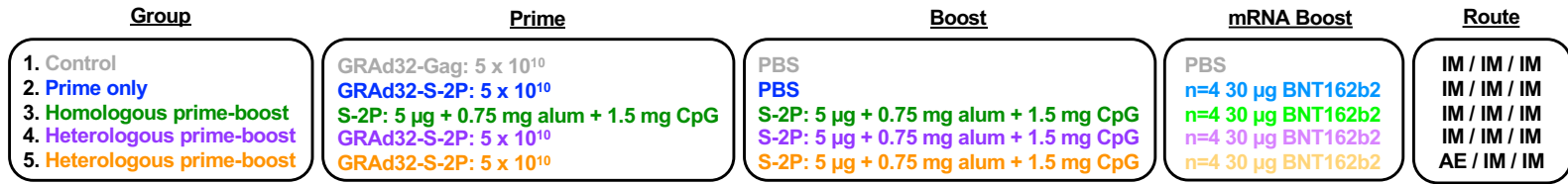


A



B

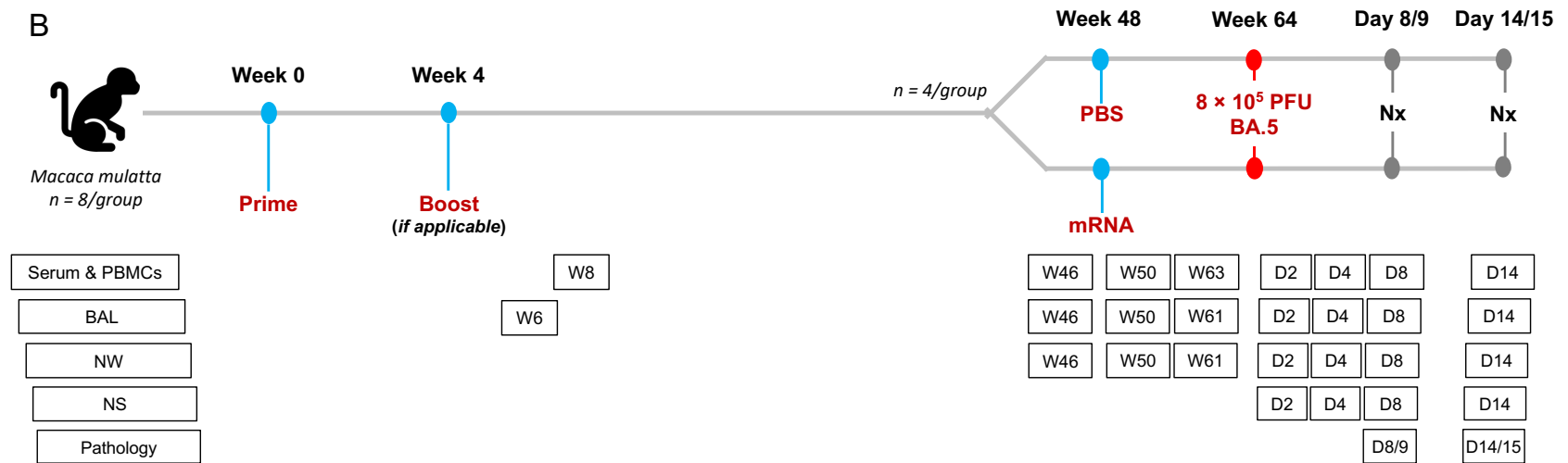


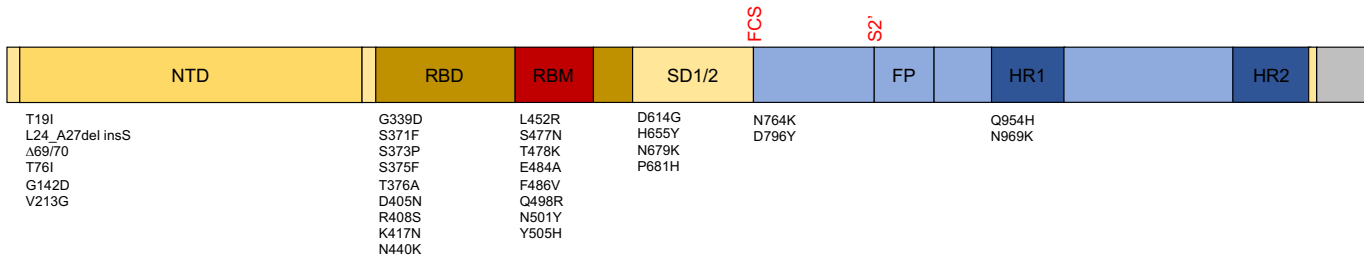
Figure S1

**Figure S1. Experimental groups, timeline and sampling schedule, related to Figure 1.**

(A) Eight NHP per group were immunized with a variety of different SARS-CoV-2 immunogens. Group one was immunized with  $5 \times 10^{10}$  GRAd32-Gag. This group served as the control. Group two was immunized with  $5 \times 10^{10}$  GRAd32-S-2P. Group three was immunized with 5  $\mu$ g adjuvanted S-2P (750  $\mu$ g alum and 1500  $\mu$ g CpG 1018; the same adjuvants were used when applicable). Group four was immunized with  $5 \times 10^{10}$  GRAd32-S-2P and with 5  $\mu$ g adjuvanted S-2P. Group five was immunized with  $5 \times 10^{10}$  GRAd32-S-2P delivered via aerosol (AE) and with 5  $\mu$ g adjuvanted S-2P. Phosphate buffered saline (PBS) was used as the placebo control as listed.

(B) NHP were primed with the selected immunogen at week 0, and boosted, if applicable, at week 4. At week 48 (44 weeks after the second immunization or 48 weeks after prime only), the eight macaques in each group were subdivided into two groups of 4 (except control group) and immunized with 30  $\mu$ g BNT162b2 (mRNA encoding S-2P). The week 0 and week 4 immunizations were delivered intramuscularly (IM) in 1 mL diluted in PBS (except aerosol GRAd32-S-2P) into the right deltoid, while the week 48 immunizations were delivered intramuscularly in 1 mL diluted in PBS into the right quadriceps. At week 64, all NHP were challenged with  $8 \times 10^5$  PFU of BA.5. Samples were collected as listed. Abbreviations: BAL – Bronchoalveolar lavage, NW – nasal wash, NS – nasal swab, Nx – necropsy.

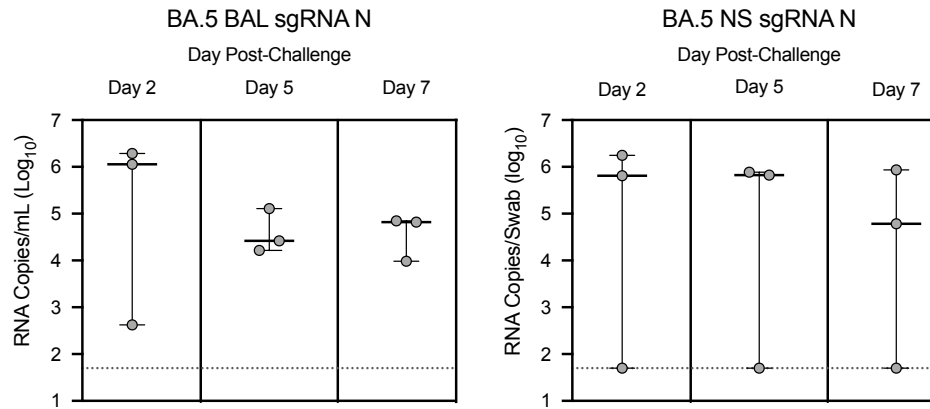
**A**



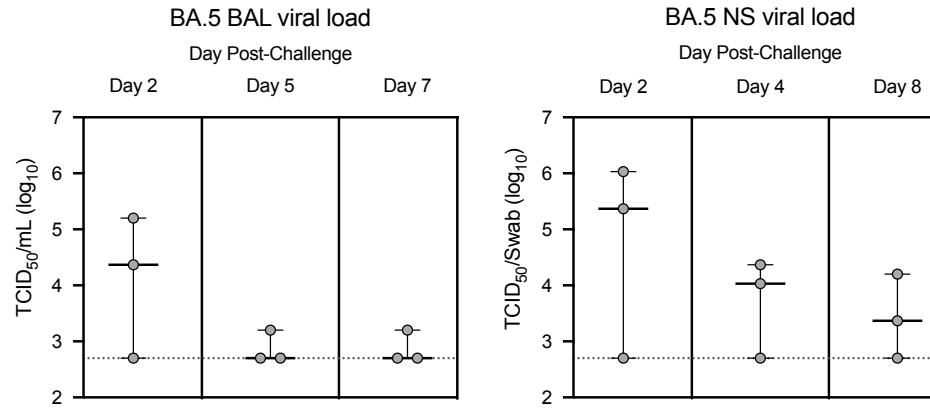
**B**

Gene	Amino acid change
ORF1ab	G82_M85del insV
ORF1ab	H83fs
ORF1ab	S135R
ORF1ab	L204F
ORF1ab	T842I
ORF1ab	G1307S
ORF1ab	L3027F
ORF1ab	T3090I
ORF1ab	T3255I
ORF1ab	P3395H
ORF1ab	L3606fs
ORF1ab	D3675/3676/3677
ORF1ab	L3829F
ORF1ab	P4715L
ORF1ab	R5716C
ORF1ab	I5967V
ORF1ab	T6564I
ORF3a	L106fs
ORF3a	T223I
E	T9I
E	Δ14
M	D3N
M	Q19E
M	A63T
N	P13L
N	Δ31/32/33
N	R203_G204del insKR
N	S413R

**C**



**D**



**Figure S2**

**Figure S2. BA.5 challenge stock sequence and titration in NHP, related to Figure 1.**

(A and B) BA.5 stock was sequenced and aligned with Wuhan-Hu-1.

