Title: Robust antibody and cellular responses induced by DNA-only vaccination for HIV

Authors:

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List of Supplementary Materials

Supplementary Materials

Supplemental Acknowledgments

HVTN 098 protocol team

Srilatha Edupuganti, Vickie Grimes, and Marcus Bolton, Emory University, Stephen De Rosa, Marnie Elizaga, Xuesong Yu, Yunda Huang, Eva Chung, Renee Rivers, Maija Anderson, Marianne Hansen, Huguette Redinger, Ramey Fair, Gail Broder, Adi Ferrara, and Erik Schwab, Fred Hutchinson Cancer Research Center, Amir Khan, Niranjan Y. Sardesai, Jessica Lee, and Mark Bagarazzi, Inovio, Inc., Edith Swann, Scharla Estep, Jeff Pulen, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Brian Green and Justin Wooley, HVTN Community Advisory Board members.

Supplemental Methods

The trial registration includes additional protocol details: ClinicalTrials.gov NCT02431767

HVTN 098 was a multicenter, randomized, placebo-controlled, double-blind trial that enrolled healthy, HIV–uninfected volunteers aged 18 to 55 years. The trial was conducted between August 2015 and December 2017. The primary objective was to evaluate the safety and tolerability (Edupuganti et al., submitted). The secondary objective was to characterize and rank the immunogenicity of 3 vaccine regimens: PENNVAX®-GP with and without *IL-12* DNA, given by intradermal injection with electroporation, and PENNVAX®-GP with *IL-12* given by intramuscular injection with electroporation.

Sample size calculation for safety: The goal of the safety evaluation for this study was to identify safety concerns associated with product administration. Sample size calculations for safety are expressed in terms of the ability to detect adverse events (AEs) requiring expedited reporting to DAIDS. The ability of the study to detect serious adverse events (SAEs) can be expressed by the true event rate above which at least one SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for the vaccine arm in Group 1 (n = 5), there is a 90% chance of observing at least 1 event if the true rate of such an event is 37% or more; and there is a 90% chance of observing no events if the true rate is 2% or less. Group 2 (n = 20), there is a 90% chance of observing at least 1 event if the true rate of such an event is 10.9% or more; and there is a 90% chance of observing no events if the true rate is 0.5% or less. For each vaccine arm in Groups 3 and 4 (n =30), there is a 90% chance of observing at least 1 event if the true rate of such an event is 7.4% or more; and there is a 90% chance of observing no events if the true rate is 0.3% or less.

Randomization: The randomization sequence was obtained by computer-generated random numbers. The randomization was done in blocks to ensure balance across arms. At each institution, the pharmacist with primary responsibility for dispensing study products was charged with maintaining security of the treatment assignments.

Blinding: Participants and site staff (except for site pharmacists) were blinded as to participant treatment arm assignments (e.g., vaccine or control) but not to group. Study product assignments were accessible to those HVTN clinical research site (CRS) pharmacists, DAIDS protocol pharmacists and contract monitors, and statistical and data management center (SDMC) staff who are required to know this information in order to ensure proper trial conduct. Any discussion of study product assignment between pharmacy staff and any other HVTN CRS staff was prohibited. The HVTN safety monitoring board (SMB) members also were unblinded to treatment assignment in order to conduct review of trial safety.

