

Supplemental Material

Supplementary Table S1. Detailed binding kinetics of Fabs and mAbs to JRFL gp140-F

	K_D (M)	on-rate (1/Ms)	off-rate (1/s)
VRC01 Fab	1.05×10^{-8}	$1.85 \times 10^4 \pm 5.38 \times 10^1^*$	$1.95 \times 10^{-4} \pm 5.53 \times 10^{-7}$
17b Fab	<i>nd</i>	<i>nd</i>	<i>nd</i>
b12 Fab	1.59×10^{-9}	$3.62 \times 10^5 \pm 5.51 \times 10^3$	$5.74 \times 10^{-4} \pm 2.63 \times 10^{-6}$
F105 Fab	4.63×10^{-9}	$2.30 \times 10^5 \pm 1.79 \times 10^3$	$1.07 \times 10^{-3} \pm 2.66 \times 10^{-6}$
b6 Fab	$<1.00 \times 10^{-12}^{**}$	$8.25 \times 10^4 \pm 6.00 \times 10^2$	$<1.00 \times 10^{-7}^{**}$
VRC03 IgG	5.87×10^{-8}	$5.31 \times 10^3 \pm 1.83 \times 10^1$	$3.12 \times 10^{-4} \pm 1.47 \times 10^{-6}$
PGV04 IgG	$<1.00 \times 10^{-12}^{**}$	$5.65 \times 10^3 \pm 8.08 \times 10^2$	$<1.00 \times 10^{-7}^{**}$
17b IgG	<i>nd</i>	<i>nd</i>	<i>nd</i>

*Standard error values are shown for on-rates and off-rates

** The kinetic constants of K_D and off-rate cannot be determined accurately by Octet because of very slow dissociation of the b6 Fab or PGV04 IgG from the trimer.

“*nd*” = no detectable binding

Supplementary Figure S1

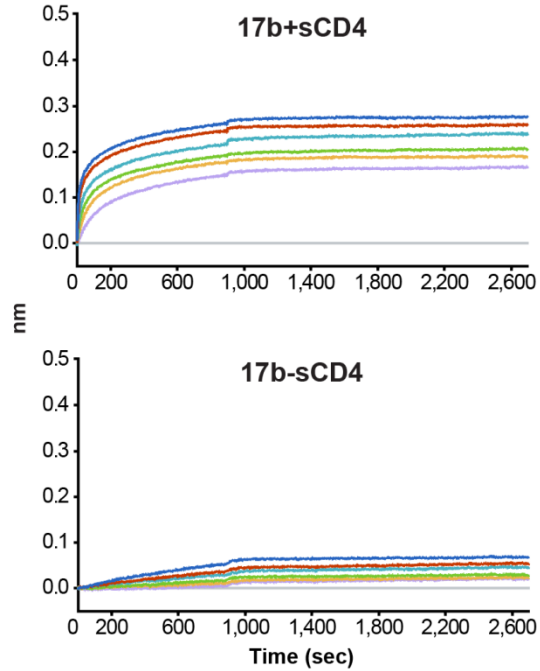


Fig S1. 17b binding to JRFL gp140-F trimmers with or without soluble CD4 pre-incubation. JRFL gp140-F trimmers were immobilized on Amine Reactive biosensors and then dipped into 50 $\mu\text{g/ml}$ sCD4 (17b+sCD4) or buffer without sCD4 (17b-sCD4) for 30 min. The association and dissociation with 17b IgG were performed as that described in the Materials and Methods section.