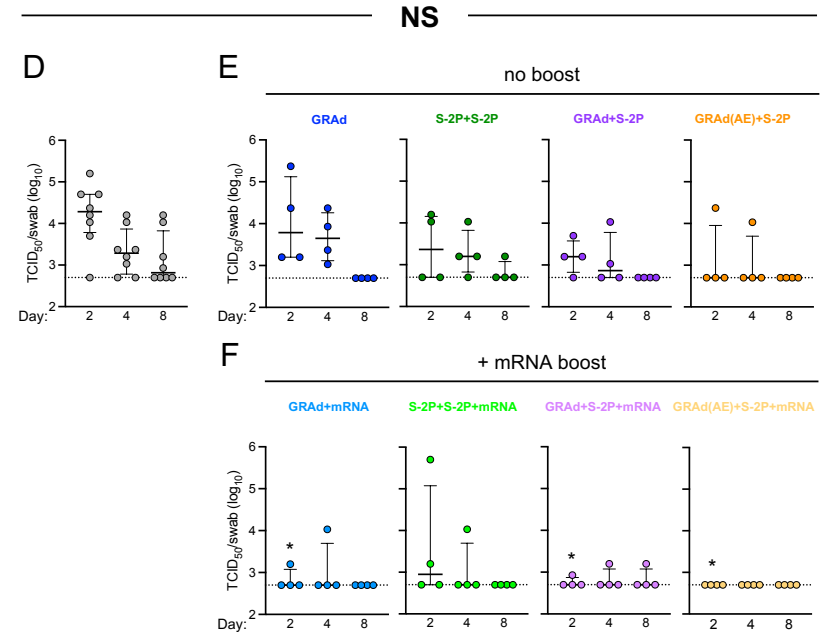
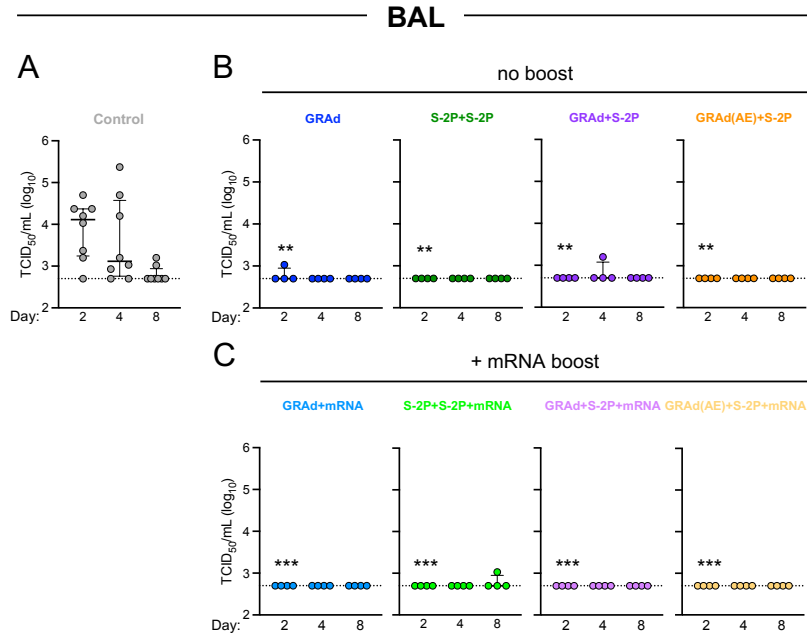
(A) S gene only. Amino acid replacements listed above graphic. NTD, N-terminal domain; RBD, receptor binding domain; RBM, receptor binding motif; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; FCS, furin cleavage site; S2', S2' site.

(B) Whole genome.

(C–D) BA.5 stock was confirmed to be virulent in NHP. Circles indicate individual NHP. Error bars represent interquartile range with the median denoted by a dotted horizontal line. Assay limit of detection indicated by a horizontal dotted line.

(C) BA.5 sgRNA<sub>N</sub> copy numbers per mL of BAL or per swab in naïve NHP.

(D) BA.5 TCID<sub>50</sub> per mL of BAL or per swab in naïve NHP.



**Figure S3**

**Figure S3. GRAd confers durable protection against BA.5 in the upper and lower airway, related to Figure 1 and 2.**

(A–F) BAL and NS was collected at days 2, 4 and 8 following challenge with  $8 \times 10^5$  PFU BA.5.

(A) BA.5 TCID<sub>50</sub> per mL in control NHP.

(B) BA.5 TCID<sub>50</sub> per mL in GRAd, S-2P+S-2P, GRAd+S-2P and GRAd(AE)-S-2P NHP.

(C) BA.5 TCID<sub>50</sub> per mL in GRAd, S-2P+S-2P, GRAd+S-2P and GRAd(AE)-S-2P NHP boosted with mRNA at week 48.

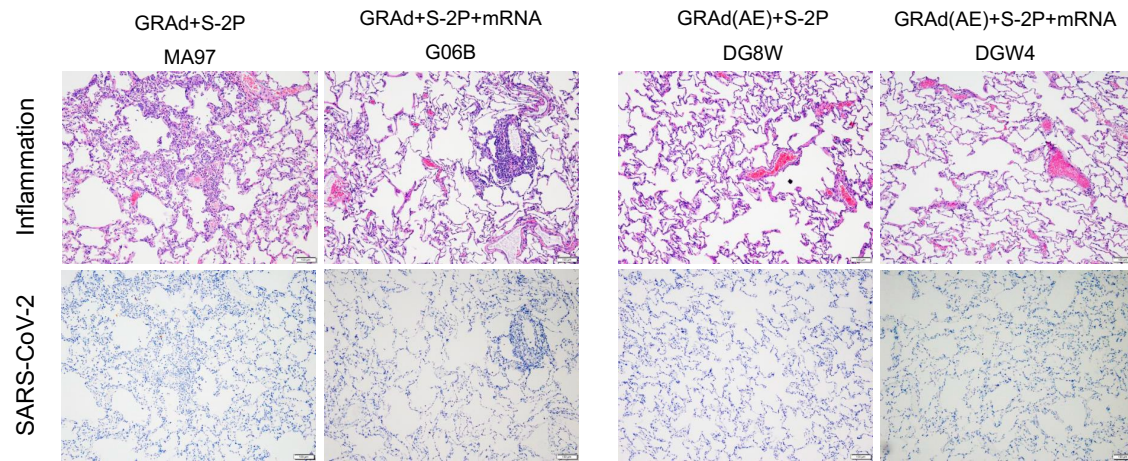
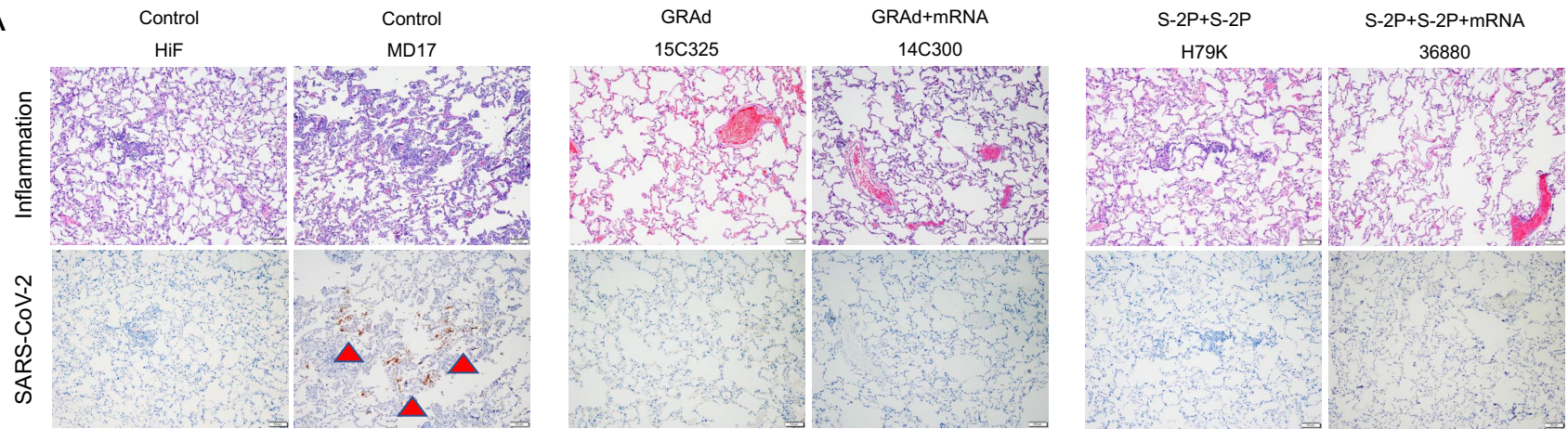
(D) BA.5 TCID<sub>50</sub> per swab in control NHP.

