Supplementary information

Materials and Methods

Ethics statement

All mouse and macaque studies were conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Science and Technology of China (Approved No. USTCACUC1801038). All procedures performed on Syrian hamster were in accordance with regulations and established guidelines, and were reviewed and approved by the Animal Ethics Committee of the Wuhan Institute of Virology, Chinese Academy of Sciences (Approved No. WIVA45202201). The animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals.

Cells and viruses

HEK293T cells, Vero cells, and Vero E6 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, ExCell Bio) and 1% penicillin-streptomycin (Gibco) at 37°C under a 5% CO_2 atmosphere. The SARS-CoV-2 WIV04 strain was initially isolated from an early severe COVID-19 patient in 2019 (GISAID accession no. EPI_ISL_402124); Delta variant (B.1.617.2; GWHBEBW01000000) by Prof. Hongping Wei; Beta variant (NPRC2.062100001) and Omicron variant (CCPM-B-V-049-2112-18) was kindly obtained from National Pathogen Resource Center. All processes in this study involving authentic SARS-CoV-2 were performed in the biosafety level 3 (BSL-3) facility in Wuhan Institute of Virology, Chinese Academy of Sciences.

mRNA design and synthesis

Spike (S) protein encoded by S_{WT}-2P vaccine was designed from original ancestral SARS-CoV-2 WA1 (GenBank MN908947.3), and $S_{Omicron}$-6P was based on a background of S sequences from SARS-CoV-2 variant Omicron (B.1.1.529) (GISAID: GR/484A). Both mRNAs were synthesized in vitro using an optimized T7 RNA polymerase-mediated transcription reaction with complete replacement of uridine by N1-methyl-pseudouridine. The reaction included a DNA template containing the open reading frame flanked by 5' untranslated region (UTR) and 3' UTR sequences and was terminated by an encoded poly A tail. The template for the S_{WT}-2P mRNA is a DNA fragment encoding SARS-CoV-2 S with K986P and V987P substitutions, while that for the $S_{Omicron}$-6P mRNA encoding Omicron variant S with F817P, A892P, A899P, A942P, K986P, and V987P substitutions.

The mRNA was purified by oligo-dT affinity purification, buffer exchanged by tangential flow filtration into sodium acetate, and sterile filtered. RNA integrity was assessed by microfluidic capillary electrophoresis (Fragment Analyzer systems 5200, Agilent), and the concentration, pH, residual DNA, proteins, and dsRNA impurities of the solution were determined. The mRNA 5' capping efficiency and 3'-polyadenosine (poly A) tail of mRNAs was studied using liquid chromatography coupled to mass spectrometry (LC-MS).

mRNA encapsulation

mRNAs were encapsulated in lipid nanoparticles (LNPs) using a modified procedure of a method previously as previously described.[1] Briefly, an ethanolic lipid mixture of ionizable

cationic lipid, phosphatidylcholine, cholesterol, and polyethylene glycol-lipid was rapidly mixed with an aqueous solution containing mRNA. The drug product underwent analytical characterization, which included the determination of particle size and polydispersity, encapsulation, pH, endotoxin, and bioburden, and the material was deemed acceptable for in vivo study.

mRNA transfection

HEK293T were seeded in 24-well plates at 1.5×10^4 cells/well, and transfected with $S_{Omicron}$-6P mRNA using Lipofectamine® Messenger MAX™ Reagent (Invitrogen) 12 h later according to the manufacturer's protocol.

Vaccine antigen detection by immunofluorescence

Transfected HEK293T cells were fixed in 4% paraformaldehyde (PFA) and permeabilized with PBS/0.1% Triton X-100. After blocked with 1% BSA for 0.5 h at room temperature, the cells were incubated with SARS-CoV-2 Omicron S protein antibody (Sino Biological, 40592-R0004). The cells were stained with a fluorescent anti-rabbit IgG antibody (Abcam, ab6717), and DAPI (Sigma-Aldrich, 28718-90-3). Images were acquired with a laser scanning confocal microscope (Nikon A1).

Mouse immunizations

All the control vaccines were generated to target the WT SARS-CoV-2 strain. CoronaVac, a 500 μL dose, contains 3 μg of SARS-CoV-2 virion with 225 μg of aluminum hydroxide for

an adult. ZF2001, a 500 μL dose, contains 25 μg RBD-dimer protein with 250 μg of aluminum hydroxide for an adult. The dosages of CoronaVac and ZF2001 applied in mice ranged from 1/50 to 1/5 corresponding human dose, that is 0.3 (50 μL) and 0.6 (100 μL) μg for CoronaVac group, 0.5 (10 μL), 2.5 (50 μL), and 5 (100 μL) μg for ZF2001 group. mRNA vaccines, including S_{WT}-2P (100 μg mRNA of S for an adult), applied in mice ranged from 1/100 to 1/10 corresponding human dose, that is 1, 5, and 10 μg for S_{WT}-2P and $S_{Omicron}$-6P groups.

