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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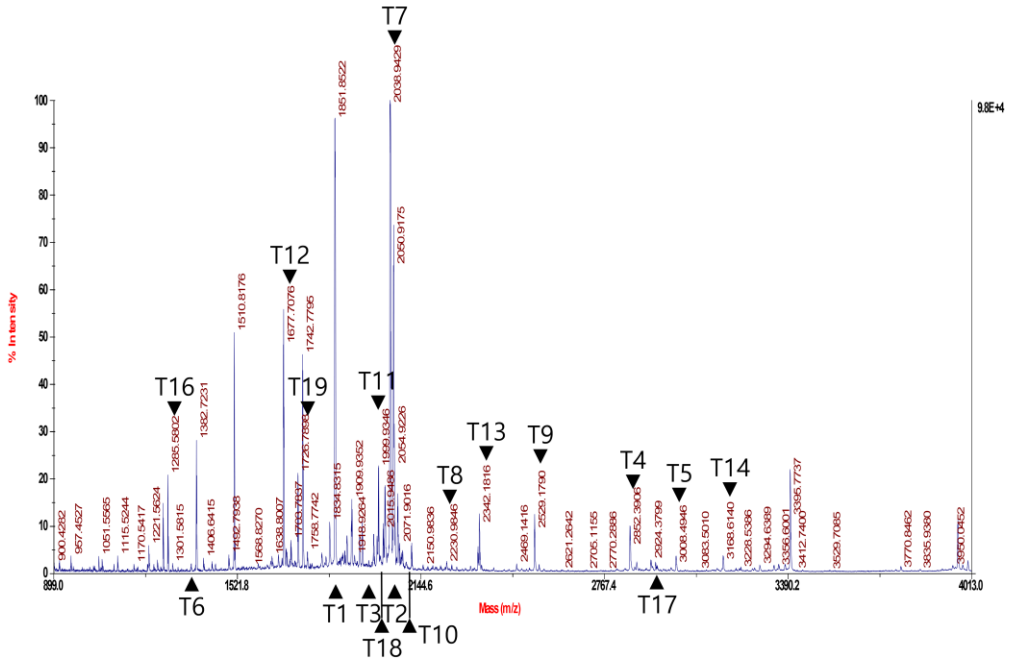

N protein [MERS-CoV]

Fragment	Region	Observed	Expected	Sequences
T1	9 - 25	1851.8967	1850.8894	R.AVSFADNNDITNTNLSR.G
T2	9 - 27	2064.9851	2063.9778	R.AVSFADNNDITNTNLSRGR.G
T3	35 - 52	1944.9475	1943.9490	R.AAPNNTVSWYTGLTQHGK.V
T4	53 - 79	2852.4336	2851.4263	K.VPLTFPPGQGVPLNANSTPAQNAGYWR.R
T5	53 - 80	3008.5459	3007.5386	K.VPLTFPPGQGVPLNANSTPAQNAGYWRR.Q
T6	85 - 97	1381.7649	1380.7576	K.INTGNGIKQLAPR.W
T7	98 - 114	2038.9905	2037.9832	R.WYFYYTGTGPEAALPFR.A
T8	98 - 117	2337.1724	2336.1651	R.WYFYYTGTGPEAALPFRAVK.D
T9	115 - 138	2529.1990	2528.1917	R.AVKDGIVWVHEDGATDAPSTFGTR.N
T10	139 - 158	2112.0432	2111.0359	R.NPNNDSAIVTQFAPGTKLPK.N
T11	156 - 174	2015.9979	2014.9906	K.LPKNFHIEGTGGNSQSSSR.A
T12	159 - 174	1677.7498	1676.7425	K.NFHIEGTGGNSQSSSR.A
T13	198 - 221	2342.2334	2341.2261	R.GTSPGPSGIGAVGGDLLYLDLLNR.L
T14	198 - 229	3168.6936	3167.6863	R.GTSPGPSGIGAVGGDLLYLDLLNRLQALESGK.V
T15	255 - 269	1686.8551	1685.8478	R.TSTKSFNMVQAFGLR.G
T16	259 - 269	1269.6205	1268.6132	K.SFNMVQAFGLR.G
T17	259 - 285	2923.4497	2922.4424	K.SFNMVQAFGLRGPGDLQGNFGDLQLNK.L
T18	396 - 413	1984.0023	1982.9950	R.TRTRPSVQPGPMIDVNTD.-
T19	398 - 413	1726.8332	1725.8260	R.TRPSVQPGPMIDVNTD.-

Figure S1. Identification of proteins binding the MERS-CoV S protein. The protein band that co-immunoprecipitated with the MERS-CoV S protein was digested in gel with trypsin. The resulting peptides were analyzed by ESI-TOF MS/MS. MS/MS analyses of the mass peaks (arrow) obtained from the ~45 kDa band revealed the peptide spectra of the MERS-CoV N protein.

