

Supplemental information

SARS-CoV-2 ferritin nanoparticle vaccines produce hyperimmune equine sera with broad sarbecovirus activity

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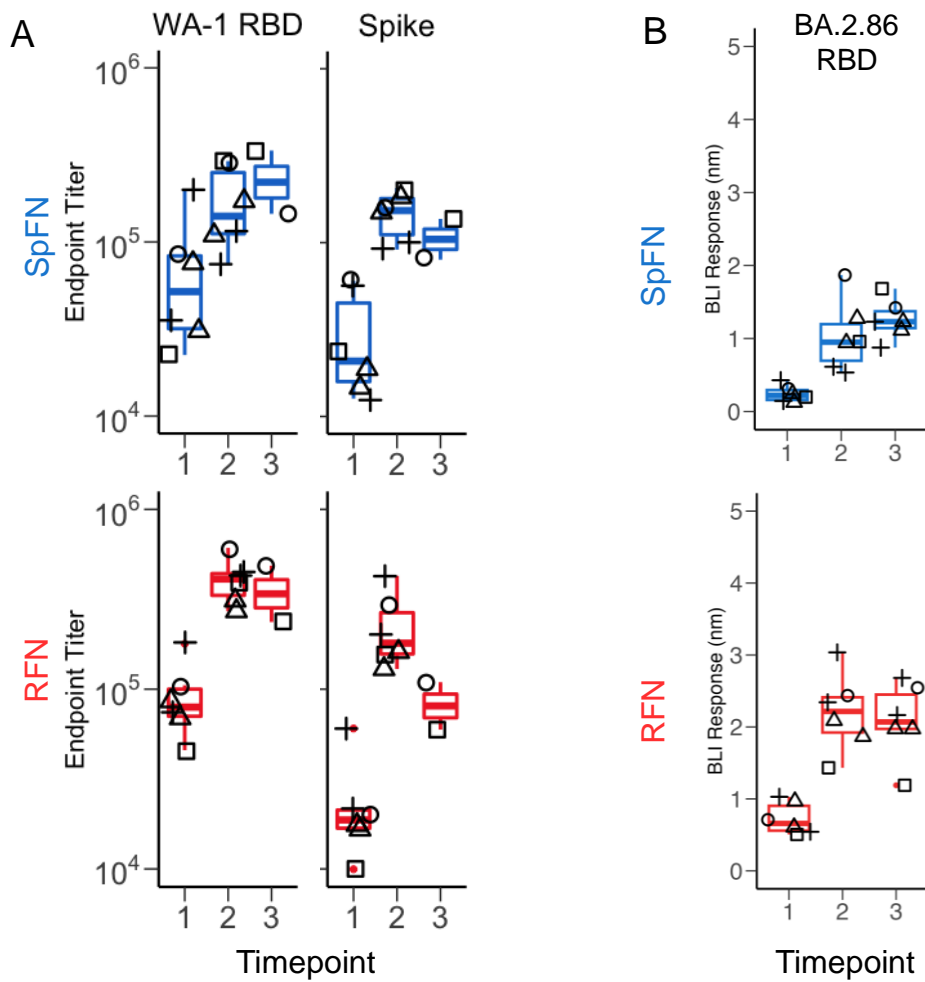


Figure S1. Antigen binding responses elicited in the equine model, Related to Figure 1.

(A) SARS-CoV-2 WA-1 RBD and Spike binding measured by ELISA. (top: SpFN immunized group (blue), bottom: RFN immunized group (red)). Symbols are matched to the immunization schema shown in Fig. 1B.

(B) SARS-CoV-2 Omicron BA.2.86 RBD binding levels measured by BLI. SpFN immunized group (blue), RFN immunized group (red). Symbols are matched to the immunization schema shown in Figure 1.

