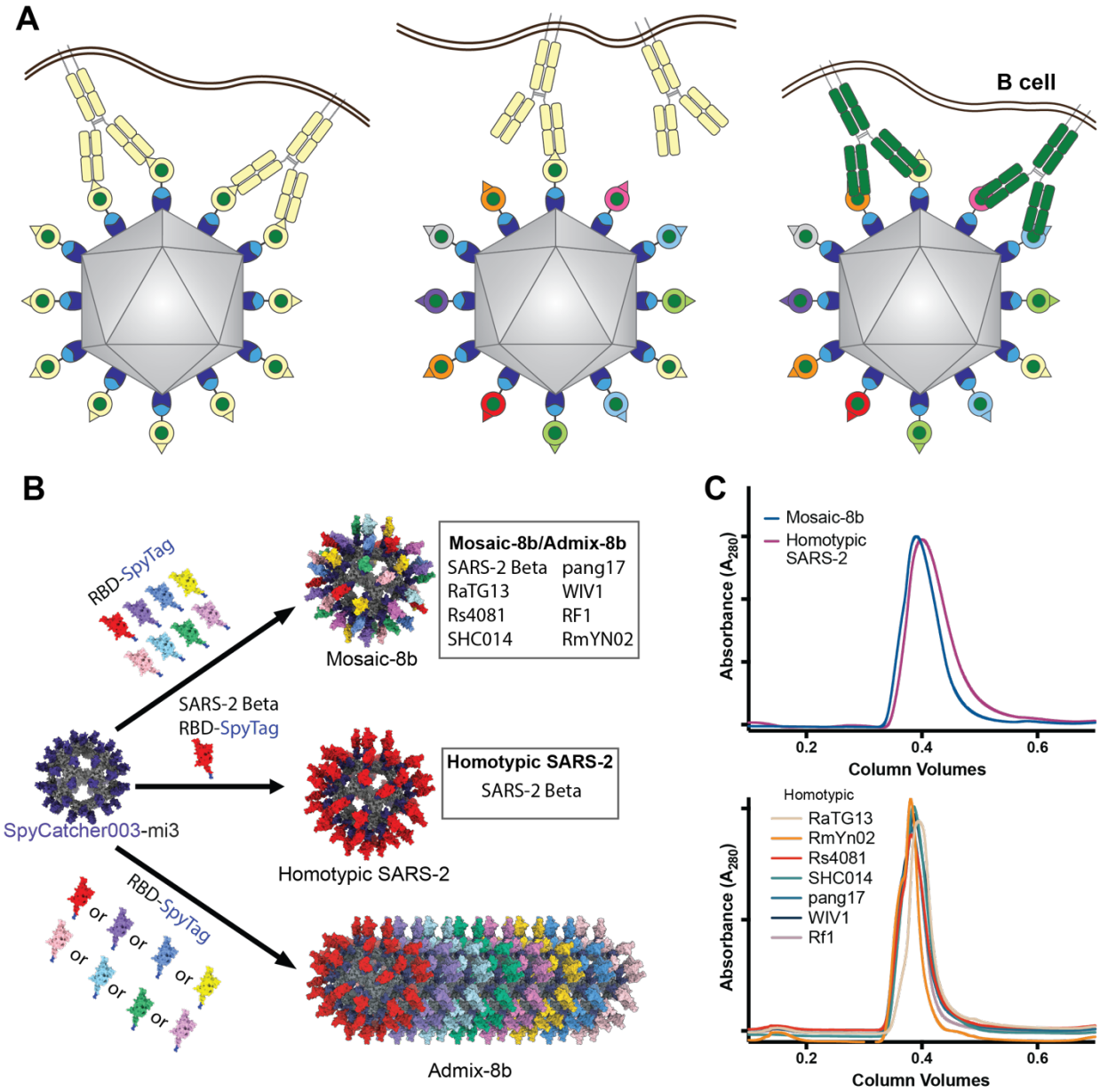

Figure S1 Preparation of RBD-mi3 nanoparticles, related to Figure 1.

(A) Hypothesis illustrating potential mechanism for mosaic RBD-nanoparticle induction of cross-reactive Abs. Left: Both Fabs of a strain-specific membrane-bound BCR can bind to a strain-specific epitope (pale yellow triangle) on yellow antigens attached to a homotypic nanoparticle. Middle: Strain-specific BCRs can only bind with one Fab to a strain-specific epitope (triangle) on yellow antigen attached to a mosaic nanoparticle. Right: Cross-reactive BCRs can bind with both Fabs to a common epitope present on adjacent antigens (green circles) attached to a mosaic particle, but not to strain-specific epitopes (triangles).

(B) Schematic of construction of mosaic-8b, homotypic SARS-2, and admix-8b RBD-mi3 nanoparticles made using models constructed with coordinates of an RBD (PDB 7BZ5), SpyCatcher (PDB 4MLI), and an i3-01 nanoparticle (PDB 7B3Y).

