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Supplemental information

**Design of a stabilized RBD enables
potently neutralizing SARS-CoV-2
single-component nanoparticle vaccines**

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Supplemental Information

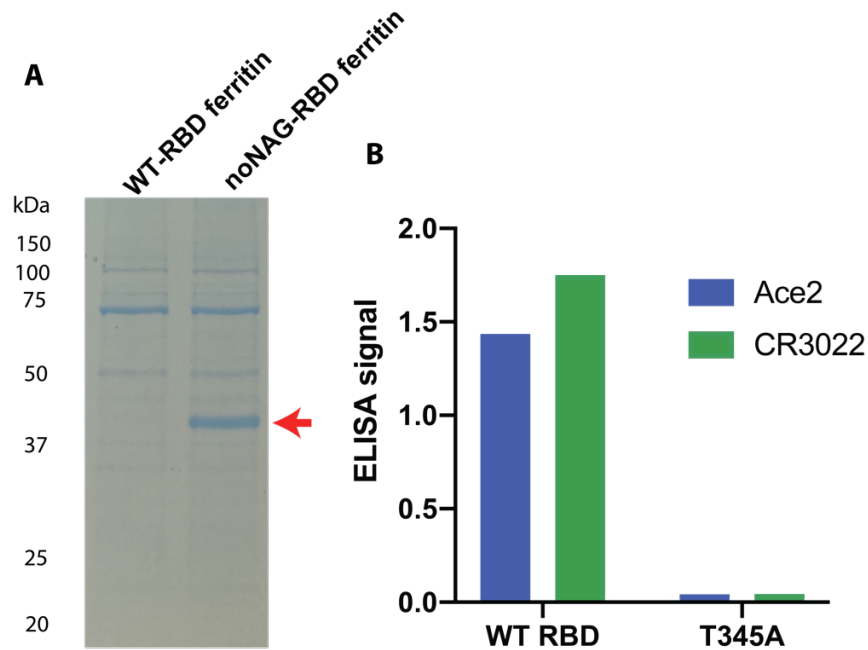


Figure S1. WT-RBD expresses poorly as a nanoparticle or non-glycosylated monomer. (A) Expi293F cell-free supernatant after 4 days expression analyzed by SDS-Page. Arrow indicates noNAG-RBD-ferritin fusion protein. (B) ELISA expression screen of cell-free supernatant using Ace2 and CR3022. WT-RBD has high expression and reacts with probes while the non-glycosylated T345A mutant does not.

