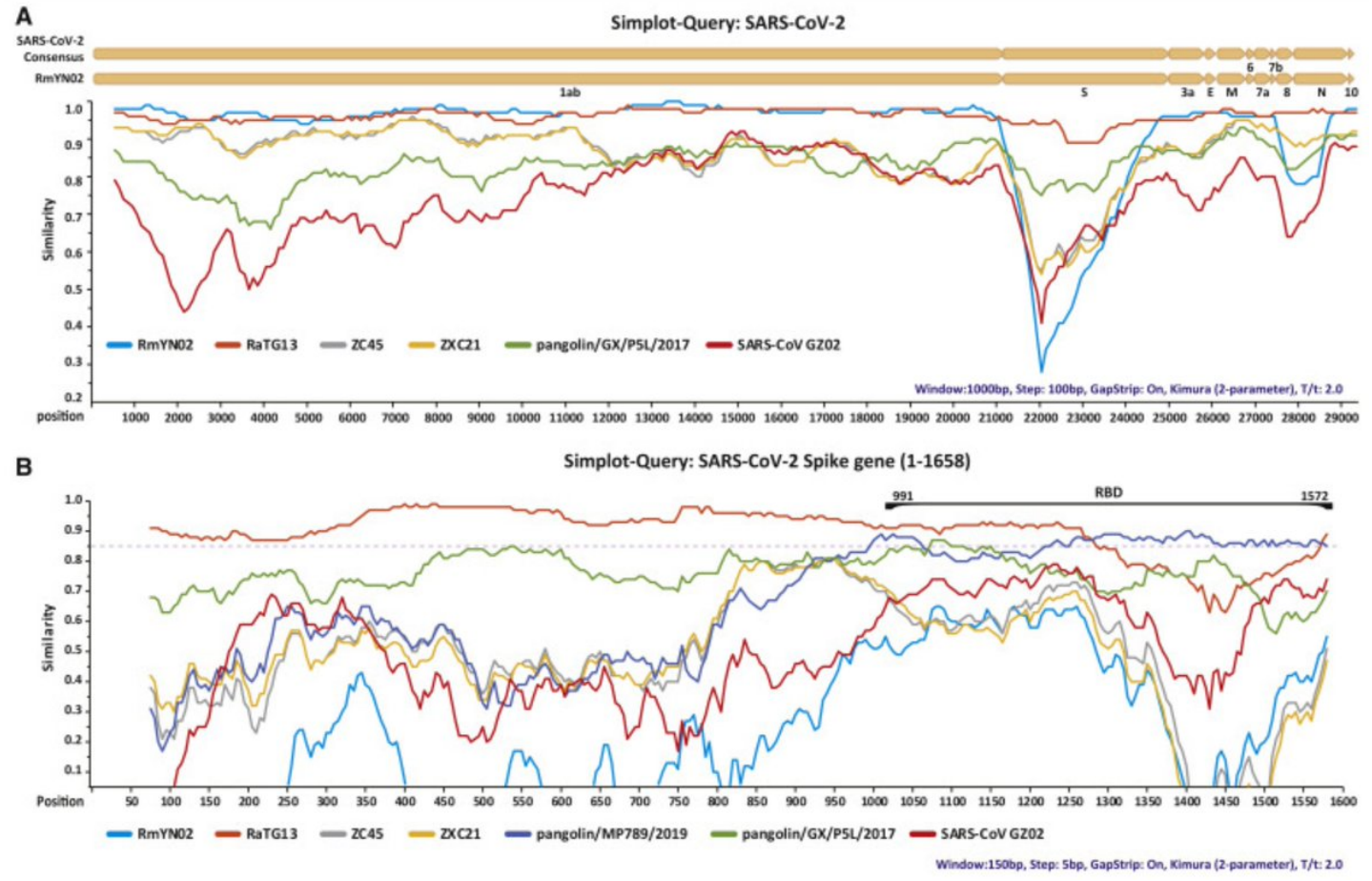


11.ORF8 or Fate! Coincidence? No, Trust a Me, it's Natural!

1. Orf8 "combined" with Orf10 to create a Porphyrin Heme Cleave and Orf8 Porphyrin Infectivity
2. WIV were already studying the RaTG13 and 7896 Clade Spike Proteins and Orf8 back in 2018

,

12. via @CZilcho
not a single live virus. genome looks like the version of a "virus" that has been compiled from known RaTG13 contigs & consensus seq from RmYN02 (ORF 1ab, 3a, E, M, 6, 7a) + some unknown seq (passaged viruses?) for S, & 3' from ORF8, N, using Shiver or similar.