(C) Superose 6 10/300 size exclusion chromatography profile after RBD conjugations to mi3 showing peaks for RBD-mi3 nanoparticles.

(D) Coomassie-stained SDS-PAGE gel of RBD-coupled nanoparticles, unconjugated RBDs, and free SpyCatcher003-mi3 particles (SC3-mi3).

Table S1 Summary of vaccines and immunogens, related to Figure 1.

Animal Study	Vaccinated with		Vaccine Source	Immunized with	Immunogen Source	Figures
pre-vaccinated non-human primates (NHPs)	WA1 Spike, Bivalent (Beta/Delta) SHARP, Trivalent (WA1/Beta/Delta) SHARP	DNA-gene gun	University of Washington, HDT Bio, University of Albany, Creative Biosolutions	mosaic-8b	Caltech	2, S2
		repRNA-LION				
		repRNA-LION				
		repRNA-gene gun				
		repRNA-gene gun				
		DNA-gene gun		homotypic SARS-2	Caltech	
		DNA-gene gun				
		repRNA-LION				
		repRNA-gene gun				
		repRNA-gene gun				
		DNA-gene gun		WA1/BA.1 rep-RNA	HDT Bio	
		DNA-gene gun				
		repRNA-LION				
		repRNA-gene gun				
pre-vaccinated BALB/c mice	Pfizer-like WA1		Helix Biotech	mosaic-8b	Caltech/Ingenza	3, S3
				admix-8b	Caltech/Ingenza	
				homotypic SARS-2	Caltech/Ingenza	
				Pfizer-like WA1 mRNA-LNP	Helix Biotech	
	Pfizer-like WA1 and WA1/BA.5 mRNA-LNP (Bivalent)			mosaic-8b	Caltech/Ingenza	
				mosaic-7	Caltech	
				Pfizer-like WA1/BA.5 mRNA-LNP (Bivalent)	Helix Biotech	
pre-vaccinated BALB/c mice	WA1 ChAdOx1		Jenner Institute, Oxford	mosaic-8b	Caltech	4, 5, S4
				admix-8b	Caltech	
				homotypic SARS-2	Caltech	
				WA1 mRNA-LNP	Rockefeller	
				WA1 ChAdOx1	Jenner Institute, Oxford	
pre-vaccinated S1pr2-IgkTag mice	WA1 mRNA-LNP		University of Pennsylvania, Acuitas	mosaic-8b	Caltech	6, S6, S7
				admix-8b	Caltech	