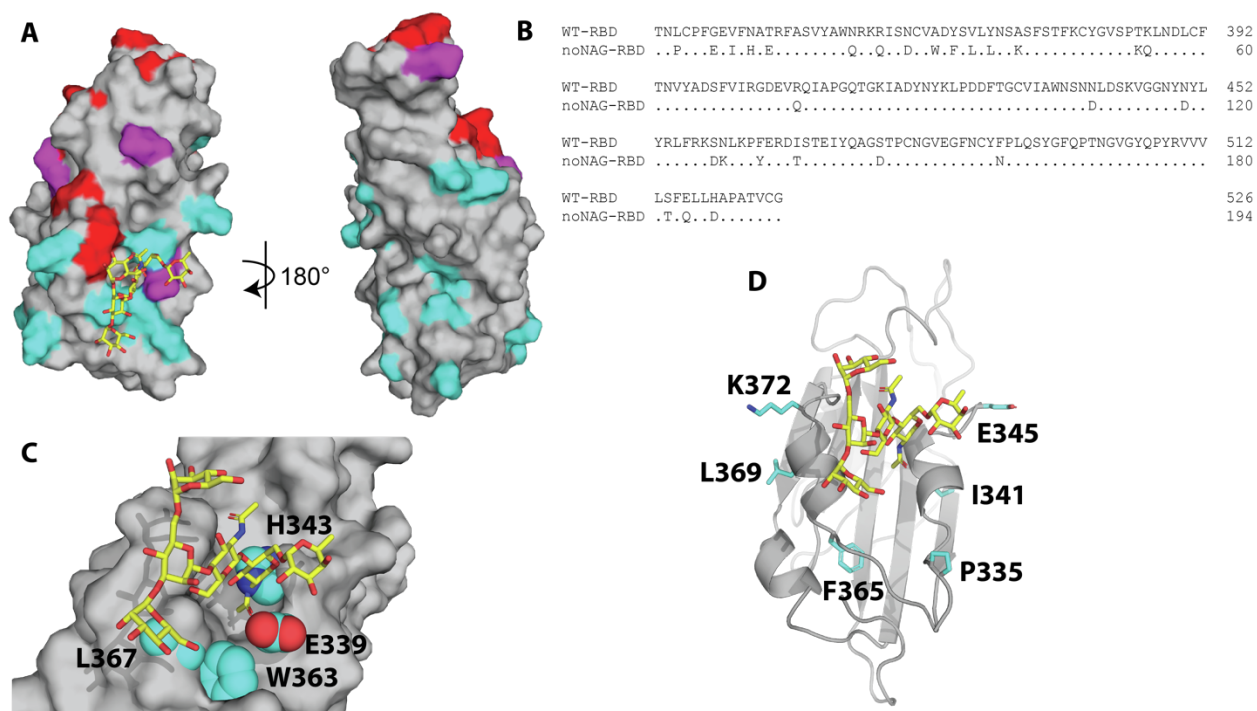


Figure S2. noNAG-RBD amino acid changes. (A) Amino acid changes mapped onto a model of noNAG-RBD. noNAG-RBD changes are indicated in cyan, omicron BA.5 changes are red, overlapping changes are magenta (G339[D]/E, R408[S]/Q, N440[K]/D, S477[N]/D, where the omicron identity is in brackets). The N-glycan is shown as a yellow sticks colored by element. (B) Sequence alignment indicating the location of amino acid changes in noNAG-RBD. (C) Cavity-filling amino acid changes G339E, N343H, A363W, V367L (cyan spheres) likely stabilize noNAG-RBD by compensating for stabilizing interactions lost upon removal of the glycan at position 343 (yellow sticks). The surface of WT RBD is depicted in grey. (D) Additional amino acid changes L335P, V341I, T345E, Y365F, Y369L, A372K (cyan sticks) likely stabilize the helices that contact the glycan in WT RBD.

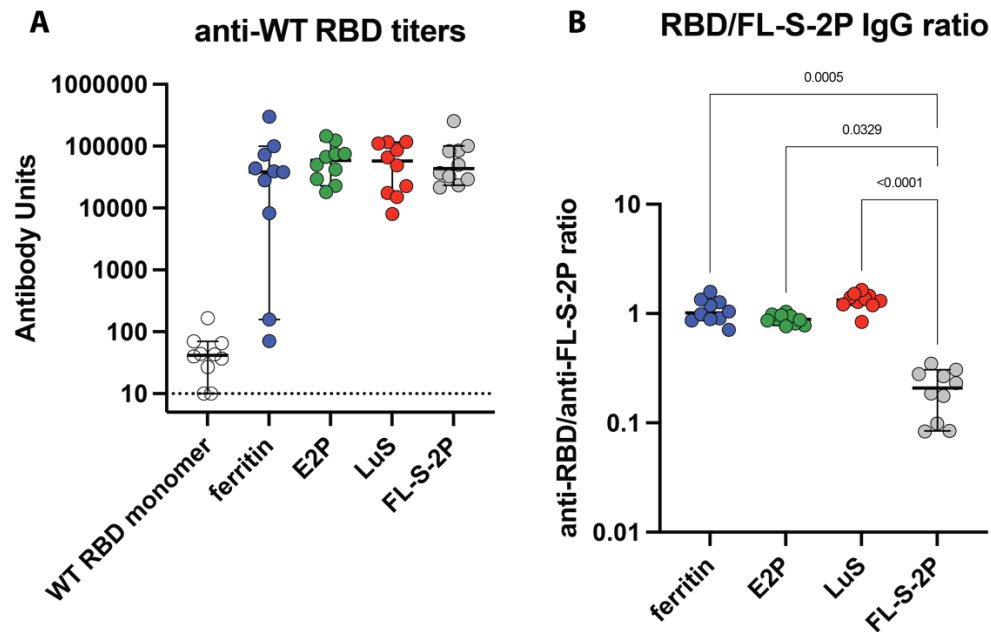


Figure S3. noNAG particles focus the immune response to the RBD in mice. (A) anti-WT RBD IgG titers in mice 14 days after a second immunization (day 35 in Figure 3A). Dashed line indicates detection limit of assay and bars represent median titers with 95% confidence interval. (B) Ratio of anti-WT RBD IgG titers to anti-FL-S-2P IgG titers at day 35, depicted as described for A. Statistical comparisons were made using a Kruskal-Wallis ANOVA followed by Dunn's comparison with FL-S-2P, corrected for multiple comparisons.

