SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

ELISA assay to detect S1-specific antibody responses. The S1-specific binding assays were coated with 100 ng/well of the SARS-CoV-2 spike S1-His Recombinant Protein (Sino Biological) using high-binding 96-well plates (Santa Cruz Biotechnology). After incubation at 4°C overnight, and 1hr. blocking with 300 μL of 2% sodium casein in 1X PBS, the concentrated BAL samples (with a series of 2-fold dilutions starting from an IgA or IgG concentration of 2 μg/mL) or nasal swab samples, or serially diluted serum samples (4-fold starting from a 1:150 dilution) were applied in duplicate. After incubation at room temperature for 1 hr., the plates were washed four times. Subsequent steps of incubation with HRP-labeled secondary antibody and TMB substrate were followed as described before. For IgG and IgA binding assay, Goat Anti-Monkey IgG (alpha-chain specific)-HRP conjugate (1:5,000 dilutions, *Alpha Diagnostic*) and were used, respectively, as a secondary antibody. Area under the curve, endpoint titer, and half-maximal binding titers were calculated by GraphPad Prism 8 software with sigmoidal nonlinear regression. Dimeric IgA in BAL and nasal swabs was measured using DuoSet ELISA Ancillary Reagent Kit 2 (R&D Systems) as described before (20). 100 ng/well of the SARS-CoV-2 spike S1 protein was coated and blocked. Original BAL samples or nasal swab flow-through from vaccinated and naïve animals were added in duplicate to the plates, followed by adding mouse anti-rhesus J chain [CA1L_33e1_A1a3] antibody (1:1000 dilutions, *NIH nonhuman primate reagent resource*), and Goat anti-mouse IgG-HRP conjugate (1:10,000 dilutions, *R&D Systems*). Each step was followed by 1 hr. incubation at room temperature and five washes.

Intracellular cytokine staining assay. SARS-CoV-2-specific T cells were measured from BAL and PBMC samples by flow cytometric intracellular cytokine analysis, as previously described (20,

47, 48). Briefly, 2 μg/ml of SARS-CoV-2 S1 overlapping peptide pools (PepTivator SARS-CoV-2 Prot_S1, and PepTivator SARS-CoV-2 Prot_S B.1.351 Mutation Pool /WT reference Pool, from *Milteny Biotech Inc.)* was incubated with PBMC and BAL cell samples at 37°C 5%CO_2 overnight in the presence of 0.15 μg/ml of brefeldin A. Negative and positive controls were stimulated with medium-only (no S1 protein) or with cell activation cocktail with PMA (20.25 pM) and ionomycin (335 pM) and 0.15 μg/ml of brefeldin A (Biolegend). Cells were stained with viability dye (Invitrogen) and the following antibody mixtures: PE-Cy7-CD3, BV605-CD4, APC-Cy7-CD8, Alexa Fluor® 700-CD45 were from BD Biosciences, FITC-CD28, Pe-Cy5-CD95, BV711- TNFα, IFNγ-PE or -PerCP, Alexa Fluor® 647-IL4, BV785-IL2, BV421-IL-17A, BV785-CD14, BV421-CD16 were from Biolegend; PE-IL13 was from Miltenyi Biotech. Detailed antibody information is listed in the previous publication (20). Data acquisition and analyses were performed using an LSRII flow cytometer with 4 lasers (BD Bioscience) and FlowJo software (Becton Dickinson).

Figure S1. Humoral immune responses against SARS-CoV-2 spike protein 1 (S1) in vaccinated macaques. (a). the kinetics of S1-specific binding IgG titers in BAL and nasal swabs. The data has been normalized to total IgG in BAL and Nasal swabs separately. Bars indicate geometric means of half-maximal binding titers and means of AUC. (b). S1-specific IgA and dimeric IgA responses in nasal swabs (NS) and BAL samples. The data has been normalized to total IgA in BAL and Nasal swabs separately. Paired t-tests were used to compare the humoral responses after the booster. WA: WA1/2020 D614G SARS-CoV-2 strain; Wu: Wuhan original strain; Beta: B.1.351 variant. The dashed lines indicate the detection limits. Data are shown as mean ± SEM. Blue color indicates the S1 protein or the virus from Wuhan or WA strain, and magenta color indicates from beta variant.