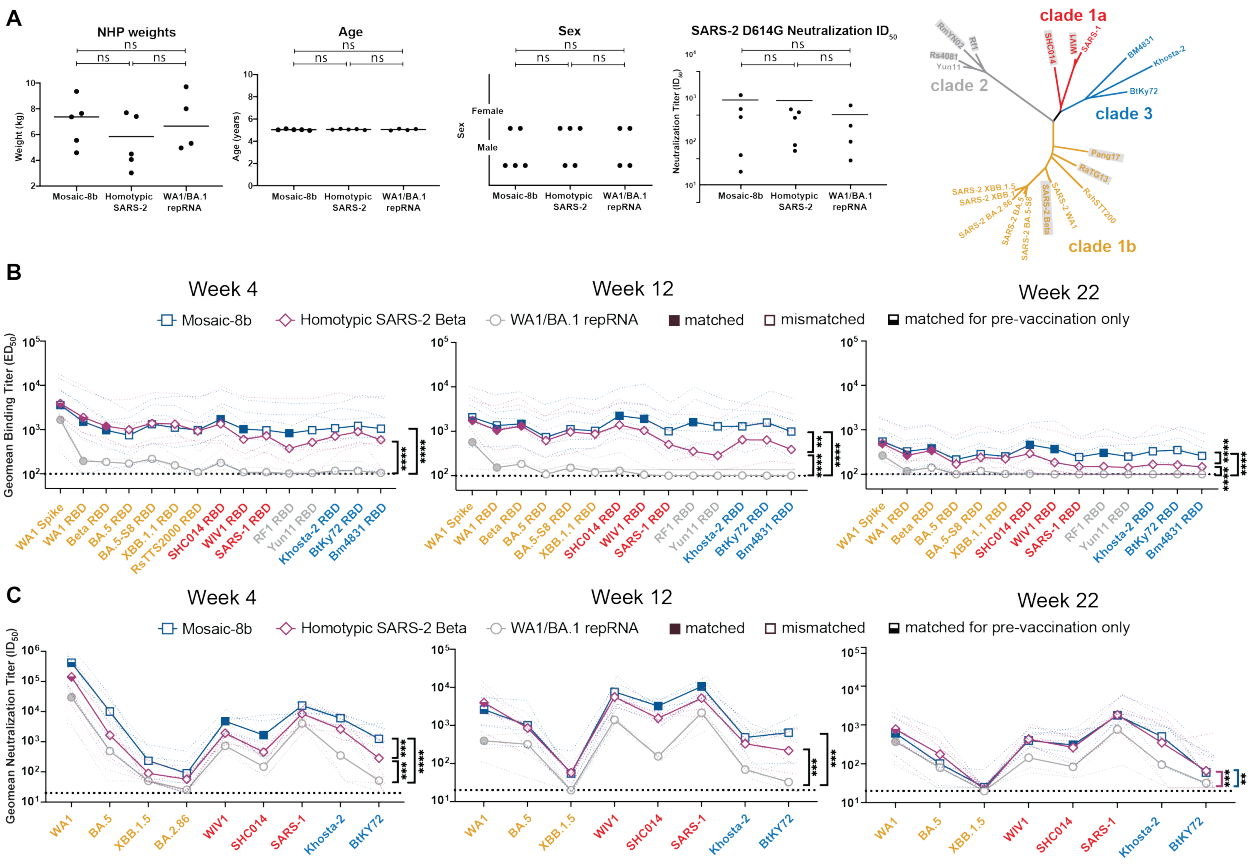

Figure S2 RBD-nanoparticle immunizations in previously-vaccinated NHPs elicit cross-reactive Ab responses, related to Figure 2.

Data are shown for ELISA and neutralization analyses for serum samples from weeks 4, 12, and 22. (Data for samples from weeks 0, 2, 8, and 10 are in Figure 2). Geometric means of ED_{50} or ID_{50} values for all animals in each cohort are indicated by symbols connected by thick colored lines; results for individual animals are indicated by dotted lines. Mean titers against indicated viral antigens or pseudoviruses were compared pairwise across immunization cohorts by Tukey's multiple comparison test with the Geisser-Greenhouse correction (as calculated by GraphPad Prism). Significant differences between cohorts linked by vertical lines are indicated by asterisks: $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, $p<0.0001$ = ****.

(A) Left: Stratification of pre-vaccinated NHPs into groups used for immunizations with mosaic-8b, homotypic SARS-2, and WA1/BA.1 repRNA based on weight, age, sex, and neutralization ID_{50} values (ns = no significant difference; Mann-Whitney test). Neutralization ID_{50}s were derived from samples taken at week -8 in the vaccination/immunization regimen in Figure 2A. Right: Phylogenetic tree of selected sarbecoviruses calculated using a Jukes-Cantor generic distance model using Geneious Prime® 2023.1.2 based on amino acid sequences of RBDs aligned using Clustal Omega.[97] RBDs included in mosaic-8b are highlighted in gray rectangles.

(B) Geometric mean ELISA binding titers at the indicated weeks after immunization with mosaic-8b, homotypic SARS-2, or bivalent WA1/BA.1 repRNA against indicated viral antigens.

(C) Geometric mean neutralization titers at the indicated weeks after immunization with mosaic-8b, homotypic SARS-2, or bivalent WA1/BA.1 repRNA against indicated sarbecovirus pseudoviruses.

