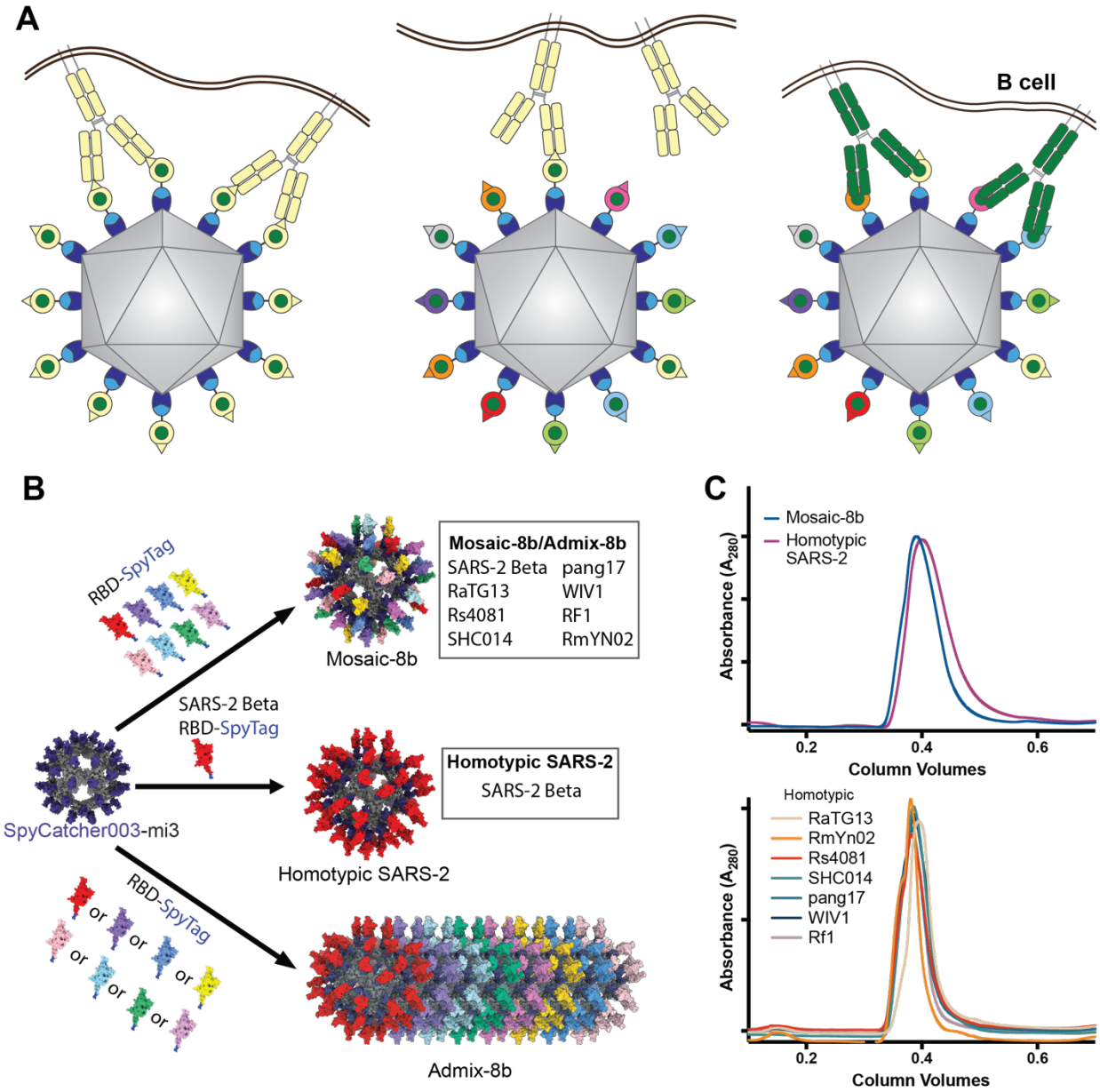


Figure S1 Preparation of RBD-mi3 nanoparticles, related to Figure 1.