Supplementary Figure S2

Overall gp41 titers to rpg41 by ELISA

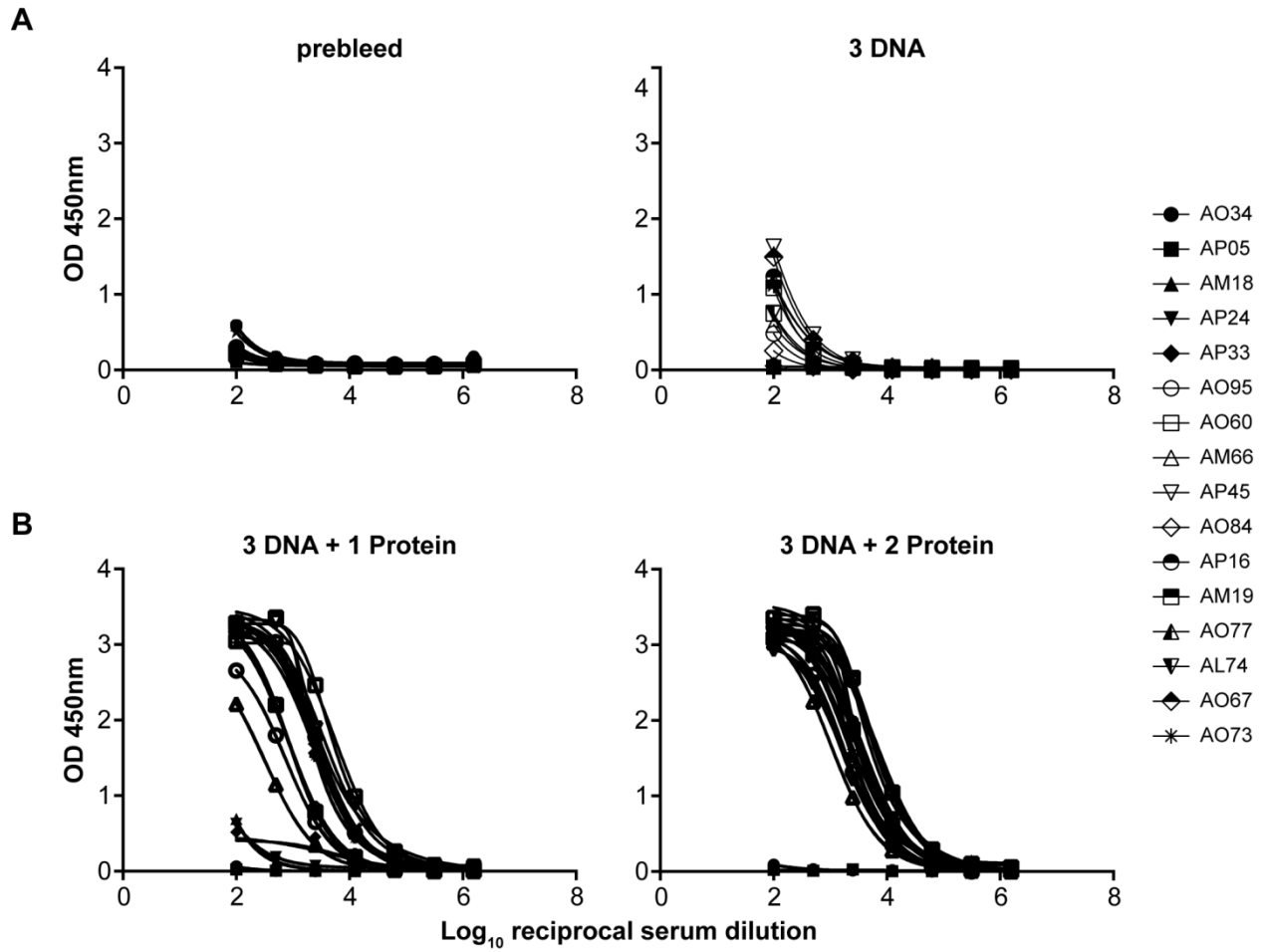


Fig S2. ELISA binding curves for the total gp41 response in the sera of JRFL Env-immunized NHPs. A. Left, the background binding curves in the pre-bleed sera; right, 3 times DNA. B. Left, 3 DNA and 2 protein; right, 3 DNA and 2 protein.

Supplementary Figure S3

Comparison of gp140 to gp41 Antibody Responses

Median EC₅₀ values of the total gp140 and gp41 titers after 3 DNA, 3 DNA + 1 protein and 3 DNA + 2 protein. (±) indicates the standard deviation associated with the median ED₅₀ values.

