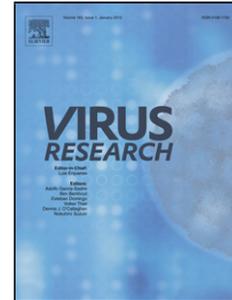


# Journal Pre-proof

Characterization of novel monoclonal antibodies against MERS-coronavirus spike protein

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PII: S0168-1702(19)30750-6  
DOI: <https://doi.org/10.1016/j.virusres.2020.197863>  
Reference: VIRUS 197863  
To appear in: *Virus Research*  
Received Date: 18 October 2019  
Revised Date: 2 January 2020  
Accepted Date: 10 January 2020

Please cite this article as: Goo J, Jeong Y, Park Y-Shin, Yang E, Jung D-Im, Rho S, Park U, Sung H, Park P-Gu, Choi J-ah, Seo SH, Cho NH, Lee H, Lee JM, Kim J-Ouk, Song M, Characterization of novel monoclonal antibodies against MERS-coronavirus spike protein, *Virus Research* (2020), doi: <https://doi.org/10.1016/j.virusres.2020.197863>

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## Characterization of novel monoclonal antibodies against MERS-coronavirus spike protein

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## Highlights

- Identification of neutralizing mAbs using MERS-CoV spike-pseudotyped virus.
- Transmembrane domain-deleted spike subunit protein induced neutralizing antibodies.
- Neutralizing antibodies could bind to RBD of MERS-CoV spike, but not vice versa.
- Mutation in residue 506-509 or 529 of S elicits neutralization escape of MERS-CoV.
- Our mAbs can be utilized for identification of specific mutation of MERS-CoV.

## Abstract

Middle East Respiratory Syndrome coronavirus (MERS-CoV) causes severe pulmonary infection, with ~35% mortality. Spike glycoprotein (S) of MERS-CoV is a key target for vaccines and therapeutics because S mediates viral entry and membrane-fusion to host cells. Here, four different S subunit proteins, receptor-binding domain (RBD; 358–606 aa), S1 (1–751 aa), S2 (752–1296 aa), and S $\Delta$ TM (1–1296 aa), were generated using the baculoviral system and immunized in mice to develop neutralizing antibodies. We developed 77 hybridomas and selected five neutralizing mAbs by immunization with S $\Delta$ TM against MERS-CoV EMC/2012 strain S-pseudotyped lentivirus. However, all five mAbs did not neutralize the pseudotyped V534A mutation. Additionally, one mAb RBD-14F8 did not show neutralizing activity against pseudoviruses with amino acid substitution of L506F or D509G (England1 strain, EMC/2012 L506F, and EMC/2012 D509G), and RBD-43E4 mAb could not neutralize the pseudotyped I529T mutation, while three other neutralizing mAbs showed broad neutralizing activity. This implies that the mutation in residue 506–509, 529, and 534 of S is critical to generate neutralization escape variants of MERS-CoV. Interestingly, all five neutralizing mAbs have binding affinity to RBD, although most mAbs generated by RBD did not have neutralizing activity. Additionally, chimeric antibodies of RBD-14F8 and RBD-43E4 with human Fc and light chain showed neutralizing effect against wild type MERS-CoV KOR/KNIH/002,

similar to the original mouse mAbs. Thus, our mAbs can be utilized for the identification of specific mutations of MERS-CoV.

**Keywords:** MERS-CoV; monoclonal antibody; neutralizing antibody; pseudovirus; neutralization; epitope

**Subject classification codes:**

Journal Pre-proof

## 1. Introduction

Middle East Respiratory Syndrome coronavirus (MERS-CoV) causes an acute and severe respiratory disease with high mortality in humans (van Boheemen et al., 2012). MERS-CoV was first identified in the Kingdom of Saudi Arabia in 2012, which is a single and positive stranded RNA virus (de Groot et al., 2013). As of 8th July 2019, 2,428 laboratory-confirmed cases of MERS worldwide, including 838 associated deaths, with a mortality rate of 34.5%, were reported. Dromedary camels are widely considered as the source of the transmission of MERS-CoV (Hemida et al., 2017). The rate of human transmission among household contacts of MERS patients has been approximately 5% based on serological analysis (Drosten et al., 2014). However, nosocomial super-spreading events occurred in South Korea in 2015 and the rapid and widespread of MERS-CoV from May to July 2015 raised strong concerns regarding the possible generation of mutations with enhanced sequential human infection (Cho et al., 2016).

The spike (S) glycoprotein of MERS-CoV is a critical viral factor for human receptor-mediated infection and is cleaved into a receptor-binding subunit S1 and a membrane-fusion subunit S2 during the infection process (Wang et al., 2013; Wang et al., 2014; Yu et al., 2015). Since the MERS outbreak in South Korea, 13 new viral genomes from 14 infected Korean patients were isolated, and 12 of these genomes were identified to possess a point mutation in the receptor-binding domain (RBD) of the S glycoprotein (Kim et al., 2016a; Kim et al., 2016b; Min et al., 2016). Specifically, 11 of these genomes showed an I529T mutation in RBD, and 1 showed a D510G mutation, which exhibits reduced affinity of RBD to its cellular receptor, human dipeptidyl peptidase 4 (DPP4; also known as CD26), compared with the wild type RBD,

suggesting that MERS-CoV adaptation during human-to-human spread may be driven to escape from neutralizing antibodies, rather than to evolve for a stronger affinity to DPP4 (Kim et al., 2016b; Park et al., 2016). Therefore, several monoclonal antibodies against different epitopes within S might be used as a prophylactic or therapeutic agent to avoid the immune escape of the virus.

Because the conformation of RBD in full-length S and its truncated versions may differ, recombinant RBD subunit protein itself may not induce neutralizing antibodies as efficiently as a larger subunit such as S1 or transmembrane deleted S (S $\Delta$ TM) (Wang et al., 2015). In this study, we produced recombinant RBD, S1, S2, and S $\Delta$ TM proteins from insect cells using baculovirus and induced neutralizing antibodies from the mice by immunization with each subunit protein. We developed monoclonal antibodies (mAbs) by hybridoma technique and several mAbs were selected and characterized for their neutralizing activity against 15 different MERS-CoV S-pseudotyped virus and wild type KOR/KNIH/002. The results of this study are expected to contribute to the development of diagnostic tools of MERS-CoV S mutation as well as for mAb-based therapeutics.

## 2. Materials and methods

### 2.1. Cells

HEK 293T/17 and 786-O cells (ATCC, Manassas, VA, USA) were grown at 37°C and 5% CO<sub>2</sub> in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Waltham, MA, USA) and 1% penicillin/streptomycin (Invitrogen). *Spodoptera frugiperda* (Sf9) cells (Thermo Fisher, Waltham, MA, USA) were cultured in Sf-900™ II SFM medium (Gibco) and 1%

penicillin/streptomycin (WELGENE, Daegu, South Korea) at 27°C, non-humidified, non-CO<sub>2</sub> incubator with 120 rpm suspension.