13. ORF8 or Fate? ORF8 similarities

Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus

Ben Hu (2017)

https://t.co/foBWRXc0Fb

Peppered with 91 references to Orf8

Abstract

Go to:

A large number of SARS-related coronaviruses (SARSr-CoV) have been detected in horseshoe bats since 2005 in different areas of China. However, these bat SARSr-CoVs show sequence differences from SARS coronavirus (SARS-CoV) in different genes (S, ORF8, ORF3, *etc*) and are considered unlikely to represent the direct progenitor of SARS-CoV. Herein, we report the findings of our 5-year surveillance of SARSr-CoVs in a cave inhabited by multiple species of horseshoe bats in Yunnan Province, China. The full-length genomes of 11 newly discovered SARSr-CoV strains, together with our previous findings, reveals that the SARSr-CoVs circulating in this single location are highly diverse in the S gene, ORF3 and ORF8. Importantly, strains with high genetic similarity to SARS-CoV in the hypervariable N-terminal domain (NTD) and receptor-binding domain (RBD) of the S1 gene, the ORF3 and ORF8 region, respectively, were all discovered in this cave. In addition, we report the first discovery of bat SARSr-CoVs highly similar to human SARS-CoV in ORF3b and in the split ORF8a and 8b. Moreover, SARSr-CoV strains from this cave were more closely related to SARS-CoV in the non-structural protein genes ORF1a and 1b compared with those detected elsewhere. Recombination analysis shows evidence of frequent recombination events within the S gene and around the ORF8 between these SARSr-CoVs. We hypothesize that the direct progenitor of SARS-CoV may have originated after sequential recombination events between the precursors of these SARSr-CoVs. Cell entry studies demonstrated that three newly identified SARSr-CoVs with different S protein sequences are all able to use human ACE2 as the receptor, further exhibiting the close relationship between strains in this cave and SARS-CoV. This work provides new insights into the origin and evolution of SARS-CoV and highlights the necessity of preparedness for future emergence of SARS-like diseases.

14. ORF8 or Fate? WIV ORF8 Experiments (2017)

Same paper as above

https://t.co/MMO6Br7XQK

Activation of activating transcription factor 6 (ATF6) by the ORF8 proteins of different bat SARSr-CoVs

The induction of the ATF6-dependent transcription by the ORF8s of SARS-CoV and bat SARSr-CoVs were investigated using a luciferase reporter, 5×ATF6-GL3. In HeLa cells transiently transfected with the expression plasmids of the ORF8s of bat SARSr-CoV Rf1, Rf4092 and WIV1, the relative luciferase activities of the 5×ATF6-GL3 reporter was enhanced by 5.56 to 9.26 folds compared with cells transfected with the pCAGGS empty vector, while it was increased by 4.42 fold by the SARS-CoV GZ02 ORF8. As a control, the treatment with tunicamyxin (TM) stimulated the transcription by about 11 folds (Fig 9A). The results suggests that various ORF8 proteins of bat SARSr-CoVs can activate ATF6, and those of some strains have a stronger effect than the SARS-CoV ORF8.

15. ORF8 or Fate?

1. ORF8 enhances the viral replication.

2. enhanced ORF8s are main factors contributing to transmission, virulence and host adaptability of CoVs.

3. enhanced ORF8s increase the efficiencies in viral entry into cells and replication

https://t.co/spkLguszNZ

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for its function. In addition, we reported—for the first time—a recombination event of *ORF8* at the whole-gene level in a bat and ultimately determined that ORF8 enhances the viral replication. In conjunction with our previous discoveries, we found that receptor binding abilities, junction furin cleavage sites (FCSs), strong first ribosome binding sites (RBSs) and enhanced *ORF8*s are main factors contributing to transmission, virulence and host adaptability of CoVs. Junction FCSs and enhanced *ORF8*s increase the efficiencies in viral entry into cells and replication, respectively while strong first RBSs enhance the translational initiation. The

16. ORF8 or Fate?

Back in Time to an ORF8 rich bat cave with WIV and @amicocolorido

Distribution of SARSr-CoVs highly similar to SARS-CoV in the variable S, ORF3 and ORF8 genes in the single cave.

https://t.co/1jU1PaWVoP

S2 Table Distribution of SARSr-CoVs highly similar to SARS-CoV in the variable S, ORF3 and ORF8 genes in the single cave

Gene	SARSr-CoV strains highly similar to SARS-CoV	Sampling time (No. of strains)	Bat species (No. of strains)
S-RBD	**WIV1**, **Rs3367**, Rs4079, Rs4087, Rs4090, Rs4105, Rs4230, Rs4829, Rs4832, **WIV16**, **Rs4874**, Rs4952, Rs7326, **Rs7327**, **Rs9401**, Rs9403	May 2012 (2) Sep 2012 (4) Apr 2013 (1) Jul 2013 (5) Oct 2014 (2) Oct 2015 (2)	*Rhinolophus sinicus* (16)
S-NTD	**Rs4231**, **WIV16**, **Rs4874**, Rs4952	Apr 2013 (1) Jul 2013 (3)	*Rhinolophus sinicus* (4)
ORF8	Rs3261, Rf4075, **Rs4084**, Rs4091, **Rf4092**, Rs4110, Rf4122, Rs4832, Rs4943	Oct 2011 (1) Sep 2012 (6) Jul 2013 (2)	*Rhinolophus sinicus* (6) *Rhinolophus ferrumequinum* (3)
ORF3a	**RsSHC014**, **WIV1**, **Rs3367**, Rs3369, **Rs4084**, Rs4087, Rs4090, Rs4105, **Rs4231**, Rs4829, **WIV16**, **Rs4874**, Rs4900, Rs4952, Rs7326, **Rs7327**, **Rs9401**	Apr 2011 (1) May 2012 (3) Sep 2012 (4) Apr 2013 (1) Jul 2013 (5) Oct 2014 (2) Oct 2015 (1)	*Rhinolophus sinicus* (17)
ORF3b	Rs7326, Rs7327	Oct 2014 (2)	*Rhinolophus sinicus* (2)

