

SUPPLEMENTAL MATERIALS

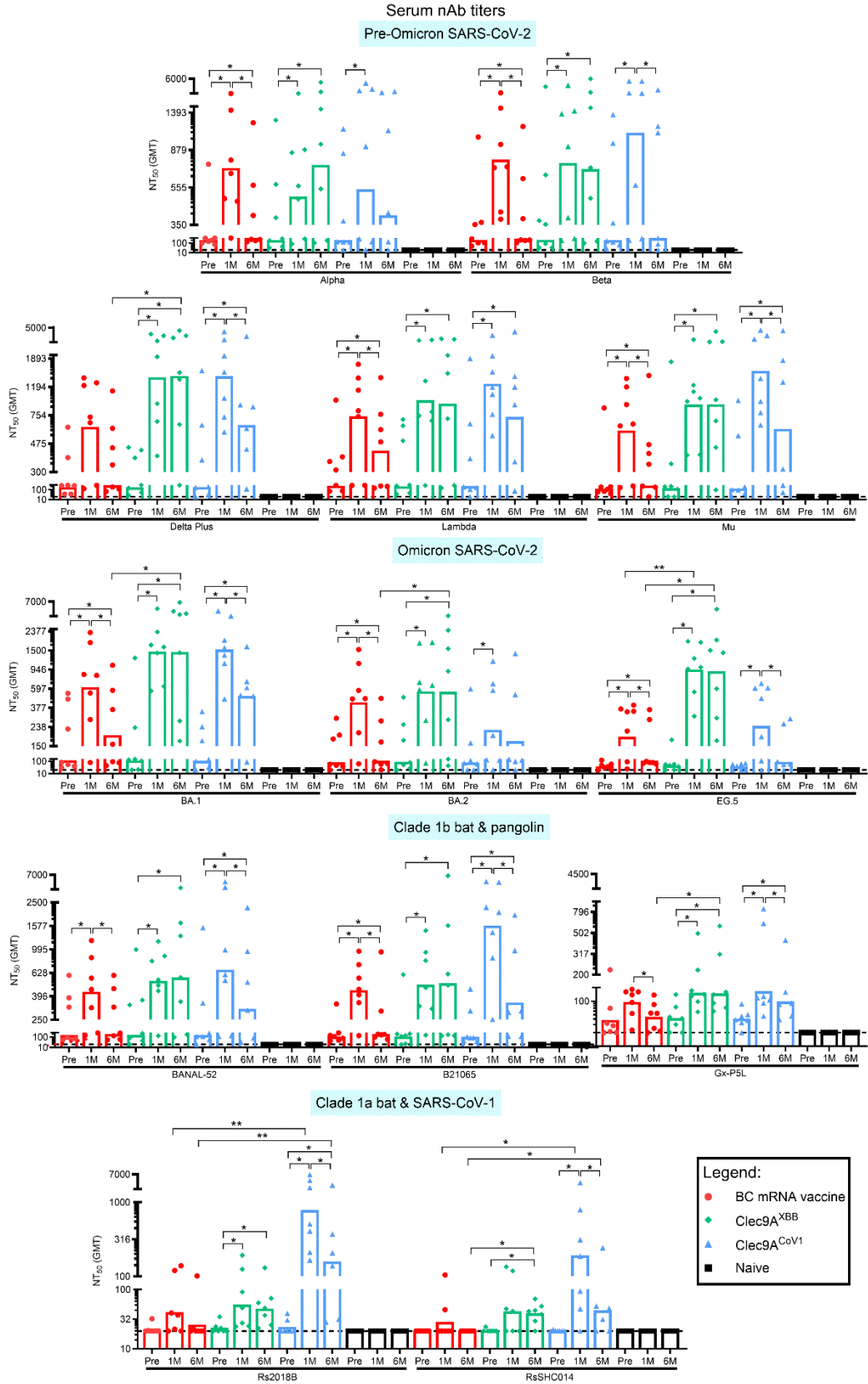


Figure S1. Serum neutralizing antibody titers upon systemic booster with Clec9A^{XBB}, Clec9A^{CoV1} or bivalent Comirnaty (BC) mRNA vaccine. Five to six-week-old BALB/c mice were immunized twice three weeks apart (0.05 µg per dose; i.m) with Pfizer-BioNTech original Comirnaty mRNA vaccine. Three months after the last immunization dose, mice were boosted either with Pfizer-BioNTech BA.4/5 bivalent Comirnaty (BC) mRNA vaccine (0.05 µg; i.m), Clec9A^{XBB} (10 µg adjuvanted with 50 µg poly I:C; s.c) or Clec9A^{CoV1} (10 µg adjuvanted with 50 µg poly I:C; s.c). A control group of non-immunized mice (naïve) was also included for baseline. The serum nAb titers against 13 sarbecoviruses representative of clades 1b and 1a at pre-boost, one- and six months post-boost were determined by multiplex sVNT. Data are from one representative experiment performed twice with similar results, n = 6-7 per group/experiment. Symbols represent individual animals and data shown are geometric means. Statistical analysis: Non-parametric two-tailed Kruskal Wallis test with Dunn's multiple-comparison test and Friedman test with Dunn's multiple-comparison test. *p < 0.05, **p < 0.01.

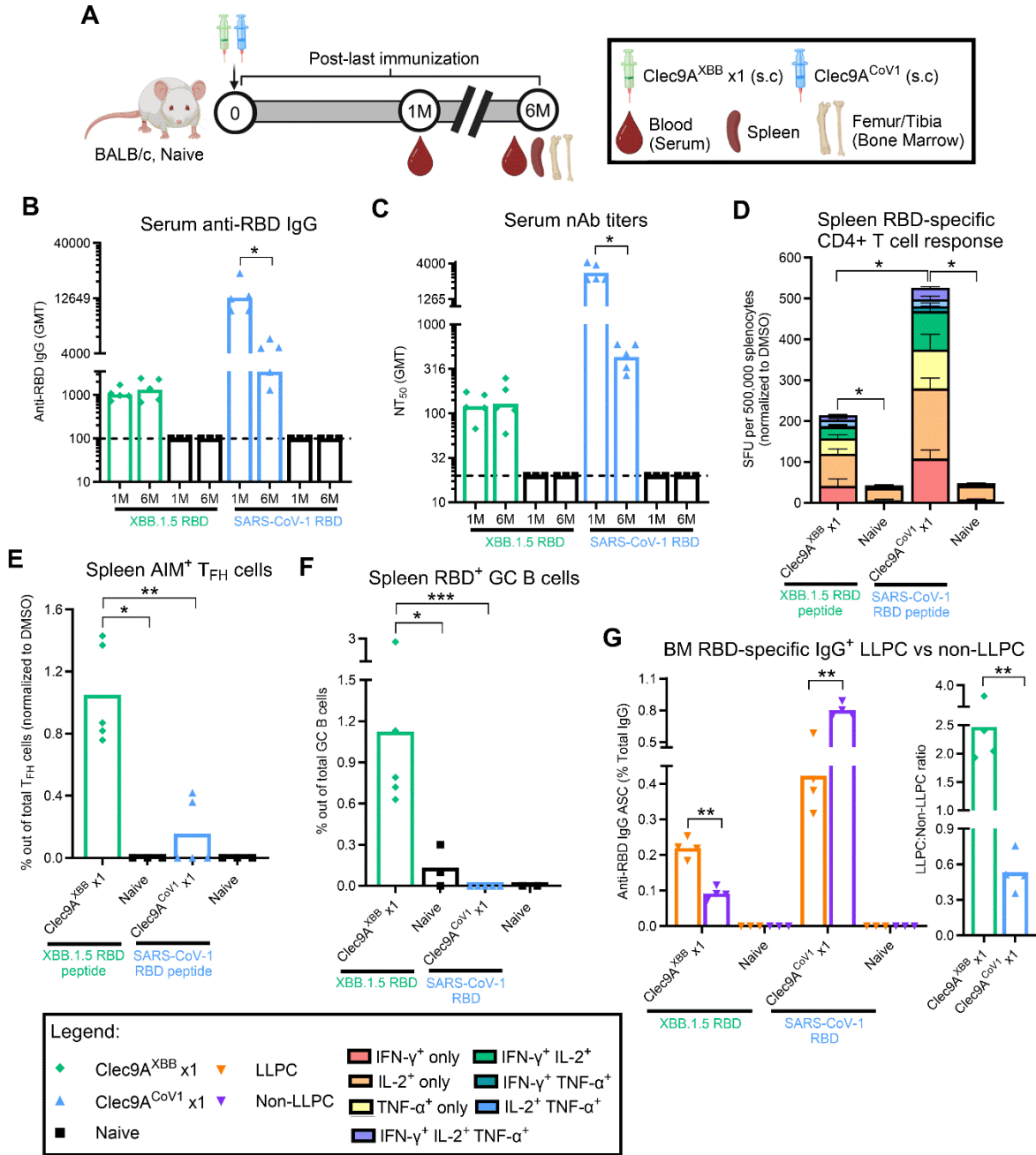


Figure S2. Durability of immune responses upon single shot immunization with Clec9A^{XBB} or Clec9A^{CoV1} in naïve mice. **A**) Five to six-week-old BALB/c mice were immunized with a single dose (2 μ g adjuvanted with 50 μ g poly I:C; s.c) of Clec9A^{XBB} or Clec9A^{CoV1}. **(B, C)** Blood was collected one- and six months after immunization. **(B)** Serum anti-RBD IgG titers against XBB.1.5 and SARS-CoV-1 RBD 1 at one- and six months post-immunization were determined by ELISA. **(C)** Serum nAb titers against