## 2.2. Construction of recombinant mammalian expression vectors containing MERS-CoV EMC/2012 SΔER sequence

Human codon-optimized gene encoding SΔER (1-1338 aa) of MERS-CoV EMC/2012 isolate (GenBank accession number: JX869059), mammalian codon-optimized SΔER of KOR/KNIH/002 strain (GenBank accession number: KT029139) and England1 strain (GenBank accession number: KC164505) synthesized by Genscript (NJ, USA) were cloned into the pCMV/R 8κB (Rao et al., 2008) using *SalI* and *BamHI* sites. Other mutated genes encoding substituted residues in RBD of S were generated by site-directed mutagenesis (QuikChange II XL Site-Directed Mutagenesis. Kit; Agilent Technologies, Santa Clara, CA, USA) using the EMC/2012 strain S gene as a template. The sequences of strains with mutations in MERS-CoV RBD residues were obtained from the GenBank database (Table 1, 2). All insert genes in the recombinant plasmids were verified by sequencing (Cosmogenetech, Seoul, Korea).

## 2.3. Generation of recombinant baculovirus containing MERS-CoV spike subunit protein genes

Insect cell codon-optimized gene for S of EMC/2012 strain was synthesized by Bioneer (Daejeon, Korea) and used as a PCR template. Four MERS-CoV spike subunit genes, SΔTM (1-1296 aa), S1 (1-751 aa), S2 (752-1296 aa), and RBD (358-606 aa), were PCR-amplified with primers containing *BamHI* site in the forward primer and 6× His-tag sequence and *SalI* site in the reverse primer. For S2 and RBD, the gp67 signal peptide sequence was also included in the forward primer following the *BamHI* site.

Each PCR product was cloned into the pFastBac donor vector (Bac-to-Bac™ Baculovirus Expression System, Thermo Fisher) using *Bam*HI and *Sal*I restriction sites. Infectious recombinant baculovirus was prepared as described previously (Yang et al., 2015a).

#### *2.4. Production and purification of recombinant S subunit proteins from insect cells*

For recombinant protein expression, Sf9 insect cells ( $2 \times 10^6$  cells/ml, 200 ml) were infected with the supernatants containing recombinant baculovirus and cultured for three days (27°C, 120 rpm), and then the culture media were collected and concentrated by the ammonium sulfate precipitation method (7M ammonium sulfate). The precipitate was re-dissolved in a buffer solution [20mM Tris-HCl (pH 8.0), 0.5M NaCl, 5mM imidazole], and then histidine-tagged proteins were purified using a metal affinity column with immobilized nickel as a ligand (ADAR Biotech, Rehovot, Israel). The purified protein was eluted from the column with elution buffer [20mM Tris-HCl (pH 8.0), 0.5M NaCl, 250mM imidazole], subjected to dialysis in 20mM Tris-HCl buffer at pH 8.0 containing 10% glycerol, and then concentrated to 0.25 mg/ml.

#### *2.5. Immunization and hybridoma generation*

Female Balb/c mice were purchased from Nara Biotech, Korea. The animal experiments for immunization and collecting ascites were approved by the IACUC (IACUC protocol number ATGen 2016-0113-06 and ATGen 2016-0113-07, respectively). Mice were immunized twice with a 2-week interval with 25µg MERS-CoV recombinant S subunit protein (SATM, S1, S2, and RBD) combined with 150µL Freund's complete adjuvant (Sigma, St Louis, CA, USA) via the subcutaneous route. The mice were sacrificed 3 or 4 days after the last immunization, and their splenocytes were fused with mouse

myeloma cells FO at a 5:1 ratio in the presence of PEG1500 (Roche, Basel, Switzerland). After 10 days of culture with hypoxanthine-aminopterin-thymidine medium, ELISA-positive hybridomas were subjected to limited dilutions and further selected based on pseudovirus neutralization results. For large-scale mAb production, ascites fluid from mice inoculated with the hybridomas was collected and purified using a rProtein A Agarose Resin (Amicogen, Jinju, Korea). Isotype classification of the purified mAbs was performed using the Pierce Rapid ELISA Mouse mAb Isotyping Kit (Thermo) according to the manufacturer's instructions.

#### 2.6. Production of chimeric monoclonal antibodies h14F8 and h43E4

The chimeric monoclonal antibodies for RBD-14F8 and RBD-43E4 were produced as described previously (Dang et al., 2013). Briefly, RNA was extracted from hybridoma cells and the first-strand cDNA was produced. The  $V_H$  and  $V_L$  regions were amplified using primers restricted with *Sfi*I and *Nhe*I or *Bgl*II and cloned into the expression vector to produce chimeric IgG. The sequences of the primers are as follows: 14F8-HC-sfiF, 5'-TTGGTGGCCACAGCGGCCGATGTCCACTCGgatgtgaagcttcaggagtcggg-3'; 14F8-HC-NheR, 5'-GAGGAGGCTAGCtgcagagacagtgaccagagtcc-3'; 14F8-LC-sfiF, 5'-TTGGTGGCCACAGCGGCCGATGTCCACTCGgacattgtgatgaccagcttccatc-3'; 14F8-LC-BglIIR, 5'-GAGGAGAGATCTatttattccagcttggtccccctccg-3'; 43E4-HC-sfiF, 5'-TTGGTGGCCACAGCGGCCGATGTCCACTCGgaggtgcagctggaggagtcag-3'; 43E4-HC-NheR, 5'-GAGGAGGCTAGCtgcagagacagtgaccagagtcc-3'; 43E4-LC-sfiF, 5'-TTGGTGGCCACAGCGGCCGATGTCCACTCGgatatccagatgacacagatctccat-3'; 43E4-LC-BglIIR, 5'-GAGGAGAGATCTtttgattccagcttggtgctcca-3'. Chimeric antibody for RBD-14F8 was named h14F8 possessing human IgG1 Fc and human lambda1 light chain. Chimeric antibody for RBD-43E4 was named h43E4 with human IgG1 Fc and

human kappa 1 light chain. Each prepared DNA plasmid was transiently transfected into HEK 293-FT cells (Invitrogen) using polyethyleneimine, and cell culture supernatant (Freestyle 293 Expression media, Thermo Fisher Scientific) was harvested after 7 days. Antibody was purified using Poly-Prep chromatography column (Bio-Rad, Hercules, CA, USA) packed with Protein A. Purified h14F8 (5.37 mg) and h43E4 (2.9 mg) were obtained.

### 2.7. *Pseudovirus production*

Lentivirus based MERS-CoV S-pseudotyped particles were generated. HEK 293T/17 cells ( $4 \times 10^6$ ) were seeded in DMEM without antibiotics into 100-mm Petri dishes at a ratio that will yield 70–90% confluence at the time of transfection. Lentiviral pseudovirions expressing MERS-CoV S protein were produced by co-transfection of HEK 293T cells with three plasmids: 7 $\mu$ g of pHR'CMV-Luc, 7 $\mu$ g of pCMV  $\Delta$ R8.2 (Naldini et al., 1996), and 100ng of pCMV/R 8 $\kappa$ B-S encoding plasmids using Lipofectamine 2000 (Invitrogen). The medium was replaced after overnight incubation. Supernatants containing the viral pseudotyped particles were harvested 48–72 h after transfection, filtered using a 0.45- $\mu$ m filter syringe and stored at -80°C as aliquots (Kim et al., 2007). Titration of pseudovirus was performed using Lentivirus-Associated p24 ELISA Kit (Cell Biolabs, San Diego, CA, USA).