Female BALB/c mice (8–12 weeks old) were randomly allocated to groups. Mice were intramuscularly (i.m.) immunized with the same dose at 21-day intervals for mRNA vaccines or 28-day intervals for inactivated vaccine or protein subunit vaccine. Sera were collected on day 0, 21, 28, 35, and 42 after the first immunization to detect SARS-CoV-2 neutralizing antibodies (nAbs) titers as described below.

Hamster immunization and challenge experiments

Four groups of female Syrian hamsters (6–7 weeks old) were vaccinated on day 0 and day 21 with 1, 10, 25, or 50 μg of $S_{Omicron}$-6P for prime-boost vaccine regimens by intramuscular injection to each hind thigh, while control hamsters were administered with PBS. On day 30, all animals were challenged intranasally with 1×10^4 PFU of authentic SARS-CoV-2 Omicron virus after anesthetization with isoflurane.

Macaque immunizations

Male macaques (3–5 years old) were randomly assigned to receive $S_{Omicron}$-6P (20 or 100 μg) on day 0 and 21. The vaccine was i.m. administered in the quadriceps muscle. Sera were

collected on day 0, 21, 28, and 35 after the first immunization.

Plaque reduction neutralization assay

The plaque reduction neutralization assay was carried out as described previously.[2] Briefly, sera were inactivated at 56°C for 30 min before use. The sera were 3-fold serially diluted (started at 1:150) with DMEM containing 2.5% FBS, and mixed with an equal volume of authentic SARS-CoV-2 viruses incubated at 37 °C for 1 h. The mixture was added to Vero E6 monolayer cells in 24-well plates. After another 1 h incubation at 37 °C, the mixture was replaced with DMEM containing 2.5% FBS and 0.9% carboxymethy lcellulose. The plates were incubated in a 5% CO_2-air incubator at 37°C for 4 days, then fixed with 8% paraformaldehyde and stained with 0.5% crystal violet. The neutralization percentage was calculated as: (1- plaque number / plaque number without serum) × 100%. The IC_{50} was analyzed using GraphPad Prism software with the "inhibitor vs normalized response (Variable slope)" model.

Viral RNA load by RT-qPCR

Quantitative reverse transcription PCR (qRT-PCR) was applied to measure the viral RNA loads in infected tissues.[3] Briefly, viral RNA were extracted from nasal turbinate and lung tissue homogenates with the QIAamp Viral RNA Mini Kit (Qiagen), and quantified using HiScript® II One Step qRT-PCR SYBR® Green Kit (Vazyme Biotech) with the primers ORF1a/b-F (5'-CCCTGTGGGTTTTACACTTAA-3') and ORF1a/b-R (5'-ACGATTGTGCATCAGCTGA-3'). ORF1a/b spans 16 non-structural proteins (NSPs) that are related to the replication-transcription complex of SAR-CoV-2. The primer-probe sets were based on sequence from

China CDC and have been demonstrated as the most sensitive one for molecular diagnosis of SARS-CoV-2 using qRT-PCR. The amplification procedures were set up as the following: 50 °C for 3 min, 95 °C for 30 s followed by 40 cycles consisting of 95 °C for 10 s, 60 °C for 30 s, and a default melting curve step in CFX96 System (Bio-Rad). The standard curve was generated using serial dilutions of SARS-CoV-2 ORF1ab gene control plasmid. The detection limit was determined by the standard curve and was about 120 copies per gram biopsy samples. The viral loads were calculated as the genome copies of SARS-CoV-2 in one gram tissues.

Infectious virus titer by plaque assay

Infectious virus titer was determined with plaque assay as previously described with slight modification.[4] Briefly, animal tissue homogenates were serially 10-fold diluted with DMEM containing 2.5% FBS, and inoculated to Vero E6 seeded overnight at 1.5×10^5 /well in 24-well plates; after incubated at 37°C for 1 h, the inoculate was replaced with DMEM containing 2.5% FBS and 0.9% carboxymethyl-cellulose. The plates were fixed with 8% paraformaldehyde and stained with 0.5% crystal violet 4 days later. Virus titer was calculated with the dilution gradient with 10~100 plaques.