XBB.1.5 and SARS-CoV-1 at one- and six months post-immunization were determined by sVNT. **(D-G)** Mice were euthanized at six months post-immunization, and spleen and BM from femur/tibia were harvested. **(D)** Frequency of IFN- γ , IL-2 and/or TNF- α secreting CD4⁺-enriched splenocytes at six months post-immunization was determined by FluoroSPOT upon re-stimulation with XBB.1.5 and SARS-CoV-1 RBD peptides. **(E, F)** Percentage of **(E)** AIM⁺ T_{FH} and **(F)** RBD⁺ GC B cells in spleen at six months post-immunization was determined by flow cytometry. **(G)** Frequency of BM RBD-specific IgG⁺ LLPC and non-LLPC (normalized to total IgG) at six months post-immunization was determined by B cell ELISPOT. **(B-G)** Data are from one representative experiment performed twice with similar results, n = 4-5 per group/experiment. **(B, C, E-G)** Symbols represent individual animals and data shown are **(B, C)** geometric means and **(D-G)** means \pm **(D)** SD. Statistical analysis: Non-parametric two-tailed **(B, C)** Wilcoxon matched-pairs signed rank test and **(D-G)** Mann-Whitney test. *p < 0.05, **p < 0.01, ***p < 0.001.

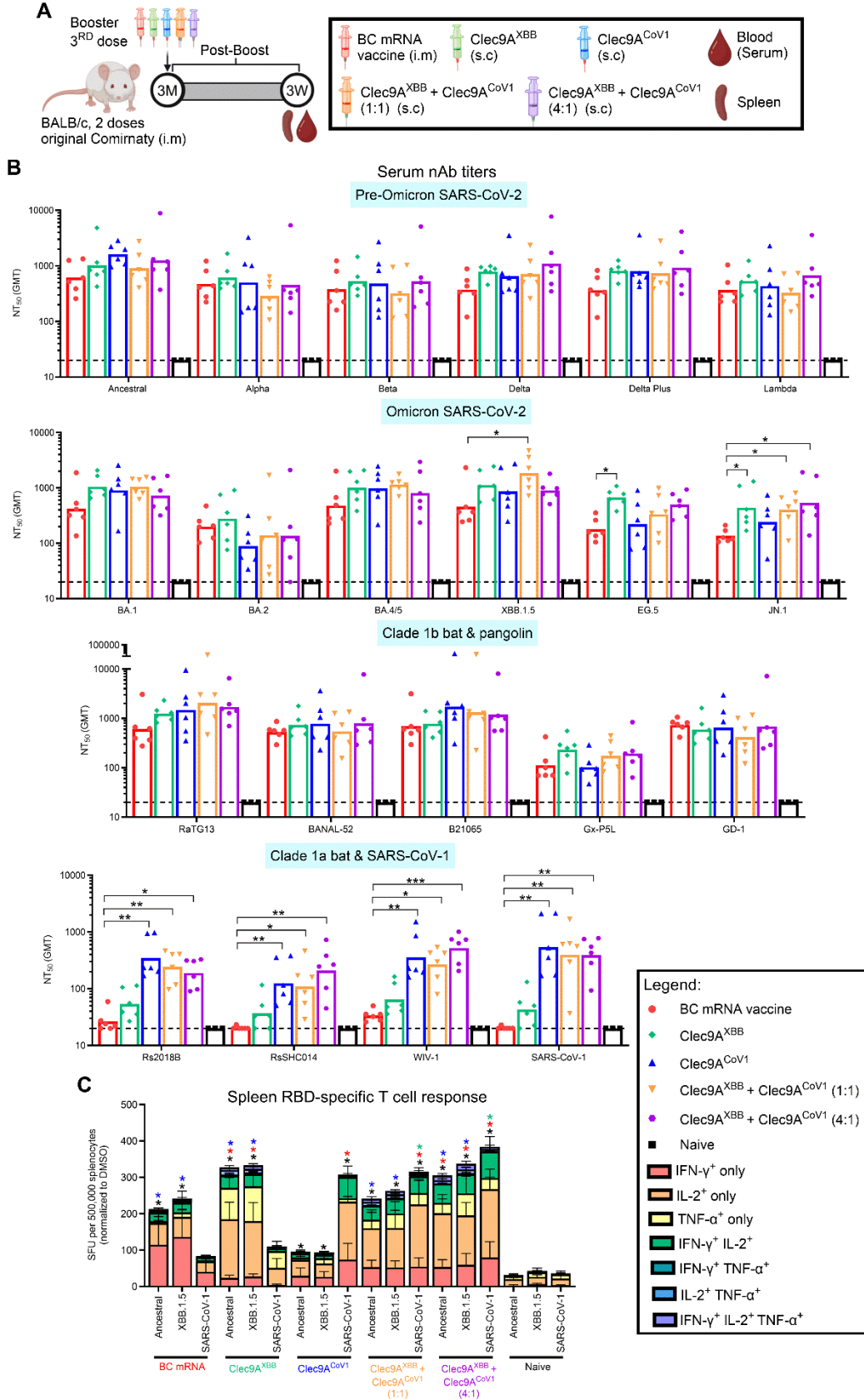


Figure S3. Dose optimization of [Clec9A^{XBB} + Clec9A^{CoV1}] booster. (A) Five to six-week-old BALB/c mice were immunized twice three weeks apart (0.05 µg per dose; i.m) with original Comirnaty mRNA vaccine. Three months after the second immunizing dose, mice were boosted with either BC mRNA vaccine (0.05 µg; i.m), Clec9A^{XBB} (10 µg), Clec9A^{CoV1} (10 µg), [Clec9A^{XBB} + Clec9A^{CoV1}] (5 µg + 5 µg) or [Clec9A^{XBB} + Clec9A^{CoV1}] (8 µg + 2 µg). All Clec9A-based formulations were adjuvanted with 50 µg poly I:C and administered s.c. At three weeks post-boost, blood was collected, and mice were euthanized to harvest spleen. (B) Serum nAb titers against 21 sarbecoviruses from clades 1a and 1b at three weeks post-boost was determined by multiplex sVNT. (C) Frequency of IFN-γ, IL-2 and/or TNF-α secreting splenocytes at three weeks post-boost was determined by FluoroSPOT upon re-stimulation with ancestral SARS-CoV-2, XBB.1.5 and SARS-CoV-1 RBD peptides. (B, C) Data are from one representative experiment performed twice with similar results, n = 5-6 per group/experiment. (B) Symbols represent individual animals and data shown are (B) geometric means and (C) means ± SD. Statistical analysis: Non-parametric two-tailed Kruskal Wallis test with Dunn's multiple-comparison test. *p < 0.05, **p < 0.01, ***p < 0.001. (C) Asterisk colors represent statistical significance between corresponding groups.

