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Supplemental Information

Immune Monitoring Reveals Fusion Peptide Priming to Imprint Cross-Clade HIV-Neutralizing Responses with a Characteristic Early B Cell Signature

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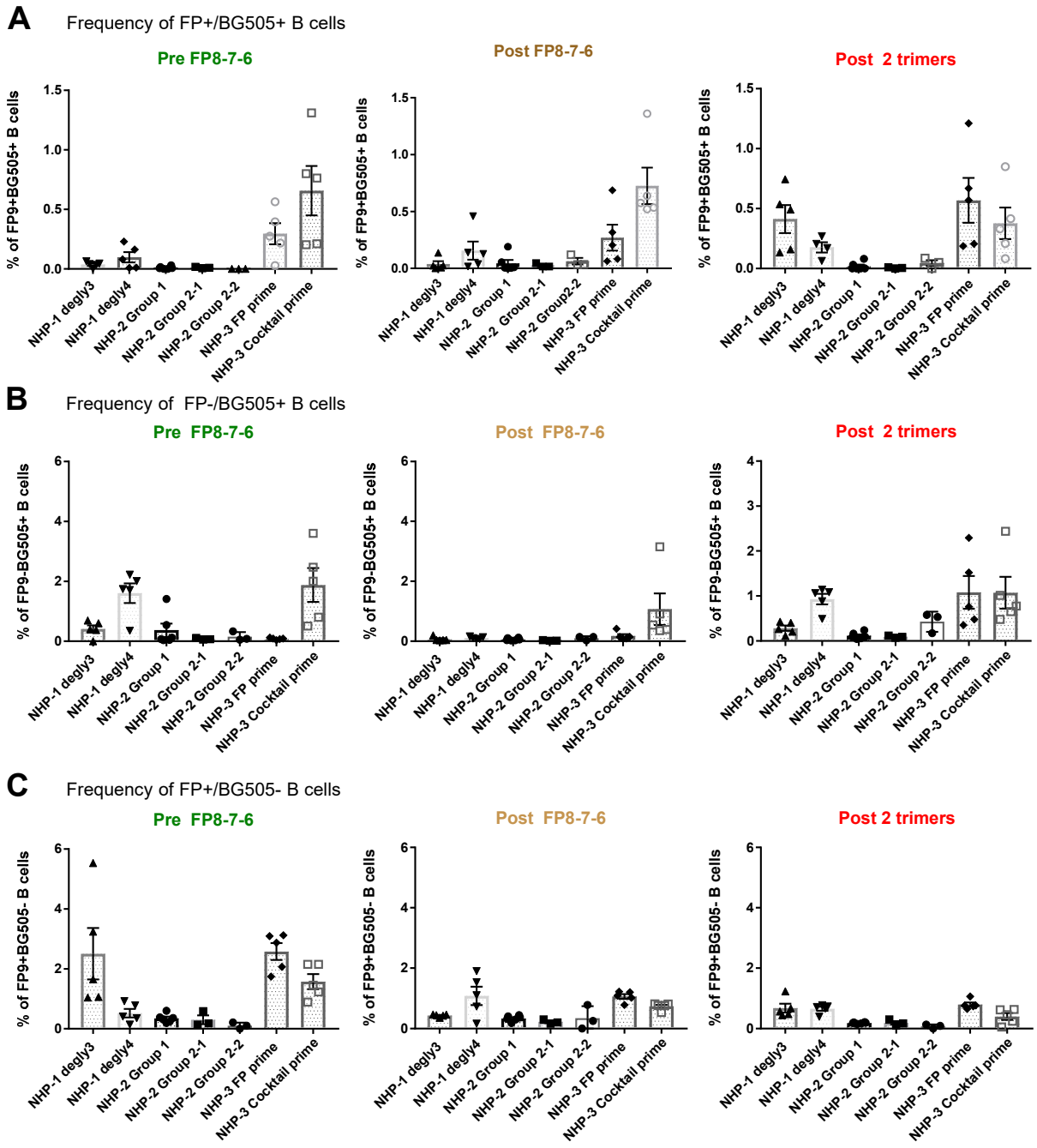
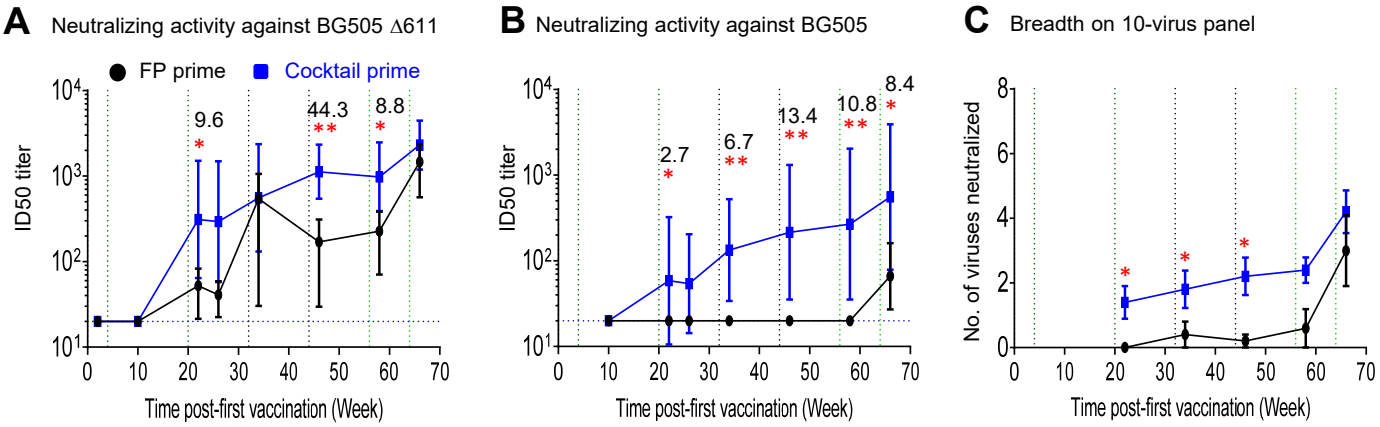


Figure S1. Frequency of Antigen Specific B Cells at Three Key Time Points, Related to Figures 2-5. Antigen specific B cell population among IgG+ B cells were characterized as dual FP+/BG505+ (**A**), single BG505+ (**B**), or single FP+ (**C**) at pre FP8-7-6, post FP8-7-6 and post 2 trimers time points. B cell frequencies were calculated as mean ± SEM.



