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Supplemental Information

Co-immunization of DNA and Protein in the Same Anatomical Sites Induces Superior Protective Immune Responses against SHIV Challenge

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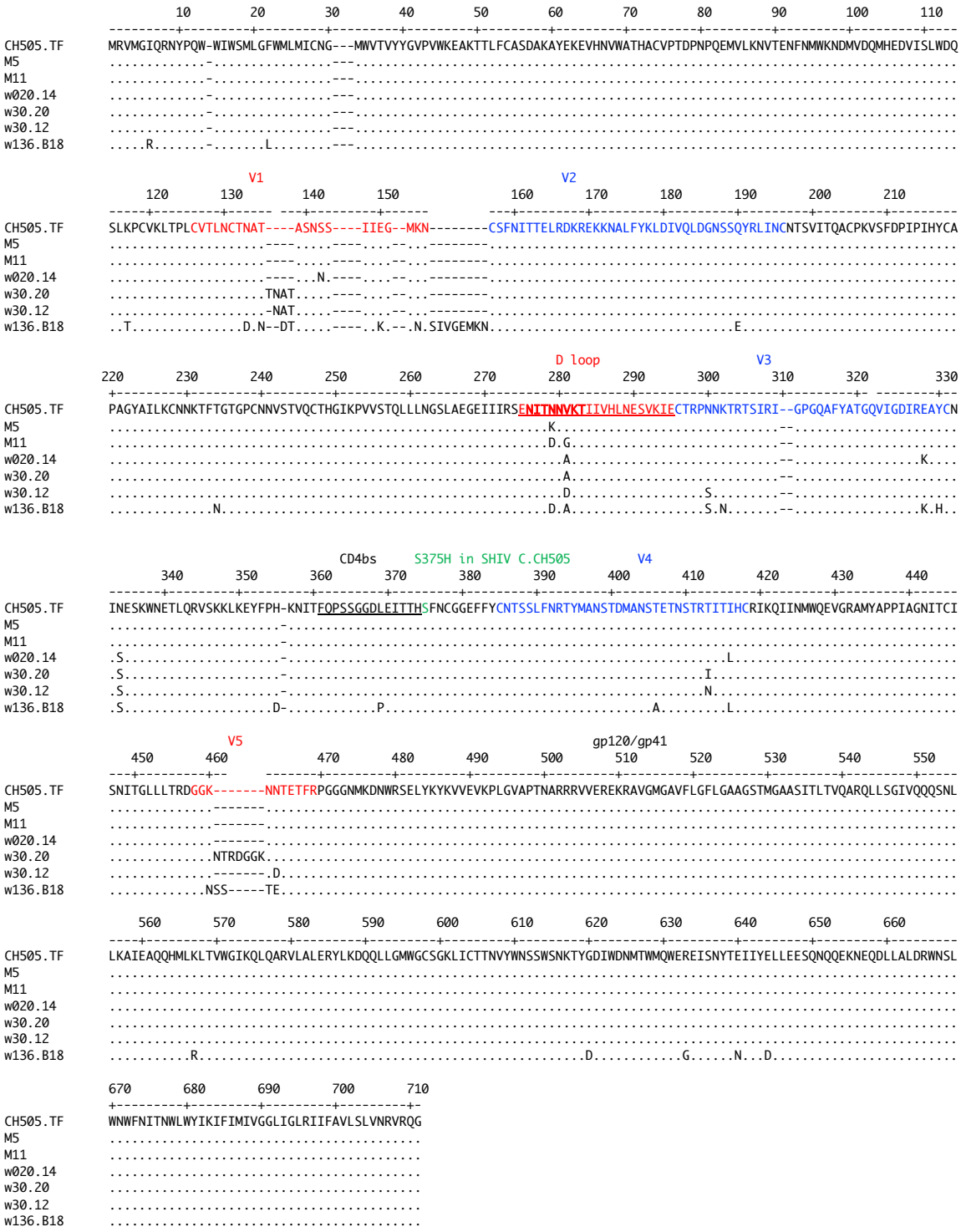
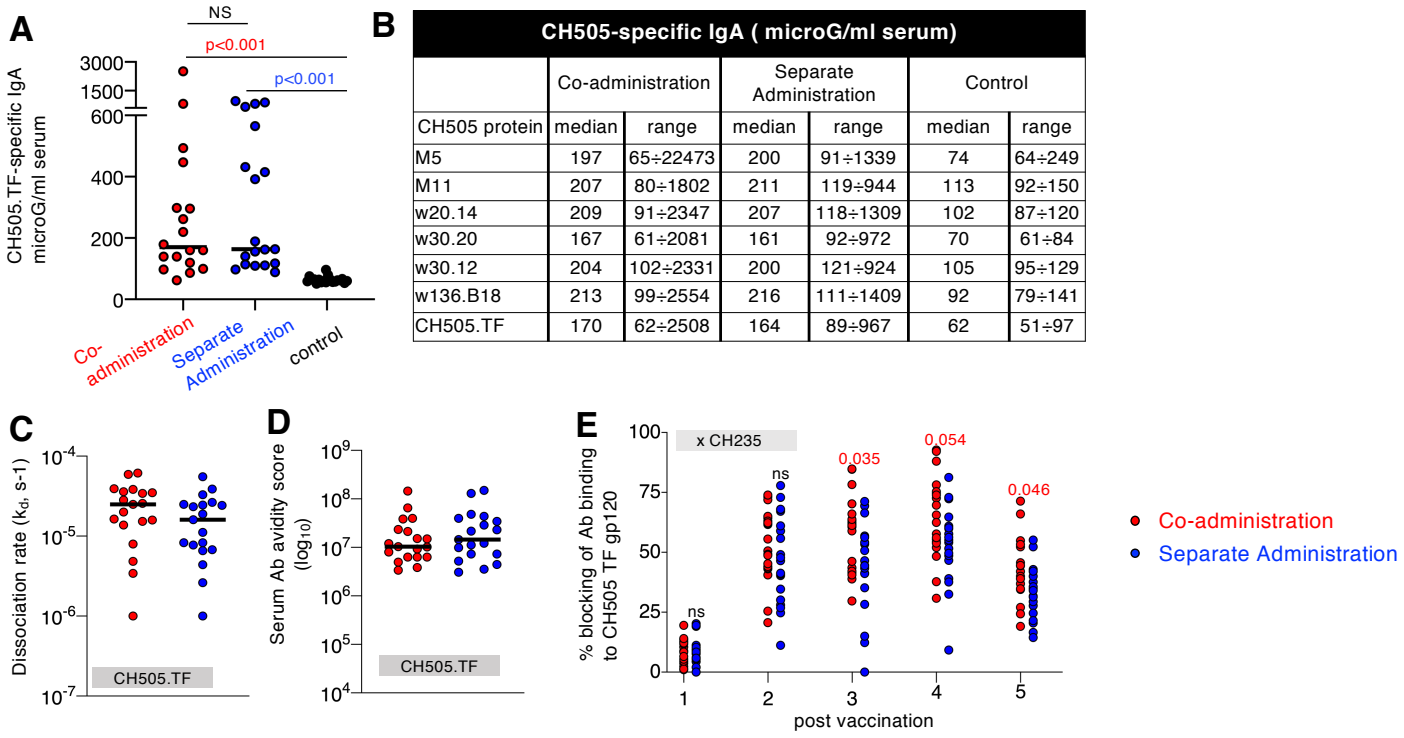