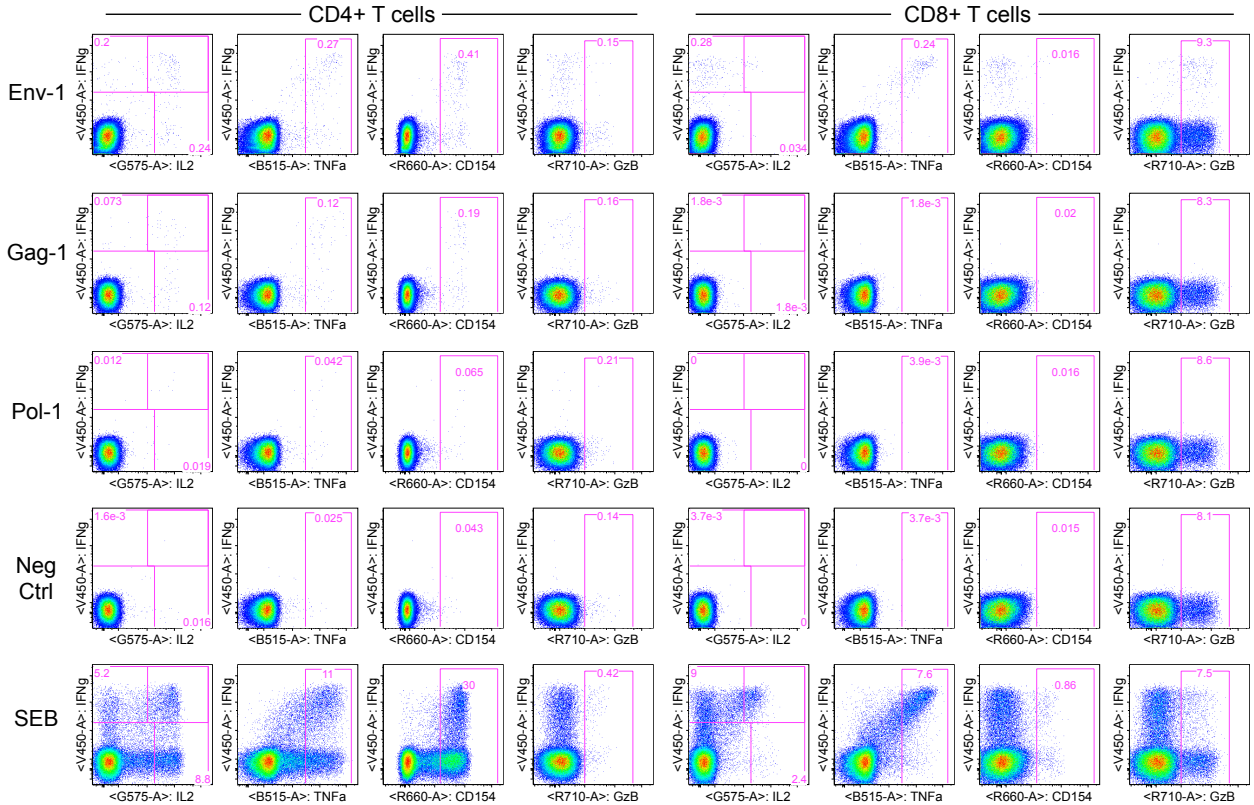

Fig. S1. Flow cytometry staining profiles for intracellular cytokine staining (ICS) for one vaccine recipient. Expression for five functional markers (IFN-γ, IL-2, TNF-α, CD154, and granzyme B) is shown for CD4+ and CD8+ T cells for stimulation with the first PTE_g peptide pool for Env, Gag, and Pol, for the negative control (DMSO, the peptide diluent) and for staphylococcus entertoxin B (SEB), the positive control). Numbers within the graphs show the percent of either CD4+ or CD8+ T cells for the cells within the region as gated.

