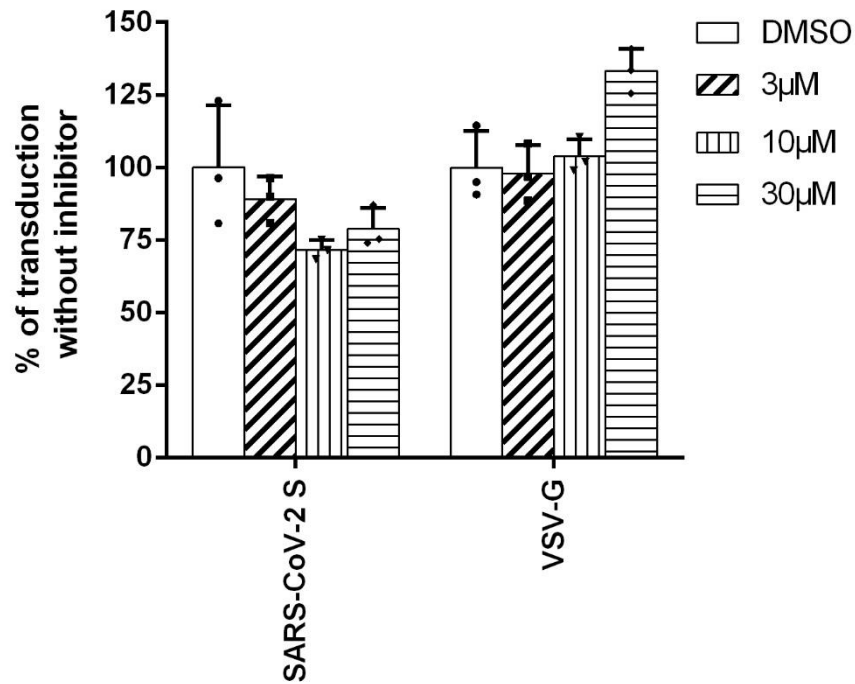


## **Supplementary information**

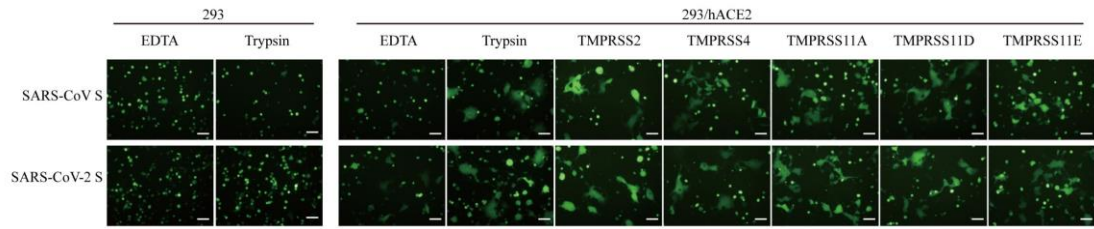
**Characterization of spike glycoprotein of SARS-CoV-2 on virus entry  
and its immune cross-reactivity with SARS-CoV**

