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**Supplementary Data**

**Cytoplasmic domain and enzymatic activity of ACE2 are not required for PI4KB dependent endocytosis entry of SARS-CoV-2 into host cells**

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**Supplementary Table S1** Primers used for DNA construct preparation.

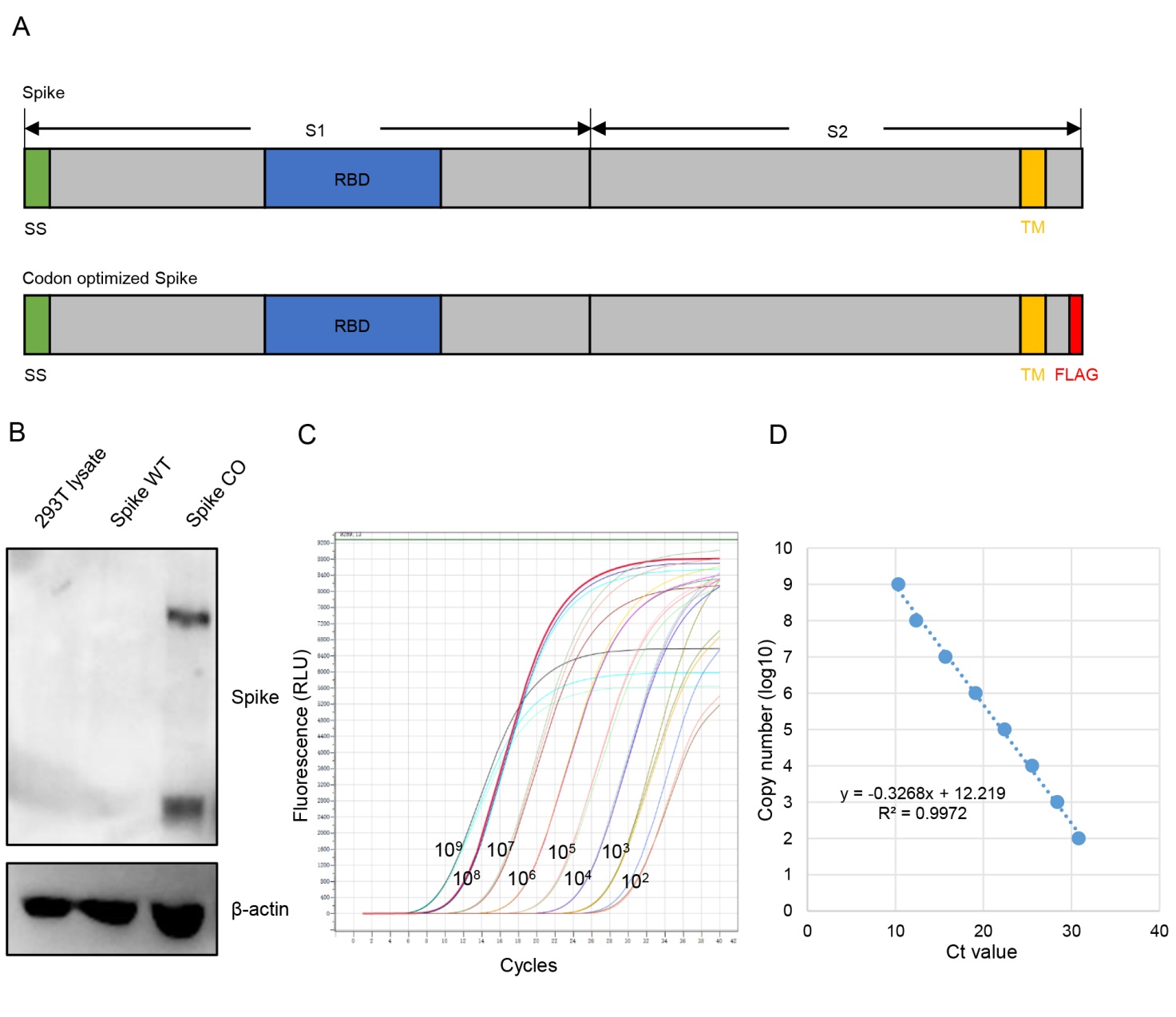
|  |  |  |  |
| --- | --- | --- | --- |
| Construct | Fragment | Forward/  Reverse | Sequence |
| ACE2 | | F | AATC ACGCGT GCCACC atgtcaagctcttcctggctc |
| R | GATTCGTACG AAAGGAGGTC TGAACATCAT CAG |
| Sac1(cytoplasmic domain) | | F | GCTA cgtacg ATGGCGACGGCGGCCTACGAG |
| R | AGCGTTAATT AATCATGGAA CACTTAAAGG ACTATG |
| INPP5E 5-ptase domain | | F | GCT GCTAGC tcggatcttgcagacta |
| R | AGCTGAATTC CTAAGAAACG GAGGCGATGG TG |
| ACE2-TM | | F | AATC ACGCGT GCCACC atgtcaagctcttcctggctc |
| R | GCATCGTACG GATCAGGATG ACAATGCCAA C |
| ACE2-ACE chimera | | F | AATC ACGCGT GCCACC atgtcaagctcttcctggctc |
| R | GGAGTGTCTCAGCTCCACCTCGGAGCCGAACTGGGGCCCG TGGGAGTGCCGGTGGAGGCTGCGGTGGCGGATGCTGAAGA GCCGCTGGAT CAGGATGACA ATGCCAAC |
| ACE2-EGFR chimera | | F | AATC ACGCGT GCCACC atgtcaagctcttcctggctc |
| R | CGATCGTACGCATGAAGAGGCCGATCCCCAGGGCCACCACCAGCAGCAAGAGGAGGGCCCCCACCATCCCAGTGGCTATG GAAACAGGGG GCTGG |