17. Some naughty but nice ORF8y images collated by @BidoliNicola
https://t.co/8RQNrSndAs

> \u27a1\ufe0fFor you, @McWLuke \U0001f447
> The insertion of the \U0001f449ORF8 protein, in SARS 2, which made \U0001f449SARS COV 2-ORF8 extremely more virulent, infectious and mutable, thanks to a mutated deletion by recombination, in Lab, obtained with the use of\U0001f449 transgenic HIV / Hu -mice.\U0001f447 pic.twitter.com/LkZMuIlB1H
>
> — Nicola Bidoli \U0001f6e1\U0001f6e1\U0001f6e1 (@BidoliNicola) January 6, 2021

18. ORF8 or Fate? Back to SARS
https://t.co/PDxyBfpGVg
Severe Acute Respiratory Syndrome (SARS) Coronavirus ORF8 Protein Is Acquired from SARS-Related Coronavirus from Greater Horseshoe Bats through Recombination
https://t.co/c5Sxc23SiG

19. ORF8 or Fate?
Latest Findings - ORF8's secrets exposed
" two unique regions: one that is only present in SARS-CoV-2 and its immediate bat ancestor, and one that is absent from any other coronavirus,"
https://t.co/W1xdkYNBRc

In a paper published in *mBio*, lead author Russell Neches and his colleagues show that ORF8 evolved from another coronavirus protein called ORF7a, and that both proteins have folds similar to that of a human antibody. This finding helps to explain how the virus avoids immune detection and is able to escalate into a severe infection in some hosts.

"By exploring the structural and functional characteristics of ORF8, and using supercomputers to look at the genomes of over 200,000 viruses, we discovered a striking and highly unusual evolutionary strategy," said co-author Nikos Kyrpides, a computational biologist at the DOE Joint Genome Institute (JGI). "Amazingly, it seems that within the SARS clade, the gene encoding ORF7a is used as a 'template' gene, remaining stable, with a duplicate copy of this gene evolving to a point almost beyond recognition." SARS-CoV-2 arose and exploded into a pandemic when a SARS strain's duplicate ORF7a gene happened to mutate leading to a new protein (which we now call ORF8) that gave it the ability to interfere with immune cells.

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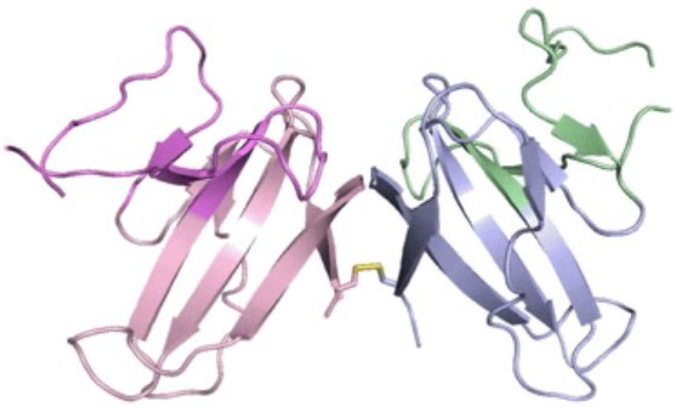
20. ORF8 or Fate? WTF is this?
Paper referenced in above article:
The odd structure of ORF8: Mapping the coronavirus protein linked to disease severity
https://t.co/KXtjwScOJ3



A ribbon diagram rendering of the ORF8 structure, which is composed of two protein units with identical ami…

21. ORF8 or Fate? via @Harvard2H

Divergence between Orf7a & Orf8 is exceptionally idiosyncratic, as Orf7a is more constrained, whereas Orf8 is subject to rampant change, a peculiar feature that may be related to hitherto unknown viral infection strategies

https://t.co/rskaNzlbXC

ABSTRACT Orf8, one of the most puzzling genes in the SARS lineage of coronaviruses, marks a unique and striking difference in genome organization between SARS-CoV-2 and SARS-CoV-1. Here, using sequence comparisons, we unequivocally reveal the distant sequence similarities between SARS-CoV-2 Orf8 with its SARS-CoV-1 counterparts and the X4-like genes of coronaviruses, including its highly divergent "paralog" gene Orf7a, whose product is a potential immune antagonist of known structure. Supervised sequence space walks unravel identity levels that drop below 10% and yet exhibit subtle conservation patterns in this novel superfamily, characterized by an immunoglobulin-like beta sandwich topology. We document the high accuracy of the sequence space walk process in detail and characterize the subgroups of the superfamily in sequence space by systematic annotation of gene and taxon groups. While SARS-CoV-1 Orf7a and Orf8 genes are most similar to bat virus sequences, their SARS-CoV-2 counterparts are closer to pangolin virus homologs, reflecting the fine structure of conservation patterns within the SARS-CoV-2 genomes. The divergence between Orf7a and Orf8 is exceptionally idiosyncratic, since Orf7a is more constrained, whereas Orf8 is subject to rampant change, a peculiar feature that may be related to hitherto-unknown viral infection strategies. Despite their common origin, the Orf7a and Orf8 protein families exhibit different modes of evolutionary trajectories within the coronavirus lineage, which might be partly attributable to their complex interactions with the mammalian host cell, reflected by a multitude of functional associations of Orf8 in SARS-CoV-2 compared to a very small number of interactions discovered for Orf7a.