***Ou et al.***



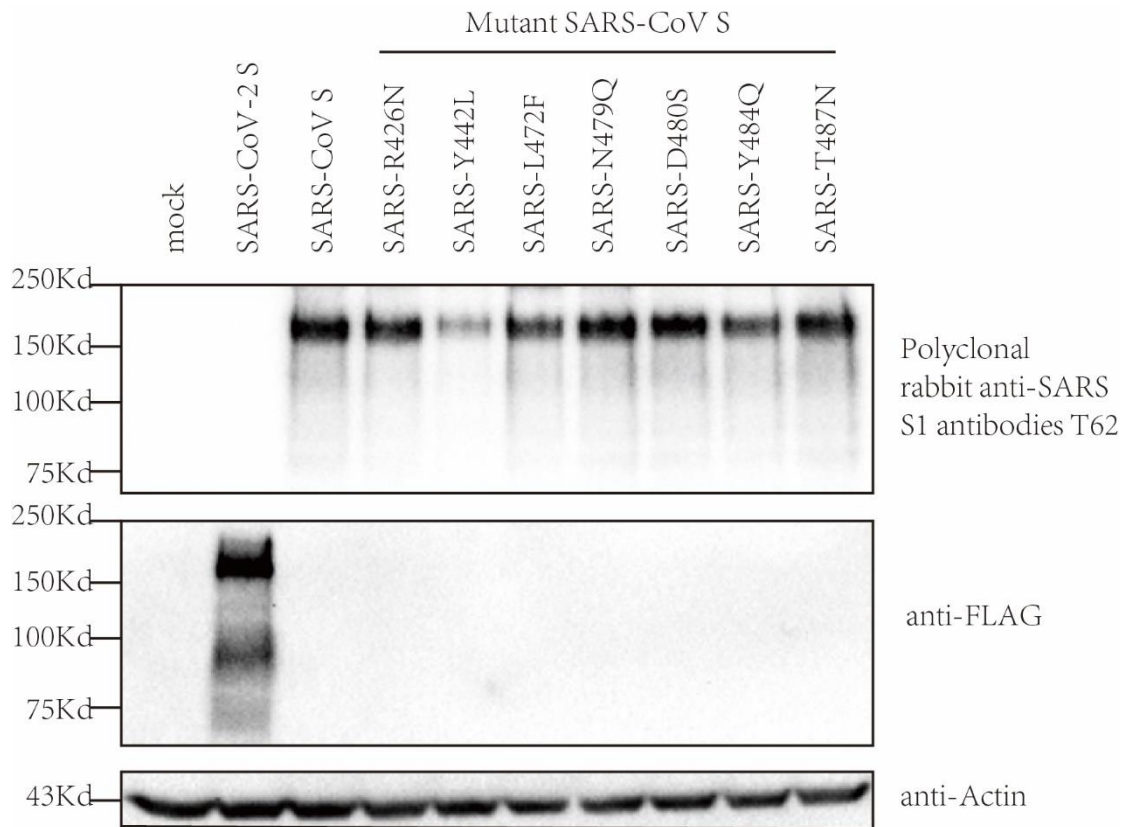
**Supplementary Figure 1. Effect of entry of SARS-CoV-2 S pseudovirions on 293/hACE2 by TRPML1 inhibitor.**

HEK 293/ACE2 cells were transduced with either SARS-CoV-2 S pseudovirions or VSV-G pseudovirions in the presence of 3, 10, 30µM 130. The luciferase activity were measured at 40 hrs post inoculation. The experiments were done triplicate and repeated three times.



**Supplementary Figure 2. Effect of type II membrane serine proteases (TMPRSS) on SARS-CoV-2 S protein mediated cell-cell fusion.**

HEK-293T cells transiently expressing CoV S glycoprotein and eGFP were lifted with either trypsin (0.25%) or 1mM EDTA, then overlaid on 293/hACE2 cells transiently expressing type II membrane serine protease (TMPRSS) 2, 4, 11A, 11D, or 11E. After 4 hrs incubation, images of syncytia were captured.



**Supplementary Figure 3. Western blot analysis of expression of SARS-CoV mutant S proteins.**

Top panel: polyclonal rabbit anti-SARS S1 antibodies T62; middle panel: anti-FLAG  
bottom panel: anti-actin.

Supplementary Table 1. Primers used in this study.

Constructs used in mutagenesis study	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
SARS-CoV-2 S	CCGAAGCTTCACCATGTTC GTTTCCTTGTTTC	CCGGGATCCGCAACATGATCCGCAA GAGCAGC
SARS-CoV S- R426N	GTGCTGGCTTGGAACACTA ACAACATTGATGCTACCTC	GAGGTAGCATCAATGTTGTTAGTGT TCCAAGCCAGCAC
SARS-CoV S- Y442L	CTACAACACTACAAGTATCGT CTGCTGCGTCATGGTAAGC TG	CAGCTTACCATGACGCAGCAGACG ATACTTGTAGTTGTAG
SARS-CoV S- L472F	CTTGCACTCCTCCAGCTTT CAACTGCTACTGGCCTC	GAGGCCAGTAGCAGTTGAAAGCTG GAGGAGTGCAAG
SARS-CoV S- N479Q	CTGCTACTGGCCTCTGCAG GATTACGGTTTCTACAC	GTGTAGAAACCGTAATCCTGCAGA GGCCAGTAGCAG
SARS-CoV S- D480S	CTACTGGCCTCTGAACTCT TACGGTTTCTACACC	GGTGTAGAAACCGTAAGAGTTCAG AGGCCAGTAG
SARS-CoV S- Y484Q	GAACGATTACGGTTTCCAG ACCACTACCGGCATTGG	CCAATGCCGGTAGTGGTCTGGAAA CCGTAATCGTTC
SARS-CoV S- T487N	GTTTCTACACCACTAACGG CATTGGCTACC	GGTAGCCAATGCCGTTAGTGGTGTA GAAAC

Supplementary Table 2. Codon-optimized DNA sequences of spike gene.