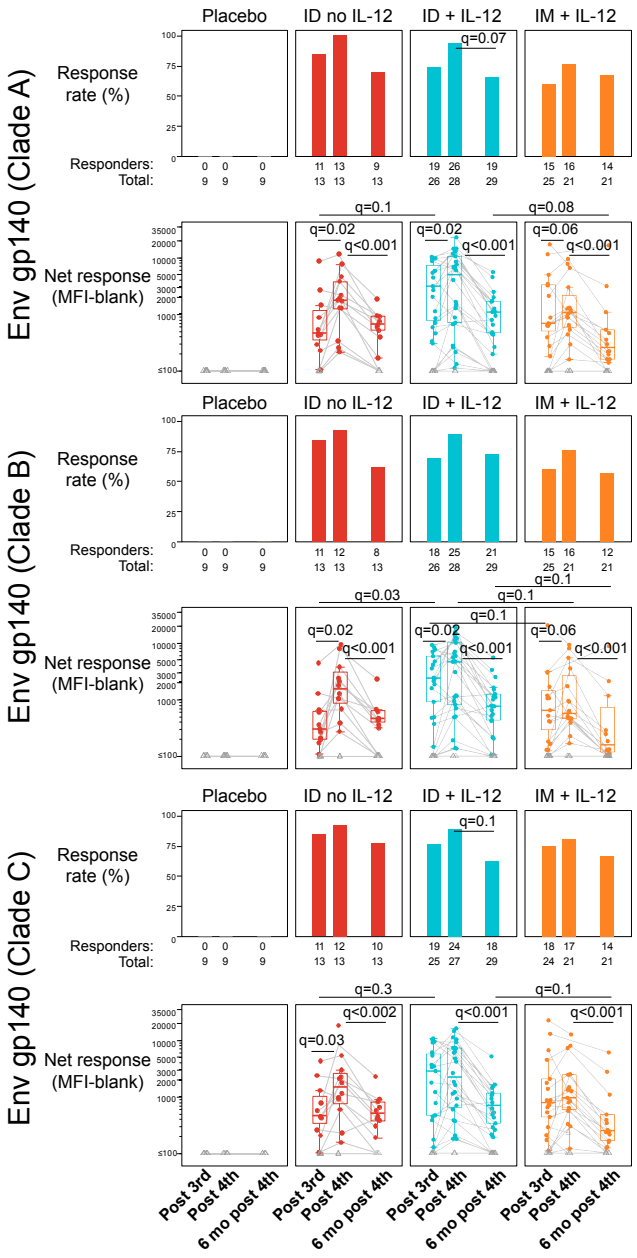

Fig. S2. IgG binding antibody responses as measured by binding antibody multiplex assay (BAMA) against consensus Env gp140 antigens for clades A, B and C. Assays were performed two weeks after the third and fourth vaccinations and six months after the fourth vaccination. Positive responses are shown in filled circles in color, negative responses are shown in open gray triangles. Box-plots represent the distribution for the positive responders only. Bar plots show response rates. Numbers below the bars indicate numbers of positive responders and total participants. Positive response rates were compared using the Fisher's

exact test for unpaired data (between treatment groups) and the McNemar test for paired data (between visits). Response magnitudes among positive responders were compared using the Wilcoxon rank sum test for unpaired data and the Wilcoxon signed rank test for paired data. All p values are two-sided. False-discovery rate adjusted q values were calculated to account for multiple antigens, multiple timepoints, or treatment groups.

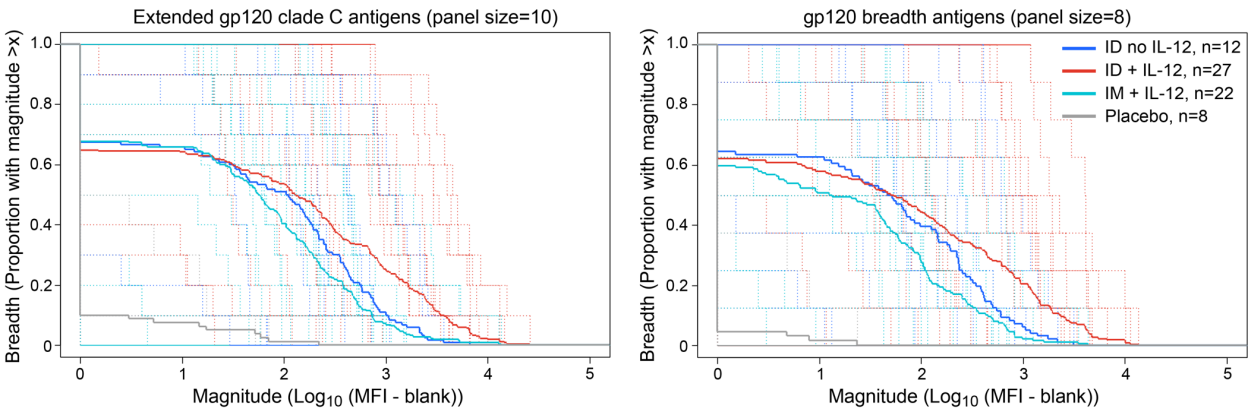

Fig. S3. IgG binding antibody responses as measured by binding antibody multiplex assay (BAMA) for an extended panel of gp120 clade C antigens and a gp120 breadth panel of antigens, measured at two weeks after the fourth vaccination. The 18 HIV proteins used as antigens are listed in S3 Table. Magnitude-breadth (MB) plots characterize the magnitude (x= MFI minus Blank) and breadth (proportion of antigens with magnitude > x) of each individual serum sample assayed against a panel of antigens. MB curves show, for each possible magnitude threshold, the fraction of assays with magnitudes greater than this threshold. In addition to the individual sample-specific curves, the group-specific curve displays the average MB across all subjects in that group.