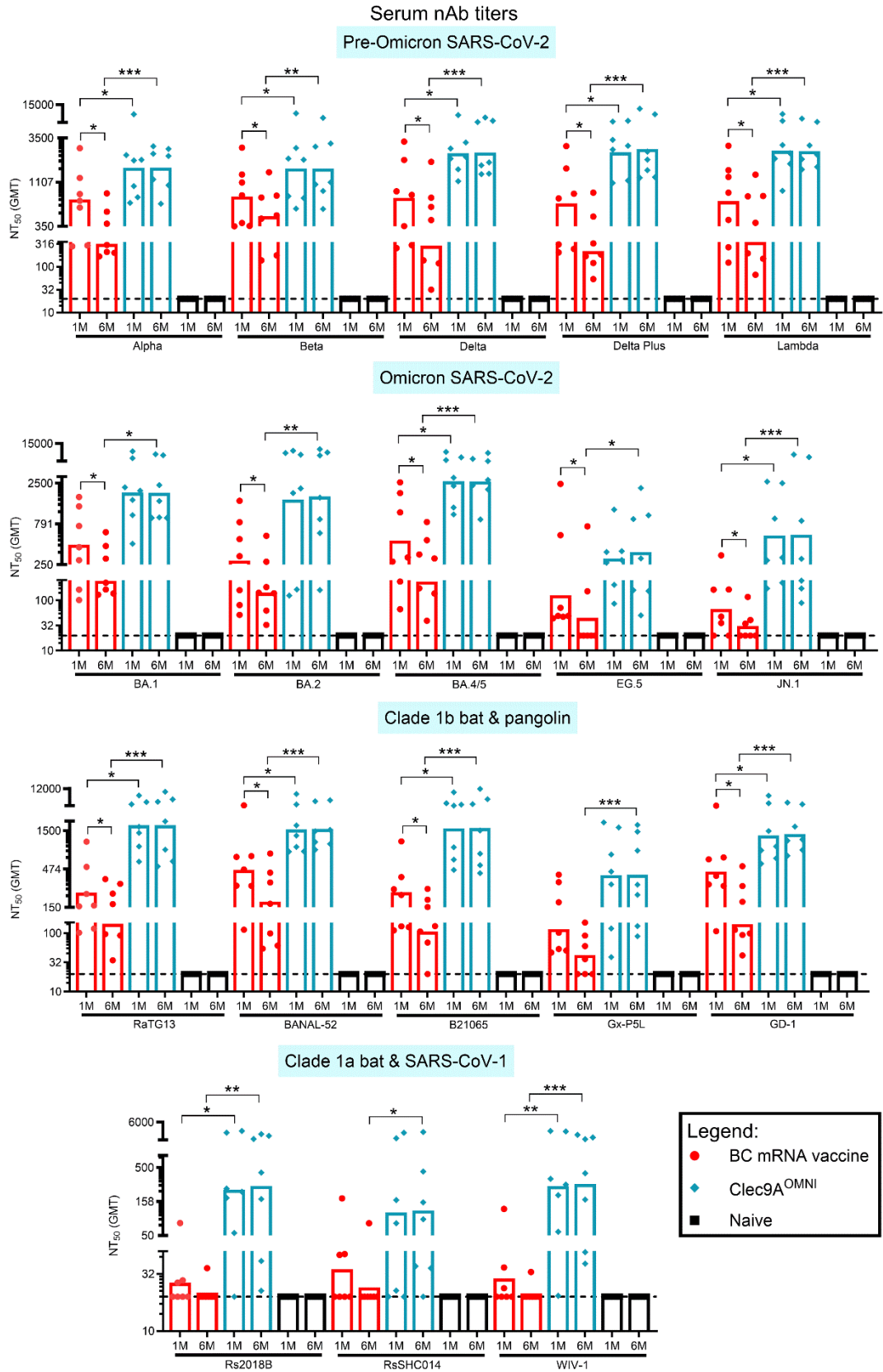


Figure S4. Serum neutralizing antibody responses upon systemic booster with Clec9A^{OMNI} versus BC mRNA vaccine. Five to six-week-old BALB/c mice were immunized twice three weeks apart (0.05 µg per dose; i.m) with original Comirnaty mRNA vaccine. Three months after the second immunization dose, mice were boosted with either BC mRNA vaccine (0.05 µg; i.m), or Clec9A^{OMNI} (8 µg Clec9A^{XBB} + 2 µg Clec9A^{CoV1} adjuvanted with 50 µg poly I:C; s.c). Serum nAb titers against 18 sarbecoviruses from clades 1a and 1b at one- and six months post-boost were determined by multiplex sVNT. Data are from one representative experiment performed twice with similar results, n = 5-6 per group/experiment. Symbols represent individual animals and data shown are geometric means. Statistical analysis: Non-parametric two-tailed Mann-Whitney test, and Friedman test with Dunn's multiple-comparison test. *p < 0.05, **p < 0.01, ***p < 0.001.

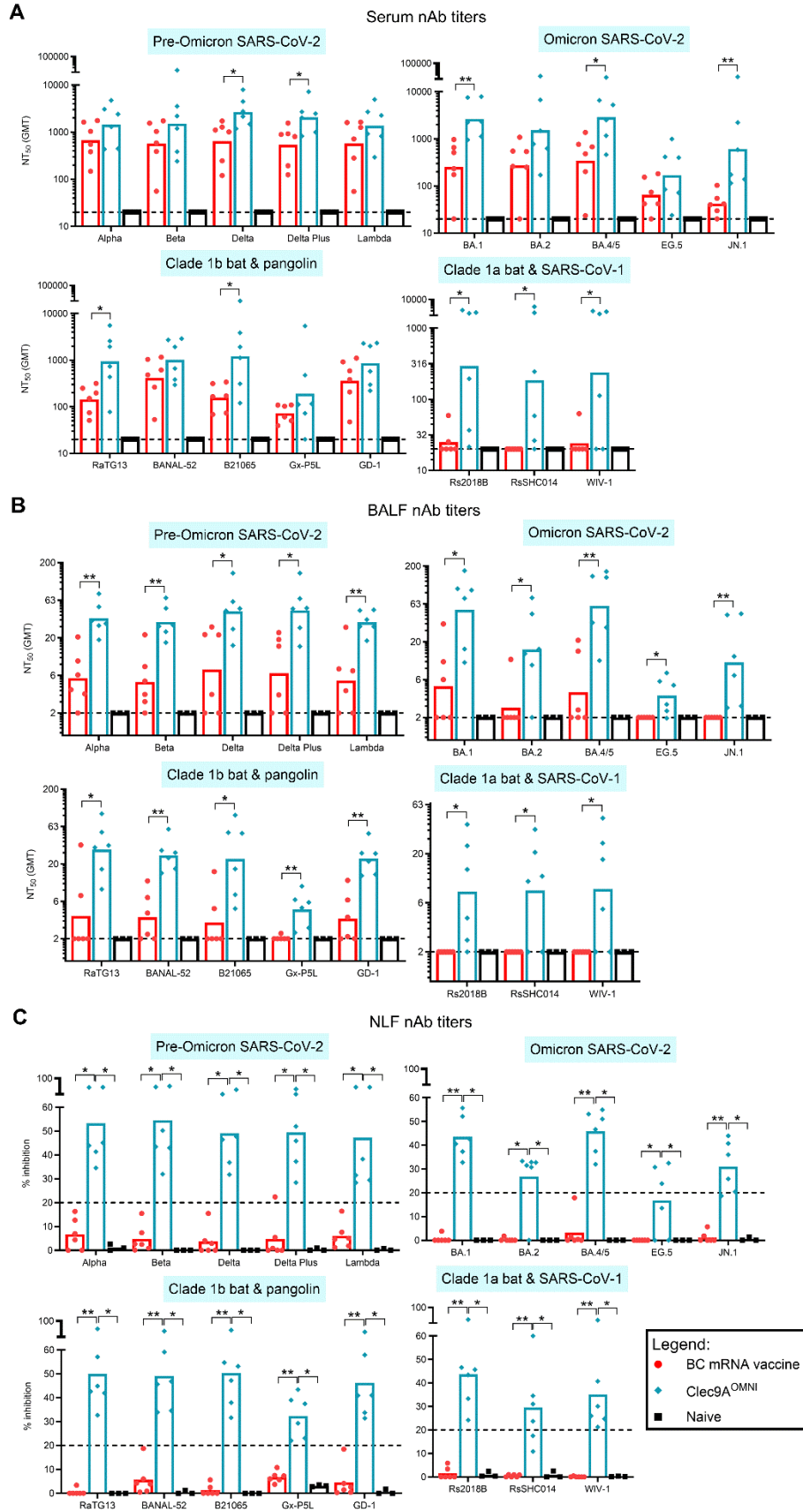


Figure S5. Neutralizing antibody responses upon nasal booster with Clec9A^{OMNI} versus systemic booster with BC mRNA vaccine. Five to six-week-old BALB/c mice were immunized twice three weeks apart with original Comirnaty mRNA vaccine (0.05 µg per dose; i.m). Three months after the second immunization dose, mice were boosted with either BC mRNA vaccine (0.05 µg; i.m), or Clec9A^{OMNI} (4 µg Clec9A^{XBB} + 1 µg Clec9A^{CoV1} adjuvanted with 50 µg poly I:C; i.n). **(A)** Serum, **(B)** BALF and **(C)** NLF nAb titers against 18 sarbecoviruses from clades 1a and 1b at one month post-boost were determined by multiplex sVNT. **(A-C)** Data are from one representative experiment performed twice with similar results, n = 5-6 per group/experiment. Symbols represent individual animals and data shown are **(A, B)** geometric means and **(C)** means. Statistical analysis: Non-parametric two-tailed **(A, B)** Mann-Whitney test and **(C)** Kruskal Wallis test with Dunn's multiple-comparison test. *p < 0.05, **p < 0.01.