22. "Barring a Technical Artefact"

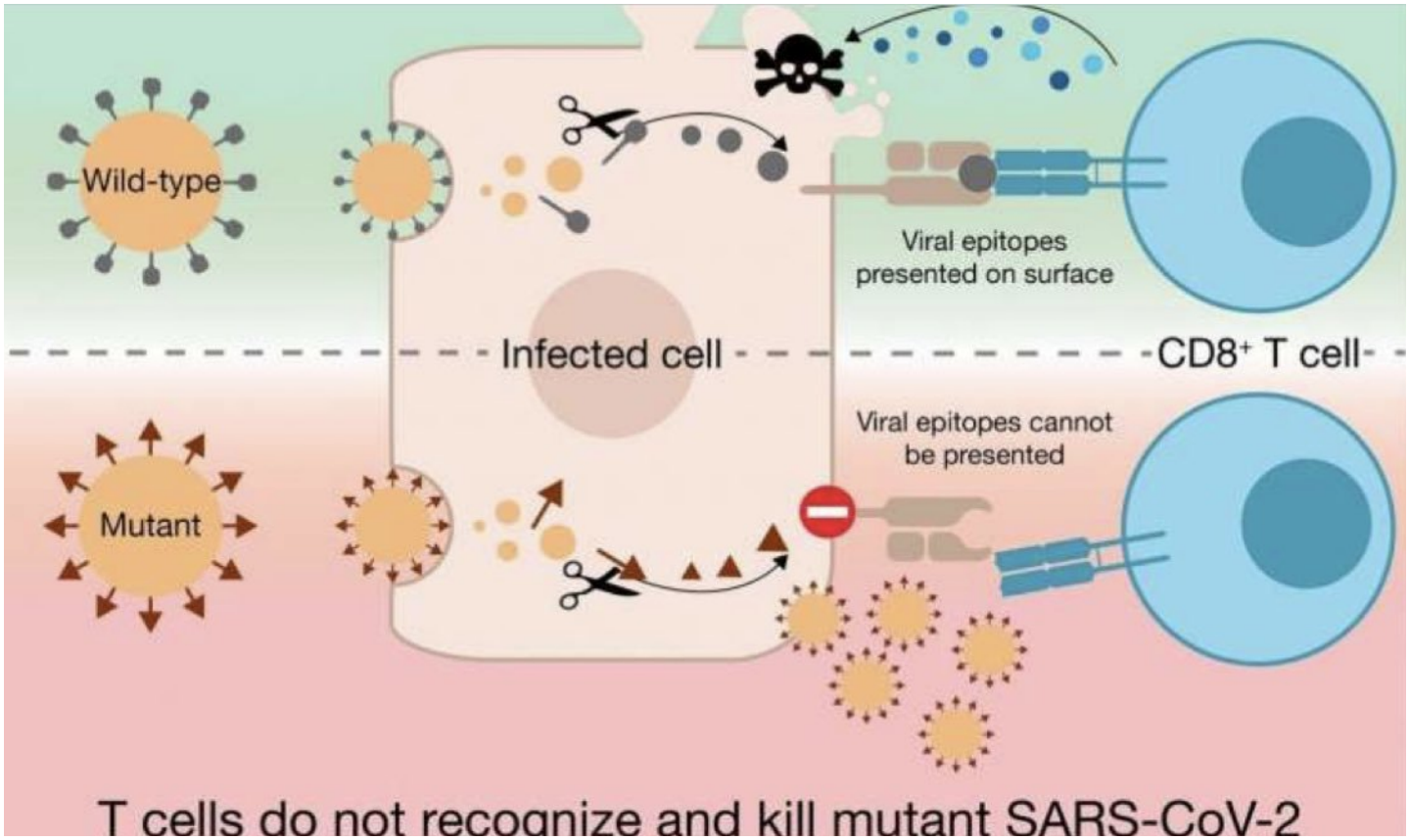
"Protein interactions of SARS-CoV-2 exhibit such a sharp contrast between Orf7a and Orf8 that, barring a technical artifact with respect to coverage..Orf7a generate variants, such as Orf8, wreaking havoc through immune evasion in the host cell"

We provide strong evidence for the peculiar divergence of Orf8 from Orf7a, within an otherwise dense viral genome and delimit the phylogenetic distribution of these two genes across coronaviruses. Neither of these genes is found in the gamma or delta coronavirus groups, suggestive of a likely loss in those coronavirus sublineages (see Fig. S4BC). It is quite perplexing that no member of this family is present in the MERS clade (23). Given that Orf7a with an Ig-like structure is potentially an immune antagonist with a pivotal role in the viral infection strategy and the recent observation that Orf8 downregulates MHC-I (46), the Orf7a/Orf8 superfamily might be a key system for immune evasion, known for other analogous cases, including herpesviruses, poxviruses, and adenoviruses (47). The detected protein interactions of SARS-CoV-2 exhibit such a sharp contrast between Orf7a and Orf8 (43) that, barring a technical artifact with respect to coverage, the hypothesis arises for Orf7a being used as a conserved template, to generate variants, such as Orf8, wreaking havoc through immune evasion in the host cell.

January/February 2021 Volume 12 Issue 1 e03014-20

23. ORF8 or Fate? via @Undergroundsar3

ORF8 is involved somehow in apoptosis/viral cytolysis pathway and functions to optimize spread of virons while cloaking the infected host cell from CD8+ killer T-cell surveillance.