Statistical analysis

All data were analyzed with GraphPad Prism 8.0 software. Unless specified, data are presented as mean ± SEM in all experiments. Analysis of variance (ANOVA) or t-test was used to determine statistical significance among different groups (*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ****$P < 0.0001$).

REFERENCES

1. Maier, M. A. et al. *Mol. Ther.* **21**, 1570-1578 (2013).
2. Wang, Z. J. et al. *Emerg. Microbes. Infect.* **9**, 2606-2618 (2020).
3. Feng, L. et al. *Nat. Commun.* **11**, 4207 (2020).
4. Zhang, Q. et al. *Emerg. Microbes. Infect.* **9**, 2013-2019 (2020).

Table S1. Amino acid sequence alignment of the full S protein of $S_{Omicron}$-6P.

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLP
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LIVNNATNVVIKVCEFQFCNDPFLDHKNNKSWMESEFRVYSSANNCTFEYVSQPFL
MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPIIVRDLPQGFSALEPLVDLPIGINI
TRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDC
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EVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNKLDSKVSGNYNYLYRLFRKSN
LKPFERDISTEIYQAGNKPCNGVAGFNCYFPLRSYSFRPTYGVGHQPYRVVVLSFEL
LHAPATVCGPKKSTNLVKNKCVNFNFNGLKGTGVLTESNKKFLPFQQFGRDIADTT
DAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTP
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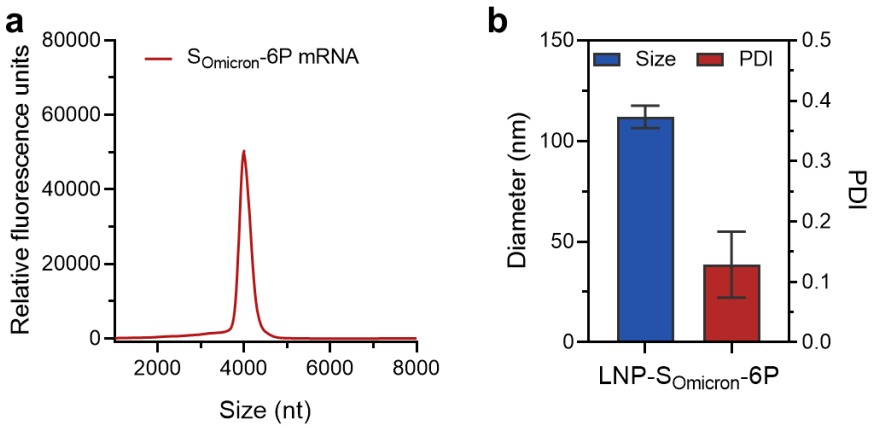


Fig. S1. SARS-CoV-2 Omicron mRNA vaccine characterization.

a Liquid capillary electropherograms of in vitro-transcribed $S_{Omicron}$-6P mRNA.

b Sizes and polydispersity index (PDI) values of lipid nanoparticles (LNP) encapsulated with $S_{Omicron}$-6P mRNA. Data are shown as mean ± SD.

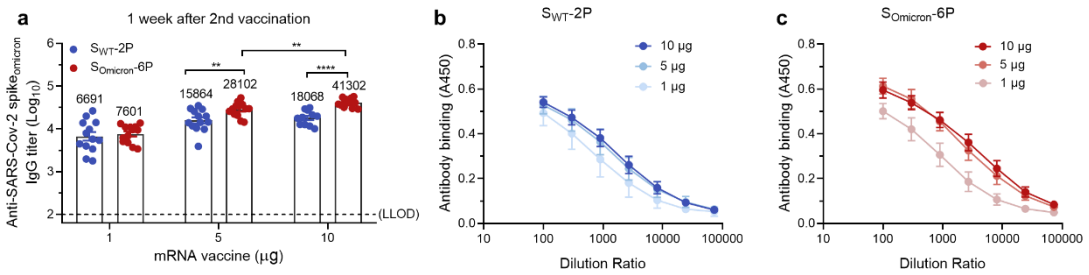


Fig. S2. $S_{Omicron}$-6P or S_{WT}-2P elicits binding antibodies in mice.

a The Omicron SARS-CoV-2 variant specific IgG antibody titers were determined by ELISA (lower limit of detection (LLOD) = 100) (n = 13).

b, c ELISA binding curves of S_{WT}-2P (a) or $S_{Omicron}$-6P (b) induced binding antibodies in mouse sera (n = 13).