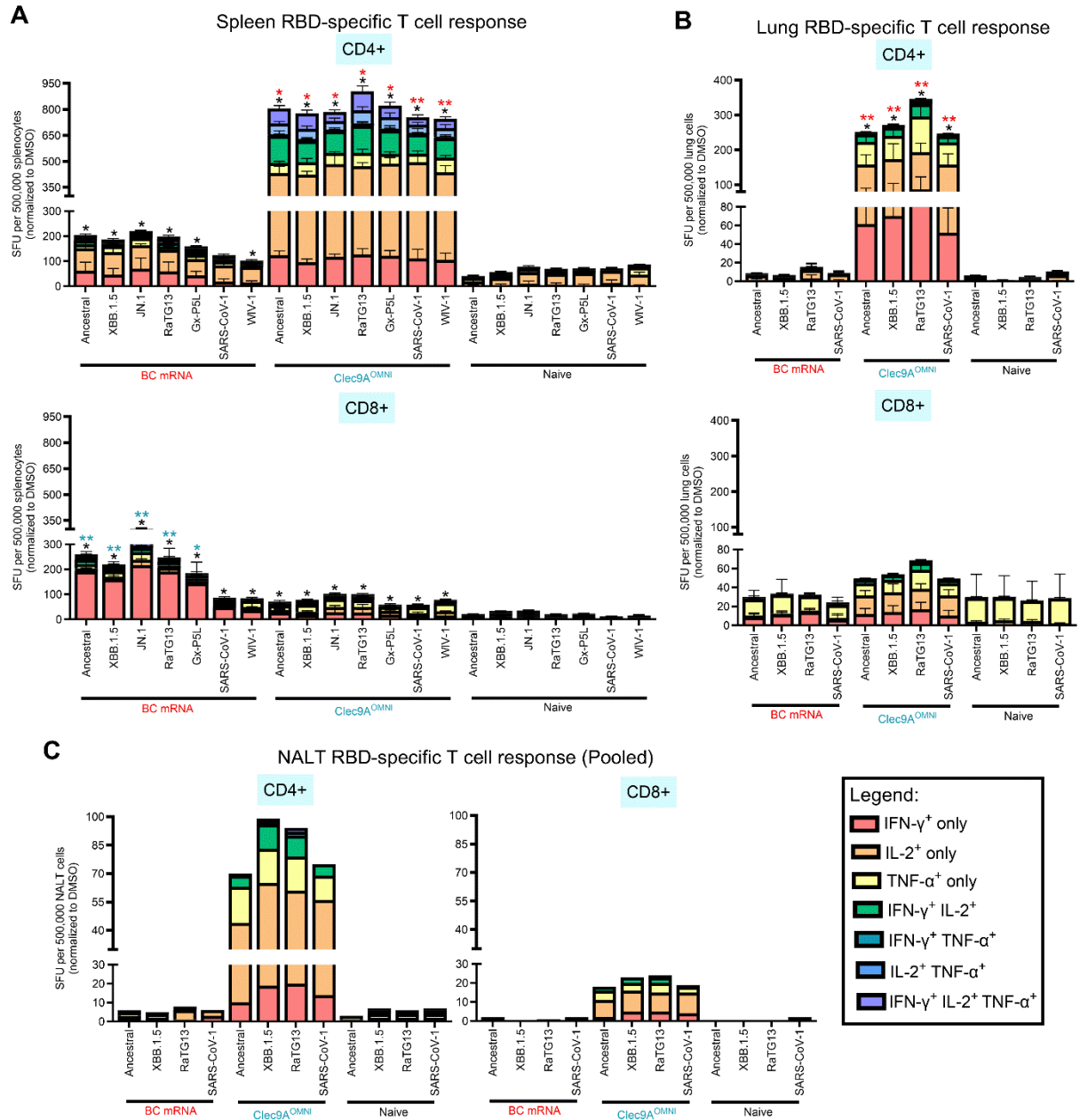


Figure S6. CD4⁺ and CD8⁺ T cell responses upon nasal booster with Clec9A^{OMNI} versus systemic booster with BC mRNA vaccine. Five to six-week-old BALB/c mice were immunized twice three weeks apart with original Comirnaty mRNA vaccine (0.05 μ g per dose; i.m). Three months after the second immunization dose, mice were boosted with either BC mRNA vaccine (0.05 μ g; i.m), or Clec9A^{OMNI} (4 μ g Clec9A^{XBB} + 1 μ g Clec9A^{CoV1} adjuvanted with 50 μ g poly I:C; i.n). (A-C) At two weeks post-boost, mice were euthanized and the frequencies of IFN- γ , IL-2 and/or TNF- α secreting CD4⁺ and CD8⁺ subsets from

(A) spleen, (B) lungs, and (C) NALT were determined by FluoroSPOT upon restimulation with ancestral SARS-CoV-2, XBB.1.5, JN.1, RaTG13, Gx-P5L, SARS-CoV-1 and WIV-1 RBD peptides. (A-C) Data are from one representative experiment performed twice with similar results, n = 5 per group/experiment. Data shown are means \pm (A, B) SD. Statistical analysis: (A, B) Non-parametric two-tailed Kruskal Wallis test with Dunn's multiple-comparison test. *p < 0.05, **p < 0.01. Asterisk colors represent statistical significance between corresponding groups.