(E) BA.5 TCID<sub>50</sub> per swab in GRAd, S-2P+S-2P, GRAd+S-2P and GRAd(AE)-S-2P NHP.

(F) BA.5 TCID<sub>50</sub> per swab in GRAd, S-2P+S-2P, GRAd+S-2P and GRAd(AE)-S-2P NHP boosted with mRNA at week 48.

Circles (A–F) indicate individual NHP. Error bars represent interquartile range with the median denoted by a horizontal line. Assay limit of detection indicated by a dotted horizontal line. Statistical analysis shown for corresponding timepoints between control and test group (e.g., ‘\*’ symbols denote comparisons at day 2). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Eight control NHP and 4 immunized NHP per cohort.

**A**



B

			SARS-CoV-2			Inflammation						SARS-CoV-2			Inflammation		
Group	Nx	ID	Lc	Rmid	Rc	Lc	Rmid	Rc	Group	Nx	ID	Lc	Rmid	Rc	Lc	Rmid	Rc
GRAd	8	H64X	-	-	-	+	+	-	GRAd	14	H62F	-	-	-	-	+	+/-
	9	15C325	-	-	-	-	-	-		15	36887	-	-	-	-	-	-
S-2P+S-2P	8	G25K	-	-	-	-	-	+	S-2P+S-2P	14	36290	-	-	-	-	+	-
	9	H79K	-	-	-	+	-	+/-		15	0HG	-	-	-	-	-	-
GRAd+S-2P	9	MA97	-	-	-	+++	-	+	GRAd+S-2P	14	36282	-	-	-	-	-	-
	9	H83N	-	-	-	-	-	++		15	37893	-	-	-	+/-	++	+
GRAd(AE)+S-2P	8	DHGK	-	-	-	+/-	-	+	GRAd(AE)+S-2P	14	DGW7	-	-	-	+	-	-
	9	DG8W	-	-	-	-	+/-	-		15	36331	-	-	-	+/-	+/-	-
GRAd+mRNA	8	14C300	-	-	-	-	-	-	GRAd+mRNA	14	0H5	-	-	-	-	-	-
	9	LV84	-	-	-	++	+/-	-		15	MH63	-	-	-	-	-	-
S-2P+S-2P+mRNA	8	36880	-	-	-	-	-	-	S-2P+S-2P+mRNA	14	MG68	-	-	-	+/-	-	-
	9	MB39	-	-	-	-	-	++		15	14C315	-	-	-	+	+/-	+/-
GRAd+S-2P+mRNA	8	G06B	-	-	-	-	-	+/-	GRAd+S-2P+mRNA	14	H73T	-	-	-	-	-	-
	8	MA83	-	-	-	+/-	-	-		15	HD4	-	-	-	+/-	-	-
GRAd(AE)+S-2P+mRNA	8	DGW4	-	-	-	-	-	-	GRAd(AE)+S-2P+mRNA	14	DGR7	-	-	-	+/-	+/-	-
	9	DHGX	-	-	-	++	+/-	++		15	DGC8	-	-	-	-	-	+
Controls	8	MD17	-	+	+	-	++	++	Controls	14	36890	-	-	-	-	+	-
	8	H83X	-	-	-	+/-	++	+/-		14	H46Y	-	-	-	+	-	+/-
	9	HiF	-	+/-	-	+	++	+/-		15	0J2	-	-	-	-	+/-	-
	9	31930	+/-	-	-	+++	-	-		15	MA74	-	-	-	-	-	-

Figure S4

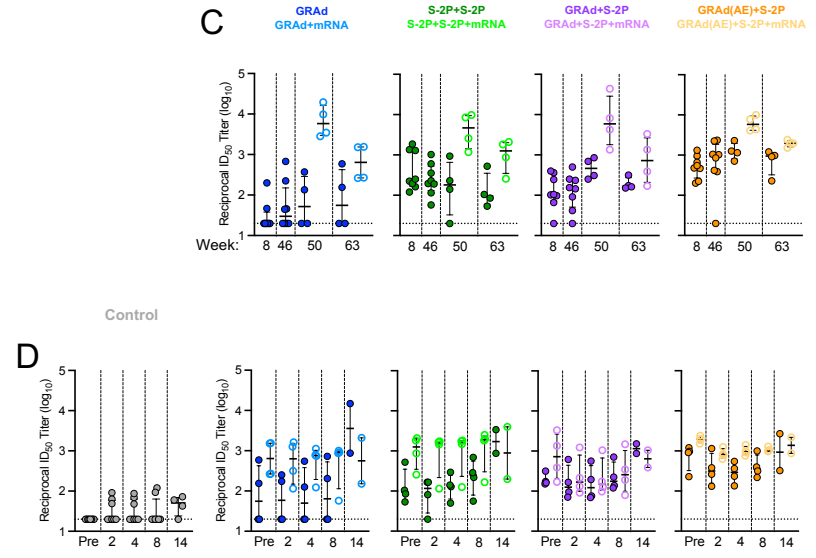
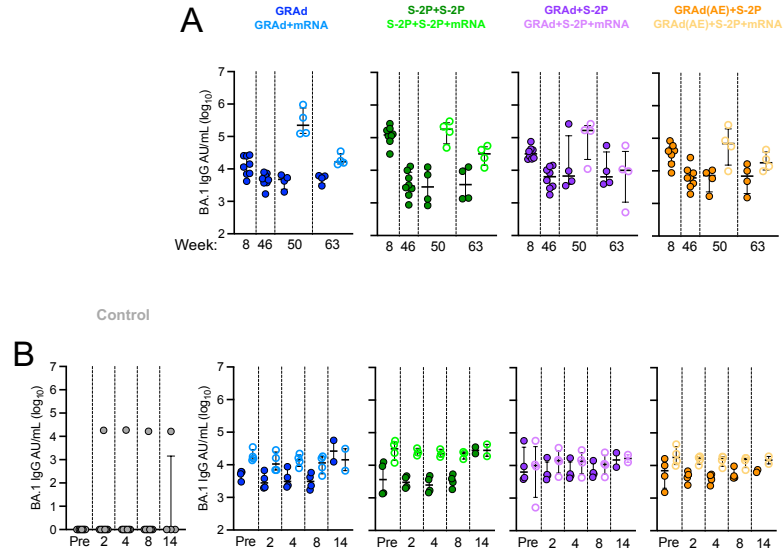


**Figure S4. Viral antigen and pathology in the lung after challenge, related to Figure 1.**

(A and B) 2 NHP per group were euthanized on day 8 or 9 and 14 or 15 following challenge and tissue sections taken from the lung.

(A) Top: Hematoxylin and eosin stain illustrating the extent of inflammation and cellular infiltrates. Bottom: Representative images indicating detection of SARS-CoV-2 N antigen by immunohistochemistry with a polyclonal anti-N antibody. Antigen-positive foci are marked by a red arrow. Images at 10x magnification with black bars for scale (100  $\mu$ m).

(B) SARS-CoV-2 antigen and inflammation scores in the left caudal lobe (Lc), right middle lobe (Rmid), and right caudal lobe (Rc) of the lung at days 8 or 9, and the left cranial lobe (Lc), right middle lobe (Rmid), and right cranial lobe (Rc) of the lung at days 14 or 15. Antigen scoring legend: – no antigen detected; +/- rare to occasional foci; + occasional to multiple foci; ++ multiple to numerous foci; +++ numerous foci. Inflammation scoring legend: – absent to minimal inflammation; +/- minimal to mild inflammation; + mild to moderate inflammation; ++ moderate-to-severe inflammation; +++ severe inflammation. Horizontal rows correspond to individual NHP.



**Figure S5**

**Figure S5. BA.1 binding and neutralization titers in serum pre-challenge and post-challenge, related to Figure 3.**

(A and C) Sera were collected at week 8, 46, 50 and 63.

(B and D) Sera were collected at days 2, 4, 8 and 14 following challenge with BA.5.

(A) IgG-binding titers to BA.1 S expressed in AU/mL pre-challenge.

(B) IgG-binding titers to BA.1 S expressed in AU/mL post-challenge.

(C) Neutralizing titers to BA.1 lentiviral pseudovirus expressed as the reciprocal ID<sub>50</sub> pre-challenge.

(D) Neutralizing titers to BA.1 lentiviral pseudovirus expressed as the reciprocal ID<sub>50</sub> post-challenge.

Circles (A–D) represent individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Assay limit of detection indicated by a horizontal dotted line which may fall below the depicted range. Vertical dashed lines are for visualization purposes only. Eight immunized NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight control NHP (4 at day 14), 8 immunized NHP at week 8 and 46, 4 immunized NHP at week 50 and 63, 4 immunized NHP at days 2, 4 and 8, and 2 immunized NHP at day 14.

