ADVANCED MATERIALS

Supporting Information

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Rational Development of a Polysaccharide–Protein-Conjugated Nanoparticle Vaccine Against SARS-CoV-2 Variants and *Streptococcus pneumoniae*

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Methods

Facility and ethics statement: All experiments with live SARS-CoV-2 were performed in the biosafety level 3 (BSL3) facilities and approved by the Animal Experiment Committee of Laboratory Animal Center, Beijing Institute of Microbiology and Epidemiology (approval number: IACUC-DWZX-2020-002). All animal experiments were conducted according to the Chinese animal use guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of our institute (approval number: ACU21-929).

Reagents, cells and viruses: Specific reagents were listed: Luciferase Assay System (E1501) and Passive Lysis 5× Buffer (E1941) were purchased from Promega. PMA (P1585), ionomycin (Sigma-Aldrich, 407953), and paraformaldehyde were purchased from Sigma-Aldrich. eBioscience™ Brefeldin A solution (1000×, 00-4506-51) was from Invitrogen. HRP-conjugated polyclonal Goat against monkey IgG was from Abcam. mCD4-R711/FITC (50134-R711-F), mCD8α-R208-APC (50389-R208-A) antibodies were purchased from Sino Biological. Monkey IFN-γ and IL-4 ELISpot^{PLUS} (ALP) kits (3421M-4AST-2), mouse IFN-γ ELISpot^{PLUS} (ALP), strips (3321-4AST-2), mouse IL-4 ELISpot^{PLUS} (ALP) (3311-4APW-2) were purchased from Mabtech. RBD peptides (15-mer peptides overlapping by 11 amino acids) were synthesized by Scilight-Peptide, China. Leukocyte Activation Cocktail and BD GolgiPlug™ (550583) were from BD. FBS, 10X HBSS (without Ca^{2+}, Mg^{2+}, phenol red, 14185-052), 10X HBSS (with Ca^{2+}, Mg^{2+}, without phenol red, 14065-056), and 100X GlutaMax-1 (35050-061) were purchased from Gibco. THB Broth (HB0311-3) was from Shanghai Canspec Scientific & technology Co., China. Bacto Agar (214010) and Bacto Yeast Extract (212750) were from Becton-Dickinson. Gelatin (48722-500G-F) and N, N-Dimethylformamide (DMF) (D4551) were from Sigma-Aldrich and Baby Rabbit Complement (31064-2) was from Pel-freez Biologicals, Rogers. Cell lines included: Calu-3 cells (Center for Cell Resource, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences), HEK293T, Vero E6, HL60 and CHO cells (ATCC), 293FT-ACE2

cells (SinocellTech) and Huh-7 cell (CCTCC, China). Mono-clone *S. pneumoniae* strain purchased from National Institutes for Food and Drug Control of China.

Animals: Female hACE2-KI/NIFDC C57BL/6 mice at 8 weeks of age, aged BALB/c (at 7-8 months of age) and adult BALB/c (at 8-12 weeks of age) mice were purchased from National Institute for Food and Drug Control (NICPBP, Beijing, China). Sprague-Dawley (SD) rats at 6-9 weeks of age were from Beijing Vital River Laboratory Animal Technology, China. The animals were housed in a specific pathogen-free facility until parts of them transferring into a BSL3 animal facility for virus challenge. Rhesus macaques (Macaca mulatta) at 3-5 years old were obtained from Joinn Laboratories (China) and housed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility, Beijing, China.

Protein expression and purification: The DNA fragment for the expression of RBD (RBD amino acid 319 to 531) of Wuhan-Hu-1 was amplified by PCR using a 699-bp full-length sequence as the template. The amplified DNA fragment was cloned into the plasmid of pc3-L1+SV40 polyA-R at sites of *KpnI* & *NotI* and the recombinant plasmid was characterized by DNA sequencing. Subsequently, the plasmid was transfected into Chinese hamster ovary (CHO) cells and followed by glutamine synthetase (*GS*)-based selection, a RBD stably expressing CHO cell line was established for the production of the RBD proteins.

Reduced polyacrylamide gel electrophoresis: The recombinant RBD protein was characterized by polyacrylamide gel electrophoresis on gradient gels (4%~12%) using the Novex NuPAGE SDS-PAGE (NuPAGE) gel system and a low pH buffer. The samples (5 μg each) were tested in triplicate at 200V constant voltage for 35 min. Subsequently, the gels were stained with Coomassie brilliant blue and after being decolorized, the protein bands were analyzed by a gel imaging system.