A

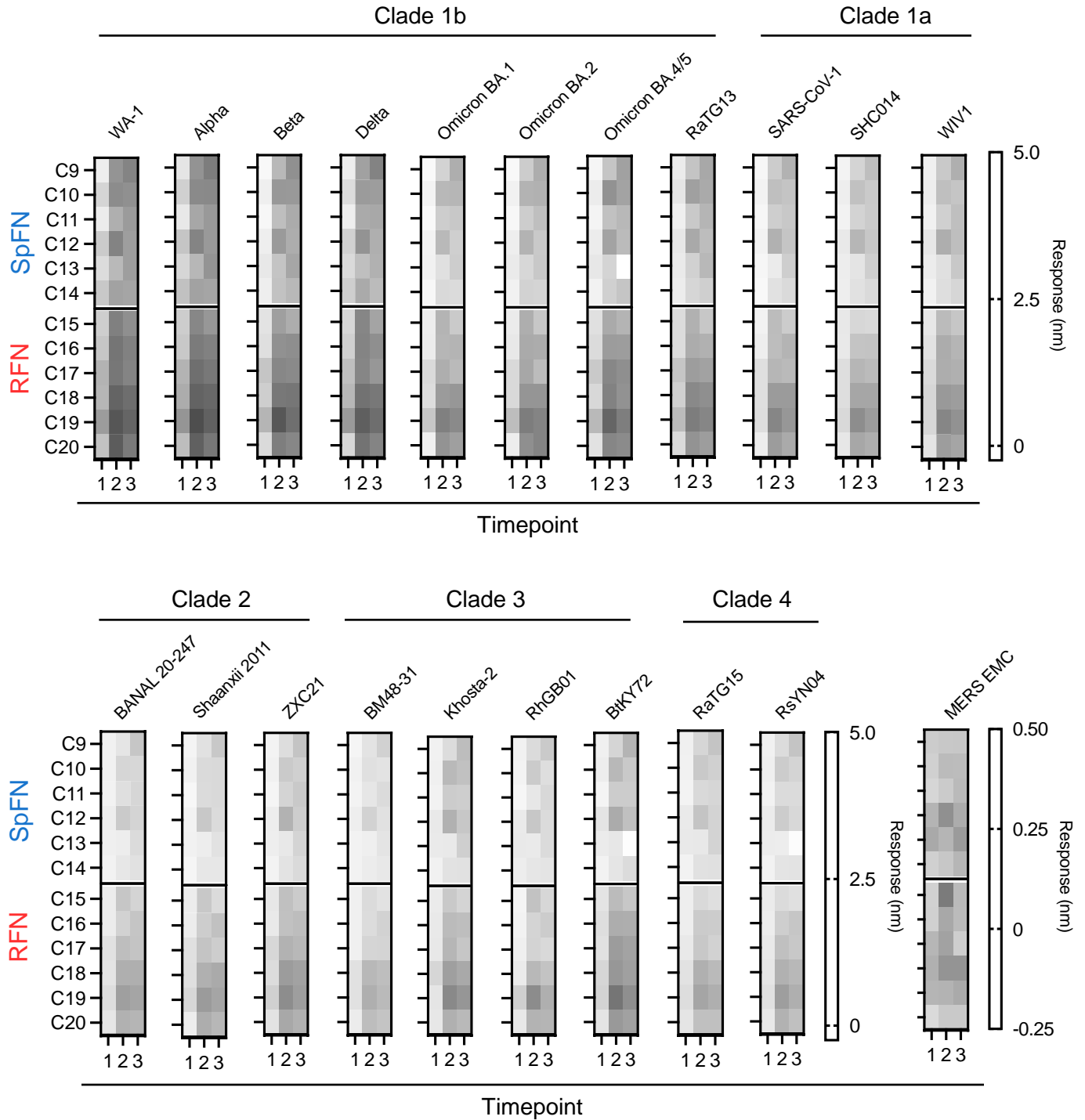


Figure S2. BLI binding responses for individual animal sera to a sarbecovirus and MERS RBD panel following immunization (1st, 2nd, and 3rd dose), Related to Figure 1 and Figure 3. Timepoints are indicated on the X-axis.