D NHP-3 serum neutralizing activity against the Montefiori 12-strain panel

ID_{50} Week66	Clade Viruses	B TRO11	B X2278	BC BJOX2000	BC CH119	C CE1176	C CE0217	C 25710	AC 246F3	A 398F1	G X1632	AE CNE55	AE CNE8
FP prime	DFTX	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	DF9L	<20	<20	<20	<20	<20	<20	25	<20	<20	<20	<20	<20
	DFIXA	<20	<20	<20	<20	<20	<20	280	<20	<20	<20	<20	<20
	DFIV	<20	<20	<20	<20	<20	<20	41	<20	<20	<20	<20	<20
	HLP	<20	<20	<20	<20	<20	<20	116	<20	21	<20	<20	<20
Cocktail prime	DFL7	<20	<20	<20	<20	<20	<20	88	<20	43	<20	<20	<20
	DFTN	<20	<20	<20	<20	<20	<20	127	<20	<20	<20	<20	<20
	DFTG	<20	<20	<20	<20	<20	<20	127	<20	<20	<20	<20	<20
	DFWB	<20	<20	<20	<20	<20	<20	43	<20	27	<20	<20	<20
	04L	<20	<20	<20	<20	<20	<20	34	<20	86	<20	<20	<20

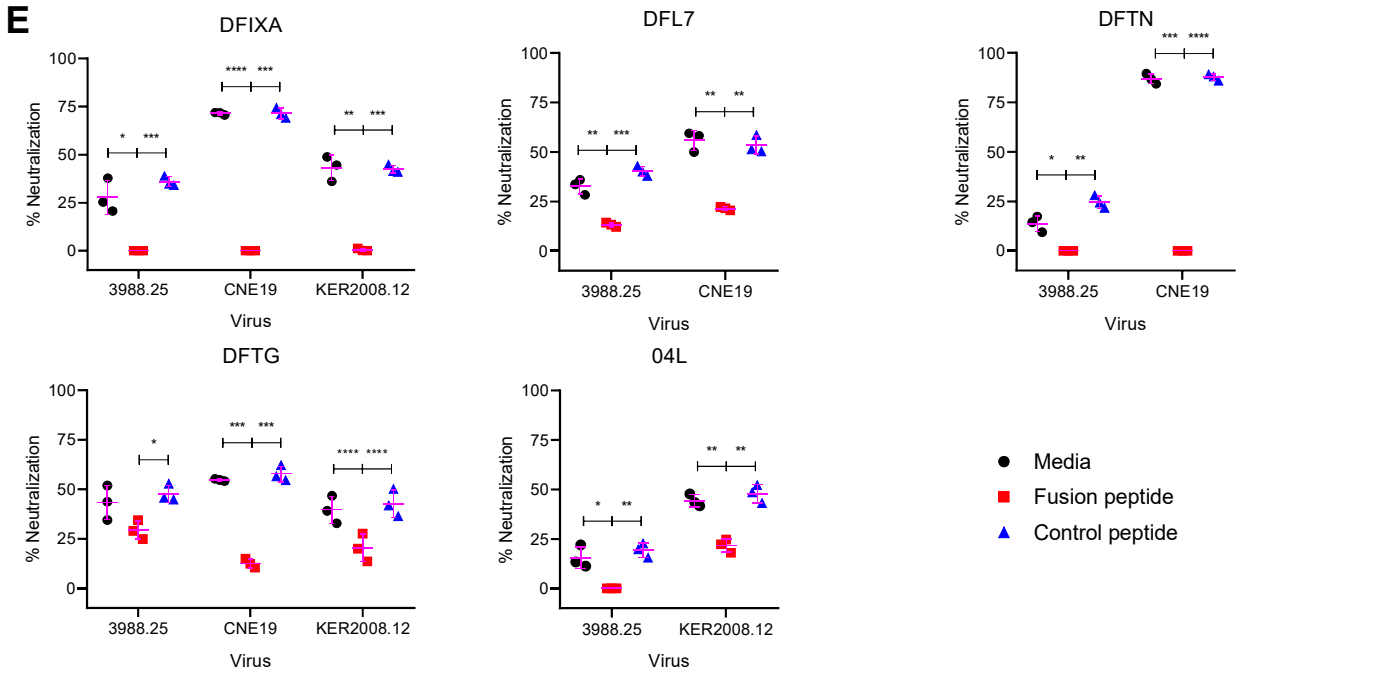


Figure S2. Cocktail-Prime Elicits Earlier and More Potent Responses than FP-Only Prime, with Both Eliciting Responses Directed Against FP, Related to Figures 4 and 5. (A-C) Cocktail-primed NHPs show earlier plasma neutralization activity with higher potency and breadth than FP-primed group in NHP-3 study. (**A**) Neutralization ID_{50} against BG505 Δ611. (**B**) Neutralization ID_{50} against wild-type BG505. (**C**) Neutralization breadth on a 10-strain panel of wild-type viruses. Data shown represent geometric mean±95% CI for panels (**A**) and (**B**) and mean±SEM for (**C**), with p values calculated with Mann-Whitney 2-tailed t test. Numbers on panels (**A**) and (**B**) indicate the ratio of GMT titers between cocktail-primed and FP-primed groups. ID_{50} values of <20 were treated as 20. (**D**) Neutralization IC50 of NHP-3 sera at week 66 on the Montefiori 12-strain panel (deCamp et al., 2014). **(E)** FP competition reveals plasma neutralizing activity against heterologous viruses to be directed against FP. Percent plasma neutralization by plasma from NHPs DFIXA (FP prime), and DFL7, DFTN, DFTG, and 04L (cocktail prime) in NHP-3 study at week 66 were assessed in media (control), fusion peptide + media (FP), and control peptide + media (non-FP control). Plasma samples were diluted 20-fold. Each data point represents an independent assay, and mean and standard deviation indicated by red bars. P values were calculated with paired parametric two-tailed t test. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$.