SARS-CoV-2 S	<p> ATGTTTCGTTTTCTTGTCTGTTGCCTCTCGTTAGTAGCCAATGCGTCAAC  CTTACTACTAGAACCCAGCTCCCTCCAGCATATACCAACTCTTTCACCAGG  GGCGTATATTACCCGGACAAAGTGTTCGCTCAAGTGTGCTGCATTCTAC  GCAGGACCTTTTCTTGCCCTTTTTTCAGTAATGTTACTTGGTTTCATGCTATC  CATGTGTCTGGAACCTAACGGAACCAAGCGCTTTGACAACCCCGTCCTCC  CTTTCAACGATGGCGTGTACTTCGCTTCCACGGAAAAGTCAAACATAATT  CGCGGCTGGATCTTTGGTACAACACTCGACTCAAAGACGCAGAGCCTGC  TGATCGTTAATAACGCTACAAATGTTGTGATAAAGGTGTGTGAATTTAGT  TCTGCAATGATCCCTTCCTGGGTGTGTACTACCATAAGAATAACAAGAGC  TGGATGGAATCCGAATTTAGGGTTACAGTTCCGCTAACAACCTGCACATT  CGAATACGTAAGCCAGCCATTTCTTATGGATCTTGAGGGCAAGCAAGGAA  ACTTCAAGAACTTGAGGGAGTTCGTGTTCAAAAATATCGACGGCTATTTT  AAGATATATAGCAAGCACACTCCAATAAACTTGGTGCGCGACCTGCCCA  GGGATTCTCTGCTCTGGAGCCCCTGGTGGATCTGCCATTGGAATAACA  TAACTCGCTTTCAAACACTGCTCGCCCTGCATCGCAGTTACCTACCCCT  GGTGATAGTAGTTCAGGATGGACAGCAGGAGCCGCCGATACTACGTCG  GCTACCTGCAGCCTAGGACCTTCTTGCTGAAGTACAACGAGAACGGTAC  AATAACTGACGCTGTGGACTGCGCTCTGGACCCTCTGTCCGAGACGAAG  TGCACCCTGAAGAGCTTTACTGTTGAAAAAGGCATTTACCAAACCAGCA  ACTTCCGCGTCCAGCCAACCGAGAGCATCGTCAGATTTCCCAACATTACA  AATCTGTGTCCCTTCGGCGAGGTGTTCAACGCCACACGCTTCGCTTCAGT  GTACGCATGGAACCGCAAGCGCATATCTAACTGCGTTCGCGGATTATTCTG  TCCTCTACAACCTCCGCTCTTTCTCCACCTTCAAGTGCTACGGAGTGCA  CCGACTAAGCTGAACGATCTCTGCTTTACCAACGTCTACGCGGACTCCTT  CGTGATAAGAGGTGATGAAGTGAGACAAATAGCCCCAGGTCAGACTGGT  AAGATCGCAGATTACAACCTACAAATTGCCTGATGATTTCACTGGTTGCGT  TATCGCGTGGAACCTCTAATAACCTCGATTCTAAGGTCGGTGGTAACTACA  ATTACCTGTACCGCTTGTTTAGGAAGTCAAACCTGAAGCCTTTCGAGAGG  GATATTTCAACCGAAATCTATCAAGCGGGTTCAACACCGTGTAACGGTGT  GGAAGGATTTAACTGCTACTTCCCCCTGCAGTCTTACGGATTCCAGCCAA  CCAATGGCGTGGGTACCAACCTTATCGCGTGGTGGTTCTGAGTTTCGAA  CTGTTGCACGCTCCCGCCACGGTATGCGGTCCCAAGAAGAGCACTAACT  TGGTGAAGAATAAGTGCGTGAATTTCAATTTCAATGGCCTCACTGGAAC  GGAGTGCTGACCGAATCCAATAAGAAGTTCTTGCCCTTCCAGCAGTTTCGG  AAGAGACATTGCTGACACAACCGACGCGGTGCGCGATCCTCAGACTCTG  GAGATATTGGACATTACCATGTTCTTTCGGCGGTGTGTCTGTCATTACT  CCGGGCACGAATACTAGCAACCAGGTAGCCGTGCTGTACCAAGACGTGA  ATTGCACAGAGGTTCCCGTCGCAATTCACGCTGACCAGCTGACCCCCAC  GTGGAGGGTTTACAGCACTGGTAGTAACGTCTTCCAGACGAGAGCCGGT  TGCTTGATCGGAGCGGAACATGTGAATAACTCCTACGAGTGCGACATCCC  CATCGGAGCCGGTATATGCGCCTCTTATCAGACACAACTAACTACCCCA  GGAGAGCCCGCAGTGTGGCTTCTCAAAGCATTATAGCATACACTATGTCT  CTTGGTGCCGAAAATTCCGTGGCCTATTCTAACAATTCAATCGCCATCCCA </p>