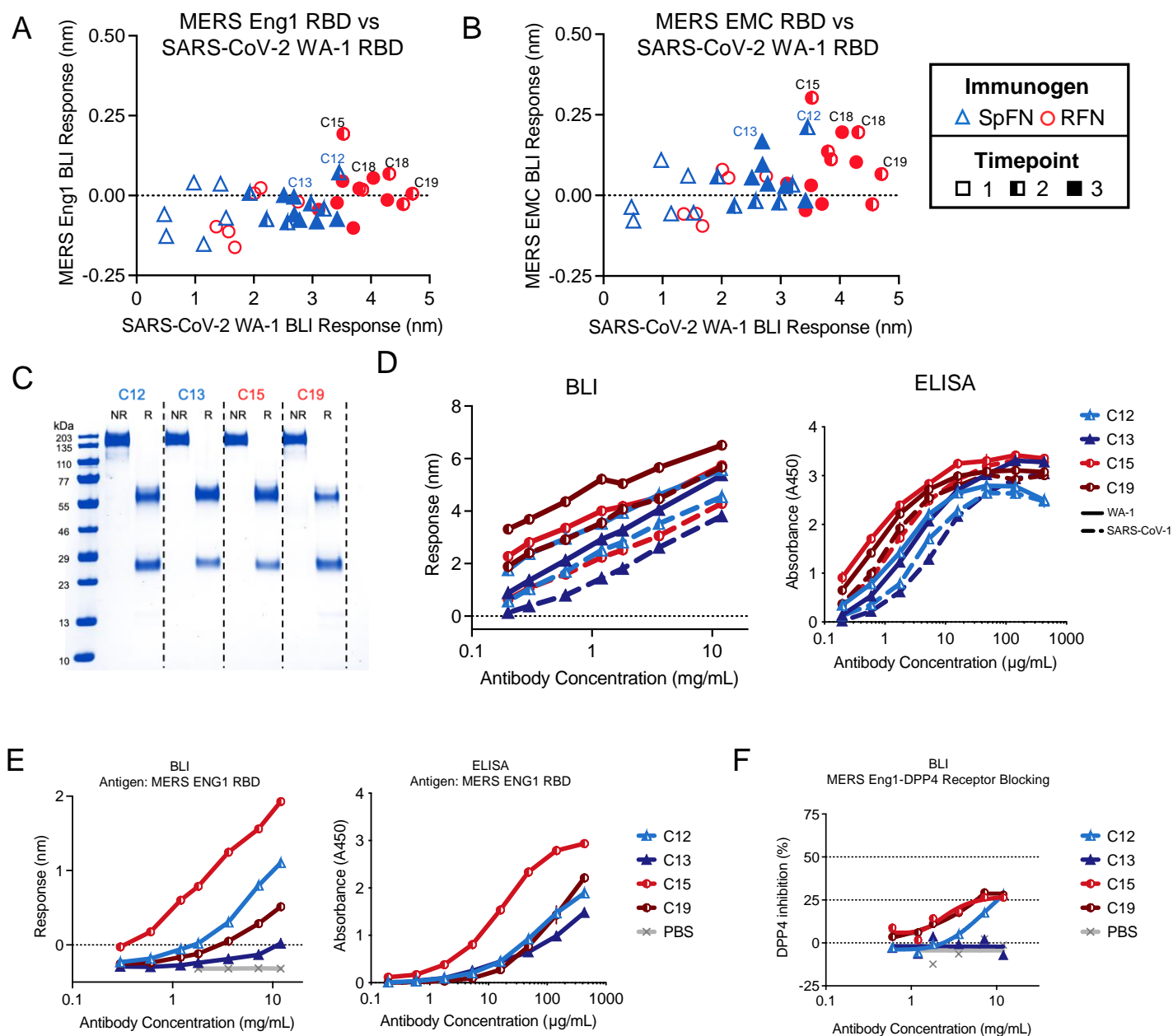


Figure S3. Purified equine IgG binding responses, Related to Figure 3.

(A-B) Serum binding response to MERS EMC and Eng1 RBDs and SARS-CoV-2 WA-1 RBD (A) MERS Eng1 RBD and (B) MERS EMC RBD; SpFN immunized group (blue) and RFN immunized group (red).

(C) SDS-PAGE of purified polyclonal IgG in both non-reducing (NR) and reducing (R) conditions for four animal sera samples. SpFN immunized samples are C12 and C13 and RFN immunized samples are C15 and C19.

(D) Binding responses of purified polyclonal IgG to SARS-CoV-2 WA-1 RBD (solid line) and SARS-CoV-1 Urbani RBD (dashed line). Responses measured by BLI (left) and ELISA (right).