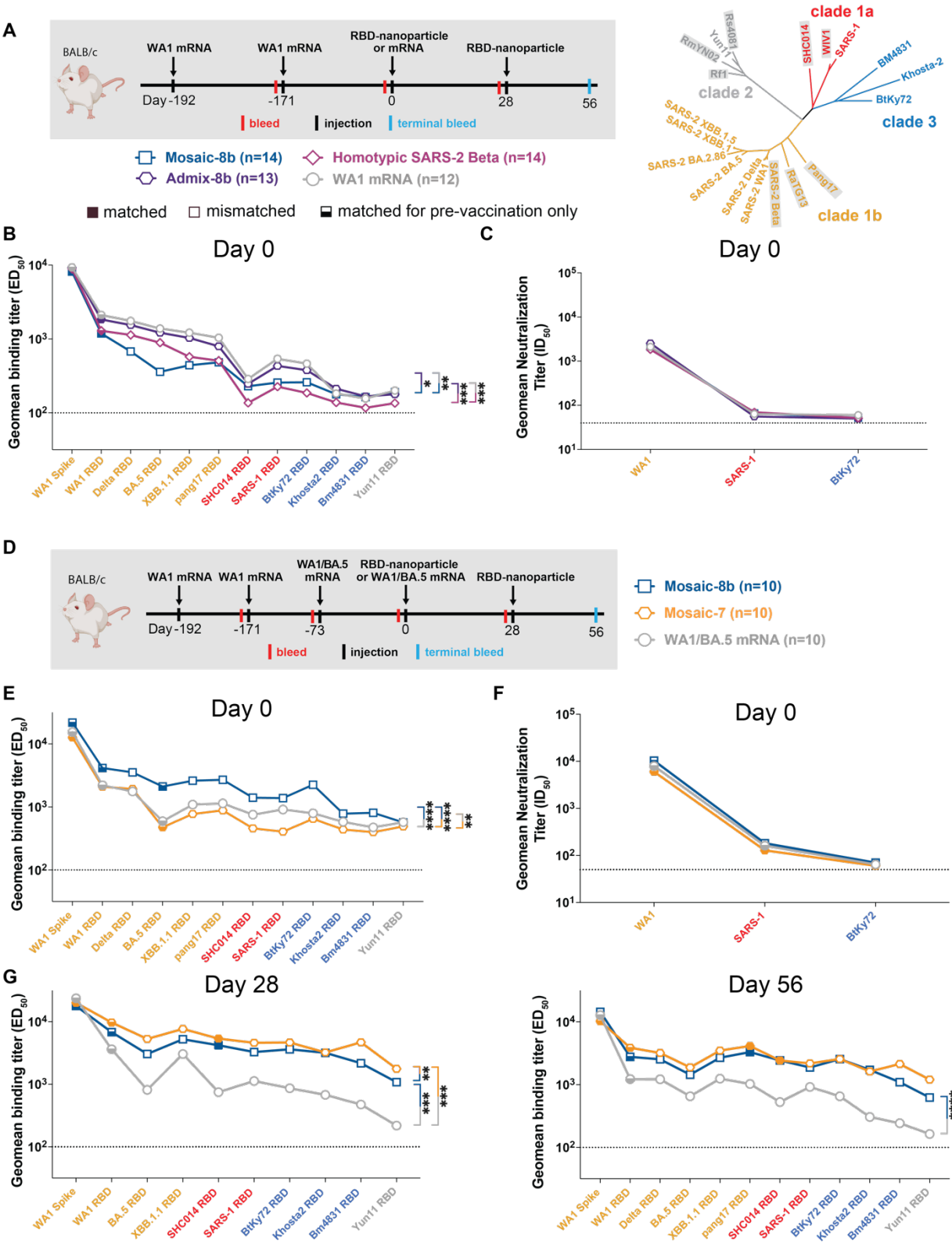

Figure S3 Cohorts of mRNA-LNP vaccinated mice showed significant differences in midpoint ED_{50} titers at day 0 (prior to RBD-nanoparticle immunizations), related to Figure 3.

Geometric means of ED_{50} or ID_{50} values for all animals in each cohort are indicated by symbols connected by thick colored lines. Mean titers against indicated viral antigens or pseudoviruses were compared pairwise across immunization cohorts by Tukey's multiple comparison test with the Geisser-Greenhouse correction (as calculated by GraphPad Prism). Significant differences between cohorts linked by vertical lines are indicated by asterisks: $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, $p<0.0001$ = ****.

(A) Left: Schematic of vaccination/immunization regimen for panels B and C. Mice were vaccinated at the indicated days prior to RBD-nanoparticle prime and boost immunizations at days 0 and 28 or mRNA-LNP prime immunization at day 0. Right: Phylogenetic tree of selected sarbecoviruses calculated using a Jukes-Cantor generic distance model using Geneious Prime® 2023.1.2 based on amino acid sequences of RBDs aligned using Clustal Omega.[97] RBDs included in mosaic-8b are highlighted in gray rectangles.

(B) Geometric mean ELISA binding titers of serum from mice assigned to each cohort at day 0 (192 and 171 days after the first and second mRNA-LNP vaccinations but prior to nanoparticle or additional mRNA-LNP immunizations) showing significant differences between cohorts prior to immunizations.

(C) Geometric mean neutralization binding titers of serum from mice assigned to each cohort at day 0 (192 and 171 days after the first and second mRNA-LNP vaccinations but prior to nanoparticle or additional mRNA-LNP immunizations).

(D) Schematic of vaccination/immunization regimen for panels E-G. Mice were vaccinated at the indicated days prior to RBD-nanoparticle prime and boost immunizations at days 0 and 28 or mRNA-LNP prime immunization at day 0.

(E) Geometric mean ELISA binding titers of serum from mice assigned to each cohort at day 0 (192, 171, and 73 days after the first, second, and third mRNA-LNP vaccinations but prior to nanoparticle or additional mRNA-LNP immunizations) showing significant differences between cohorts prior to immunizations.

(F) Geometric mean neutralization binding titers of serum from mice assigned to each cohort at day 0 (192, 171, and 73 days after the first, second, and third mRNA-LNP vaccinations but prior to nanoparticle or additional mRNA-LNP immunizations).

(G) Non-baseline corrected geometric mean ELISA binding titers at the indicated days after immunization with mosaic-8b, mosaic-7, or WA1/BA.5 mRNA-LNP against indicated viral antigens. Compare with Figure 3E (baseline corrected geometric mean ELISA binding titers).