Receptor binding of RBD to human ACE2 by BLI: The binding affinity and kinetics of recombinant RBD to biotinylated human ACE2-His were determined by bio-layerinterferometry (BLI, Octet RED96e, Fortebio) performed by PALL Corporation's biomolecular interaction instrument. The assays used 5 μg/mL of biotinylated ACE2-His in PBST solution. The streptavidin probe was to capture biotinylated ACE2-His and different concentrations (52.3 nM~837 nM) of recombinant RBD protein for a reaction time of 60 seconds and dissociation time of 60 seconds. The affinity constant was analyzed using the data analysis software provided.

Preparation of pneumococcal capsular polysaccharides: S. pneumoniae serotype 14 was cultured in Hoeprich's medium at 37°C for 10-16 hours in a shaker for fermentation. When

the culture reached at OD_{600}>1.0, sodium deoxycholate was added to lyze the bacteria. After being centrifuged and ultra-filtrated, the concentrated lysate pH was adjusted to 3.5 using 36% acetic acid. After further centrifugation, chromatography and ethanol washing, the sediments were dissolved with pure water to obtain the PPS14 solution.

Nuclear magnetic resonance (NMR): ^{1}H NMR spectroscopy was performed as previously described [1]. Prior to NMR, 3 mg polysaccharides were dissolved in 99.9% deuterated water (D_2O) for exchange by lyophilization and re-suspended in D_2O for NMR data acquisition. The NMR spectra were taken at 600 MHz.

Conjugation RBD with PPS14: The conjugation procedures were conducted by reductive amination method as previously described. [2] Briefly, 100 mg of PPS14 was oxidized by 100 mg $NaIO_4$ which proceeded for 1 hour in the dark. To conjugate recombinant RBD with PPS14, RBD protein (5 mg) was mixed with PPS14 (3 mg) in Na_2HPO_4 buffer at pH 7.0, which was reacted with 0.5 μl of 5M sodium cyanoborohydride solution in rotation for 16 hours at room temperature. The mixture was further treated with 0.3 ml of 10 mg/mL sodium borohydride solution for another 1 hour to reduce any remaining aldehyde and ultra-filtrated with a cut-off molecule of 100,000 MW. The conjugated samples were further filtered with a 0.22 μm filter and stored at 4°C.

RBD and PPS14 concentration determination: RBD concentration of SCTV01B was estimated by measuring the UV absorbance at 280 nm. Extinction coefficient of SCTV01B was calculated by Vector NTI Advance™ 11.0 software. The phenol-sulfuric acid (PHS) was a rapid colorimetric method used to determine the polysaccharide content of SCTV01B[3]. Briefly, the polysaccharide content is first hydrolyzed into monosaccharide in the sulfuric acid buffer, and then dehydrated to form uronic derivative rapidly. After added with phenol, the solution formed an orange compound which was determined by colorimetry at 492 nm.

Size Exclusion Chromatography (SEC-HPLC): The purity of SCTV01B was analyzed by Size Exclusion Chromatography using Agilent 1260 with an analytic TSKgel G3000 SWXL column (Tosoh, 7.8×300mm, 5μm) and SEC buffer (200 mM disodium hydrogen phosphate, 100 mM arginine, 1% isopropanol, pH 6.5) was used as mobile phase under a 280 nm UV detector, at a flow rate of 0.5 mL/min at 25°C.

Transmission electron microscopy (TEM): The SCTV01B nanoparticle vaccine at 0.3 mg/ml of proteins were loaded on the copper net and after being dried, the samples were stained with phosphotungstic acid. The samples were observed and photo-imaged under a transmission electron microscope (Hitachi, model: H-7650).

Thermal stability assessment: The melting temperature (T_m) and aggregation temperature (T_{agg}) of SCTV01B were measured using differential scanning fluorimetry (DSF, UNCLE-0330, Unchained Labs, Pleasanton, CA). 9 μL SCTV01B solution was heated from 25 to 95 °C, with an increment of 0.3 °C/min. At each temperature, the barycentric mean (BCM) of the intrinsic fluorescence spectra was measured and plotted against temperature to generate the BCM curve. The temperature corresponding to the first peak of the first-order derivative curve was defined as Tm1, and the second as Tm2. Aggregation onset temperature ($T_{agg}266$) of each sample was calculated by Uncle Analysis software. At each temperature, the intensity of static light scattering at 266 nm (as represented by SLS 266 nm) was measured and plotted against temperature to generate an aggregation curve.

Adjuvant: Sodium citrate solution mixed with squalene (Merck) and Span 85 was homogenized by a high-pressure homogenizer (AH-PILOTATS) to obtain a uniform oil in water emulsion.