Figure S1, Related to Figure 1: Alignment of CH505 Env Variants.

Alignment of the CH505.TF gp145 and the sequentially isolated CH505 gp145 proteins used as immunogens in this study. The key differences among the immunogens in V1, loop D and V5 (indicated in red lettering) were associated with development of CD4bs NAb (Bonsignori et al., 2016; Liao et al., 2013). Based on inferred unmutated common ancestor (UCA) analysis, a CH505 Env that differs from the TF Env by a single amino acid N279K change in the D loop suggested that this variant (called M5) initiated the CH235 lineage, whereas two amino acid changes in the D loop (N279D and V281G) suggested that this variant (called M11) initiated the CH103 lineage (Bonsignori et al., 2016; Fera et al., 2014; Liao et al., 2013). Co-evolution of two the CH103 and CH235B cell lineages has been associated with the development and maturation of the CD4 binding site (CD4bs) in patient CH505 (Bonsignori et al., 2017; Bonsignori et al., 2016; Gao et al., 2014; LaBranche et al., 2019; Saunders et al., 2017). The amino acid (AA) numbering is according to HXB2. The amino acid S375H (indicated in green) indicates change in SHIV.CH505 Env.



CH505-specific IgA (microG/ml serum)						
	Co-administration		Separate Administration		Control	
CH505 protein	median	range	median	range	median	range
M5	197	65÷22473	200	91÷1339	74	64÷249
M11	207	80÷1802	211	119÷944	113	92÷150
w20.14	209	91÷2347	207	118÷1309	102	87÷120
w30.20	167	61÷2081	161	92÷972	70	61÷84
w30.12	204	102÷2331	200	121÷924	105	95÷129
w136.B18	213	99÷2554	216	111÷1409	92	79÷141
CH505.TF	170	62÷2508	164	89÷967	62	51÷97

Figure S2, Related to Figure 2: Characterization of CH505 Env Vaccine-induced Ab

(A, B) CH505 Env-specific IgA in serum. IgA antibodies recognizing different Env proteins were measured in serum (1:250 fold dilution) and plotted as antigen-specific IgA (microG per ml), using a multiplex assay as described (Brown et al., 2017; Brown et al., 2015) Samples analyzed include Co-administration vaccine group (N=18); Separate Administration vaccine group (N=19); and controls (N=20). There was a significant difference comparing pre samples to 2 weeks post 6th vaccination for all RMs except the control group. (A) The panel shows the comparison of the CH505.TF-specific IgA measured in both vaccine groups and the controls. (B) Summary of the analysis of the median and range of CH505-specific IgA. **(C, D)** Avidity of vaccine-induced serum CH505-Env specific IgG. The binding magnitude (in response units [RU]) and dissociation rate constant (K_d) **(C)** were measured and the avidity score **(D)** was calculated by determining the RU/K_d value with a slower dissociation rate resulting in higher avidity score. **(E)** CH505 vaccine-induced Env-specific antibodies recognize CD4 binding site on gp120 using CD4bs competition assays. Plasma Ab recognized the CD4bs in CH505 TF gp120 Env and this interaction was blocked by CD4bs-specific monoclonal antibodies (CH106, CH235) or soluble CD4 (sCD4). The assay showing CH235 competition performed over the course of the vaccination period is shown. The % inhibition is plotted. p values (Mann Whitney test) are given (color of p value indicates vaccine group with higher values).