(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 Studies	Vacinated with	Vaccine Source	Immunized with	Immunogen Source	Figures
pre-vax NHPs	WA1 Spike, Bivalent (Beta/Delta) SHARP, Trivalent (WA1/Beta/Delta) SHARP Delivered via: repRNA-LION, repRNA gene gun, or DNA	UW, HDT, U Albany, Creative Biosciences	mosaic-8b	Caltech	2, S2
			homotypic SARS-2	Caltech	
			WA1/BA.1 rep-RNA	HDT	
pre-vax BALB/c mice	Pfizer-like WA1	Helix Biotech	mosaic-8b	Caltech	3, S3
			admix-8b	Caltech	
			homotypic SARS-2	Caltech	
			Pfizer-like WA1	Helix Biotech	
	Pfizer-like WA1 and WA1/BA.5 mRNA-LNP		mosaic-8b	Caltech	
			mosaic-7	Caltech	
			Pfizer-like WA1 and WA1/BA.5 mRNA-LNP	Helix Biotech	
pre-vax 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-vax Tg mice	WA1 mRNA-LNP	UPenn, Acuitas	mosaic-8b	Caltech	6, S6, S7
			homotypic SARS-2	Caltech	

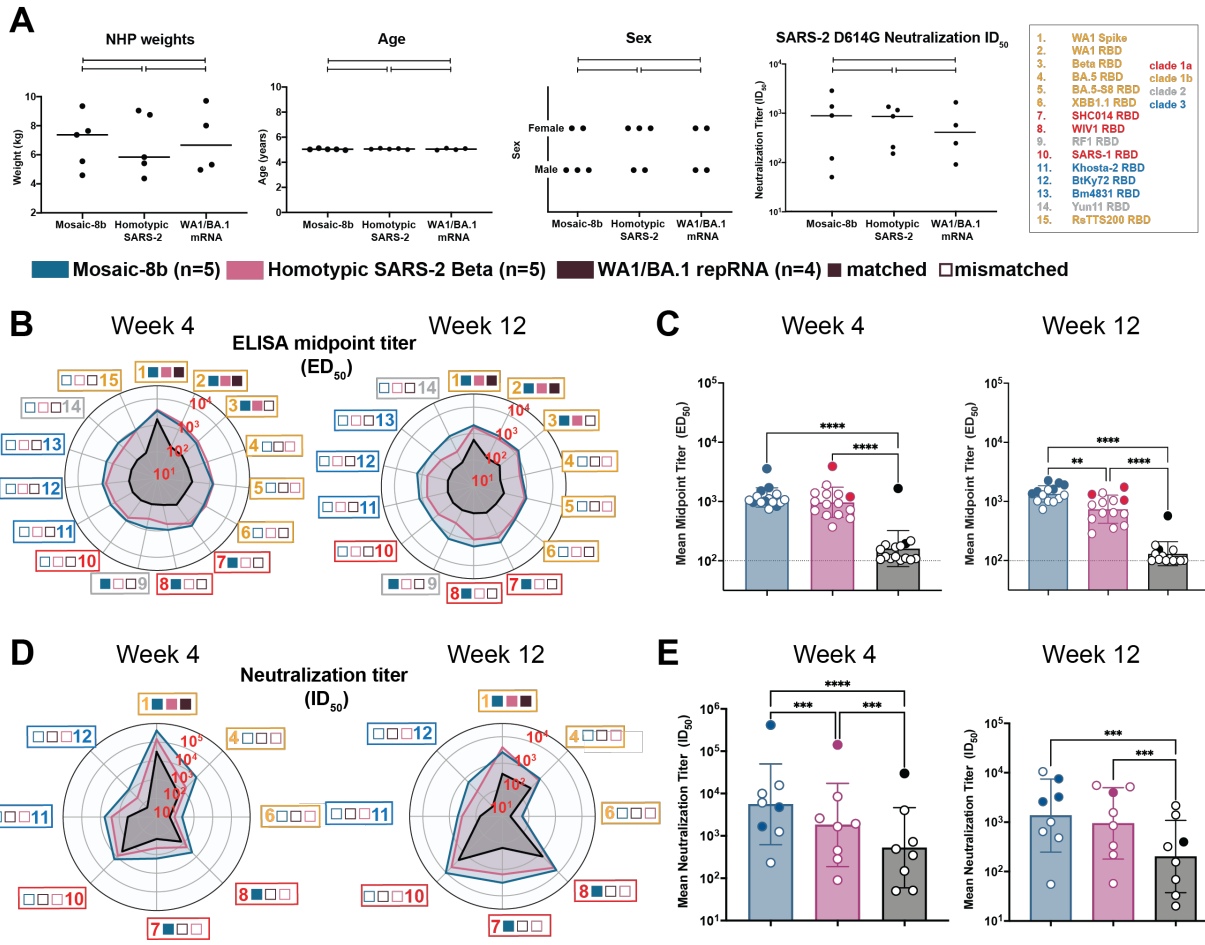


Figure S2 Mosaic-8b immunizations in previously-vaccinated NHPs elicits cross-reactive Ab responses, related to Figure 2.