Immunogenicity analysis of SCTV01B in rodents: The hACE2 transgenic C57BL/6 (*n*=6-16/group, 8-12 week), aged BALB/c (*n*=4-27/group, 7-8 month) , adult BALB/c mice (*n*=6/group; 6-8 weeks old) and SD rats (*n*=10/group, 6-9 weeks) were intramuscularly immunized with various doses of SCTV01B (1, 3, 10 μg for hACE2 mice; 1 or 3 μg for aged BALB/c mice; 1 or 10 μg for adult BALB/c mice formulated with 2 mg adjuvant, and 20 or 60 μg for SD rats formulated with 10 mg adjuvant) and equal volume of adjuvant alone used as the negative control for three times with an interval of 21 days. Their tail vein blood samples were collected after each vaccination on schedule to prepare serum samples. At eight days (hACE2 mice) or twenty-five days (aged BALB/c) post the second immunization, these animals were euthanized and their spleens were dissected for enzyme-linked immunospot (ELISpot) and intracellular cytokine staining (ICS) assays.

To compare the immunogenicity between RBD and SCTV01B, adult BALB/c mice (*n*=8/group; 6 weeks old) were intramuscularly immunized with 1 μg RBD or SCTV01B formulated with 2 mg adjuvant, their tail vein blood samples were collected after prime and boost vaccinations. At thirteen days post boosting, these animals were euthanized and their spleens were dissected for ELISpot assay.

Immunogenicity analysis of SCTV01B in non-human primates: Equal numbers of female and male Rhesus macaques (Macaca mulatta at 3-5 years of age) were randomized into the control (*n*=2) and vaccine dose groups (*n*=6) and intramuscularly immunized with SCTV01B and 10 mg adjuvant or 10 mg adjuvant alone on day 0 and 21. Their peripheral blood samples were collected longitudinally at day 0, 14 and 21 post prime immunization and days 7, 14 and

21 post boosting to detect the RBD-specific IgG titer and PSV NT_{50}. At D0, D21 post prime dose and D7 post boosting, PBMC of these animals were isolated for ELISpot. Functional bactericidal antibodies were conducted by opsonophagocytic killing assay at 14 days post boosting.

ELISA analysis of SARS-CoV-2 RBD-specific IgG and PPS14 titer: The levels of serum SARS-CoV-2 RBD-specific IgG or PPS14-specific IgG titer in individual samples were quantified by ELISA. Briefly, individual wells in 96-well plates were coated with recombinant SARS-CoV-2 spike RBD protein (500 ng, 100 μL) or PPS14 (5 μg/μL) in Na_2CO_3 buffer (pH 9.6) overnight at 2-8℃. After being washed, these wells were blocked with 2% BSA in blocking solution at room temperature for at least 1 hour. Subsequently, the wells were added in triplicate with 100 μL of serum samples at different dilutions and incubated at room temperature for 1 hour. The serum samples from unimmunized same species of animals were used as negative controls. After being washed, the bound antibodies were detected with 100 μL of HRP-conjugated rabbit anti-mouse or anti-rat IgG $F(ab)_2$ (80 ng/mL) for 1 hour at room temperature, and developed with substrate TMB (3,3',5,5'-tetramethylbenzidine) solution. Finally, the plates were measured for absorbance at 450 nm in a microplate reader (BioTek) and the titers of each serum sample were calculated using the formula of antibody titer = the highest dilution factor with OD_{450} of 2.1-fold greater than negative control. Similar method was used to detect anti-RBD antibodies in monkey serum samples, except for using 200 ng/100 μl of recombinant RBD protein (Sino Biological) for coating, 2% casein in PBST as blocking solution. Serum samples were diluted in 0.1% casein-PBST at 1:1000 and further diluted in 1‰ serum from unimmunized animals. The secondary antibodies were 15 ng/mL of HRP-conjugated polyclonal goat anti-monkey IgG (Abcam, ab112767).

Construction of the pseudovirus: Pseudoviruses used in neutralization assays were replication-deficient vesicular stomatitis virus (VSV△G-Luc-G) with its VSV-G protein gene replaced by a luciferase reporter gene in the genome. Construction of these pseudoviruses were described previously[4]. Briefly, expression vector pCMV3-2019-nCoV-spike encoding spike protein of Wuhan-Hu-1 strain (GISAID accession: EPI_ISL_402125) or vector of variants were transfected into 293T cells and cultured in CO_2 incubator for 16-24 hours. VSV △G-Luc-G was added into the above cell supernatant by a certain multiplicity of infection (MOI). After infection of cells, supernatant was discarded and cells were washed to remove the uninfected virus. Fresh medium was added for further culture. 24-48 hours after infection, virus was collected and filtered to discard cell debris. Accession ID of VOC/VOI/VUM:

D614G (EPI_ISL_406862), B.1.1.7(EPI_ISL_764238), B.1.351(EPI_ISL_736940), P.1(EPI_ISL_792680), B.1.617.2(EPI_ISL_1999775), AY.1(EPI_ISL_2597889), AY.2(EPI_ISL_3331050), AY.3(EPI_ISL_2978186), Omicron (EPI_ISL_6640917), C.37(EPI_ISL_2756117), B.1.621(EPI_ISL_3856732), B.1.526(EPI_ISL_1588435), B.1.429(EPI_ISL_730092), B.1.617.1(EPI_ISL_1704611).