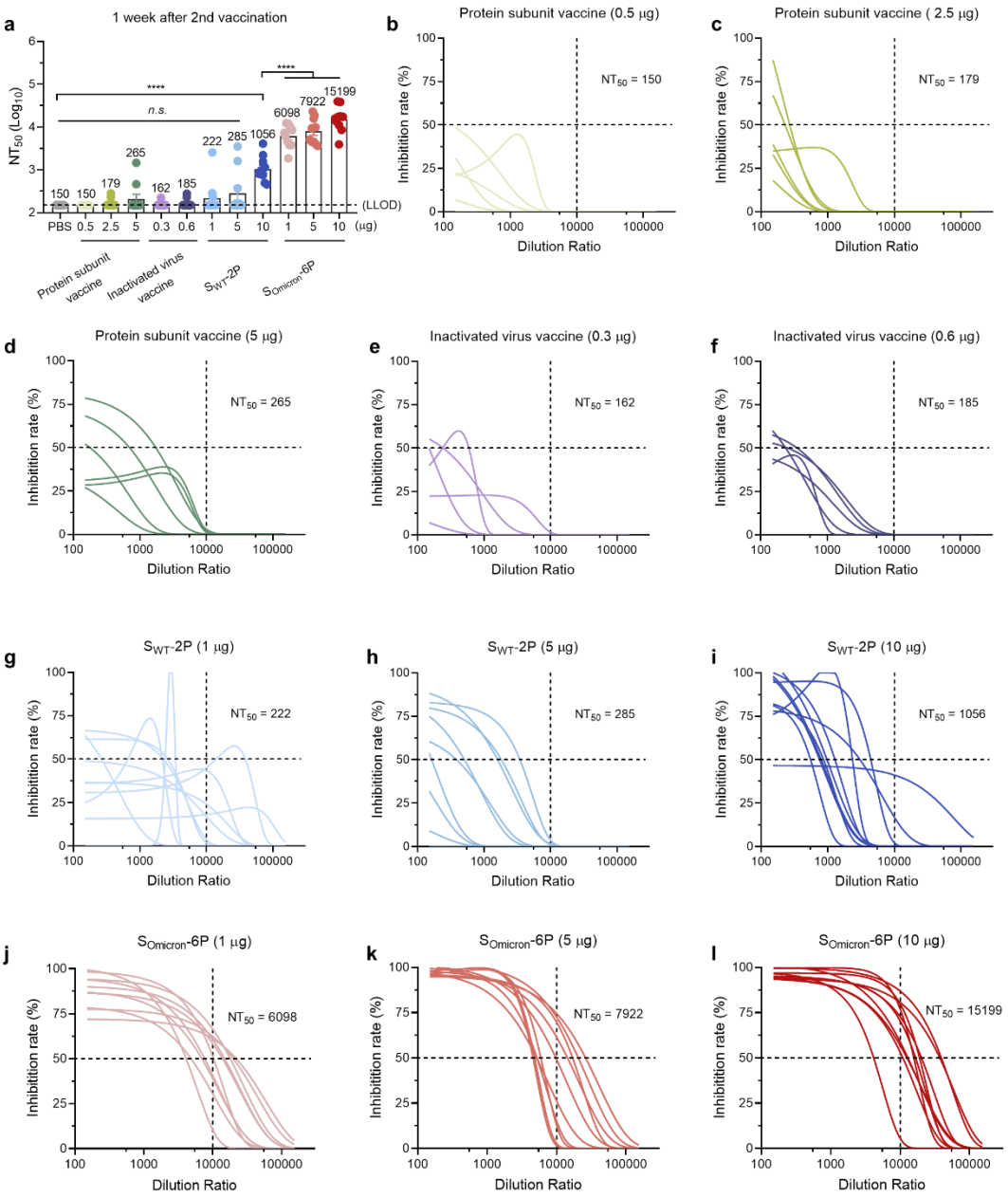


Fig. S3. $S_{Omicron}$-6P induces high levels of nAbs against pseudovirus of Omicron variant in mice.

a Neutralization titers (NT_{50}) were determined by recombinant vesicular stomatitis virus (VSV)-based pseudovirus (Omicron variant) neutralization assay (LLOD = 150) (n = 6~10).

b-d Neutralization curves of 0.5 (b), 2.5 (c), and 5 (d) μg protein subunit vaccine (25 μg/vial for an adult) induced nAbs against pseudotyped and replication-deficient SARS-CoV-2 Omicron (n = 6).

e, f Neutralization curves of 0.3 (e), and 0.6 (f) μg inactivated virus vaccine (3 μg/vial for an adult) induced antibodies against pseudotyped and replication-deficient SARS-CoV-2 Omicron (n = 6).

g-i Neutralization curves of 1 (g), 5 (h), and 10 (i) μg S_{WT}-2P induced antibodies against

pseudotyped and replication-deficient SARS-CoV-2 Omicron (*n* =10)

j-l Neutralization curves of 1 (j), 5 (k), and 10 (l) μg $S_{Omicron}$-6P induced antibodies against pseudotyped and replication-deficient SARS-CoV-2 Omicron (*n* =10).