### 2.8. *Pseudovirus neutralization assay*

The 786-O cells were plated at a density of  $1 \times 10^4$  cells/well in 96-well plate with complete DMEM (10% FBS, 1% penicillin/streptomycin) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> the day before pseudo-viral infection until 95% confluence was achieved. After incubation of 50 $\mu$ L of  $2 \times 10^6$  MERS-CoV S-pseudotyped particles and

20 $\mu$ L of serially diluted mouse immune serum or neutralizing antibody as positive control (Sino Biological Inc., Beijing, China) for 1 h at room temperature, the mixtures were added to the cells and wells were replaced with 100 $\mu$ L of fresh media after 6 h of incubation. Cells were lysed after 72 h with 20 $\mu$ L of lysis buffer (Promega, Promega, Madison, WI, USA) and transferred to Opaque plate (Perkin-Elmer, Waltham, MA, USA) (Kim et al., 2007). Luciferase activity was measured by adding 40 $\mu$ L of the substrate (Promega) using SpectraMax L Microplate Reader (Molecular Devices, San Jose, CA, USA).

### *2.9. Plaque reduction neutralization test (PRNT) of wild type MERS-CoV*

MERS-CoV (0.004 m.o.i. in 24 well) KOR/KNIH/002, provided by the Korean Centers for Disease Control (Yang et al., 2015b), was pre-incubated with each mAb and then added to Vero cells. After 1 h of incubation, cells were overlaid with a medium containing 1% Sea-plaque agarose (Lonza, Basel, Switzerland). At 48 h after MERS-CoV infection, cells were fixed and stained with anti-MERS-CoV N protein antibody (Sino Biological Inc.) to count the viral plaques.

### *2.10. Enzyme-linked immunosorbent assay (ELISA)*

RBD-, S1-, S2-, and S $\Delta$ TM-specific antibody in the hybridoma culture supernatant was measured by ELISA as described previously (Kim et al., 2015; Shere et al., 1997).

### *2.11. Statistical analysis*

Analyses were performed using Prism 5 (GraphPad, San Diego, CA) or Excel program (Microsoft office). Differences between groups were analyzed by one-way analysis of

variance (ANOVA) followed by Tukey's test. *P* values of <0.05 were considered statistically significant.

### 3. Results

#### 3.1. MERS-CoV S-specific antibody generation from mice immunized with recombinant S subunit proteins

To develop neutralizing mAbs with different epitopes, several S subunit proteins were designed as antigen. The MERS-CoV S glycoprotein consists of a globular S1 domain at the N-terminal region containing the RBD that is responsible for binding to the host cellular receptor DPP4, followed by membrane-proximal S2 domain and a transmembrane domain (Du et al., 2013) (Fig. 1A). Recombinant proteins of S $\Delta$ TM (1–1296 aa), S1 (1–751 aa), S2 (752–1296 aa), and RBD (358–606 aa) corresponding to the amino acid sequence of MERS-CoV EMC/2012 strain were produced from the Sf9 insect cells using baculovirus expression system (Fig. 1B). These proteins were immunized into Balb/c mice, and the hybridoma fusion was performed using spleen cells. Then, the culture supernatant of hybridoma cells was subjected to ELISA to assess whether they secrete the antibody that can bind to S subunit proteins (data not shown). A total of 77 hybridomas secreted antibodies binding to S subunit proteins; 25 from S $\Delta$ TM, 29 from S1, 11 from RBD, and 12 mAbs from S2.

#### 3.2. Identification of neutralizing mAbs against MERS-CoV S using pseudovirus system

MERS-CoV S-pseudotyped lentivirus was produced to evaluate the neutralizing activity of antibodies secreted by hybridoma cells. Pseudovirus expressing the MERS-CoV spike protein was generated by co-transfection of the plasmids of HIV-1 Gag/pol, luciferase-expressing HIV-1, and S into HEK 293T cells. We used S genes without an

endoplasmic reticulum signal (*SΔER*) (Kim et al., 2016b). *SΔER* gene of EMC/2012, England 1, and KOR/KNIH/002 strains were cloned (Table 1) and the other 13 RBD genes of naturally occurring strains of MERS-CoV were cloned based on the EMC/2012 strain *S* gene, except for the RBD region (Table 2).

Next, the neutralizing activity of a panel of ELISA-positive 77 hybridomas was evaluated against MERS-CoV EMC/2012 strain *S*-pseudotyped virions. The inhibition of the pseudovirus infection by antibodies was quantified by luciferase activity in pseudovirus-infected cells (Fig. 2A). Selected clones were further tested against *S*-pseudotyped KOR/KNIH/002 strain (Fig. 2B), and seven clones were selected: six clones (6, 23, 25, 40, 43, and 14) and one clone (14S2) generated by  $S\Delta TM$  and S2 immunization, respectively. Among these clones, 6 ( $S\Delta TM$ ) did not neutralize both EMC/2012 and KOR/KNIH-002 strains, and 14 (S2) inhibited the entry of both pseudotyped strains to the target 786O cells with approximately 50% activity. All these seven clones were further sub-cloned and finally, mAbs S1-6E6, RBD-14F8, RBD-23D3, RBD-25E4, RBD-40G7, RBD-43E4 by  $S\Delta TM$ , and S2-14H8 by S2 were purified and characterized for IgG subclass and light chain type (Table 3). Upon examination of the binding domain of the  $S\Delta TM$ -generated mAbs, S1-6E6 bound to non-RBD S1 and the other neutralizing mAbs showed affinity to RBD (Fig. 2C). Taken together, several neutralizing mAbs were generated by  $S\Delta TM$  immunization, although the antibodies developed by RBD immunization did not induce a neutralization effect under our experimental condition (Fig. 2A).

### 3.3. Neutralizing effect of mAbs against various MERS-CoV *S* pseudoviruses

To evaluate whether the mAbs have cross-protective capability against various MERS-CoV strains, we further examined neutralizing activity against our MERS-CoV

S pseudovirus, in which the S gene contained different RBD region of England1/England-Qatar (L506F), Qatar3 (S460F), Riyadh1/Bisha1 (D509G), Riyadh2 (V534A), Al-Hasa8c (N582I), Riyadh9 (A431P and A482V), Asir2f (A434V), Riyadh345/Riyadh59 (T424I), Jeddah\_C9313 (Q522H), KOR/CNUH\_SNU/122 (D510G), KOR/CNUH\_SNU/016\_06 (V529T), or KOR/KNIH/002 (V530L) strains (Table 2). Of these, the EMC/2012-V534A (Riyadh2) variant exhibited neutralization resistance to our mAbs (data not shown).

Among five mAbs that could bind to RBD, three mAbs (RBD-23D3, RBD-25E4, and RBD-40G7) showed broad neutralizing activity against 14 different S-pseudotyped viruses (Fig. 3 and Fig. 4). However, RBD-14F8 mAb did not neutralize three pseudoviruses possessing amino acid substitution of L506F or D509G (*SΔER* of England1 and *RBD* gene of England1/ England-Qatar and Riyadh1/Bisha1 strain based on EMC/2012 *SΔER* gene), implying that 506–509 residue mutation of EMC/2012 S can elicit neutralization escape from RBD-14F8 (Fig. 3). RBD-43E4 mAb did not neutralize KOR/CNUH\_SNU/016\_06 RBD (I529T) pseudotype (Fig. 4). As expected, S1-6E6 that bound to the non-RBD region of S1 did not show any neutralizing activity even at the highest antibody dose of 20 ng. S2-14H8 mAb, which showed binding affinity to S2, showed partial neutralizing effect compared with other RBD-binding neutralizing mAbs (Fig. 3 and Fig. 4).