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<p>SARS- CoV S/nRBD</p>	<p>ATGTTTCATCTTTCTGCTGTTTCTGACACTGACCAGCGGCTCCGACCTGGA  TAGGTGCACCACATTTGACGATGTGCAGGCCCCCAACTATAACCAGCACA  CAAGCTCCATGCGGGGCGTGTACTATCCTGATGAGATCTTCAGATCCGAC  ACCCTGTATCTGACACAGGACCTGTTTCTGCCCTTTTACTCTAATGTGACC  GGCTTCCACACAATCAATCACACCTTTGGCAACCCTGTGATCCCATTCAA  GGATGGCATCTACTTTGCCGCCACAGAGAAGAGCAACGTGGTGTAGGGGC  TGGGTGTTTCGGCTCCACCATGAACAATAAGTCTCAGAGCGTGATCATCAT  CAACAATTCCACCAACGTGGTCATCCGCGCCTGCAATTTTGTGCTGTGCG  ACAACCCATTCTTTGCCGTGTCTAAGCCCATGGGCACACAGACCCACACA  ATGATCTTTGATAATGCCTTCAACTGTACCTTTGAGTATATCTCCGATGCCT  TTTCTCTGGACGTGTCTGAGAAGAGCGGCAACTTCAAGCACCTGCGGGA  GTTTCGTGTTTAAGAATAAGGACGGCTTCCTGTACGTGTATAAGGGCTACC  AGCCAATCGATGTGGTGAGAGACCTGCCAGCGGCTTCAACACCCTGAA  GCCTATCTTTAAGCTGCCACTGGGCATCAATATCACAAACTTCAGGGCCAT  CCTGACCGCCTTTTCTCCTGCACAGGACATCTGGGGCACCAGCGCCGCA  GCATATTTTCGTGGGCTACCTGAAGCCAACCACCTTCATGCTGAAGTATGAT  GAGAACGGCACCATCACAGACGCCGTGGATTGCAGCCAGAATCCTCTGG  CCGAGCTGAAGTGTTCGTGAAGTCTTTTCGAGATCGACAAGGGCATCTA  CCAGACATCCAACCTTTCCGGGTGCAGCCAACCGAGTCTATCGTGAGATTCC  CTAATATCACAAACCTGTGCCCATTCGGCGAGGTGTTTAAATGCCACCCGC  TTTGCCTCCGTGTACGCCTGGAATCGGAAGAGAATCAGCAACTGCGTGG  CCGACTATTCCGTGCTGTACAACCTCCGCCTTTTCAGCACCTTTAAGTGCT  ATGGCGTGAGCCCCACAAAGCTGAATGACCTGTGCTTCACCAACGTGTA  CGCCGATTCCCTTTGTGATCAGGGGCGACGAGGTGCGCCAGATCGCACCT  GGACAGACAGGCAAGATCGCCGACTACAACCTATAAGCTGCCAGACGATT  TCACCGGCTGCGTGATCGCCTGGAATAGCAACAATCTGGATTCCAAAGTG  GGCGGCAACTACAATTATCTGTACAGGCTGTTCCGCAAGAGCAACCTGA  AGCCATTTGAGCGGGACATCAGCACAGAGATCTACCAGGCAGGATCCAC  CCCATGCAATGGAGTGGAGGGCTTCAACTGTTATTTTCTCTGCAGTCCT  ACGGCTTCCAGCCAACCAATGGCGTGGGCTATCAGCCCTACAGAGTGGT  GGTGTCTCTTTTGTGCTGTGCACGCACCAGCAACAGTGTGCGGACCT</p>