SARS-CoV-2 pseudovirus-based neutralization assay: For the quantitative measurement of the neutralizing antibody titer, vaccine immunized serum samples were treated at 56 ℃ for 30 min to inactivate. The serum samples were serially diluted, incubated with 100 or 200 $TCID_{50}$ of SARS-CoV-2 pseudovirus of Wuhan-Hu-1 or different variants at 37 ℃ for 1 hour, and co-cultured with 3×10^4 293FT-ACE2 or 2×10^4 Huh-7 cells at 37 ℃ for 20 hours. After incubation, the culture supernatant was removed and 1×Passive lysis buffer was added at 50 μL/well to lyse the cells. Relative light unit (RLU) of the cell lysate was measured to evaluate luciferase activity (LB960 Microplate Luminometer CentroXS3). The neutralizing antibody titer (50% inhibitory dilution, NT_{50}) was defined as the serum dilution at which the RLUs were reduced by 50% when compared with the positive control wells. The NT_{50} of each serum sample was calculated as the highest diluted sample that led to 50% of reduction in RLU, compared with the positive control of virus alone.

Opsonophagocytic assay: The DMF-differentiated HL60 cells in Opsonization Buffer B (OBB) were added to 96-well plate at 1×10^5 /well and 20 μL serum samples at a dilution of 1:10 or 1:50 were added. Then 20 μL Pneumococcal Polysaccharide Powder Type 14 were added to incubate for 30 minutes at RT and room air on a mini-orbital shaker (600 rpm). After incubation, 50 μL HL60/complement mixture (4:1) was added and incubated on a mini-orbital shaker (600 rpm) for 45 minutes at 37 ºC with 5% CO_2. After the incubation, the 96-well plate was placed on ice for 20 minutes. 10 μL of reaction mixture was spotted from each well onto THYA plates, and the plates were tilted to shape the spots into a small strip of fluid (~2-3 cm long). The THYA plates were leaved at RT for ~20 minutes to let the excess fluid seep into the agar. Following, the 15 mL of overlay agar containing TTC was added to THYA plate. Incubate the plates at RT for ~20 minutes. After the overlay has solidified, the THYA plates were incubated at 37℃ in 5% CO_2 incubator for 16~18 hours. After overnight incubation, the colonies (Colony Forming Unit, CFU) were counted using an automated counter and the data was analyzed.

Enzyme-linked immune absorbent spot (ELISpot) assay: The frequency of RBD-specific T cells with secretion of IFN-γ or IL-4 were quantified by ELISpot assay using IFN-γ and IL-4 ELISpot kits (Mabtech), according to the manufacturer's instruction. Briefly, splenocytes

were isolated from immunized mice, and the peripheral blood mononuclear cells (PBMCs) were isolated from immunized rhesus macaques using Ficoll-Paque density gradient centrifugation. Splenocytes and PBMCs were cultured into ELISpot plate at a density of 2×10^5 and 2.5×10^5 cells per well (100μL/well), respectively. Then, the pooled RBD peptides spanning RBD domain with a concentration of 2 μg/mL in a volume of 100 μL per well were added or not (for negative control wells). Assay plates were incubated overnight at 37ºC in a 5% CO_2 incubator and developed using substrate until distinct spots emerged. The spots were counted in the enzyme-linked spot analyzer (ImmunoSpot® S6, CTL). The number of IFN-γ and IL-4 secreting cells was obtained by subtracting the negative control number. Values below zero were presented as zero. The results were reported as spot forming cells (SFCs) per million Splenocytes or PBMCs and the data was analyzed by GraphPad Prism.

Intracellular cytokine staining: Frequencies of RBD-specific $CD4^+$ and $CD8^+$ T cells positive for two T cell activation markers (IFNγ and CD154) were measured by intracellular cytokine staining (ICS). Briefly, splenocytes were isolated from immunized mice, added into 48-well plates at a density of 3×10^6 cells/well in 200 μL, and cultured in the presence or absence (for negative control wells) of the pooled RBD peptides at the concentration of 2 μg/mL at 37°C in 5% CO_2 overnight. Leukocyte Activation Cocktail, with BD GolgiPlug™ was added, and cells were further incubated for 5 hours. Cells were washed and stained for surface markers (CD3, CD4, and CD8). After fixation and permeabilization, cells were stained with antibodies to IFNγ and CD154, washed and analyzed by flow cytometry (BD FACSCelesta).