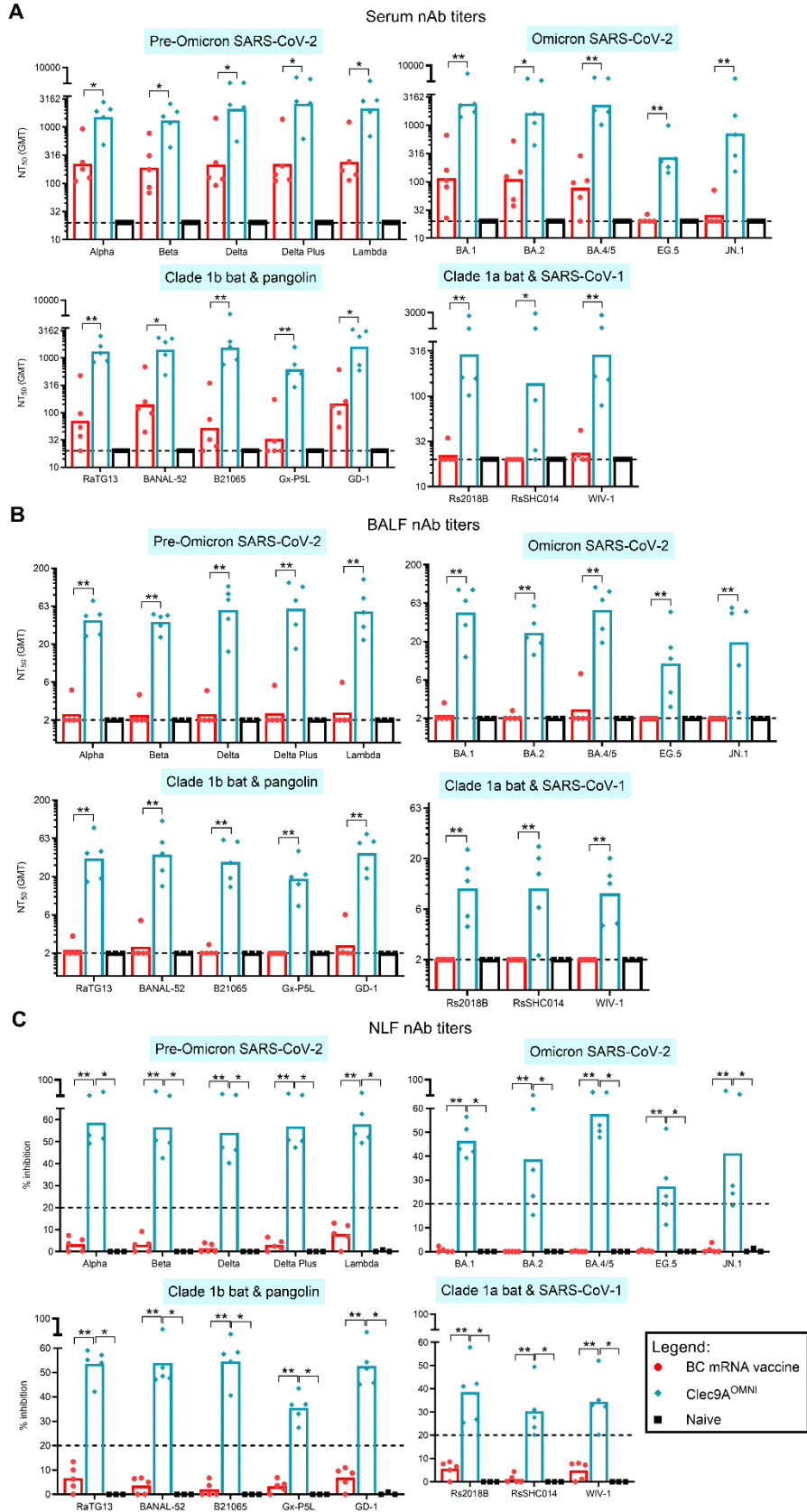


Figure S7. Long-term neutralizing antibody responses upon nasal booster with Clec9A^{OMNI} versus systemic booster with BC mRNA vaccine. Five to six-week-old BALB/c mice were immunized twice three weeks apart with original Comirnaty mRNA vaccine (0.05 µg per dose; i.m). Three months after the second immunization dose, mice were boosted with either BC mRNA vaccine (0.05 µg; i.m), or Clec9A^{OMNI} (4 µg Clec9A^{XBB} + 1 µg Clec9A^{CoV1} adjuvanted with 50 µg poly I:C; i.n). **(A)** Serum, **(B)** BALF and **(C)** NLF nAb titers against 18 sarbecoviruses from clades 1a and 1b at six months post-boost were determined by multiplex sVNT. **(A-C)** Data are from one representative experiment performed twice with similar results, n = 5-6 per group/experiment. Symbols represent individual animals and data shown are **(A, B)** geometric means and **(C)** means. Statistical analysis: Non-parametric two-tailed **(A, B)** Mann-Whitney test and **(C)** Kruskal Wallis test with Dunn's multiple-comparison test. *p < 0.05, **p < 0.01.

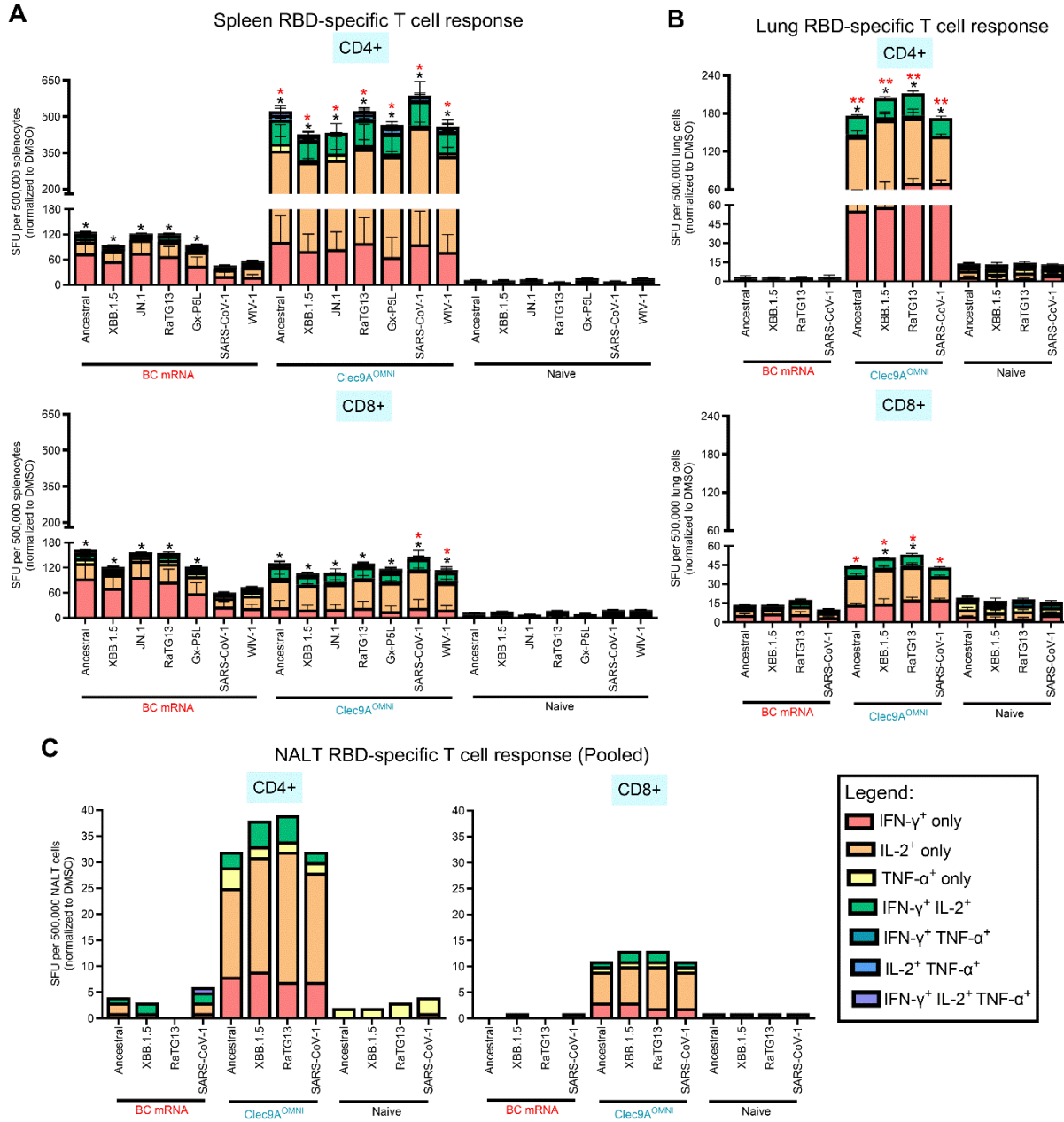


Figure S8. Long-term CD4⁺ and CD8⁺ T cell responses upon nasal booster with Clec9A^{OMNI} versus systemic booster with BC mRNA vaccine. Five to six-week-old BALB/c mice were immunized twice three weeks apart with original Comirnaty mRNA vaccine (0.05 μ g per dose; i.m). Three months after the second immunization dose, mice were boosted with either BC mRNA vaccine (0.05 μ g; i.m), or Clec9A^{OMNI} (4 μ g Clec9A^{XBB} + 1 μ g Clec9A^{CoV1} adjuvanted with 50 μ g poly I:C; i.n). (A-C) Frequencies of IFN- γ , IL-2 and/or TNF- α secreting CD4⁺ and CD8⁺ subsets in (A) spleen, (B) lungs, and (C) NALT were determined

at four months post-boost by FluoroSPOT upon restimulation with ancestral SARS-CoV-2, XBB.1.5, JN.1, RaTG13, Gx-P5L, SARS-CoV-1 and WIV-1 RBD peptides. **(A-C)** Data are from one representative experiment performed twice with similar results, $n = 5$ per group/experiment. Data shown are means \pm **(A, B)** SD. Statistical analysis: **(A, B)** Non-parametric two-tailed Kruskal Wallis test with Dunn's multiple-comparison test. * $p < 0.05$, ** $p < 0.01$. Asterisk colors represent statistical significance between corresponding groups.

