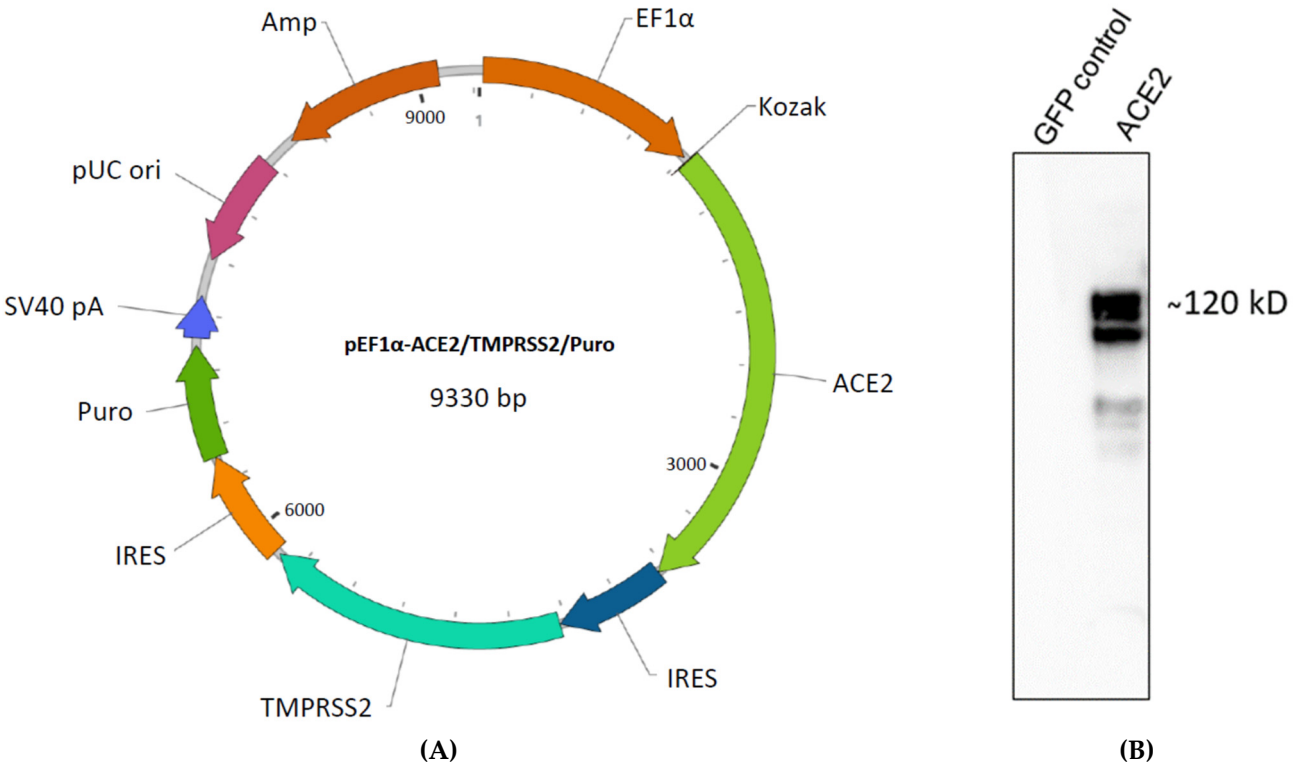

Figure S1. ACE2/TMPRSS2 expressing construct. (**A**) Diagram not on scale of pEF1α-ACE2/TMPRSS2/Puro construct, where functional elements of the construct are indicated and highlighted with different colours. EF1α (human Elongation Facto 1 alpha promoter), Kozak (Kozak's translation initiation sequence), ACE2 (Human Angiotensin Converting Enzyme 2 ORF), IRES (Encephalomyocarditis virus internal ribosomal entry site), TMPRSS2 (human Transmembrane Protease Serine 2), Puro (Puromycin resistant gene ORF), pUC ori (High copy number pUC origin of replication) SV40 early pA (Simian virus 40 early polyadenylation signal) and Amp (Ampicillin resistance gene, comprising the prokaryotic promoter and ORF). (**B**) Western immunoblotting of pEF1α-ACE2/TMPRSS2/Puro stably transfected HEK293T cells expressing ACE2. Negative control was established with HEK293T cell transfected with pEGFP-C1 plasmid. Each lane was loaded with 40 μg of cell protein extract.

pEF1A-ACE2/TMPRRS2/Puro complete sequence.

CAACTTTGTATAGAAAAGTTGGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCC
ACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGG
CGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG
GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCC
GCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTAT
GGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAG
CTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCT
CGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCAC
CTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCT
GCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACT
GGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGT
TCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCT
GGCCGGCCTGCTCTGGTGCCTGGTCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCA
AGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCT
GCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACA
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GCAAGAGCAAATCATTATGAGGACTATGGGGATTATTGGAGAGGAGACTATGAAGTAAAT
GGGGTAGATGGCTATGACTACAGCCGCGGCCAGTTGATTGAAGATGTGGAACATACCTTT
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AGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTT
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TGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTC
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CAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAG
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CCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT
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ACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCAC
GAGGCCCTTTCGTCGGCGCGCCGCGGCCGC

Figure S2. S-ΔRS-HA human adapted ORF sequence.

ATGTTCGTGTTCCTGGTGCTGCTGCCCCTGGTGAGCAGCCAGTGCGTGAACCTGACCACCCGCACCCAGCTGCCCCCCGCCTACACCAACAGCTTC
ACCCGCGGCGTGTACTACCCCGACAAGGTGTTCCGCAGCAGCGTGCTGCACAGCACCCAGGACCTGTTCCTGCCCTTCTTCAGCAACGTGACCTG
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AGAAGAGCAACATCATCCGCGGCTGGATCTTCGGCACCACCCTGGACAGCAAGACCCAGAGCCTGCTGATCGTGAACAACGCCACCAACGTGGT
GATCAAGGTGTGCGAGTTCCAGTTCTGCAACGACCCCTTCCTGGGCGTGTACTACCACAAGAACAACAAGAGCTGGATGGAGAGCGAGTTCCGC
GTGTACAGCAGCGCCAACAACTGCACCTTCGAGTACGTGAGCCAGCCCTTCCTGATGGACCTGGAGGGCAAGCAGGGCAACTTCAAGAACCTGC
GCGAGTTCGTGTTCAAGAACATCGACGGCTACTTCAAGATCTACAGCAAGCACACCCCCATCAACCTGGTGCGCGACCTGCCCCAGGGCTTCAGC
GCCCTGGAGCCCCTGGTGGACCTGCCCATCGGCATCAACATCACCCGCTTCCAGACCCTGCTGGCCCTGCACCGCAGCTACCTGACCCCCGGCGAC
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Biscistronic turboGFP-IRES-Luc2 ORF

ATGCCCGCCATGAAGATCGAGTGCCGCATCACCGGCACCCTGAACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGAGAGGGCACCCCCGAGCAG
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TCGCCAGATCTCGAGCTCGATGAACCCAGCTTTCTTGTACAAAGTGGGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAA
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AACGTGCAAAAGAAGCTACCGATCATACAAAAGATCATCATCATGGATAGCAAGACCGACTACCAGGGCTTCCAAAGCATGTACACCTTCGTGAC
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TTGTGTTCGTGGACGAGGTGCCTAAAGGACTGACCGGCAAGTTGGACGCCCGCAAGATCCGCGAGATTCTCATTAAGGCCAAGAAGGGCGGCAA
GATCGCCGTGTAA

