Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission

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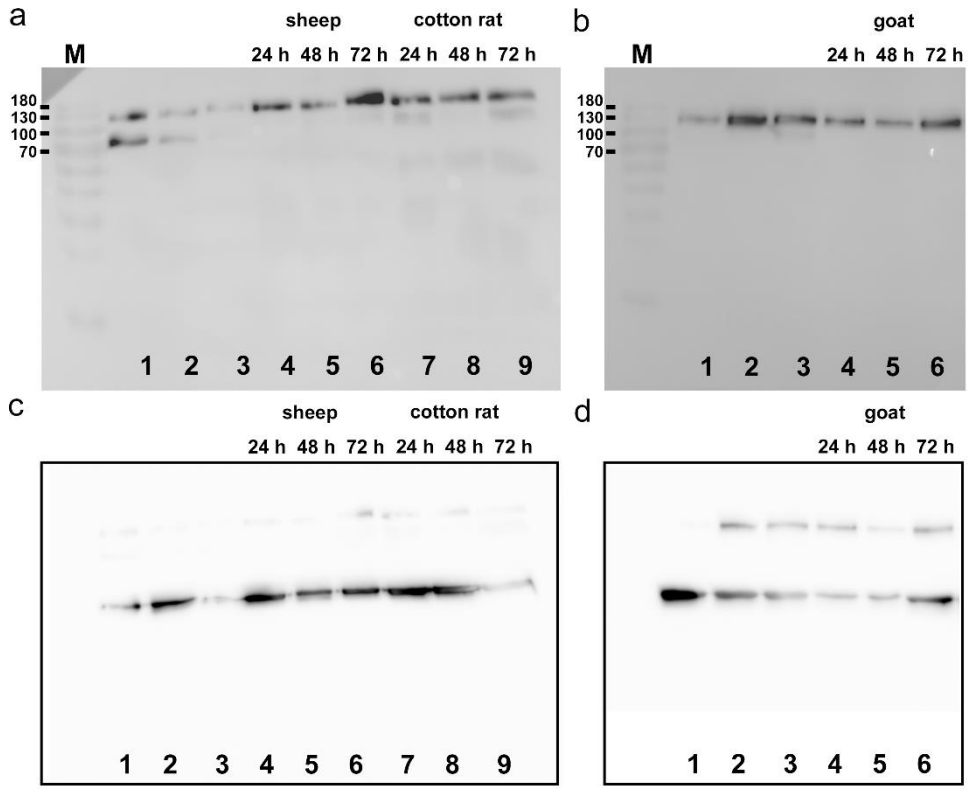
Supplementary Fig. S1: Expression of hACE2 after transduction of "non-SARS-CoV-host" cell lines. Cells from sheep, cotton rat and goat were seed in 24-well plates to reach 80% confluency for infection with lentiviruses (total of 50 ng reverse transcriptase activity) to induce transient expression of hACE2. Protein expression was analysed at 24, 48 and 72 h post infection. HACE2 (ca. 140 kDa) was detected using anti-hACE2 Ig (1:1,000; R&D Systems, Wiesbaden-Nordenstadt, Germany) and a goat anti-mouse horseradish peroxidase (HRP)-conjugated Ig (1:20,000) (in a) lanes 4-9, in b) lane 4-6). The expression of β-actin (ca. 42 kDa) served as a loading control using a rabbit anti-β-actin Ig (1:2,000; Sigma-Aldrich, Munich, Germany) and a goat anti-rabbit horseradish peroxidase-labeled Ig (1:20,000) (lanes 4-9 in c) and lanes 4-6 in d). Lanes 1-3 of a), b), c) and d) carry samples non-relevant for the publication. Chemiluminscence signals were detected using SuperSignal® West Femto Chemiluminescence Substrate (Fisher Scientific, Schwerte, Germany) after 1 min (a), 10 sec (b), 10 sec (c) and 1 min (d) exposure time in a FusionFX7 (PeqLab/Vilbert Lourmat, Erlangen, Germany). HACE2 signals were visible during actin detection because Western Blots were not stripped between incubation with different primary antibodies. M, PageRuler Prestained Protein Ladder (Fisher Scientific).


a
sheep
cotton rat
M
24 h 48 h 72 h 24 h 48 h 72 h
180
130
100
70
1 2 3 4 5 6 7 8 9
b
goat
M
24 h 48 h 72 h
180
130
100
70
1 2 3 4 5 6
c
sheep
cotton rat
24 h 48 h 72 h 24 h 48 h 72 h
1 2 3 4 5 6 7 8 9
d
goat
24 h 48 h 72 h
1 2 3 4 5 6