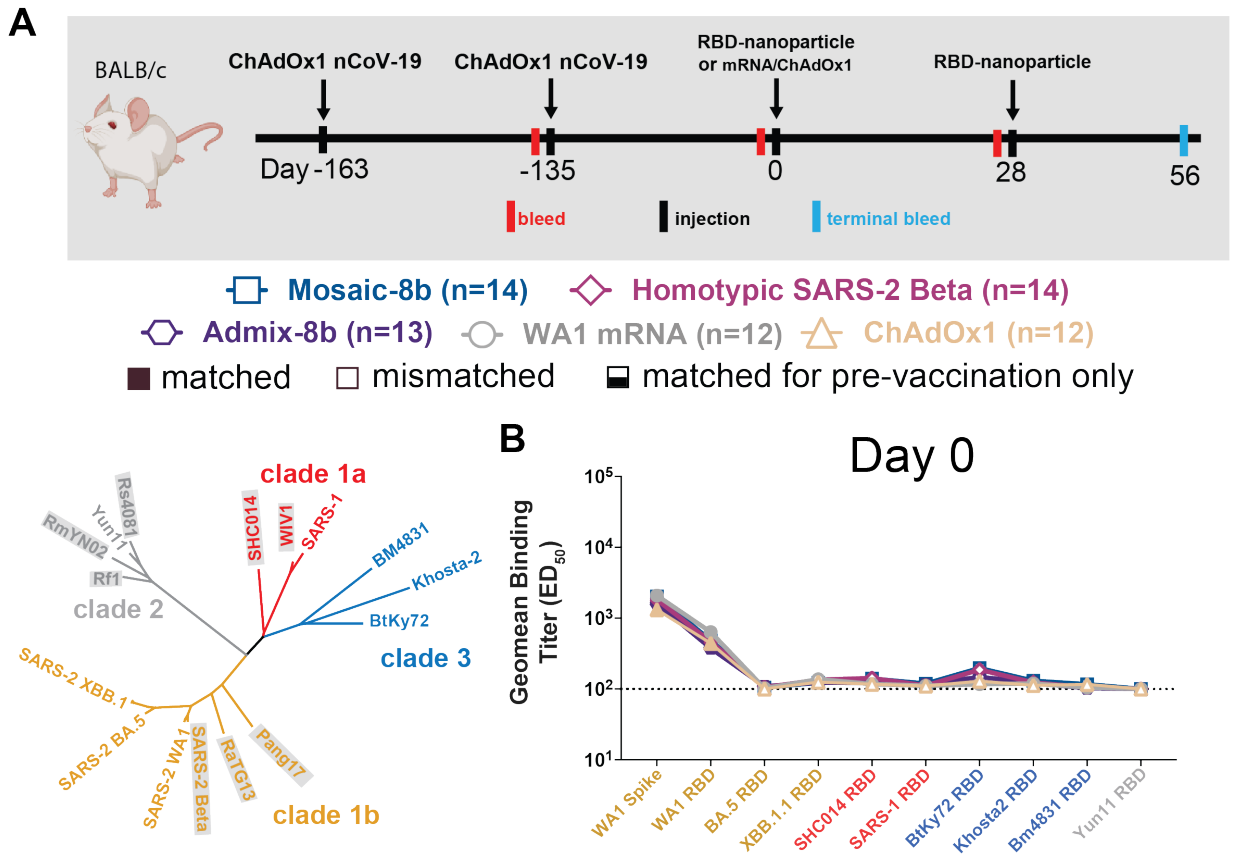

Figure S4 Cohorts of ChAdOx1 vaccinated mice showed no significant differences in midpoint ED_{50} titers at day 0, related to Figure 4.

Geometric means of ED_{50} or ID_{50} values for all animals in each cohort are indicated by symbols connected by thick colored lines. Mean titers against indicated viral antigens or pseudoviruses were compared pairwise across immunization cohorts by Tukey's multiple comparison test with the Geisser-Greenhouse correction (as calculated by GraphPad Prism). Significant differences between cohorts linked by vertical lines are indicated by asterisks: $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, $p<0.0001$ = ****.

(A) Top: Schematic of vaccination/immunization regimen. Mice were vaccinated at the indicated days prior to RBD-nanoparticle prime and boost immunizations at days 0 and 28 or mRNA-LNP or ChAdOx1 prime immunizations at day 0. Bottom: Phylogenetic tree of selected sarbecoviruses calculated using a Jukes-Cantor generic distance model using Geneious Prime® 2023.1.2 based on amino acid sequences of RBDs aligned using Clustal Omega.[97] RBDs included in mosaic-8b are highlighted in gray rectangles.

(B) Geometric mean ELISA binding titers of serum from mice assigned to each cohort at day 0 (163 and 134 days after the first and second ChAdOx1 vaccinations but prior to nanoparticle, mRNA-LNP, or additional ChAdOx1 immunizations). Binding titers are represented as mean ED_{50} values for serum IgG binding to RBD or spike proteins from the indicated sarbecovirus strains.