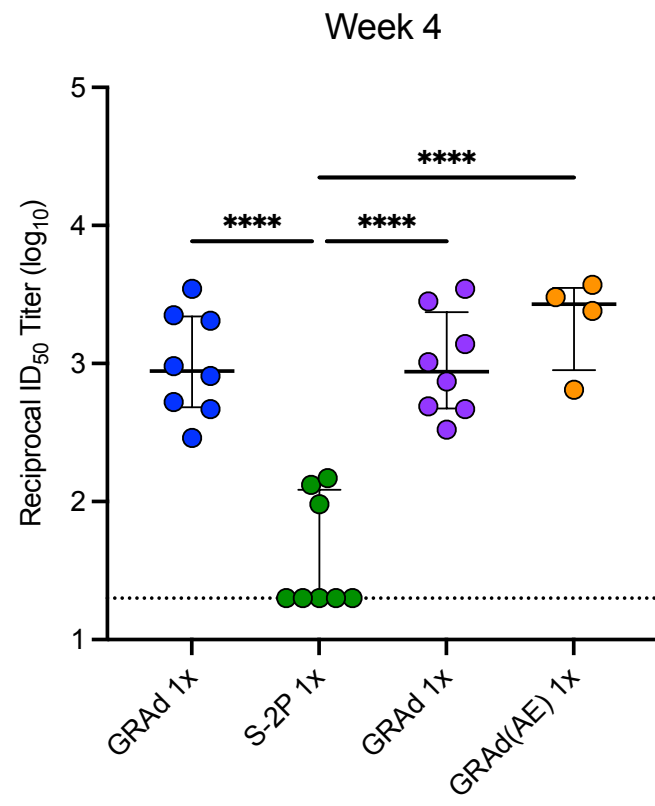
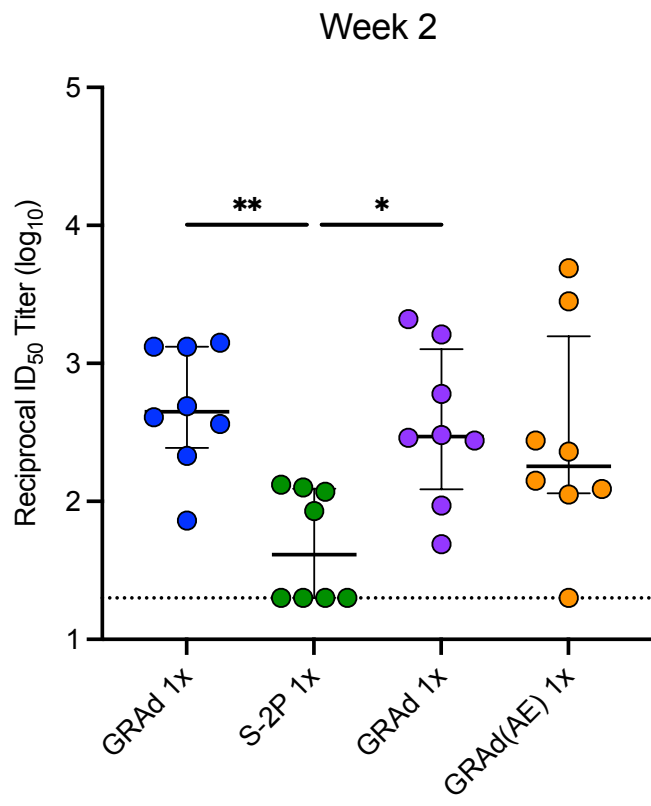
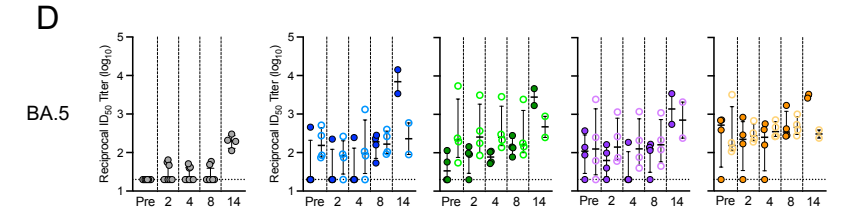
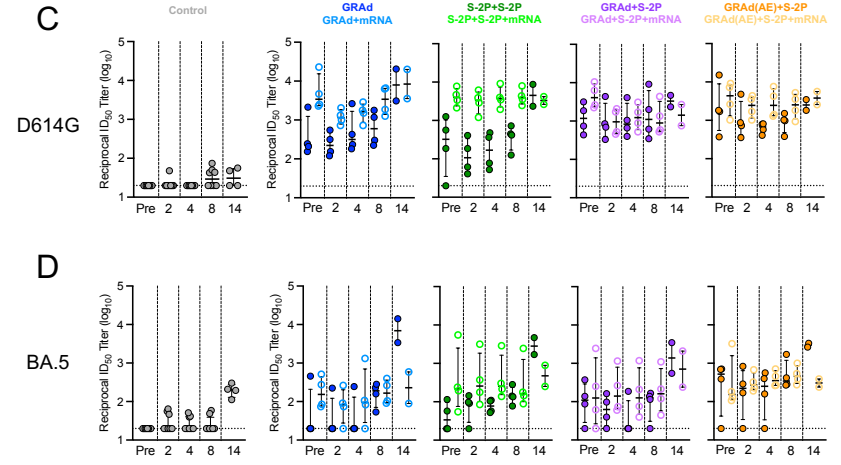
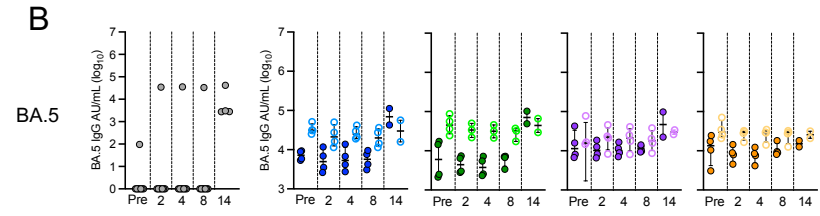
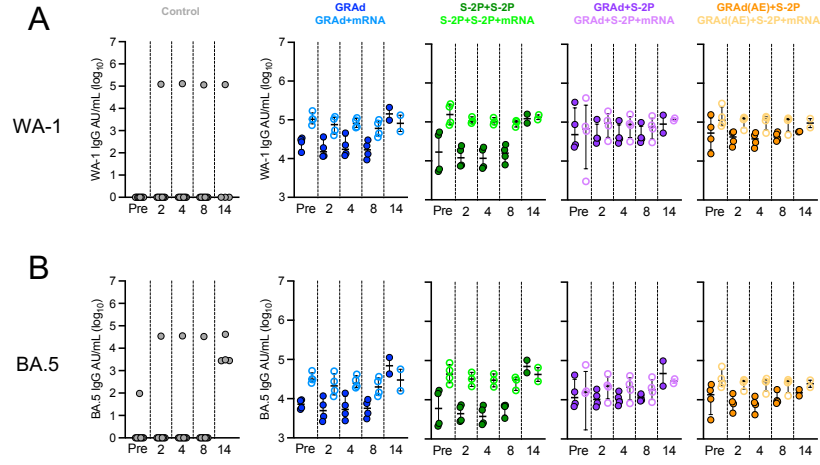


Figure S6

**Figure S6. D614G neutralization titers in serum at week 2 and 4, related to Figure 3.**

Sera were collected at week 2 and 4. Neutralizing titers to ancestral D614G lentiviral pseudovirus expressed as the reciprocal ID<sub>50</sub>.

Circles represent individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Assay limit of detection indicated by a horizontal dotted line. Eight immunized NHP (except for week 4 GRAd(AE) 1x, only 4 out of the 8 NHP were not bled at this timepoint due to blood sampling limits). Statistical analysis shown for corresponding timepoint between groups. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ .



