

# Supplementary Materials

## S2 Peptide-Conjugated SARS-CoV-2 Virus-Like Particles Provide Broad Protection against SARS-CoV-2 Variants of Concern

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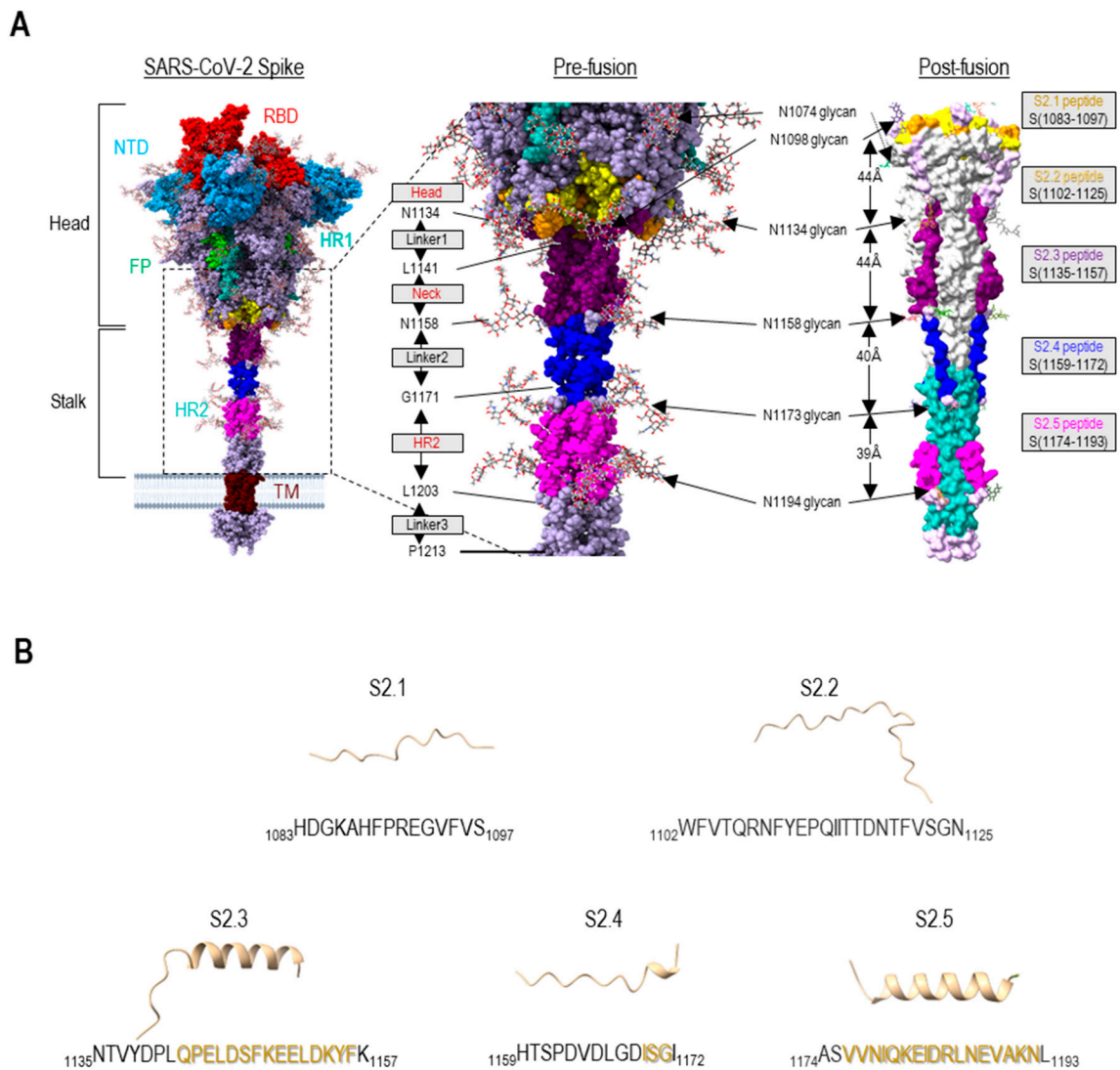
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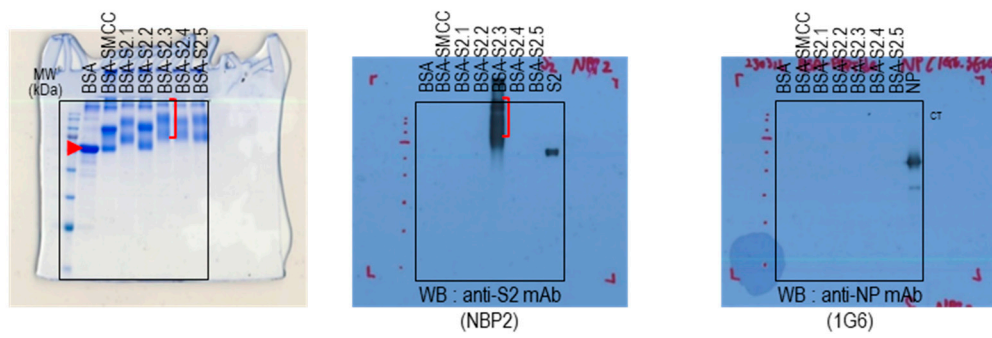
<sup>6</sup> College of Medicine, Chungbuk National University, Cheongju, Chungcheongbuk-do, Republic of Korea; goldjdj@cbnu.ac.kr (D.J.K.)

