Supplementary Materials for

Vaccine-Induced Env V1-V2 IgG3 Correlates with Lower HIV-1 Infection Risk and Declines Soon After Vaccination

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The PDF file includes:

Fig. S1

A.

RV144

A244 gp120 IgG3	AE.A244 V1-V2 tags IgG3	gp70.B.CaseA2 V1-V2 IgG3
p-value: 0.000017	**p-value: 0.0000024**	**p-value: 0.02**

B.

VAX003

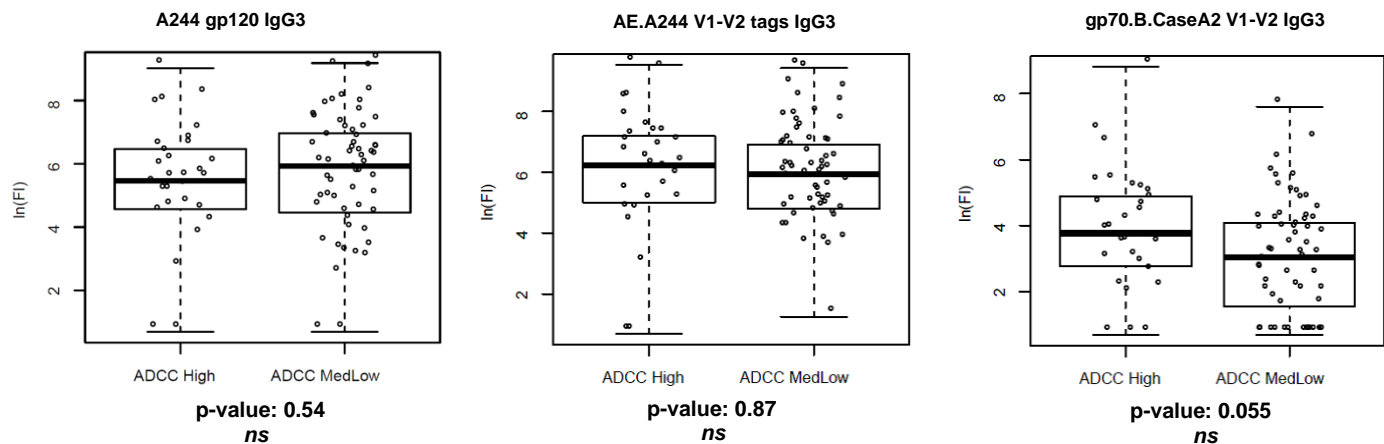|		
A244 gp120 IgG3	AE.A244 V1-V2 tags IgG3	gp70.B.CaseA2 V1-V2 IgG3
p-value: 0.54 *ns*	**p-value: 0.87** *ns*	**p-value: 0.055** *ns*

Fig. S1. Significant correlation of Env IgG3 and V1-V2 with ADCC in RV144, but not VAX003.
IgG3 responses to A244 gp120, AE.A244 V1-V2 tags, and gp70.B.CaseA2 V1-V2 are shown for RV144 (N=>200)(A) and VAX003 (N=87) (B). The RV144 data are representative of tests with 9 antigens (3 different envelopes and 6 V1/V2 antigens) that all showed significant correlations with ADCC. The VAX003 data are representative of tests with 11 antigens (7 different envelopes and 4 V1/V2 antigens). All tests for the VAX003 comparison were not significant (*ns*) for any antigen. Only one showed a trend (gp70.B.CaseA2 V1-V2: P-value 0.055) All p-values for the RV144 comparison were significant (p-values: 0.03 to <0.00001). The p values are t-test, respectively (Wilcoxon rank tests gave the same significance result).
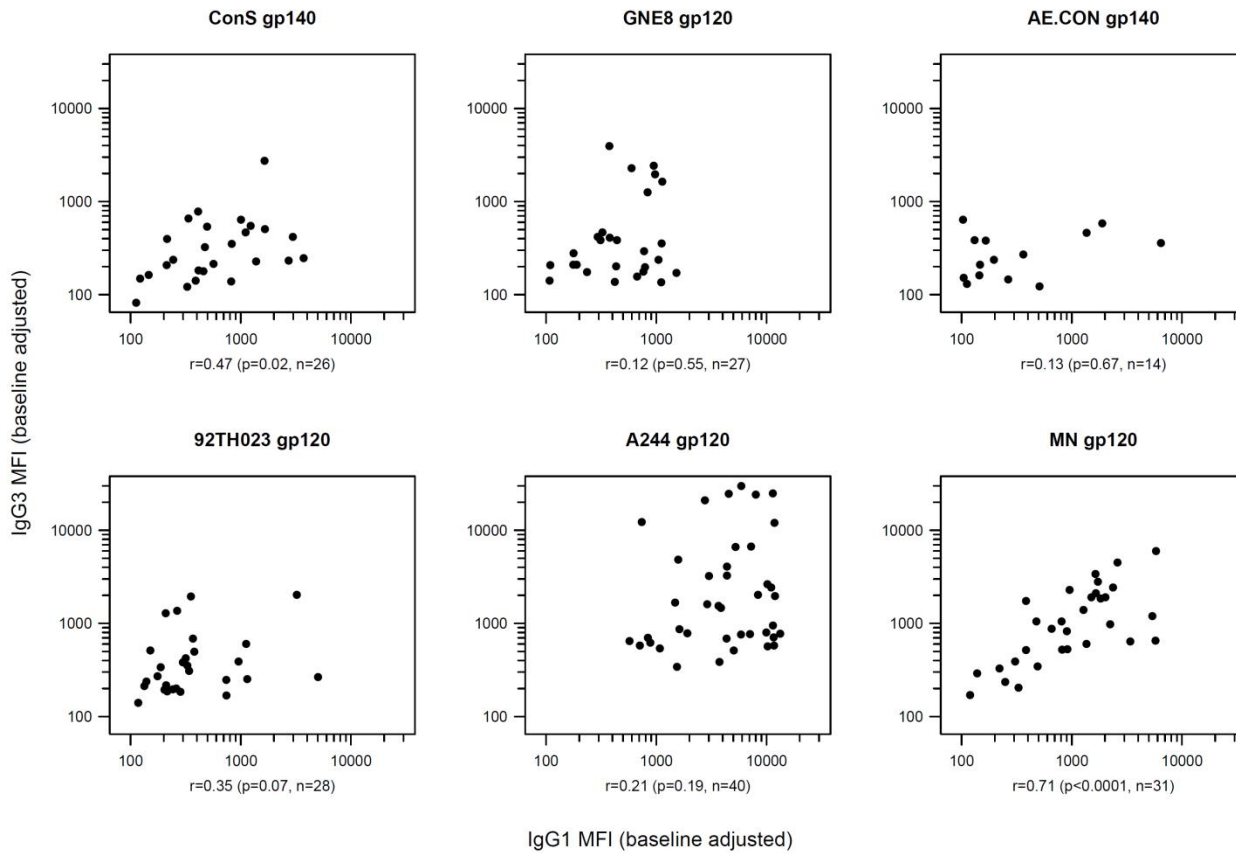
Fig. S2