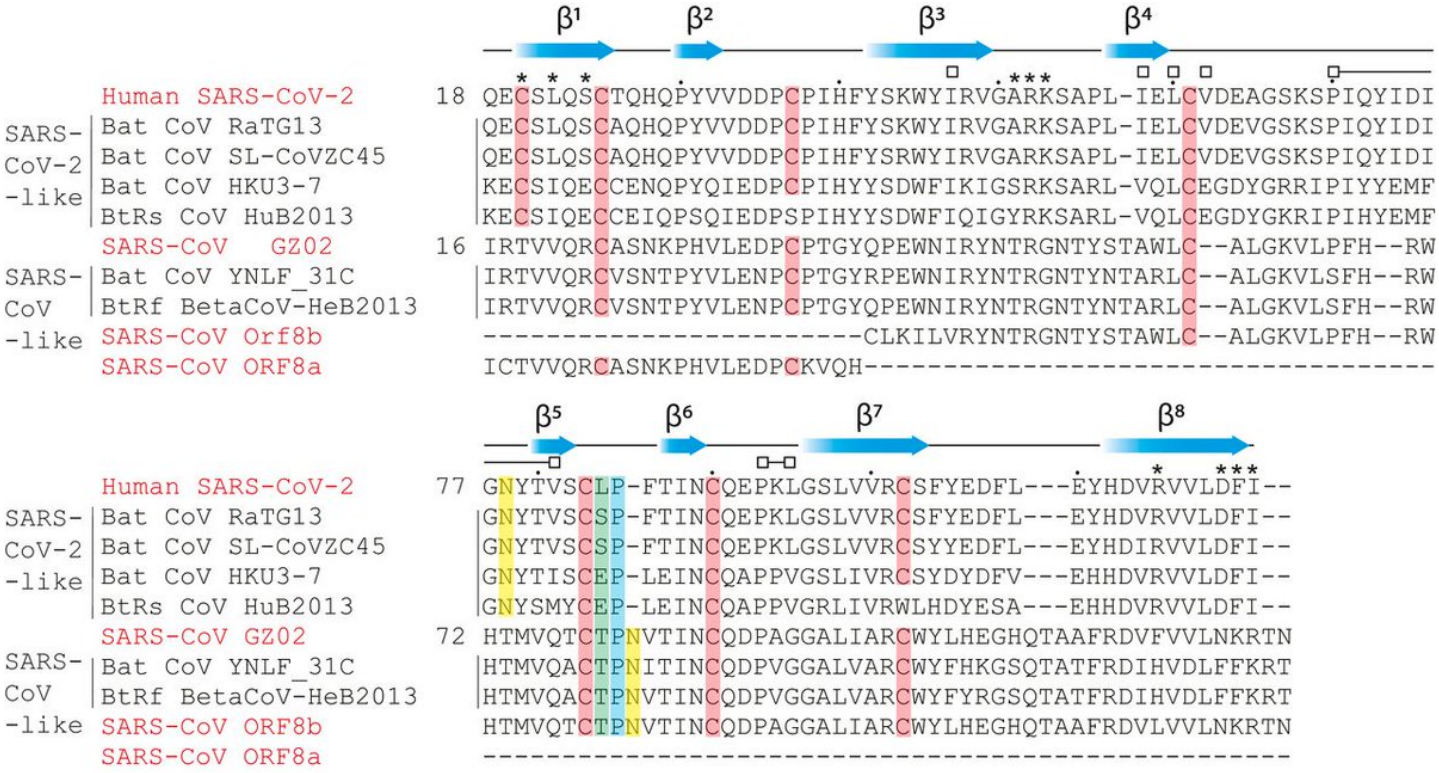


24. ORF8 or Fate? @SBgrid

1. Solved structure of SARS-CoV-2 ORF8 uncovers vital role in immune suppression & evasion https://t.co/DNHkoOG9it

2. Covalent dimer is an "evolutionarily" recent addition unique to SARS-CoV-2

3. SARS-CoV-2 ORF8 contains a large, unstructured insertion



25. ORF8 or Fate?

Question for Hans Kristian from @Undergroundsar3

What are your thoughts on the CD8+ t-cell story in the milieu of the host (vs. test tube humoral only neutralization assays) where ORF8 is expressed?