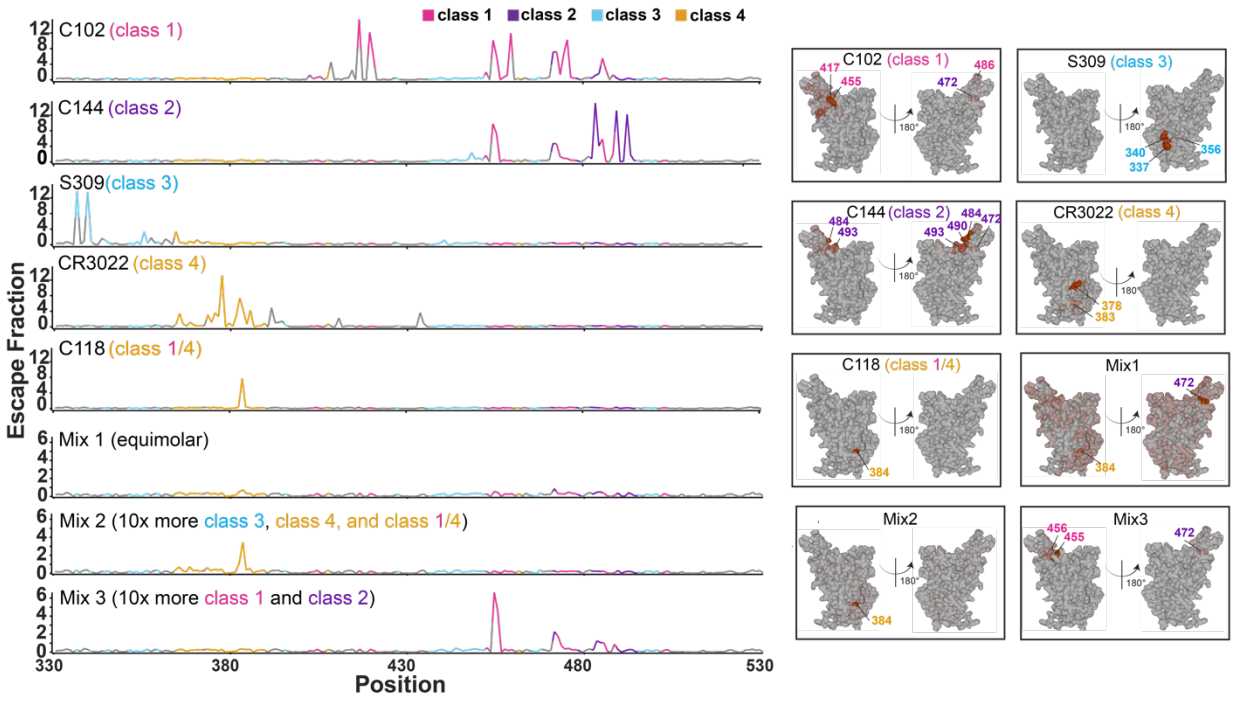

Figure S5 Comparison of DMS profiles of individual mAbs and mAb mixtures, related to Figure 5.

Left: Line plots for DMS results from individual mAbs or the indicated mAb mixtures recognizing different RBD epitopes (epitopes defined in Figure 1B). C102, C118, C144, and S309 are mAbs that were derived from COVID-19 convalescent donors.[60,70] CR3022 is an anti-SARS-CoV mAb that binds to SARS-2 RBD.[66] Epitope assignments for these mAbs were previously described.[90] DMS was conducted for the mAb reagents using a WA1 RBD library. The x-axis shows the RBD residue number and the y-axis shows the sum of the Ab escape of all mutations at a site (larger numbers indicating more Ab escape). Each line represents one antiserum with heavy lines showing the average across the n=3 sera in each group. Lines are colored differently for RBD epitopes within different classes[17] (epitopes defined in Figure 1B); gray for residues not assigned to an epitope. Right: The average site-total Ab escape for the indicated mAbs and mAb mixtures (Mix 1 = equimolar mixture of all five mAbs; Mix 2 = 10-fold more S309, C118, and CR3022 than C102 and C144; Mix 3 = 10-fold more C102 and C144 than S309, C118, and CR3022) mapped to the surface of the WA1 RBD (PDB 6M0J). The locations of individual residues are highlighted on the RBD surfaces in colors corresponding to their epitope.