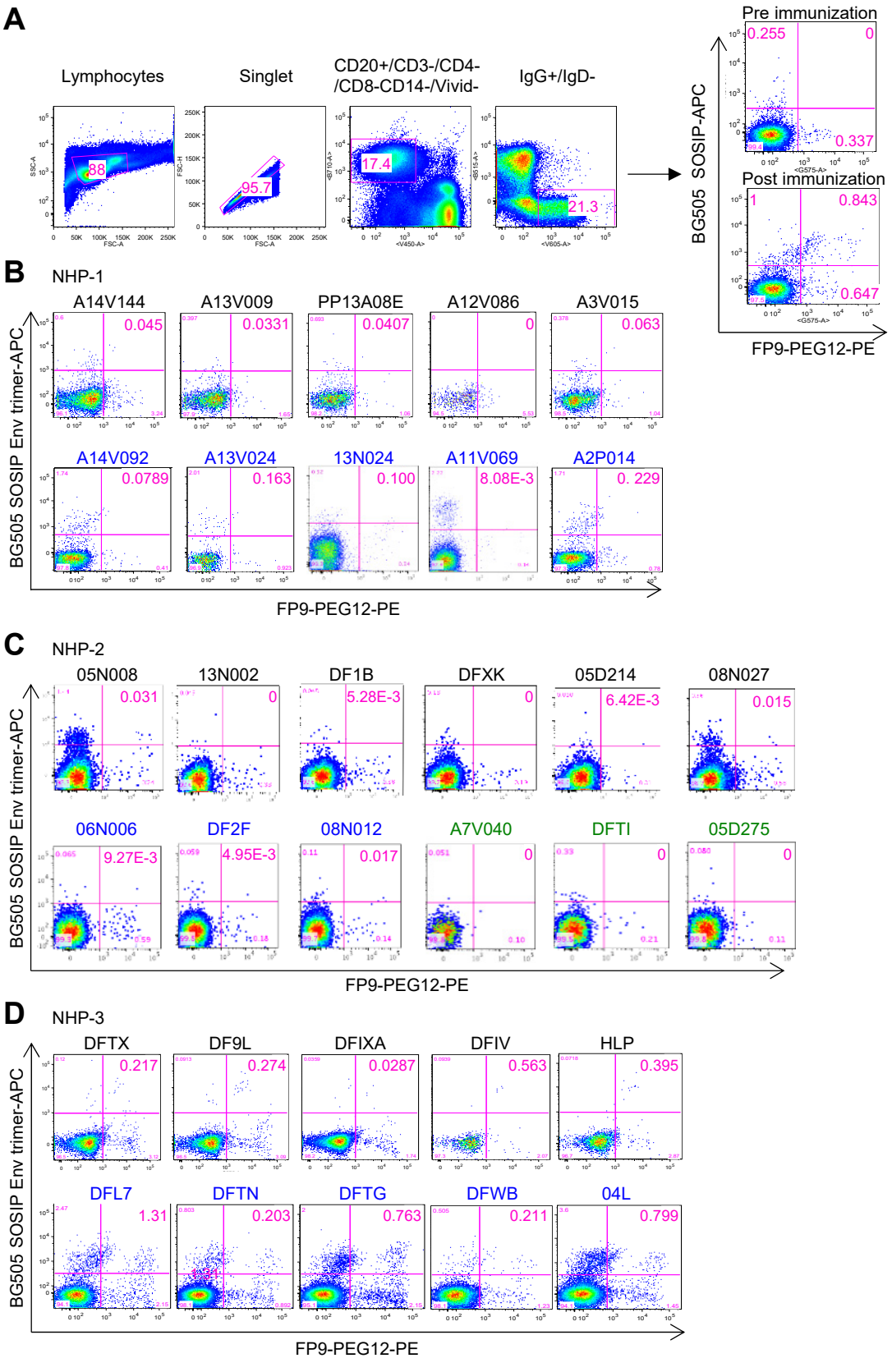


Figure S3. FACS Analysis of PBMCs at the Pre FP8-7-6 Time Point, Related to Figures 2-6. (**A**) Gating strategy for FP+BG505+ memory B cells in naïve and immunized PBMCs. PBMCs from the pre immunization time points were used as a control to set up gating for the antigen-specific B cells. (**B-D**) FACS analysis of PBMCs. NHP IDs were colored in the same pattern as in Figures 2-4. Pseudocolor graphs are shown for IgG+ B cells gated with the two probes based on gating setups using PBMCs from each monkey at pre vaccination as the control.

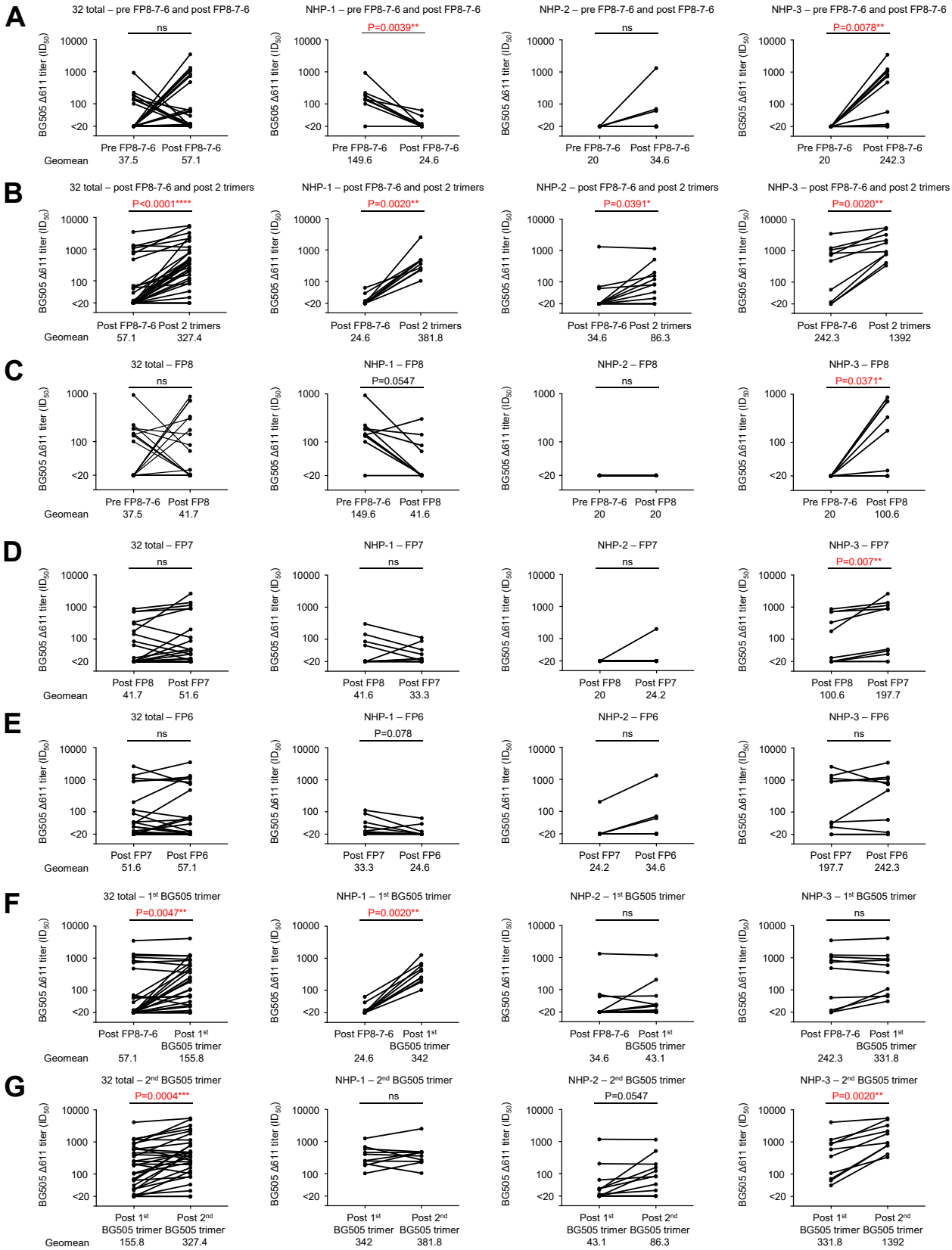
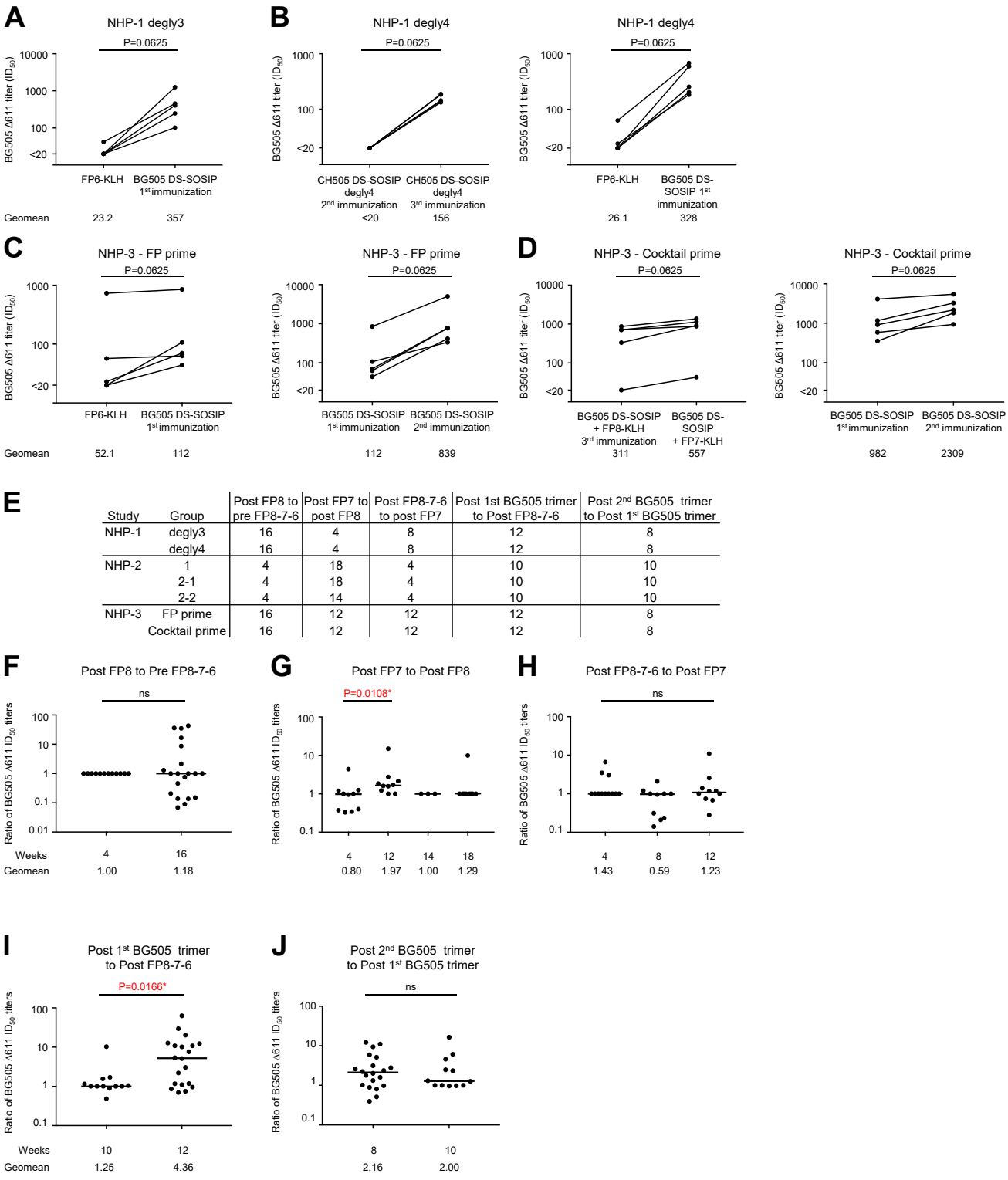