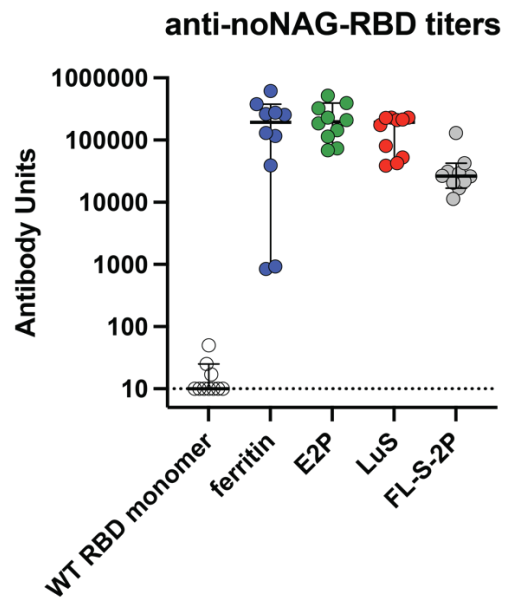


Figure S4. Anti-noNAG-RBD IgG titers in mice 14 days after a second immunization (day 35 in Figure 3A). Dashed line indicates detection limit of assay and bars represent median titers with 95% confidence interval.

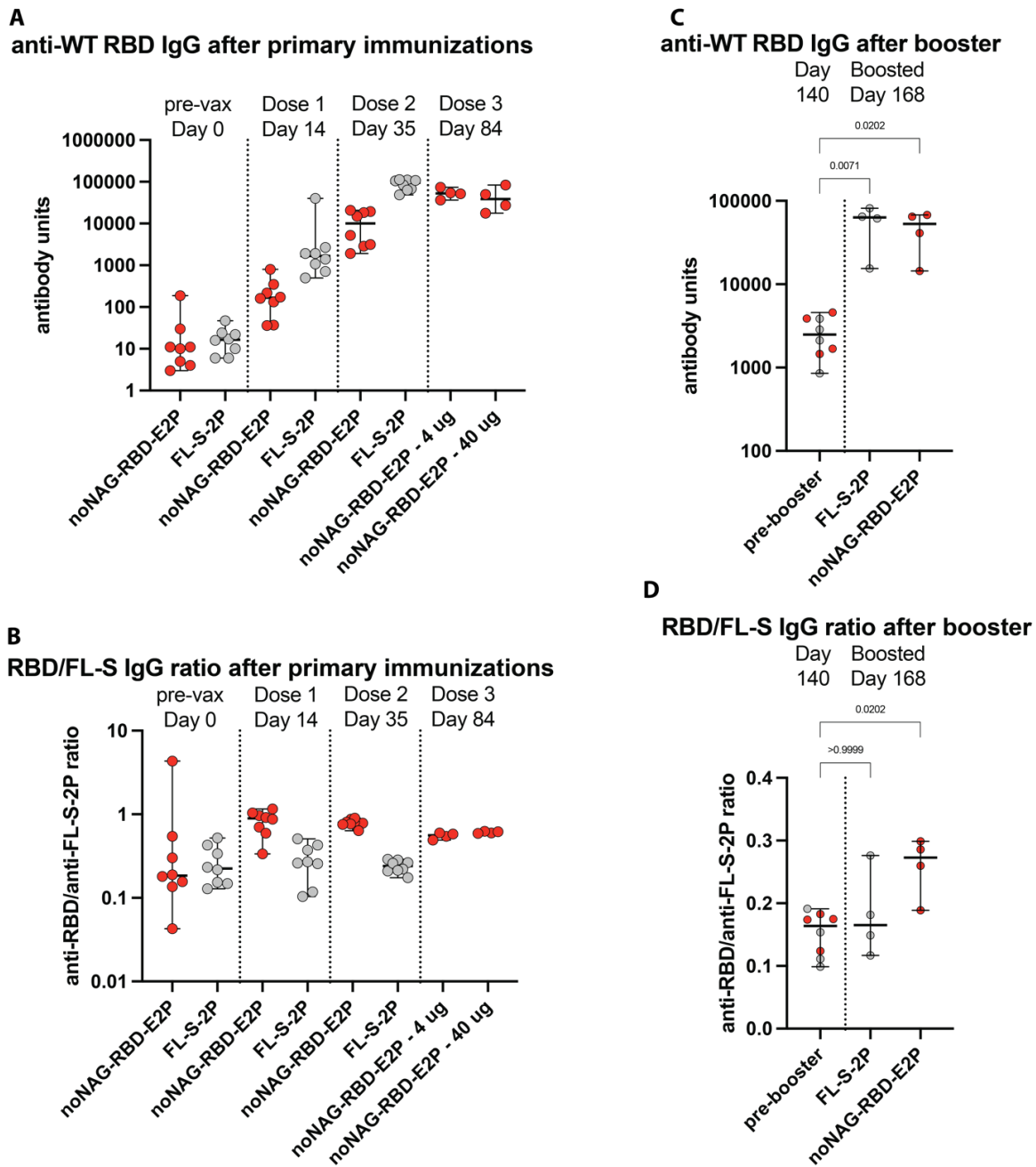


Figure S5. Anti-WT RBD IgG titers in NHPs. (A) anti-WT RBD IgG titers according to the immunization schedule in Figure 4A. Bars represent median titers with 95% confidence interval. (B) Ratio of anti-WT RBD IgG titers to anti-FL-S-2P IgG titers, depicted as described for A. (C) anti-WT RBD IgG titers before and after boosting, according to the