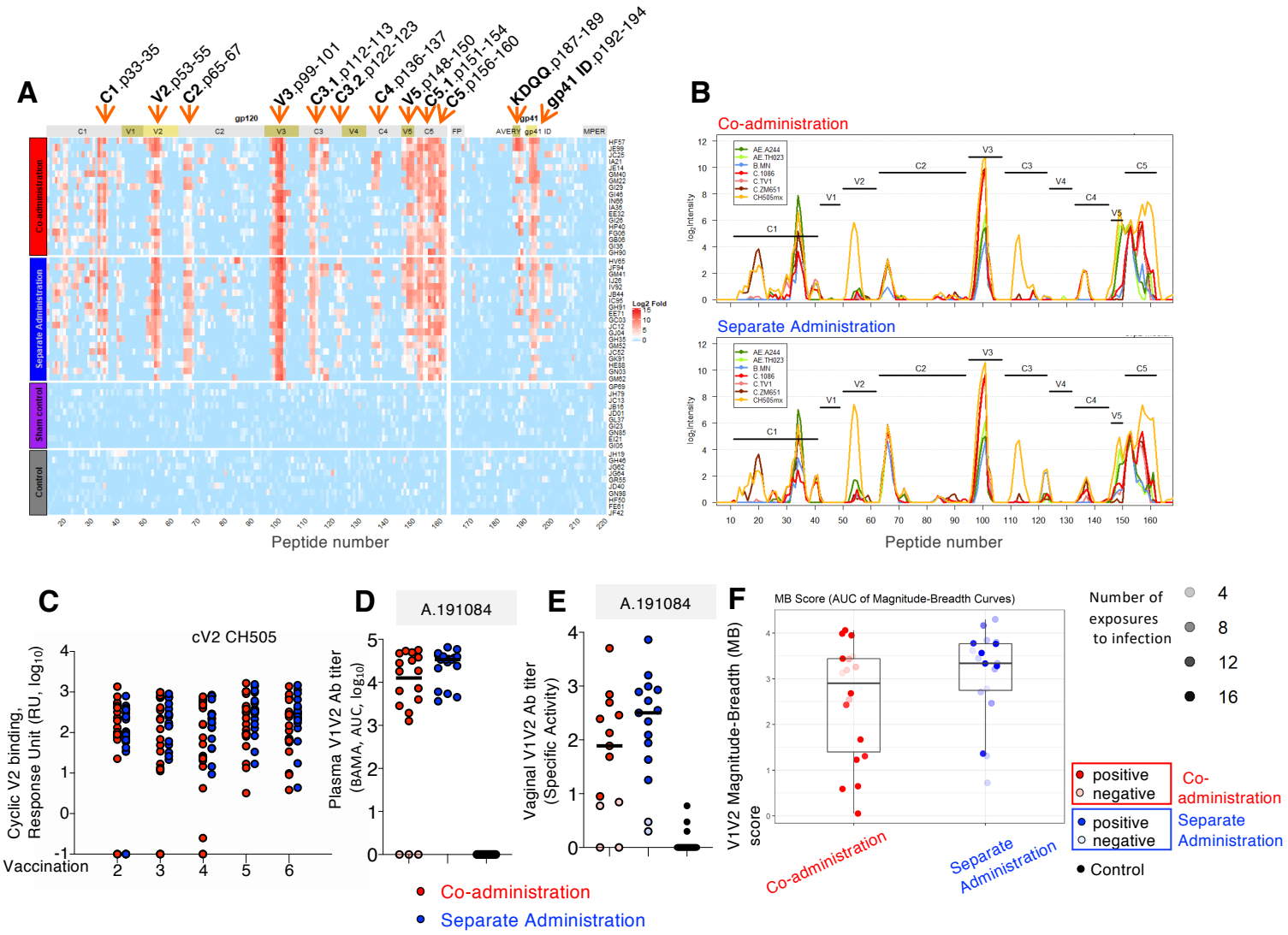


Figure S3, Related to Figure 2. Env Ab Responses to Linear Peptide, Cyclic V2 and V1V2 Scaffold.

(A, B) Plasma samples collected at 2 weeks post the 5th vaccination were tested for their ability to recognize overlapping linear peptides from CH505 TF gp145. **(A)** The binding to individual peptides is shown as heatmap. Responses targeting mainly V3, typical for HIV Env responses in macaques, in accord with our previous report (Shen et al., 2015), with a strong recognition of the V3 peptides (peptides 96-99 containing the V3 tip GPG AA 312-314). Most of the animals showed binding to the V2 region (peptides 53-55), containing the α4β7 integrin binding site (LDI/V AA179-181). Linear peptide mapping of plasma Ab showed overall comparable binding specificities to CH505 peptides in both vaccine groups, except higher binding to the C2 and C3.2 regions in the animals from the Separate Administration group. **(B)** The binding to peptides of different virus strains shows cross-clade responses including to peptides from different clades and CH505mx (max of all CH505 strains) with unique recognition of CH505 V2 and C3 regions. Values plotted are group medians. Each line represents a different sequence. The array data were processed using pepStat (Imholte and Gottardo, 2016). Binding magnitude: Log_2-transformed, baseline (pre-bleed binding) subtracted signal intensity. **(C)** Env-specific Ab recognize CH505 V2 in plasma. Starting with samples collected after the 2nd vaccination, the vaccine-induced Ab recognized cyclic V2 peptide (cV2) from different clades (CH505, C.1086 and AE.92TH23) measured overtime by SPR using a 1:50 dilution of the plasma. The example of CH505 is shown. Low binding to cV2 MN was found (data not shown). (D-F) Antibody to a panel scaffolded gp70-V1V2 Env proteins (strains A, AE, B, C) were measured in **(D)** plasma and **(E)** vaginal secretion and the example of A.191084 is shown. Values from plasma are given as AUC (log_{10}), values from mucosal samples are given as SA (Specific Activity). Mann-Whitney tests showed no significant differences between the groups. **(F)** Magnitude-Breadth (MB) scores for individual animals (He and Fong, 2019), of the two groups are shown (AUC of MB curves). The number of exposures to infection is indicated by transparency of the red or blue coloring.

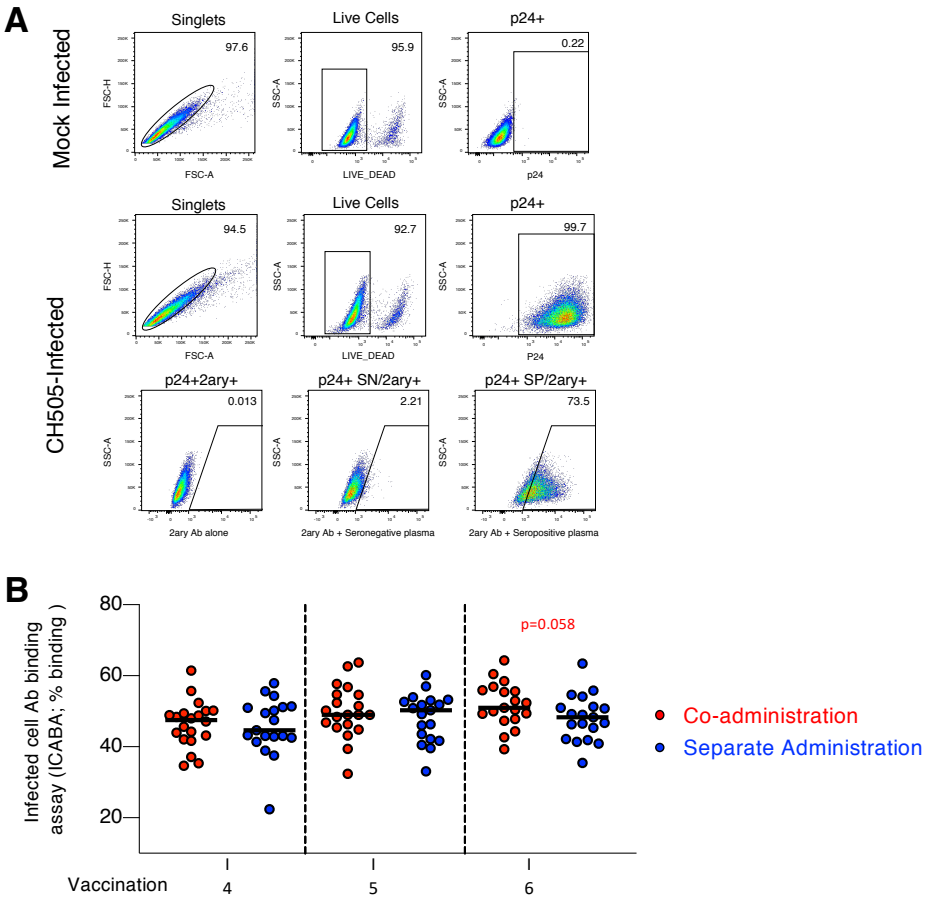


Figure S4, Related to Figure 3: Infected Cell Antibody Binding Assay (ICABA) Detects Binding of the Vaccine-induced Env-specific Antibodies to the Surface of HIV.CH505 Infected Cells.