#### *3.4. Neutralizing effect of humanized RBD-14F8 and RBD-43E4 against pseudovirus and wild type MERS-CoV*

To make mAbs available for human clinical use or standard of human sample analysis, RBD-14F8 and RBD-43E4 were genetically engineered to increase their similarity to antibody variants produced naturally in humans; their Fc region was

replaced by human IgG1 Fc, and the light chain was replaced with human lambda1 and kappa1 chain (Table 4). The humanized antibody for 14F8 and 43E4 was produced using 293F cells by transfection of cloned antibody DNA plasmids and purified with Protein A column (Fig. 5A). These chimeric mAbs neutralized the pseudoviruses with S of EMC/2012, KOR/KNIH/002, and V530L variant of EMC/2012. However, h14F8 showed lower neutralization activity against pseudotyped D509G, D510G, and especially L506F variant, and h43E4 did not neutralize the pseudotyped I529T variant at all (Fig. 5B), which were also observed in mouse mAb RBD-14F8 and RBD-43E4, respectively.

Neutralizing activity of both mouse and chimeric mAbs of RBD-14F8 and RBD-43E4 were verified by PRNT assay against wild type MERS-CoV KOR/KNIH/002 strain (Fig. 5C). As controls, S2-6E6 and RBD-23D3 were included in PRNT. S1-6E6 did not neutralize, but RBD-23D3 neutralized the wild type strain in a dose-dependent manner, as in previous pseudovirus neutralization. Collectively, chimeric RBD-14F8 and RBD-43E4 mAb showed similar neutralization effect as the original mouse mAb RBD-14F8 and RBD-43E4, respectively, suggesting that these chimeric mAbs can be used to distinguish mutations in specific residue 509 or 529 of MERS-CoV clinical isolates.

#### **4. Discussion**

In this study, we aimed to develop mAbs specific to MERS-CoV to utilize them as therapeutics or diagnostic tools of variant MERS-CoV strains. MERS-CoV is a single, positive stranded RNA virus of approximately 30kb, which encodes four major viral structural proteins, including S, envelope, membrane, and nucleocapsid as well as several accessory proteins. Since MERS-CoV S mediates viral attachment and fusion to

human cells via human cellular receptor DPP4, also known as CD26, we considered S as a target for mAb development including neutralizing antibodies.

We generated mAbs by mouse hybridoma technique using recombinant S subunit proteins as antigens. We selected the 7 mAbs from 77 hybridomas based on the specific binding ability to each S subunit and characterized their neutralizing activity against diverse MERS-CoV S pseudoviruses: six mAbs were developed by  $\Delta$ TM showing specific binding affinity to non-RBD S1, and RBD; the other one mAb was produced by S2 subunit protein immunization. Polyclonal serum developed by S1 showed high neutralizing activity against both EMC/2012 and KOR/KNIH/002 S pseudovirions, which was comparable to  $\Delta$ TM immunization (data not shown). However, we failed to obtain potent neutralizing mAbs from S1-immunized mice under our experimental condition. Our RBD-specific mAbs, RBD-14F8, -23D3, -25E4, -40G7, and -43E4, showed higher neutralizing potency than other non-RBD-binding mAbs (S1-6E6 for non-RBD S1, and S2-14H8 for S2), which is consistent with the fact that most reported neutralizing mAbs are RBD-specific (Pascal et al., 2015; Wang et al., 2018; Ying et al., 2014). The mAbs developed by other groups, CDC-C2, m336, and REGN3051, also bind to RBD and potently neutralize MERS-CoV (de Wit et al., 2018; Pascal et al., 2015; Wang et al., 2018; Ying et al., 2014). Meanwhile, it has also been reported that non-RBD S1-specific mAbs, G2 and 5F9, and S2-specific mAb G4 neutralized MERS-CoV (Chen et al., 2017; Pallesen et al., 2017; Wang et al., 2015; Zhang et al., 2018).

In our study, the RBD subunit protein itself could not induce sufficient neutralizing activity, unlike  $\Delta$ TM protein, which suggests that the design of antigen to possess an appropriate structural conformation is crucial for developing vaccines and therapeutic neutralizing antibodies. The baculoviral expression system produced all

recombinant S subunit proteins in this study. Hence, the baculovirus-expressed SATM can be considered as a promising MERS-CoV vaccine antigen given its ability to induce potent neutralizing antibodies, although it does not have trimerization tag for pre-fusion conformation (Pallesen et al., 2017) and may have different glycosylation patterns as compared to mammalian-expressed S proteins (Li et al., 2016).

Our mAbs RBD-23D3, RBD-25E4, and RBD-40G7 targeting RBD showed high cross-neutralizing activities against 14 different MERS-CoV S pseudoviruses. In contrast, the transduction of pseudovirions with the change of V534A in RBD of EMC/2012 was not inhibited by any of our mAbs. Interestingly, two mAbs, RBD-14F8 and RBD-43E4, did not neutralize another pseudovirion with specific mutation in RBD region: RBD-14F8 could not neutralize the pseudovirions with changes in the amino acid residue at 506, 510, and particularly at 509 site (D509G); meanwhile, RBD-43E4 did not neutralize pseudovirions with the mutation at 529 amino acid residue (I529T) compared with the EMC/2012 strain S sequence. A single amino acid change in the RBD region was identified in 2015 Korea outbreak patients and it was reported that the mutation of either D510G or I529T reduces the viral affinity to human cellular receptor DPP4 (Kim et al., 2016b). In our experiments, lower luciferase activity was also observed in 786O target cells after pseudovirus infection with D510G or I529T mutation. The mAb CDC-C2 developed by US NIH possessed binding affinity to RBD and lost neutralizing effect against pseudovirions with mutations at 509, 512, 534, 536, 539, 540, or 542 amino acid residues from EMC/2012 strain S amino acid sequence (Wang et al., 2018). The common amino acid that influences the loss of neutralization between CDC-C2 and our RBD-14F8 mAbs is the 509 residue. S2-binding S2-14H8 showed partial neutralizing activity against all tested pseudoviruses, and non-RBD S1-binding S1-6E6 did not hinder cell entry of the entire tested pseudoviruses at all. More

structural data are needed to determine precise epitopes for each neutralizing mAb and the mechanisms by which combining the mAbs might delay viral escape.

Wild type MERS-CoV KOR/KNIH/002 strain also showed susceptibility to RBD-23D3, -14F8, and -43E4 mAbs, regardless of its origin (either mouse or Fc-/light chain-humanized chimeric antibody). Humanized mAb may be utilized as caliber to measure human immune responses or therapeutic purposes in humans. However, symptoms often appear after the peak of viremia; combined neutralizing mAbs therapy would have to be used before the onset of symptoms to be early enough to avoid viremia. In addition, mAbs targeting non-RBD epitopes of MERS-CoV S may have potential clinical applications in the prevention and/or treatment of disease. With animal models that reflect human MERS-CoV infection, the protective effect of these mAbs may be better assessed.

In conclusion, several mAbs developed by murine hybridoma technique may be utilized as MERS-CoV diagnostics distinguishing S 509 and 529 escape mutant strains or combined therapeutic antibodies with humanized forms.

#### **Author statement**

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### **Disclosure of potential conflicts of interest**

The authors declare they have no potential conflicts of interest to disclose.

### **Funding**

This work was supported by a grant from the Korea Ministry of Health and Welfare (HI15C2971).