	3 DNA	3 DNA + 1 Protein	3 DNA + 2 Protein
Response to gp140	413.1±303	8540±2970	12995±4339
Response to gp41	38.14±12.7	2293±1113.4	3323±1656
Ratio of responses (gp140/gp41)	10.82	3.72	3.91

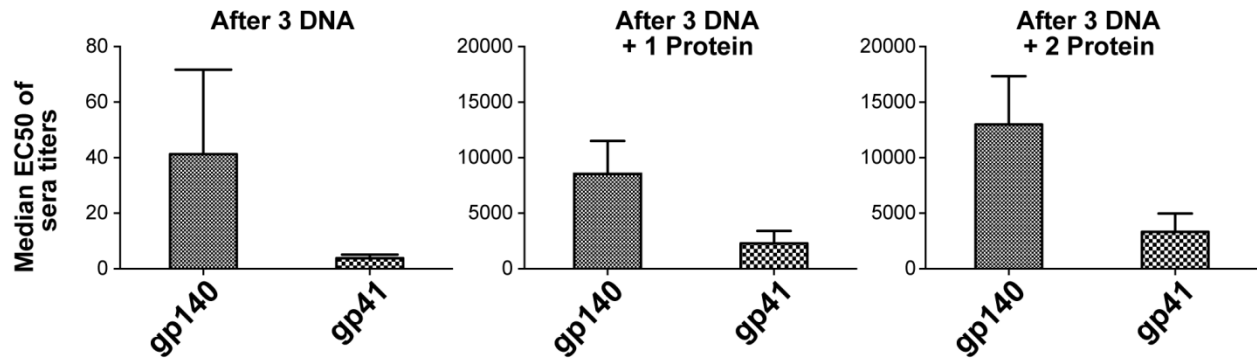


Fig S3. Comparison of the total gp140 and gp41 response after 3 DNA, 3 DNA and 1 protein, and 3 DNA and 2 protein inoculations into NHPs as indicated.