*Mouse protection efficacy of SCTV01B :*hACE2-KI/NIFDC C57BL/6 mice (abbreviated to hACE2 mice)[5] immunized i.m. with 1 or 3 μg SCTV01B formulated with 2 mg adjuvant for three times with an interval of 21 days were randomly selected from immunogenicity study (*n*=6/each group). The control mice received PBS (*n*=12). Two months after the 3rd vaccination, individual mice were anesthetized and inoculated intranasally with 5.6×10^5 PFU in 30 μl of the Beijing isolate of SARS-CoV-2 (BetaCoV/Beijing/IMEBJ05/2020). On the 5rd day and 7th day post virus infection, the mice were euthanized. Their lung tissues were dissected for RNA extraction and histological analysis.

Similarly, the protection of SCTV01B against a lethal mouse-adapted SARS-CoV-2 strain MASCp36 was tested in aged BALB/c mice. Individual aged BALB/c mice (*n*=12) immunized i.m with 1 μg SCTV01B formulated with 2 mg adjuvant in PBS with an interval of 21 days were randomly selected from immuogenecity study. Control mice received PBS (*n*=9). One month after 2nd vaccination mice were challenged with 6×10^3 PFU of mouse-

adapted SARS-CoV-2 MASCp36 and on the 3rd day post virus infection, some mice were randomly selected from the control (*n*=3) and vaccinated groups (*n*=6) and euthanized. Their lung tissues were dissected. One lobe of each lung tissue was homogenized in AVL buffer to extract RNAs and the remaining part of the lung was fixed with 4% PFA for H&E staining. The animal behaviors and death were monitored daily for 15 consecutive days among the left mice (*n*=6 per group).

Viral nucleic acid extraction: Total viral RNA was extracted using QIAampViral RNA Mini Kit (QIAGEN), according to the manufacturer's protocol. Briefly, the tissue homogenates were mixed with AVL buffer and homogenized with stainless steel beads in a Tissuelyser-24 (Shanghai Jingxin Industrial Development) in 1 ml of DMEM and centrifuged. The supernatants were mixed with 560 μL of absolute ethanol and centrifuged. The supernatants were loaded on the unsoaked QIAamp column, centrifuged at 8000 rpm/min for 1 min, washed with AW1 buffer and AW2 buffer, followed by centrifuging. The bound RNAs were eluted with 60 μL of AVE elution buffer and centrifuged at 8000 rpm/min for 1 min. The eluents containing viral RNA were tested immediately or stored below -80°C.

Quantitative real-time polymerase chain reaction (qRT-PCR): Viral RNAs in the lung tissues from the virus-infected mice were analyzed by quantitative real-time PCR (RT-qPCR). Briefly, the extracted SARS-CoV-2 RNAs were reversely transcribed into cDNAs at 42 ℃ for 5 min using One Step PrimeScript RT-PCR Kit (Takara) and PCR-amplified using the SARS-CoV-2 specific primers and probes: CoV-F3 (5'-TCCTGGTGATTCTTCTTCAGGT-3'), CoV-R3 (5'-TCTGAGAGAGGGTCAAGTGC-3'), and CoV-P3 (5'-FAM-AGCTGCAGCACCAGCTGTCCA-BHQ1-3'). The PCR reactions were performed in duplicate at 95 ℃ for 10 sec; and subjected to 40 cycles of 95 ℃ for 5 sec, 60 ℃ for 20 sec. The virus loads were determined, according to the standard curve established by serial ten-fold dilutions of SARS-CoV-2 RNA.

Hematoxylin and eosin (H&E) staining: The collected lung tissues were fixed in 4% paraformaldehyde and paraffin-embedded. The tissue sections (3 μm) were dewaxed, rehydrated and regularly stained with H&E. The tissue sections were examined and photo imaged under a light microscope.