Figure S4. Comparison of BG505 Δ611 Titers (ID_{50}) Before and After Each Immunization in the FP8-7-6-Trimer-Trimer Boosting Module, Related to Figures 2-4. Impact of FP and trimer boosts on neutralization activity was evaluated by comparing BG505 Δ611 titers (ID_{50}) before and after (**A**) FP8-7-6-KLH boost and (**B**) two rounds of BG505 DS SOSIP boosting. Neutralization responses were further broken down by comparing BG505 Δ611 titers before and after boosting with each immunogen: FP8-KLH (**C**), FP7-KLH (**D**), FP6-KLH (**E**), BG505 DS SOSIP (**F**), and second round of BG505 DS-SOSIP (**G**). Responses are pooled for 32 NHPs and divided into NHP studies NHP-1, NHP-2 and NHP-3, in that order from left to right. P values were calculated with 2-tailed non-parametric Wilcoxon matched pairs signed rank test, *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001 .



Study	Group	Post FP8 to pre FP8-7-6	Post FP7 to post FP8	Post FP8-7-6 to post FP7	Post 1st BG505 trimer to Post FP8-7-6	Post 2nd BG505 trimer to Post 1st BG505 trimer
NHP-1	degly3	16	4	8	12	8
	degly4	16	4	8	12	8
NHP-2	1	4	18	4	10	10
	2-1	4	18	4	10	10
	2-2	4	14	4	10	10
NHP-3	FP prime	16	12	12	12	8
	Cocktail prime	16	12	12	12	8

Figure S5. Impact of Variations in Immunogens and Time Intervals on BG505 Δ611 Neutralization Titers (ID_{50}), Related to Figures 2 and 4. Immunizations with statistically significant changes to BG505 Δ611 titers (ID_{50}) were observed for groups (**A**) NHP-1 degly3; (**B**) NHP-1 degly4; (**C**) NHP-3 FP prime; (**D**) NHP-3 Cocktail prime. P values were calculated with 2-tailed non-parametric Wilcoxon matched pairs signed rank test. Note that the minimum p value for a sample size of 5 is 0.0625; since all samples show an increase between the first and second values, all are statistically equivalent and equal with p = 0.0625. (**E**) Summary of time intervals in weeks for study groups. Impact of immunization time interval on neutralization activity was evaluated by examining the ratio of BG505 Δ611 titers (ID_{50}) from (**F**) post FP8 to pre FP8-7-6, (**G**) post FP7 to post FP8, (**H**) post FP8-7-6 to post FP7, (**I**) post 1st BG505 trimer to post FP8-7-6, and (**J**) post 2nd BG505 trimer to post 1st BG505 trimer. P values were calculated with 2-tailed, non-parametric Mann-Whitney test with the exception of the lower two panels in (**K**) and (**L**) which were calculated with non-parametric Kruskal-Willis test, *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001 .