immunization schedule in Figure 5A. Bars represent median titers with 95% confidence interval. Grey circles in the pre-booster sample are animals subsequently boosted with FL-S-2P and red circles were subsequently boosted with FL-S-2P. Statistical comparisons were made using a Kruskal-Wallis ANOVA followed by Dunn's comparison with the pre-booster group, corrected for multiple comparisons. (D) Ratio of anti-WT RBD IgG titers to anti-FL-S-2P IgG titers, depicted as described for C.

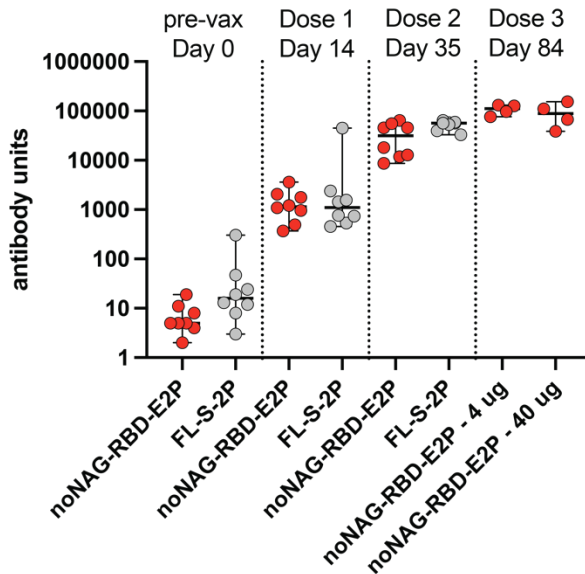
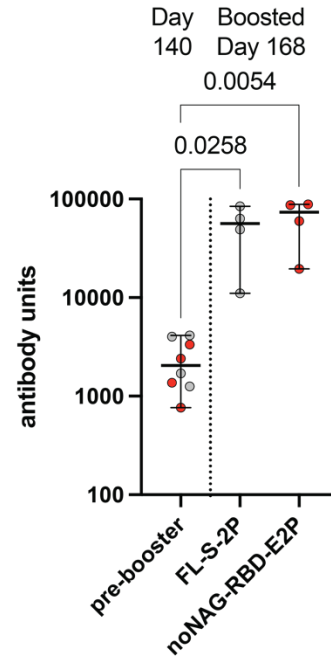
A**anti-noNAG-RBD IgG after primary immunizations****B anti-noNAG-RBD IgG after booster**