Supplementary Figures

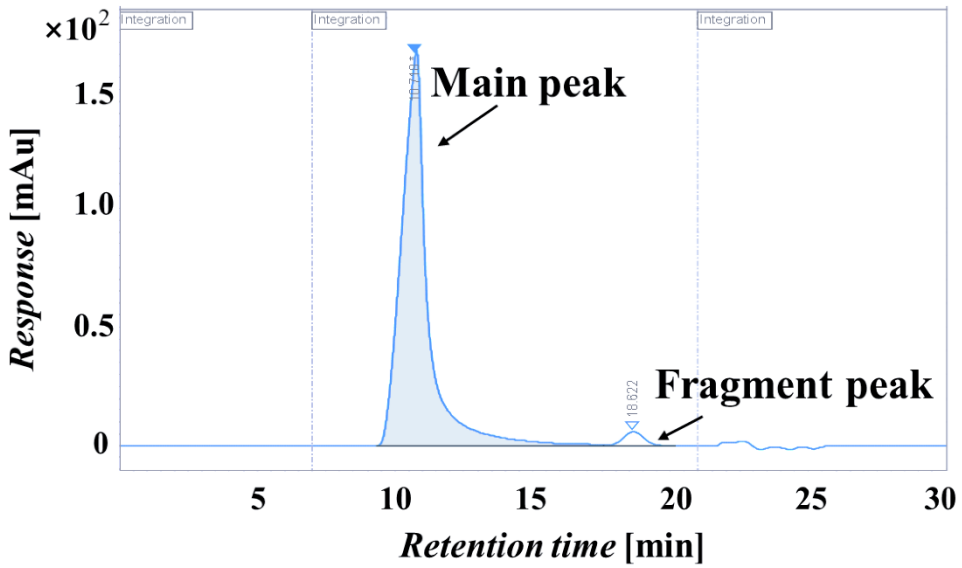

Figure S1. SEC-HPLC of SCTV01B. Main peak represented the target PPS14 conjugated components with main percentage of high molecular particles, and the fragment peak represented the residual RBD.

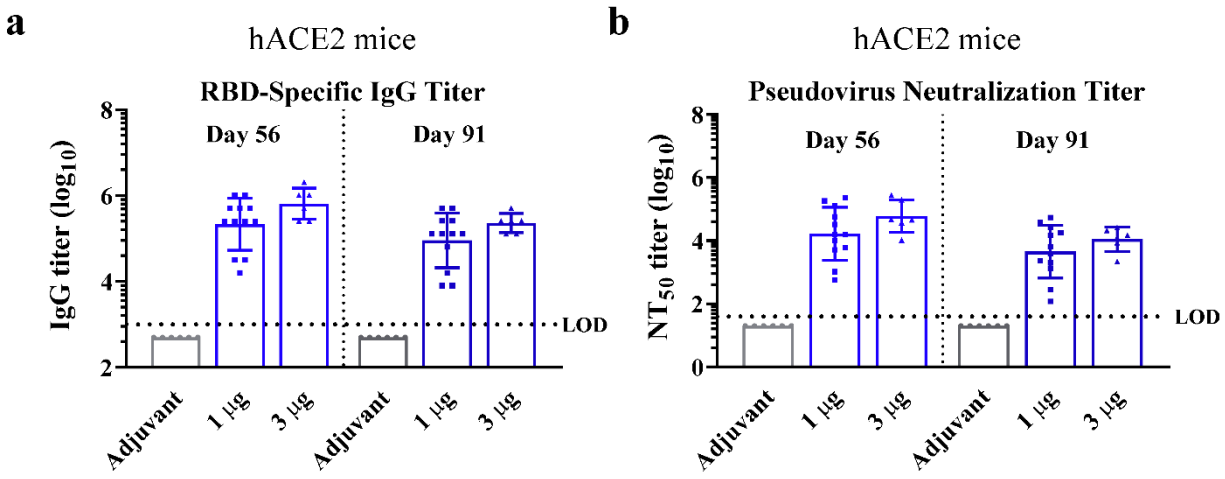

Figure S2. Durability of humoral response after vaccination of SCTV01B.hACE2 mice (*n*=6-12/group) were immunized with 1 and 3 μg of SCTV01B for 3 injections. RBD-specific antibody titer (a) and NT_{50} titer against Wuhan-Hu-1 strain (b) were monitored at 56 day and 91 day after the 3rd vaccination of SCTV01B. The dashed black lines indicate the limit of detection (LOD).