Supplementary Figure S4

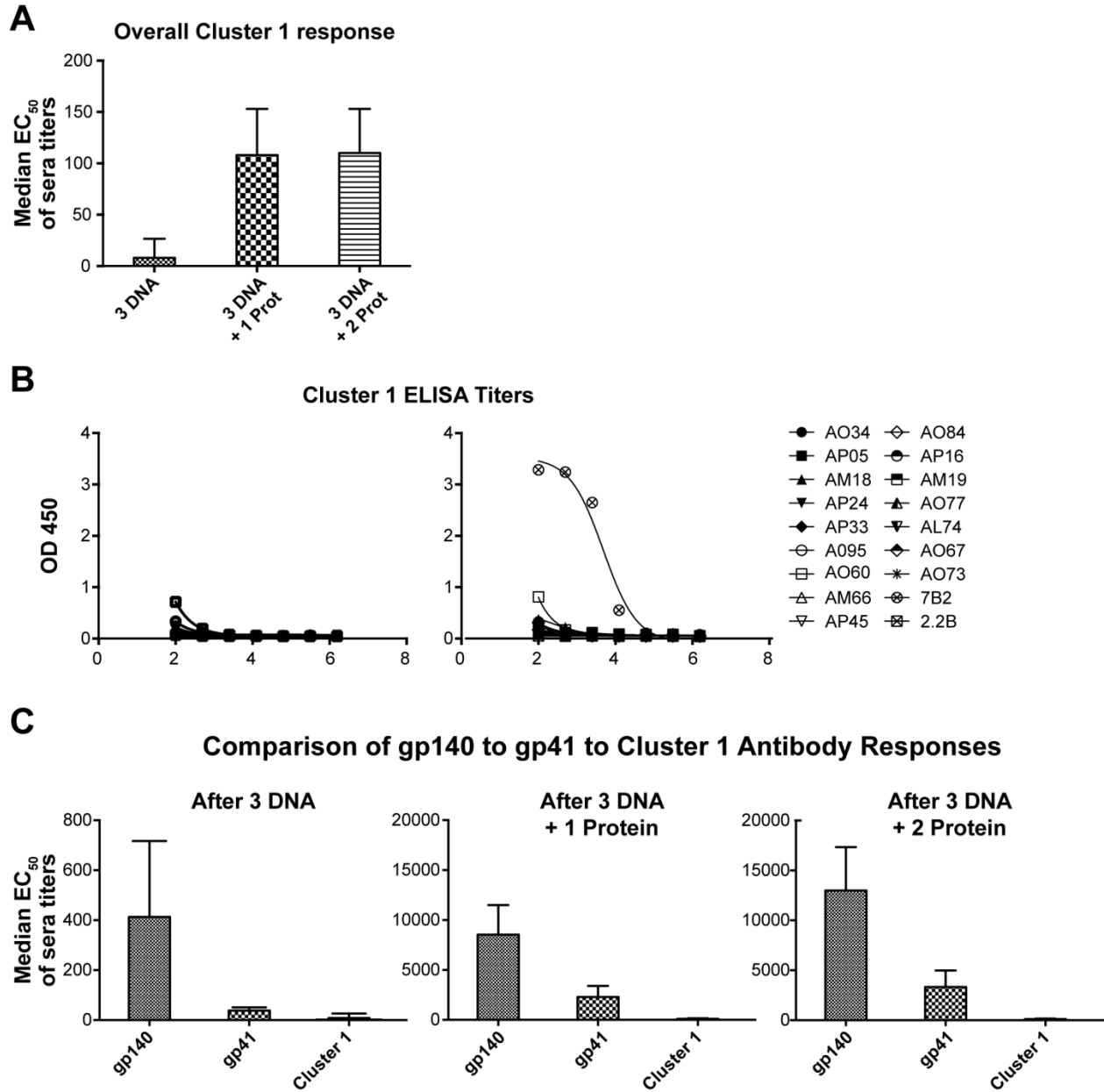


Fig S4. Cluster I IgG responses in Env-immunized NHPs. A. The median ED₅₀ values of total cluster I responses after 3 DNA, 3 DNA + 1 protein and 3 DNA + 2 protein. B. Low ELISA titers to cluster I after 3 DNA + 1 protein and 3 DNA and 2 protein boost. C. Comparison of the total gp140 and gp41 and cluster I responses after 3 DNA immunization, 3 DNA and 1 protein

and 3 DNA and 2 protein. The error bars represent the standard deviations associated with the median EC₅₀ values.

Supplementary Figure S5

TZM-bl Assay: JRFL Trimer-elicited Neutralizing Titers ID₅₀

		Clade B virus											Clade C virus		
		Tier 1					Tier 2								
Inoculation/ Bleed	Animal	MN	HXB2	SF162	BaL	SS1196	JRFL	YU2	SC422	6535	THRO	MW965	ZM109	JRFL Δ301	
Controls	PBS	AO34	<5	<5	<5	<5	19	8	<5	<5	<5	18	<5	6	<5
	PBS		40	<5	<5	<5	21	20	24	19	<5	19	<5	20	18
	PBS		<5	<5	<5	<5	17	17	13	11	<5	16	<5	11	<5
	PBS	AP05	<5	<5	<5	<5	27	16	11	14	<5	14	<5	15	<5
	PBS		<5	<5	<5	<5	42	22	19	22	<5	24	<5	20	5
	PBS		<5	<5	<5	<5	38	27	15	26	<5	25	<5	16	<5
Protein Trimers	PBS	AM18	<5	<5	<5	<5	16	7	5	<5	<5	17	<5	<5	<5
	1 Protein		<5	<5	<5	14	26	20	22	20	<5	20	<5	20	13
	2 Protein		32918	65	2677	282	227	28	12	31	7	22	1592	23	213
	PBS	AP24	<5	<5	<5	<5	15	7	7	6	<5	9	<5	5	<5
	1 Protein		<5	<5	<5	<5	31	25	27	25	<5	25	<5	24	16
	2 Protein		24690	382	1413	124	99	37	17	24	9	23	1935	20	137
Non-Codon optimized DNA Prime	PBS	AP33	<5	<5	<5	<5	20	6	9	29	20	20	<5	19	19
	1 Protein		<5	<5	<5	<5	24	20	21	16	<5	20	<5	18	13
	2 Protein		16614	121	1073	102	70	16	8	22	<5	22	629	14	71
	3XDNA	AO95	<5	<5	<5	<5	<5	6	7	<5	<5	<5	<5	<5	<5
	3XDNA+1Protein		6101	10	62	19	51	14	17	25	5	22	84	21	79
	3XDNA+2Protein		34690	434	781	39	96	14	10	10	<5	23	3564	26	97
Codon-optimized DNA Prime	3XDNA	AO60	32	<5	<5	<5	18	11	17	17	<5	12	<5	15	8
	3XDNA+1Protein		8976	33	577	52	69	23	5	19	11	20	835	20	144
	3XDNA+2Protein		34578	75	1892	59	75	64	5	20	10	16	1383	16	290
	3XDNA	AM66	78	<5	<5	<5	16	<5	<5	10	<5	14	<5	11	<5
	3XDNA+1Protein		7627	12	329	22	65	23	22	23	8	23	121	19	67
	3XDNA+2Protein		32311	116	7382	173	146	35	14	25	19	25	7658	19	168
Protein Trimers	3XDNA	AP45	<5	<5	<5	<5	18	10	19	15	<5	10	<5	<5	<5
	3XDNA+1Protein		3465	14	121	15	48	24	33	37	<5	27	34	22	41
	3XDNA+2Protein		32935	381	1507	66	104	27	13	65	42	36	1256	30	145
	3XDNA	AO84	75	<5	<5	<5	15	8	18	12	<5	9	<5	6	<5
	3XDNA+1Protein		10931	17	441	64	66	33	38	30	<5	25	471	30	102
	3XDNA+2Protein		24928	659	4398	476	221	35	17	129	<5	30	4759	53	215
Non-Codon optimized DNA Prime	3XDNA	AP16	16	<5	5	<5	20	11	25	20	<5	14	8	10	14
	3XDNA+1Protein		6566	20	786	72	125	39	40	37	5	42	532	22	165
	3XDNA+2Protein		33019	288	6123	295	190	139	21	12	33	33	4209	53	391
	3XDNA	AM19	230	<5	5	<5	28	10	18	21	<5	19	<5	13	9
	3XDNA+1Protein		3509	129	553	37	58	29	42	31	9	31	557	28	81
	3XDNA+2Protein		19805	923	6756	154	83	21	14	34	33	24	8121	38	163
Codon-optimized DNA Prime	3XDNA	AO77	1390	<5	28	<5	21	17	14	18	<5	19	15	9	17
	3XDNA+1Protein		8146	162	147	31	73	21	41	21	<5	32	673	25	181
	3XDNA+2Protein		42282	1605	6073	260	157	26	19	62	15	27	15919	20	541
	3XDNA	AL74	3156	<5	27	7	43	13	15	28	<5	16	32	13	16
	3XDNA+1Protein		9926	115	1135	82	96	44	40	35	25	42	1003	43	207
	3XDNA+2Protein		27049	2361	6758	368	297	55	13	75	28	32	6024	74	428
Protein Trimers	3XDNA	AO67	463	<5	5	<5	22	1	17	17	<5	19	7	1	17
	3XDNA+1Protein		5486	59	402	36	74	22	28	24	5	32	444	21	86
	3XDNA+2Protein		33110	697	3993	135	146	29	1	244	88	40	4566	25	264
	3XDNA	AO73	270	<5	13	<5	35	15	18	18	<5	18	20	12	10
	3XDNA+1Protein		3103	27	751	62	65	30	30	20	11	25	1352	30	73
	3XDNA+2Protein		49704	1636	19890	486	272	38	15	63	25	27	91920	30	437