**Figure S7**

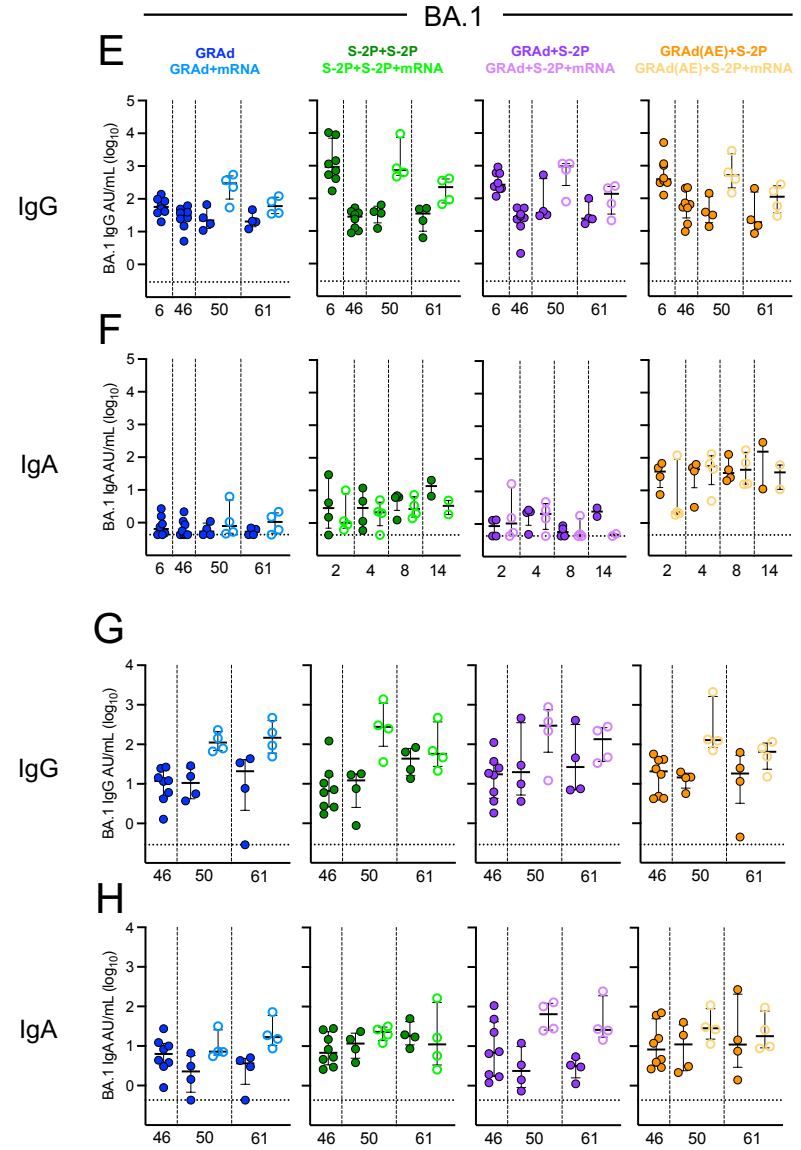
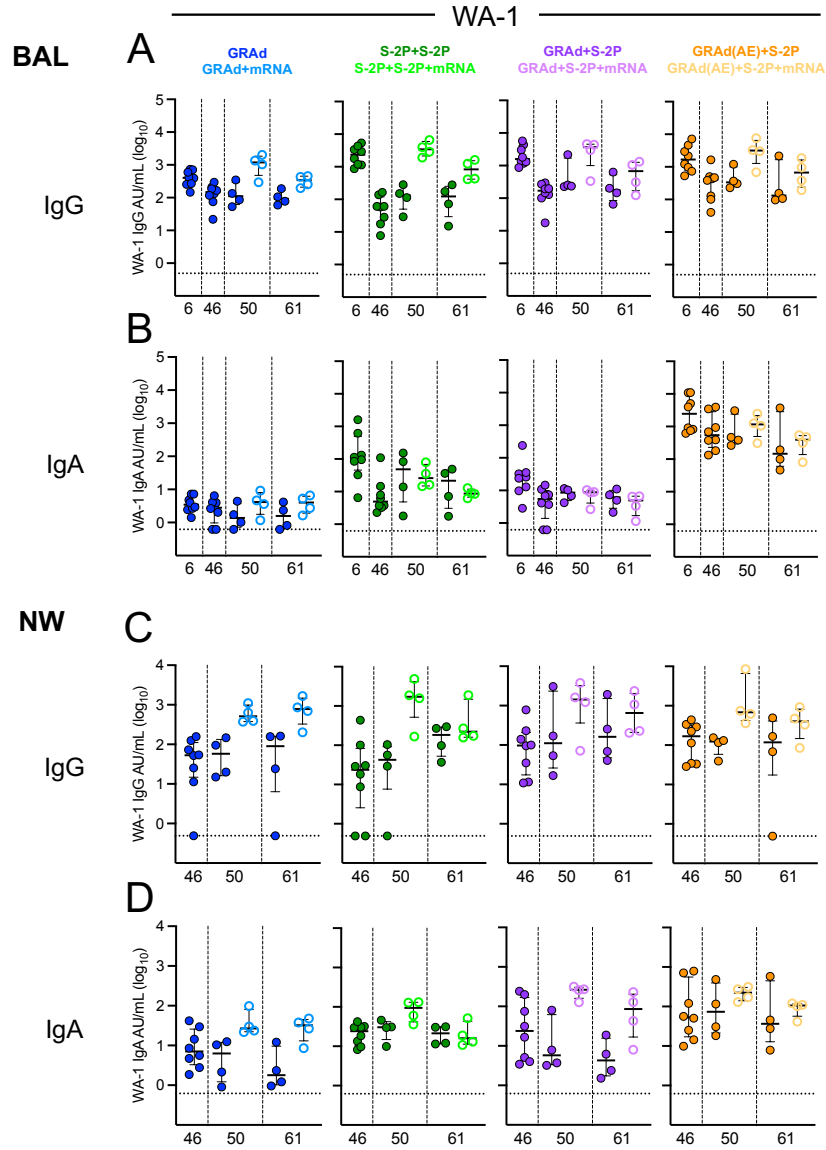
**Figure S7. WA-1 or D614G and BA.5 binding and neutralization titers in serum following challenge, related to Figure 3.**

(A–D) Sera were collected at days 2, 4, 8 and 14 following challenge with BA.5.

(A and B) IgG-binding titers to WA-1 and BA.5 S expressed in AU/mL post-challenge.

(C and D) Neutralizing titers to WA-1 and BA.5 lentiviral pseudovirus expressed as the reciprocal ID<sub>50</sub> post-challenge.

Circles (A–D) represent individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Assay limit of detection indicated by a horizontal dotted line which may fall below the depicted range. Vertical dashed lines are for visualization purposes only. Eight immunized NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight control NHP (4 at day 14), 8 immunized NHP at week 8 and 46, 4 immunized NHP at week 50 and 63, 4 immunized NHP at days 2, 4 and 8, and 2 immunized NHP at day 14.



**Figure S8**



**Figure S8. WA-1 and BA.1 IgG and IgA binding titers in BAL and NW prior to challenge, related to Figure 4.**

(A–H) BAL (A and B) was collected at week 6, 46, 50 and 61 and NW (C and D) was collected at week 46, 50, and 61.

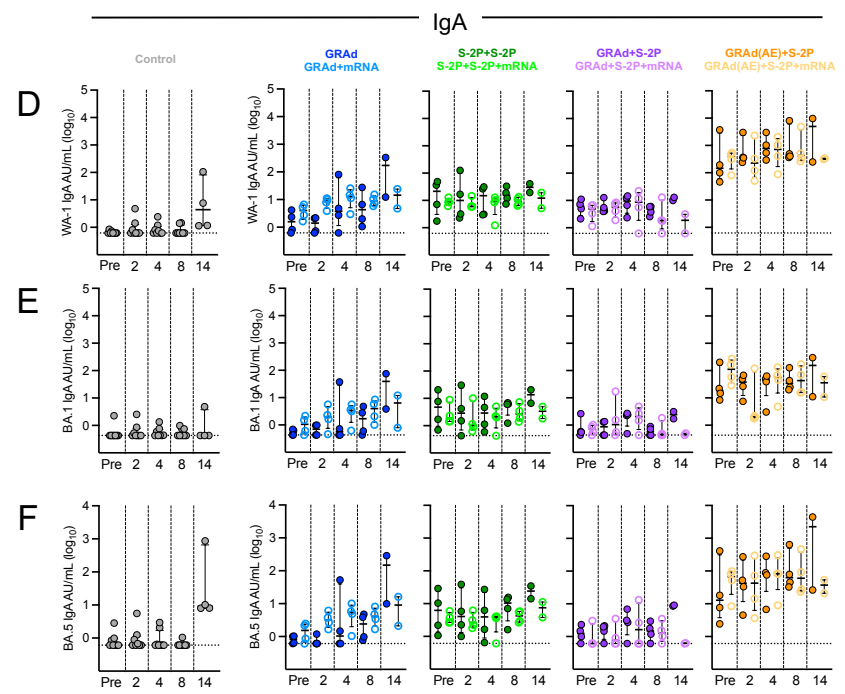
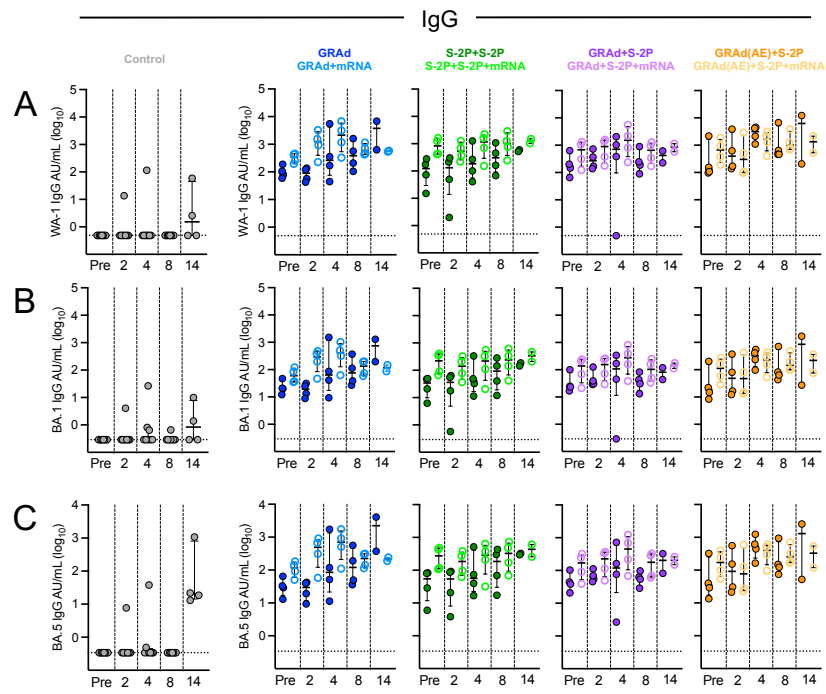
(A and B) IgG (A) and IgA (B) antibody binding titers to WA-1 expressed in AU/mL in BAL.

(C and D) IgG (C) and IgA (D) antibody binding titers to WA-1 expressed in AU/mL in NW.

(E and F) IgG (A) and IgA (B) antibody binding titers to BA.1 expressed in AU/mL in BAL.