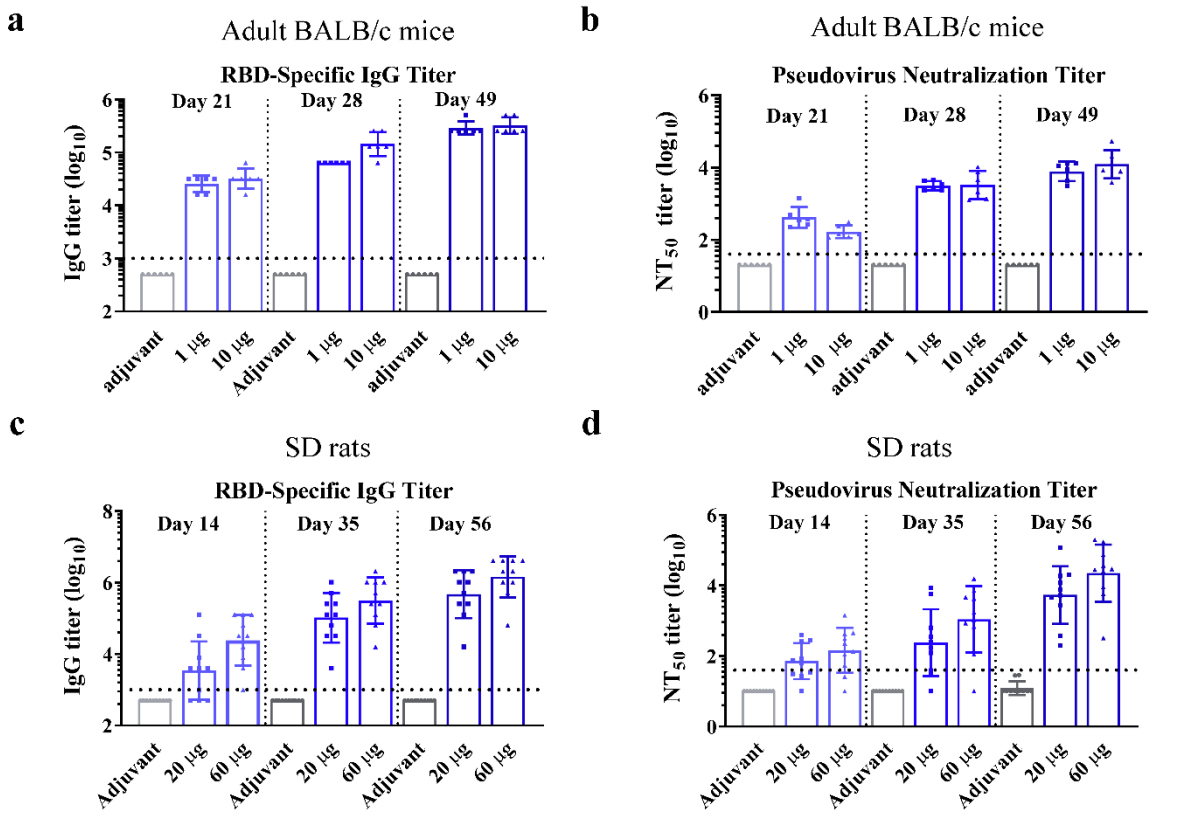

Figure S3. SCTV01B stimulated potent humoral responses in mice and rats. Adult BALB/c mice (*n*=6/group) were immunized with 1 and 10 μg SCTV01B for three doses. SD Rats (*n*=10/group) were immunized with 20 and 60 μg of SCTV01B for three doses. The serum RBD-specific antibody titer (a, c) and NT_{50} titer against Wuhan-Hu-1 strain (b, d) were analyzed at the indicated times. The dashed black lines indicate the limit of detection (LOD).