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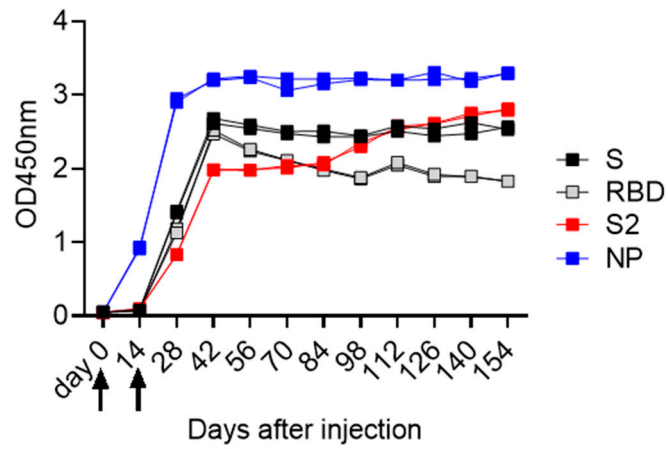


Prediction of S2 peptide structure with AlphaFold2 [AlphaFold2.ipynb - Colaboratory \(google.com\)](https://colab.research.google.com/github/alphafold/alphafold2.ipynb)

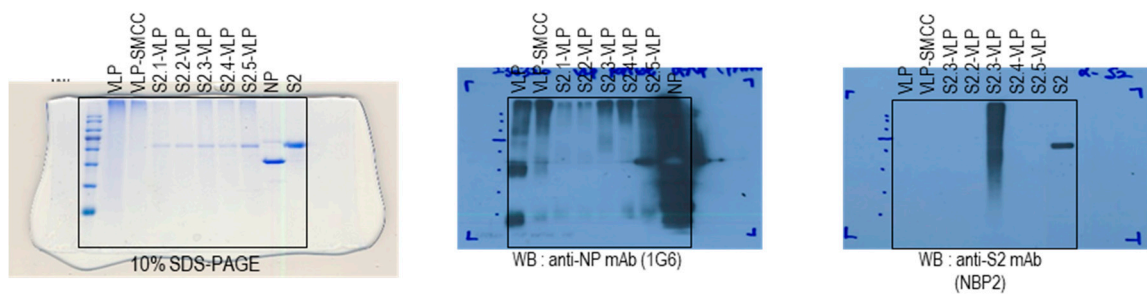
**Figure S1.** Structural features of the SARS-CoV-2 S2 antigenic peptides used in this study. **(A)** Modeled structure of the full-length glycosylated spike in its pre- and postfusion states as determined by Cryo-EM (PDB ID 6VSB and 6XRA, respectively). The head, linker, and neck domains in the prefusion state structure were annotated based on a previous study [25]. In the postfusion state, five N-glycans were arranged on one side of the 6-helical bundle structure, with approximately 40 Å of space between these glycans. Five peptide sequences corresponding to the regions between N-glycans (S2.1, S2.2, S2.3, S2.4, and S2.5) were selected as antigenic epitope candidates, which are depicted in distinct colors. **(B)** Prediction of the S2 peptide structure was performed using AlphaFold2 protein structure prediction software. The structure of each S2 peptide, corresponding to the sequences provided, was predicted using AlphaFold2 ([AlphaFold2.ipynb-Colaboratory \(google.com\)](https://colab.research.google.com/github/alphafold/alphafold2.ipynb)), and molecular graphics were visualized using the molecular visualization program UCSF ChimeraX. The helical structures of each peptide are highlighted using bold brown characters.