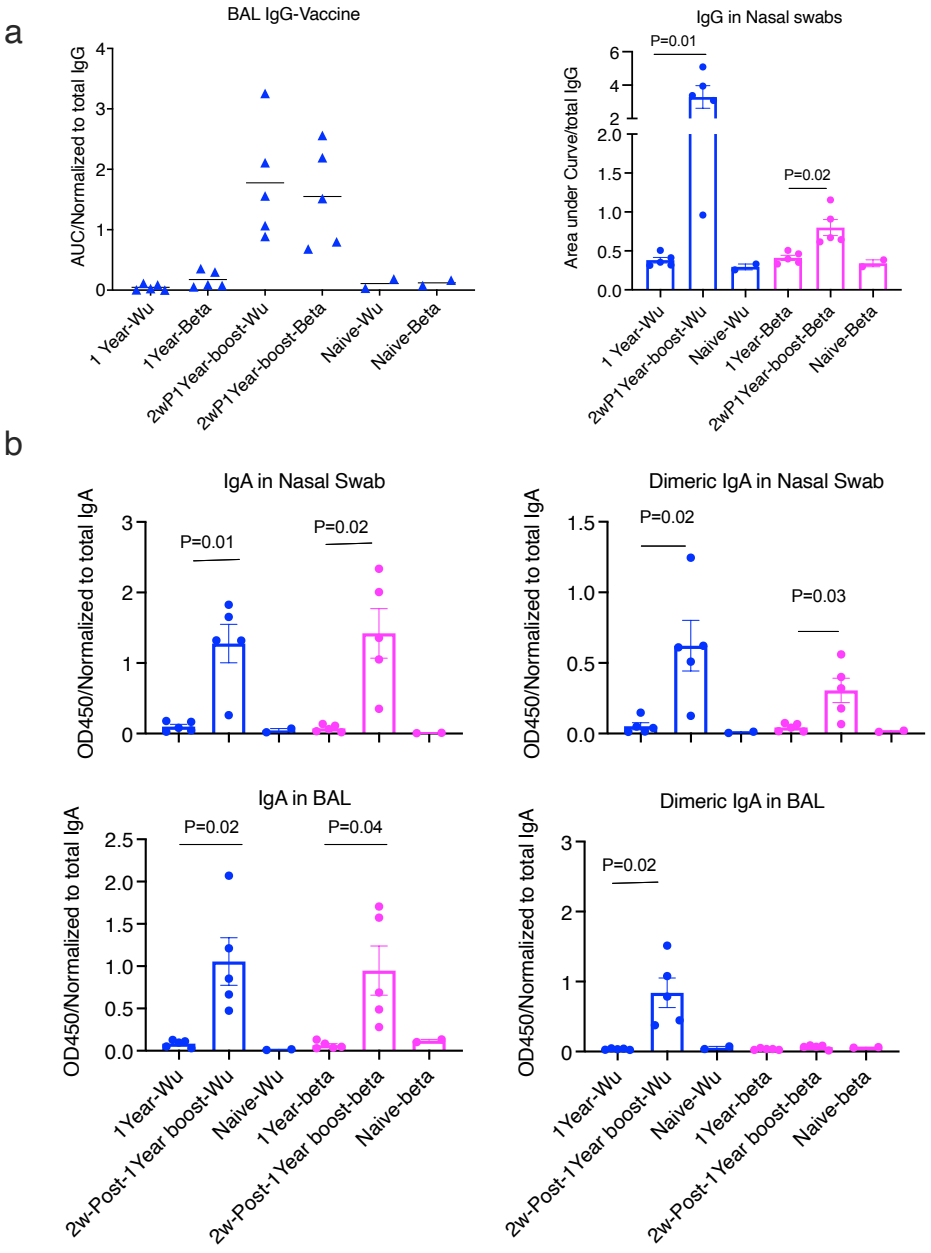

Figure S2. SARS-CoV-2 spike protein 1 (S1)-specific binding antibody responses against original strain and Omicron variant in serum, BAL and nasal swab samples collected at 2-week post one year booster. S1-specific IgG, IgA and dimeric IgA responses against original strain (Wu) and Omicron variant in serum and mucosal samples were measured by ELISA. Paired t-tests were used to compare the humoral responses after the booster. Bars indicate geometric means of half-maximal binding titers. Wu: Wuhan original strain; Omicron: B.1.1.529 variant. Data are shown as mean ±SEM.