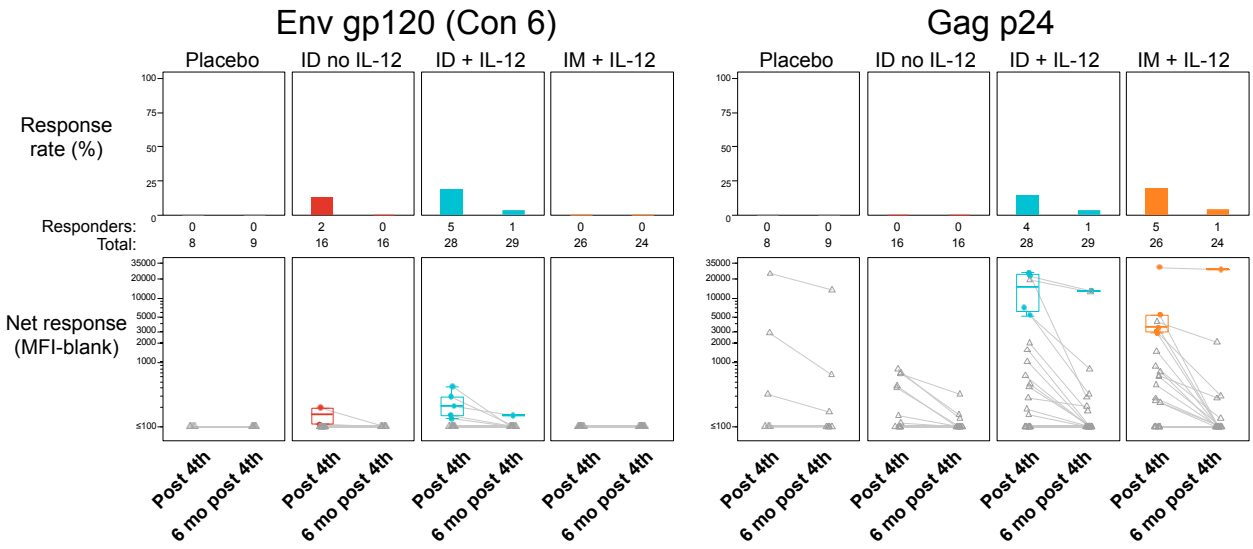

Fig. S4. IgG3 binding antibody responses as measured by binding antibody multiplex assay (BAMA) against consensus Env gp120 and Gag p24. Positive responses are shown in filled circles in color, negative responses are shown in open gray triangles. Box-plots represent the distribution for the positive responders only. Bar plots show response rates. Numbers below the bars indicate numbers of positive responders and total participants.