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MERS-CoV S	ATGATTCACTCCGTGTTCTCCTGATGTTCTGCTCACTCCTACCGAAAGT TATGTGGATGTGGGTCCCGATAGCGTCAAGTCCGCATGCATCGAGGTGGA TATTCAGCAGACATTCTTTGACAAGACCTGGCCTCGGCCAATCGATGTCT CCAAAGCCGACGGCATCATTTACCCTCAGGGACGCACCTATAGCAACATC ACTATTACATACCAGGGCCTGTTCCCATATCAGGGCGACCACGGAGATATG TACGTGTATTCTGCCGGACATGCTACTGGCACCCTCCTCAGAACTGTT CGTGGCAAATACTCCCAGGACGTCAAGCAGTTCGCCAACGGTTTTGTG GTCAGGATCGGCGCCGCTGCAAACCTTACC GGCACTGTGATCATTAGCCC TTCTACTTCAGCCACAATCAGAAAAATTTACCCAGCTTTTATGCTGGGCTC CAGCGTGGGAACTTCTCCGATGGCAAGATGGGCCGTTTCTTTAATCACA CTCTGGTCCTGCTCCCTGACGGATGCGGTACTGCTCCGCGCTTTCTAC TGTATCCTGGAGCCCAGGAGCGGAAATCACTGCCCTGCCGGTAACTCTTA CACCTCATTCGCCACCTATCATACTCCAGCTACAGACTGTTCTGATGGCAA TTATAACAGAAATGCCAGTCTGAACTCCTTCAAGGAATACTTTAATCTCCG TAACTGCACATTCATGTACACCTATAATCACTGAGGACGAAATCTGGA GTGGTTTCGGTATCACACAGACCGCTCAGGGCGTGCACCTGTTTTCTTAC GCTACGTCGATCTCTATGGCGGAAACATGTTCCAGTTTGCCACTCTGCC GTGTACGACACAATCAAGTACTATTCCATCATTCTCATAGCATCCGGTCT ATTCAGTCAGACCGCAAGGCTTGGGCCGCTTTCTACGTGTATAAACTGCA GCCTCTCACCTTCTGCTCGATTTTTCCGTGGACGGATACATCCGCAGGG CCATTGATTGCGGTTTCAACGACCTGAGCCAGCTCCACTGTAGTTATGAA TCCTTCGATGTGGAGAGTGGTGTGTACTCCGTCAGTTCCTTTGAGGCTAA GCCCAGTGGTTCGTTGGTTCGAGCAGGCAGAAGGCGTGGAGTGCAGCTTC AGCCCTCTGCTCTCTGGCACACCCCCTCAGGTGTATAACTTCAAAGGCT GGTCTTTACCAACTGTAATTACAACCTGACTAAGCTGCTCAGCCTCTTCTC TGTGAACGACTTTACCTGCTCACAGATCAGTCCCGCAGCCATTGCTTCAA ATTGTTACAGCTCTCTGATCCTCGATTACTTCAGTTATCCTCTGTCCATGAA AAGCGACCTCTCTGTGTCAAGTGCTGGCCCAATCTCCAGTTTAATTACA AGCAGAGCTTCTCTAACCCTACCTGCCTGATTCTCGCCACTGTGCCACAC AACCTGACAACCATCACAAAGCCCCCTCAAGTACAGCTACATCAACAAGT GCTCAAGGCTGCTCAGTGACGATAGAACCGAAGTGCCACAGCTGGTCAA TGCCAACCAGTACTCCCCATGTGTGAGCATCGTCCCCTTACCCTGTGGG AAGACGGAGATTACTATAGGAAGCAGCTGTCCCCCTGGAGGGTGGAGG ATGGCTGGTGGCATCAGGTAGTACAGTCGCCATGACCGAGCAGCTCCAG ATGGGCTTCGGAATCACTGTGCAGTACGGCACTGATACAAATTCCGTCTG CCCAAAGCTGGAATTTGCTAACGACACAAAAATCGCAAGCCAGCTGGGC AATTGCGTGGAGTACTCCCTCTATGGAGTGAGCGGTTCGTGGCGTCTTCCA GAACTGTA CTGCAGTGGGAGTCAGGCAGCAGAGGTTTGTGTACGATGCT TATCAGAACCTGGTCCGCTACTATAGCGACGATGGAAATTA CTATTGCCTC CGCGCTTGTGTGTCAGTCCCCGTGAGTGT CATCTACGACAAGGAAACCA AAACTCACGCAACCCTGTTCCGGCTCTGTGGCCTGCGAGCATATTTCCAGC ACTATGTCACAGTACTCCAGGAGCACAAGAAGTATGCTGAAGAGACGTG ACTCCACATACGGACCCCTCCAGACCCCTGTGGGTTGCGTCCTGGGCCTC

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MHV S	<p>ATGCTGTTTCGTGTTTCATCCTGTTCCCTGCCAGCTGCCTGGGCTACATCGGC  GACTTCCGGTGCATCCAGCTGGTGAACAGCAACGGAGCAAACGTGAGC  GCACCAAGCATCAGCACCGAGACCGTGGAGGTGAGCCAGGGACTGGGC  ACCTACTACGTGCTGGACAGAGTGTACCTGAACGCCACCCTGCTGCTGA  CCGGCTACTACCCCGTGGACGGCAGCAAGTTCAGGAACCTGGCCCTGAC</p>

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