ID50 20-99 ID50 100-999 ID50 >1000

Fig S5. Shown are the ID₅₀ neutralization values in the TZM-bl assay elicited by the selected regimens of JRFL trimers inoculated into NHPs. The first two animals (A034, AP05) are controls that received 5 inoculations of PBS. The next three animals (AM18, AP21 and AP33) received three PBS “primers” in lieu of Env plasmid DNA and were inoculated once (1 Protein) and twice (2 Protein) with JRFL gp140-F trimer protein in adjuvant. The next 6 animals were primed three times with non-codon optimized cell-surface JRFL Env plasmid DNA and pCTat at 1:1 ratio of the relative concentrations (A095, A060 and AM66) or non-codon optimized JRFL Env plasmid to pCTat at a 1:20 ratio (AP45, AO84 and AP16) followed by 1 Protein and 2

Protein boosts of JRFL gp140-F trimers in adjuvant as shown. Then next three animals (AM19, AO77 and AL74) were primed with 3 inoculations of codon-optimized JRFL cell-surface plasmid DNA followed by 1 Protein and 2 Protein boosts of JRFL gp140-F trimers in adjuvant. The last two animals (AO67 and AO73) were primed 3 times with codon-optimized DNA expressing soluble gp140-F trimers followed by Protein 1 and Protein 2 boosts of JRFL gp140-F trimers in adjuvant as shown.

