Characterization of spike activation and temperature dependence of SARS-CoV-2 and other human coronaviruses

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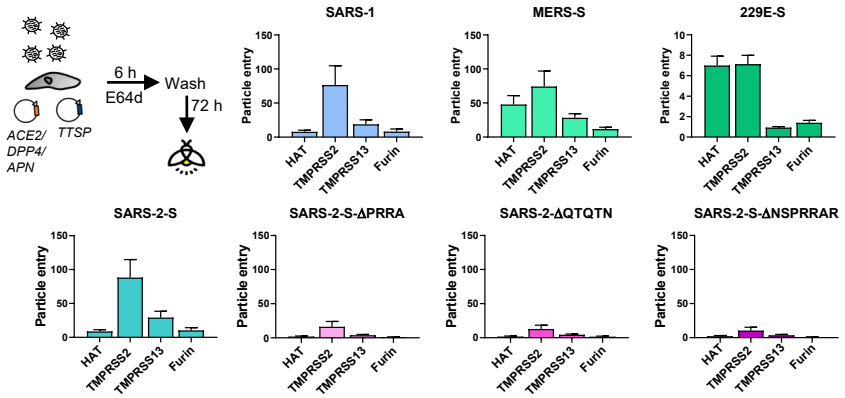
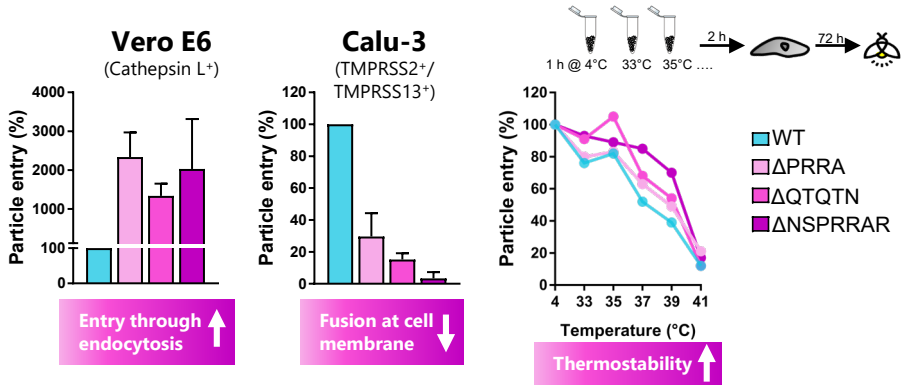
1| Spike activation by human airway proteases



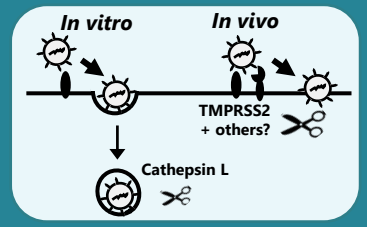
Panel 1| Other proteases than TMPRSS2 activate CoVs. All three highly virulent CoVs are recognized by TMPRSS13. HAT (human airway trypsin-like protease) activates MERS and HCoV-229E. The SARS-2-S furin mutants do not require activation by membrane-bound proteases.

2| The SARS-2 furin mutants enter via endosomes and are more stable

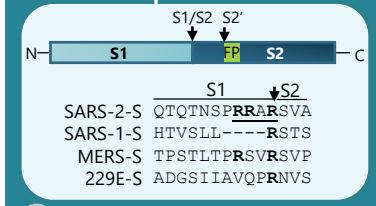


Panel 2| SARS-2-S furin mutants enter very efficiently in Vero E6 cells that have high levels of cathepsin L, but entry in the TMPRSS2+/TMPRSS13+ human airway epithelial cell line Calu-3 is abrogated. The absence of furin priming increases thermostability of the spike protein.