(A) The dot plots of the top and middle rows illustrate the gating strategy to identify the singlets, live and infected p24Gag+ cells (left to right) among the mock and CH505-infected cells, respectively. The bottom row illustrates the percentage of infected $p24^{Gag+}$ cells detected in presence of the secondary (2ary) Ab alone (left panel) or in presence of the seronegative (center) and seropositive (right) plasma. **(B)** Binding of anti-Env Ab exposed on the surface of HIV.CH505 infected cells was measured by ICABA showing % binding in the samples from the two vaccine groups. Data from the same analysis are shown as MFI in Figure 3D.

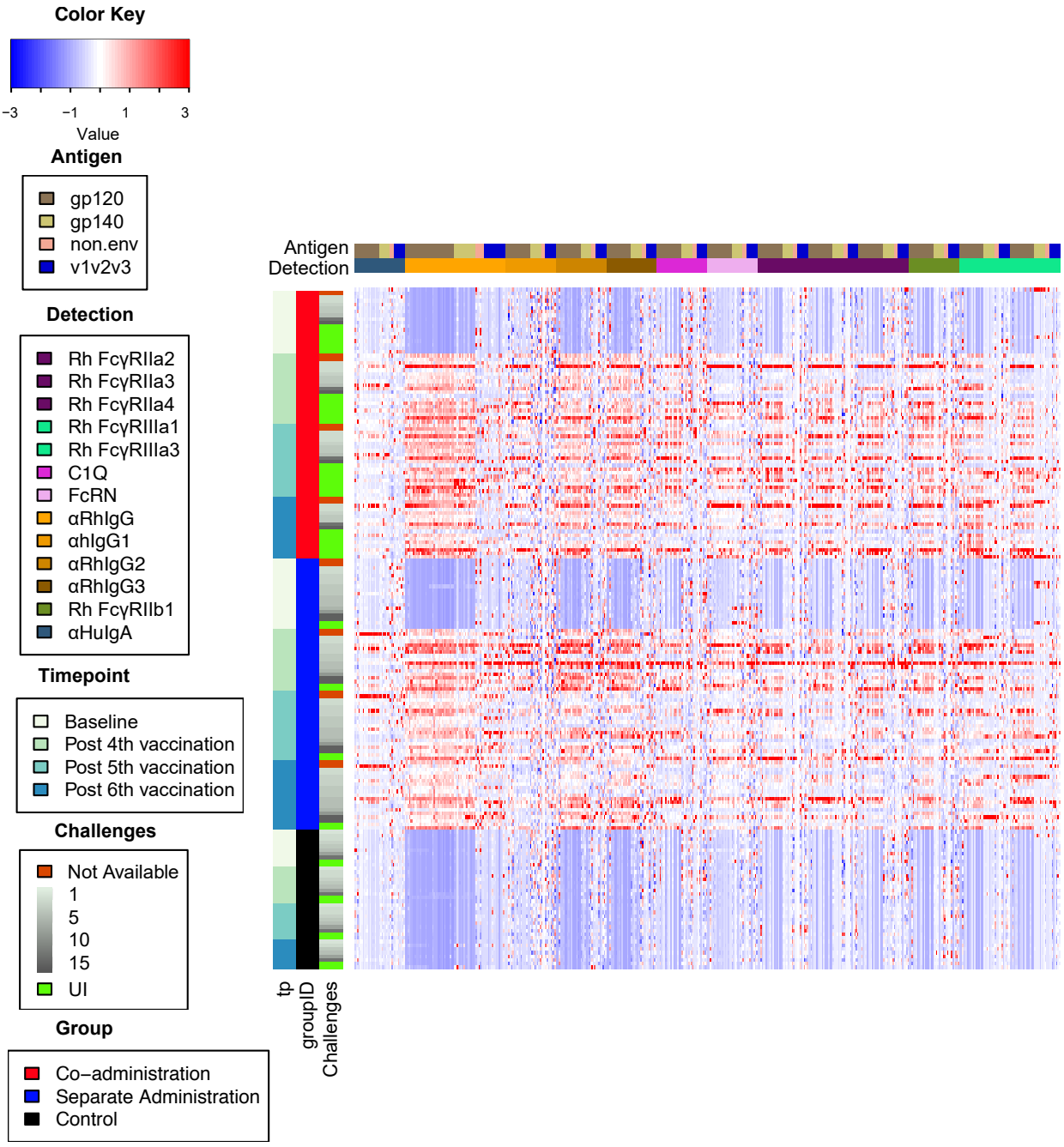
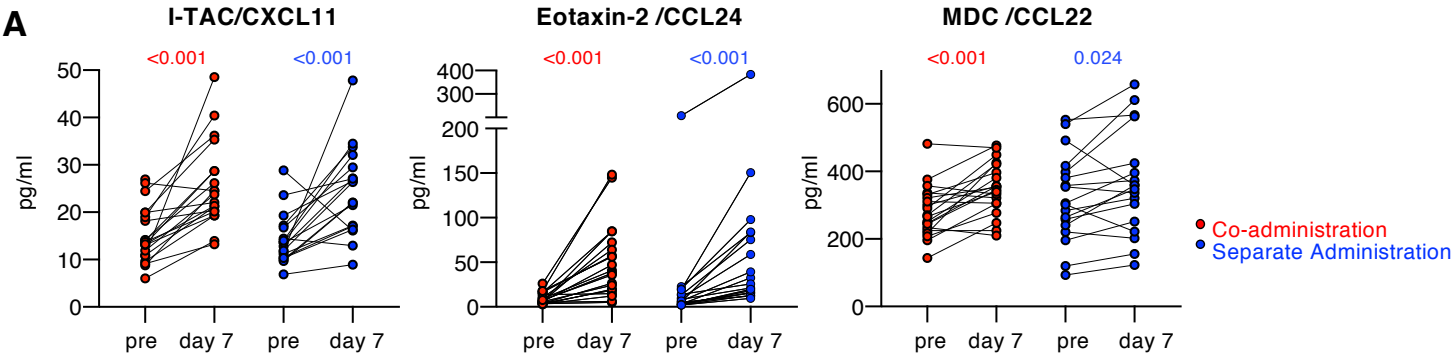