Figure S6. Anti-noNAG-RBD IgG titers in NHPs. (A) anti-noNAG-RBD IgG titers according to the immunization schedule in Figure 4A. Bars represent median titers with 95% confidence interval. (B) anti-noNAG-RBD IgG titers before and after boosting, according to the immunization schedule in Figure 5A. Bars represent median titers with 95% confidence interval. Grey circles in the pre-booster sample are animals subsequently boosted with FL-S-2P and red circles were subsequently boosted with FL-S-2P. Statistical comparisons were made using a Kruskal-Wallis ANOVA followed by Dunn's comparison with the pre-booster group, corrected for multiple comparisons.

Table S1. NHP immunology data

Gro up #	Immunizations	ani mal #	day	blocking ID50 - WA1	blocking ID50 - Beta	blocking ID50 - Delta	PRNT 50 - WA1	spike-binding IgG titer	Ab quality (PRNT50/IgG ratio)	FRNA WA1 ID50	FRNA BA.5 ID50
1b	none	2	0					77			
1b	none	5	0					77			
1a	none	6	0					47			
1b	none	7	0					26			
1a	none	8	0					41			
1b	none	9	0					146			
1a	none	12	0					91			
1a	none	14	0					55			
2	none	1	0					36			
2	none	3	0					43			
2	none	4	0					54			
2	none	10	0					62			
2	none	11	0					59			
2	none	13	0					23			
2	none	15	0					33			
2	none	16	0					56			
1b	1x 4 ug FL-S-2P	2	14					5,280			
1b	1x 4 ug FL-S-2P	5	14					156,500			
1a	1x 4 ug FL-S-2P	6	14					4,480			
1b	1x 4 ug FL-S-2P	7	14					7,133			
1a	1x 4 ug FL-S-2P	8	14					4,230			
1b	1x 4 ug FL-S-2P	9	14					1,913			
1a	1x 4 ug FL-S-2P	12	14					4,133			
1a	1x 4 ug FL-S-2P	14	14					13,517			
2	1x 4 ug noNAG-RBD-E2P	1	14					309			
2	1x 4 ug noNAG-RBD-E2P	3	14					828			
2	1x 4 ug noNAG-RBD-E2P	4	14					334			
2	1x 4 ug noNAG-RBD-E2P	10	14					109			
2	1x 4 ug noNAG-RBD-E2P	11	14					144			
2	1x 4 ug noNAG-RBD-E2P	13	14					198			