Supplementary Figure S6

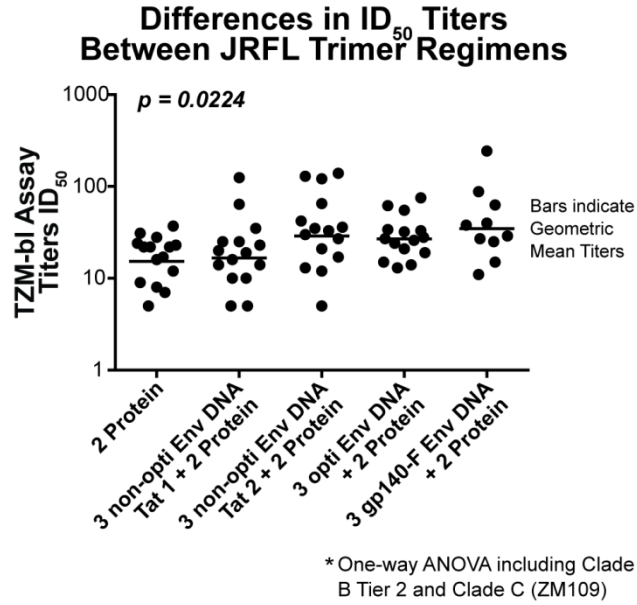


Fig S6. Statistical analysis of the TzM-bl ID₅₀ neutralization values elicited by the 5 different inoculation regimens described in Fig S4 and the Results. The one-way ANOVA analysis revealed a statistically significant difference between the 5 regimens.

Supplementary Figure S7

A3R5 Assay: JRFL Trimer-elicited Neutralizing Titers ID₅₀

		Inoculation/ Bleed	Clade B viruses						Clade C viruses					
			Animal	SC22	RHPA	CH58	THRO	WITO	1051	CAP45	CEe1086	Du151	Ce2010	
Controls		PBS	AO34	22	<20	31	22	23	36	<20	21	37	34	
		PBS		<20	<20	27	<20	<20	25	<20	<20	<20	<20	
		PBS		<20	<20	28	<20	<20	<20	37	<20	<20	21	<20
		PBS	AP05	<20	<20	29	<20	<20	<20	30	<20	<20	32	28
		PBS		23	<20	28	21	<20	<20	34	<20	<20	42	38
		PBS		<20	<20	34	23	<20	<20	32	<20	21	28	<20
Protein Trimers		PBS	AM18	23	<20	<20	<20	<20	26	<20	<20	<20	<20	
		1 Protein		31	<20	29	80	<20	38	28	30	23	NS	
		2 Protein		329	1980	62	162	36	50	<20	29	40	29	
		PBS	AP24	<20	<20	<20	<20	<20	24	<20	<20	26	<20	
		1 Protein		24	<20	28	24	23	32	<20	<20	NT [†]	NT	
		2 Protein		415	1258	104	145	45	93	372	91	61	102	
Non-Codon optimized		PBS	AP33	<20	<20	20	23	<20	31	<20	<20	50	29	
		1 Protein		<20	<20	20	<20	<20	23	<20	<20	35	NT	
		2 Protein		399	1105	121	143	34	67	84	183	83	57	
		3XDNA	AO95	<20	<20	22	21	21	26	<20	<20	23	<20	
		3XDNA+1Protein		163	203	50	69	55	51	<20	24	29	33	
		3XDNA+2Protein		521	2124	101	140	105	99	52	67	54	47	
Codon-optimized		3XDNA	AO60	<20	<20	27	35	24	23	<20	24	32	23	
		3XDNA+1Protein		584	1959	107	332	92	63	380	156	62	77	
		3XDNA+2Protein		715	1845	75	135	73	58	234	78	46	41	
		3XDNA	AM66	<20	<20	29	23	<20	34	<20	<20	27	21	
		3XDNA+1Protein		159	430	49	76	31	49	<20	77	30	53	
		3XDNA+2Protein		503	2043	69	167	48	86	50	91	64	85	
		3XDNA	AP45	<20	<20	28	20	<20	22	<20	<20	22	<20	
		3XDNA+1Protein		302	693	82	103	28	46	195	69	63	57	
		3XDNA+2Protein		314	1588	86	124	37	33	398	88	86	90	
		3XDNA	AO84	<20	<20	<20	<20	21	<20	<20	<20	<20	<20	
		3XDNA+1Protein		64	543	31	55	36	<20	<20	27	<20	26	
		3XDNA+2Protein		450	1957	77	167	65	78	131	47	31	32	
3XDNA	AP16	<20	<20	26	23	<20	23	<20	24	23	27			
3XDNA+1Protein		346	2737	97	184	41	65	<20	37	30	28			
3XDNA+2Protein		775	5228	121	180	90	43	219	51	36	27			
Codon-optimized		3XDNA	AM19	<20	<20	22	<20	<20	<20	<20	<20	<20		
		3XDNA+1Protein		881	1752	84	158	30	33	<20	46	47	32	
		3XDNA+2Protein		1289	2865	94	114	<20	41	144	48	51	31	
		3XDNA	AO77	<20	<20	23	<20	<20	<20	<20	<20	<20	<20	
		3XDNA+1Protein		390	1217	43	37	<20	56	<20	<20	<20	<20	
		3XDNA+2Protein		2075	4335	80	63	<20	50	<20	21	29	<20	
		3XDNA	AL74	<20	<20	24	<20	<20	<20	<20	<20	NT	NT	
		3XDNA+1Protein		295	1839	81	135	66	44	61	70	NT	NT	
		3XDNA+2Protein		1297	3152	148	135	ns	ns	285	99	NT	NT	
		3XDNA	AO67	<20	<20	21	<20	<20	<20	<20	<20	<20	<20	
		3XDNA+1Protein		429	690	59	78	52	57	<20	31	32	26	
		3XDNA+2Protein		2450	1967	195	286	55	72	251	94	50	83	
3XDNA	AO73	<20	<20	27	<20	<20	<20	<20	<20	<20	<20			
3XDNA+1Protein		204	465	60	67	39	30	<20	29	28	25			
3XDNA+2Protein		889	4541	90	149	89	32	36	57	39	<20			