Figure S5, Related to Figures 4-6: Heatmap of Fc Array Humoral Response Features.
Animals (rows) are ordered by group, then timepoint, and finally in ascending order of time-to-infection. Each antibody response feature (columns) is comprised of an Fv (antigen) specificity and Fc (detection) reagent, such as rhesus (Rh) Fc receptors, and are sorted first by detection reagent, then by antigen. Antibody features are centered, scaled and truncated at +/- 3 standard deviations from the column mean, with high responses indicated in red and low responses in blue.

A



B

Analyte	Co-administration (N=20) (pg/ml plasma)			Separate site administration (N=18) (pg/ml plasma)			Difference between vaccine groups	
	pre (median)	day 7 (median)	Wilcoxon T test, p value	pre (median)	day 7 (median)	Wilcoxon T test, p value	pre t test, p value	day 7 t test, p value
I-TAC /CXCL11	13.5	22.9	**<.001**	13.2	24.2	<.001	NS	NS
IP-10 /CXCL10	327.7	423.5	**0.009**	324.1	370.2	**0.014**	NS	NS
IL-1Ra	45.5	89.9	**0.005**	48.6	70.9	**0.03**	NS	NS
Eotaxin /CCL11	184.9	249.7	**0.027**	192.5	229.5	**0.081**	NS	NS
Eotaxin-2 /CCL24	6.7	39.2	**<.001**	6.0	29.2	**<.001**	NS	NS
FLT3L	11.0	17.9	**<.001**	11.3	13.81	**<.001**	NS	NS
MIP-1α /CCL3	11.9	14.5	**0.022**	10.3	14.0	**0.009**	NS	NS
MIP-1β /CCL4	71.1	78.6	**0.033**	72.8	93.4	**0.005**	NS	NS
MDC /CCL22	268.4	349.3	**<.001**	304.2	353.1	**0.024**	NS	NS

C

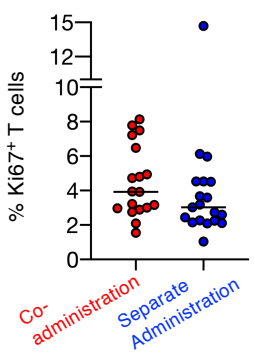


Figure S6, Related to Figures 1-3. Measurements of Factors Associated with Immune Activation.
(A, B) Plasma concentration of chemokines associated with immune activation. Comparison of pre-vaccination samples (pre) and plasma samples collected at day 7 after the 1st vaccination shows significant increases in the chemokines shown, using the NHP Meso Scale Discovery multiplex assay. **(A)** Change in I-TAC/CXCL11 levels. **(B)** Summary of comparison of median levels of these analytes at pre-vaccination and day 7 and p values (Wilcoxon T test). Mann-Whitney tests did not show differences between the groups. **(C)** Comparison of Ki67 levels in total T cells from PBMC collected 2 weeks after the 6th vaccination shows similar low levels of T cell proliferation.

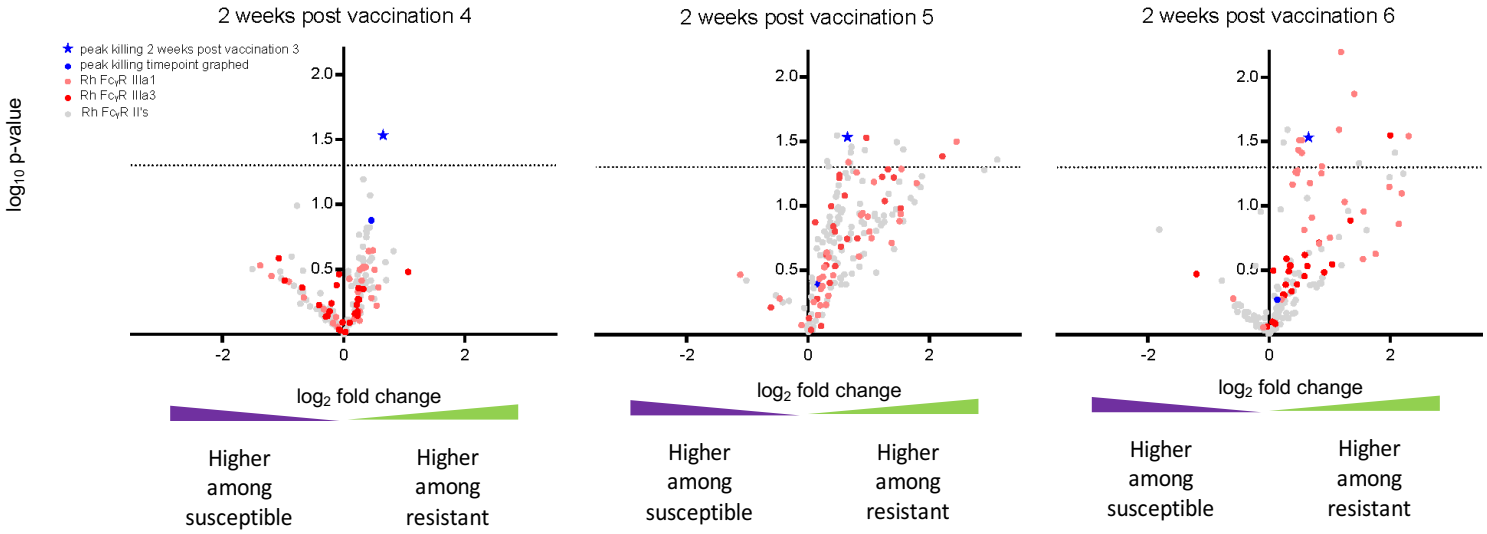


Figure S7, Related to Figure 6: Variation between Resistant and Susceptible Animals among Select Features Over Time.