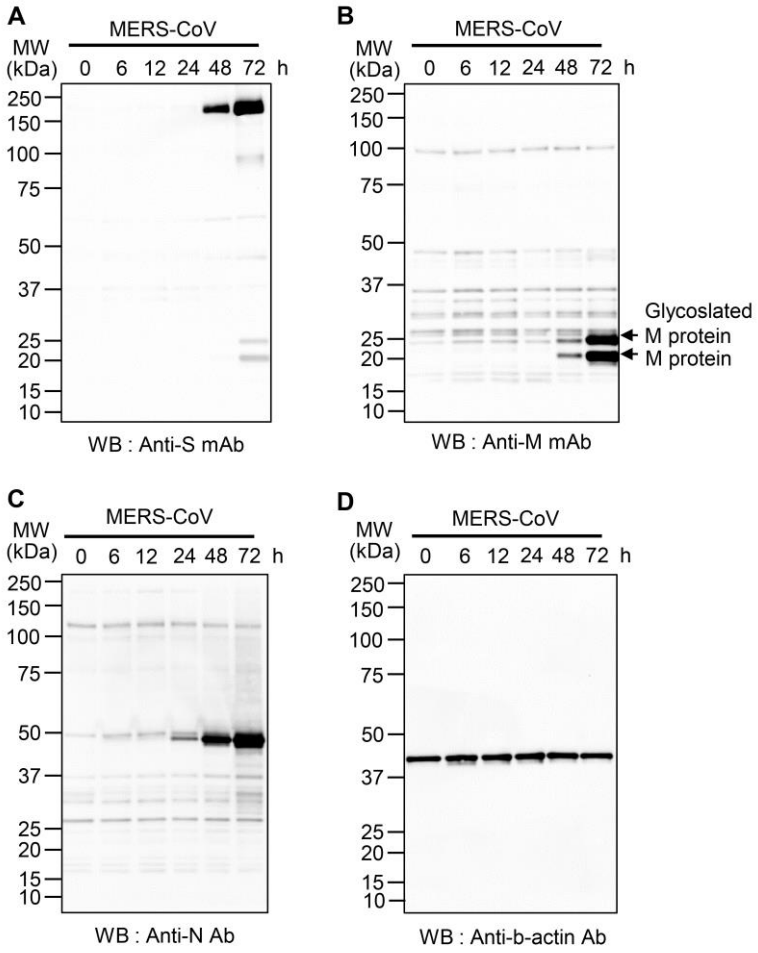

Figure S2. Expression of S, M, and N proteins in MERS-CoV-infected cells at 72 h after infection. Cell lysates were prepared from uninfected and MERS-CoV (0.1 MOI)-infected Vero cells. Cell lysates including 50 μg (**A, B, D**) and 25 μg (**C**) proteins were resolved by 4-12% gradient SDS-PAGE and analyzed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

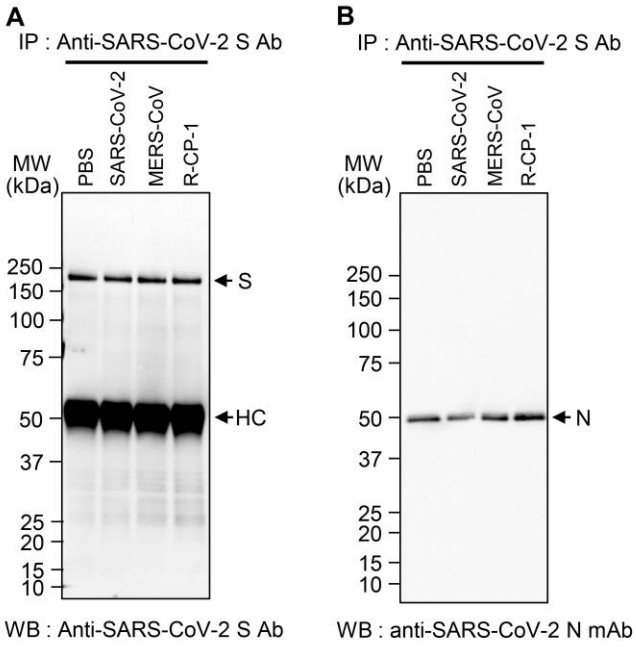

Figure S3. Interaction of SARS-CoV-2 Spike CD with N protein. Vero cells were infected with SARS-CoV-2 (0.1 MOI) for 72 h and then cell lysates were prepared. The cell lysates were mixed with Spike CD-SARS-CoV-2 peptide, Spike CD-MERS-CoV peptide, or R-CP-1 peptide (5 μg peptide/each reaction) and then 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.