Data for ELISA and neutralization analyses for serum samples from weeks 4 and 12. Data for samples from weeks 0, 2, 8, 10, and 22 are shown in Figure 2. The mean of mean titers in panels C and E is compared across immunizations by Tukey's multiple comparison test with the Geisser-Greenhouse correction (as calculated by GraphPad Prism), with pairing by viral strain. Significant differences between cohorts linked by horizontal lines are indicated by asterisks: $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, $p<0.0001$ = ****.

(A) Left: Top: Stratification of pre-vaccinated NHPs into groups used for immunizations with mosaic-8b, homotypic SARS-2, and WA1/BA.1 mRNA. Neutralization ID_{50} values derived from samples taken at week -8 in Figure 2A. Right: Numbers and color coding used to indicate sarbecovirus strains within clades throughout the figure. (Bottom) Colors and symbols used to indicate different immunizations (colors) and matched versus mismatched viral strains (symbols).

(B) ELISA binding titers at the indicated weeks after priming with mosaic-8b, homotypic SARS-2, or bivalent WA1/BA.1 mRNA-LNP represented as mean ED_{50} values for serum IgG binding to RBD or spike proteins from the indicated sarbecovirus strains (numbers and color coding as in panel A).

(C) Means of all ELISA binding titers for each type of immunization at the indicated weeks after priming. Each circle represents the mean ED_{50} titer against a single viral strain of sera from NHPs that were immunized with the specified immunogen (solid circles=matched; open circles=mismatched; colors for different strains defined in panel A).

(D) Neutralization titers at the indicated weeks after priming with mosaic-8b, homotypic SARS-2, or bivalent WA1/BA.1 mRNA-LNP represented as mean ID_{50} values for serum IgG neutralization of pseudoviruses derived from the indicated sarbecovirus strains (numbers and color coding as in panel A).

(E) Means of all neutralization titers for each type of immunization at the indicated weeks after priming. Each circle represents the mean neutralization ID_{50} titer against a single viral strain of sera from NHPs that were immunized with the specified immunogen (solid circles=matched; open circles=mismatched; colors for different strains defined in panel A).