| ACE2CM1 | F1 | F | AATC ACGCGT GCCACC atgtcaagctcttcctggctc |
| R | TATCATACTGGATATTCCCCATCTCATTATGAGCTGTCAG GAAGTCGTC |
| F2 | F | CCTGACAGCTCATAATGAGATGGGGAATATCCAGTATGATA TGGCATATGC |
| R | GATTCGTACG AAAGGAGGTC TGAACATCAT CAG |
| ACE2CM2 | F1 | F | AATC ACGCGT GCCACC atgtcaagctcttcctggctc |
| R | GAATGAGTAA TCATTAGAAA CAAGGAACAG AGATGCGGGG TC |
| F2 | F | CCGCATCT CTGTTCCTTG TTTCTAATGA TTACTCATTC ATTCG |
| R | GATTCGTACG AAAGGAGGTC TGAACATCAT CAG |
| ACE2CM3 | F1 | F | AATC ACGCGT GCCACC atgtcaagctcttcctggctc |
| R | GAATGAGTAA TCATTAGAAA CAAGGAACAG AGATGCGGGG TC |
| F2 | F | CCGCATCT CTGTTCCTTG TTTCTAATGA TTACTCATTC ATTCG |
| R | GATTCGTACG AAAGGAGGTC TGAACATCAT CAG |
| Spikefur/mut | F1 | F | GTGAACGCGTgccaccatgttcgtcttcctggtcc |
| R | CCACGCTCCG AAGGATTGTC TGGGTCTGGT AAGAGGC |
| F2 | F | acccagaca ATCCTT cggagcgtggcctcccag |
| R | GTCCGTAGCACAGCAGGAGCCACAGC |
| Spikedelta | F1 | F | CATTTCAGGTGTCGTGAAC |
| R | CGAAGGGCTTCAGATTGCTCTTTCTAAACAGCCGGTACCG ATAATTGTAG TTGCCGCCC |
| F2 | F | gggacatctctacagaaatctaccaggccggcagcaAGccttgcaatggcgtggag |
| R | GCGAGAGTTT GTCTGGGTCT GG |
| F3 | F | ccagacccagacaaactctcGc agaagagcccggagcgtg |
|  | R | CGTCATCGTC TTTGTAGTCC |

