Supporting Information

**RBD-modified Bacterial Vesicles Elicited Potential Protective Immunity Against SARS-CoV-2**

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RBD-BBV Vaccines Showed Excellent Biocompatibility

We conducted a preliminary evaluation of the safety of RBD-BBVs in vivo. No significant fluctuation in body temperature (Figure S7A) or decrease in body weight (Figure S7B) was observed during immunization with the RBD-BBV vaccine at a dose of 5 µg. No significant tissue lesions were observed in major organs after completing RBD-BBV immunizations at 0.5 µg or 5 µg (Figure S7C). We measured the levels of serum cytokines 2 h and 24 h after immunization with 5 µg and 0.5 µg of RBD-BBVs, and no significant increase in serum IFN-γ was observed (Figure S7D). The serum level of IL-6 increased 2 h after injection of 5 µg and 0.5 µg of RBD-BBVs, but the level returned to normal at 24 h (Figure S7E). The level of TNF-α increased 2 h after injecting 5 µg of RBD-BBVs, and the level returned to normal at 24 h, while no significant increase in TNF-α was observed after injecting 0.5 µg of RBD-BBVs (Figure S7F). Because RBD-BBVs contain PAMP components, slight inflammatory responses are unavoidable. However, the response faded after 24 h. Therefore, the results suggest that RBD-BBVs have excellent biocompatibility.

Materials and Methods

Ethics and Biological Safety

All animal procedures were approved by the animal care and use committee at the Institute of Medical Biology of the Chinese Academy of Medical Sciences, and every effort was made to minimize animal suffering. All work with infectious SARS-CoV-2 was performed with approval (by the Institutional Biosafety Committee of Institute of Medical Biology (IMB)) under biosafety level-3 (BSL3) conditions. The BSL3 facilities were designed to conform to the safety requirements recommended by the China National Accreditation Service for Conformity Assessment (CNAS) and the National Health Commission (NHC) of the People’s Republic of China (PRC). The facility and laboratory safety plans were approved for use by the NHC and CNAS. Experiments with infectious viruses were performed in a certified Class IIB biosafety cabinet in BSL3.

Bacteria, Mice, Cell Lines, and Viruses

*E. coli* BL21 was incubated in Luria-Bertani (LB) media (1% tryptone, 0.5% yeast extract, and 1% NaCl) at 37°C under shake culture conditions. Female 6- to 8-week-old SPF BALB/c mice were obtained from the Laboratory Animal Center of the Institute of Medical Biology of the Chinese Academy of Medical Sciences. None of the mice were involved in any other experiment, and all mice were maintained in an SPF laboratory animal facility at the Institute of Medical Biology of the Chinese Academy of Medical Sciences. Vero E6 African green monkey kidney cells (KCB 92017YJ) were obtained from the Conservation Genetics CAS Kunming Cell Bank (Kunming, China) and maintained in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin (BI) at 37°C in the presence of 5% CO2. A clinical SARS-CoV-2 isolate was propagated in Vero E6 cells; amplified SARS-CoV-2 was confirmed via qRT-PCR, sequencing, and TEM and titrated via a plaque assay (10^5 pfu/mL)^2.