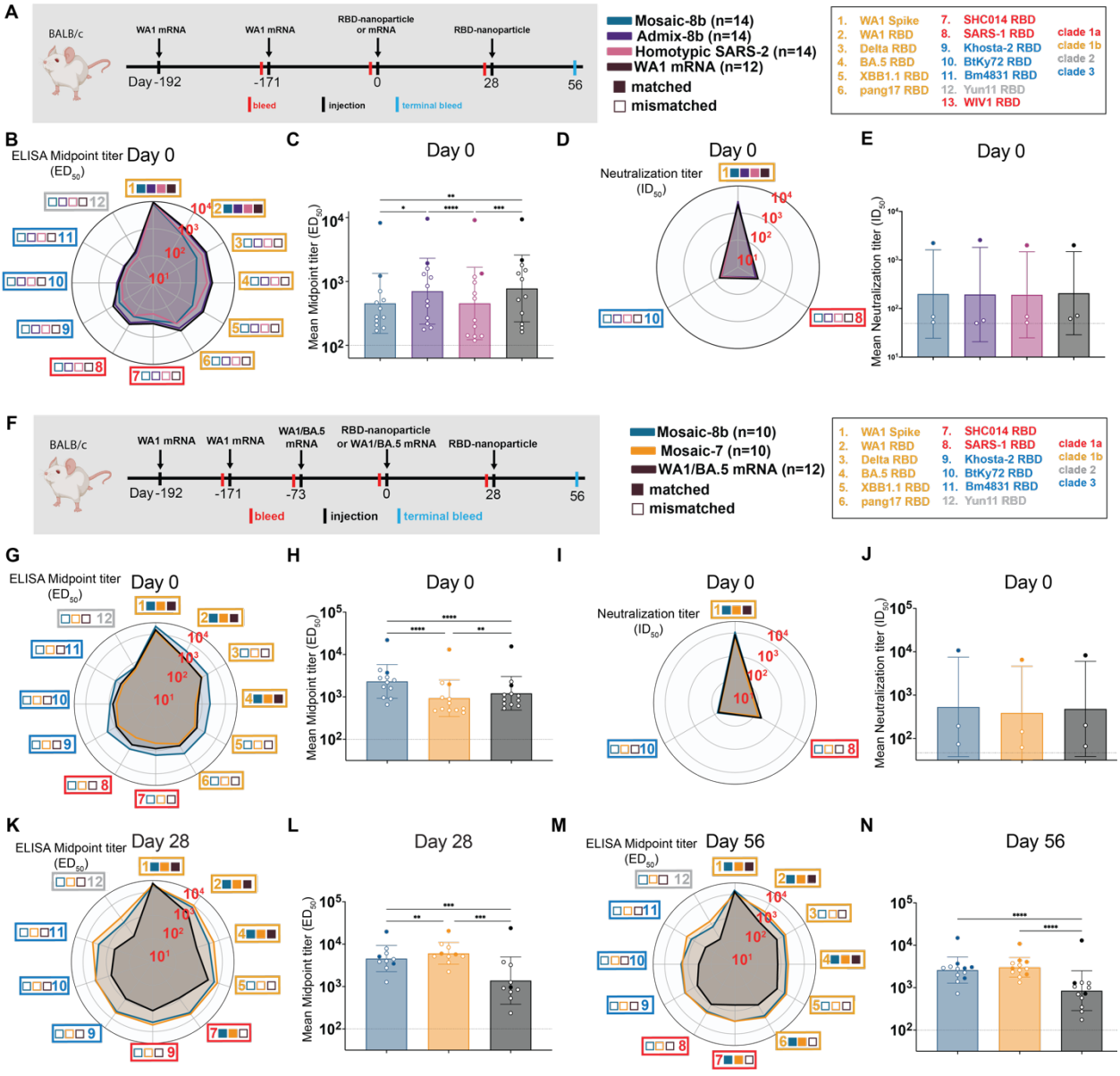


Figure S3 Cohorts of mRNA-LNP vaccinated mice showed significant differences in midpoint ED_{50} titers at day 0 (prior to RBD-nanoparticle immunizations), related to Figure 3.

Binding responses at day 0 (immediately prior to nanoparticle or other vaccine immunizations) showed significant differences across cohorts in titers elicited by the pre-vaccinations. We therefore applied baseline corrections to account for different mean responses at day 0 prior to immunizations in each of the four groups (see Methods). Non-baseline corrected binding data for panels Figure 3G,H are shown elsewhere.[72]

The mean of mean titers is compared in panels C,E,H, and J across immunizations by Tukey's multiple comparison test with the Geisser-Greenhouse correction (as calculated by GraphPad Prism), with

pairing by viral strain. Significant differences between cohorts linked by horizontal lines are indicated by asterisks: $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, $p<0.0001$ = ****.

(A) Left: (Top) Schematic of immunization regimen. Mice were vaccinated with a Pfizer-equivalent mRNA-LNP vaccine encoding WA1 spike at the indicated days prior to prime and boost immunizations with RBD-nanoparticle at day 0 and day 28 or with an additional WA1 mRNA-LNP immunization at day 0. (Bottom) Colors and symbols used to indicate different immunizations (colors) and matched versus mismatched viral strains (symbols). Right: Numbers and color coding used to indicate sarbecovirus strains within clades throughout the figure.

(B) 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). Binding titers are represented as mean ED_{50} values for serum IgG binding to RBD or spike proteins from the indicated sarbecovirus strains (numbers and color coding as in panel A).

(C) Means of 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) (solid circles=matched; open circles=mismatched; colors for different strains defined in panel A).

(D) 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). Neutralization titers are represented as mean ID_{50} values for serum against the indicated sarbecovirus strains (numbers and color coding as in panel A).

(E) Means of 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) (solid circles=matched; open circles=mismatched; colors for different strains defined in panel A).

(F) Left: (Top) Schematic of vaccination regimen for panels G-J. Mice were vaccinated twice with a Pfizer-equivalent mRNA-LNP vaccine encoding WA1 spike and once with a WA1/BA.5 mRNA-LNP at the indicated days prior to prime and boost immunizations with RBD-nanoparticles at day 0 and day 28 or with an additional WA1/BA.5 mRNA-LNP immunization at day 0. (Bottom) Colors and symbols used to indicate different immunizations (colors) and matched versus mismatched viral strains (symbols). Right: Numbers and color coding used to indicate sarbecovirus strains within clades throughout the figure.