**Supplementary Table S2** Primer sequences for qPCR.

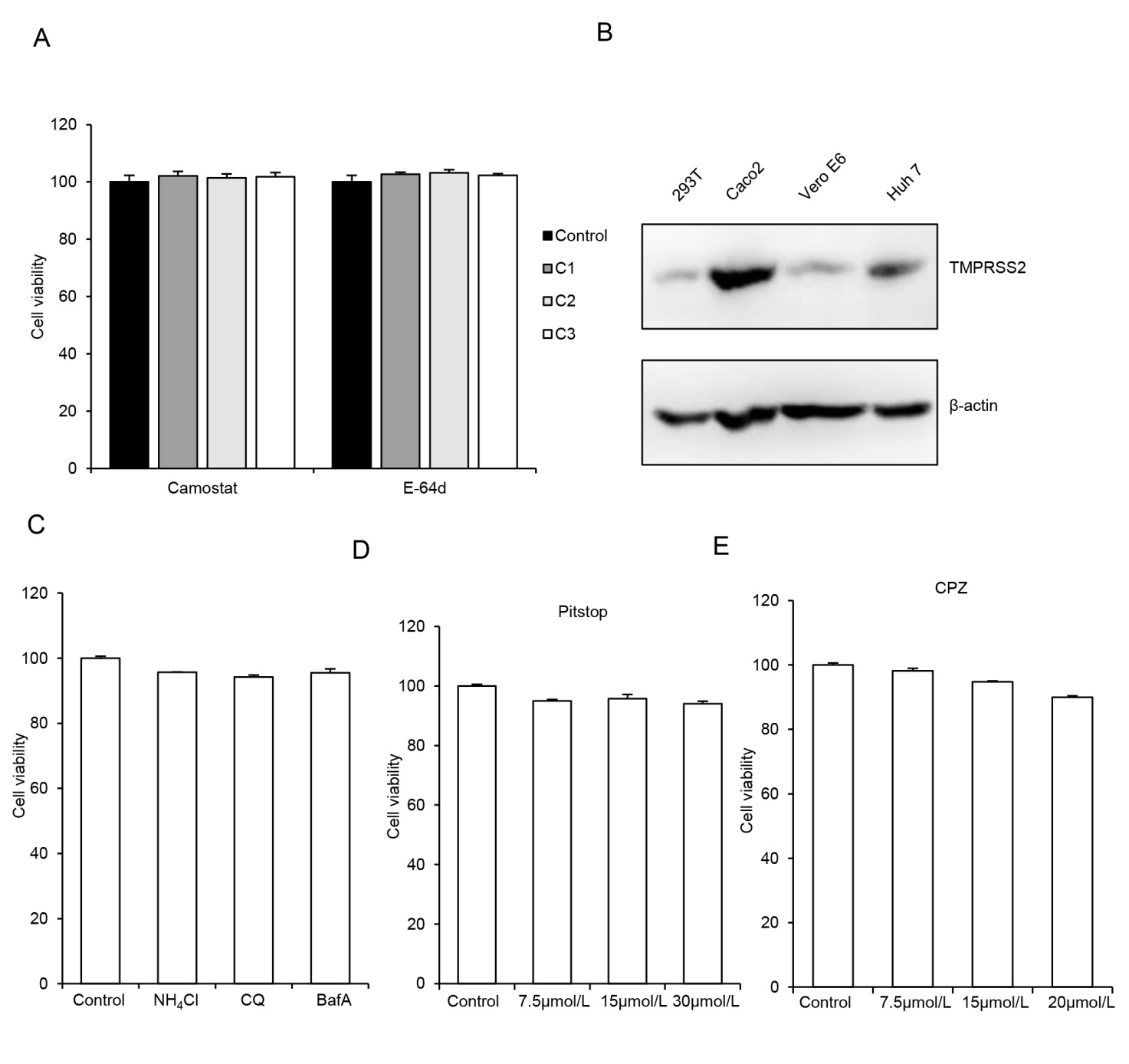
|  |  |
| --- | --- |
| Gene | Sequence |
| PI4KB-F | AACTTGCCCGAATGTGGTATT |
| PI4KB-R | GCCTATGTCATCCACCGACC |
| GAPDH-F | CTCTGCTCCTCCTGTTCGAC |
| GAPDH-R | TTAAAAGCAGCCCTGGTGAC |
| Gag-F | AGGCTGTAGACAAATACTGGG |
| Gag-R | GCACACAATAGAGGGTTGC |

