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https://t.co/TVXC4QOjt0
https://t.co/QnMiOrdNhx
https://t.co/ZK2vfwYEFj
in fact, adaptation in CaLu-3 actually reverses changes that happened in VERO E6. P681 and RRAR is fine-tuned to growth in CaLu-3 cell cultures. P681 guards the cardin-weintraub motif against cleavage in

cell lines.

https://t.co/vytn6YVRYQ
https://t.co/fjg6ZXc2KN
the FCS is perfectly stable in anything that isn't VERO E6 classic or 293T-ACE2. anything that had TMPRSS2 and grown in trypsin-free media stably maintains the FCS.

https://t.co/NUrJ8AndTx
in fact, the PRRARS, as opposed to other mutated cleavage sites--even the "perfect" H5CS--confers the greatest infectivity in CaLu-3 cells.

https://t.co/TVXC4QOjt0
in fact, even P681R or S686G changes were less fit in CaLu-3 compared to PRRA virus--the P681R virus show either no difference or is slightly less effective compared to the P681 virus, and the S686G virus
https://t.co/DNtIR17r4S
https://t.co/nDNNw9OaWd

show significantly less entry compared to PRRAR/S681 virus.https://t.co/9HCxNjyNAC similarily, both CaLu-3 and HELA-hACE2 show significantly reduced viral entry upon mutation of the FCS.

https://t.co/tAP9OQ9FYl
P681 is favored specifically becuse of the cardin-weintraub motif it leaves behind (pRRaRs(O-hexNAc)) compared to R681.

https://t.co/LqKsreg1tu
in deed, even the optimized RRRKR site fares LESS well compared to the unmodified PRRAR site for CaLu-3.

https://t.co/6XFCMUPUqe
once agaiP681 and P681 with RRARS in particular, optimizes CaLu-3 cell entry for the SARS-CoV-2 S.

https://t.co/9HX97A9T5S

https://t.co/H7UqiJ15v9
The final piece of the puzzle: it turned out that CaLu-3 cells DO glycosylate the S1-S2 in the presence of P681, which is necessary for all the previous mechanisms to function—in deed the live SARS-CoV-2 virus on CaLu-3

Cells leads to a significant fraction of S proteins that have not been cleaved, whereas almost no S remain uncleaved on pseudoparticles produced on HEK293T cells. https://t.co/UDOU3kDGlT
These uncleaved fractions indicate that intact cardin-weintraub motifs are formed during

CaLu-3 cell culture, and that these motifs are formed as the result of interference of furin cleavage in the cells by P681-mediated glycosylation of T678 and S685.

https://t.co/qbaazFZTIF

https://t.co/9O3WLoNiY0

In addition, G614 eliminated these motifs by allowing complete cleavage of the S protein, which is why it will not form during cell passage in CaLu-3. As O-linked glycosylation in The live virus is incomplete, it can not perform any of the proposed immunoevasive function

As claimed by Anderson et al, as it (with fraction <25% on CaLu-3) will not be able to shield most of the S trimers on virion (only <1/64 of the S trimers will be fully shielded given the fraction observed on CaLu-3) against Antibody or T cell recognition on the S1-S2 junction,

Whereas the intact Cardin-Weintraub motifs formed on each virion (~50 Heparan Sulfate binding sites formed for 100 S trimers per virion), alongside with the O-glycan epitopes ares sufficient to both confer an selective advantage (inhibition of cleavage in at least 20% of the S,

Binding to Heparan Sulfate proteoglycan coreceptors in culture) in the CaLu-3 cell line and confer an selective disadvantage in the presence of an innate immune system (SIGLEC binding by O-linked HexNAc glycans, PRR recognition of intact cardin-weintraub motif by macrophages) in

Live hosts—if P681 is so critical to shield the virus from the immune system, why it is destroyed in humans by P681R and P681H mutations? If P681 can not arise in passage, why it is so conserved in CaLu-3 cells? It is important to notice that while CaLu-3 cells are used

As a model for airway epithelial cells for passage experiments, viral stock preparation (after each passage) is still only performed in VERO E6 cells. This cell line had a much more pronounced preference of P681 over R681 compared to CaLu-3, especially if TMPRSS2 is expressed in

The cell line—therefore, if virus stocks are regularly prepared between passage experiments, per the PREEMPT proposal, the conjugate potential formed by VERO E6 and CaLu-3 cell lines will fixate P681 on the PRRA insert even faster than CaLu-3 alone, while the CaLu-3 passages

In between completely eliminates any VERO-derived mutations between passages.

In fact, the presence of mutations that both abolish S1-S2 glycosylation (destruction of the P681 residue) and disrupts the Cardin-Weintraub motif in all major human Variants-Of-Concern (B.1.1.7, B.1.617.2,A.23.1) in the form of an additional basic residue appended before the

XBBXBX motif, during the course of immune selection in live human hosts, runs directly against the idea that P681 and cell-specific O-linked glycosylation associated with it could “only be the result of immune selection in the presence of a functional immune system”—in stead it

is the exact opposite, specifically favored in the two PREEMPT-specified cell types (airway epithelial and VERO) and abhorred by immune selection in-vivo. https://t.co/elcdQBF9z3
https://t.co/tAP9OQ9FYl

O-linked glycosylation is only found in uncleaved fractions, which contains an intact XBBXBX Cardin-weintraub Heparan Sulfate binding motif that is favored above furin cleavage specifically in cell cultures. Uncleaved fraction is found both in VERO and CALU-3 cells, which

O-glycosylates the S1-S2 with fractions that are consistent with the uncleaved S in total S for these cell lines.