(G) 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). Binding titers are represented as mean ED_{50} values for serum IgG binding to RBD or spike proteins from the indicated sarbecovirus strains (numbers and color coding as in panel A).

(H) Means of 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) (solid circles=matched; open circles=mismatched; colors for different strains defined in panel F).

(I) 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). Neutralization titers are represented as mean ID_{50} values for serum against the indicated sarbecovirus strains (numbers and color coding as in panel F).

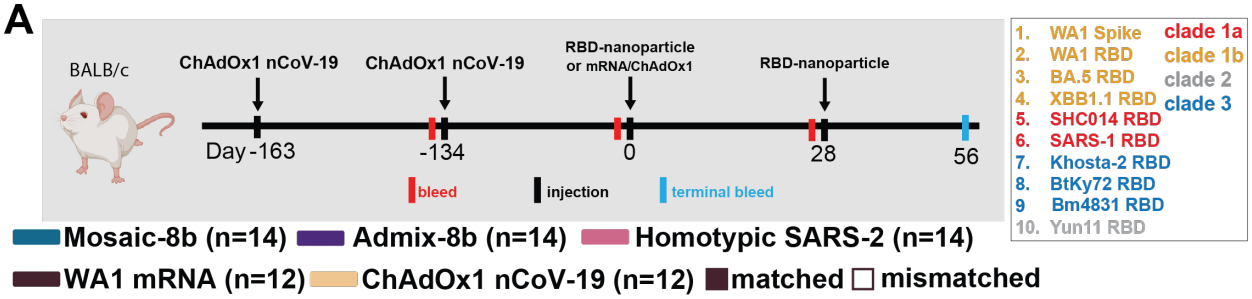
(J) Means of neutralization binding titers of serum from mice assigned to each cohort at day 0 (192, 171, and 73 days after the first, second, and third mRNA-LNP vaccinations but prior to nanoparticle or additional mRNA-LNP immunizations) (solid circles=matched; open circles=mismatched; colors for different strains defined in panel F).

(K) ELISA binding titers of serum from mice assigned to each cohort at day 28. Binding titers are represented as mean ED_{50} values for serum IgG binding to RBD or spike proteins from the indicated sarbecovirus strains (numbers and color coding as in panel A).

(L) Means of ELISA binding titers of serum from mice assigned to each cohort at day 28 (solid circles=matched; open circles=mismatched; colors for different strains defined in panel F).

(M) ELISA binding titers of serum from mice assigned to each cohort at day 56. Binding titers are represented as mean ED_{50} values for serum IgG binding to RBD or spike proteins from the indicated sarbecovirus strains (numbers and color coding as in panel A).

(N) Means of ELISA binding titers of serum from mice assigned to each cohort at day 56 (solid circles=matched; open circles=mismatched; colors for different strains defined in panel F).