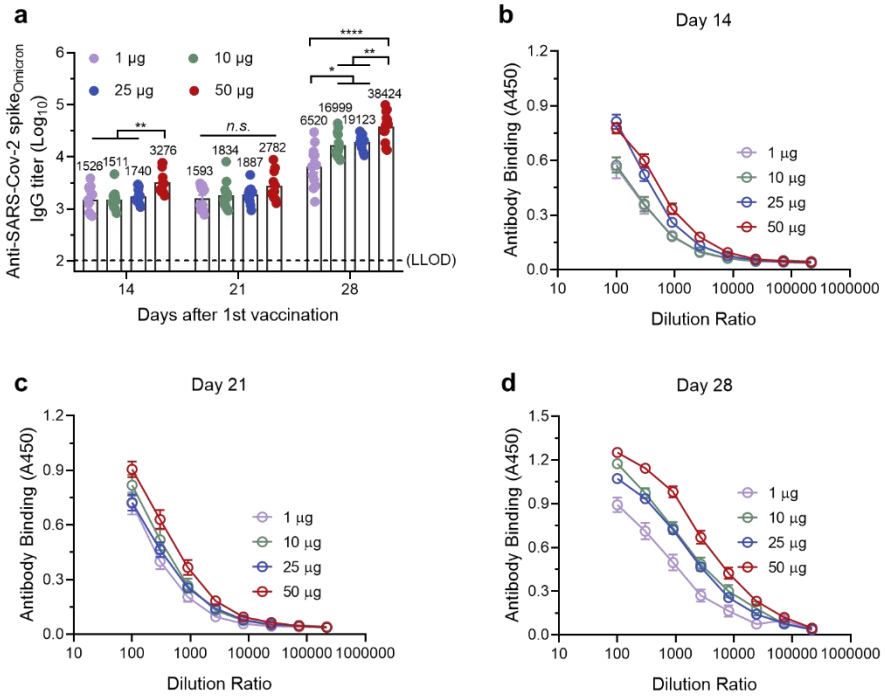
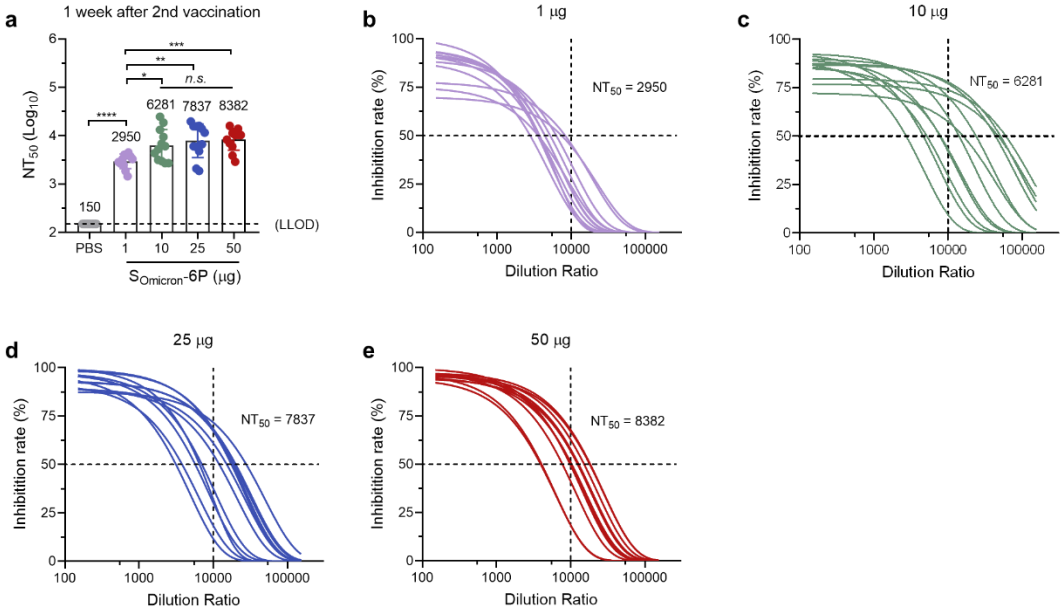


Fig. S4. S$_{Omicron}$-6P elicits binding antibodies in hamsters.

a The Omicron SARS-CoV-2 variant specific IgG antibody titers were determined by ELISA (LLOD = 100) (*n* = 12).

b-d ELISA binding curves of S$_{Omicron}$-6P induced antibodies in hamster sera on day 14 (b), day 21 (c), and day 28 (d) (*n* = 12).