(G and H) IgG (C) and IgA (D) antibody binding titers to BA.1 expressed in AU/mL in NW.

Circles (A–G) represent individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Assay limit of detection indicated by a horizontal dotted. Vertical dashed lines are for visualization purposes only. Eight vaccinated NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight immunized NHP at week 6 and 46, 4 immunized NHP at week 50 and 61.



**Figure S9**

**Figure S9. IgG and IgA binding titers in BAL following challenge, related to Figure 4.**

(A–F) BAL was collected at days 2, 4, 8 and 14 following challenge.

(A–C) IgG antibody binding titers to WA-1 (A), BA.1 (B) and BA.5 (C) expressed in AU/mL in BAL.

(D–F) IgA antibody binding titers to WA-1 (A), BA.1 (B) and BA.5 (C) expressed in AU/mL in BAL.

Circles (A–F) represent individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Assay limit of detection indicated by a horizontal dotted line. Vertical dashed lines are for visualization purposes only. Eight vaccinated NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight control NHP (4 at day 14), 4 immunized NHP at days 2, 4 and 8, 2 immunized NHP at day 14. For reference, the week 61 BAL IgG or IgA titer was included in the graphs as “pre”.



**Figure S10. IgG and IgA binding titers in NW following challenge, related to Figure 4.**

(A–F) NW was collected at days 2, 4, 8 and 14 following challenge.

(A–C) IgG antibody binding titers to WA-1 (A), BA.1 (B) and BA.5 (C) expressed in AU/mL in NW.

(D–F) IgA antibody binding titers to WA-1 (A), BA.1 (B) and BA.5 (C) expressed in AU/mL in NW.

Circles (A–F) represent individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Assay limit of detection indicated by a horizontal dotted line. Vertical dashed lines are for visualization purposes only. Eight vaccinated NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight control NHP (4 at day 14), 4 immunized NHP at days 2, 4 and 8, 2 immunized NHP at day 14. For reference, the week 61 NW IgG or IgA titer was included in the graphs as “pre”.

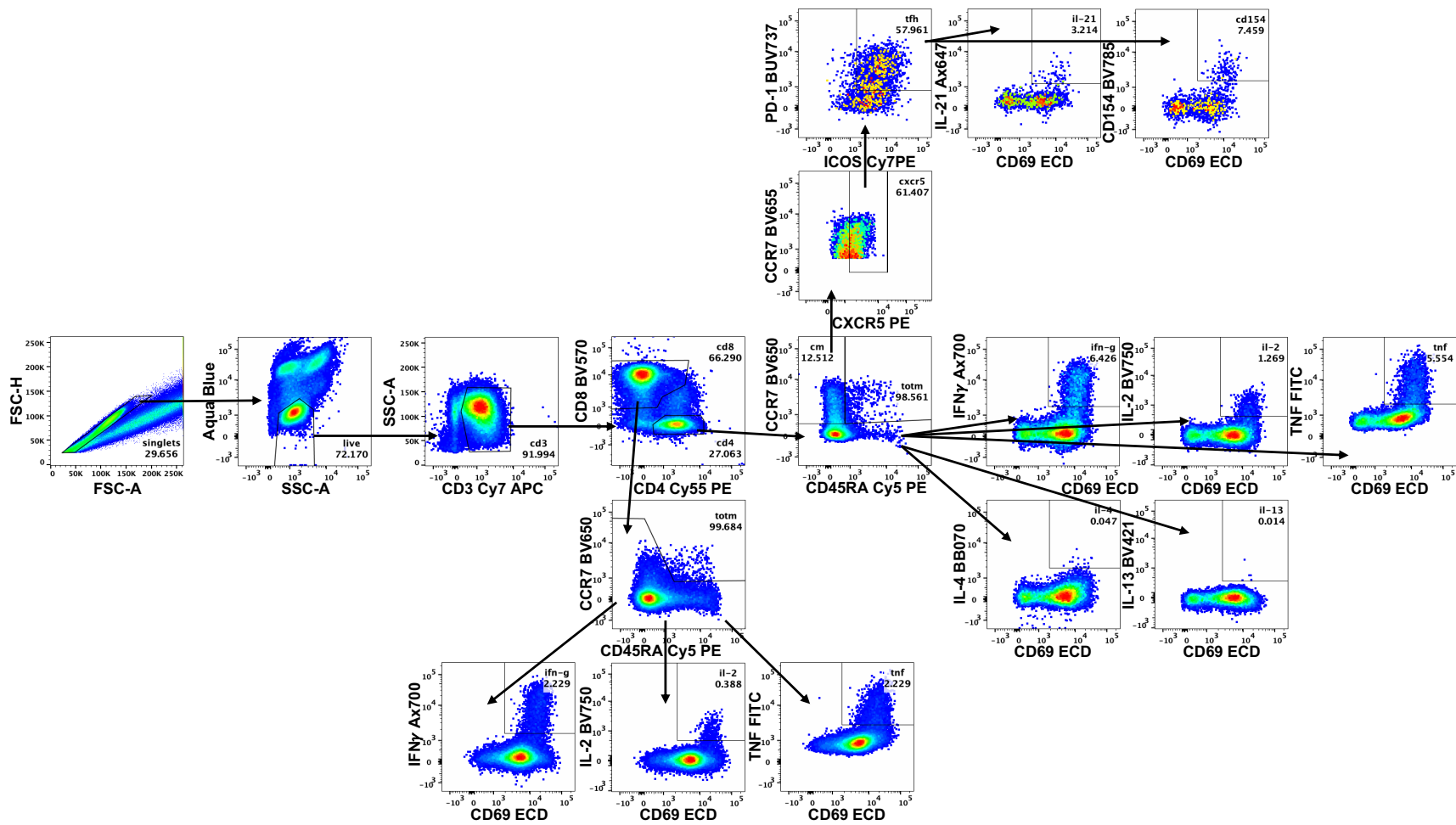


Figure S11

**Figure S11. T cell gating strategy, related to Figure 5.**

Representative flow cytometry plots showing gating strategy for T cells in Figures 5, S11 and S12. Cells were gated as singlets and live cells on forward and side scatter and a live/dead aqua blue stain. CD3<sup>+</sup> events were gated as CD4<sup>+</sup> or CD8<sup>+</sup> T cells. Total memory CD8<sup>+</sup> T cells were selected based on expression of CCR7 and CD45RA, and SARS-CoV-2 S-specific memory CD8<sup>+</sup> T cells were gated according to co-expression of CD69 and IL-2, TNF or IFN $\gamma$ . The CD4<sup>+</sup> events were defined as naive, total memory, or central memory according to expression of CCR7 and CD45RA, and CD4<sup>+</sup> cells with a T<sub>h</sub>1 phenotype were defined as memory cells that co-expressed CD69 and IL-2, TNF or IFN $\gamma$ . CD4<sup>+</sup> cells with a T<sub>h</sub>2 phenotype were defined as memory cells that co-expressed CD69 and IL-4 or IL-13. T<sub>fh</sub> cells were defined as central memory CD4<sup>+</sup> T cells that expressed CXCR5, ICOS, and PD-1. T<sub>fh</sub> cells were further characterized as IL-21<sup>+</sup> and CD69<sup>+</sup> or CD40L<sup>+</sup> (CD154) and CD69<sup>+</sup>.

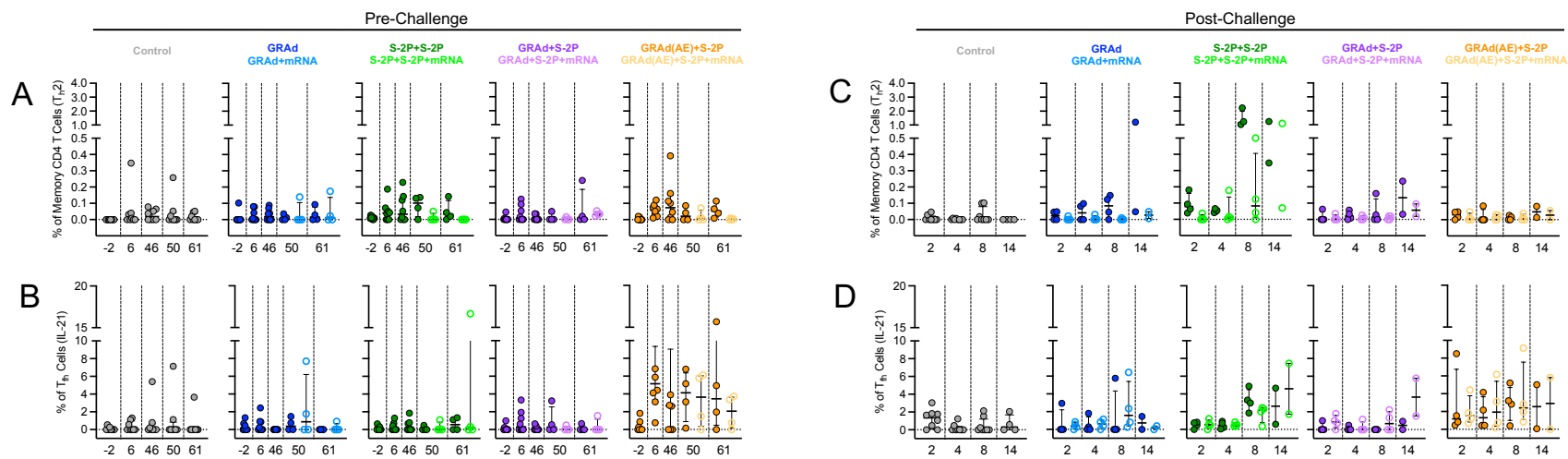


Figure S12



**Figure S12. T<sub>h</sub>2 and IL-21+ T<sub>fh</sub> cells in BAL, related to Figure 5.**

(A–B) BAL was collected at week -2, 6, 46, 50 and 61. Cells were stimulated with SARS-CoV-2 S1 and S2 peptide pools (WA1) and then measured by intracellular cytokine staining.

