

## Electronic Supplementary Material

### Inefficiency of Sera from Mice Treated With Pseudotyped SARS-CoV to Neutralize 2019-nCoV Infection

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#### Materials and Methods

##### Animal, reagents and cells

Specific pathogen-free (SPF) 6 to 8-week-old female Balb/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co. (Beijing, China). All animal experiments were approved by the Institutional Laboratory of Animal Care at Fudan University. Freund's complete and incomplete adjuvants were from Sigma (USA). The luciferase assay systems were from Promega. Huh-7, HEK-293T and HEK-293 T/ACE2 were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA).

##### SARS-PsV and MERS-PsV production and concentration

Production of SARS-PsV and MERS-PsV was conducted as previously described (Xia *et al.* 2019). Briefly, high-quality plasmids of pNL4-3.luc.RE and pcDNA3.1-SARS or MERS-CoV-S were cotransfected with HEK-293T cells. After incubation at 37 °C for 48 h, the supernatants were collected, concentrated and purified by ultrafiltration using Amicon Ultra-50 filters (Millipore).

##### Mice immunization

Fifteen female Balb/c mice were randomly assigned to three groups, and each group had five mice. Freund's complete adjuvant was emulsified with SARS-PsV, MERS-PsV or PBS at a volume ratio of 1: 1. The mice were subcutaneously injected with SARS-PsV, MERS-PsV or PBS for the first prime immunization. After 4 weeks, Freund's incomplete adjuvant was mixed with SARS-PsV, MERS-PsV or PBS, and the mice were

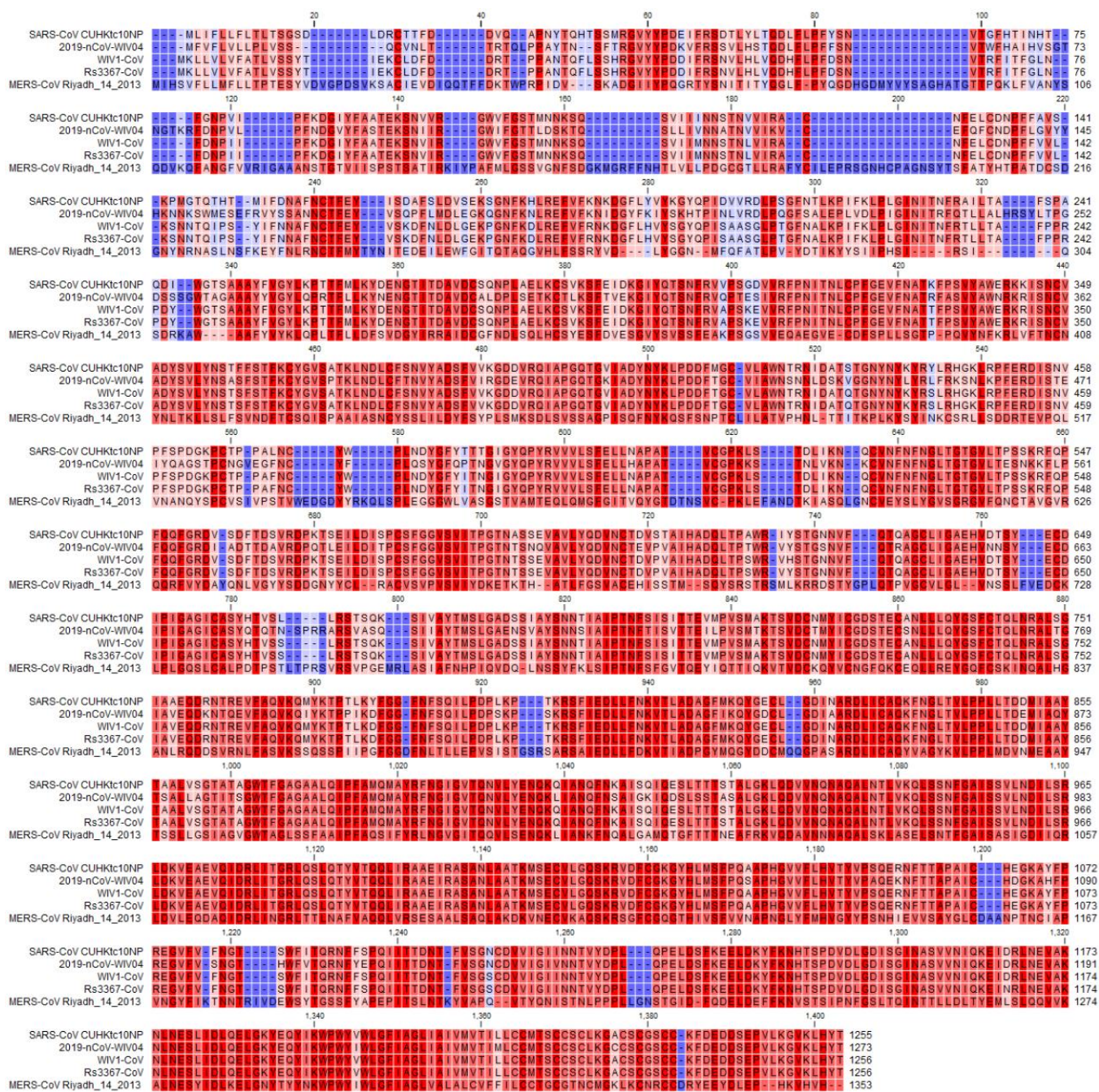
immunized subcutaneously for the boost immunization. Mice were bled at 7 days post- last immunization. Then, the serum was separated from the blood and inactivated at 56 °C for 30 min. The sera were stored at -80°C until use.

### **Neutralization assay**

A serial dilution of sera was mixed with SARS-PsV , 2019-nCoV-PsV , MERS-PsV, WIV1-PsV and Rs3367-PsV for 30 min at 37 °C before applying to target cells. HEK-293T cells expressing ACE2 were used as target cells for SARS-PsV, WIV1-PsV and Rs3367-PsV infection. Huh-7 cells were used as the target cells for 2019-nCoV-PsV and MERS-PsV infection. After incubation with the target cells for 12 h, the supernatants were replaced with fresh DMEM and incubated another 24h. Then, the cells were washed with PBS and lysed with lysis reagent (Promega). The cell lysates were transferred to 96-well Costar flat-bottom luminometer plates (Corning Costar), followed by detection of relative light units using a Firefly Luciferase Assay Kit (Promega).

### **Reference:**

Xia S, Yan L, Xu W, Agrawal AS, Algaissi A, Tseng CK, Wang Q, Du L, Tan W, Wilson IA, Jiang S, Yang B, Lu L (2019) A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci Adv* 5: eaav4580



**Fig. S1.** Amino acid alignment of SARS-CoV (CUHKtc10NP) with 2019-nCoV (WIV04), Rs3367-CoV, W1-CoV and MERS-CoV (Riyadh\_14\_2013).

**Table S1.** Comparison of amino acid sequence identity of SARS-CoV (CUHKtc10NP) with 2019-nCoV (WIV04), Rs3367-CoV, WIV1-CoV and MERS-CoV (Riyadh\_14\_2013).

Virus strain	Sequence identities with SARS-CoV CUHKtc10NP
WIV1-CoV	92.28%
Rs3367-CoV	92.28%
2019-nCoV (WIV04)	75.88%
MERS-CoV Riyadh_14_2013	28.67%