(E) Binding responses of purified IgG measured by BLI (left) and ELISA (right).

(F) Assessment of purified equine polyclonal IgG for human DPP4-RBD blocking capability.

Table S1. Immunization study design, Related to Figure 1.

This study was an unblinded randomized control study, with six horses per immunogen, SpFN and RFN

Group	N= No. of Horses	Animal Identifier	Treatment	Vaccine Dose (mg)	Immunization Schedule
1	1	C9	SpFN_1B-06-PL	150	Weeks 0, 3, 6
2	1	C12	SpFN_1B-06-PL	500	Weeks 0, 3, 6
3	2	C10, C11	SpFN_1B-06-PL	150	Weeks 0, 4, 8
4	2	C13, C14	SpFN_1B-06-PL	500	Weeks 0, 4, 8
5	1	C15	RBD-Ferritin_pCoV131	150	Weeks 0, 3, 6
6	1	C18	RBD-Ferritin_pCoV131	500	Weeks 0, 3, 6
7	2	C16, C17	RBD-Ferritin_pCoV131	150	Weeks 0, 4, 8
8	2	C19, C20	RBD-Ferritin_pCoV131	500	Weeks 0, 4, 8

Table S2. SARS-CoV-2 WA-1 RBD and Spike ELISA binding titers, Related to Figure 1 and Figure 3.

N.T.: not tested.

Animal Identifier	Immunogen	Vaccine Dose (µg)	Immunization Schedule	Week 2		Week 5/6*		Week 8/10*	
				RBD	Spike	RBD	Spike	RBD	Spike
C9	SpFN	150	Weeks 0, 3, 6	22,407	23,067	291,553	206,084	335,183	136,922
C10	SpFN	150	Weeks 0, 4, 8	76,185	18,782	171,629	186,703	N.T.	N.T.
C11	SpFN	150	Weeks 0, 4, 8	30,673	15,002	110,224	147,151	N.T.	N.T.
C12	SpFN	500	Weeks 0, 3, 6	85,698	60,207	283,804	158,832	145,172	79,805
C13	SpFN	500	Weeks 0, 4, 8	35,279	12,672	74,010	101,034	N.T.	N.T.
C14	SpFN	500	Weeks 0, 4, 8	199,978	56,164	115,223	91,181	N.T.	N.T.

C15	RFN	150	Weeks 0, 3, 6	45,780	9,964	395,984	155,760	236,386	59,564
C16	RFN	150	Weeks 0, 4, 8	69,764	16,637	313,564	160,912	N.T.	N.T.
C17	RFN	150	Weeks 0, 4, 8	85,977	17,485	270,875	129,427	N.T.	N.T.
C18	RFN	500	Weeks 0, 3, 6	105,137	20,053	610,557	291,996	486,492	109,321
C19	RFN	500	Weeks 0, 4, 8	178,805	60,421	424,121	201,893	N.T.	N.T.
C20	RFN	500	Weeks 0, 4, 8	73,403	21,686	444,655	427,060	N.T.	N.T.

Table S3. RBD binding response by BLI (nm at 180s) at first timepoint , Related to Figure 1 and Figure 3.

Vaccine Dose (mg)	Immunization Schedule	Timepoint 1									
		WA-1	Alpha	Beta	Delta	BA.1	BA.2	BA.4/5	XBB.1.5	BA.2.86	SARS-CoV-1
150	Weeks 0, 3, 6	0.472	0.523	-0.062	0.233	-0.095	-0.057	0.038	0.178	0.198	0.002
150	Weeks 0, 4, 8	1.146	1.145	0.215	0.771	0.082	0.135	0.289	0.268	0.245	0.116
150	Weeks 0, 4, 8	0.507	0.431	-0.028	0.224	-0.085	-0.035	0.051	0.098	0.126	-0.014
500	Weeks 0, 3, 6	1.433	1.474	0.248	1.086	0.107	0.234	0.305	0.356	0.310	0.114
500	Weeks 0, 4, 8	0.976	0.973	0.408	0.824	0.154	0.351	0.427	0.464	0.428	-0.007
500	Weeks 0, 4, 8	1.526	1.575	0.229	1.183	-0.018	0.136	0.122	0.212	0.145	-0.064
150	Weeks 0, 3, 6	1.359	1.234	0.55	0.9	0.255	0.321	0.651	0.551	0.506	0.116
150	Weeks 0, 4, 8	1.572	1.368	0.685	1.115	0.282	0.417	0.797	0.666	0.607	0.22
150	Weeks 0, 4, 8	2.119	2.021	1.436	1.599	0.815	0.862	1.33	0.901	0.969	0.683
500	Weeks 0, 3, 6	2.014	2.217	1.187	1.859	0.739	0.863	0.873	0.660	0.711	0.677
500	Weeks 0, 4, 8	2.754	2.908	1.897	2.711	1.401	1.684	1.793	1.492	1.030	0.693
500	Weeks 0, 4, 8	1.678	1.711	0.832	0.92	0.172	0.257	0.582	0.412	0.543	0.251