**Supplementary Table S3** shRNA, sgRNA target sequences.

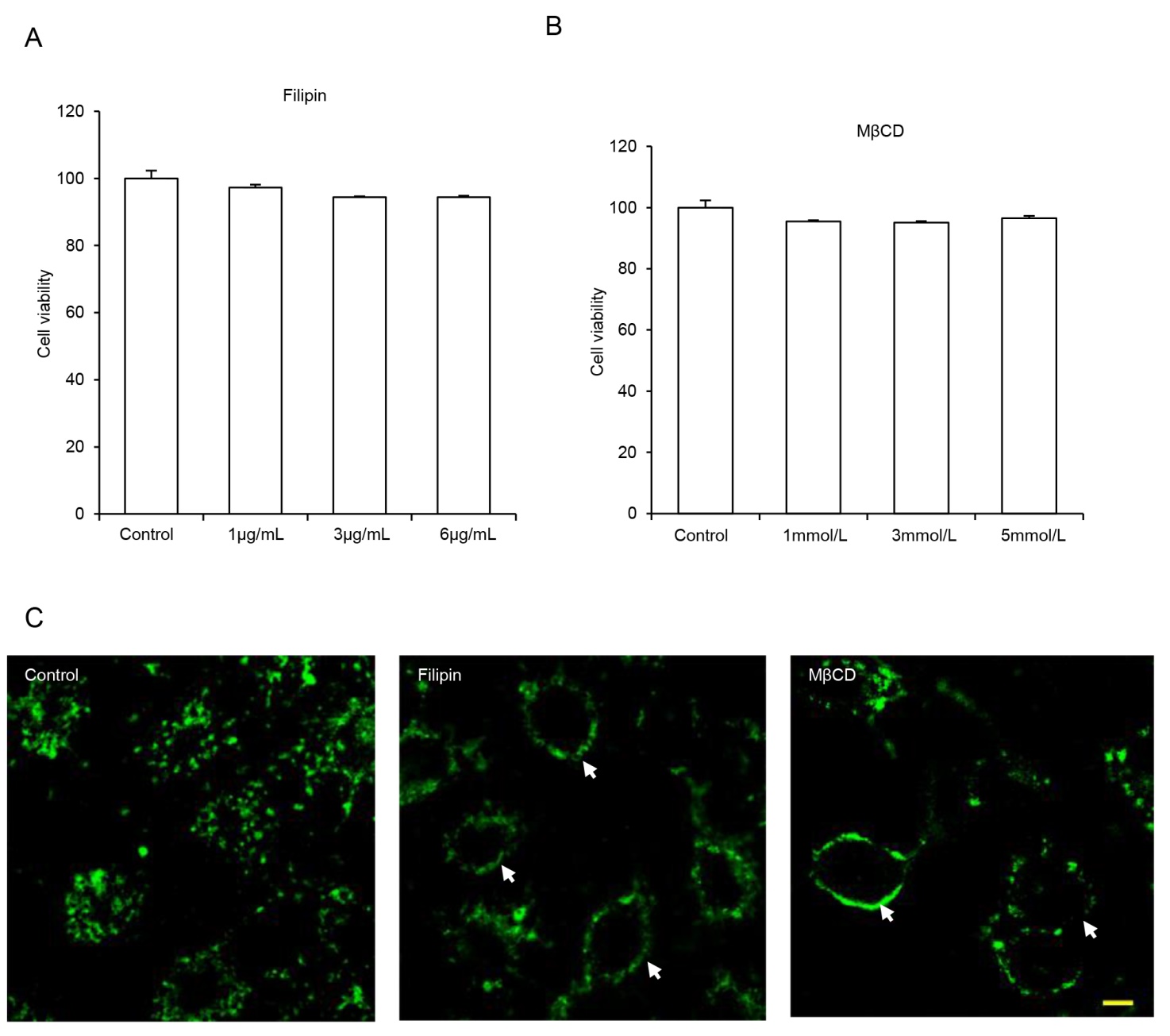
|  |  |
| --- | --- |
| Gene | Sequence |
| CHC sgRNA-1 | TGCAGCTATATTCTGTAGAT |
| CHC sgRNA -2 | ATGGCGCTGTCTGCTGAAAT |
| CHC sgRNA -3 | GAATACGGTTGCTCTTGTTA |
| CAV1 sgRNA -1 | AGTGTACGACGCGCACACCA |
| CAV1 sgRNA -2 | TAAACACCTCAACGATGACG |
| CAV1 sgRNA -3 | CCTTCACTGTGACGAAATAC |
| PI4KB shRNA-1 | GATGGCAGTATGCGGTCTATC |
| PI4KB shRNA-2 | GCAAGAAACACGAAGGATCAT |
| PI4KB shRNA-3 | TCTCGGTACTTAGGACTTGAT |

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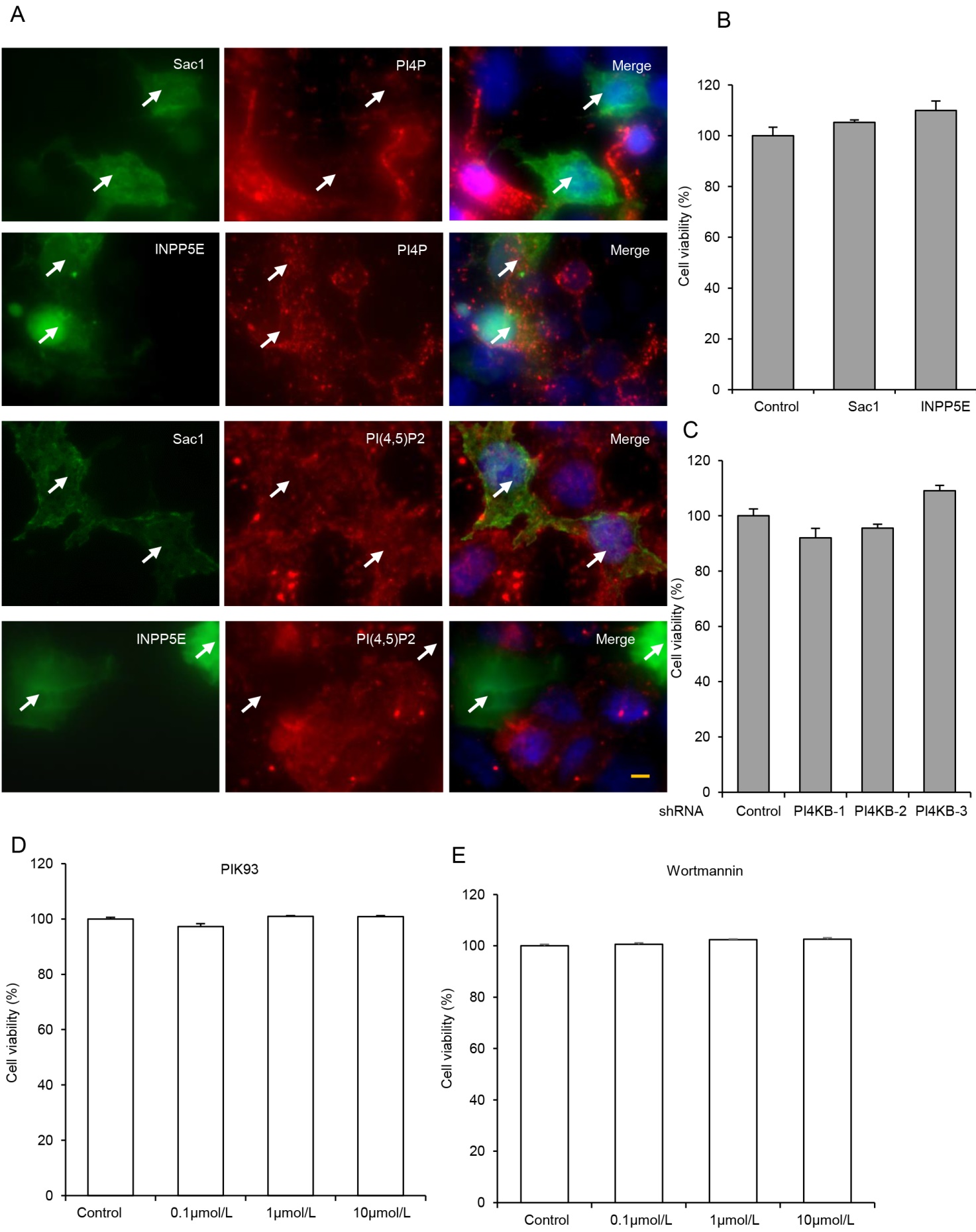
**Supplementary Fig. S1**  S pseudovirus preparation and characterization. **A** Diagram of SARS-CoV-2 Spike domains and codon optimized spike used for pseudovirus package. SS, signal sequence; RBD, receptor-binding domain; TM, transmembrane domain. **B** Wild type or codon optimized spike construct was transfected into 293T cells and S expression was determined with immunoblotting. **C** Serial dilutions of psPAX2 construct were quantitated with qPCR and amplification curves are shown. **D** Standard curve was plotted for virus titer calculation.