Figure S3. (**A**) pLV-CMV-(S-ΔRS-HA)-IRES-Puro-WPRE complete sequence

AATGTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACA
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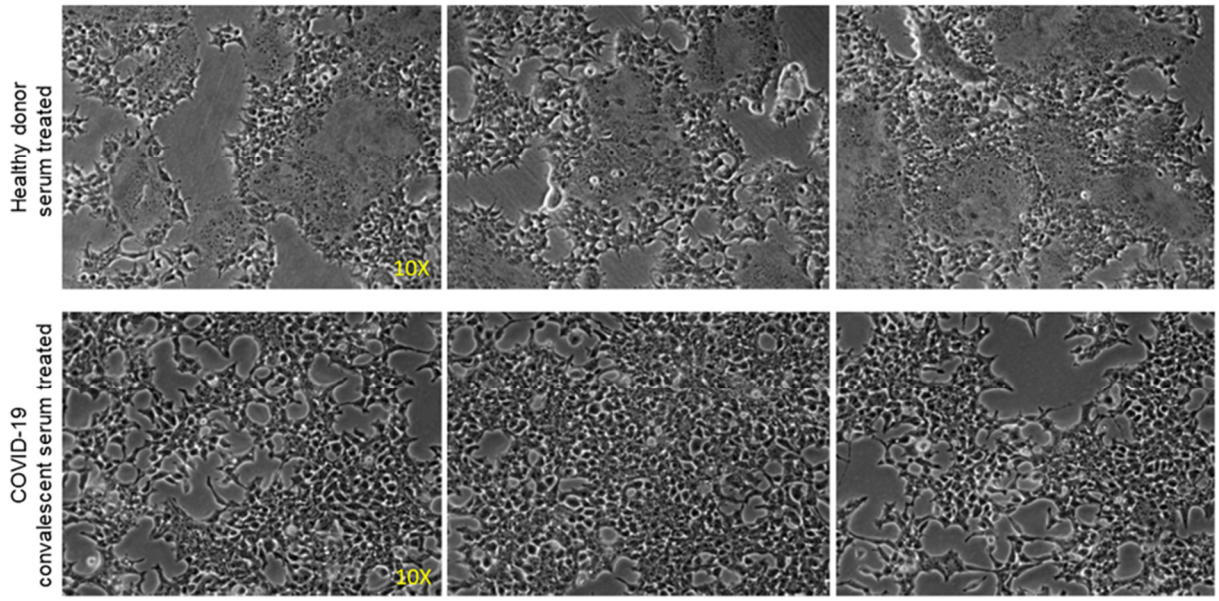

(**B**) Three representive phase contrast images (10X) of healthy donor serum treated pLV-CMV-(S-ΔRS-HA)-IRES-Puro-WPRE transfected HEK/ACE2/TMPRRS2/Puro cells (large syncytia formation) and COVID-19 convalescent serum treated pLV-CMV-(S-ΔRS-HA)-IRES-Puro-WPRE transfected HEK/ACE2/TMPRRS2/Puro cells (absence of syncytia). HEK/ACE2/TMPRRS2/Puro cells were treated with serum before and after transfection with pLV-CMV-(S-ΔRS-HA)-IRES-Puro-WPRE. The experiment was repeated 4 times with identical results