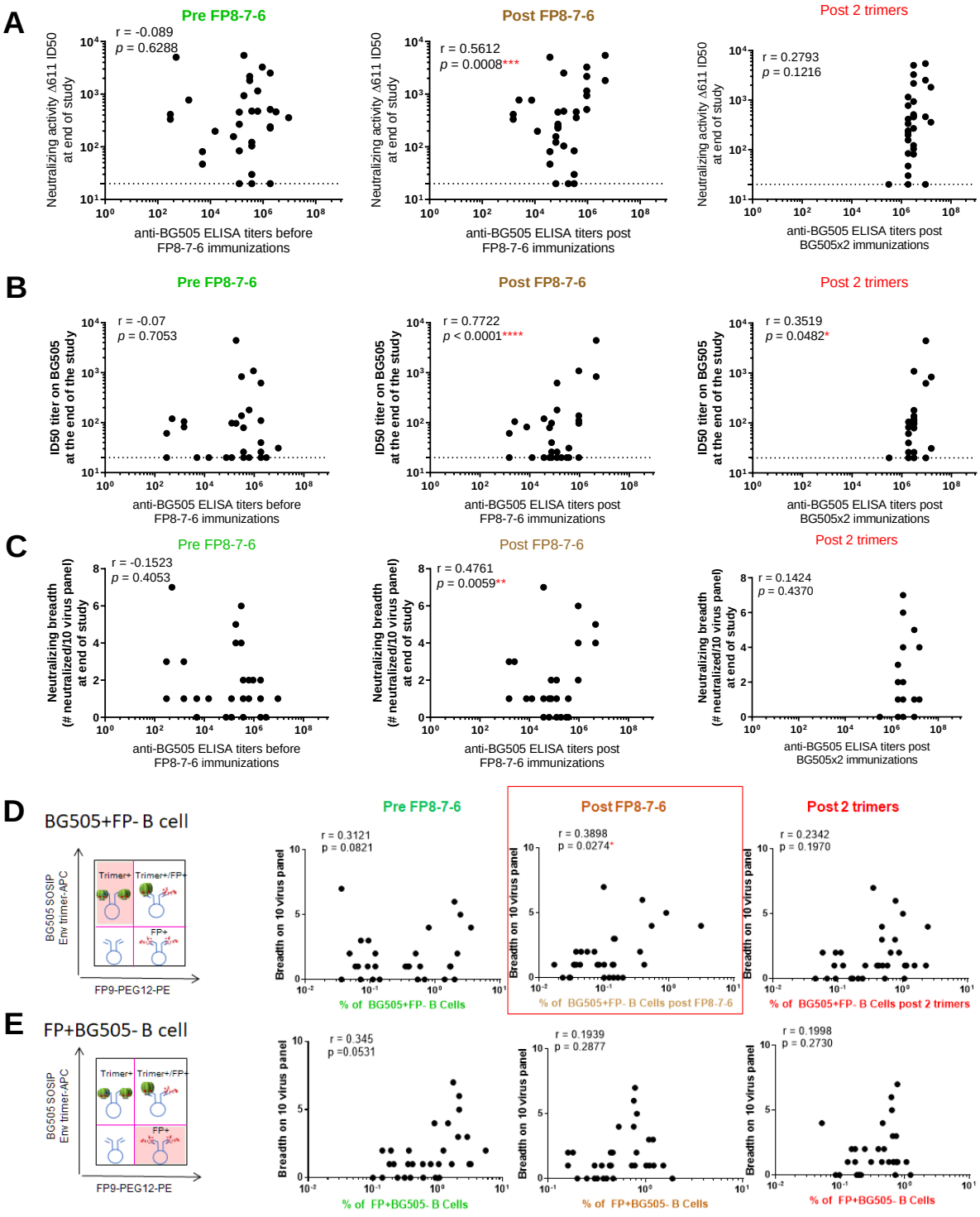


Figure S6. Correlation of Anti-BG505 ELISA Titer or Single-Positive B Cell Frequency with Vaccine Outcome, Related to Figures 2-6. Correlation of anti-BG505 ELISA endpoint titers at pre-FP8-7-6, post FP8-7-6 and post 2 trimer boost with neutralizing activity against BG505 Δ611 (**A**), BG505 (**B**) or 10 virus panel (**C**) at the end of the study. Correlation of single-positive B cell frequency, BG505+/FP9- (**D**) and FP9+/BG505- (**E**), at pre-FP8-7-6, post FP8-7-6 and post 2 trimer boost with neutralizing breadth at the end of the study. r and p values were calculated with 2-tailed Pearson coefficiency analysis, *: p<0.05, **: p <0.01, ***: p <0.001, ****: p <0.0001 .

Table S1. ELISA Endpoint Titers of NHP Plasma Binding to FP8 and BG505 DS-SOSIP Trimer, Related to Figures 2-4

(A) Plasma binding to FP8 and BG505 DS-SOSIP trimer in NHP-1 study detected with ELISA endpoint titers.

Week post immunization	FP8 ELISA endpoint titers										BG505 DS-SOSIP ELISA endpoint titers									
	Group 1					Group 2					Group 1					Group 2				
	PP13A0										PP13A0									
	A14V144	A13V009	8E	A12V086	A3V015	A14V092	A13V024	13N024	A11V069	A2P014	A14V144	A13V009	8E	A12V086	A3V015	A14V092	A13V024	13N024	A11V069	A2P014
0	300	100	100	100	100	100	100	300	100	100	500	1500	300	300	2500	500	300	1500	300	300
2	300	100	300	100	100	100	500	500	1500	500	7500	2500	12500	1500	7500	2500	2500	7500	12500	7500
6	1500	100	12500	300	7500	2500	7500	937500	37500	37500	4687500	4687500	1562500	312500	4687500	937500	1562500	1562500	937500	937500
10	300	100	2500	300	1500	1500	1500	312500	7500	7500	1562500	937500	312500	62500	312500	187500	1562500	937500	187500	187500
14	300	100	500	300	300	500	500	62500	2500	1500	375000	125000	375000	15000	3000	75000	375000	125000	75000	75000
18	300	500	1500	1500	500	7500	7500	62500	7500	1500	1875000	1875000	1875000	3125000	3125000	3125000	3125000	1875000	1875000	3125000
30	100	100	1500	500	500	500	500	12500	37500	1500	1875000	3125000	3125000	625000	625000	1875000	3125000	625000	625000	375000
34	937500	312500	312500	312500	937500	937500	312500	1562500	312500	187500	125000	625000	625000	375000	375000	625000	625000	375000	625000	375000
38	7500	7500	7500	1500	37500	187500	187500	937500	187500	37500	25000	5000	625000	125000	375000	375000	375000	125000	75000	75000
42	2500	300	7500	500	300	12500	37500	187500	62500	7500	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
46	12500	37500	12500	2500	37500	12500	37500	62500	12500	12500	125000	375000	375000	75000	75000	125000	125000	75000	75000	125000
54	2500	7500	7500	500	12500	1500	37500	37500	12500	12500	25000	375000	375000	75000	75000	375000	125000	75000	75000	75000
58	7500	62500	187500	500	12500	7500	187500	187500	37500	187500	3125000	15625000	9375000	1875000	1875000	3125000	3125000	3125000	3125000	9375000
66	12500	12500	62500	500	12500	7500	187500	937500	312500	37500	9375000	15625000	9375000	1875000	1875000	3125000	3125000	3125000	3125000	3125000

(B) Plasma binding to FP8 and BG505 DS-SOSIP trimer in NHP-2 study detected with ELISA endpoint titers.