## References

- Chen, Y., Lu, S., Jia, H., Deng, Y., Zhou, J., Huang, B., Yu, Y., Lan, J., Wang, W., Lou, Y., Qin, K., Tan, W., 2017. A novel neutralizing monoclonal antibody targeting the N-terminal domain of the MERS-CoV spike protein. *Emerg Microbes Infect* 6(6), e60.
- Cho, S.Y., Kang, J.M., Ha, Y.E., Park, G.E., Lee, J.Y., Ko, J.H., Kim, J.M., Kang, C.I., Jo, I.J., Ryu, J.G., Choi, J.R., Kim, S., Huh, H.J., Ki, C.S., Kang, E.S., Peck, K.R., Dhong, H.J., Song, J.H., Chung, D.R., Kim, Y.J., 2016. MERS-CoV outbreak following a single patient exposure in an emergency room in South Korea: an epidemiological outbreak study. *Lancet* 388(10048), 994-1001.
- Dang, V.T., Mandakhalikar, K.D., Ng, O.W., Tan, Y.J., 2013. A simple methodology for conversion of mouse monoclonal antibody to human-mouse chimeric form. *Clin Dev Immunol* 2013, 716961.
- de Groot, R.J., Baker, S.C., Baric, R.S., Brown, C.S., Drosten, C., Enjuanes, L., Fouchier, R.A., Galiano, M., Gorbalenya, A.E., Memish, Z.A., Perlman, S., Poon, L.L., Snijder, E.J., Stephens, G.M., Woo, P.C., Zaki, A.M., Zambon, M., Ziebuhr, J., 2013. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *J Virol* 87(14), 7790-7792.
- de Wit, E., Feldmann, F., Okumura, A., Horne, E., Haddock, E., Saturday, G., Scott, D., Erlandson, K.J., Stahl, N., Lipsich, L., Kyratsous, C.A., Feldmann, H., 2018. Prophylactic and therapeutic efficacy of mAb treatment against MERS-CoV in common marmosets. *Antiviral Res* 156, 64-71.
- Drosten, C., Meyer, B., Muller, M.A., Corman, V.M., Al-Masri, M., Hossain, R., Madani, H., Sieberg, A., Bosch, B.J., Lattwein, E., Alhakeem, R.F., Assiri, A.M., Hajomar, W., Albarrak, A.M., Al-Tawfiq, J.A., Zumla, A.I., Memish, Z.A., 2014. Transmission of MERS-coronavirus in household contacts. *N Engl J Med* 371(9), 828-835.
- Du, L., Zhao, G., Kou, Z., Ma, C., Sun, S., Poon, V.K., Lu, L., Wang, L., Debnath, A.K., Zheng, B.J., Zhou, Y., Jiang, S., 2013. Identification of a receptor-binding domain in the S protein of the novel human coronavirus Middle East respiratory syndrome coronavirus as an essential target for vaccine development. *J Virol* 87(17), 9939-9942.
- Hemida, M.G., Elmoslemay, A., Al-Hizab, F., Alnaeem, A., Almathen, F., Faye, B., Chu, D.K., Perera, R.A., Peiris, M., 2017. Dromedary Camels and the Transmission of Middle East Respiratory Syndrome Coronavirus (MERS-CoV). *Transbound Emerg Dis* 64(2), 344-353.
- Kim, D.W., Kim, Y.J., Park, S.H., Yun, M.R., Yang, J.S., Kang, H.J., Han, Y.W., Lee, H.S., Kim, H.M., Kim, H., Kim, A.R., Heo, D.R., Kim, S.J., Jeon, J.H., Park, D., Kim, J.A., Cheong, H.M., Nam, J.G., Kim, K., Kim, S.S., 2016a. Variations in Spike Glycoprotein Gene of MERS-CoV, South Korea, 2015. *Emerg Infect Dis* 22(1), 100-104.

- Kim, J.-O., Rho, S., Kim, S.H., Kim, H., Song, H.J., Kim, E.J., Kim, R.Y., Kim, E.H., Sinha, A., Dey, A., 2015. Shigella outer membrane protein PSSP-1 is broadly protective against Shigella infection. *Clinical and Vaccine Immunology* 22(4), 381-388.
- Kim, J.O., Chakrabarti, B.K., Guha-Niyogi, A., Louder, M.K., Mascola, J.R., Ganesh, L., Nabel, G.J., 2007. Lysis of human immunodeficiency virus type 1 by a specific secreted human phospholipase A2. *J Virol* 81(3), 1444-1450.
- Kim, Y., Cheon, S., Min, C.K., Sohn, K.M., Kang, Y.J., Cha, Y.J., Kang, J.I., Han, S.K., Ha, N.Y., Kim, G., Aigerim, A., Shin, H.M., Choi, M.S., Kim, S., Cho, H.S., Kim, Y.S., Cho, N.H., 2016b. Spread of Mutant Middle East Respiratory Syndrome Coronavirus with Reduced Affinity to Human CD26 during the South Korean Outbreak. *MBio* 7(2), e00019.
- Li, D., von Schaewen, M., Wang, X., Tao, W., Zhang, Y., Li, L., Heller, B., Hrebikova, G., Deng, Q., Ploss, A., Zhong, J., Huang, Z., 2016. Altered Glycosylation Patterns Increase Immunogenicity of a Subunit Hepatitis C Virus Vaccine, Inducing Neutralizing Antibodies Which Confer Protection in Mice. *J Virol* 90(23), 10486-10498.
- Min, C.K., Cheon, S., Ha, N.Y., Sohn, K.M., Kim, Y., Aigerim, A., Shin, H.M., Choi, J.Y., Inn, K.S., Kim, J.H., Moon, J.Y., Choi, M.S., Cho, N.H., Kim, Y.S., 2016. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. *Sci Rep* 6, 25359.
- Naldini, L., Blomer, U., Gage, F.H., Trono, D., Verma, I.M., 1996. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc Natl Acad Sci U S A* 93(21), 11382-11388.
- Pallesen, J., Wang, N., Corbett, K.S., Wrapp, D., Kirchdoerfer, R.N., Turner, H.L., Cottrell, C.A., Becker, M.M., Wang, L., Shi, W., Kong, W.P., Andres, E.L., Kettenbach, A.N., Denison, M.R., Chappell, J.D., Graham, B.S., Ward, A.B., McLellan, J.S., 2017. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc Natl Acad Sci U S A* 114(35), E7348-E7357.
- Park, S.H., Kim, Y.S., Jung, Y., Choi, S.Y., Cho, N.H., Jeong, H.W., Heo, J.Y., Yoon, J.H., Lee, J., Cheon, S., Sohn, K.M., 2016. Outbreaks of Middle East Respiratory Syndrome in Two Hospitals Initiated by a Single Patient in Daejeon, South Korea. *Infect Chemother* 48(2), 99-107.
- Pascal, K.E., Coleman, C.M., Mujica, A.O., Kamat, V., Badithe, A., Fairhurst, J., Hunt, C., Strein, J., Berrebi, A., Sisk, J.M., Matthews, K.L., Babb, R., Chen, G., Lai, K.M., Huang, T.T., Olson, W., Yancopoulos, G.D., Stahl, N., Frieman, M.B., Kyratsous, C.A., 2015. Pre- and postexposure efficacy of fully human antibodies against Spike protein in a novel humanized mouse model of MERS-CoV infection. *Proc Natl Acad Sci U S A* 112(28), 8738-8743.