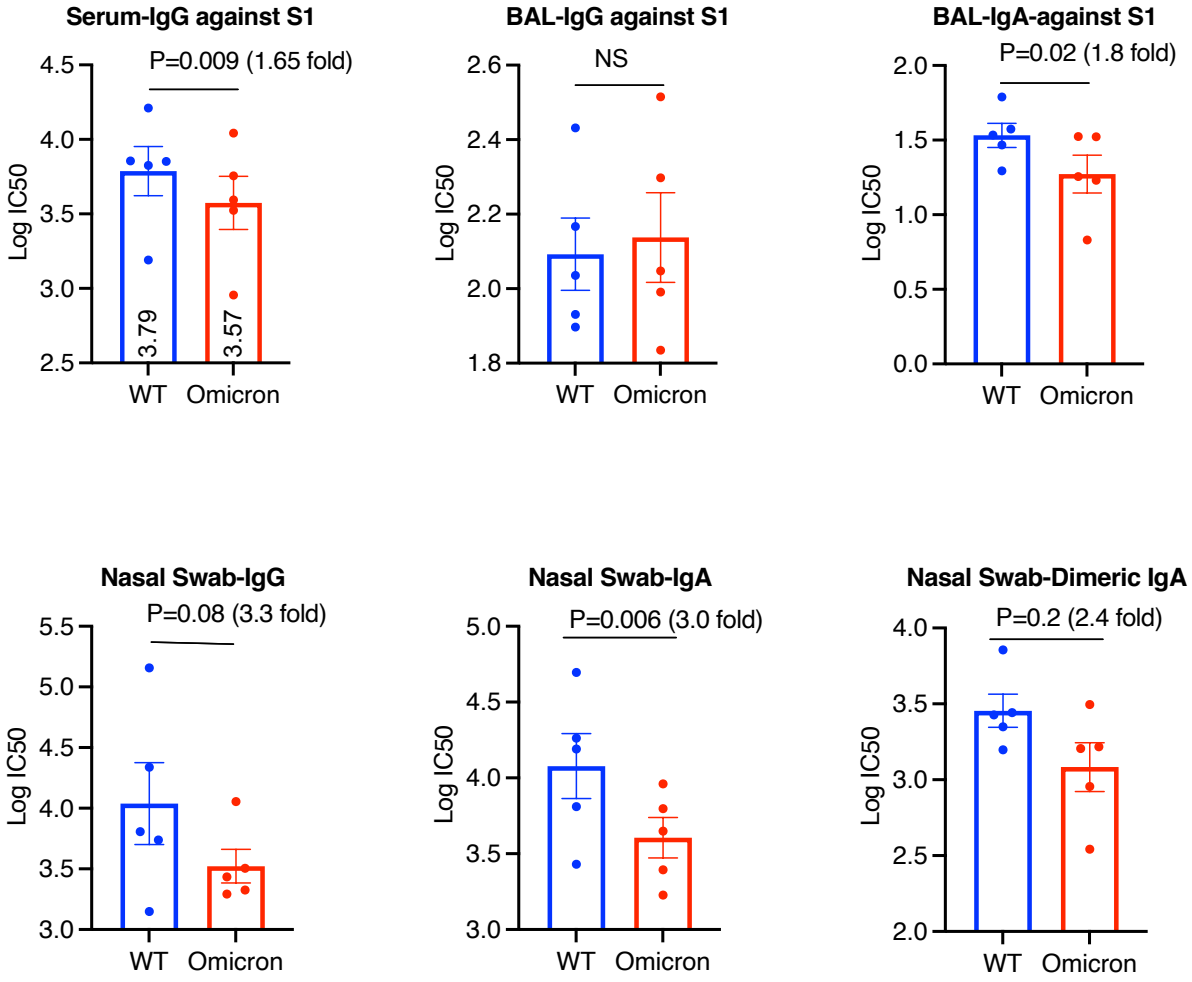

Figure S3. T helper subsets in PBMC samples of the vaccinated macaques. The frequencies of IFNγ$^+$, TNFα$^+$, or IL4$^+$ CD4$^+$ T cells were stained and measured after stimulation with PMA + ionomycin for 18hrs in PBMCs from different time-points. Flow cytometry plots of IFNγ$^+$, TNFα$^+$ CD4$^+$ T cells were shown below. Wilcoxon tests were used for comparisons.