A

XBB.1.5 RBD	1	KSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFHEVFNATTFASVYAWNRRKRISNCVA	60
		KSF ++KGIYQTSNFRV P+ +VREFPNITNLCPF EVFNAT F SVYAW RK+ISNCVA	
SARS-CoV-1 RBD	1	KSFEIDKGIYQTSNFRVVPDGDVVRFPNITNLCPFGEVFNATKFPSSVYAWERKKISNCVA	60
XBB.1.5 RBD	61	DYSVIYNFAPFFAFKCYGVSPTKLNLDLCTNVYADSFVIRGNEVSQIAPGQTGNIADYNY	120
		DYSV+YN F FKCYSVS TKLNLDLCF+NVIYADSFV++G++V QIAPGQTG IADYNY	
SARS-CoV-1 RBD	61	DYSVLYNSTFFSTFKCYGVSATKLNLDLCSNVYADSFVVKGDVVRQIAPGQTGVIADYNY	120
XBB.1.5 RBD	121	KLPDDFTGCVIAWNSNKLDSPSGNYNYLYRLFRKSKLKPPERDISTEIQAGNKPCNGV	180
		KLPDDF GCV+AWN+ +D+ +GNYNY YR R KL+PPERDIS + KPC	
SARS-CoV-1 RBD	121	KLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPPERDISNVVFPDGGKPCCTPP	180
XBB.1.5 RBD	181	AGPNCYSPLQSYGFRPTYGVGHQPYRVVVLSEFELLHAPATVCGPKKSTNLVKNKCVNFNF	240
		A NCY PL YGF T G+G+QPYRVVVLSEFELL+APATVCGPK ST+L+KN+CVNFNF	
SARS-CoV-1 RBD	181	A-LNCYWPLNDYGFYTTTGIGYQPYRVVVLSEFELLNAPATVCGPKLSTDLIKNQCVNFNF	239
XBB.1.5 RBD	241	NGLTGTG	247
		NGLTGTG	
SARS-CoV-1 RBD	240	NGLTGTG	246

B

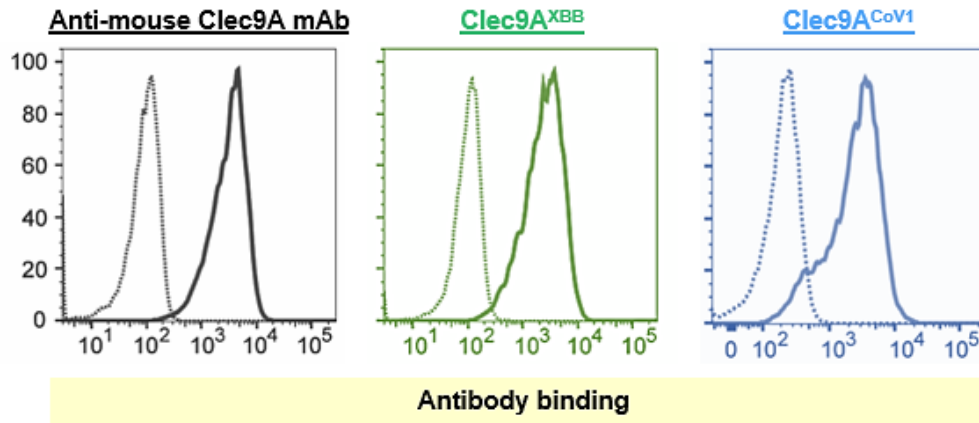


Figure S9. Antigenic sequence and validation of Clec9A-RBD constructs. (A) BLAST amino acid sequence alignment for RBD antigens (XBB.1.5 and SARS-CoV-1) used for expression of Clec9A-RBD constructs (Clec9A^{XBB} and Clec9A^{CoV1}). **(B)** Binding of Clec9A-RBD constructs to mouse Clec9A was verified by flow cytometry. CHO-K1 (dotted lines) or CHO-Clec9A cells (solid lines) were incubated with control anti-mouse Clec9A mAb (black, left panel), purified Clec9A^{XBB} construct (green, middle panel) or

Clec9A^{CoV1} construct (blue, right panel). Binding was detected with PE-conjugated anti-rat Ig and dead cells were excluded by forward/side scatter and live/dead staining.

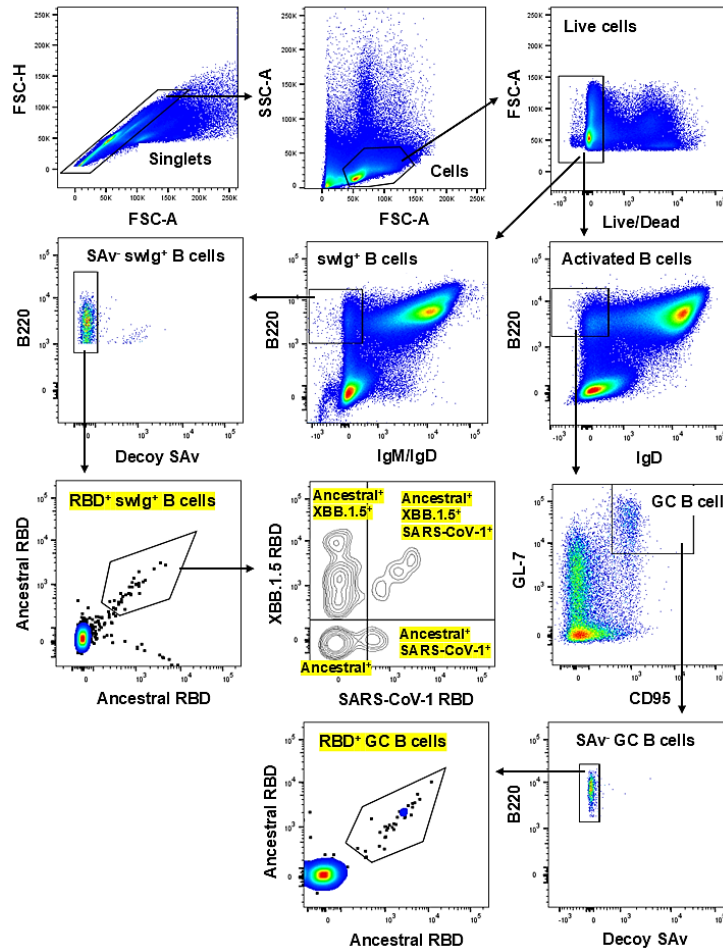


Figure S10. Gating strategy to identify antigen-specific swIg⁺ and GC B cell subsets. Single cells were first identified via FSC-H/FSC-A and SSC-A/FSC-A. Dead cells were excluded with eFluor780 Fixable Viability Dye, and swIg⁺ (B220⁺ IgD⁻ IgM⁻) and activated (B220⁺ IgD⁺) B cells were identified from live cell population (Live/Dead). For the former, swIg⁺ B cells that bind non-specifically to SA_v were excluded using decoy SA_v probe, and RBD-specific swIg⁺ B cells were identified from the SA_v⁻ swIg⁺ B cell population via a double discrimination gate where cells must be double positive for both ancestral RBD-BV421 and -PE to be considered as antigen-specific. Cross-reactivity to XBB.1.5 and SARS-CoV-1 RBD were further determined from the gated total RBD⁺ swIg⁺ B cell population. For the latter, GC B cells (GL-7⁺ CD95⁺) were identified from the activated B cell population, and those binding non-specifically to SA_v were excluded using decoy SA_v probe. RBD-specific GC B cells were subsequently identified from the SA_v⁻ GC B cell population via double discrimination gating.