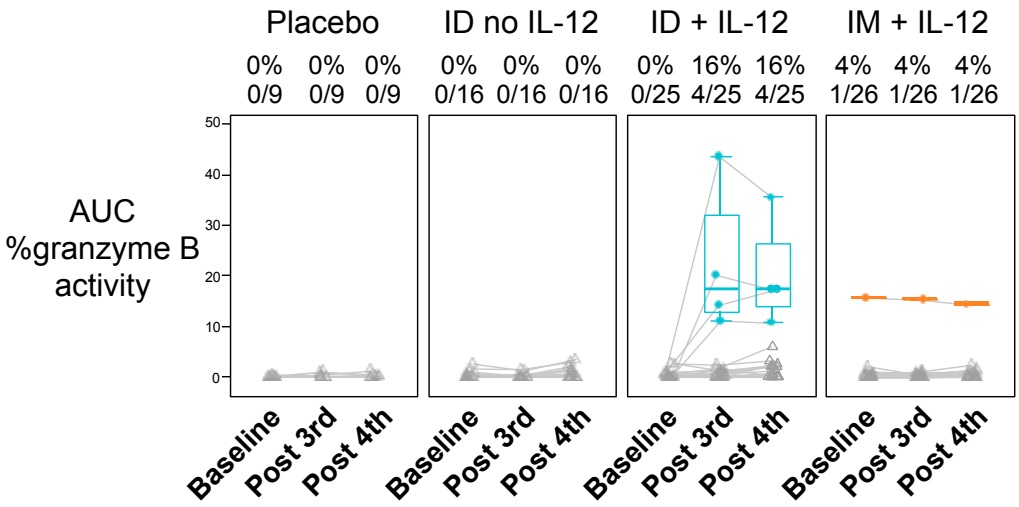

Fig. S5. Antibody-dependent cell mediated cytotoxicity (ADCC) as determined by the gp120-coated cells target assay for a clade C viral isolate (TV1). Positive responses are shown in filled circles in color, negative responses are shown in open gray triangles. Box-plots represent the distribution for the positive responders only. Response rates are listed above each graph along with numbers of positive responders and total participants.

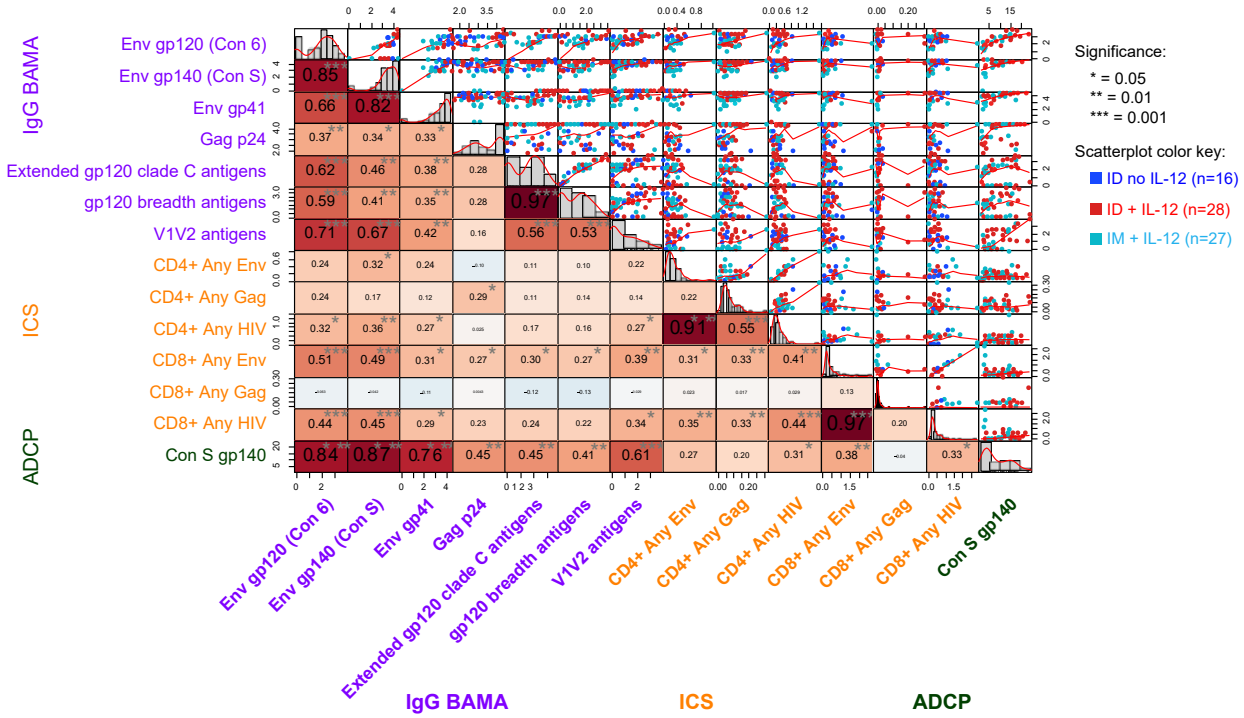

Fig. S6. Cross-assay correlations at two weeks after the fourth vaccination including the three treatment groups. The upper right part of the matrix shows Scatterplots of each pair of assay readouts with points color-coded by treatment group. The correlation coefficients are displayed on a gradient color scale, overlaid by statistical significance stars for unadjusted p-values from testing a zero correlation coefficient in the lower left. The un-scaled response magnitudes are used in these plots.