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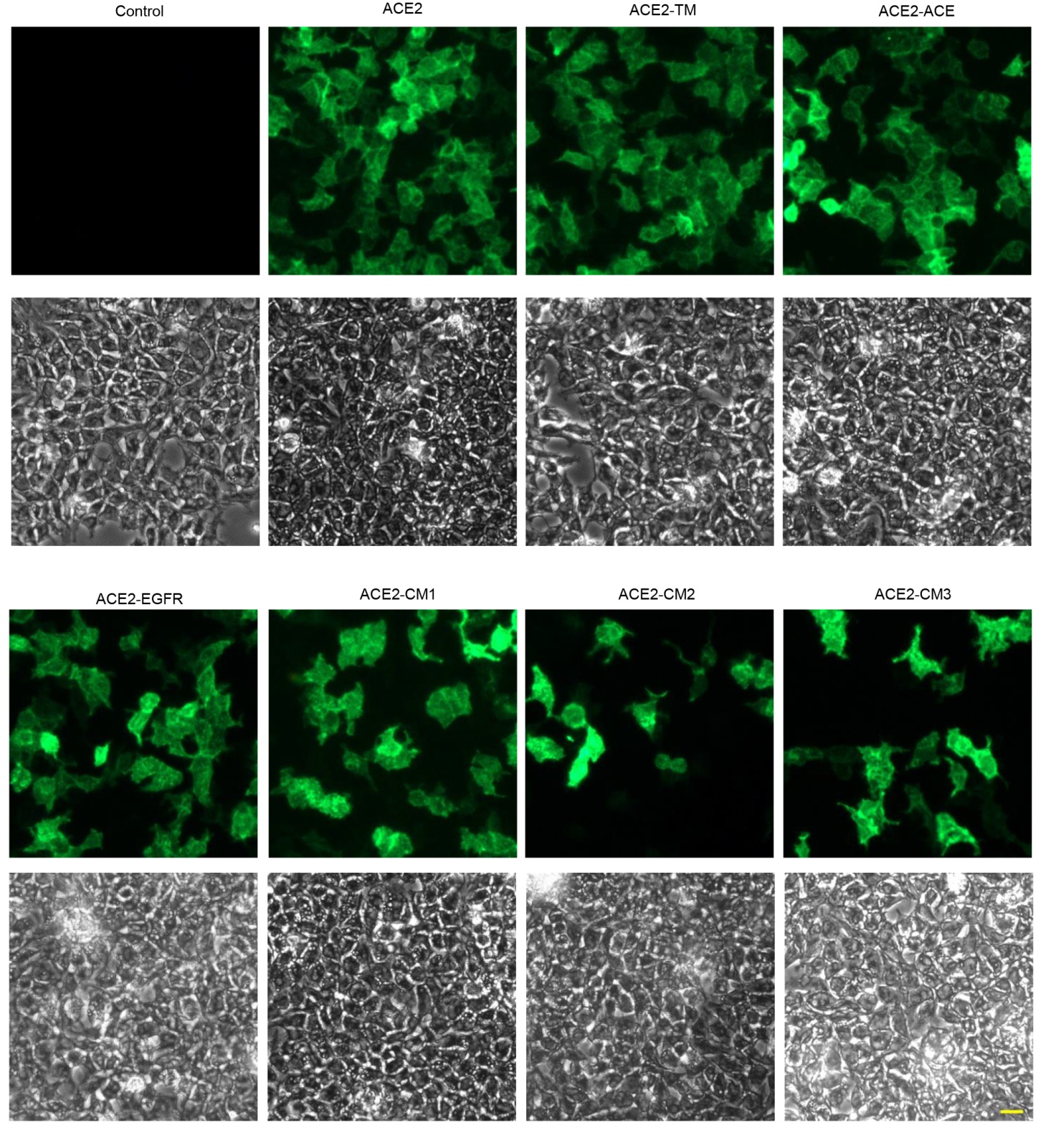
**Supplementary Fig. S2** SARS-CoV-2 enters Huh 7 cells via clathrin-mediated endocytosis **A** Huh 7 cells were mock treated or treated with 5, 15, 50 μmol/L camostat mesylate or 5, 10, 25 μmol/L E-64d for 30 h and cell viability was determined with Cell Titer Glo. Values were normalized to control and expressed as mean ± SD. **B** TMPRSS2 expression in 293T, Caco2, Vero E6 or Huh 7 cells was determined by immunoblotting. **C–E** Huh 7 cells were mock treated or treated with 50 mmol/L NH4Cl, 100 μmol/L chloroquine or 100 nmol/L bafilomycin A (**C**), or indicated amount of pitstop (**D**), or CPZ (**E**) for 30 h and cell viability was measured with Cell Titer Glo. Values were normalized to control and expressed as mean ± SD. SD, standard deviation.

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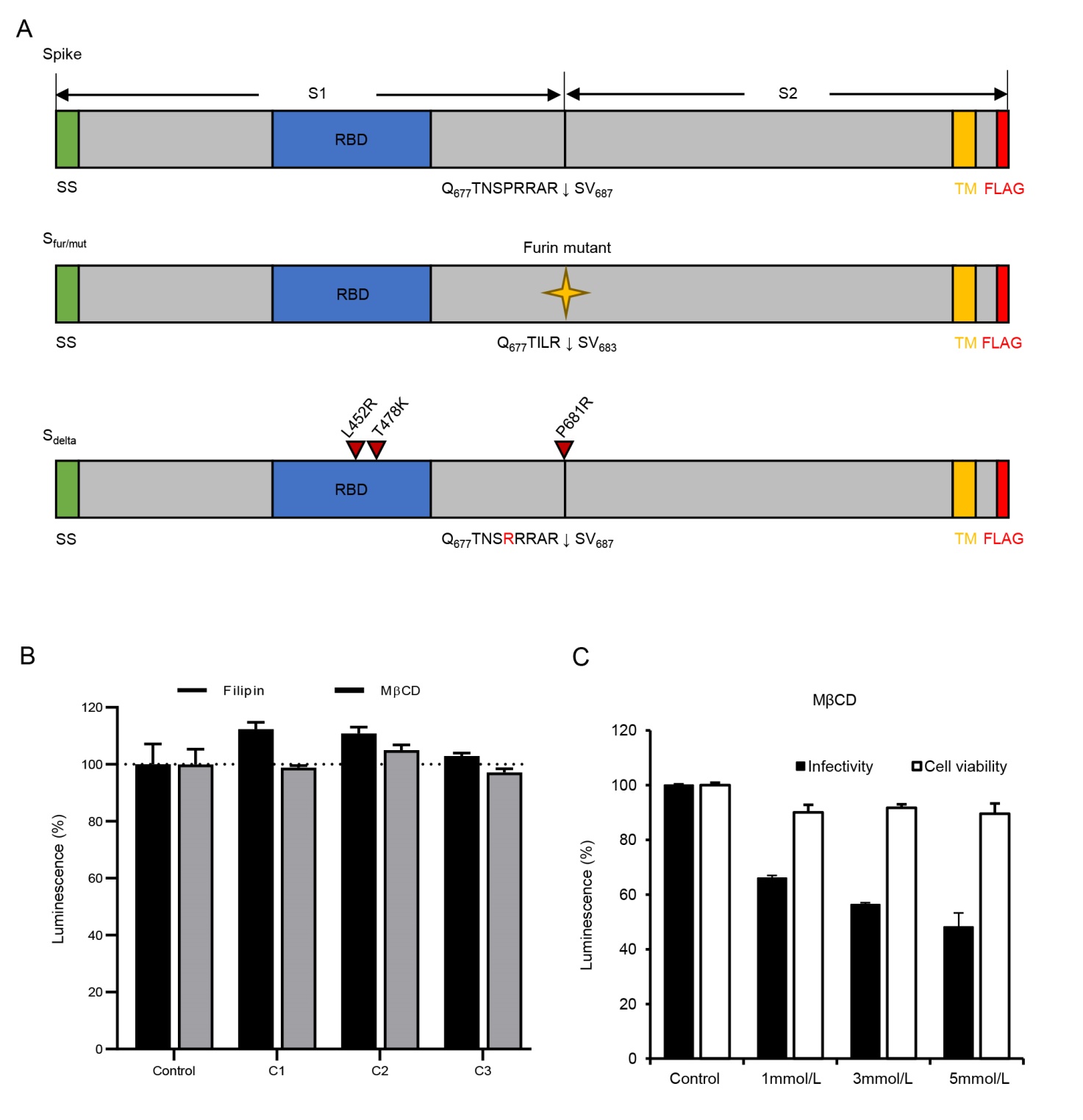
**Supplementary Fig. S3**  SARS-CoV-2 enters Huh 7 cells independent of caveolae-mediated endocytosis. **A–B** Huh 7 cells were mock treated or treated with indicated amount of filipin (**A**), or MβCD (**B**) for 30 h and cell viability was measured with Cell Titer Glo. Values were normalized to control and expressed as mean ± SD. SD, standard