Volcano plots for ADCC and antigen-specific antibody interactions with FcγRII, and FcγRIII between resistant and susceptible animals at 2 weeks post 4th, 5th and 6th vaccination, depicting $\log_2$ fold change in medians between groups and unadjusted $\log_{10}$ p values. Contemporaneous peak ADCC and that observed at post 3rd vaccination are indicated with blue dots and stars, respectively. Each red and grey dot represents Fc receptor binding for a different individual antigen specificity for a given FcγR allotype.

Table S1, Related to Figure 1: Description of 60 Female Macaques Enrolled in the Vaccine Study

vaccination	Animal ID	Age at study start (yr)[1]	selected MHC class I haplotypes[2]			necropsy during study	SHIV CH505 acquisition
			A*01	B*08	B*17		
Co-administration (N=20)[3]	EE32	13	P	N	N		>15
	EI94	12	N	N	N		>15
	FG06	11	N	N	N	yes[4]	N/A
	GB06	10	N	N	N		13
	GH90	9	N	N	N		>15
	GI26	9	**P**	N	N		3
	GI29	9	N	N	N		>15
	GI36	9	N	N	N		>15
	GI46	9	N	N	N		>15
	GM16	9	N	N	N		2
	GM22	9	N	N	P		>15
	GM40	9	N	N	N		5
	HF57	8	N	N	N		>15
	HP40	7	N	N	N		15
	IA21	7	N	N	N		2
	IA36	7	N	N	N		2
	IN66	6	N	N	N		>15
	JC25	5	N	N	N		4
	JE14	5	P	N	N	yes[4]	N/A
	JE99	5	N	N	N		4
Separate Administration (N=20)[5]	EE71	13	P	N	N		4
	FE35	11	N	N	N	yes[6]	N/A
	GC03	10	N	N	N		5
	GH35	9	N	N	N		15
	GH91	9	P	N	N	yes[4]	N/A
	GJ04	9	N	N	N		3
	GK91	9	N	N	N		2
	GM41	9	N	N	N		5
	GM52	9	N	N	N	yes[4]	N/A
	GM62	9	P	N	N		6
	GN03	9	N	N	N		4
	HE88	8	N	N	N		4
	HV65	7	N	N	P		2
	IC95	7	N	N	N		9
	IJ26	6	N	N	N		>15
	IV92	5	P	N	N		>15
	JB44	5	N	N	N		5
	JC12	5	N	N	N		3
	JC52	5	N	N	N		3
	JF94	5	N	N	N		15
sham control (N=10)	EI21	12	N	N	N	yes[7]	14
	GI05	9	N	N	N		1
	GI23	9	N	N	N		9
	GL37	9	P	N	N		5
	GN85	9	N	N	N		5
	GP69	9	N	N	N		9
	JB16	5	P	N	N		3
	JC13	5	N	N	N		1
	JD01	5	N	N	N		4
	JH79	5	N	N	N		4
treatment-naïve control (N=10)	FE61	11	N	N	N		13
	GH46	9	P	N	N		3
	GN98	9	N	N	N		2
	GR55	9	N	N	N		8
	HF50	8	N	N	N		1
	JD40	5	N	N	N		4
	JF42	5	N	N	N		5
	JG62	5	N	N	N		>15
	JG64	5	N	N	N		2
	JH19	5	N	N	P		>15

[1]age range 5-13 years; median 9 years
[2]per group: 3-4 mamuA*01; no mamuB*08; 1 mamuB*17
[3]challenge study was performed with 18 animals
[4]elected necropsy at 2 weeks post 6th vaccination
[5]challenge study was performed with 17 animals
[6]health-related necropsy after the 2nd vaccination
[7]sacrificed 9 weeks post-infection due to development of AIDS-related disease including weight loss, low albumin levels, opportunistic viral infection, and secondary amyloidosis

Table S2, Related to Figures 2-6: Listing of Env-specific Humoral Immune Response Assays

ASSAY		Time points analyzed during the study (2 weeks post vaccination)				
		2	3	4	5	6
Ab, plasma	Env	X	x	x	x	x
	Env on cell-surface ICABA			x	x	x
	V1V2					x
	cV2	X	x	x	x	x
	linear peptide				x	
	CD4 binding site blocking assay	X	x	x	x	
	Avidity				x	x
Ab, mucosal	Env, V1V2					x
NAb	Env		x	x	x	x
Ab, function targeting CH505 gp120:	ADCC (antibody-dependent cellular cytotoxicity)		x	x	x	x
	ADCD (antibody-dependent complement deposition)					x
	ADCP (antibody-dependent cellular phagocytosis					x
	ADNP (antibody-dependent neutrophil phagocytosis					x
	ADNKA (antibody dependent NK activation)					x
Ab, IgG glycan structures	galactosylated, sialylated, fucosylated, bisecting					x
Ab, Fc Array detection: Ab subclass	aRh IgG, aRh IgG1, aRh IgG2, aRh IgG3			x	x	x
Ab isotype	aHu IgA			x	x	x
Rh Fc receptors	FcγRIIa1, FcγRIIa2, FcγRIIa3, FcγIIb1, FcγRIIIa1, FcγRIIIa3, Hu FcRn			x	x	x
complement	C1q			x	x	x