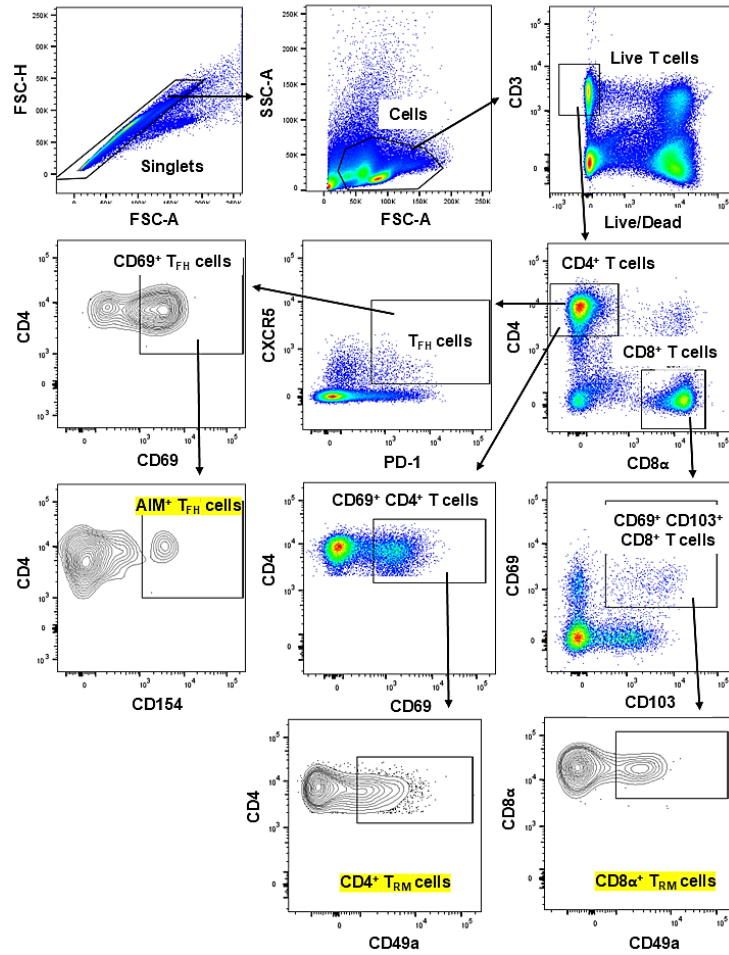


Figure S11. Gating strategy to identify AIM⁺ T_{FH} and T_{RM} cells. Single cells were first identified via FSC-H/FSC-A and SSC-A/FSC-A. Dead cells were excluded with eFluor780 Fixable Viability Dye, and CD4⁺ and CD8⁺ T cells were identified from the total live T cell (Live/Dead⁻ CD3⁺) population. AIM⁺ T_{FH} cells were examined by first identifying total T_{FH} cells (CXCR5⁺ PD-1⁺) from the CD4⁺ T cell population, followed by gating on those that are double positive for both CD69 and CD154 AIM. T_{RM} cells in lung and NALT tissues were identified from total CD4⁺ and CD8⁺ T cells by gating into CD69⁺ and CD69⁺ CD103⁺ populations respectively, followed by further gating into cells that were also CD49a⁺ (CD4⁺: CD69⁺ CD49a⁺, CD8⁺: CD69⁺ CD103⁺ CD49a⁺).

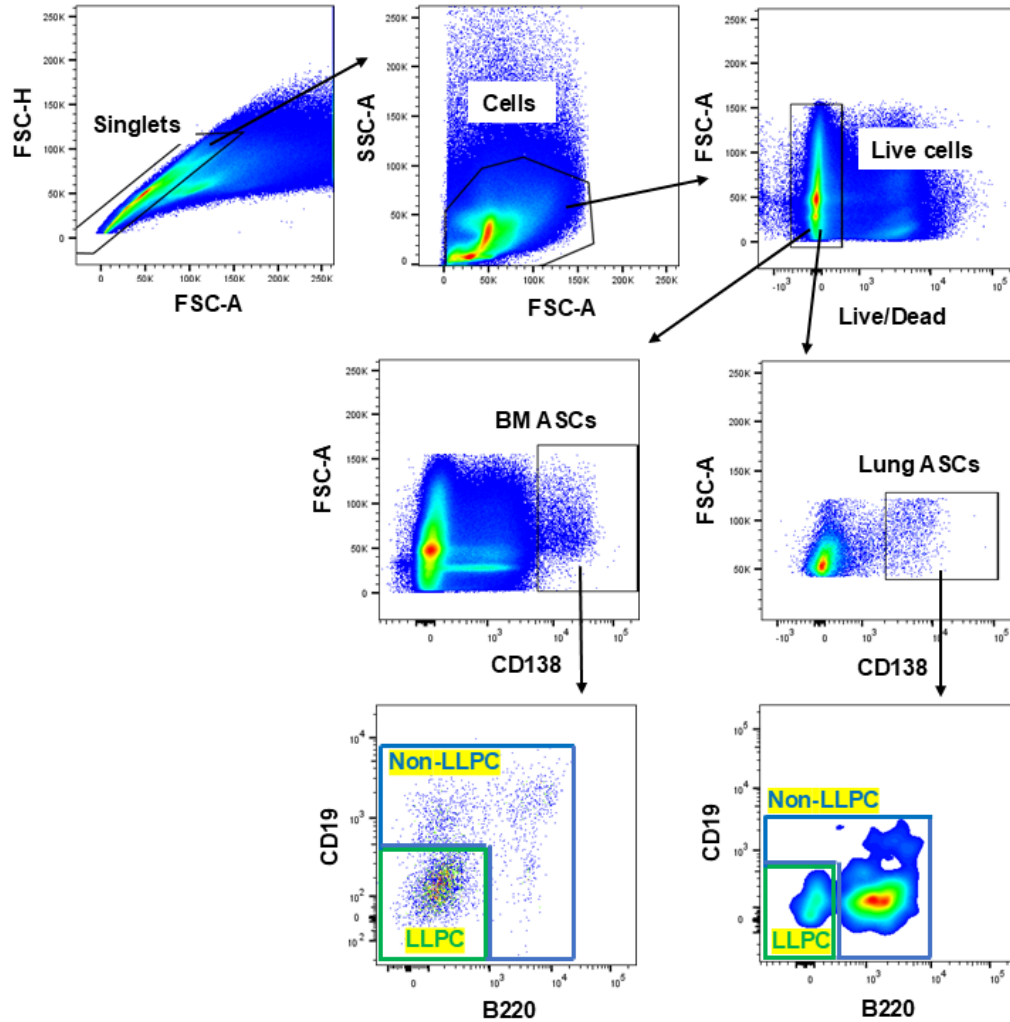


Figure S12. Gating strategy for sorting BM and lung LLPC and non-LLPC ASC subsets. Single cells were first identified via FSC-H/FSC-A and SSC-A/FSC-A. Dead cells were excluded with eFluor780 Fixable Viability Dye, and BM and lung ASC populations were identified as live cells that highly express CD138 (Live/Dead⁻ CD138^{hi}). Boolean gating was subsequently applied to identify LLPC and non-LLPC ASC subsets based on their expression of B220 and CD19; LLPC = CD138^{hi} B220^{lo} CD19^{lo} (green), non-LLPC = inverse of LLPC gating (blue).

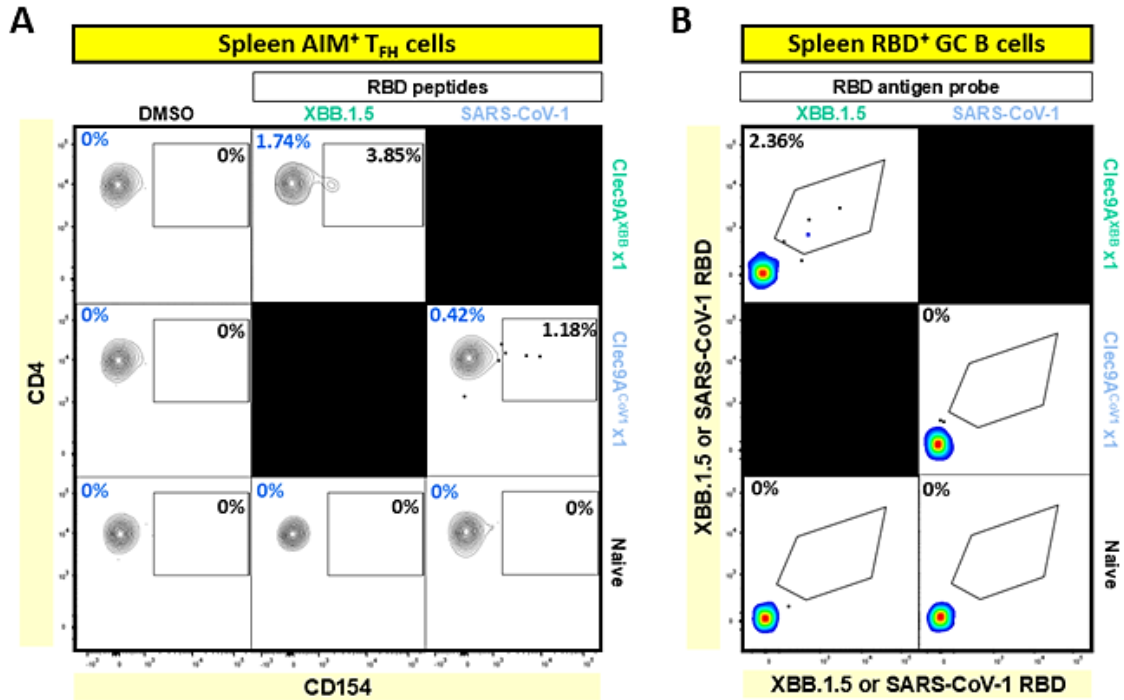


Figure S13. Representative flow cytometric analysis results of AIM⁺ T_{FH} and RBD⁺ GC B cells following single dose Clec9A^{XBB} or Clec9A^{CoV1} immunization. (A) Representative plots of spleen AIM⁺ T_{FH} cells at six months post-boost. Values indicated in black and blue represent percentage AIM⁺ T_{FH} cells out of CD69⁺ T_{FH} cells and total T_{FH} cells respectively. **(B)** Representative plots of RBD-specific spleen GC B cells at six months post-boost.