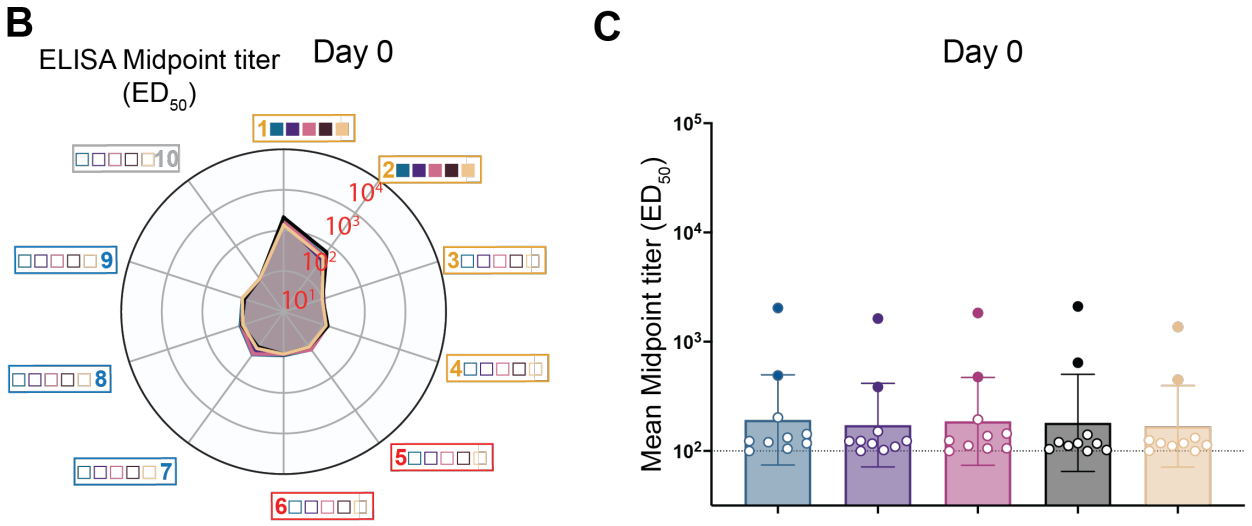


Figure S4 Cohorts of ChAdOx1 vaccinated mice showed no significant differences in midpoint ED_{50} titers at day 0, related to Figure 4.

(A) Left: (Top) Schematic of vaccination regimen. Mice were vaccinated with a ChAdOx1 nCoV-19 viral vectored vaccine encoding WA1 spike at the indicated days prior to prime and boost immunizations with RBD-nanoparticles at day 0 and day 28 or with another dose of ChAdOx1 at day 0 or with a Moderna mRNA-LNP vaccine encoding WA1 spike at day 0. (Bottom) Colors and symbols used to indicate different immunizations (colors) and matched versus mismatched viral strains (symbols). Right: Numbers and color coding are used to indicate sarbecovirus strains within clades throughout the figure.

(B) 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). Binding titers are represented as mean ED_{50} values for serum IgG binding to RBD or spike proteins from the indicated sarbecovirus strains (numbers and color coding as in panel A).

(C) Means of 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) (solid circles=matched; open circles=mismatched; colors for different strains defined in panel A).

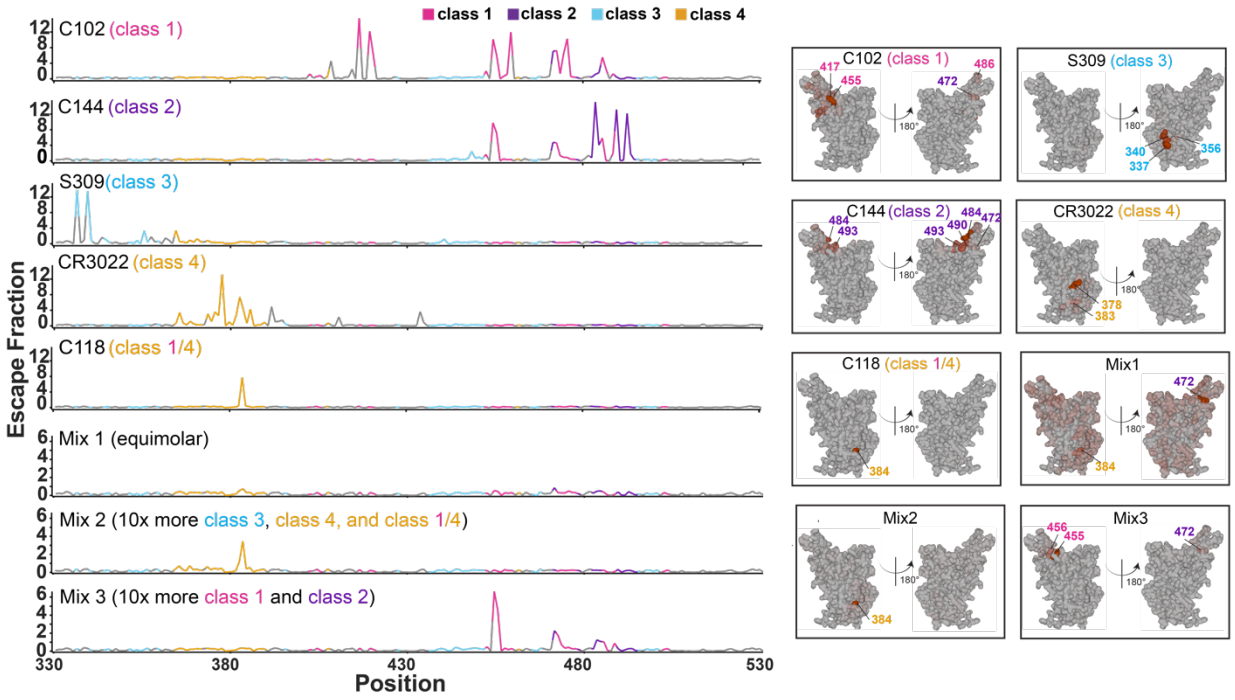


Figure S5 Comparison of DMS profiles of individual mAbs and mAb mixtures, related to Figure 5.