Generation and Analysis of RBD-BBVs

The DNA sequences encoding the recombinant protein containing the SARS-CoV-2 RBD (Asn331-Val524; YP_009724390) and ClyA (AAL55667) were obtained by direct synthesis. The RBD gene was linked to the 3’ end of the ClyA gene and cloned into the pThioHisA plasmid by gene engineering
technology. Then, positive plasmids were identified through restriction enzyme analysis and sequencing. The recombinant plasmids were transformed into E. coli BL21 and cultured in LB media. When the optical density at a wavelength of 600 nm (OD_{600}) of the bacterial suspension reached 0.4-0.6, isopropyl-β-d-1-thiogalactopyranoside (IPTG, Solarbio) was added at 1 mM, and the expression of the recombinant ClyA-RBD protein was induced overnight at 30°C. On the following day, 2 mM Na_2EDTA (Sigma) was added, and the incubation was continued for 2 h. Bacteria were collected after centrifugation at 14,000×g for 30 min at 4°C. Wild-type cells and E. coli BL21 cells overexpressing the recombinant ClyA-RBD protein were resuspended in Hank’s balanced salt solution (HBSS) buffer (Servicebio). A high-pressure homogenizer (APV-2000) was used to drive the bacteria through gaps for budding (conditions: 200, 400, 800, and 1200 bar, three times, 4°C). The sample that passed through gaps was centrifuged at 6,000×g for 30 min at 4°C to pellet the bacteria, and the supernatant was collected. The supernatant was ultracentrifuged at 100,000×g for 30 min at 4°C. The pellet was resuspended in HBSS, and RBD-BBVs were purified through gradient centrifugation using OptiPrep™ (STEMCELL) media. The gradient levels from top to bottom were 45%, 35%, 30%, 25%, 20%, 15%, and 10% OptiPrep™/HBSS and RBD-BBVs. Centrifugation was carried out at 130,000×g for 3-4 h, and sample layers were collected. Samples were stained using a Fast Silver Stain Kit (Beyotime), and the RBD load in the BBV sample was analyzed with Image Lab software (Bio-Rad). The BBV yield was analyzed using the Bradford protein assay kit (Sangon Biotech). The particle diameter of BBVs was measured using NTA (NTA LM-2000) was used to drive the bacteria through gaps for budding (conditions: 200, 400, 800, and 1200 bar, three times, 4°C). The sample that passed through gaps was centrifuged at 6,000×g for 30 min to hydrolyze proteins exposed on the membrane surface. After incubation, the sample was placed on ice, and phenylmethylsulfonyl fluoride (PMSF) (Meilunbio) was added to a final concentration of 1 mM to stop the reaction. Proteinase K-treated samples were analyzed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot using rabbit anti-SARS-CoV-2 S1 polyclonal antibody (Sino Biological, 1:2000) and goat anti-rabbit IgG-HRP (Sino Biological, 1:10,000) as the primary and secondary antibodies, respectively.

Preparation of Bacterial Membranes by Sonication

E. coli BL21 cells overexpressing the recombinant ClyA-RBD protein were resuspended in HBSS buffer. The cells were sonicated for 5 and 10 min (15% power, sonication time: 10 s, interval: 10 s) and then centrifuged at 6,000×g for 30 min at 4°C to remove bacteria. The supernatant was ultracentrifuged at 100,000×g for 30 min at 4°C. The pellet was resuspended in HBSS as the bacterial membrane sample.

Generation and Analysis of RBD-OMVs

The OMVs were prepared as previously described. Briefly, expression was induced as documented above, and the supernatant after expression induction was filtered through a 0.45-μm membrane (Millipore). The filtrate was concentrated by ultrafiltration using a 500-kDa column (Millipore), and the concentrate was ultracentrifuged at 120,000×g for 2 h at 4°C. The vesicle pellet was resuspended in phosphate-buffered saline (PBS) and washed twice with PBS before additional filtration through a 0.45-μm membrane. The filtrate underwent density gradient centrifugation as described above, and the purified RBD-OMV sample was analyzed via SDS-PAGE and Western blot as previously described.

Transmission Electron Microscopy (TEM)
TEM was carried out as described previously. Briefly, the sample was mounted on a carbon-coated copper grid, and negative staining was performed using 0.75% (w/v) uranyl formate. The sample was then observed and imaged under a transmission electron microscope (Hitachi).

**DNA Electrophoresis**

The bacterial nucleic acids in the RBD-BBV, RBD-OMV, and RBD-whole cell (WC) samples were extracted with a Wizard Genomic DNA purification kit (Promega). Nucleic acid loading buffer (TaKaRa) was added to the samples, and then the samples were loaded onto a 1% agarose gel containing ethidium bromide. Electrophoresis was carried out at 120 V for 30 min, and images were obtained using a gel imaging system (CexdocXR+, Bio-Rad).