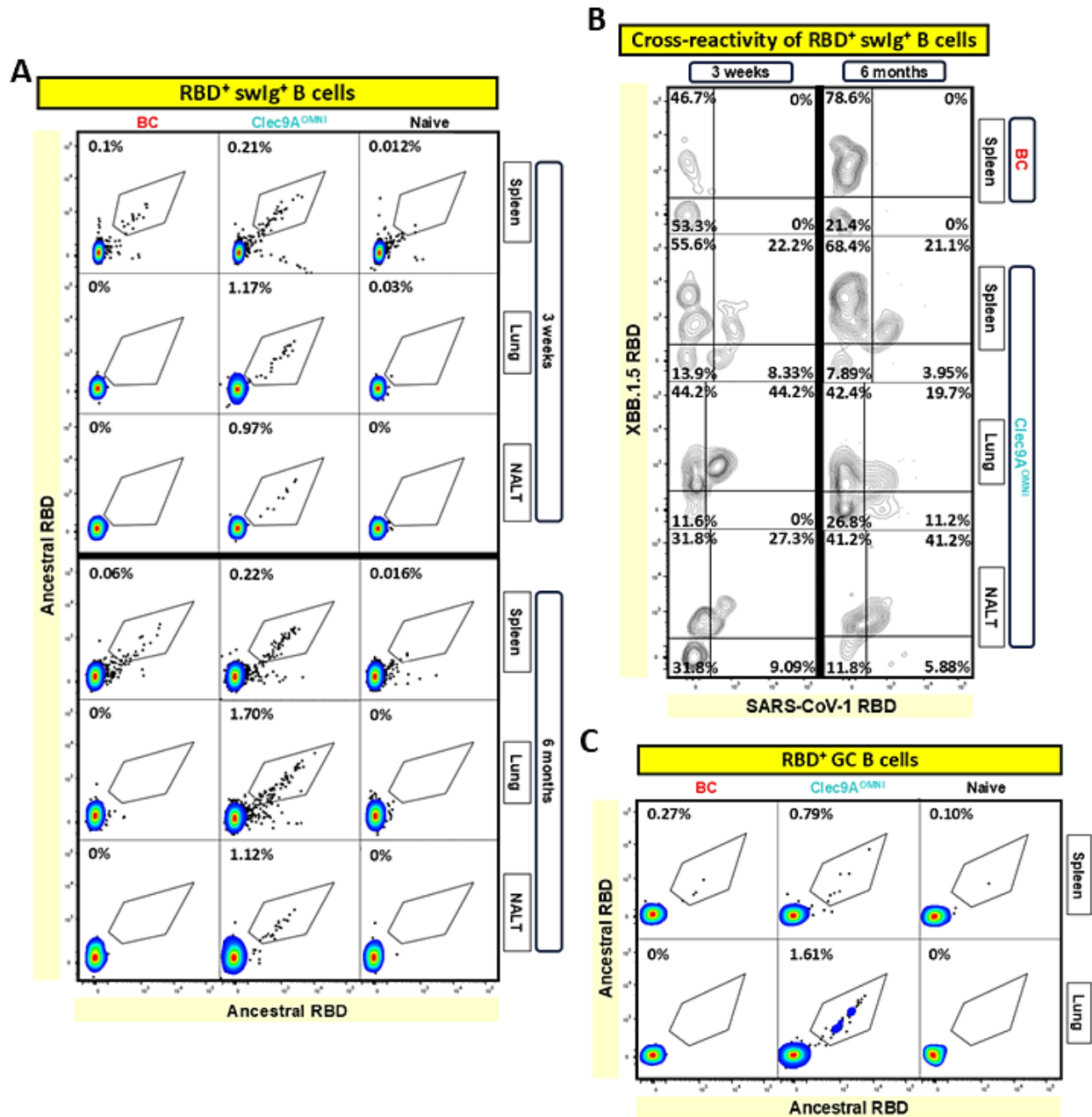


Figure S14. Representative flow cytometric analysis results of antigen-specific B cell subsets following BC mRNA vaccine and Clec9A^{OMNI} booster immunization. (A, B) Representative plots of ancestral SARS-CoV-2 RBD-specific spleen, lung and NALT (A) swIg⁺ B cells and their (B) cross-reactivity to XBB.1.5 and SARS-CoV-1 RBD, at three weeks and six months post-boost. (C) Representative plots of RBD-specific spleen and lung GC B cells at six months post-boost.

CD4⁺ and CD8⁺ T_{RM} cells at one-month post-boost. Values indicated in black and blue represent percentage T_{RM} cells out of CD69⁺ CD4⁺ or CD69⁺ CD103⁺ CD8⁺ T cells, and total CD4⁺ or CD8⁺ T cells respectively.

Table S1. Sarbecovirus RBD antigens used in the Multiplex sVNT.

Clade	Sarbecovirus	Variant/Strain	Source
1B	Pre-Omicron SARS-CoV-2	Ancestral	Custom made by Genscript
		Alpha	
		Beta	
		Delta	ACROBiosystems, SPD-C82Ed
		Delta Plus	Produced in-house in HEK293T cells as described previously (2)
		Lambda	
		Mu	
	Omicron SARS-CoV-2	BA.1	ACROBiosystems, SPD-C522K
		BA.2	ACROBiosystems, SPD-C82Eq
		BA.4/5	ACROBiosystems, SPD-C82Ew
		XBB.1.5	Produced in-house in HEK293T cells as described previously (2)
		EG.5	
		JN.1	
	Bat	RaTG13	Custom made by Genscript
		BANAL-52	Produced in-house in HEK293T cells as described previously (2)
		B21065	
	Pangolin	Gx-P5L	Custom made by Genscript
GD-1		Produced in-house in HEK293T cells as described previously (2)	
1A	Bat	Rs2018B	Produced in-house in HEK293T cells as described previously (2)
		RsSHC014	
		WIV-1	
	SARS-CoV-1	SARS-CoV-1	Custom made by Genscript

Table S2. Antibodies used for flow cytometry staining of antigen-specific B cell subsets, AIM⁺ T_{FH} cells, T_{RM} cells, and sorting of LLPC and non-LLPC ASC subsets.

Marker	Fluorophore	Manufacturer & catalogue #	Dilution/Concentration
RBD⁺ swIg⁺ B cells			
B220	Alexa Fluor 488	BD Biosciences, 557669	1:200
IgM	BUV395	BD Biosciences, 564025	
IgD		BD Biosciences, 564274	
Decoy (SAv)	BV605	BD Biosciences, 563260	1 µg/mL
Ancestral RBD	BV421		
	PE		
XBB.1.5 RBD	BV711		
SARS-CoV-1 RBD	APC		
RBD⁺ GC B cells			
B220	BUV395	BD Biosciences, 563793	1:200
IgD	BV711	BD Biosciences, 564275	
GL-7	Alexa Fluor 647	BD Biosciences, 561529	
CD95	BV605	Biologend, 152612	
Decoy (SAv)	BB515	BD Biosciences, 564453	1 µg/mL
Ancestral RBD	BV421		
	PE		
AIM⁺ T_{FH} cells			
CD3	FITC	BD Biosciences, 555274	1:200
CD4	BUV395	BD Biosciences, 563790	
CXCR5	APC	BD Biosciences, 560615	
PD-1	BV605	BD Biosciences, 563059	
CD69	BV421	BD Biosciences, 562920	
CD154	PE	BD Biosciences, 553658	
T_{RM} cells			
CD3	FITC	BD Biosciences, 555274	1:200
CD4	BUV395	BD Biosciences, 563790	
CD8α	BV711	BD Biosciences, 563046	
CD69	BV421	BD Biosciences, 562920	
CD103	APC	BD Biosciences, 562772	
CD49a	PE	BD Biosciences, 562115	
LLPC and non-LLPC ASC subset sorting			
CD138	BV605	BD Biosciences, 563147	1:200
B220	Alexa Fluor 488	BD Biosciences, 557669	
CD19	BUV395	BD Biosciences, 563557	