(C–D) BAL was collected at days 2, 4, 8, 50 and 14 following challenge. Cells were stimulated with SARS-CoV-2 S1 and S2 peptide pools (WA-1) and then measured by intracellular cytokine staining.

(A, C) Percentage of memory CD4+ T cells with T<sub>h</sub>2 markers (IL-4 or IL-13) following stimulation.

(B, D) Percentage of T<sub>fh</sub> cells that express IL-21.

Circles in (A–D) indicate individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Dotted lines set at 0%. Reported percentages may be negative due to background subtraction and may extend below the range of the y axis. Eight vaccinated NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight control NHP at days 2, 4 and 8, four at day 14.

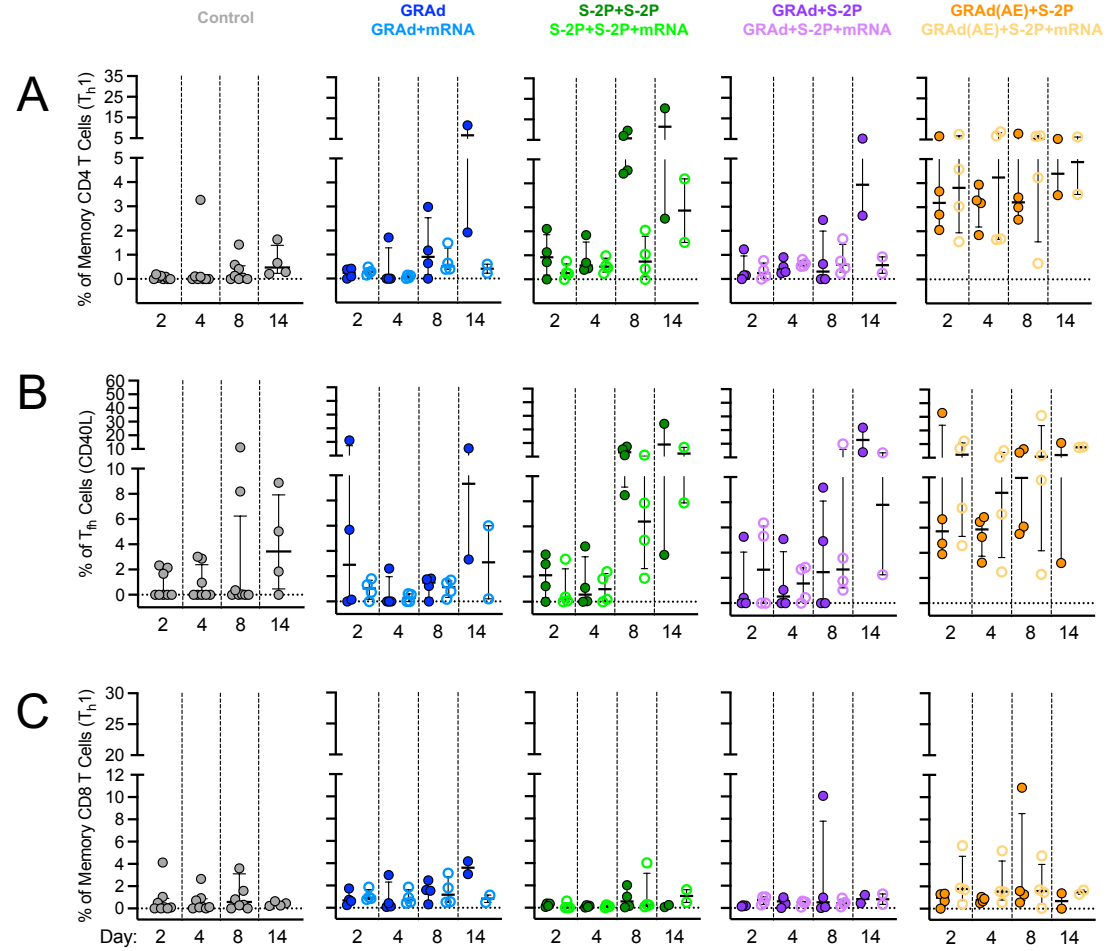


Figure S13

**Figure S13. T cell in BAL following challenge, related to Figure 5.**

(A–C) BAL was collected at days 2, 4, 8 and 14 following challenge. Cells were stimulated with SARS-CoV-2 S1 and S2 peptide pools (WA-1) and then measured by intracellular cytokine staining.

(A) Percentage of memory CD4<sup>+</sup> T cells with Th1 markers (IL-2, TNF, or IFN $\gamma$ ).

(B) Percentage of T<sub>fh</sub> cells that express CD40L.

(C) Percentage of CD8 T cells expressing IL-2, TNF, or IFN $\gamma$ .

Circles in (A–D) indicate individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Dotted lines set at 0%. Reported percentages may be negative due to background subtraction and may extend below the range of the y axis. Eight vaccinated NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight control NHP (4 at day 14), 4 immunized NHP at days 2, 4 and 8, 2 immunized NHP at day 14.

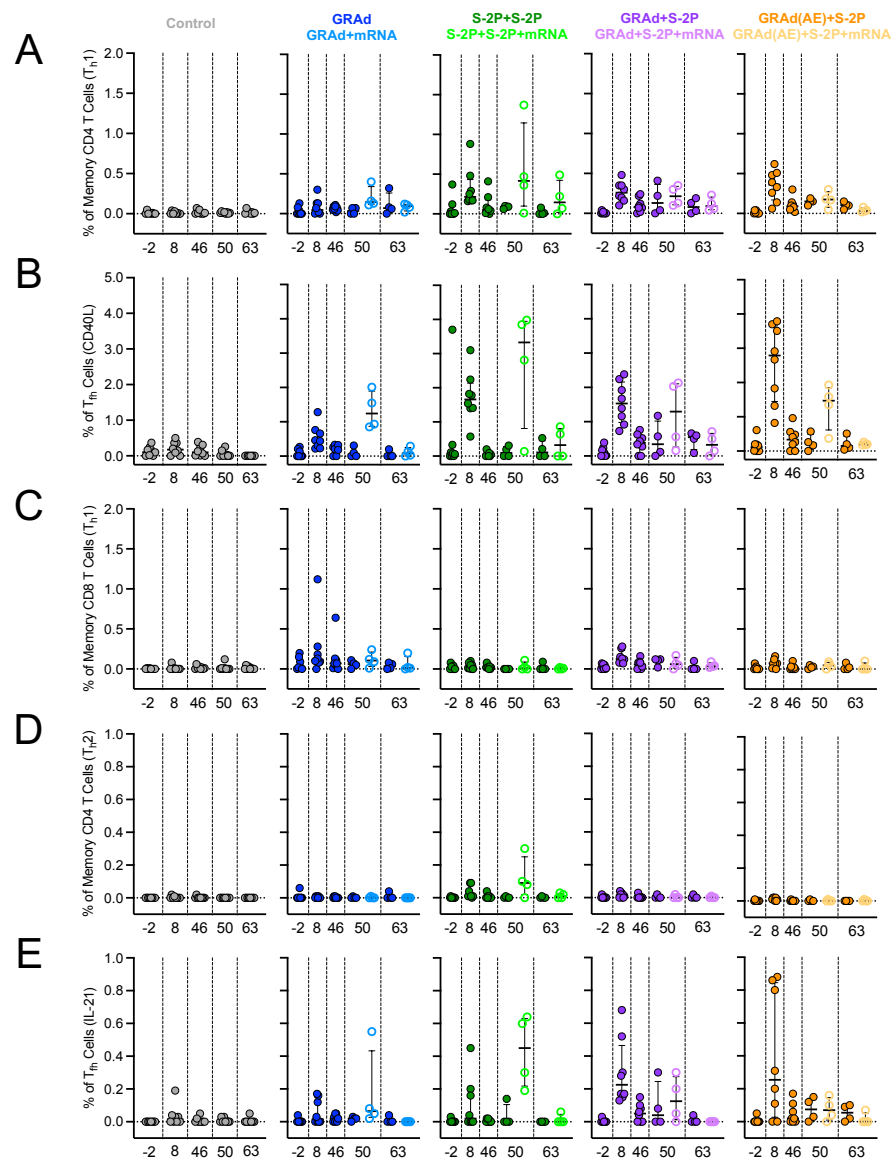


Figure S14

**Figure S14. T cell in blood prior to challenge, related to Figure 5.**

(A–E) Peripheral blood mononuclear cells (PBMCs) were collected at week -2, 8, 46, 50 and 63. Cells were stimulated with SARS-CoV-2 S1 and S2 peptide pools (WA-1) and then measured by intracellular cytokine staining.

(A) Percentage of memory CD4<sup>+</sup> T cells with Th1 markers (IL-2, TNF, or IFN $\gamma$ ).