Table S1. HVTN 098 study schema

				PENNVAX®-GP					IL-12
Group [A]	N	Administration [B]	Volume per injection	env A	env C	gag	pol	Total PENNVAX®-GP	Total IL-12 DNA
1	5	ID + IL-12 (low dose)	0.1 mL	0.1 mg	0.1 mg	0.2 mg	0.2 mg	0.6 mg	0.2 mg
	1	Placebo (ID)	0.1 mL	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
2	20	ID no IL-12	0.1 mL (2 sites)	0.6 mg	0.6 mg	0.2 mg	0.2 mg	1.6 mg	0 mg
	2	Placebo (ID)	0.1 mL (2 sites)	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
3	30	ID + IL-12	0.1 mL (2 sites)	0.6 mg	0.6 mg	0.2 mg	0.2 mg	1.6 mg	0.4 mg
	3	Placebo (ID)	0.1 mL (2 sites)	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
4	30	IM + IL-12	1 mL	3 mg	3 mg	1 mg	1 mg	8 mg	1 mg
	3	Placebo (IM)	1 mL	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
Total 94 (85 vaccine/9 placebo)									

[A] Groups 2 and 3 received the doses of PENNVAX®-GP and IL-12 DNA indicated administered via EP over two injection sites on the arm(s). Each of the two ID injection sites received 0.1 mL.
[B] Participants in all groups receive injections at four timepoints: day 0, months 1, 3, and 6.
Abbreviations: ID, intradermal; IM, intradermal

164 **Table S2. Intracellular cytokine staining (ICS) staining panel**
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Antibody	Antibody clone	Manufacturer	Catalog number
AViD[A]	NA	Molecular Probes/Invitrogen	L34957
CD14 BV510[A]	M5E2	BioLegend	301842
CD56 BV570	HCD56	BioLegend	318330
CD3 BUV737	UCHT1	BD Biosciences	564307
CD4 BUV395	SK3	BD Biosciences	563550
CD8 BV650	RPA-T8	BD Biosciences	563821
CD45RA APC H7	HI100	BD Biosciences	560674
CCR7 BV786	G043H7	BioLegend	353229
CXCR5 PE-Dazzle594	J252D4	BioLegend	356928
PD-1 BV605	EH12.2H7	BioLegend	329924
ICOS BV711	DX29	BD Biosciences	563833
IFNγ V450	B27	Becton Dickinson	560371
IL2 PE	MQ1-17H12	BD Biosciences	559334
IL4 PerCP-Cy5.5	MP4-25D2	BioLegend	500822
IL-17a PE-Cy7	BL168	BioLegend	512315
TNFα FITC	Mab11	eBioscience	11-7349-82
CD40L APC	TRAP1	BD Biosciences	555702
Granzyme B Alx700	GB11	BD Biosciences	560213
[A]AVID and CD14 are detected in the same channel			

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Table S3. Details of the BAMA, ICS, and nAb antigens, including HIV-1 viral strain information.