**Structure Modeling**

The structural model of the recombinant ClyA-RBD protein was built using MODELLER 9.24. The templates used for the modeling were the crystal structures of *E. coli* hemolysin E (PDB ID: 1QOY; 2.0 Å resolution) and the SARS-CoV-2 RBD extracted from a Fab-RBD complex (PDB ID: 6YLA; 2.4 Å resolution). The sequence identities of the templates 1QOY and 6YLA (chain E) to the corresponding portions of ClyA-RBD are 98.7% and 100.0%, respectively, thus ensuring the quality of the constructed model. Specifically, the alignment between the target and templates was obtained using the ‘align2d()’ command in MODELLER, followed by a manual check and adjustment to avoid errors at the junction between ClyA and the RBD. Ten 3D models of the target were then built using the ‘automodel’ module, with each model being optimized and refined by a slow library schedule protocol and a slow molecular dynamics simulated annealing level, respectively. Finally, the structural model with the lowest discrete optimized protein energy (DOPE) score was selected as a representative.

**Determination of the Binding of RBD-BBVs to hACE2**

As discussed earlier, the osmotic shock method was used to obtain periplasmic components, and the ClyA-RBD monomer was purified. SARS-CoV-2 S1-Fc (Sino Biological) and RBD-BBV (2 µg/ml) were produced using 50 mM Na2CO3-NaHCO3 coating buffer, and the samples were added to 96-well flat-bottom plates. Then, His-tagged hACE2 protein (Sino Biological, 2 µg/ml) was added. After incubation at 37°C for 1 h, HRP-conjugated antibodies against the His tag (Sino Biological, 0.2 µg/ml) were added, followed by incubation at RT for 1 h. After 1.0 M H2SO4 was added to stop the reaction, color development was performed using TMB (Solarbio) for 10 min. The OD450 was measured using a microplate reader (Thermo).

**Neutralizing Antibody Binding Assay for RBD-BBVs**

The SARS-CoV-2 spike neutralizing antibodies MM43, MM57, R001, R004, D001, and D002 were developed and provided by Sino Biological. Different concentrations of neutralizing antibodies were added to ELISA plates, and each well contained 100 ng of SARS-CoV-2 S1-His protein (Sino Biological) or RBD-BBV. The RBD content in RBD-BBV was calculated based on RBD load%. HRP-conjugated antibodies against human IgG, mouse IgG, and rabbit IgG (Sino Biological, 1:10,000) were used as the secondary antibodies to detect the binding of neutralizing antibodies to the antigens. TMB (Solarbio) was used to observe the reaction. After the reaction was terminated with H2SO4, a microplate reader was used to measure the OD450.
Live Imaging *In Vivo*

RBD-BBs and the SARS-CoV-2 recombinant spike RBD (Sino Biological) were labeled with Cy7-NHS (Amersham Biosciences). Excess free dye was removed with a Sephadex G-25 PD 10 desalting column (GE Healthcare) and ultracentrifugation. The protein and Cy7 concentrations were determined by measuring the absorbance at 280 nm and 747 nm. Cy7-labeled RBD or RBD-BBs (5 µg) were administered subcutaneously into mice at the tail base. Fluorescent signals were obtained at 0 h, 6 h, 12 h, 24 h, and 48 h after injection with the In Vivo FX PRO imaging system (Bruker) using a 760-nm excitation filter and 790-nm emission filter. Twelve hours after subcutaneous injection of Cy7-labeled RBD or RBD-BBs, inguinal lymph nodes of mice were collected and imaged with an In Vivo FX PRO imaging system (Bruker). Molecular imaging software was used to determine the radiant efficiency and relative fluorescence intensity.

Analysis of Cell Types for Antigen Uptake in Lymph Nodes

Twelve hours after 5 µg of Cy7-labeled RBD or RBD-BBs were injected subcutaneously into mice, the inguinal lymph nodes of the mice were collected and treated with 0.5 mg/ml collagenase I (Sigma) at 37°C for 1 h. Lymphocytes were isolated from the lymph nodes by passage through a 70-µm cell strainer (FALCON) and centrifuged at 500 xg for 5 min. The collected cells were stained with anti-mouse CD11c and CD11b (BioLegend) monoclonal antibodies, incubated at 4°C for 30 min, and centrifuged to remove excess antibodies. The cells were analyzed with a CytoFLEX flow cytometer (Beckman Coulter), and data analysis was performed using FlowJo software.

