SPIKE MUTATION T403R ALLOWS BAT CORONAVIRUS RATG13 TO USE HUMAN ACE2

00165

Fabian Zech[1], Daniel Schniertshauer[1], Christoph Jung[2,3,4], Alexandra Herrmann[5], Arne Cordsmeier[5], Janis Müller[1], Jana Krüger[1], Sandra Heller[1], Steffen Stenger[2], Alexander Kleger[1], Timo Jacob[2,3,4], Karl-Klaus Conzelmann[6], Armin Ensser[5], Konstantin M.J. Sparrer[1] and Frank Kirchhoff[1]

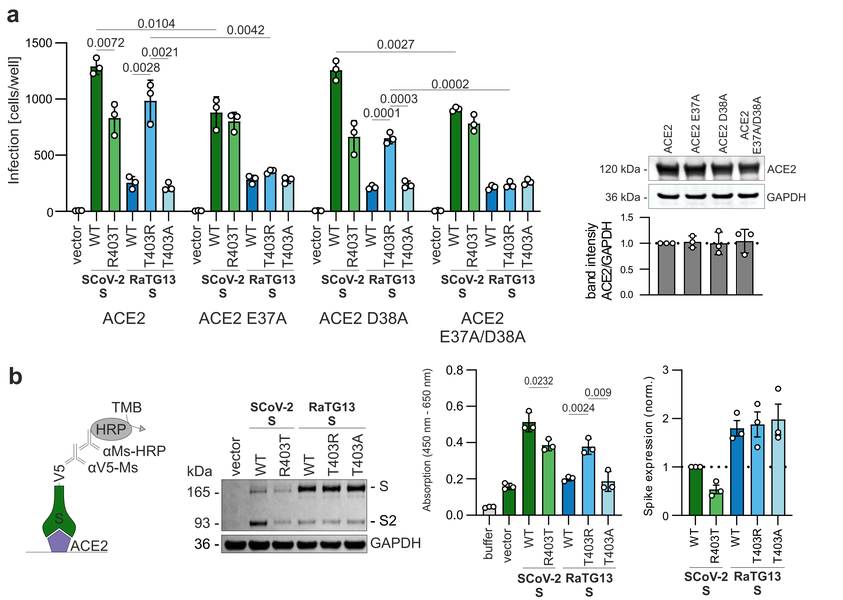
[1]Ulm University Medical Center, Ulm, Germany; [2]Ulm University, Ulm, Germany; [3]Helmholtz Institute, Ulm, Germany; [4]Karlsruhe Institute of Technology, Karlsruhe, Germany; [5]University Hospital Erlangen, Erlangen, Germany [6]Medical Faculty, and Gene Center, Ludwig Maximilians-Universität München, Munich, Germany.

BACKGROUND

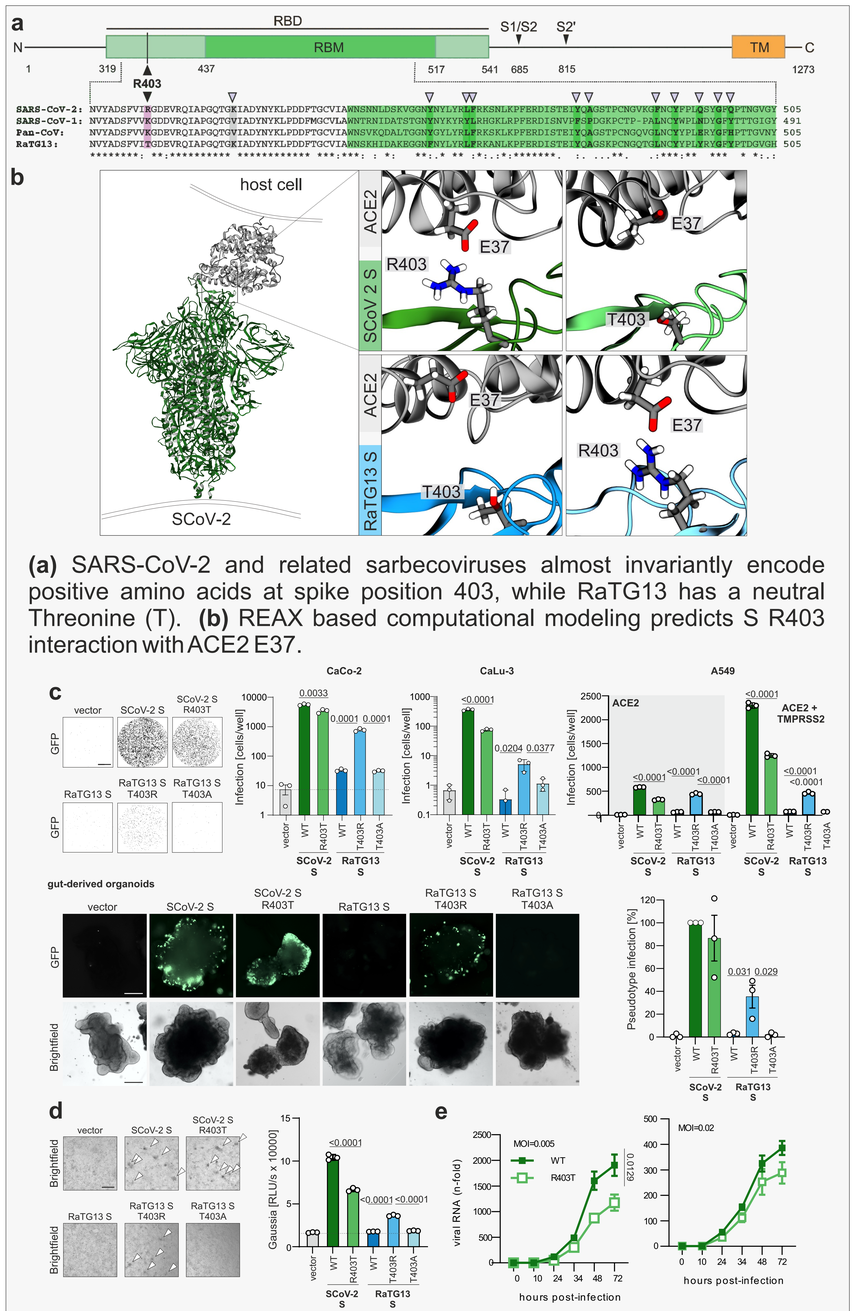
The bat coronavirus RaTG13 shares 96% sequence identity to SARS-CoV-2, the causative agent of the COVID-19 pandemic. However, the RaTG13 Spike (S) protein interacts only weakly with the human SCoV-2 receptor Angiotensin-converting Enzyme 2 (ACE2) and does not mediate efficient infection of human cells. Here, we examined which alterations are required to allow the RaTG13 S protein to use human ACE2 for efficient entry into human cells. (Zech et al., *Nat Commun.* 2021, https://doi.org/10.1038/s41467-021-27180-0)