Table S3, Related to Figures 2-6: Listing of Assays and Targets

ASSAYS	TARGETS	clade
binding Ab	CH505.TF.gp120	C
	CH0505.TF	C
	CH0505.TF.d371	C
	CH505TF_D7gp120	C
	CH505.M5.D8	C
	CH0505.M11.D8	C
	CH505.w020.14D8	C
	CH505.w030.12D8	C
	CH505.w030.20.D8	C
	CH505.w136.18D8	C
	RSC3	B
	RSC3.d371	B
	YU2	B
	YU2 D368R	B
	HxBc2 Core Ds109/428/GnTI-	B
	CH0505_CON D7 gp120	C
	CH505w100 B6 6R SOSIP	C
	BG505 gp140 SOSIP T332N	A
	Con.6.gp120.B	B
	Con.S.gp140.CFI	M
	p27gag	NA
competition	CH106xCH0505TF_D7 gp120.avi/293F	C
	CH106xCH0505TF_D7 gp120.avi/293F	C
	CH235xCH0505TF_D7 gp120.avi/293F	C
	sCD4xCH0505TF_D7 gp120.avi/293F	C
	sCD4xB.63521_gp120delta11/293F	B
	2G12 x M.CON-S gp140CFI/trimer	M
Avidty	1086C_gp120	C
	1086 gp140C	C
	B63521_gp120delta11	B
	CH505_TF D7gp120	C
	CH505w53 e16 D8gp120	C
	CH505 M5D8gp120	C
	CH505TFchim.6R.SOSIP.664_avi-Bio	C
	CH505TF.6R.SOSIP.664.v4.1_avi.2_Bio	C
gp70 V1V2	gp70.191084_B7.V1V2	A
	gp70.C2101.c01_V1V2	AE
	gp70.CM244.ec1.V1V2	AE
	gp70.GNE5	AE
	gp70.62357.14.V1V2	B
	gp70.700010058.V1V2	B
	gp70_B.CaseA_V1_V2	B
	gp70.RHPA4259.7.V1V2	B
	gp70.TT31P.2F10.2792.V1V2	B
	gp70.BJOX002000.03.2.V1V2	BC
	gp70.001428.2.42.V1V2	C
	gp70.7060101641.V1V2	C
	gp70.96ZM651.02.V1V2	C
	gp70.BF1266_431a_V1V2	C
	gp70.CAP210.2.00.E8.V1V2	C
	gp70.Ce1086_B2.V1V2	C
	gp70.TV1.21.V1V2	C
	gp70.1394C9G1.V1V2	C
	gp70.CAP45.2.00.G3.V1V2	C
	gp70.Ce1176.V1V2	C
	gp70.Ce704010042_2ES.V1V2	C
	gp70.ConC.V1V2	C
	gp70.Du156.12.V1V2	C
tags	AE.A244.V1V2.Tags.293F	AE
	AE.A244.V2.tags.293F	AE
	C.1086.V2.tags.293F	C
	C.1086C_V1_V2.Tags	C
cyclic V2	CH505	C
	1086C	C
	92TH23	AE
	MN	B
ICABA	CH505	C
Ab function :		
ADNKA	CH505 M5 gp120	C
ADCC	CH505 M5 gp120	C
ADCD	CH505 M5 gp120	C
ADCP	CH505 M5 gp120	C
ADNP	CH505 M5 gp120	C

ASSAYS	TARGETS	clade
linear peptide	A.con	A
	AE.con	AE
	AG.con	AG
	B.con	B
	C.con	C
	D.con	D
	M.con	M
	B.MN	B
	AE.A244	AE
	AE.92TH023	AE
	C.1086	C
	C.TV1	C
	C.ZM651	C
	CH505TF.gp160	C
	CH505TFD8gp120	C
	CH505.M5.gp160	C
	CH505.M5D8gp120	C
	CH505.M6 gp160	C
	CH505.M11.gp160	C
	CH505.M11D8gp120	C
	CH505w020.14.gp160	C
	CH505w020.14D8gp120	C
	CH505w0.30.12.gp160	C
	CH505.w30.12D8gp120	C
	CH505w0.30.20.gp160	C
	CH505w030.20D8gp120	C
	CH505w030.21 gp160	C
	CH505w30.25gp145	C
	CH505w030.25D8gp120	C
	CH505w030.28 gp160	C
	CH505w053.16 gp160	C
	CH505w053.16D8gp120	C
	CH505w53.25gp145	C
	CH505w053.25D8gp120	C
	CH505w53.29gp145	C
	CH505w053.29D8gp120	C
	CH505w053.31 gp160	C
	CH505w078.15 gp160	C
	CH505w078.33 gp160	C
	CH505w078.33D8gp120	C
	CH505w100.B6 gp160	C
	CH505w100.B6D8gp120	C
	CH505w0.136.B18.gp160	C
	CH505w136.B18D8gp120	C
	CH505TFchim.6R.SOSIP.664	C
	CH505TFchim.DS.6R.SOSIP.664	C
	CH505TFchim.6R.SOSIP.664v4.1	C
	CH505TFchim.6R.SOSIP.664v4.2	C
	CH505M5chim.6R.SOSIP.664v4.1	C
	CH505M5chim.6R.SOSIP.664v4.2	C
	CH505M11chim.6R.SOSIP.664v4.1	C
	CH505M11chim.6R.SOSIP.664v4.2	C
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	CH505w53.16chim.6R.SOSIP.664	C
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	CH505w78.33.6R.SOSIP.664	C
	CH505w78.33.DS.6R.SOSIP.664	C
	CH505w100.B6.6R.SOSIP.664	C
	CH505w100.B6.DS.6R.SOSIP.664	C
	CH505w100.B6chim.6R.SOSIP.664v4.1	C
	CH505w100.B6chim.6R.SOSIP.664v4.2	C
	CH505w136.B6chim.6R.SOSIP.664v4.1	C
	CH505w136.B6chim.6R.SOSIP.664v4.2	C