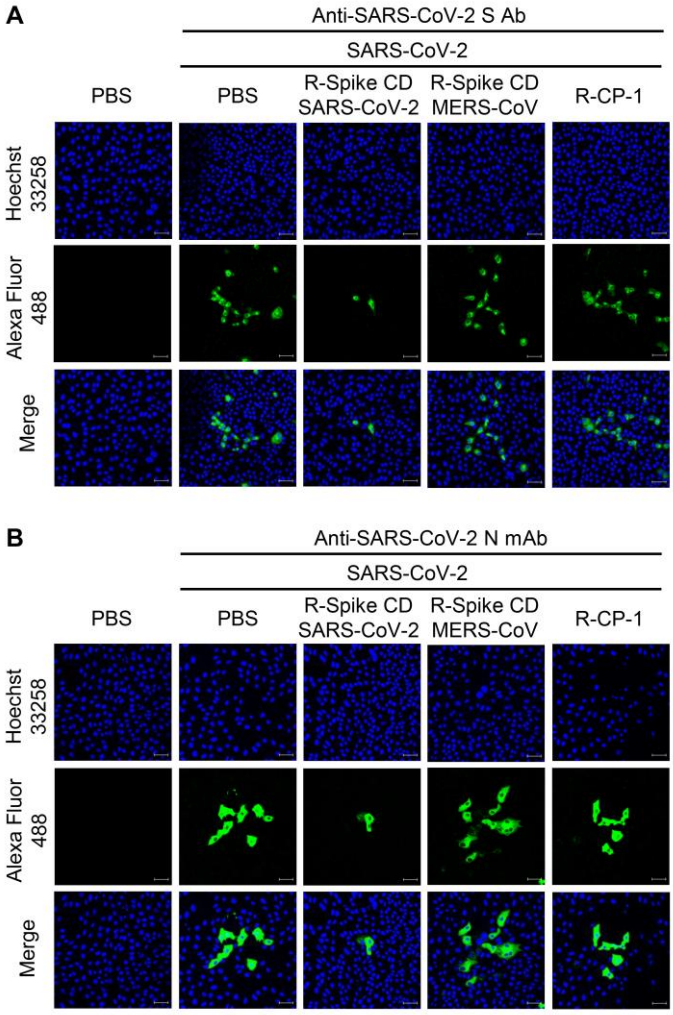

Figure S4. Effects of cell-penetrating Spike CD peptides of coronaviruses on SARS-CoV-2 protein production. Vero cells were infected with SARS-CoV-2 (0.1 MOI) and then treated with PBS or 2 μM of cell-penetrating peptides (R-Spike CD-SARS-CoV-2, R-Spike CD-MERS-CoV, or R-CP-1) at 6 h after virus infection (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 μm.

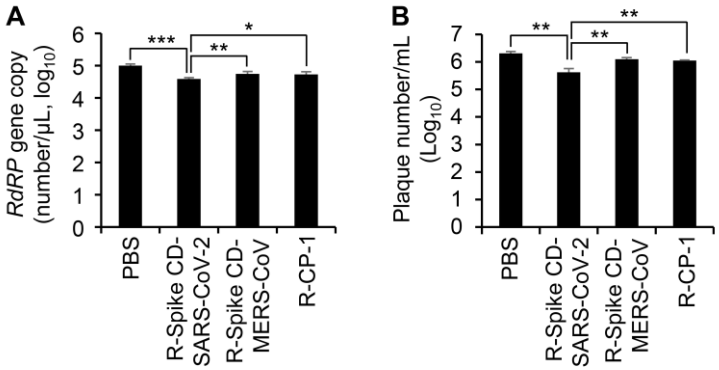

Figure S5. Effect of R-Spike CD-MERS-CoV peptide on the replication of SARS-CoV-2. Vero cells infected with SARS-CoV-2 (0.1 MOI) and then treated with PBS or 2 μM of cell-penetrating peptides (R-Spike CD-SARS-CoV-2, R-Spike CD-MERS-CoV, or R-CP-1) at 6 h after virus infection (n = 3). Supernatants of 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**). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

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

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

	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