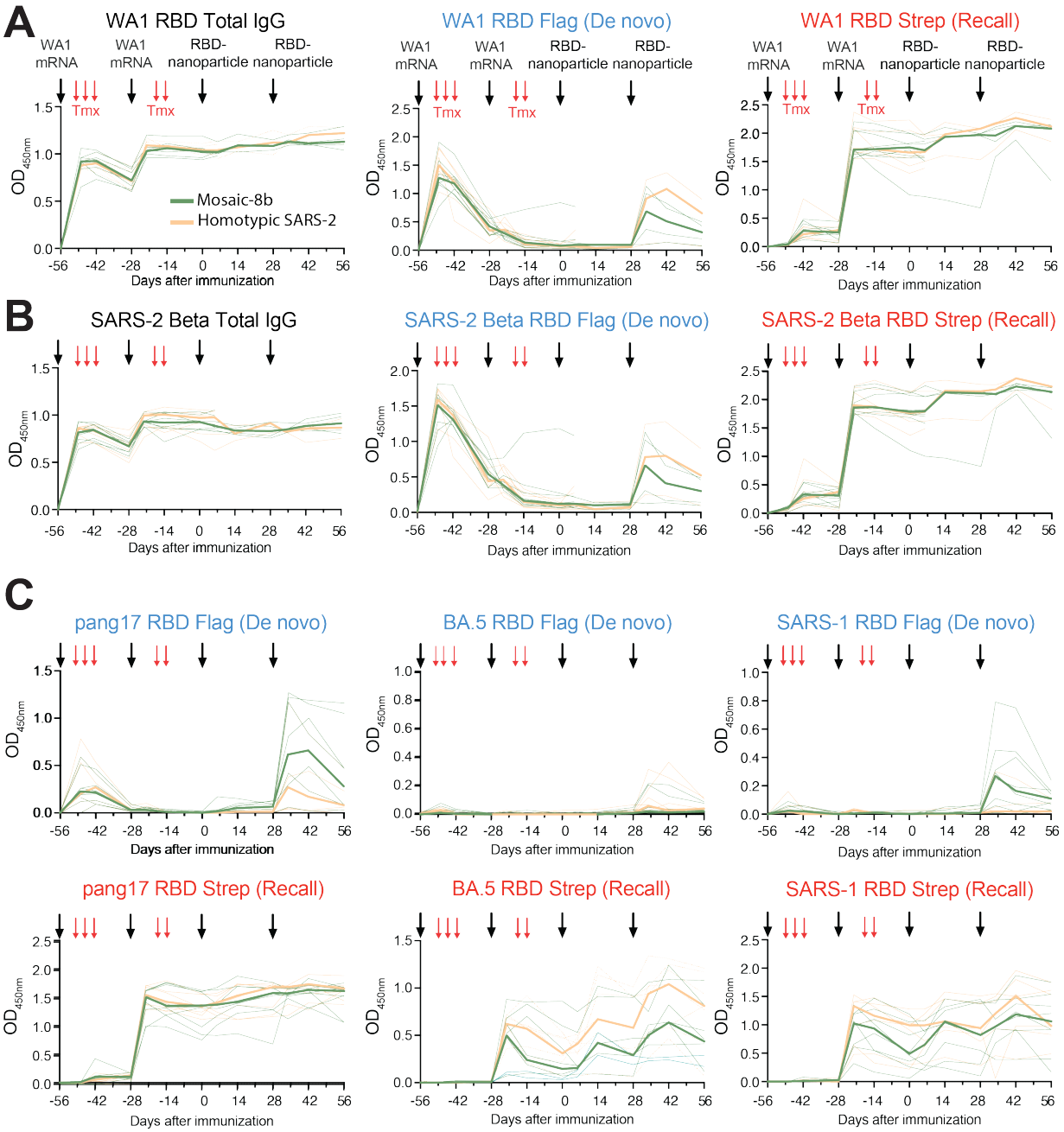

Figure S6 Time course of serum from fate mapping studies reveals differences in Ab responses before RBD-nanoparticle immunization, related to Figure 6.

Time course of serum antibody responses. Flag$^+$/de novo (blue) or Strep$^+$/recall (red) Abs after immunization with RBD-nanoparticles. Red arrows indicate tamoxifen (Tmx) treatment in all panels; black arrows indicate vaccination with WA1 mRNA-LNP or the indicated RBD-nanoparticles, as denoted in the upper left panel. Mice immunized with mosaic-8b are indicated in green; mice immunized with homotypic SARS-2 Beta are indicated in orange. Individual mice are indicated by thin lines; median responses for groups are shown in thick lines.

(A-B) Time course of levels of total IgG (left), Flag$^+$/de novo Igκ (center), or Strep+/recall Igκ (right) Ab responses after immunization with mosaic-8b (green) or homotypic SARS-2 Beta (orange) RBD-nanoparticles measured against RBDs of (A) SARS-2 WA1, (B) SARS-2 Beta. (C) Time course of Flag$^+$/de novo Igκ (top), or Strep+/recall Igκ (bottom) Ab responses after immunization with mosaic-8b (green) or homotypic SARS-2 Beta (orange) RBD-nanoparticles measured against RBDs of pang17, SARS-2 BA.5, or SARS-1. ELISA absorbance values ($OD_{450\ nm}$) are shown for serum samples diluted at 1:100 (the first dilution for endpoint titer ELISAs shown in Figure 6). Data up to day 0 are from the two independent experimental cohorts shown in Figure 6. Data from one of the experimental cohorts was collected up to day 56. For accurate comparisons, each datapoint within each plot is from samples analyzed together in one assay.

