## Supplementary information

## MERS-CoV and SARS-CoV-2 virus replication can be inhibited by targeting the interaction between the viral spike protein and the nucleocapsid protein

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Figure S1

Figure S2

Figure S3

Figure S4

Figure S5

Table S1







Figure S2. Expression of S, M, and N proteins in MERS-CoV-infected cells at 72 h after infection. Cell lysates were prepared from uninfected and MERS-CoV (0.1 MOI)-infected Vero cells. Cell lysates including 50  $\mu$ g (A, B, D) and 25  $\mu$ g (C) proteins were resolved by 4-12% gradient SDS-PAGE and analyzed by western blotting with the indicated antibodies. The exposure time for signal detection was 60 s (A, B, D) and 5 s (C).



**Figure S3. Interaction of SARS-CoV-2 Spike CD with N protein.** Vero cells were infected with SARS-CoV-2 (0.1 MOI) for 72 h and then cell lysates were prepared. The cell lysates were mixed with Spike CD-SARS-CoV-2 peptide, Spike CD-MERS-CoV peptide, or R-CP-1 peptide (5 μg peptide/each reaction) and then incubated for 2 h at 4°C. Anti-SARS-CoV-2 S Ab was added to each lysate and then co-immunoprecipitated proteins were collected with Protein A bead. Co-immunoprecipitated samples were analyzed by Western blotting using anti-SARS-CoV-2 S Ab (A) and anti-SARS-CoV-2 N mAb (B). SARS-CoV-2, Spike CD-SARS-CoV-2 peptide; MERS-CoV, Spike CD-MERS-CoV peptide.



Figure S4. Effects of cell-penetrating Spike CD peptides of coronaviruses on SARS-CoV-2 protein production. Vero cells were infected with SARS-CoV-2 (0.1 MOI) and then treated with PBS or 2  $\mu$ M of cell-penetrating peptides (R-Spike CD-SARS-CoV-2, R-Spike CD-MERS-CoV, or R-CP-1) at 6 h after virus infection (n = 3) in DMEM medium containing 2% FBS. The cells were cultured for 48 h and then analyzed by confocal microscopy after staining with anti-SARS-CoV-2 S Ab (A) or anti-SARS-CoV-2 N mAb (B) and then Alexa Fluor 488-conjugated secondary antibody. Scale bar, 20  $\mu$ m.



Figure S5. Effect of R-Spike CD-MERS-CoV peptide on the replication of SARS-CoV-2. Vero cells infected with SARS-CoV-2 (0.1 MOI) and then treated with PBS or 2  $\mu$ M of cell-penetrating peptides (R-Spike CD-SARS-CoV-2, R-Spike CD-MERS-CoV, or R-CP-1) at 6 h after virus infection (n = 3). Supernatants of virus-infected cell cultures were collected at 24 h after virus infection. Virus replication was quantified by qRT-PCR analysis of the SARS-CoV-2 *RdRP* gene (**A**) and plaque formation assay (**B**). \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

| Protein   | Strain   | Accession number |
|-----------|--|------------------|
| S protein |  |                  |
|           | KOR-KNIH-002/05/2015                               | AKL59401         |
|           | Human betacoronavirus 2c Jordan-N3/2012            | AHY21469         |
|           | Human betacoronavirus 2c England-Qatar/2012        | AGG22542         |
|           | Human betacoronavirus 2c EMC/2012                  | AFS88936         |
|           | Betacoronavirus England 1                          | YP_007188579     |
|           | Hypsugo bat coronavirus HKU25                      | ASL68953         |
|           | BtVs-BetaCoV/SC2013                                | AHY61337         |
|           | Erinaceus hedgehog coronavirus HKU31               | QGA70702         |
|           | Betacoronavirus Erinaceus/VMC/DEU/2012             | YP_009513010     |
|           | Coronavirus Neoromicia/PML-PHE1/RSA/2011           | AGY29650         |
|           | Betacoronavirus PREDICT/PDF-2180                   | YP 009361857     |
|           | BtPa-BetaCoV/GD2013                                | AIA62343         |
|           | Bat coronavirtus HKU5-5                            | ABN10902         |
|           | Bat coronavirtus HKU5-3                            | ABN10893         |
|           | Bat coronavirtus HKU5-2                            | ABN10884         |
|           | Bat coronavirtus HKU5-1                            | ABN10875         |
|           | Pipistrellus abramus bat coronavirus HKU5-related  | QHA24687         |
|           | Betacoronavirus BtCoV/KW2E-F93/Nyc spec/GHA/2010   | AGC51116         |
|           | Bat coronavirtus HKU4-1                            | ABN10839         |
|           | Bat coronavirtus HKU4-2                            | ABN10848         |
|           | Bat coronavirtus HKU4-3                            | ABN10857         |
|           | Bat coronavirtus HKU4-4                            | ABN10866         |
|           | BtTp-BetaCoV/GX2012                                | AIA62352         |
|           | Bat coronavirtus (BtCoV/133/2005)                  | ABG47052         |
|           | Tylonycteris pachypus bat coronavirus HKU4-related | QHA24678         |
| N protein |  |                  |
|           | KOR-KNIH-002/05/2015                               | AGN70936.1       |
|           | Human betacoronavirus 2c EMC/2012                  | AFS88943.1       |
|           | Human betacoronavirus 2c Jordna-N3/2012            | AHY21476.1       |
|           | Human betacoronavirus 2c England-Qatar/2012        | AGG22549.1       |
|           | Betacoronavirus Erinaceus/VMC/DEU/2012             | YP 007188586.1   |
|           | Coronavirus Neoromicia/PML-PHE1/RSA/2011           | AIG13103.1       |
|           | Bat coronavirus                                    | YP 009361864.1   |
|           | Bat coronavirus HKU4-2                             | ABN10855.1       |
|           | Bat coronavirus HKU4-3                             | ABN10864.1       |
|           | Bat coronavirus HKU4-1                             | ABN10846.1       |
|           | Bat coronavirus HKU4-4                             | ABN10873.1       |
|           | Bat coronavirus (BtCoV/133/2005)                   | ABG47058.1       |
|           | BtTp-BetaCoV/GX2012                                | AIA62359.1       |
|           | Tyonycteris pachypus bat coronavirus HKU4-related  | QHA24685.1       |
|           | BtPa-BetaCoV/GD2013                                | AIA62350.1       |
|           | Pipistrellus abramus bat coronavirus HKU5-related  | QHA24694.1       |
|           | Bat coronavirus HKU5-1                             | ABN10882.1       |
|           | Bat coronavirus HKU5-2                             | ABN10891.1       |
|           | Bat coronavirus HKU5-3                             | ABN10900.1       |
|           | Bat coronavirus HKU5-5                             | ABN10909.1       |

## Table S1. Accession number of MERS-CoV S and N protein amino acids used in this study

| Betacoronavirus Erinaceus/VMC/DEU/2012 | YP 009513018.1 |
|--|----------------|
| Erinaceus hedgehog coronavirus HKU31   | QGA70699.1     |
| BtVs-BetaCoV/SC2013                    | AHY61344.1     |
| Hypsugo bat coronavirus HKU25          | ASL68960.1     |