2	1x 4 ug noNAG-RBD-E2P	15	14					60			
2	1x 4 ug noNAG-RBD-E2P	16	14					139			
1b	2x 4 ug FL-S-2P	2	35	735.9	135.5	658.6	1994	387,333	0.0019	1280	160
1b	2x 4 ug FL-S-2P	5	35	1826	235.2	1719	10190	324,667	0.0056	10240	1280
1a	2x 4 ug FL-S-2P	6	35	676.3	151.3	616	1534	366,667	0.0018	640	80
1b	2x 4 ug FL-S-2P	7	35	1215	215.1	1174	3349	408,333	0.0030	1280	320
1a	2x 4 ug FL-S-2P	8	35	748.4	175.3	623.6	1183	321,333	0.0023	640	40
1b	2x 4 ug FL-S-2P	9	35	603.1	80.76	452.6	2334	220,333	0.0027	1280	160
1a	2x 4 ug FL-S-2P	12	35	753.6	116.9	607.2	2859	365,333	0.0021	1280	80
1a	2x 4 ug FL-S-2P	14	35	1178	267.9	1004	2725	531,667	0.0022	1280	40
2	2x 4 ug noNAG-RBD-E2P	1	35	20.8	9.416	19.92		3,667	0.0057		
2	2x 4 ug noNAG-RBD-E2P	3	35	96.01	28.44	75.35		24,193	0.0040		
2	2x 4 ug noNAG-RBD-E2P	4	35	108.1	24.92	81.85		28,650	0.0038		
2	2x 4 ug noNAG-RBD-E2P	10	35	9.985	10	9.645		2,190	0.0046		
2	2x 4 ug noNAG-RBD-E2P	11	35	66.02	8.673	36.91		18,580	0.0036		
2	2x 4 ug noNAG-RBD-E2P	13	35	21.46	9.88	16.49		3,510	0.0061		
2	2x 4 ug noNAG-RBD-E2P	15	35	24.66	9.512	16.77		6,840	0.0036		
2	2x 4 ug noNAG-RBD-E2P	16	35	133.6	16.33	80.48		27,297	0.0049		
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	1	84	655.3	68.81	347.4	1632	66,800	0.0098	1280	40
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	3	84	767	175.5	476.9	2056	82,900	0.0093	2560	160
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	4	84	864.8	269	362.2	3181	126,567	0.0068	1280	80
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	10	84	227.6	30.32	134.8	438.8	28,567	0.0080	320	20
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	11	84	1039	170.5	586.8	2134	90,600	0.0115	1280	40
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	13	84	583.4	180.8	343.2	360.8	43,933	0.0133	640	40
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	15	84	1067	303.9	692.7	1072	104,533	0.0102	640	160
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	16	84	950.5	236.5	588.4	2251	140,833	0.0067	1280	160
1b	2x 4 ug FL-S-2P	2	140	406.7	182.1	439.2		25,133	0.0162		
1b	2x 4 ug FL-S-2P	5	140	303.6	79.84	382.5		22,367	0.0136		
1a	2x 4 ug FL-S-2P	6	140	262	123.8	344		14,967	0.0175		
1b	2x 4 ug FL-S-2P	7	140	326	136.6	412.1		11,800	0.0276		
1a	2x 4 ug FL-S-2P	8	140	150.5	47.45	158.5		7,667	0.0196		
1b	2x 4 ug FL-S-2P	9	140	162.6	54.39	154.6		9,633	0.0169		

1a	2x 4 ug FL-S-2P	12	140	480.4	168.1	420.5		13,800	0.0348		
1a	2x 4 ug FL-S-2P	14	140	348.9	141.5	271.8		39,067	0.0089		
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	2	168	4436	1475	4643	8538	158,333	0.0280	5120	1280
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	5	168	2668	761	3175	6203	216,333	0.0123	2560	320
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	6	168	4574	1475	6833	6804	325,667	0.0140	2560	320
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	7	168	5264	2085	7692	15863	237,667	0.0221	10240	2560
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	8	168	6610	2169	8322	23854	547,000	0.0121	20480	5120
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	9	168	1794	606.8	3272	2538	52,333	0.0343	1280	160
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	12	168	3861	1933	6702	13407	132,333	0.0292	10240	1280
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	14	168	4245	1841	5032	10709	356,333	0.0119	10240	5120

Table S2. NHP history

Group #	immunizations	animal #	Sex	Age	Weight (kg)	History
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	6	M	7	7.62	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	8	M	7	7.49	10/2016 inoculated w/Leishmania major
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	12	M	5	13.55	inoculated w/Malaria & zika Pfs2301M-EPA/LS130
1a	2x 4 ug FL-S-2P + 4 ug FL-S-2P boost	14	M	7	8.52	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	2	M	7	8.66	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	5	M	7	8.74	10/2016 inoculated w/Leishmania major
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	7	M	7	8.45	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
1b	2x 4 ug FL-S-2P + 4 ug noNAG-RBD-E2P boost	9	M	9	11.65	inoculated w/CMV & Epstein barr
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	1	M	7	8.72	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	4	M	7	7.71	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	11	M	6	12.88	inoculated w/Malaria & zika Pfs2301M-EPA/LS130
2a	2x 4 ug + 4 ug noNANG-RBD-E2P	15	M	7	9.77	10/2016 inoculated w/Leishmania major
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	3	M	7	8.16	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	10	M	6	11.7	inoculated w/Malaria Pfs230D1M-EPA-
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	13	M	7	9.09	10/2016 inoculated w/Leishmania major; 12/2020 inoculated w/ VSV-ZEBOV
2b	2x 4 ug + 40 ug noNANG-RBD-E2P	16	M	7	10.39	10/2016 inoculated w/Leishmania major