Supplementary Material

A Pan-sarbecovirus Vaccine Induces Highly Potent and Durable Neutralizing Antibody Responses in Non-human Primates against SARS-CoV-2 Omicron Variant

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Methods:

Cells

HEK293T cells and Huh-7 cells were obtained from the American Type Culture Collection (ATCC). VeroE6-TMPRSS2 cells were maintained in our laboratories. Dulbecco's Modified Eagle's Medium (DMEM) (Meilun, China) supplemented with 10% fetal bovine serum (FBS) was used to culture the HEK293T, Huh-7 and VeroE6-TMPRSS2 cells.

Animals

Rhesus macaques with good health were purchased from the Beijing Institute of Xieerxin Biology Resource and these macaques did not conduct any other experiment before. All animal experiments were conducted at Beijing Institute of Xieerxin Biology Resource and approved by the Institutional Animal Care and Use Committee (E20201101).

Immunization

Six rhesus macaques were randomly assigned to two groups and each group contain 3 macaques. The first group of macaques was intramuscularly immunized with 100 μg RBD-Fc formulated with 400 μg CF501 at days 0, 21 and 115. The second group of macaques was intramuscularly immunized with 100 μg RBD-Fc formulated with an equal volume of Imject Alum adjuvant (Thermo Scientific, USA) at days 0, 21 and 115.

The sera were collected on days 28, 51, 64, 78, 100, 113, 122, 136, 150, 178, 191 respectively.

ELISA

Enzyme-linked immunosorbent assay (ELISA) was used to test the SARS-CoV-2 wildtype (WA1)- or Omicron-specific IgG endpoint titer as previously described with minor modification.[1] Briefly, the ELISA plates were coated with 1 μg/ml Omicron RBD-His protein (Kactus Biosystems) at 4 °C overnight. After blocking with the blocking buffer (PBS contains 5% BSA), the plates were incubated with the serially diluted pooled sera from the immunized macaques at 37°C for 45 min. And then, the HRP-conjugated goat anti-monkey IgG (Abcam, UK) was added and incubated at 37 °C for another 45 min. Finally, 3,3',5,5'-tetramethylbenzidine (TMB) was used to show the reaction and the H_2SO_4 was used to stop the reaction. A microplate reader (Infinite M200PRO, Switzerland) was used to read the absorbance at 450 nm (A450). The endpoint titer was considered as the highest serum dilution exhibiting their A450>2.1-fold of background values.

Production of the pseudovirus

PsVs of SARS-CoV-2 WA1 strain, Omicron variant and Mu variants were produced as previously described.[1-3] For the production of the HIV backbone PsV, HEK293T cells were co-transinfected with the plasmids of pNL4-3.Luc.R−E− and PcDNA3.1-SARS-CoV-2 WA1/Omicron/Mu-S. After 60 h incubation, the supernatants were collected and used for the serum neutralization assay. For the production of the VSV backbone PsV, HEK293T cells were transfected with the plasmid carrying the SARS-CoV-2 WA1/Omicron spike. After that, the cells were transfected with the G* ΔG-VSV (VSV G pseudotyped virus). The supernatant was collected at 24 h post the infection and used for the serum neutralization assay.

Pseudovirus neutralization assay

The serum neutralization assay using pseudovirus was conducted as previously described.[1,3] Briefly, 1×10^4 cells/well were seeded into the 96-well plates. After 8 h,

the serially diluted sera from the immunized macaques were pre-incubated with the Omicron/WA1 PsV at 37°C for 30 min. After that, the mixture was added into the Huh-7 cells and incubated for 12 h. The cell supernatants were replaced with the fresh DMEM and furtherly incubated at 37°C for 48 h. Finally, a Firefly Luciferase Assay Kit (Promega, USA) was used to detect the luciferase activity. NT_{50} was defined as the dilution of the antiserum that reduces the relative luminescence units by 50%, compared with the relative luminescence units in the virus control wells containing no antiserum.

Authentic virus neutralization assay

The authentic Omicron (hCoV-19/Hong Kong/HKU-344/2021; GISAID accession number EPI_ISL_7357684) was isolated from the respiratory tract specimens of a COVID-19 patient in Hong Kong.[4] Vero-E6-TMPRSS2 cells were used as the target cells to evaluate the serum neutralization activity against the SARS-CoV-2 HKU-001a (GenBank accession number MT230904) and Omicron infection. Briefly, the 1×10^4 cells/well Vero-E6-TMPRSS2 cells were seeded in a 96 well-plate overnight at 37 °C. After 12 h, the pooled sera from the immunized macaques were diluted into indicated concentrations. And then the diluted sera were incubated with 0.01 MOI of SARS-CoV-2 HKU-001a or Omicron variant at 37 °C for 1 h. Afterward, the mixture of serum and virus was overlaid onto the Vero-E6-TMPRSS2 cells and further incubated at 37 °C for approximately 48 h. Finally, cytopathic effect (CPE) was observed for all wells. The CPE was scored as either positive (0% inhibition) or negative (100% inhibition) in a blinded manner as previously described.[4] The NT50 was defined as the dilution of the antiserum that reduces CPE by 50%, compared with the CPE in virus control wells containing no antiserum. GraphPad Prism was used for calculating the NT50 value.