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180 **Fig. S5. $S_{Omicron}$-6P induced high levels of nAbs against pseudovirus of Omicron variant in**

181 **hamsters.**

182 **a** NT_{50} values were determined by VSV-based pseudovirus (Omicron variant) neutralization

183 assay (LLOD = 150) (n = 12).

184 **b-e** Neutralization curves of 1 (a), 10 (b), 25 (c), and 50 (d) μg $S_{Omicron}$-6P induced antibodies

185 against pseudotyped and replication-deficient SARS-CoV-2 Omicron at 1 week after second

186 vaccination (n = 12).

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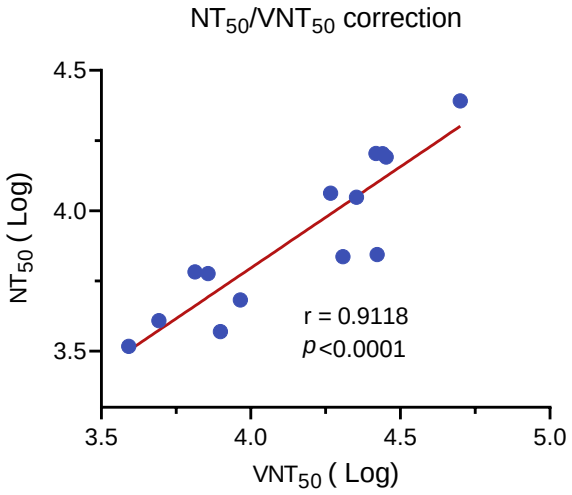

Fig. S6. Pearson correlation of VSV-SARS-CoV-2 (Omicron variant) VNT_{50} with live SARS-CoV-2 (Omicron variant) VNT_{50} for *n* = 14 random selected serum samples from hamsters immunized with $S_{Omicron}$-6P.

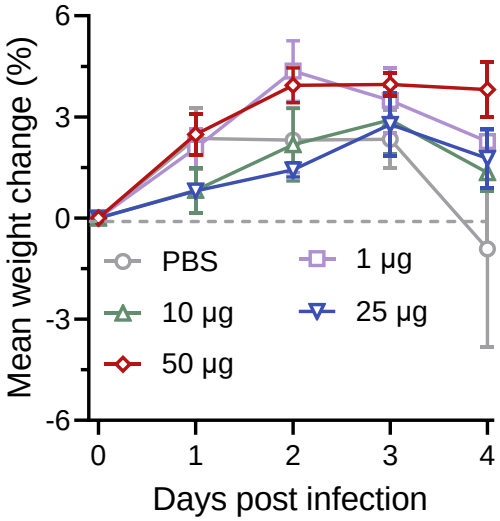

Fig. S7. Body weight change in hamsters immunized with PBS or 1, 10, 25, 50 μg $S_{Omicron}$-6P from day 0 to day 4 after challenge with Omicron (*n* = 3~4).