(B) Percentage of T<sub>fh</sub> cells that express CD40L.

(C) Percentage of CD8 T cells expressing IL-2, TNF, or IFN $\gamma$ .

(D) Percentage of memory CD4<sup>+</sup> T cells with T<sub>h</sub>2 markers (IL-4 or IL-13).

(E) Percentage of T<sub>fh</sub> cells that express IL-21.

Circles in (A–E) indicate individual NHP. Error bars represent the interquartile range with the median denoted by a horizontal black line. Dotted lines set at 0%. Reported percentages may be negative due to background subtraction and may extend below the range of the y axis. Eight vaccinated NHP, split into 2 cohorts of 4 NHP post mRNA boost. Eight control NHP at days 2, 4 and 8, four at day 14.

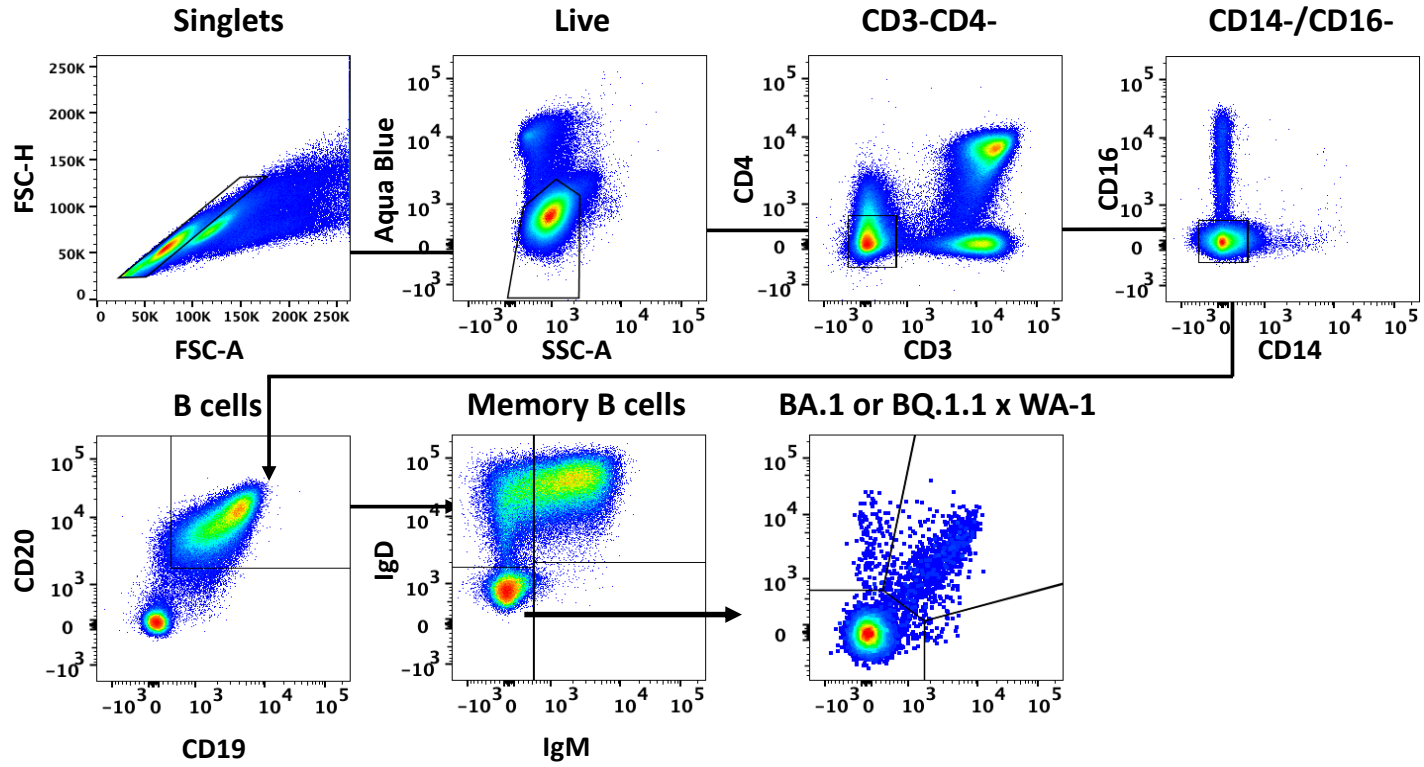


Figure S15

**Figure S15. B cell gating strategy, related to Figure 6.**

Representative flow cytometry plots showing gating strategy for B cells in Figures 6 and S16. Cells were gated as singlets and live cells on forward and side scatter and a live/dead aqua blue stain. CD3-, CD4-cells were then gated on absence of CD14 and CD16 expression and positive expression of CD20 and CD19. Memory B cells were selected based on lack of IgD or IgM. Finally, memory B cells of variant S-2P (WA-1 and BA.5 or WA-1 and BQ.1.1) probes were used to determine binding specificity.

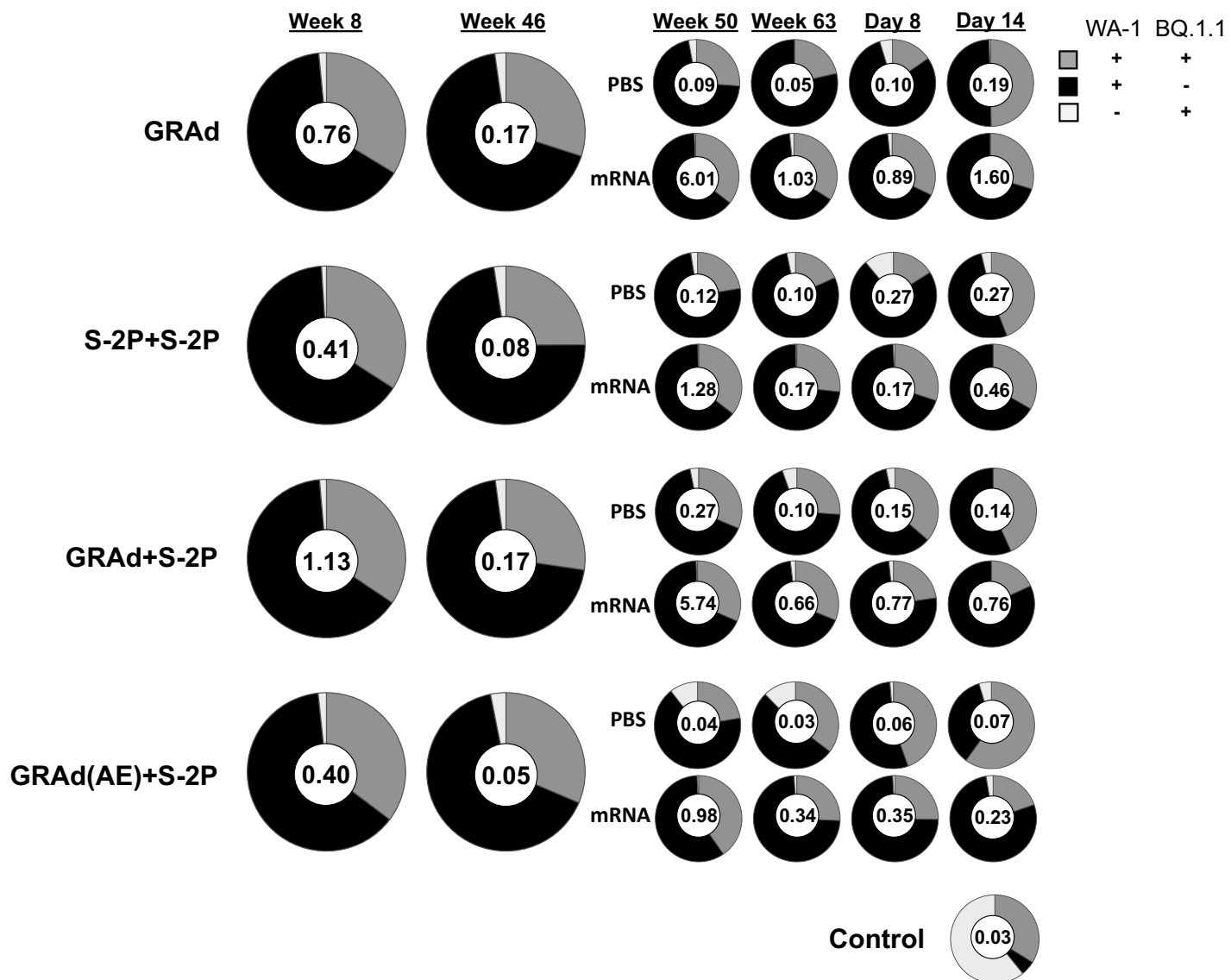


Figure S16



**Figure S16: WA-1 and BQ.1.1 cross-reactive S-2P specific memory B cells following immunization, related to Figure 6.**

Pie charts indicate the frequency (numbered circle at the center) and proportion of total S-binding memory B cells that are dual specific for WA-1 and BQ.1.1 (dark gray), specific for WA-1 (black), or specific for BQ.1.1 (light gray) for all NHP in each group and timepoint at week 8, 46, 50 and 63 post-immunization, and days 8 and 14 post-challenge. Seven or eight NHP per group at week 8 and 46, 3-4 NHP per group at week 50 and 63, and day 8, 1-2 NHP at day 14.