Table S4. RBD binding response by BLI (nm at 180s) at second timepoint , Related to Figure 1 and Figure 3.

Animal Identifier	Immunogen	Vaccine Dose (mg)	Immunization Schedule	Timepoint 2									
				WA-1	Alpha	Beta	Delta	BA.1	BA.2	BA.4/5	XBB.1.5	BA.2.86	SARS-CoV-1
C9	SpFN	150	Weeks 0, 3, 6	2.973	2.961	1.812	2.485	1.032	1.236	1.435	0.876	0.957	1.234
C10	SpFN	150	Weeks 0, 4, 8	3.196	3.238	2.743	2.674	1.832	1.961	2.925	1.686	1.276	1.621
C11	SpFN	150	Weeks 0, 4, 8	2.215	2.377	1.630	2.218	1.209	1.206	1.556	0.813	0.942	1.176
C12	SpFN	500	Weeks 0, 3, 6	3.454	3.417	2.688	2.915	1.786	1.965	2.438	1.966	1.869	2.030
C13	SpFN	500	Weeks 0, 4, 8	1.930	1.881	1.471	1.621	0.645	0.896	1.003	0.819	0.534	0.333
C14	SpFN	500	Weeks 0, 4, 8	2.574	2.522	1.533	2.229	0.842	1.045	1.101	0.785	0.613	0.623
C15	RFN	150	Weeks 0, 3, 6	3.526	3.327	2.553	3.296	1.947	2.125	2.241	1.989	1.432	1.688
C16	RFN	150	Weeks 0, 4, 8	3.851	3.690	2.960	3.334	1.885	2.222	2.638	1.628	1.867	1.675
C17	RFN	150	Weeks 0, 4, 8	3.799	4.034	3.246	3.281	2.114	2.302	3.259	1.909	2.089	1.873
C18	RFN	500	Weeks 0, 3, 6	4.311	4.404	3.701	3.875	2.669	2.857	3.406	2.521	2.436	2.655
C19	RFN	500	Weeks 0, 4, 8	4.703	5.013	4.671	4.431	3.451	3.535	4.242	2.887	3.036	3.282
C20	RFN	500	Weeks 0, 4, 8	4.549	4.482	3.519	3.851	2.901	3.015	3.276	2.997	2.343	2.637

Table S5. RBD binding response by BLI (nm at 180s) at third timepoint, Related to Figure 1 and Figure 3.

