Vaccination against SARS-CoV-2 Using Extracellular Blebs Derived from Spike Protein-Expressing Dendritic Cells

Jee Young Chung[1], Melissa N. Thone[1], Jenny E. Davies[2], Johannes S. Gach[3], D. Huw Davies[2], Donald N. Forthal[3], and Young Jik Kwon[1,4,5,6*]

[1]Department of Pharmaceutical Sciences, University of California, Irvine, CA 92697, United States; [2]Vaccine Research and Development Center, Department of Physiology and Biophysics, University of California, Irvine, CA 92697, United States; [3]Division of Infectious Diseases, Department of Medicine, University of California, Irvine, CA 92697, United States; [4]Department of Chemical and Biomolecular Engineering, University of California, Irvine, CA 92697, United States; [5]Department of Biomedical Engineering, University of California, Irvine, CA 92697, United States; [6]Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697, United States.

*Corresponding author:

Young Jik Kwon, Ph.D.
132 Sprague Hall, Irvine, CA 92697
Tel: +1 949 824 8714; Fax: +1 949 824 4023
E-mail: kwonyj@uci.edu

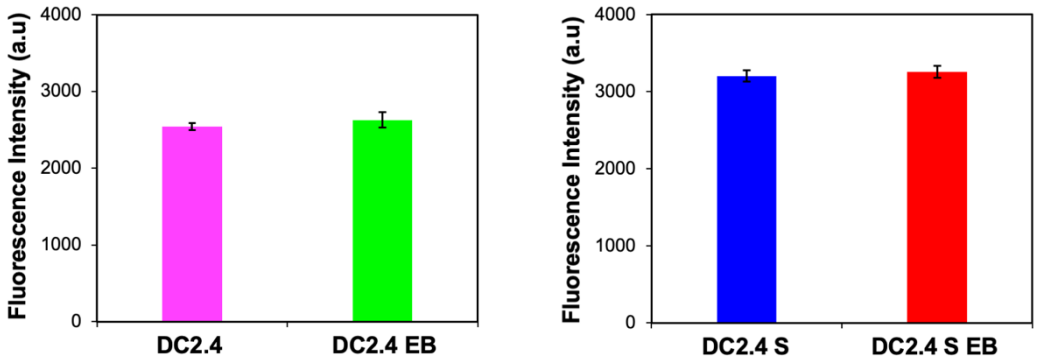

Figure S1. **Surface area of DC2.4 and DC2.4 S cells and their extracellular blebs (EBs).** The cells were labeled with PKH26 before blebbing. After isolation and purification, EBs produced from 5×10^5 DC2.4 and DC2.4 S cells were lysed and quantified by the fluorescence of PKH26 at 570 nm Em/590 nm Ex. The EBs showed similar fluorescence intensity to their parent cells, indicating highly preserved membrane conversion.

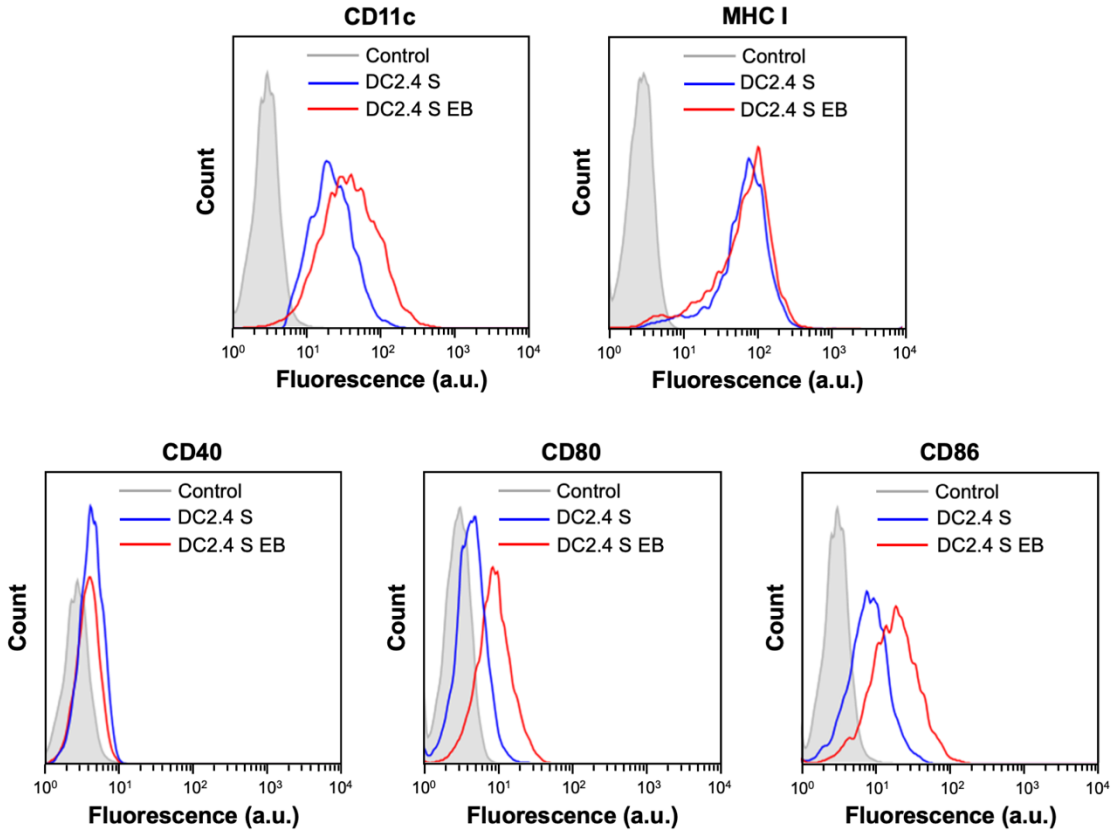

Figure S2. Surface marker analysis of DC2.4 S cells and DC2.4 S EBs. DC2.4 cells were transduced using a SARS-CoV-2 spike protein (S)-expressing lentivirus and used for the preparation of DC2.4 S EBs. Both DC2.4 S cells and DC2.4 S EBs were labeled for CD11c, MHC I, CD40, CD80, and CD86 (Biolegend, CA, USA) by staining with fluorescently labeled antibodies before analysis by flow cytometry. Notably, the expression of CD11c, CD80, and CD86 was slightly higher on DC2.4 S EBs than on the DC2.4 S cells.