Week post immunization	FP8 ELISA endpoint titers												BG505 DS-SOSIP ELISA endpoint titers											
	Group 1						Group 2-1			Group 2-2			Group 1						Group 2-1			Group 2-2		
	05N008	13N002	DF1B	DFXK	05D214	08N027	06N006	DF2F	08N012	A7V040	DFTI	05D275	05N008	13N002	DF1B	DFXK	05D214	08N027	06N006	DF2F	08N012	A7V040	DFTI	05D275
0	300	300	100	100	100	300	300	300	100	100	100	100	2500	1500	300	1500	1500	7500	2500	500	1500	500	500	7500
2	300	1500	100	100	100	1500	1500	1500	1500	100	100	100	12500	1500	7500	12500	2500	187500	7500	37500	7500	300	12500	2500
6	100	100	100	100	100	100	2500	12500	12500	100	100	100	9375000	1875000	1875000	9375000	1875000	1875000	3000	15000	15000	1875000	1875000	1875000
10	12500	62500	312500	937500	937500	312500	312500	937500	937500	100	500	300	1875000	375000	6250000	1875000	125000	375000	5000	15000	5000	375000	625000	125000
14	62500	37500	62500	62500	12500	12500	12500	187500	62500	100	1500	500	125000	75000	75000	375000	25000	125000	3000	15000	5000	125000	375000	75000
18	7500	12500	37500	12500	7500	12500	62500	62500	37500	37500	62500	37500	46875000	46875000	46875000	46875000	46875000	46875000	937500	937500	937500	62500	62500	7500
28	500	1500	2500	1500	500	1500	12500	7500	7500	1500	37500	500	46875000	15625000	15625000	15625000	312500	187500	37500	62500	62500	62500	62500	12500
32	2500	7500	7500	7500	7500	12500	37500	12500	37500	7500	37500	1500	312500	937500	312500	937500	187500	187500	62500	37500	37500	62500	37500	37500
36	12500	62500	62500	62500	62500	62500	312500	62500	187500	62500	62500	187500	187500	312500	937500	937500	312500	312500	37500	12500	37500	62500	62500	62500
44	1500	1500	7500	7500	7500	37500	62500	12500	7500	2500	12500	7500	75000	75000	15000	25000	75000	375000	15000	15000	15000	375000	75000	15000
46	2500	2500	62500	12500	12500	62500	62500	62500	12500	2500	12500	12500	3125000	625000	3125000	3125000	625000	1875000	1875000	3125000	625000	3125000	9375000	1875000
52	2500	12500	62500	37500	37500	187500	187500	62500	37500	12500	12500	37500	625000	625000	625000	3125000	625000	625000	125000	625000	625000	3125000	625000	125000
56	7500	12500	62500	62500	37500	62500	187500	37500	12500	12500	12500	62500	1875000	1875000	1875000	3125000	1875000	3125000	3125000	1875000	1875000	9375000	3125000	1875000

(C) Plasma binding to FP8 and BG505 DS-SOSIP trimer in NHP-3 study detected with ELISA endpoint titers.

Week post immunization	FP8 ELISA endpoint titers										BG505 DS-SOSIP ELISA endpoint titers									
	FP Prime					Cocktail Prime					FP Prime					Cocktail Prime				
	DFTX	DF9L	DFIXA	DFIV	HLP	DFL7	DFTN	DFTG	DFWB	04L	DFTX	DF9L	DFIXA	DFIV	HLP	DFL7	DFTN	DFTG	DFWB	04L
0	100	300	300	100	100	100	100	100	100	100	300	300	500	300	300	300	500	500	7500	500
2	62500	37500	62500	62500	187500	37500	12500	37500	187500	62500	500	500	1500	500	300	300	300	500	2500	500
6	312500	187500	312500	312500	4687500	187500	62500	312500	312500	187500	1500	300	500	300	1500	187500	187500	312500	937500	312500
10	187500	62500	187500	187500	187500	187500	37500	187500	187500	37500	500	300	1500	500	500	37500	12500	62500	62500	37500
14	37500	37500	62500	62500	37500	12500	12500	37500	12500	1500	500	300	1500	300	1500	37500	7500	12500	37500	12500
18	37500	37500	62500	62500	12500	12500	7500	37500	37500	1500	500	300	1500	500	1500	12500	2500	7500	12500	12500
22	4687500	312500	937500	937500	312500	625000	75000	375000	625000	375000	2500	1500	1500	2500	1500	1562500	937500	937500	1562500	937500
26	937500	187500	187500	187500	62500	312500	62500	187500	187500	62500	7500	1500	12500	1500	7500	937500	187500	312500	937500	312500
30	312500	62500	62500	62500	37500	187500	12500	187500	187500	37500	7500	1500	7500	1500	2500	937500	187500	187500	312500	187500
34	937500	312500	937500	187500	312500	187500	62500	312500	187500	37500	18750	1250	93750	750	6250	937500	187500	187500	312500	312500
42	1562500	187500	312500	62500	187500	312500	37500	187500	187500	62500	7500	1500	7500	500	2500	187500	62500	62500	62500	187500
46	1562500	937500	937500	937500	937500	937500	187500	4687500	312500	312500	7500	1500	37500	1500	2500	4687500	937500	937500	937500	4687500
50	937500	937500	937500	187500	187500	187500	62500	937500	312500	62500	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
54	312500	62500	312500	62500	62500	312500	37500	312500	312500	62500	5000	1000	15000	1000	1000	1875000	125000	375000	375000	1875000
58	1562500	1562500	1562500	312500	937500	937500	62500	312500	1562500	187500	75000	75000	75000	75000	75000	3125000	1875000	3125000	3125000	9375000
62	937500	312500	937500	312500	187500	312500	62500	312500	312500	187500	75000	75000	125000	75000	75000	9375000	1875000	1875000	1875000	9375000
66	3125000	3125000	312500	375000	625000	1875000	125000	1875000	375000	125000	1875000	1875000	3125000	1875000	1875000	9375000	3125000	3125000	3125000	15625000

N.T.: not tested.

100-1000 1000-1000 10000-10000 100000-1000000 >1000000

Table S2. Immunization with CH505 FP-Deglycan Variants (NHP-1 Study) Elicited Immune Responses Against the FP Site but Failed To Neutralize Wild-Type BG505 or CH505 Viruses, Related to Figure 2. Neutralizing activity against BG505 and its mutants, or CH505 virus post two (week6) or three (week18) trimer immunizations was shown as ID_{50}.