https://t.co/pka3kgY9kA

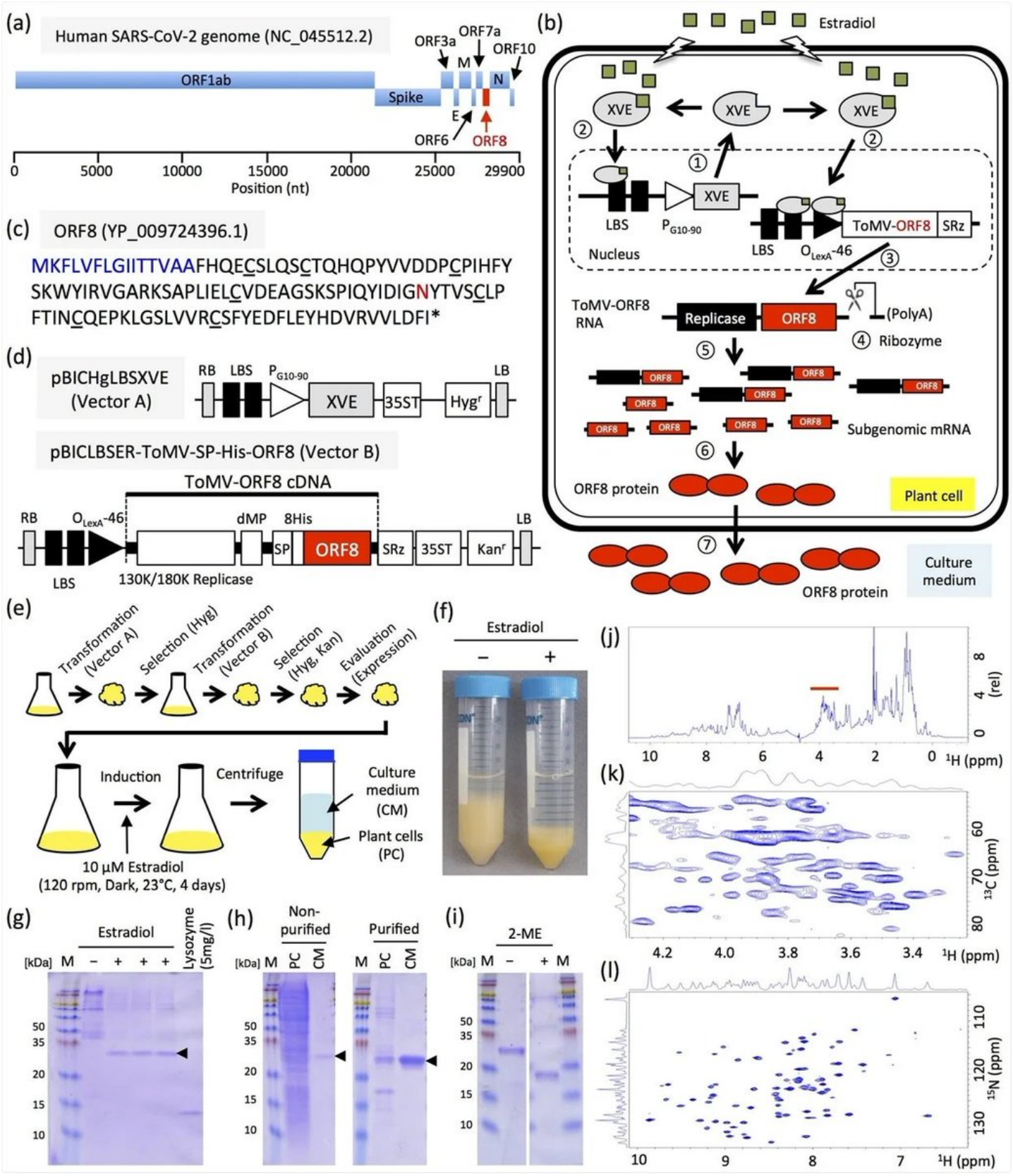
Referenicing:

https://t.co/cc4hZdk3SY

> What are your thoughts on the CD8+ t-cell story in the milieu of the host (vs. test tube humoral only neutralization assays) where ORF8 is expressed?https://t.co/Rgd0rSVahN
>
> — Jaded Court Lady #239842309 (@Undergroundsar3) March 4, 2021