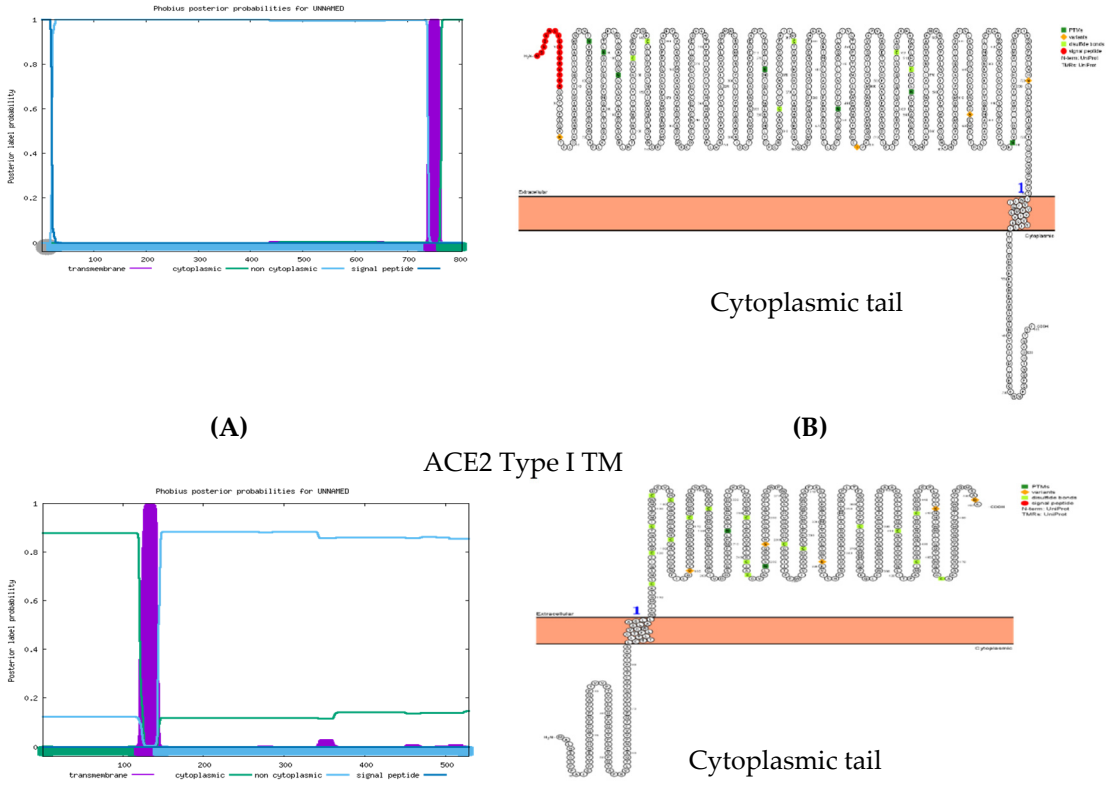

Figure S4. ACE2 and TMPRSS2 topology: (**A**) A combined transmembrane topology and signal peptide prediction of ACE2 and TMPRSS2 as performed by Phobius (https://phobius.sbc.su.se). (**B**) A topological and subcellular localization prediction of ACE2 and TMPRSS2 as performed by Protter (https://wlab.ethz.ch/protter/start/). Open-source tool for visualization of proteoforms and interactive integration of annotated and predicted sequence features together with experimental proteomic evidence.

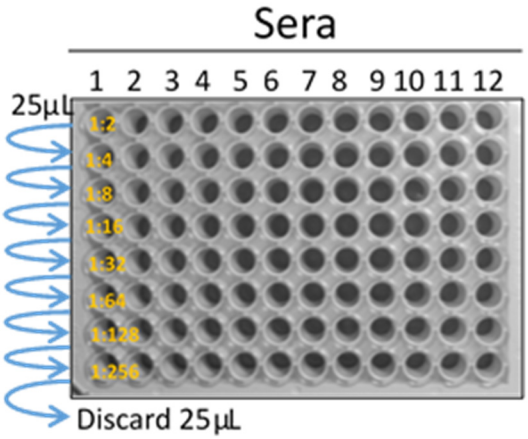

Figure S5. Serum Neutralization Flow-chart.

(1) Dilution of the sera

25µL of complete medium are added to each well of the microplate and 25 µL of each serum is added to each well of the first line of wells. Than 25 µL of the first dilution of the sera (1:2) are mixed and passed to the subsequent lines of wells. 25µL from the last line of wells are discarded. Therefore, the final volume for each well is 25µL.

(2) Adding the pseudovirus

25µL of pseudovirus in complete medium (corresponding to 104 RLUs; ~3-5µL of the initial preparation) are added to each well and left at room temperature for 1,5 hours. Final volume for each well is 50µL, therefore the sera dilution is doubled (1:4-1:8-1:16-1:32-1:64-1:128-1:256-1:512).

(3) Adding the cells

50µL of complete medium containing 10^4 HEK/ACE2/TMPRRS2/Puro cells are added to each well and left for 60 h at 37 °C and 5% CO_2. Final volume for each well is 100µL.

(4) Adding the Luciferine and reading

25µL of complete medium containing Luciferin (20µL of complete medium plus 5µL of 15mg/mL Luciferin in PBS) are added to each well just before the luminometric reading of the microplate. Final volume for each well is 125µL.

RLUs obtained were compared and normalized to those derived from wells where pseudovirus were added in the absence of plasma/sera (100%). Neutralization titer 50 (NT50/mL) was expressed as the maximal dilution of the sera where the reduction of the signal is ≥ 50%. This titer has to be multiplied per 40 because the initial volume of the sera tested is 0,025mL and it has to be normalized to 1mL. This is a very important issue because SN protocol could differ from each lab and the initial amount of sera tested could be different in volume. However, in most of the case, operators tend to indicate SN50 and not SN50/mL.