		ID_{50}	Virus	BG505.W6M.C2.SG3		BG505.W6M.C2.N88Q.SG3		BG505.W6M.C2.N611Q.SG3		BG505.W6M.C2.N88Q.N611Q.SG3		CH0505s.T/F.SG3	
Group	Vaccine		Animal ID	wk 6	wk 18	wk 6	wk 18	wk 6	wk 18	wk 6	wk 18	wk 6	wk 18
1	Week 0, 4, 16: CH505 DS-SOSIP degly3		A14V144	<20	<20	<20	<20	<20	930	<20	3,091	<20	<20
			A13V009	<20	<20	<20	<20	<20	<20	<20	75	<20	<20
			PP13A08E	<20	<20	<20	<20	<20	222	<20	727	<20	70
			A12V086	<20	<20	<20	<20	<20	145	<20	329	<20	<20
			A3V015	<20	<20	<20	<20	<20	101	<20	152	<20	58
2	Week 0, 4, 16: CH505 DS-SOSIP degly4		A14V092	<20	<20	<20	<20	<20	133	<20	446	<20	<20
			A13V024	<20	<20	<20	<20	<20	188	<20	715	<20	<20
			13N024	<20	<20	<20	<20	<20	144	<20	594	<20	25
			A11V069	<20	<20	<20	<20	<20	140	<20	244	<20	<20
			A2P014	<20	<20	<20	<20	<20	184	<20	676	<20	<20

Table S3. Priming with CD4bs-Deglycan Immunogen Elicited CD4bs-Directed Neutralizing Activity Against CH505 CD4bs-degly4 Virus, but Generated Minimum Neutralizing Activity Against CH505 and Other Wild-Type Viruses (NHP-2 Study), Related to Figure 3. Serum neutralizing activity (ID_{50}) against BG505 and its glycan mutants, CH505 and its glycan mutants, and three other clade C viruses are shown at weeks 10 and 18.

ID50	Clade A										Clade C									
Virus	BG505.W6M.C2.SG3		BG505.W6M.C2.CD4bs degly4.SG3		BG505.W6M.C2.N88Q.SG3		BG505.W6M.C2.N611Q.SG3		BG505.W6M.C2.N88Q.N611Q.SG3		BI369.9A.SG3		96ZM651.02.SG3		CH0505s.T/F.SG3		CH505 CD4bs degly4		MW965.26.SG3	
Week	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18	wk 10	wk 18
05N008	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	9,515	65,254	<20	93
13N002	<20	<20	<20	27	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	1,480	7,727	<20	50
DF1B	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	1,190	29,885	31	32
DFXK	<20	<20	<20	71	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	653	18,038	<20	<20
05D214	<20	<20	<20	52	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	32	8,300	<20	<20
08N027	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	71	6,783	<20	<20
06N006	<20	<20	<20	<20	<20	<20	<20	<20	<20	117	<20	<20	<20	<20	<20	<20	45	16,236	<20	20
DF2F	<20	<20	<20	<20	<20	<20	<20	<20	<20	227	<20	<20	<20	<20	<20	<20	<20	3,250	<20	127
08N012	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	33,548	<20	74
A7V040	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	896	569	<20	<20
DFTI	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	189	407	<20	<20
05D275	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	177	326	<20	<20

Table S4. Neutralization Titers of FP-Primed and Cocktail-Primed Groups Against BG505 Δ611, BG505 WT and 10-Strain Virus Panel at Various Time Points (NHP-3), Related to Figures 4 and 5.

(A) Neutralization titers against BG505 Δ611 and BG505 WT at various time points. NHPs with week 66 plasma tested in FP competition assay are highlighted in red letters.

ID_{50} on BG505.Δ611	Animal ID.	wk 10	wk 22	wk 26	wk 34	wk 46	wk 54	wk 58	wk 62	wk 66
FP prime	DFTX	<20	26	30	48	57	20	63	31	770
	DF9L	<20	<20	<20	<20	<20	<20	107	35	334
	DFIXA	<20	175	114	2,605	732	116	851	925	5,037
	DFIV	<20	<20	<20	<20	<20	<20	44	<20	414
	HLP	<20	<20	<20	34	23	<20	70	48	775
Cocktail prime	DFL7	<20	866	1,633	1,371	3,493	931	4,120	2,808	5,459
	DFTN	<20	704	393	1,132	841	91	586	403	936
	DFTG	<20	332	335	914	1,067	429	914	1,805	2,164
	DFWB	<20	721	509	879	1,222	212	1,170	758	3,267
	04L	<20	<20	<20	43	474	43	354	353	1,816

ID_{50} on BG505	Animal ID.	wk 10	wk 22	wk 26	wk 34	wk 46	wk 54	wk 58	wk 62	wk 66
FP prime	DFTX	<20	<20	<20	<20	<20	<20	<20	<20	82
	DF9L	<20	<20	<20	<20	<20	<20	<20	<20	<20
	DFIXA	<20	<20	<20	<20	<20	<20	<20	25	120
	DFIV	<20	<20	<20	<20	<20	<20	<20	<20	61
	HLP	<20	<20	<20	<20	<20	<20	<20	<20	105
Cocktail prime	DFL7	<20	125	104	550	1,208	366	1,889	1,530	4,428
	DFTN	<20	<20	25	53	36	<20	35	35	97
	DFTG	<20	30	35	75	72	<20	80	59	138
	DFWB	<20	463	254	347	606	89	787	305	1,085
	04L	<20	<20	<20	56	247	22	334	180	835

(B) ID_{50} on 10 strain panel at various time points after 1st immunization.

	Clade A					CLADE AE				
Virus	BG505.W6M.C2.SG3					CNE56				
glycan	missing 241					Missing 241 and 611				
Week	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66
DFTX	<20	<20	<20	<20	82	<20	<20	<20	<20	<20
DF9L	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFIXA	<20	<20	<20	<20	120	<20	<20	<20	<20	<20
DFIV	<20	<20	<20	<20	61	<20	<20	<20	<20	<20
HLP	<20	<20	<20	<20	105	<20	<20	<20	<20	<20
DFL7	125	550	1,208	1,889	4,428	<20	<20	<20	<20	<20
DFTN	<20	53	36	35	97	<20	<20	<20	<20	<20
DFTG	30	75	72	80	138	<20	<20	<20	<20	<20
DFWB	463	347	606	787	1,085	<20	<20	<20	<20	<20
04L	<20	56	247	334	835	<20	<20	<20	<20	<20

	CLADE BC					CLADE C				
Virus	CNE19					25710-2.43				
glycan	Missing 448					Missing 241				
Week	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66
DFTX	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DF9L	<20	<20	<20	<20	<20	<20	<20	<20	<20	25
DFIXA	<20	53	<20	31	115	<20	81	66	39	280
DFIV	<20	<20	<20	<20	<20	<20	<20	<20	<20	41
HLP	<20	<20	<20	<20	<20	<20	<20	<20	<20	116
DFL7	<20	<20	<20	<20	49	<20	<20	68	59	88
DFTN	49	138	127	66	153	33	173	121	67	127
DFTG	<20	<20	26	39	92	<20	36	111	89	127
DFWB	<20	<20	<20	<20	<20	<20	<20	<20	21	43
04L	<20	<20	<20	<20	<20	<20	<20	<20	<20	34