METHODS

- REAX based computational modeling of the Spike/ACE2 interaction
- Side directed mutagenesis of position 403 in S and E37 in human ACE2
- Infection of human lung- and intestine-derived cell lines as well as hPSC-derived gut organoids with S pseudotyped VSV particles.
- Growth monitoring of replication-competent SARS-CoV-2 S R403T
- In vitro ACE2 interaction assay
- Neutralization of S pseudotyped particles by sera of vaccinated individuals



(a) The enhancing effect of T403R in RaTG13 S depends on E37 in ACE2. **(b)** RaTG13 T403R enhanced ACE2 binding without affecting S expression levels.



(a) SARS-CoV-2 and related sarbecoviruses almost invariantly encode positive amino acids at spike position 403, while RaTG13 has a neutral Threonine (T). **(b)** REAX based computational modeling predicts S R403 interaction with ACE2 E37.

(c) Spike T403R enhanced infection of VSV-based RatG13 S pseudotypes in human lung and colon cells as well as gut-derived organoids. **(d)** R403T mutation reduced infectivity of SCoV2 S pseudotypes and **(e)** recombinant SCoV2 replication.

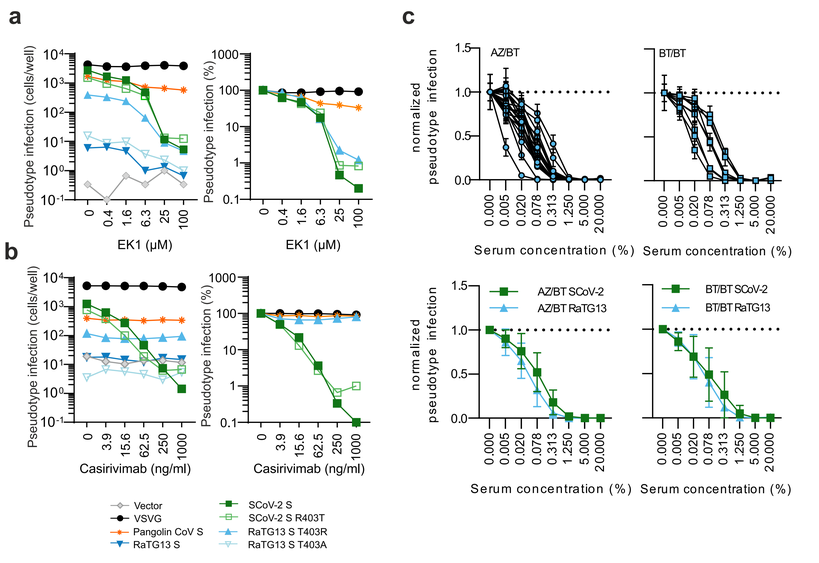
CONCLUSIONS

A positively charged amino acid at position 403 in the S protein of bat coronaviruses is critical for efficient utilization of human ACE2. Our results help to better assess the zoonotic potential of bat sarbecoviruses and suggest that COVID-19 vaccination will also protect against close relatives of SARS-CoV-2 that may emerge in the future.

ADDITIONAL KEY INFORMATION

Presenting author contact:

fabian.zech@uni-ulm.de
Institute of Molecular Virology
Ulm University Medical Center
Meyerhofstrasse 1/N27 2.042
89081 Ulm



RaTG13 T403R S-mediated infection was blocked by the **(a)** fusion inhibitor EK-1 but not by the **(b)** SCoV-2 antibody Casirivimab. **(c)** SARS-CoV-2 and the RaTG13 S T403R were equally susceptible to neutralization by sera from individuals vaccinated against COVID-19.