Animal Identifier	Immunogen	Vaccine Dose (mg)	Immunization Schedule	Timepoint 3									
				RBD binding response (nm at 180 s)									
				WA-1	Alpha	Beta	Delta	BA.1	BA.2	BA.4/5	XBB.1.5	BA.2.86	SARS-CoV-1
C9	SpFN	150	Weeks 0, 3, 6	3.421	3.61	2.99	3.364	2.124	2.13	2.488	1.844	1.682	2.008
C10	SpFN	150	Weeks 0, 4, 8	3.074	3.262	2.739	2.613	1.873	2.02	2.428	1.340	1.113	1.458
C11	SpFN	150	Weeks 0, 4, 8	2.779	2.786	2.203	2.298	1.352	1.6	1.804	1.131	1.232	1.355
C12	SpFN	500	Weeks 0, 3, 6	2.693	2.784	2.137	2.09	1.138	1.318	1.739	1.693	1.420	1.44
C13	SpFN	500	Weeks 0, 4, 8	2.685	2.946	2.354	2.017	1.214	1.353	-0.257	1.128	1.230	1.163
C14	SpFN	500	Weeks 0, 4, 8	2.512	2.434	1.808	1.727	0.783	0.989	1.437	1.107	0.875	0.821
C15	RFN	150	Weeks 0, 3, 6	3.106	2.838	2.155	2.414	1.296	1.388	1.93	1.813	1.190	1.223
C16	RFN	150	Weeks 0, 4, 8	3.515	3.501	3.047	3.01	1.923	2.158	2.6	2.055	1.974	1.936
C17	RFN	150	Weeks 0, 4, 8	3.421	3.767	3.163	2.771	1.774	1.715	3.032	2.276	1.973	1.752
C18	RFN	500	Weeks 0, 3, 6	4.04	4.247	3.752	3.682	2.78	2.856	2.92	2.843	2.545	2.676
C19	RFN	500	Weeks 0, 4, 8	4.279	4.466	3.972	3.997	3.174	3.345	3.527	2.981	2.682	2.794
C20	RFN	500	Weeks 0, 4, 8	3.698	3.793	3.243	3.343	2.562	3.343	3.143	2.461	2.165	2.227

Table S6. Pseudovirus neutralization titers at first timepoint, Related to Figure 2.

Animal Identifier	Immunogen	Vaccine Dose (mg)	Immunization Schedule	Timepoint 1								
				Neutralization ID50 (Dil ⁻¹)								
				WA-1	Alpha	Beta	Delta	BA.1	BA.5	XBB.1.5	SARS-CoV-1	
C9	SpFN	150	Weeks 0, 3, 6	1827	1126	358	778	40	40	40	193	
C10	SpFN	150	Weeks 0, 4, 8	3243	2734	633	3905	192	61	263	1156	
C11	SpFN	150	Weeks 0, 4, 8	1175	872	569	1459	230	40	40	220	
SpFN 150mg				GMT	1909.4	1389.8	505.2	1642.7	120.9	46.0	74.9	366.1
C12	SpFN	500	Weeks 0, 3, 6	4475	7109	445	4045	70	40	40	40	
C13	SpFN	500	Weeks 0, 4, 8	6442	2224	876	5580	60	40	40	161	
C14	SpFN	500	Weeks 0, 4, 8	4784	4128	284	3336	143	50	40	271	
SpFN 500mg				GMT	5166.6	4026.2	480.2	4222.7	84.4	43.1	40.0	120.4
C15	RFN	150	Weeks 0, 3, 6	360	150	40	275	40	40	40	40	
C16	RFN	150	Weeks 0, 4, 8	926	298	186	2427	40	40	40	57	
C17	RFN	150	Weeks 0, 4, 8	2853	3010	40	1080	40	40	40	40	
RFN 150mg				GMT	983.4	512.4	66.8	896.6	40.0	40.0	40.0	45.0
C18	RFN	500	Weeks 0, 3, 6	1703	3539	85	2654	329	40	40	40	
C19	RFN	500	Weeks 0, 4, 8	6524	5750	190	11589	111	118	40	969	
C20	RFN	500	Weeks 0, 4, 8	1797	1640	40	957	40	40	40	54	
RFN 500mg				GMT	2712.8	3219.6	86.4	3087.6	113.5	57.4	40.0	127.9
total				GMT	2265.0	1743.1	193.4	2093.4	82.5	46.2	46.8	126.2

Table S7. Pseudovirus neutralization titers at second timepoint, Related to Figure 2.