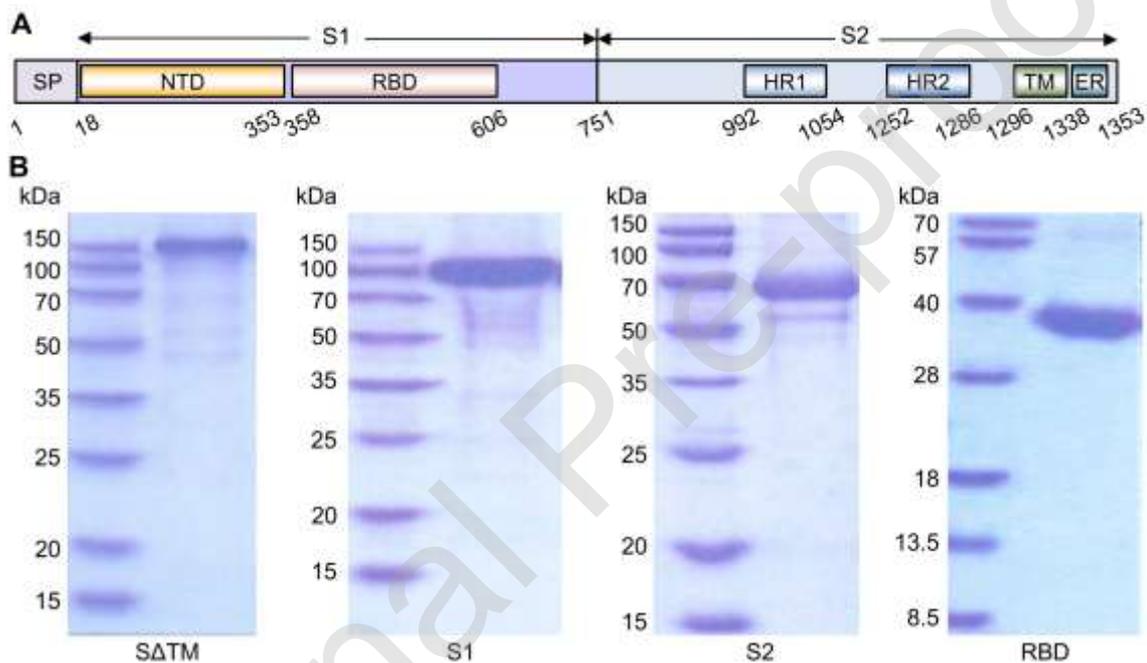
- Rao, S., Kong, W.P., Wei, C.J., Yang, Z.Y., Nason, M., Styles, D., DeTolla, L.J., Panda, A., Sorrell, E.M., Song, H., Wan, H., Ramirez-Nieto, G.C., Perez, D., Nabel, G.J., 2008. Multivalent HA DNA vaccination protects against highly pathogenic H5N1 avian influenza infection in chickens and mice. *PLoS One* 3(6), e2432.
- Shere, K.D., Sallustio, S., Manassis, A., D'Aversa, T.G., Goldberg, M.B., 1997. Disruption of IcsP, the major *Shigella* protease that cleaves IcsA, accelerates actin- based motility. *Molecular microbiology* 25(3), 451-462.
- van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T.M., Raj, V.S., Zaki, A.M., Osterhaus, A.D., Haagmans, B.L., Gorbalenya, A.E., Snijder, E.J., Fouchier, R.A., 2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *MBio* 3(6).
- Wang, L., Shi, W., Chappell, J.D., Joyce, M.G., Zhang, Y., Kanekiyo, M., Becker, M.M., van Doremalen, N., Fischer, R., Wang, N., Corbett, K.S., Choe, M., Mason, R.D., Van Galen, J.G., Zhou, T., Saunders, K.O., Tatti, K.M., Haynes, L.M., Kwong, P.D., Modjarrad, K., Kong, W.P., McLellan, J.S., Denison, M.R., Munster, V.J., Mascola, J.R., Graham, B.S., 2018. Importance of Neutralizing Monoclonal Antibodies Targeting Multiple Antigenic Sites on the Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein To Avoid Neutralization Escape. *J Virol* 92(10).
- Wang, L., Shi, W., Joyce, M.G., Modjarrad, K., Zhang, Y., Leung, K., Lees, C.R., Zhou, T., Yassine, H.M., Kanekiyo, M., Yang, Z.Y., Chen, X., Becker, M.M., Freeman, M., Vogel, L., Johnson, J.C., Olinger, G., Todd, J.P., Bagci, U., Solomon, J., Mollura, D.J., Hensley, L., Jahrling, P., Denison, M.R., Rao, S.S., Subbarao, K., Kwong, P.D., Mascola, J.R., Kong, W.P., Graham, B.S., 2015. Evaluation of candidate vaccine approaches for MERS-CoV. *Nat Commun* 6, 7712.
- Wang, N., Shi, X., Jiang, L., Zhang, S., Wang, D., Tong, P., Guo, D., Fu, L., Cui, Y., Liu, X., Arledge, K.C., Chen, Y.H., Zhang, L., Wang, X., 2013. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Res* 23(8), 986-993.
- Wang, Q., Qi, J., Yuan, Y., Xuan, Y., Han, P., Wan, Y., Ji, W., Li, Y., Wu, Y., Wang, J., Iwamoto, A., Woo, P.C., Yuen, K.Y., Yan, J., Lu, G., Gao, G.F., 2014. Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26. *Cell Host Microbe* 16(3), 328-337.
- Yang, E., Cho, Y., Choi, J.A., Choi, Y., Park, P.G., Park, E., Lee, C.H., Lee, H., Kim, J., Lee, J.M., Song, M., 2015a. Protection of mice against pandemic H1N1 influenza virus challenge after immunization with baculovirus-expressed stabilizing peptide fusion hemagglutinin protein. *J Microbiol Biotechnol* 25(2), 280-287.
- Yang, J.S., Park, S., Kim, Y.J., Kang, H.J., Kim, H., Han, Y.W., Lee, H.S., Kim, D.W., Kim, A.R., Heo, D.R., Kim, J.A., Kim, S.J., Nam, J.G., Jung, H.D., Cheong, H.M., Kim, K., Lee, J.S., Kim, S.S., 2015b. Middle East Respiratory Syndrome in 3 Persons, South Korea, 2015. *Emerg Infect Dis* 21(11), 2084-2087.

- Ying, T., Du, L., Ju, T.W., Prabakaran, P., Lau, C.C., Lu, L., Liu, Q., Wang, L., Feng, Y., Wang, Y., Zheng, B.J., Yuen, K.Y., Jiang, S., Dimitrov, D.S., 2014. Exceptionally potent neutralization of Middle East respiratory syndrome coronavirus by human monoclonal antibodies. *J Virol* 88(14), 7796-7805.
- Yu, X., Zhang, S., Jiang, L., Cui, Y., Li, D., Wang, D., Wang, N., Fu, L., Shi, X., Li, Z., Zhang, L., Wang, X., 2015. Structural basis for the neutralization of MERS-CoV by a human monoclonal antibody MERS-27. *Sci Rep* 5, 13133.
- Zhang, S., Zhou, P., Wang, P., Li, Y., Jiang, L., Jia, W., Wang, H., Fan, A., Wang, D., Shi, X., Fang, X., Hammel, M., Wang, S., Wang, X., Zhang, L., 2018. Structural Definition of a Unique Neutralization Epitope on the Receptor-Binding Domain of MERS-CoV Spike Glycoprotein. *Cell Rep* 24(2), 441-452.