Isobaric Tags for Relative and Absolute Quantitation (iTRAQ)-based Quantitative Proteomics Analysis

iTRAQ-based quantitative proteomic analysis was performed as previously described. Briefly, *E. coli* BL21 whole cells and BBVs were prepared according to a previously described method. Whole cells and BBV proteins were digested with trypsin (Promega) at 37°C overnight. The digested peptides were labeled with iTRAQ reagents (Applied Biosystems) according to the manufacturer’s instructions. The labeled sample was cleaned up with cation exchange chromatography using the Cation Exchange Cartridge System. Next, peptides were preseparated by strong cation exchange chromatography (SCX). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was performed with an Orbitrap Elite spectrophotometer (Thermo Scientific) in higher-energy collisional dissociation (HCD) mode. The data were analyzed with Proteome Discoverer™ 2.2 software (Thermo, USA).

Microarray Analysis

Twelve hours after 5 µg of RBD-BBs were injected subcutaneously into mice, the inguinal lymph nodes of the mice were collected, and RNA was isolated as described previously. Two hundred nanograms of total RNA was amplified using the Low RNA Input Fluorescent Labeling Kit (Agilent Technologies). Cyanine 3-CTP or Cyanine 5-CTP (Perkin Elmer) was directly incorporated into the cRNA during *in vitro* transcription. A total of 200 ng of cyanine-labeled RNA was cohybridized at 60°C for 17 h and subsequently washed according to the Agilent standard hybridization protocol (Agilent Oligo Microarray Kit, Agilent Technologies). Slides were scanned with a confocal laser scanner (Agilent Technologies). Fluorescence intensities on scanned images were quantified, and the values were corrected for the background level and normalized.
**DC Uptake**

Femurs and tibiae were isolated from mice. Bone marrow cells were flushed out of the bones and treated with red cell lysis buffer (Solarbio). Cells were cultured in RPMI 1640 complete media (with 10% FBS and 1% penicillin-streptomycin) supplemented with GM-CSF (20 ng/ml) and IL-4 (20 ng/ml) (PeproTech). After Dio (Beyotime)-labeled RBD-BBVs and BBVs (0.05 mg/ml) were incubated with BMDCs for 4 h, staining was performed using anti-mouse CD11c monoclonal antibodies (BioLegend) followed by nuclear staining with DAPI (Meilunbio). Imaging was performed with the ImageXpress Micro Confocal system (Molecular Devices).

**Phenotype Analysis of DCs**

After incubation of BBV and RBD-BBV samples (0.05 mg/ml) with BMDCs for 24 h, staining was performed using anti-mouse CD11c, CD86, CD80 (BioLegend) monoclonal antibodies, and the cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter). The levels of IL-6 and IL-1β in the stimulated supernatants were measured with mouse IL-6 and IL-1β ELISA kits (Invitrogen) according to the manufacturer’s instructions.

**Analysis of Lysosomal Escape**

After Dio (Beyotime)-labeled RBD-BBV and BBV samples (0.05 mg/ml) were incubated with BMDCs for 12 h and 24 h, cellular lysosomes were stained with LysoTracker Red (Beyotime) according to the manufacturer’s instructions. Additionally, the incubated cells were fixed in 4% paraformaldehyde for 30 min, followed by membrane permeabilization with 0.2% Triton X-100 for 15 min. Then, rabbit anti-SARS-CoV-S1 polyclonal antibodies (Sino Biological, 1:200) and goat anti-rabbit IgG-FITC antibodies (Proteintech, 1:50) were added, and lysosome staining was carried out with LysoTracker Red (Beyotime) according to the manufacturer’s instructions. Imaging was performed with the ImageXpress Micro Confocal system (Molecular Devices).

**Animal Immunity and the Humoral Immune Response**

Samples containing 5 µg and 0.5 µg of BBVs, samples containing 5 µg and 0.5 µg of RBD-BBVs mixed with alum (Thermo Scientific), and the alum control were injected subcutaneously into BALB/c mice on days 0, 14, and 28. Blood samples were collected 7 days after immunization, and antibody responses were analyzed by ELISA. Briefly, each well of a flat-bottom 96-well plate (Corning) was coated with 100 ng of SARS-CoV-2 S1-Fc (Sino Biological) and then coated with diluted serum at 37°C for 1 h. Incubation with biotinylated antibodies against mouse IgG (Invitrogen, 1:5,000), IgM (Invitrogen, 1:20,000), IgG1 (Biolegend, 1:8,000), and IgG2a (Biolegend, 1:8,000) was carried out at 37°C for 1 h, followed by incubation with streptavidin-HRP (Biolegend, 1:3,000) at RT for 45 min. Color development was carried out with the addition of TMB (Solarbio) for 10 min; then, 1.0 M H₂SO₄ was added to stop the color development reaction. The OD₄₅₀ was measured using a microplate reader (Thermo). The titer of specific antibodies against SARS-CoV-2 spike protein was determined with serially diluted serum samples.