ID50 20-99 ID50 100-999 ID50 >1000

Fig S7. Shown are the ID₅₀ neutralization values in the A3R5 assay elicited by the selected regimens of JRFL trimers inoculated into NHPs. The first two animals (A034, AP05) are controls that received 5 inoculations of PBS. The next three animals (AM18, AP21 and AP33) received three PBS “primes” in lieu of Env plasmid DNA and were then inoculated once (1 Protein) and twice (2 Protein) with JRFL gp140-F trimer protein in adjuvant. The next 6 animals were primed three times with non-codon optimized cell-surface JRFL Env plasmid DNA and

pCTat at 1:1 ratio of the relative concentrations (A095, A060 and AM66) or non-codon optimized JRFL Env plasmid to pCTat at a 1:20 ratio (AP45, AO84 and AP16) followed by 1 Protein and 2 Protein boosts of JRFL gp140-F trimers in adjuvant as shown. Then next three animals (AM19, AO77 and AL74) were primed with 3 inoculations of codon-optimized JRFL cell-surface plasmid DNA followed by 1 Protein and 2 Protein boosts of JRFL gp140-F trimers in adjuvant. The last two animals (AO67 and AO73) were primed 3 times with codon-optimized DNA expressing soluble gp140-F trimers followed by Protein 1 and Protein 2 boosts of JRFL gp140-F trimers in adjuvant as shown.

Supplementary Figure S8

A3R5 Assay: YU2 gp140-F Trimer-elicited Tier 2 Titers Post 5 Protein

	Inoculation/ Bleed	Animal	Clade B virus			Clade C virus		
			SC22	RHPA	CH58	CEe1086	Du151	
YU2 gp140-F Trimer	Pre	F123	23	<20	29	33	31	
	5 Protein		60	163	ND	ND	ND	
	Pre	F124	33	<20	37	38	41	
	5 Protein		282	360	272	57	44	
	Pre	F125	49	<20	31	29	35	
	5 Protein		381	483	127	58	42	
	Pre	F126	44	30	36	42	48	
	5 Protein		235	504	75	49	31	
	Pre	F127	24	38	39	33	34	
	5 Protein		243	872	344	60	43	
	Pre	F128	<20	30	50	35	28	
	5 Protein		300	1233	337	100	63	
	Adjuvant only control		F129	43	33	39	34	32
			F130	<20	36	38	28	31
		F131	35	31	42	33	26	
		F132	46	23	34	39	36	
		F133	39	34	45	37	44	
		F134	51	39	38	37	34	




ID50  20-99 ID50  100-999 ID50  >1000

Fig S8. Shown are the ID₅₀ values detected against five viruses in the A3R5 assay following 5 inoculations of the YU2 gp140-F trimeric proteins into NHPs (F123-F128). The last 6 animals are “adjuvant only” inoculated controls that were analyzed similarly.