Assay	Antigen class	Antigen label used in plots, tables and text	Full antigen name	Viral strain information: Subtype.Country.Year.Stage [A]
ICS	-	Any HIV	[sum of Any Env, Any Gag, Any Pol]	[HIV global potential T-cell epitope (PTEg) peptide pool]
	Env	Any Env	[maximum of PTEg-Env-1, PTEg-Env-2, PTEg-Env-3]	[HIV global potential T-cell epitope (PTEg) peptide pool]
	Gag	Any Gag	[maximum of PTEg-Gag-1, PTEg-Gag-2]	[HIV global potential T-cell epitope (PTEg) peptide pool]
	Pol	Any Pol	[maximum of PTEg-Pol-1, PTEg-Pol-2]	[HIV global potential T-cell epitope (PTEg) peptide pool]
BAMA	gp120	Env gp120 (Con 6)	Con 6 gp120/B	[Group M Consensus]
	gp140	Env gp140 (Con S)	Con S gp140 CFI	[Group M Consensus]
	gp140	Env gp140 (Clade A)	A1.con.en03 140 CF	A1.xx.xx.xx
	gp140	Env gp140 (Clade B)	B.con.env03 140 CF	B.xx.xx.xx
	gp140	Env gp140 (Clade C)	C.con.env03 140 CF_avi	C.xx.xx.xx
	gp41	Env gp41	gp41	B.US.xx.6
	V1V2	Clade AE (A244)	AE.A244 V1V2 Tags/293F	CRF01_AE.TH.90.6
	V1V2	Clade B (gp70 Case A)	gp70_B.CaseA_V1_V2	B.US.88.6
	V1V2	Clade C (1086)	C.1086_V1_V2 Tags	C.MW.04.1-2
	p24	Gag p24	p24	B.xx.xx.xx
	RT	[data not shown]	p66(RT)	B.FR.83.6
BAMA (IgG breadth)	gp120 (extended clade C antigen panel)	Extended gp120 clade C antigens (panel size=10)	1394C9_G1.D11gp120.avi	C.MW.04.1-2
			1428_D11gp120.avi/293F	C.IN.00.4
			1641A7_D11gp120.avi/293F	C.ZA.07.4
			96ZM651.D11gp120.avi	C.ZM.96.6
			CAP210_D11gp120.avi/293F	C.ZA.05.4
			CAP45_D11gp120.avi/293F	C.ZA.05.4
			CH505TF_D7gp120.avi/293F	C.MW.08.4
			Ce0042_D11gp120.avi/293F	C.ZA.07.5
			Du156_D11gp120.avi/293F	C.ZA.99.1-4
			TV1c8_D11gp120.avi/293F	C.ZA.98.6
	gp120 (antigen panel)	gp120 breadth antigens (panel size=8)	51802_D11gp120.avi/293F	A1.KE.09.1
			BORI_D11gp120.avi/293F	B.US.90.2
			TT31P.2792_D11gp120.avi/293F	B.TT.98.2
			B.6240_D11gp120/293F	B.US.95.2
			A244 D11gp120_avi	CRF01_AE.TH.90.6
			254008_D11gp120.avi/293F	CRF01_AE.TH.09.2
			CNE20_D11gp120.avi/293F	CRF07_BC.CN.07.6
			BJOX002_D11gp120.avi/293F	CRF07_BC.CN.07.1-2
nAb	EPV (tier 1A)	MW965.26	MW965.26	C.MW.93.6
	EPV (tier 1B)	[data not shown]	18814602_H8_F3	C.ZA.05.1-2
		[data not shown]	6535.3	B.US.95.5
		[data not shown]	BaL.26	B.US.85.6
		[data not shown]	Bx08.16	B.FR.92.6
		[data not shown]	CAP37.1.18_D2_19	C.ZA.06.4
		[data not shown]	DU156.12	C.ZA.99.1-4
	EPV (tier 2)	[data not shown]	246-F3_C10_2	AC.TZ.01.6
		[data not shown]	Ce1176_A3	C.MW.04.1-2
		[data not shown]	X1632-S2-B10	G.ES.04.xx
		[data not shown]	BJOX002000.03.2	CRF07_BC.CN.07.1-2
		[data not shown]	TRO.11	B.IT.95.3
		[data not shown]	25710-2.43	C.IN.99.5
ADCP	gp140	ConS gp140	Con S gp140 CFI	[Group M Consensus]
ADCC (gp120-gp120)	gp120	Clade C TV1	TV1c8_D11gp120.avi/293F	C.ZA.98.6
	gp120	[data not shown]	MN_gp120gDneg/293F Monomer	B.US.87.6

coated cells)	gp120	[data not shown]	Q23.17.D11 gp120.avi/293F	A1.KE.94.6
ADCC (Infected target cells)	IMC	Clade C TV1	TV1.21.LucR.T2A.ecto/293T/17	C.ZA.98.6
	IMC	[data not shown]	Q23.17.LucR.T2A.ecto/293T/17	A1.KE.94.6

[A] Subtype is denoted by a capital letter; country of origin is denoted by the 2 digit International Organization for Standardization code; year isolated is denoted by 2 digits; and stage is denoted by "a" (acute, if Fiebig stage is unknown) or "1", "2", "3", "4", "5", or "6" (acute or early chronic, where the number or range corresponds to the Fiebig stage or range of stages when known).

Abbreviations: ICS, intracellular cytokine staining; BAMA, binding antibody multiplex assay; RT, reverse transcriptase; nAb, neutralizing antibody; EPV, Env-pseudotyped virus; ADCP, antibody-dependent cellular phagocytosis; ADCC, antibody dependent cellular cytotoxicity; IMC, infectious molecular clone

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