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.[80] 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.

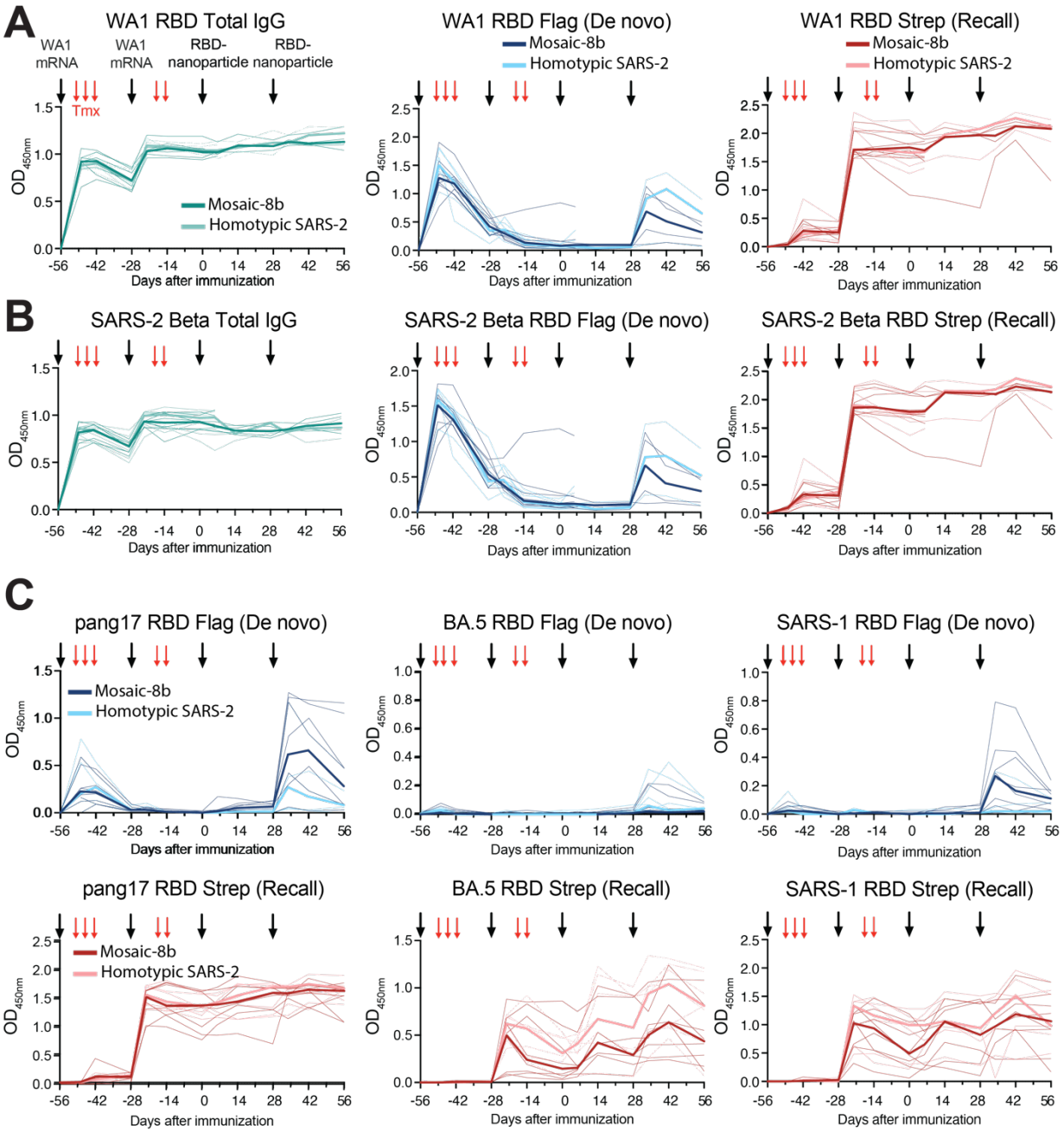


Figure S6 Time course of serum from fate mapping studies reveals differences in Ab responses before RBD-nanoparticle immunization, related to Figure 6.