ASSAYS	TARGETS	clade
Nab	CH505.TF	C
	CH505.w4.3	C
	CH505.w53.e16	C
	CH505.w78.33	C
	CH505.w100.B6	C
	SHIV CH505.375Y	C
	CH505.gly4/293T	C
	CH505.gly3.276/293T	C
	CH505.G458Y.4/GnTI-	C
	426c.DM/GnTI-	C
	426c.TM/GnTI-	C
	SHIV SF162P3	B
bAb in vaginal secretion:		
gp120	CH505.M11D8gp120/293F/Mon	C
	CH505.M5D8gp120/293F/Mon	C
	CH505.w30.12D8gp120/293F/Mon	C
	CH505w020.14D8gp120/293F Mon	C
	CH505w030.20D8gp120/293F/Mon	C
	CH505w136.B18D8gp120/Mon	C
	HxB2 new 8b core 6x His	B
	HxB2 new 8b core 1420R	B
	YU2 gp120 old core	B
	YU2 gp120 old core D368R	B
	CH505TF_D7gp120.avi/293F	C
	CH505 TF delta371Ile gp120	C
gp140	C.con_env03gp140CF_avi	C
	Con S gp140 CFI	C
gp70-V1V2	gp70_B.CaseA_V1_V2	A
	gp70-BF1266_431a_V1V2	C
	gp70-Ce1086_B2 V1V2	C
	gp70-191084_B7 V1V2	A
	gp70-TV1.21 V1V2	C
	gp70-1394C9G1 V1V2	C
	gp70-620345.c01 V1V2	AE
	gp70-CNE5_V1V2	AE
gp70-V3	B.MN V3 gp70	B
Fc Array assay:		
gp140	9004S gp140C.avi*	A
	C3347_11 gp140C.avi	AE
	CNE5 gp140C.avi/293F	AE
	QH0515.gp140C.avi	B
	B.6240 gp140C/293F	B
	gp140 SHIV SF162P3	B
gp120	Q769_D11 gp120.avi/293F	A
	X620345_D11 gp120.avi/293F	AE
	BJOX028_D11 gp120.avi/293F	AE
	B.6240 D11gp120.avi/293F*	B
	FLSC (CD4i)	B
	gp120 JRCSF	B
	gp120 SHIV SF162P3	B
	C.089_D11 gp120/293F	C
	CH0505_TF gp120/293F	C
	CH0505_TF D7gp120/293F/Mon	C
	CH505.M11 D8 gp120/293F	C
	CH505.M5D8gp120/293F	C
	CH505.w30.12D8gp120/293F	C
	CH505w020.14D8 gp120 293F	C
	CH505w030.20D8gp120/293F	C
	CH505w136.B18D8gp120/293F	C
	STG-WT	B
	STG/KLE	B
gp70	REJO4541.67 gp70-V1V2	B
	CaseA2 gp70 V1V2*	A
	62357_14 gp70-V1V2*	B
	7060101641A7 gp70-V1V2*	C
	Ce1086_B2 gp70-V1V2*	C
	Ce704010042_2E5 gp70-V1V2	C
	gp70 Scaffold (control) WT	
other	gp41 HxBc2	B
	RSC	B
	V3	
	Gag SIV mac239	
	IIIB p55 Gag; p24gag HxBc2	B

Table S4, Related to Figure 3: Neutralization Assays in TZM-bl Cells using Pseudotyped Viruses

post vaccination	vaccination group	Number of animals tested	Animal with sporadic response	ID50 in TZM-bl cells[1]											
				CH0505s (TF)	CH0505. w53.e16[2]	CH0505. w78.33[2]	CH0505. w100.B6[2]	SHIV CH0505. 375Y	SHIV SF162P3	CH0505. gly4 /293T[3]	CH0505. gly3.276 /293T[3]	426c.DM /GnTI[4]	426c.TM /GnTI[4]	CH0505. G458Y.4 /GnTI[5]	CH0505. gly3.276 /GnTI[6]
				Clade C	Clade C	Clade C	Clade C	Clade C	Clade B	Clade C	Clade C	Clade C	Clade C	Clade C	Clade C
				TF	Tier 2	Tier 2	Tier 2	Tier 2	Tier 2	Tier 1A	Tier 1A	Tier 2	Tier 2	Tier 1A	Tier 1A
3	Co-administration	20		<20	<20	<20	<20	<20	ND	ND	ND	ND	ND	ND	ND
	Separate Administration	19		<20	<20	<20	<20	<20	ND	ND	ND	ND	ND	ND	ND
4	Co-administration	20		<20	<20	<20	<20	<20	ND	ND	ND	ND	ND	ND	ND
	Separate Administration	19		<20	<20	<20	<20	<20	ND	ND	ND	ND	ND	ND	ND
5	Co-administration	20		<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	ND	ND
			GB06	<20	<20	<20	**24**	<20	<20	<20	<20	<20	<20	ND	ND
			JE99	<20	<20	<20	**23**	<20	<20	**21**	<20	<20	<20	ND	ND
	Separate Administration	19		<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	ND	ND
			HE88	<20	<20	<20	**36**	<20	<20	<20	<20	<20	<20	ND	ND
6	Co-administration	20		<20	ND	ND	ND	ND	ND	<20	<20	<20	<20	<20	<20
			FG06	<20	ND	ND	ND	ND	ND	<20	<20	<20	<20	<20	**20**
	Separate Administration	19		<20	ND	ND	ND	ND	ND	<20	<20	<20	<20	<20	<20

[1]Values are the serum dilution at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample)
[2]CH505 lineage Env CH505.w53.e16, CH505.w78.e33, CH505.w100.B6 are sensitive to CH103 lineage bNAb precursors
[3]CH505.gly3.276 and CH0505.gly4 are sensitive to CH103 and DH235 lineage bNAb precursors
[4]426c.DM and 426c.TM (Man5-enriched) are sensitive to VRC01-like CD4bs bNAbs and precursors
[5]CH0505.G458Y/GnTI-, a mutant of CH0505 that is neutralized by the DH235 fully reverted germline antibody, is sensitive to DH235 linage antibodies
[5]CH0505.gly3.276 glycan mutant grown in 293S/GnTI- cells is sensitive to V2-glycan bNAbs and CD4bs bNAbs

Table S5, Related to Figure 4: Cross-timepoint Model Accuracy for Classification of Vaccine Groups and Challenge.

Model: Timepoint 2 wks post vaccination	Input Data[1]: baseline	vaccination 1	vaccination 2	Vaccination 3	vaccination 4	vaccination 5	vaccination 6	avg 1st – 6th vaccination
Vaccine Group Classification								
1	74	80[2]	51	63	41	46	-[3]	56
2	43	51	89	82	69	79	-	74
3	54	57	89	97	66	66	42	75
4	46	-	-	-	74	72	-	73
5	40	54	74	57	74	91	-	70
6	41	-	-	-	51	64	73	58
								68
Challenge Outcome Classification – Co-Administration								
	baseline	vaccination 1	vaccination 2	vaccination 3	vaccination 4	vaccination 5	vaccination 6	avg 1st – 6th vaccination
3	-	-	-	78[2]	61	67	53	65
Randomly selected features[4]								
						Average across all models		49

[1]Model generalizability was assessed by defining the accuracy of classification when simplified final models for a given timepoint were applied to classify subjects based on data from other timepoints. For each timepoint, a simplified final model relying on the top two features and their coefficients from the global final model was used to predict class using data available at other timepoints.

[2]Performance of contemporaneous data using the simplified, two-feature models is on the diagonal, indicated in outlined boxes.

[3]Dashes indicate timepoints at which data for the relevant features were not available.

[4]In contrast, models learned from pairs of features selected at random yielded a mean classification accuracy of 49% across 100 repeats.