**Antibody Blocking of S1-hACE2 Binding**

Diluted mouse serum was added to a 96-well plate precoated with 40 ng/ml SARS-CoV-2 S1-Fc protein (Sino Biological) overnight at 4°C and incubated at 37°C for 1 h. His-tagged hACE2 protein (Sino Biological, 2 µg/ml) was added, followed by incubation for 1 h at 37°C. After incubation with an HRP-
conjugated anti-His antibody (Sino Biological, 0.2 µg/ml) at RT for 1 h, color development was carried out with TMB (Solarbio) for 10 min, and the reaction was stopped with the addition of 1.0 M H$_2$SO$_4$. The OD$_{450}$ was measured with a microplate reader (Thermo).

SARS-CoV-2 Neutralization Assay

Neutralization of SARS-CoV-2 was performed as previously described$^{10}$. Briefly, the experiment was carried out in a biosafety level-3 (BSL3) facility. Vero E6 cells were seeded in a 96-well plate and cultured overnight. One hundred TCID$_{50}$ (50% tissue culture infectious dose) of SARS-CoV-2 was preincubated with an equal volume of inactivated diluted serum for 1 h at 37°C; then, the mixture was added to Vero E6 cells. On the third day after infection, the cytopathic effects (CPEs) were observed under a microscope. The neutralizing titer was defined as the reciprocal of the serum dilution required to neutralize 50% of viral infection (EC$_{50}$).

Cellular Immune Response and Memory T Cells

Lymphocytes isolated from mice were cultured in a 96-well plate (1×10$^6$ per well). After the addition of 2 µg/ml SARS-CoV-2 S1-Fc protein (Sino Biological), incubation was carried out at 37°C and 5% CO$_2$ in a humidified incubator for 48 h. After centrifugation at 500xg, the cells were analyzed via flow cytometry using anti-mouse CD3, CD4, CD8, CD44, and CD62L monoclonal antibodies (BioLegend). Mouse GzmB, IFN-γ, and IL-2 ELISA kits (Invitrogen) were used to determine cytokine levels in the cell culture media. To stain intracellular cytokines, cells were incubated with 5 µg/ml brefeldin A (BioLegend) for 5 h. Then, surface staining was performed with anti-mouse CD3, CD4, and CD8 (BioLegend) monoclonal antibodies. Cells were analyzed with a fixation/permeabilization kit (BD Biosciences), followed by staining with an anti-mouse IFN-γ monoclonal antibody (BioLegend). Data were collected by a CytoFLEX flow cytometer (Beckman Coulter), and data analysis was performed using FlowJo software.

Enzyme-linked Immunospot Assay (ELISpot)

ELISpot assays were performed using a mouse IFN-γ ELISpot$^{\text{PLUS}}$ kit (MABTECH) according to the manufacturer’s instructions. Briefly, 3×10$^5$ lymphocytes were seeded in each well of an ELISpot plate and stimulated with 2 µg/ml SARS-CoV-2 S1-Fc protein (Sino Biological). After incubation in a humidified incubator at 37°C and 5% CO$_2$ for 16 h, the cells were removed, and detection antibodies were added. After incubation for 2 h at RT, the cells were washed, and streptavidin-HRP was added, followed by incubation at RT for 1 h. Then, TMB substrate was added, and the reaction was stopped with the addition of deionized water when spots were observable. Spots were counted with a colorimetric/fluorescent dual detection ELISpot reader (AID).

Biocompatibility Analysis of RBD-BBVs

After the immunization procedure described above, the body weight and body temperature of each mouse were measured every second day. On the 35th day after immunization, heart, liver, spleen, lung, and kidney tissues were collected, fixed in 10% formalin, and subjected to hematoxylin and eosin (H&E) staining followed by pathological examination. Images of tissue sections were obtained with a microscope (TS2, Nikon). Samples containing 0.5 µg/50 µl and 5 µg/50 µl RBD-BBVs were subcutaneously injected into BALB/c mice, and control BALB/c mice were injected with 50 µl of PBS.
Serum samples were collected 2 h and 24 h after injection. Cytokine levels in the serum samples were measured using mouse IL-6, IFN-γ, and TNF-α ELISA kits (Invitrogen).