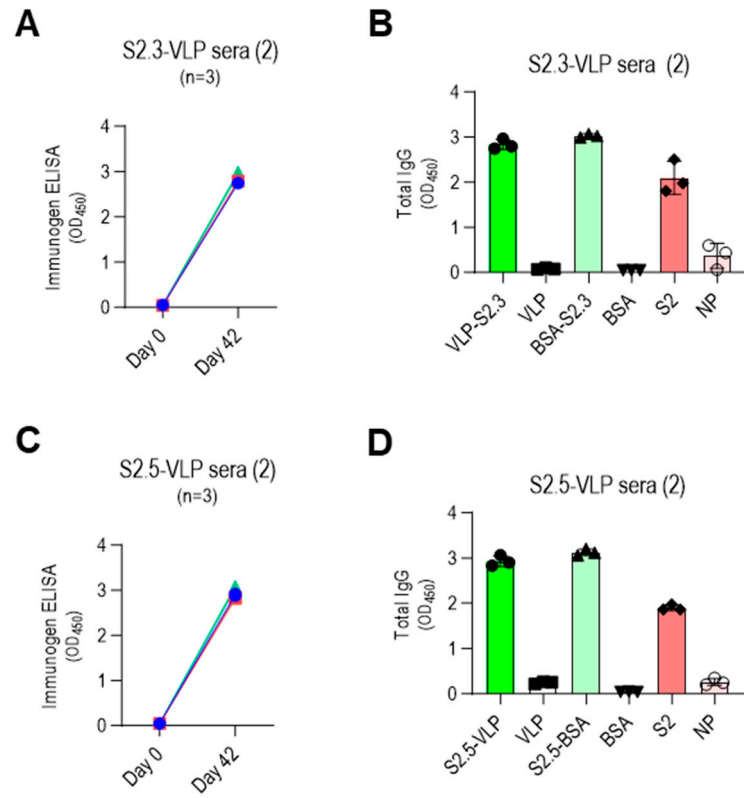
**Figure S2.** The original images of SDS-PAGE and western blots of Figure 1B.



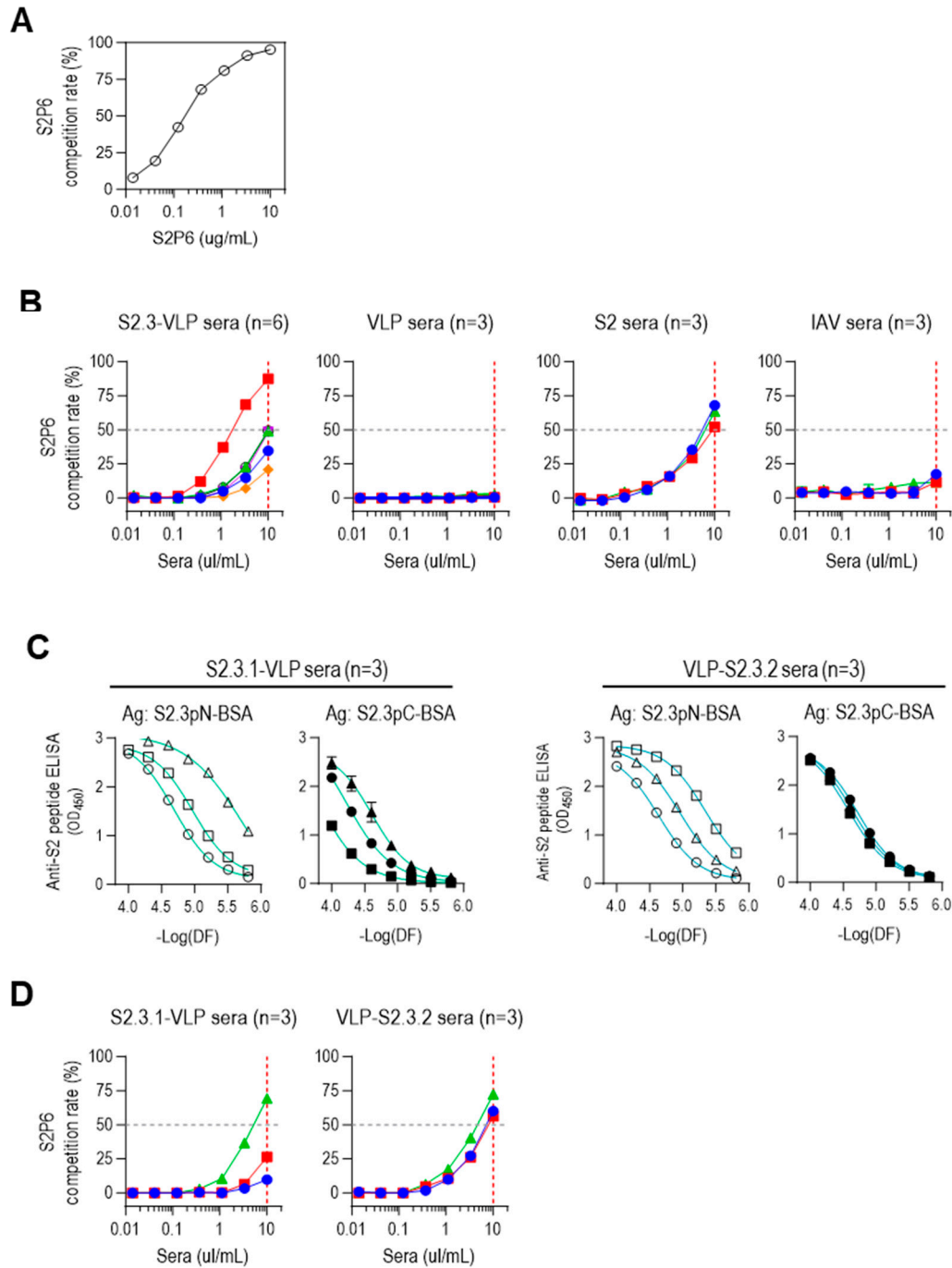
**Figure S3.** Total IgG response in sera from mice immunized with inactivated SARS-CoV-2 (IAV). Antisera from immunized mice ( $n = 3$ ) were collected at specified time points after immunization. Antibody responses against recombinant SARS-CoV-2 antigens, including S, S2, NP, and RBD, were examined. Each dot represents the measurement of an individual serum, and the graph displays the mean  $\pm$  standard deviation (SD) of replicate wells.