	CLADE A					CLADE A				
Virus	KER2008.12.SG3					Q23.17.SG3				
glycan	Complete					Complete				
Week	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66
DFTX	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DF9L	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFIXA	<20	<20	<20	20	39	<20	<20	<20	<20	<20
DFIV	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
HLP	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFL7	<20	<20	<20	<20	<20	<20	<20	<20	45	59
DFTN	<20	28	22	<20	<20	<20	<20	<20	<20	<20
DFTG	<20	<20	<20	<20	36	<20	<20	<20	<20	23
DFWB	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
04L	<20	<20	<20	<20	74	<20	<20	<20	<20	<20

	CLADE B					CLADE B				
Virus	3988.25.SG3					BL01.DG.SG3				
glycan	Complete					Complete				
Week	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66
DFTX	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DF9L	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFIXA	<20	<20	<20	<20	83	<20	<20	<20	<20	36
DFIV	<20	<20	<20	<20	21	<20	<20	<20	<20	<20
HLP	<20	<20	<20	<20	27	<20	<20	<20	<20	<20
DFL7	88	<20	<20	<20	45	<20	<20	<20	<20	<20
DFTN	49	<20	<20	<20	30	<20	<20	<20	<20	<20
DFTG	<20	<20	<20	<20	61	<20	<20	<20	<20	<20
DFWB	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
04L	<20	<20	<20	<20	48	<20	<20	<20	<20	<20

	CLADE C					CLADE C				
Virus	0077.V1.C16.SG3					286.36.SG3				
glycan	Missing 241					Complete				
Week	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66	Wk 22	Wk 34	Wk 46	Wk 58	Wk 66
DFTX	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DF9L	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFIXA	<20	<20	<20	<20	30	<20	<20	<20	<20	<20
DFIV	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
HLP	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFL7	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFTN	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFTG	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
DFWB	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
04L	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20

Table S5. Competition Assay Reveals Heterologous Viral Strains to be Neutralized by FP-Directed Plasma Responses, Related Figures 4 and 5. Animal plasma at end of study or control Abs were tested at a single-point dilution that resulted in >25% neutralization of the corresponding HIV-1 viruses (KER2008.12, Q23.17, 3988.25, BL01.DG, CNE19, 25710-2.43, or BG505.W6M.C2). Plasma and Ab samples were pre-incubated with FP (sequence AVGIGAVFL) or a non-cognate FLAG peptide or control media before mixing with virus. %Neut is the average of 3-5 independent assays with duplicates in each assay. Reduction of neut was calculated as (1-%Neut[peptide]/%Neut[media]), i.e. a value of 100% indicates complete inhibition of neutralization, whereas a value of 0% indicates no effect by peptide. Values in Reduction of neut of >30% are highlighted in red.

				Media	FP		FLAG Peptide	
Group	NHP #ID	Virus	Plasma dilution or antibody concentration	%Neut	%Neut	Reduction of Neut	%Neut	Reduction of Neut
FP prime	DFTX	BG505.W6M.C2	20	75.9	64.9	15%	75.5	1%
	DF9L	25710-2.43	20	36.4	12.2	66%	38.1	-5%
	DFIXA	KER2008.12	20	43.2	0.4	99%	42.4	2%
		3988.25	20	27.9	0.0	100%	35.9	-29%
		BL01.DG	20	30.6	0.0	100%	32.9	-8%
		CNE19	20	71.4	0.0	100%	71.5	0%
		25710-2.43	20	82.1	5.8	93%	80.9	2%
		BG505.W6M.C2	20	52.4	30.7	41%	52.6	0%
	DFIV	3988.25	20	43.7	13.9	68%	45.0	-3%
		25710-2.43	20	40.4	0.5	99%	38.4	5%
		BG505.W6M.C2	20	69.0	52.3	24%	70.5	-2%
	HLP	3988.25	20	35.7	0.0	100%	34.3	4%
		25710-2.43	20	72.4	63.5	12%	71.3	1%
		BG505.W6M.C2	20	41.4	12.1	71%	44.3	-7%
Cocktail prime	DFL7	Q23.17	20	72.6	58.6	19%	72.1	1%
		3988.25	20	32.7	13.3	59%	40.3	-23%
		CNE19	20	55.8	21.5	62%	53.4	4%
		25710-2.43	20	71.5	21.3	70%	72.0	-1%
		BG505.W6M.C2	20	96.6	94.5	2%	96.5	0%
	DFTN	CNE19	20	86.9	0.0	100%	87.7	-1%
		25710-2.43	20	76.1	9.3	88%	77.4	-2%
		BG505.W6M.C2	20	64.5	47.5	26%	66.4	-3%
	DFTG	KER2008.12	20	39.5	20.3	49%	42.8	-8%
		Q23.17	20	43.5	14.6	66%	41.7	4%
		3988.25	20	43.3	29.4	32%	47.8	-10%
		CNE19	20	54.7	12.5	77%	57.8	-6%
		25710-2.43	20	63.6	17.8	72%	64.5	-1%
		BG505.W6M.C2	20	74.4	66.2	11%	75.1	-1%
	DFWB	25710-2.43	20	40.4	18.5	54%	44.1	-9%
		BG505.W6M.C2	20	98.9	97.8	1%	98.5	0%
	04L	KER2008.12	20	44.3	21.7	51%	48.0	-8%
		25710-2.43	20	36.9	0.1	100%	40.5	-10%
		BG505.W6M.C2	20	99.8	99.5	0%	99.5	0%
FP antibody	VRC34	KER2008.12	0.250 ug/mL	79.4	3.0	96%	80.0	-1%
		Q23.17	1.2 ug/mL	82.2	19.1	77%	82.3	0%
		3988.25	0.3 ug/mL	77.4	14.8	81%	77.0	0%
		BL01.DG	0.3 ug/mL	79.6	13.0	84%	80.4	-1%
		CNE19	50 ug/mL	70.0	44.0	37%	69.8	0%
		25710-2.43	50 ug/mL	53.0	16.1	70%	51.9	2%
		BG505.W6M.C2	1.2 ug/mL	82.4	23.7	71%	81.0	2%
CD4bs antibody	VRC01	KER2008.12	2.0 ug/mL	69.0	63.3	8%	69.4	0%
		Q23.17	50 ug/mL	100.0	100.0	0%	100.0	0%
		3988.25	1.5 ug/mL	78.9	77.7	2%	78.2	1%
		BL01.DG	50 ug/mL	0.0	0.1	-	0.0	-
		CNE19	0.9 ug/mL	71.4	71.0	1%	71.8	0%
		25710-2.43	2 ug/mL	78.4	78.4	0%	78.3	0%
		BG505.W6M.C2	0.21 ug/mL	78.2	77.4	1%	77.7	1%