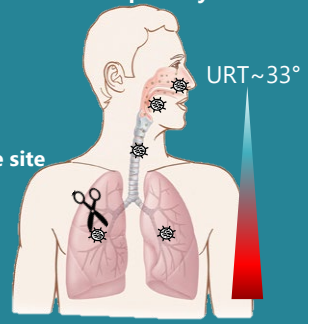
Coronaviruses can enter cells via two routes



SARS-CoV-2 spike has a furin cleavage site



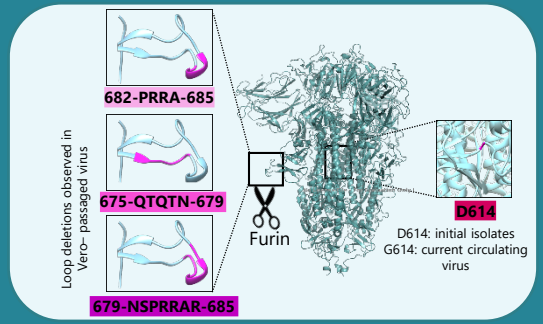
Temperature gradient in the human respiratory tract



Main research questions:

- Which proteases activate the different CoVs?
- Impact of the furin cleavage site in SARS-CoV-2?
- Role of URT versus LRT temperature?

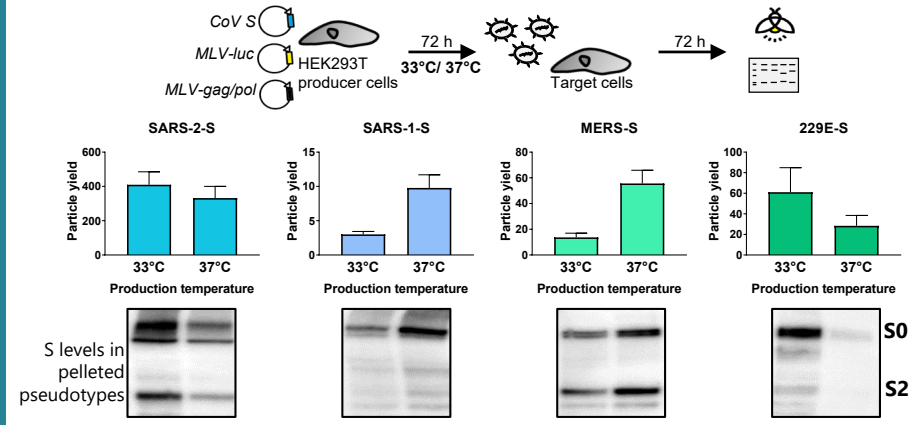
➔ Method: S-pseudotyped MLV particles



Highlights

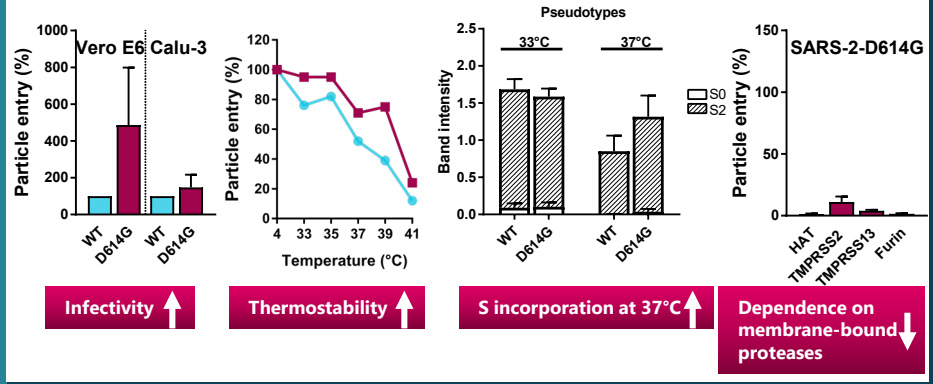
- Besides TMPRSS2, TMPRSS13 is a potent activator of highly virulent CoVs.
- Removal of the furin cleavage loop in SARS-CoV-2-S increases endosomal entry.
- Different CoVs exhibit a different temperature profile.
- Mutation D614G increases SARS-CoV-2 thermostability.

3| Spike expression is temperature-dependent



Panel 3| SARS-1 and MERS pseudotypes prefer production at 37°C, while 229E prefers 33°C. SARS-2 is efficiently produced at both temperatures, with a slight preference for 33°C.

4| Basis for higher infectivity of the SARS-2 D614G mutant?



Panel 4| Pseudotypes carrying the SARS-2-D614G mutation show higher infectivity, a potential consequence of its higher thermostability and better incorporation into pseudotypes. Reduced dependence on membrane-bound proteases suggests increased uptake by endocytosis.