Animal Identifier	Immunogen	Vaccine Dose (mg)	Immunization Schedule	Timepoint 2								
				Neutralization ID50 (Dil ⁻¹)								
				WA-1	Alpha	Beta	Delta	BA.1	BA.5	XBB.1.5	SARS-CoV-1	
C9	SpFN	150	Weeks 0, 3, 6	31704	36723	5012	24326	3275	537	1764	4655	
C10	SpFN	150	Weeks 0, 4, 8	64079	22728	4998	46367	6148	353	1527	4403	
C11	SpFN	150	Weeks 0, 4, 8	12939	9533	3442	10320	1016	231	232	1500	
SpFN 150mg				GMT	29733.3	19963.8	4417.8	22663.1	2734.9	352.5	854.5	3132.7
C12	SpFN	500	Weeks 0, 3, 6	30237	16651	4423	35334	2241	984	1074	2808	
C13	SpFN	500	Weeks 0, 4, 8	12872	5169	3597	16196	484	40	114	790	
C14	SpFN	500	Weeks 0, 4, 8	27121	25000	5727	24060	919	218	178	1698	
SpFN 500mg				GMT	21936.3	12910.1	4499.8	23968.0	998.9	204.7	279.6	1555.9
C15	RFN	150	Weeks 0, 3, 6	20432	20515	1191	22019	526	194	165	412	
C16	RFN	150	Weeks 0, 4, 8	19782	20497	4301	46478	1339	179	1307	1241	
C17	RFN	150	Weeks 0, 4, 8	31412	11903	5192	30477	2443	190	1429	1529	
RFN 150mg				GMT	23328.8	17105.6	2985.0	31477.9	1198.3	187.6	675.6	921.2
C18	RFN	500	Weeks 0, 3, 6	17989	49841	3160	29410	3152	644	616	1274	
C19	RFN	500	Weeks 0, 4, 8	219097	163180	43463	96857	29747	12411	11767	13827	
C20	RFN	500	Weeks 0, 4, 8	90762	47499	5834	37831	5615	662	2022	4578	
RFN 500mg				GMT	70987.6	72830.4	9288.047	47587.32	8074.7	1742.5	2447.3	4320.4
total				GMT	32238.2	23804.3	4845.2	30033.9	2267.5	391.9	792.8	2098.7

Table S8. Pseudovirus neutralization titers at third timepoint, Related to Figure 2.

Animal Identifier	Immunogen	Vaccine Dose (mg)	Immunization Schedule	Timepoint 3								
				Neutralization ID50 (Dil ⁻¹)								
				WA-1	Alpha	Beta	Delta	BA.1	BA.5	XBB.1.5	SARS-CoV-1	
C9	SpFN	150	Weeks 0, 3, 6	207331	48007	25192	42799	12548	1349	4129	6838	
C10	SpFN	150	Weeks 0, 4, 8	37188	26847	10087	28975	6651	2713	1759	2805	
C11	SpFN	150	Weeks 0, 4, 8	14149	10474	5037	15772	4004	871	1298	1445	
SpFN 150mg				GMT	47782.0	23810.6	10857.6	26943.1	6939.3	1471.7	2112.4	3026.3
C12	SpFN	500	Weeks 0, 3, 6	16892	14241	4233	10851	4892	721	2204	2516	
C13	SpFN	500	Weeks 0, 4, 8	17401	15368	7467	15207	4319	161	917	2136	
C14	SpFN	500	Weeks 0, 4, 8	10088	16488	4774	16836	2759	200	583	1915	
SpFN 500mg				GMT	14366.6	15338.2	5323.8	14057.8	3877.4	285.3	1056.0	2175.2
C15	RFN	150	Weeks 0, 3, 6	7764	7323	1548	11647	1722	438	524	661	
C16	RFN	150	Weeks 0, 4, 8	13919	11313	6060	16381	3414	938	1773	2795	
C17	RFN	150	Weeks 0, 4, 8	11006	28251	6293	27203	5005	325	2198	1221	
RFN 150mg				GMT	10595.2	13277.0	3893.7	17313.7	3087.2	511.1	1268.9	1311.5
C18	RFN	500	Weeks 0, 3, 6	21123	32553	11770	12815	4160	467	984	3675	
C19	RFN	500	Weeks 0, 4, 8	35436	47894	28759	33367	14342	946	7511	8157	
C20	RFN	500	Weeks 0, 4, 8	13634	28483	15730	56120	10682	303	5113	3662	
RFN 500mg				GMT	21690.7	35412.2	17462.0	28843.7	8605.7	511.5	3356.1	4788.2
total				GMT	19929.7	20356.3	7917.8	20854.6	5170.7	575.6	1755.6	2535.6