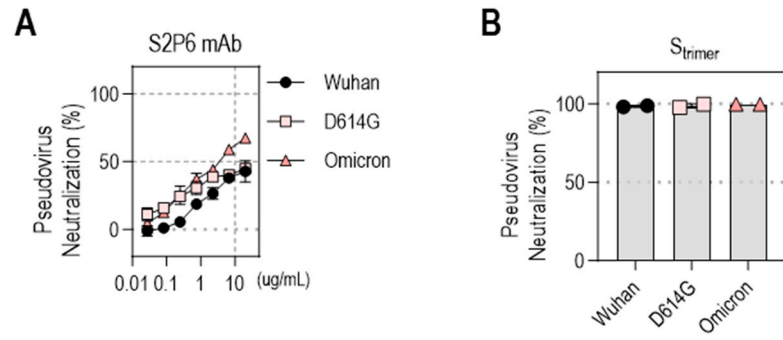
**Figure S4.** The original images of SDS-PAGE and western blots of Figure 2B.



**Figure S5.** Specific total IgG responses to immunization with S2.3- or S2.5-conjugated VLP. After confirming the immune responses to S2-VLPs (Figure 2F), additional immunization with S2.3- or S2.5-VLPs was performed following the protocol presented in Figure 2E. After the second boost, sera were collected, and ELISA was performed for immunogens (A, C) or SARS-CoV-2 antigens (B, D). Each dot represents the antibody response of individual mice to S2 peptide-VLP immunization and displays the mean  $\pm$  SD of replicate wells.



**Figure S6.** S2P6-like activity of S2.3 peptide-conjugated VLP-immunized sera. **(A)** Standard reaction of a competitive ELISA for quantifying S2P6-like activity. The recombinant S2 antigen was coated on a MaxiSorp plate. Serially diluted S2P6 antibody solutions were added to the well along with 10 ng HRP-conjugated S2P6. After incubation, S2P6-HRP activity was measured, and the S2P6 competition rate (%) was determined. **(B)** S2P6-like activity of immunized sera. Each plot represents the measurement of an individual mouse ( $n = 6$  or  $3$ ) and displays the mean  $\pm$  SD of replicate wells. **(C)** S2.3.1-VLP or VLP-S2.3.2 were immunized following the immunization regimen ( $n = 3$ /each immunogen), as shown in Figure 2E. The reactivity of antisera after the second boost against S2.3pN or S2.3pC epitopes was analyzed using serial dilutions. **(D)** S2P6-like activity of S2.3.1-VLP or VLP-S2.3.2-immunized sera. Each plot represents the measurement of an individual mouse and displays the mean  $\pm$  SD of replicate wells.



**Figure S7.** SARS-CoV-2 variant-neutralization potential of antisera against S-related antigens. **(A)** The neutralization potential of a broadly neutralizing S2-specific monoclonal antibody S2P6 was assessed against the pseudotyped SARS-CoV-2 prototype (Wuhan) or variants (D614G and Omicron BA2). S2P6 showed a pseudovirus neutralization potential of approximately 50% at a concentration of 20  $\mu$ g/mL without significant differences in virus variant types. **(B)** The neutralization potential of antisera from mice immunized with S<sub>trimer</sub> ( $n = 2$ ). The neutralization potential was evaluated using a pseudotyped SARS-CoV-2 prototype (Wuhan) or variants (D614G and Omicron BA2) and 20  $\mu$ L of serum collected on day 42 after immunization, with triplicate measurements for each sample. Each symbol displays the neutralization potential of each serum, and the values represent the mean  $\pm$  SD. The luminescence value of the pseudovirus infection without sera was used as the negative control.