## Figure legends

**Fig. 1.** Production of S $\Delta$ TM, S1, S2, and RBD subunit recombinant proteins by baculovirus system.

**A.** Schematic diagram for the domain structure of MERS-CoV Spike (S) protein. **B.** SDS-PAGE and Coomassie blue staining of purified recombinant S subunit proteins from the insect cell culture supernatant: S $\Delta$ TM (1-1296 aa), S1 (1-751 aa), S2 (752-1296 aa), and RBD (358-606 aa).



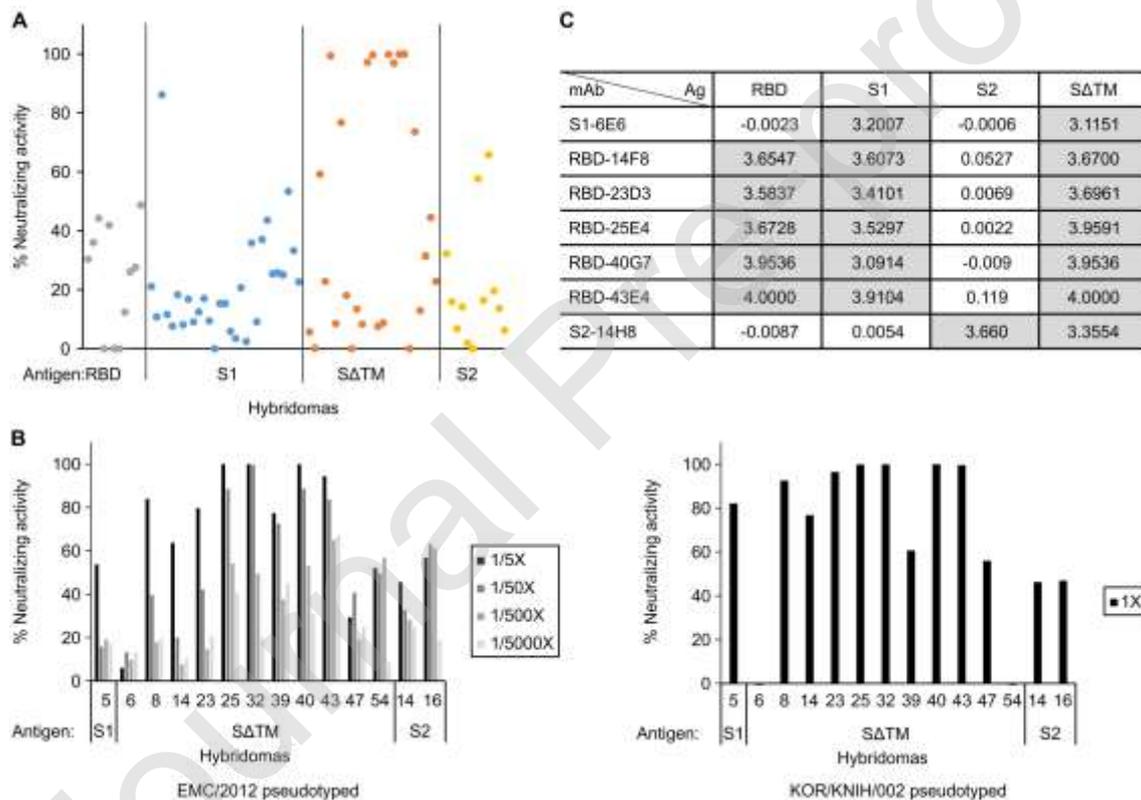
**Fig. 2.** Pseudovirus neutralizing activity of hybridomas generated using splenocytes of mice that were immunized with S $\Delta$ TM, S1, S2, and RBD recombinant protein

**A.** Distribution of neutralizing activity of 77 hybridomas against MERS-CoV EMC/2012 Spike (S)-pseudotyped lentivirus. Hybridoma culture supernatant was incubated with MERS-CoV EMC/2012 pseudovirions for 60 min at 37°C and added to 786O cells, incubated for 6 h, replaced with fresh medium, and then analyzed for luciferase-reporter activity 3 days later. The relative luminescence unit (RLU) was

converted to % neutralizing activity. **B.** Neutralizing activity of the selected hybridomas against EMC/2012 (left) and KOR/KNIH/002 (right) strain of pseudovirions.

Among 77 hybridomas, selected 15 hybridomas (1 by S1, 11 by S $\Delta$ TM, and 2 by S2) were tested for their neutralizing activity against indicated pseudovirions. Four ten-fold serial dilutions from 1/5 of hybridoma culture supernatant were used for EMC/2012 pseudovirions, and undiluted culture supernatant was used for KOR/KNIH/002 pseudovirions. **C.** Binding domain of selected mAbs within S protein.

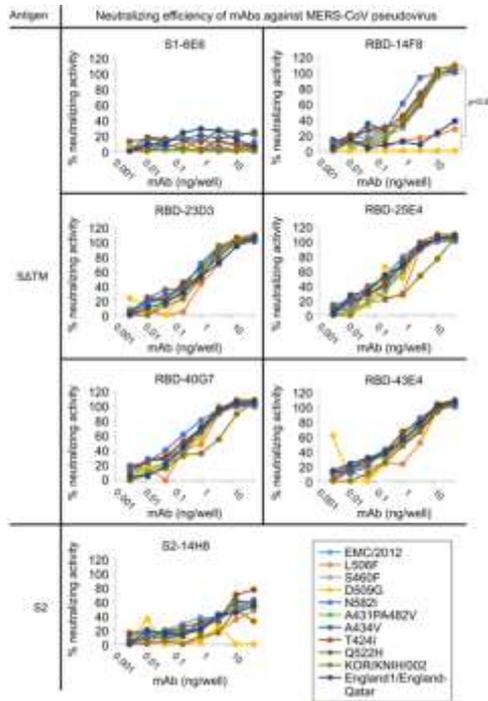
Seven mAbs were purified, and each binding region was assessed using ELISA. The value indicates the OD after coating the 96-well plate with each S subunit antigen.



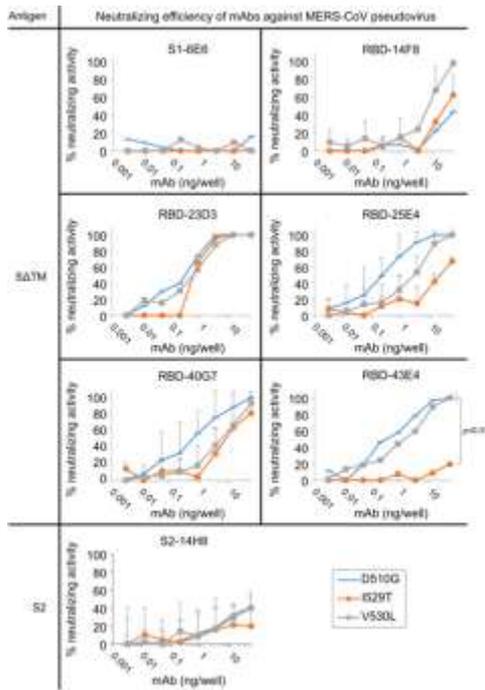
**Fig. 3.** Dose-dependent neutralizing activity of purified mAbs against 11 different MERS-CoV Spike (S)-pseudotyped virions.