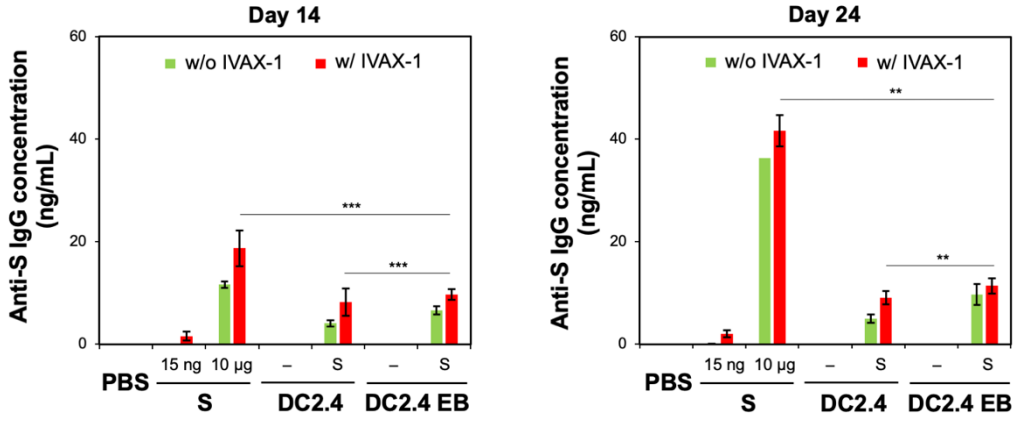

Figure S3. IgG production with or without an adjuvant, IVAX-1. The level of IgG against S in the plasma collected from mice vaccinated with PBS, S (15 ng per mouse; an equivalent S amount to that of DC2.4 S EBs), S (10 µg per mouse; a conventional dose), 2.5×10^5 DC2.4 cell, 2.5×10^5 DC2.4 S cells, DC2.4 EBs, and DC2.4 S EBs, where EBs with an equivalent surface area to 2.5×10^5 cells were used, with or without IVAX-1. The mice were immunized twice 14 days apart (prime and booster shots), and plasma were collected at the time of the booster shot (Day 14), and 10 days after the booster shot (Day 24). Data are presented as mean ± SD (n=5 per group). ** $p < 0.01$, *** $p < 0.001$ by one-way ANOVA with Tukey's post hoc test.

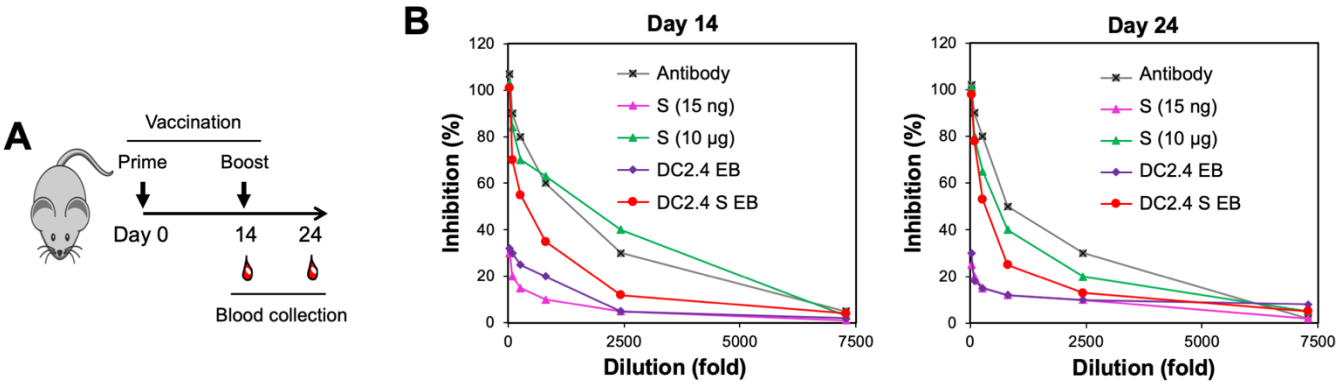

Figure S4. Production of IgG neutralizing S-pseudotyped lentivirus. (A) Plasma was collected at the time of the booster (Day 14) and 10 days after the booster (Day 24) from the mice vaccinated with PBS, free SPK (15 ng and 10 µg), DC2.4 and DC2.4 S cells, and DC2.4 and DC2.4 S EBs (n=5), without adjuvants, prior to (B) neutralization of S-pseudotyped lentivirus. Despite the noticeable difference in IgG amounts in the plasma collected from the mice vaccinated with S at a high dose (10 µg per mouse) and DC2.4 S EBs in Day 24 (**Fig. S3**), they demonstrated comparable capabilities of virus neutralization. This indicate that EBs produce IgG with a higher neutralization capability than free proteins.