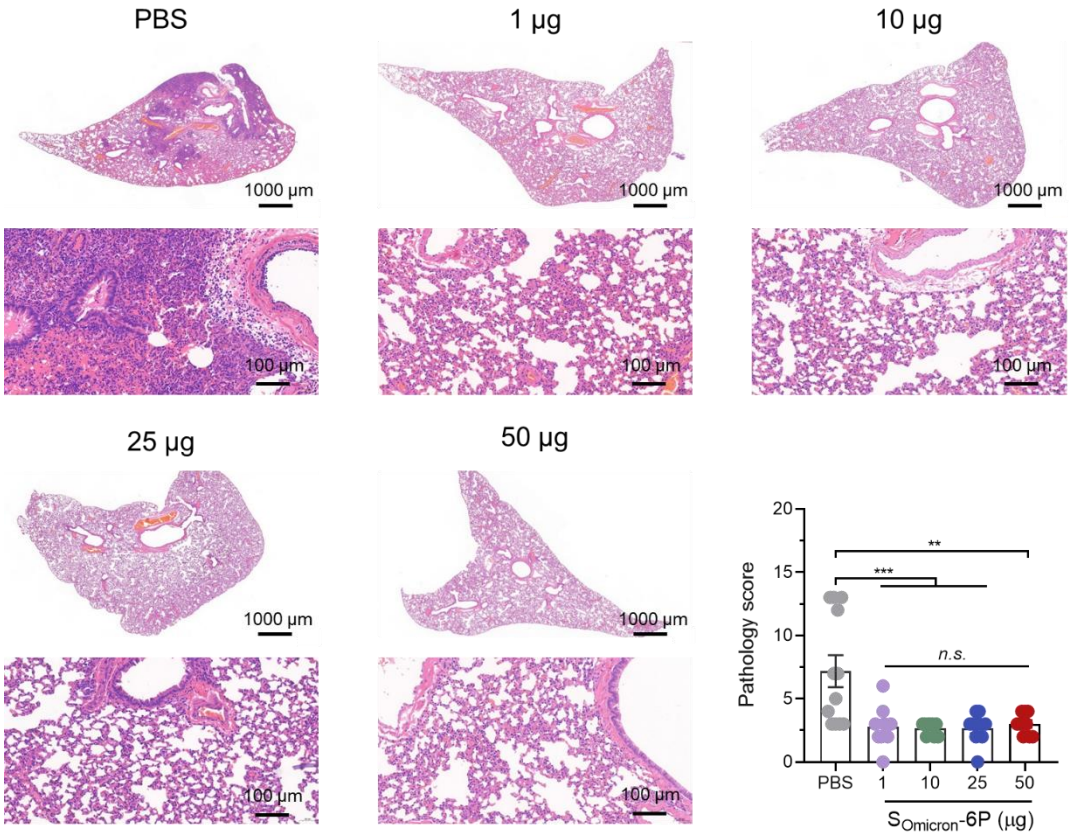

Fig. S8. Hematoxylin and eosin (H&E) staining of lung sections harvested from PBS or $S_{Omicron}$-6P (1, 10, 25, 50 μg) vaccinated hamsters after challenge with Omicron at 4 dpi. The corresponding pathology score of all lung sections from different groups ($n \geq 9$).

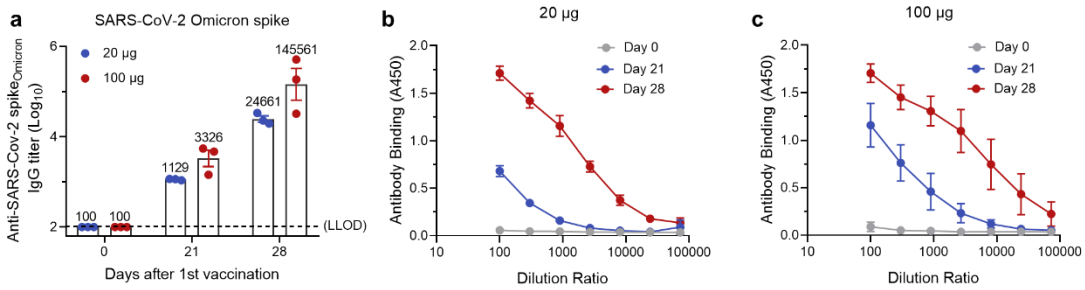

Fig. S9. $S_{Omicron}$-6P elicits binding antibodies in macaques.

a The Omicron SARS-CoV-2 variant specific IgG antibody titers were determined by ELISA (LLOD = 100) (n = 3).

b, c ELISA binding curves of 20 (b) and 100 (c) μg $S_{Omicron}$-6P induced antibodies in macaque sera on day 0, 21, 28 after the first immunization (n = 3).