Each mAb was diluted 2-fold from 50ng and tested. mAb was incubated with indicated MERS-CoV S-pseudotyped virions for 60 min at 37°C and added to 786O cells, incubated for 6 h, replaced with fresh medium, and analyzed for luciferase-reporter

activity 3 days later. The RLU value was converted to % neutralizing activity. The data is represented as the mean $\pm$ standard deviation from at least two independent experiments. Statistical significance was determined by one-way ANOVA followed by Tukey's test.



**Fig. 4.** Dose-response curve of neutralizing activity of mAbs against three pseudoviruses with MERS-CoV RBD identified in 2015 during the Korean MERS outbreak (D510G, I529T, and V530L respectively). Each mAb was diluted 2-fold from 50ng and tested. mAb was incubated with indicated MERS-CoV S-pseudotyped virions for 60 min at 37°C and added to 786O cells, incubated for 6 h, replaced with fresh medium, and analyzed for luciferase-reporter activity 3 days later. The RLU value was converted to % neutralizing activity. The data is represented as the mean $\pm$ standard deviation from at least two independent experiments. Statistical significance was determined by one-way ANOVA followed by Tukey's test.



**Fig. 5.** Epitope-specific neutralizing activity of chimeric h14F8 and h43E4 against MERS-CoV Spike (S)-pseudotyped virions

**A.** Fc/light chain-humanized chimeric h14F8 (left) and h43E4 (right) mAb purified from 293F cell culture supernatant after transfection with relevant DNA plasmids and run on the 4~20% SDS-PAGE followed by Coomassie-blue staining. Re, reducing condition; NR, non-reducing condition. **B.** h14F8 (left) and h43E4 (right) mAb was diluted 4-fold from 12.5ng and were neutralized against EMC/2012, KOR/KNIH/002, L506F, D509G, D510G, I529T, and V530L pseudotyped virions. The luciferase activity of pseudovirus was converted to % neutralizing activity. **C.** Neutralizing activity of both mouse and chimeric 14F8 and 43E4 against wild type MERS-CoV KOR/KNIH/002 strain was assessed by PRNT. mAbs were diluted 4-fold from 100ng and were tested. The data is representative of at least two independent experiments.

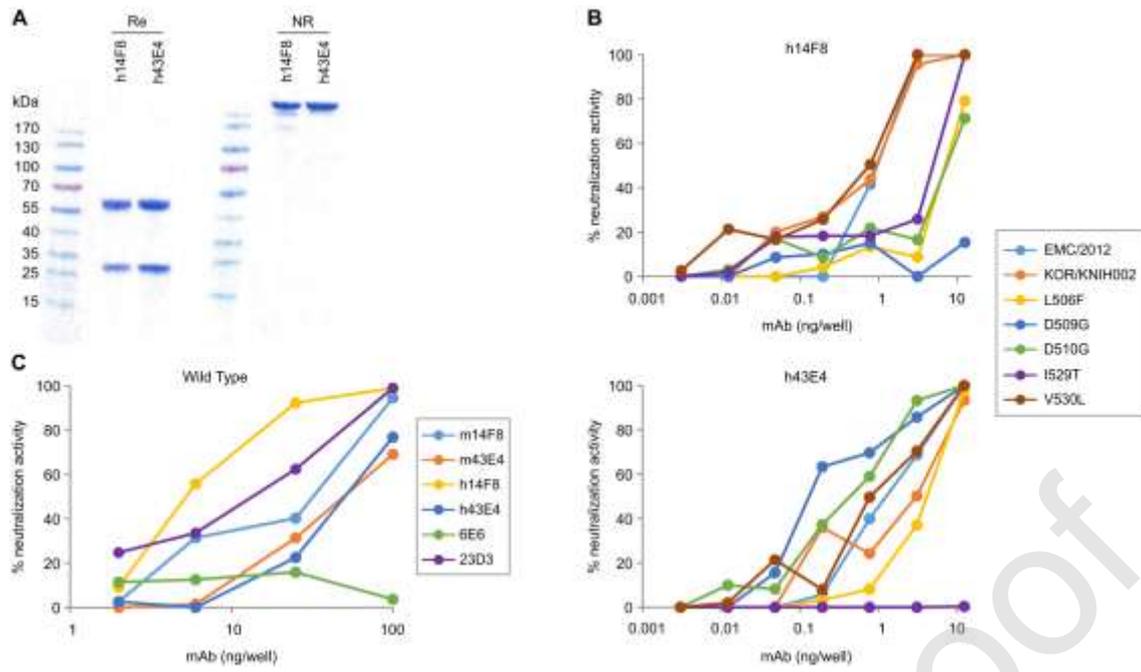


Table 1. Amino acid sequence of MERS-CoV Spike derived from EMC/2012, England1, and KOR/KNIH/002 strains.

Virus isolate	GenBank Accession No. (Protein ID)	S1						S2					
		NTD			RBD				HR1		TM		
		58 91 94 97 137 158 194 289 301	424 431 434 460 482 506 509 510 522 529 530 534 582	833 966 1020 1052 1060 1158 1199 1208 1227 1313 1347									
EMC/2012	AFS88936.1	Y H G P S D H T R	T A A S A L D D Q I V V N	Q S Q G V A S Q T C P									
England1	AFY13307.1		F				H						
KOR/KNIH/002	AKL59401.1	R		L			R						

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Table 2. Amino acid sequence variation within the receptor-binding domain of MERS-CoV Spike

Virus isolate	GenBank Accession No. (Protein ID)	Spike RBD													
		424	431	434	460	482	506	509	510	522	529	530	534	582	
	EMC/2012	AFS88936.1	T	A	A	S	A	L	D	D	Q	I	V	V	N
1	England1/ England-Qatar	AFY13307.1/ AGG22542.1							F						
2	Qatar3	AHC74088.1			F										
3	Riyadh1/Bisha1	AGV08379.1/ AGV08408.1							G						
4	Riyadh2	AGV08584.1												A	
5	Al-Hasa8c	AGV08438.1													I
6	Riyadh9	AHI48528.1	P				V								
7	Asir2f	AHI48733.1			V										
8	Riyadh345/Riyadh59	AID55095.1/ AID55090.1	I												
9	Jeddah_C9313	AID55087.1									H				
10	KOR/CNUH_SNU/122	ALK80291.1								G					
11	KOR/CNUH_SNU/016_06	ALK80192.1											T		
12	KOR/KNIH/002	AKL59401.1												L	

Table 3. Characterization of mAbs developed by MERS-CoV S subunit proteins

mAb	Isotype		Specificity	Antigen
	Heavy chain	Light chain		
<b>6E6</b>	IgG1	κ	S1-non RBD	SΔTM
<b>14F8</b>	IgG1	κ	RBD	
<b>23D3</b>	IgG1	λ	RBD	
<b>25E4</b>	IgG1	κ	RBD	
<b>40G7</b>	IgG1	κ	RBD	
<b>43E4</b>	IgG2b	κ	RBD	
<b>14H8</b>	IgG2b	κ	S2	S2

Table 4. Humanized mAb characteristics

<b>Humanized mAb</b>	<b>Isotype</b>	<b>Light chain</b>
<b>Hu-14F8</b>	hIgG1	hLambda1
<b>Hu-43E4</b>	hIgG1	hKappa1

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