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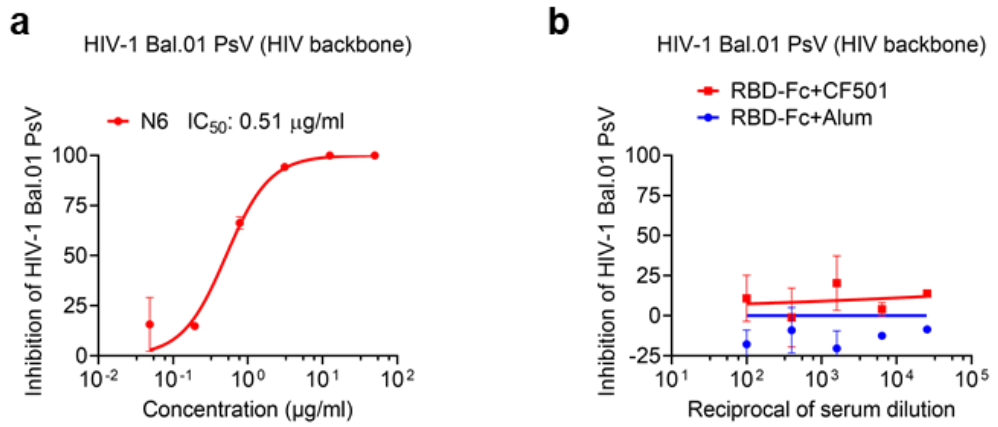

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112 **Supplementary Fig. S1. The neutralizing activity of HIV-1 neutralizing mAb N6**
113 **and the sera from the immunized macaques against HIV-1 Bal.01 PsV.**

114 a Dose-dependent curve of N6 against the infection of HIV-1 Bal.01 PsV. Data are
115 mean±SD.

116 b The neutralizing activity against HIV-1 Bal.01 PsV for the sera from CF501/RBD-
117 Fc and Alum/RBD-Fc group. Data are mean±SD.

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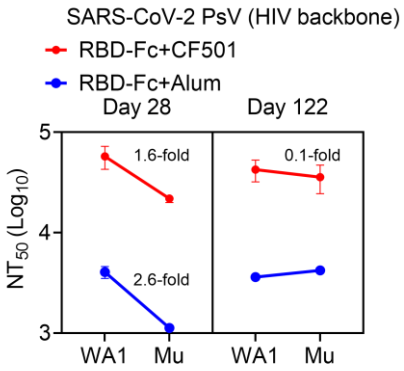

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120 **Supplementary Fig. S2.** Fold reduction of the NT_{50} in the pooled sera from the
121 immunized macaques against Mu PsV compared to the SARS-CoV-2 WA1 PsV at days
122 28 and 122. Data are mean ± SD.

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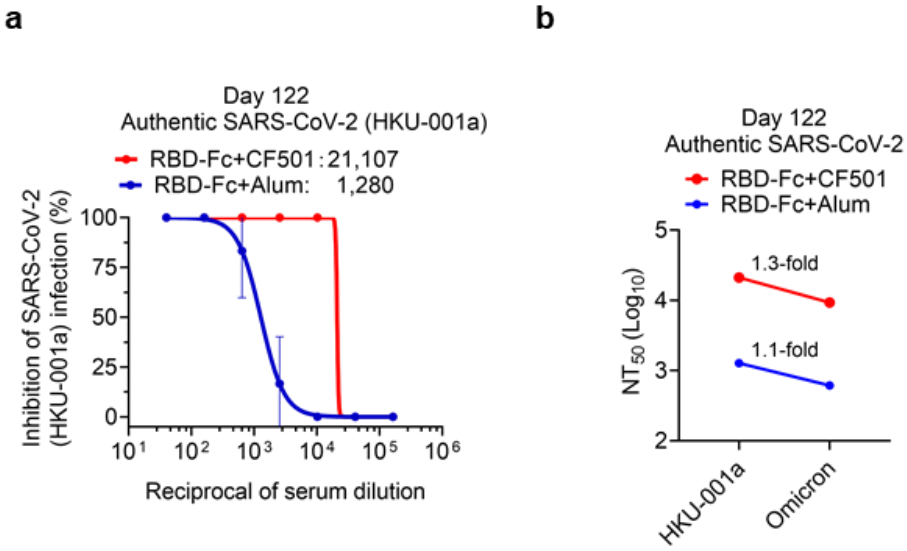

Supplementary Fig. S3. Comparison of the nAb titer of the pooled sera against the authentic SARS-CoV-2 (HKU-001a) and Omicron.

a Titers of nAbs in the pooled sera at day 122 in the CF501/RBD-Fc and Alum/RBD-Fc groups against authentic SARS-CoV-2 (HKU-001a). Data are mean ± SD.

b Fold reduction of nAb titer against authentic Omicron in the pooled sera from the immunized macaques at day 122, compared to the titer against authentic SARS-CoV-2 (HKU-001a).