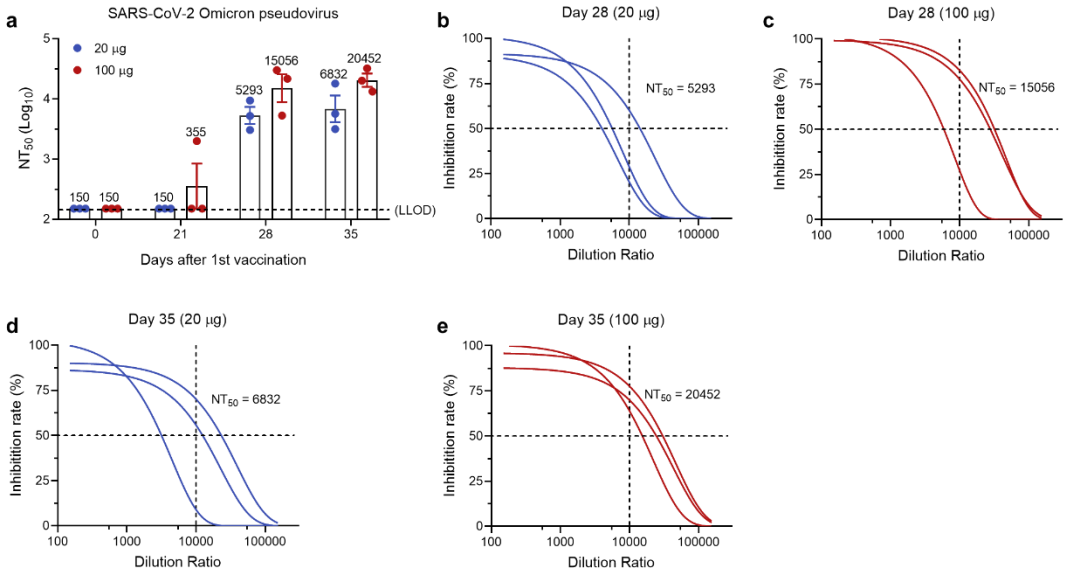

Fig. S10. $S_{Omicron}$-6P induced high levels of nAbs against pseudovirus of Omicron variant in macaques.

a NT_{50} values were determined by VSV-based pseudovirus (Omicron variant) neutralization assay (LLOD = 150) (n = 3).

b-e Neutralization curves of $S_{Omicron}$-6P induced antibodies against pseudotyped and replication-deficient SARS-CoV-2 Omicron 1 week (b, c) and 2 weeks (d, e) after the second vaccination (n = 3).

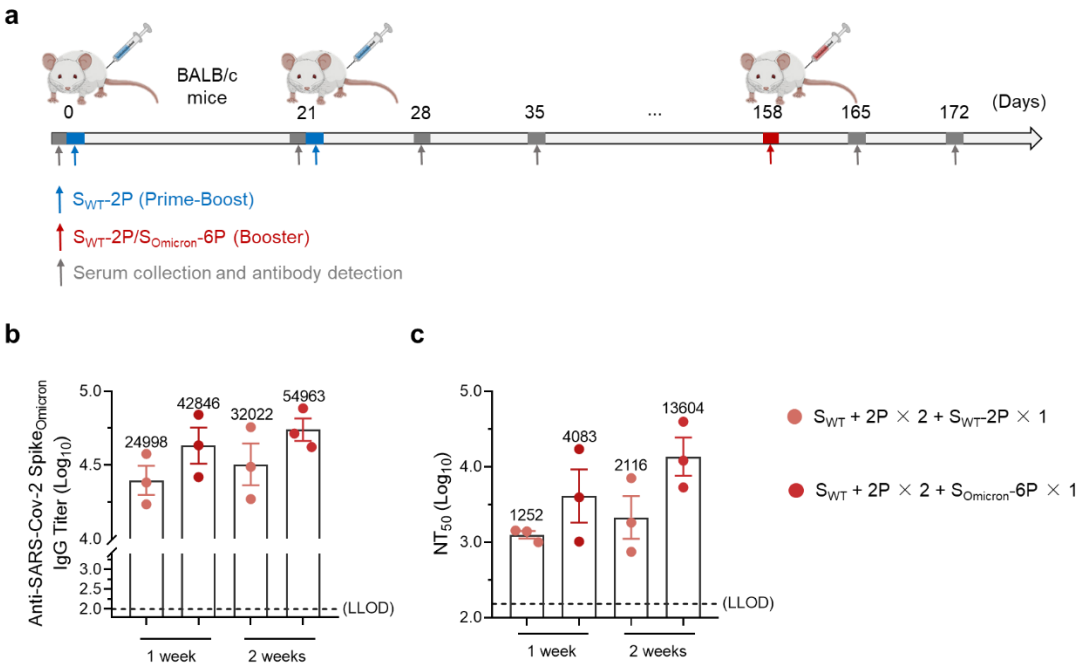

Fig. S11 Boosting WT mRNA vaccines with $S_{Omicron}$-6P increases protection immune response against the Omicron variant.

a. Schematics showing the immunization and blood sampling schedule of mice immunized with heterologous or homologous mRNA vaccines. Female BALB/c mice were first immunized with 10 μg S_{WT}-2P on day 0 and day 21. On day 158, the mice were immunized with 1 μg $S_{Omicron}$-6P or S_{WT}-2P as a booster shot.

b The Omicron SARS-CoV-2 variant specific IgG antibody titers were determined by ELISA (LLOD = 100) (n = 3).

c NT_{50} values were determined by VSV-based pseudovirus (Omicron variant) neutralization assay (LLOD = 150) (n = 3).