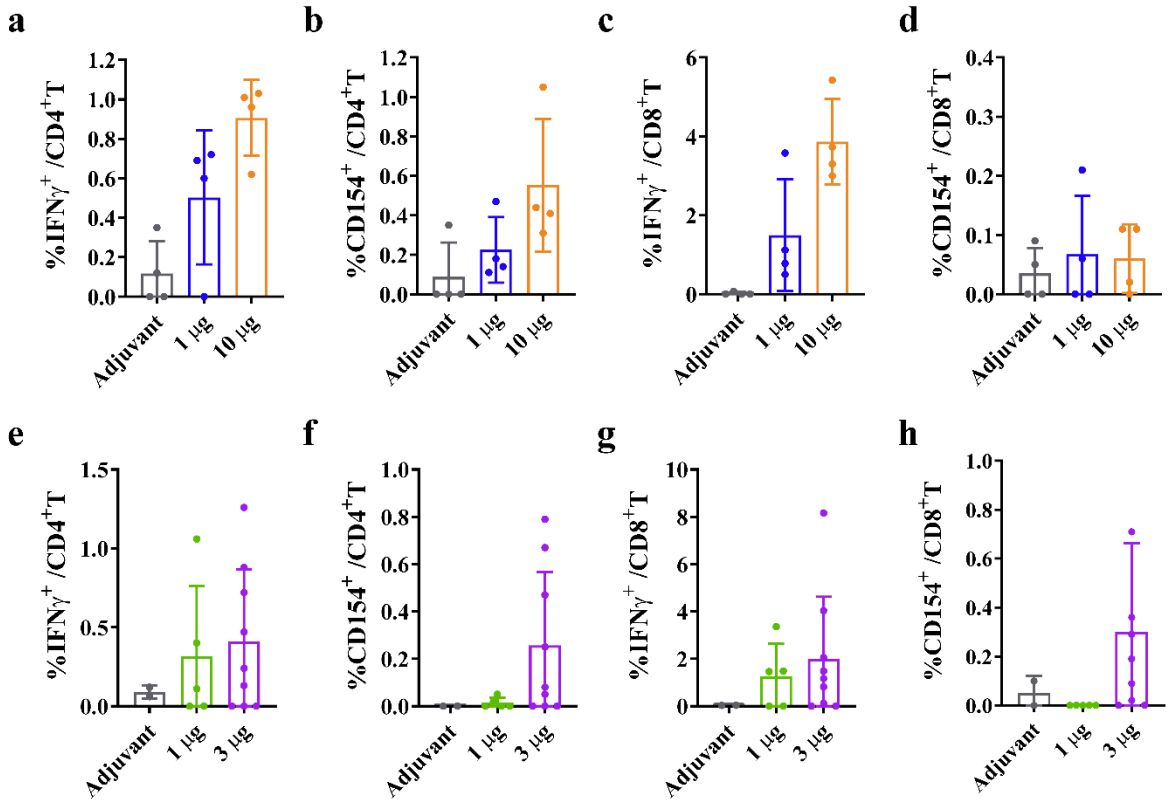

Figure S4. ICS analysis of CD4$^+$ and CD8$^+$ T cells induced by stimulation with RBD peptides

a)-d): The hACE2 mice (n=4/group) were immunized with 1 and 10 μg of SCTV01B for two doses. The percentage of RBD-specific IFNγ$^+$, CD154$^+$ CD4$^+$T (a, b) and CD8$^+$T (c, d) cells were determined by ICS. e)-h): Aged BALB/c mice (n=2-9/group) were immunized with 1 and 3 μg of SCTV01B for two doses. The percentage of RBD-specific IFNγ$^+$, CD154$^+$ CD4$^+$T (e, f) and CD8$^+$T (g, h) cells was determined by ICS.

Supplementary Table

Table S1. Anti-RBD IgG and NT_{50} titer induced by SCTV01B in hACE2 mice

	Day 21				Day 35				Day 56		
	Adjuvant	1 μg	3 μg	10μg	Adjuvant	1 μg	3 μg	10 μg	Adjuvant	1 μg	3 μg
N	16	16	16	16	12	12	12	12	6	12	6
IgG Titer											
Geomean	500	2954	5417	24675	500	67806	128000	215826	500	215269	645080
SD	/	5851	5007	30481	/	97444	83371	58312	/	345962	680530
NT_{50} Titer											
Geomean	20	58	98	451	20	2985	26262	17614	20	16593	59970
SD	/	257	151	576	/	5828	73581	11761	/	78397	106558

Table S2. Anti-RBD IgG and NT_{50} titer induced by SCTV01B in aged BALB/c mice

	Day 21			Day 35			Day 56		
	Adjuvant	1 μg	3 μg	Adjuvant	1 μg	3 μg	Adjuvant	1 μg	3 μg
N	16	27	27	16	27	27	4	10	10
IgG Titer									
Geomean	500	13368	21773	500	40317	56290	500	111430	147033
SD	/	11218	14476	/	37350	34753	/	73901	176726
NT_{50} Titer									
Geomean	20	61	69	20	1356	1920	20	16706	10825
SD	/	100	66	/	2528	2961	/	21446	45364

Table S3. IFNγ and IL-4 induced by SCTV01B in hACE2 mice

	Adjuvant	1 μg	3 μg	10 μg
N	4	4	4	4
IFNγ				
Mean	4	11	19	100
SD	/	12	21	35
IL-4				
Mean	1	13	32	66
SD	/	21	26	46

Table S4. IFNγ and IL-4 induced by SCTV01B in aged BALB/c mice

	Adjuvant	1 μg	3 μg

N	2	5	9
IFNγ			
Mean	1	32	43
SD	/	24	23
IL-4			
Mean	8	86	66
SD	/	57	56

Table S5. Viral loads of hACE2 mice vaccination with SCTV01B after virus challenge

Vaccination	PBS	SCTV01B-1μg	SCTV01B-3μg
5 dpi	8.09	6.93	5.14
7 dpi	8.26	5.72	5.14

Table S6. NT_{50} titers induced by SCTV01B against pandemic variants

Strain	Wuhan	D614G	B.1.617.2	AY.1	AY.2	AY.3	B.1.1.7	B.1.351
NT_{50}	7943	7494	3090	1791	4888	1889	4714	1019
Fold decrease (/Wuhan)	/	1.1	2.6	4.4	1.6	4.2	1.7	7.8
Strain	**P.1**	**B.1.1.529**	**C.37**	**B.1.621**	**B.1.429**	**B.1.617.1**	**B.1.526**	
NT_{50}	651	323	2165	475	3964	1031	522	
Fold decrease (/Wuhan)	12.2	24.6	3.7	16.7	2.0	7.7	15.2	

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