Time course for levels of 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. Individual mice are indicated by thin lines; median responses for groups are shown in thick lines.

(A-C) Time course of levels of total IgG (teal), $Flag^+$/de novo Igκ (blue), or Strep+/recall Igκ (recall/red) Ab responses after immunization with RBD-nanoparticles measured against RBDs of (A) SARS-2 WA1, (B) SARS-2 Beta, or (C) 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.

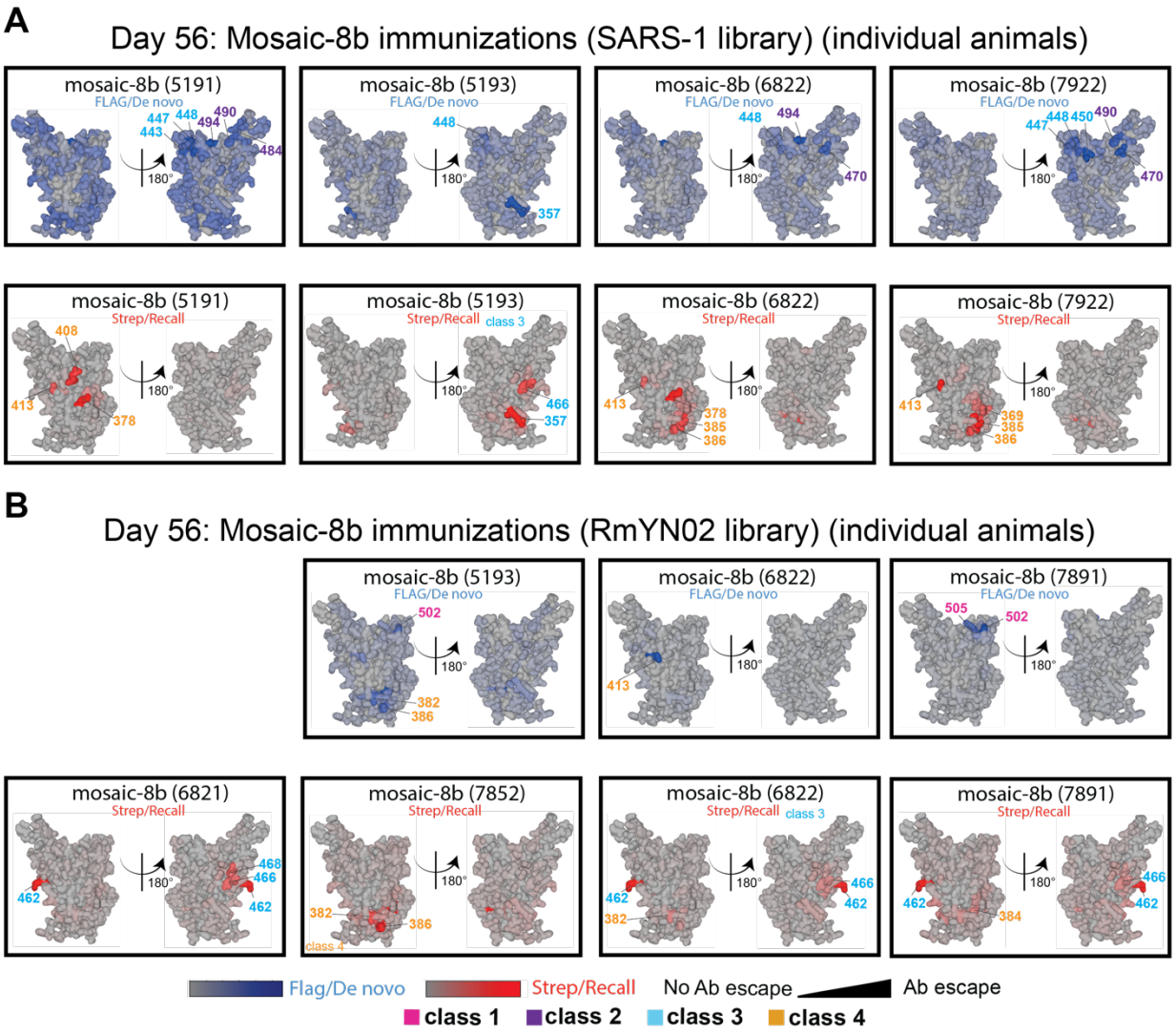


Figure S7 Individual animal DMS results, related to Figure 6.

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.