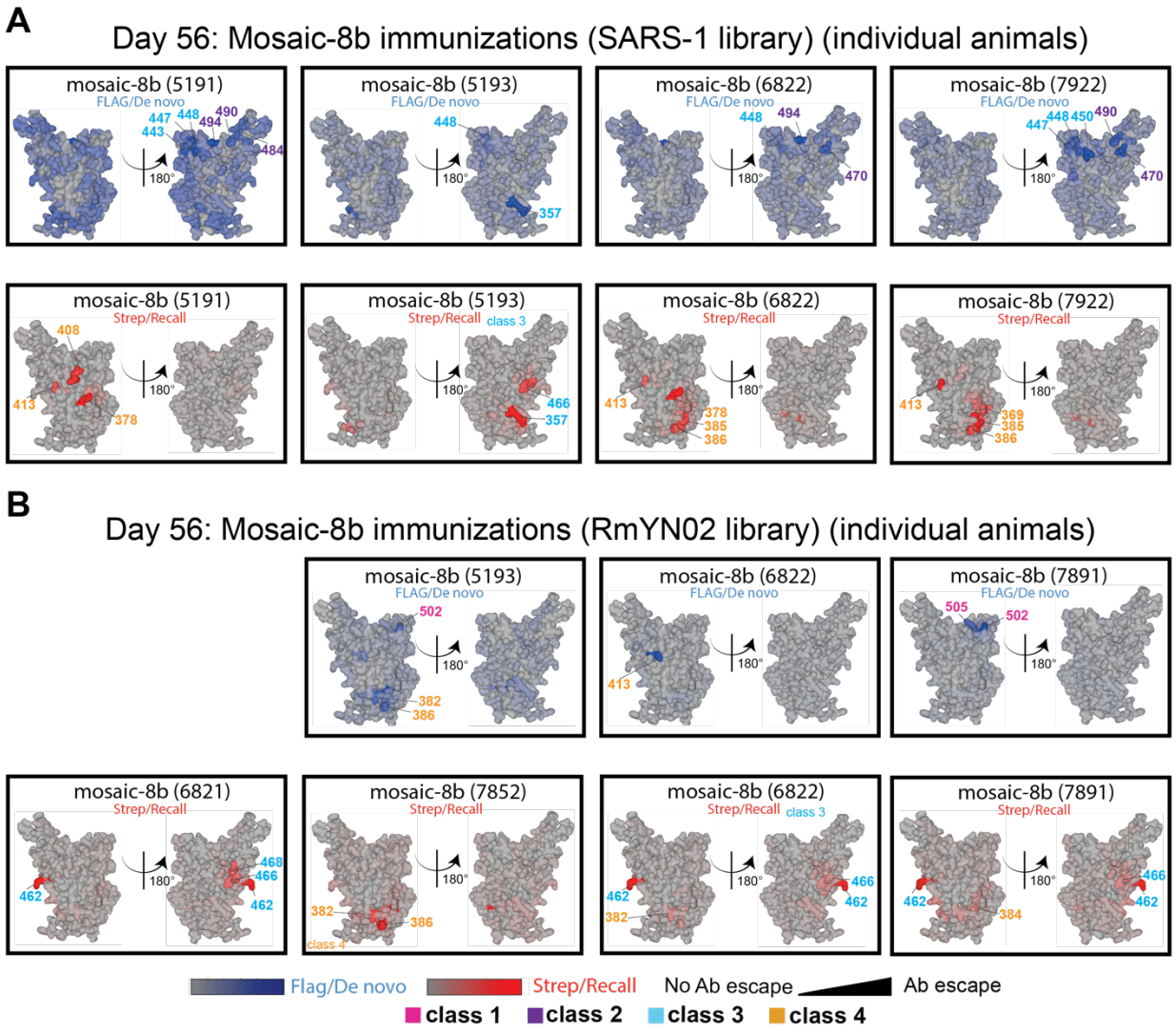

Figure S7 Individual animal DMS results, related to Figure 6.

DMS analyses shown for individual animals for which we had sufficient sera and the sera exhibited detectable levels of Flag^{+} or Strep^{+} Abs.

(A) DMS analyses for individual animals (indicated by 4-digit numbers) for compiled results shown in Figure 6F of day 56 serum from RBD-nanoparticle immunized mice using SARS-1 (antigenic distance score = 0.81) RBD mutant library. Ab binding sites are shaded according to degree of Ab escape, with blue for Flag/de novo responses and red for Strep/recall responses, on the surface of the WA1 RBD (PDB 6M0J). Comparisons are made for Flag/de novo and Strep/recall elicited by mosaic-8b and for Strep/recall elicited by homotypic SARS-2 (there were weak to no Flag/de novo responses after homotypic SARS-2 immunization).

(B) DMS analyses for individual animals (indicated by 4-digit numbers) for compiled results shown in Figure 6F of day 56 serum from RBD-nanoparticle immunized mice using RmYN02 (antigenic

distance score = 0.63) RBD mutant library. Ab binding sites are shaded according to degree of Ab escape, with blue for Flag/de novo responses and red for Strep/recall responses, on the surface of the WA1 RBD (PDB 6M0J). Comparisons are made for Flag/de novo and Strep/recall elicited by mosaic-8b and for Strep/recall elicited by homotypic SARS-2 (there were weak to no Flag/de novo responses after homotypic SARS-2 immunization). RmYN02 RBD is included on the mosaic-8b nanoparticle.