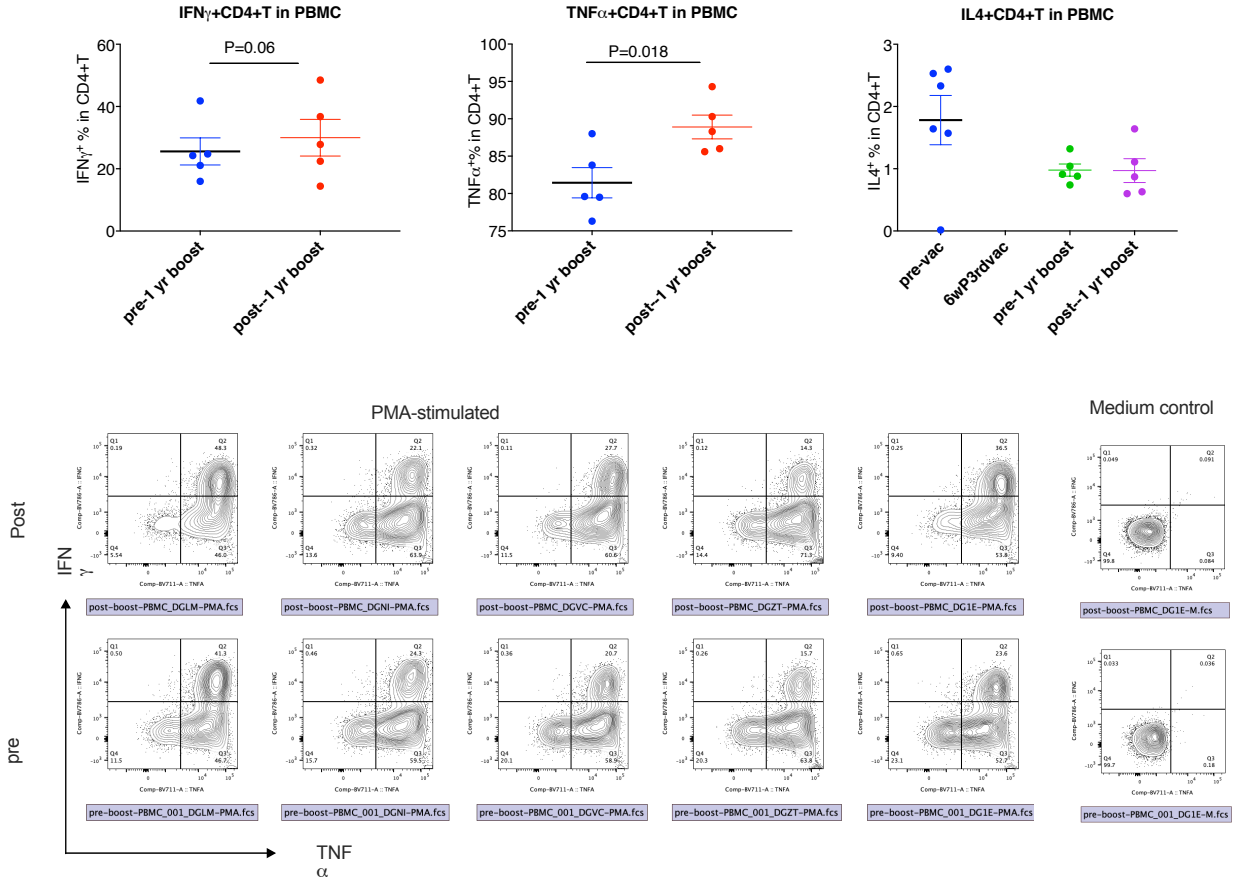

Table S1. Basic information of the animals enrolled in the study

Group	Animal ID	Sex	Date birth	Weight
Vaccine	DGVC	Male	05/01/2016	5.7
Vaccine	DGNi	Male	03/18/2016	6.5
Vaccine	DG1E	Male	04/07/2016	6.8
Vaccine	DGZT	Male	04/04/2016	5.5
Vaccine	DGLM	Male	04/14/2015	5.1
Control	14D044	Female	06/19/2014	4.07
Control	14D064	Female	06/19/2014	4.8
Control	14D080	Female	08/08/2014	4.28
Control	14D065	Female	06/29/2014	4.68
Control	14D068	Female	07/15/2014	4.8

Table S2. Inflammation and immunohistochemistry evaluation of the lung sections (necropsy at day 7 post SARS-CoV-2 challenge)

Animal ID	Group	H&E (Lc; Rm; Rc)	COVID-19 IHC (Lc; Rm; Rc)
DGVC	Vaccine	+/-; +/-; +/-	-; -; -
DGNi	Vaccine	+/-; +/-; +	-; -; -
DG1E	Vaccine	+/-; +/-; +/-	-; -; -
DGZT	Vaccine	+; + ; +	-; -; -
DGLM	Vaccine	+/-; +/-; +/-	-; -; -
14D044	Control	+/-; +; +	-; -; -
14D064	Control	++; +-; ++	-; -; +
14D080	Control	+/-; +; ++	+; -; +/-
14D065	Control	+; + ; ++	-; -; +/-
14D068	Control	+/-; +; +	-; +/-; -

H&E (inflammation) severity scale: normal= - (0); <10% (tissue affected) = +/- (1); >10-<25% = + (2) ; >26-<50% = ++ (3); >50%= +++ (4). Three parts of the lung lobes: Left caudal [Lc], Right Middle [Rm], and Right caudal [Rc].

IHC (COVID + foci)
Scoring: - = no SARS-CoV-2 Ag detected; +/- = rare- occasional; + = occasional-multiple; ++ = multiple- numerous (foci often larger); +++ = numerous