deviation. **C** Filipin or MβCD blocked the uptake of CTB. Huh 7 cells pretreated with 5 μg/mL filipin or 5 mmol/L MβCD were incubated with Alexa Fluor™ 488 Conjugated CTB for 1 h before images were taken. Arrows indicate blocked CTB on cell surface. Scalebar, 10 μm.

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**Supplementary Fig. S4**  PI4KB is relevant to S pseudovirus infection. **A** Effect of Sac1 and INPP5E on PI4P and PI(4,5)P2 expression. 293T-ACE2 cells transfected with HA-Sac1 or HA-INPP5E were immunolabelled with anti-HA (green) and anti-PI4P (red, upper panels) or anti-PI(4,5)P2 antibody (red, lower panels). Cell nuclei was counterstained with DAPI. Arrows indicate cells with HA expression. Scalebar, 10 μm. **B** 293T cells were mock transfected or transfected with construct expressing HA-Sac1 or HA-INPP5E, and cell viability was determined with Cell Titer Glo after 30 h. Values were normalized to control and expressed as mean ± SD. **C** Huh 7 cells stably transduced with the indicated shRNAs were tested for cell viability with Cell Titer Glo. Values were normalized to control and expressed as mean ± SD. **D–E** Huh 7 cells were mock treated or treated with indicated amount of PIK93 (**D**), or wortmannin (**E**) for 30 h and cell viability was measured with Cell Titer Glo. Values were normalized to control and expressed as mean ± SD. SD, standard deviation.

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**Supplementary Fig. S5**  ACE2 mutants express on cell surface. 293T cells were mock transfected or transfected with ACE2 or indicated mutant for 30 h before cells were fixed and ACE2 cell surface expression was determined with immunofluorescence (upper panel). Corresponding DIC images were shown below to indicate the presence of cells. Scalebar, 20 μm.

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**Supplementary Fig. S6**  Delta variant enters Huh 7 cells via clathrin-mediated endocytosis. **A** Diagram of Spike and Spike mutants. The amino acid sequences of furin cleavage site are shown for each spike protein. SS, signal sequence; RBD, receptor-binding domain; TM, transmembrane domain. **B** Huh 7 cells pretreated with 1, 3, 6 μg/mL filipin or 1, 3, 5 mmol/L MβCD were transduced with Sdelta pseudovirus and luciferase was measured. Values were normalized to control and expressed as mean ± SD. **C** 293T-ACE2 cells pretreated with 1, 3, 5 mmol/L MβCD were transduced with S pseudovirus and luciferase as well as cell viability were measured. Values were normalized to control and expressed as mean ± SD. SD, standard deviation.