26. Added Bonus: DIY ORF8

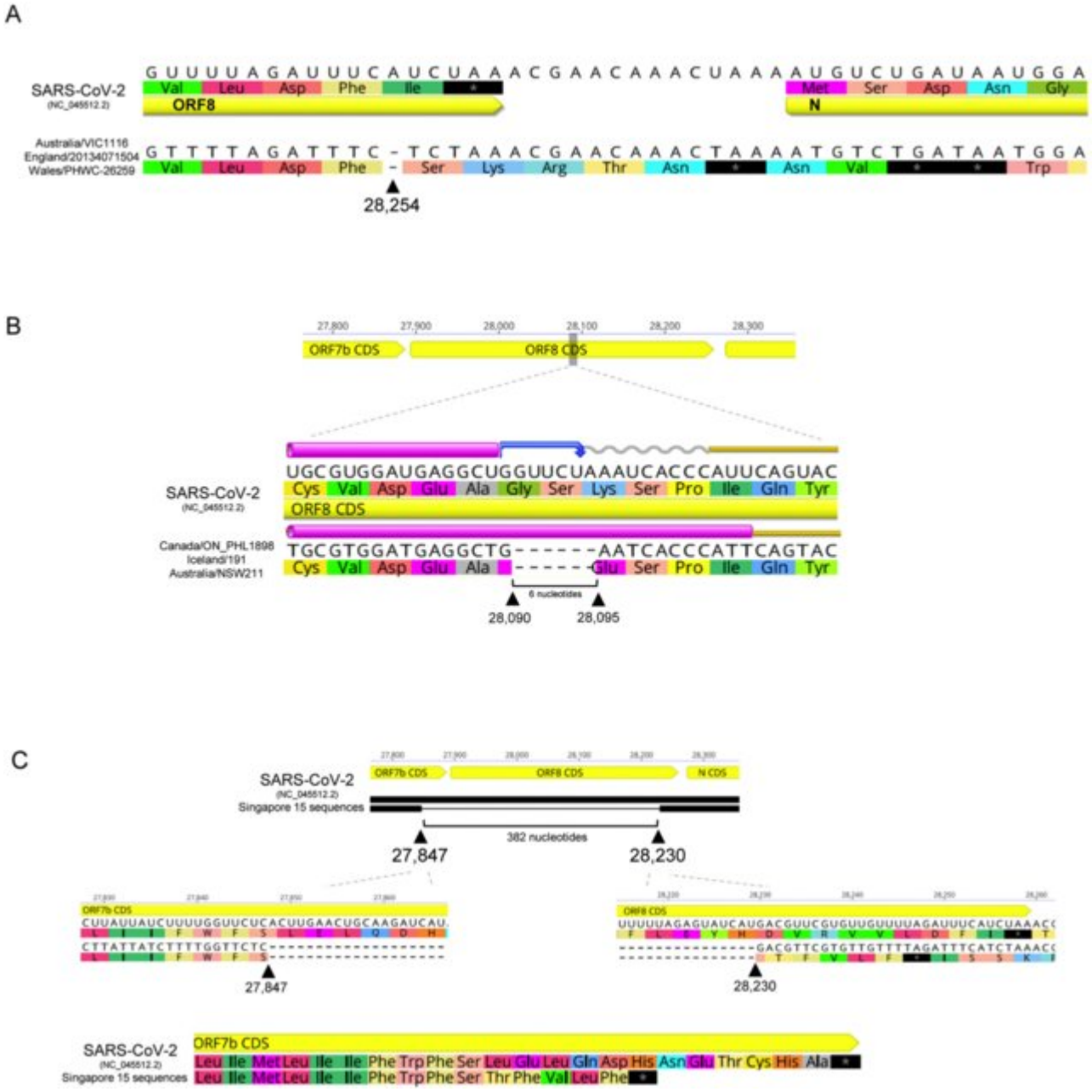
https://t.co/R5ZUPJNMFf



27. ORF8 or Fate?

Evolutionary dynamics of the SARS-CoV-2 ORF8 accessory gene

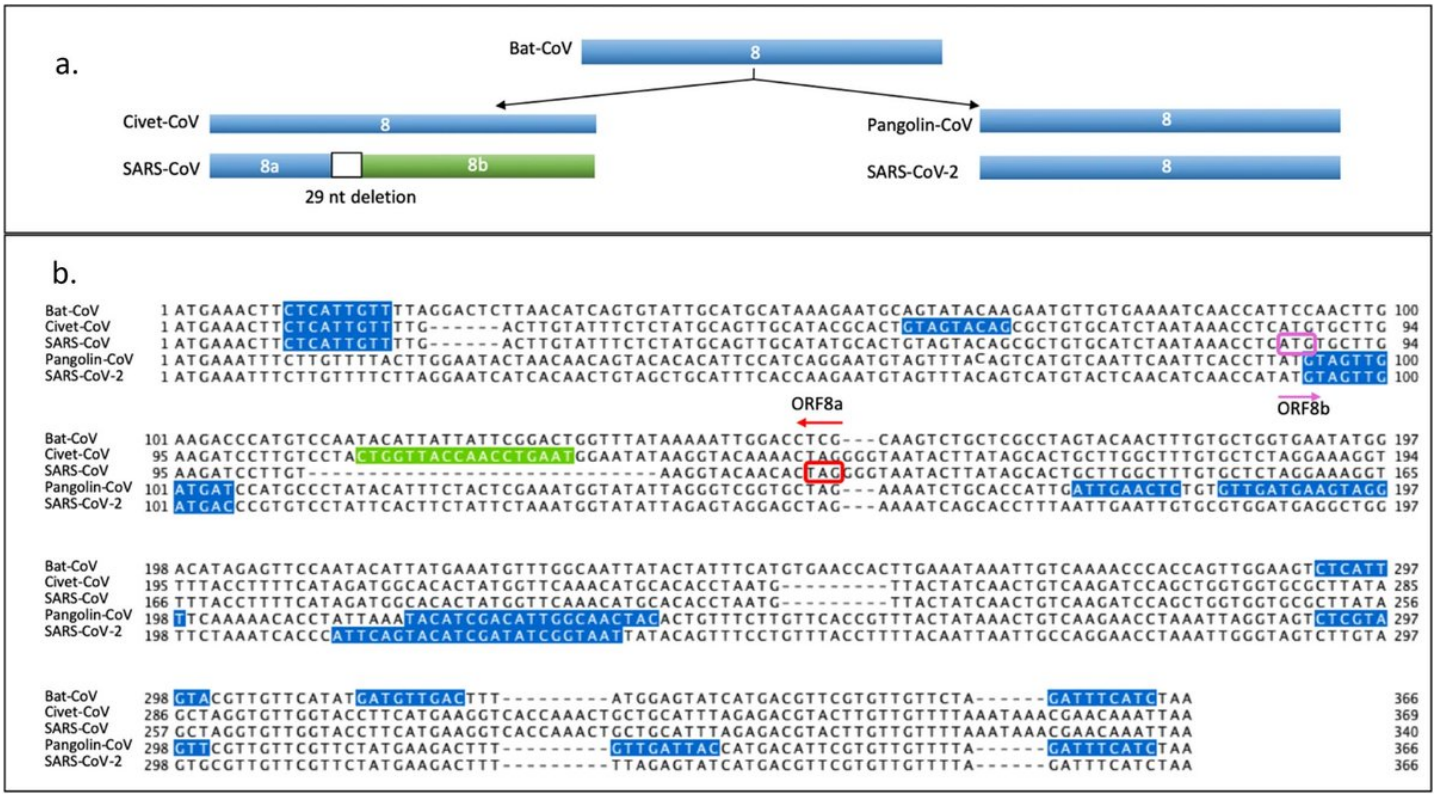
https://t.co/R2PvNsVleu



28. ORF8 or Fate?

Characterization of accessory genes in coronavirus genomes

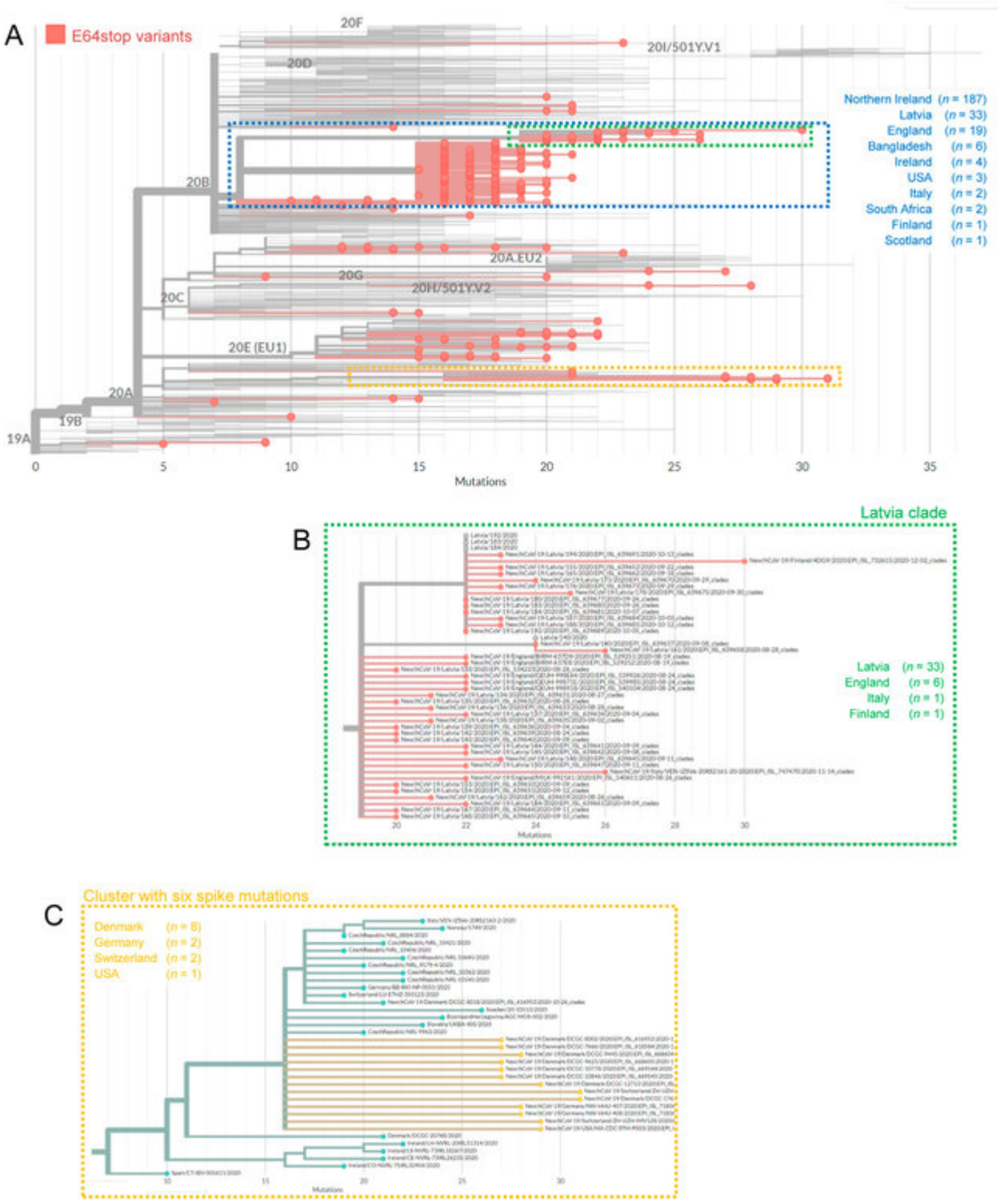
https://t.co/Rwehz9SEDS



29. ORF8 or Fate? To be or not to be?

SARS-CoV-2 variants combining spike mutations & absence of ORF8 may be more transmissible & require close monitoring https://t.co/yWNSdWqMBr

SARS-CoV-2 phylogeny highlighting ORF8-deficient variants resulting from nonsense mutation E64stop.


A
E64stop variants
20F
20I/501Y.V1
20D
20B
20A.EU2
20G
20H/501Y.V2
20C
20E (EU1)
20A
19B
19A
Northern Ireland (n = 187)
Latvia (n = 33)
England (n = 19)
Bangladesh (n = 6)
Ireland (n = 4)
USA (n = 3)
Italy (n = 2)
South Africa (n = 2)
Finland (n = 1)
Scotland (n = 1)
0
5
10
15
20
25
30
35
Mutations
B
Latvia clade
Latvia (n = 33)
England (n = 6)
Italy (n = 1)
Finland (n = 1)
20
22
24
26
28
30
Mutations
C
Cluster with six spike mutations
Denmark (n = 8)
Germany (n = 2)
Switzerland (n = 2)
USA (n = 1)
10
15
20
25
30
Mutations