Statistical Analysis
Statistical analyses were performed using Prism 8.0 (GraphPad software). Comparisons among multiple groups were performed using one-way ANOVA followed by Tukey’s multiple comparison posttest. Comparisons between two groups were performed using unpaired Student’s t-tests. P values < 0.05 were considered significant (*P < 0.05, **P < 0.01 and ***P < 0.001), and “ns” indicates no significance.
Figure S1. Pathway analysis of differential proteins between BBVs and whole bacteria was performed using the KEGG database. The signaling pathways associated with upregulated or downregulated proteins are shown on the left and right, respectively.

Figure S2. Structural model of the ClyA-RBD recombinant protein obtained using the homology modeling method. The domains of the RBD and ClyA are colored blue and green, respectively, and the linker between these two domains is colored red.
Figure S3. Microarray analysis was used to analyze the immune response caused by RBD-BBVs in lymph nodes. (A) Gene Ontology (GO) bar graph. The Y axis in the figure is -$\log_{10}(P$ value); the higher the bar graph height is, the smaller the corresponding $P$ value. (B) KEGG bubble chart. The X axis in the figure represents the enrichment degree, and the Y axis represents the enrichment pathway. A larger dot in the figure corresponds to more genes in the pathway, and the color of the bubble changes from purple-blue-green-red. A smaller enrichment $P$ value corresponds to greater significance.
Figure S4. Analysis of the antibody subtype response induced by RBD-BBV. (A) The levels of IgG1, (B) IgG2a, and (C) IgM against SARS-CoV-2 spike protein in sera after 50× dilution were determined with ELISA, and the results are presented as OD_{450} values (n=3).

Figure S5. Representative photos of the neutralization experiment. (A) Schematic diagram of antibody blocking of the invasion of Vero cells by live SARS-CoV-2. (B) Antisera were diluted 64-fold and used to block the invasion of Vero cells by live SARS-CoV-2. Images of randomly selected fields are shown.
Figure S6. RBD-BBVs can stimulate virus-specific cellular immunity in the lungs. (A) Flow cytometry analysis of the ability of RBD-BBVs to induce SARS-CoV-2-specific CD4+ and (B) CD8+ T cells in the lungs of mice (n=3). (C) Representative flow cytometry images of SARS-CoV-2 S1-specific Th1 cells (CD4+IFN-γ+) and (D) CTLs (CD8+IFN-γ+) induced by RBD-BBVs (n=3). (E) Representative images from the ELISpot analysis of the number of lung lymphocytes secreting IFN-γ after stimulation with S1-Fc protein following vaccine immunization. Statistical analysis of the number of spots from the ELISpot analysis of the lungs (n=3). (F) ELISAs of the functional cytokines GzmB and (G) IFN-γ (n=3) and (H) pro-T cell proliferation cytokine IL-2 (n=3) secreted by lymphocytes of immunized mice after stimulation with S1-Fc of SARS-CoV-2. (I) Statistical analysis of the flow cytometry results for CD4+ TCM cells (CD4+CD44hiCD62Llo) and (J) CD8+ TCM cells (CD8+CD44hiCD62Llo) (n=3) in the lungs and (K) CD4+ TEM cells (CD4+CD44hiCD62Llo) and (L) CD8+ TEM cells (CD8+CD44hiCD62Llo) (n=3) in the lungs.
Figure S7. The RBD-BBV vaccines showed excellent biocompatibility. (A) Body temperature and (B) body weight measurements of mice after immunization (3 doses) with 5 µg of RBD-BBVs and BBVs. (C) Pathological examination results for major organs 7 days after three immunizations with RBD-BBVs at doses of 0.5 µg and 5 µg. Representative images are shown. (D) ELISA assessment of the serum levels of IFN-γ, (E) IL-6, and (F) TNF-α at 2 h and 24 h after mice were injected with 0.5 µg and 5 µg of RBD-BBVs (n=3).
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