Fig. S2. Lack of direct correlations for all HIV-1 Env IgG1 and IgG3 antibody responses. Env specific IgG and IgG3 responses from RV144 were measured for six HIV-1 envelope antigens: the vaccine boost envelopes (A244 gp120, MN gp120), the vaccine prime envelope gp120 (92TH023 gp120), a clade B gp120 (GNE8 gp120), a group M consensus envelope (ConSgp140) and a clade AE consensus (representing clades circulating in Thailand). Correlation coefficients (r) range from 0.12 to 0.71 (Spearman rank).

Fig. S3

A.

gp70.B.CaseA2 V1-V2

r=0.38 (p=0.002, n=64)

B.

gp70.B.CaseA2 V1-V2 169K

r=0.39 (p<0.0001, n=114)
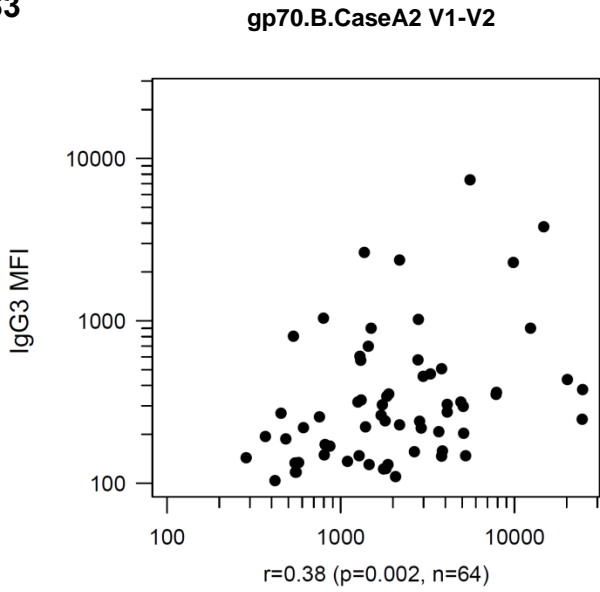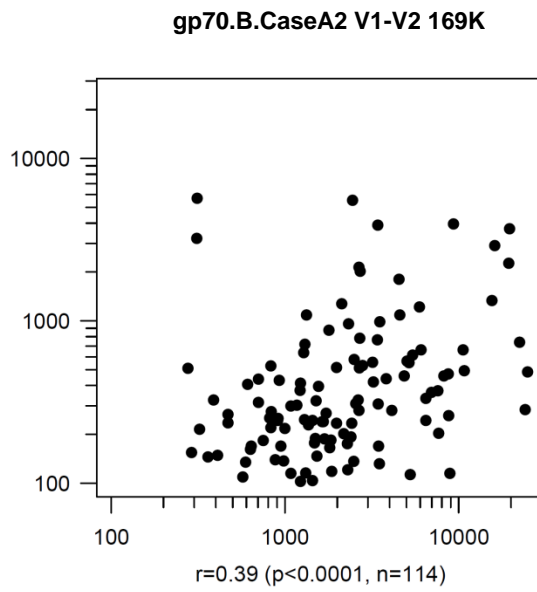
IgG MFI

Fig. S3. Weak association between V1-V2 IgG and V1-V2 IgG3 measurements. IgG and IgG3 responses from RV144 are shown for gp70.B.CaseA2 V1-V2 (A) and for gp70.B.CaseA2 V1-V2 169K in panel (B). Correlation coefficients (r) = 0.38 and 0.39, respectively (Spearman rank).
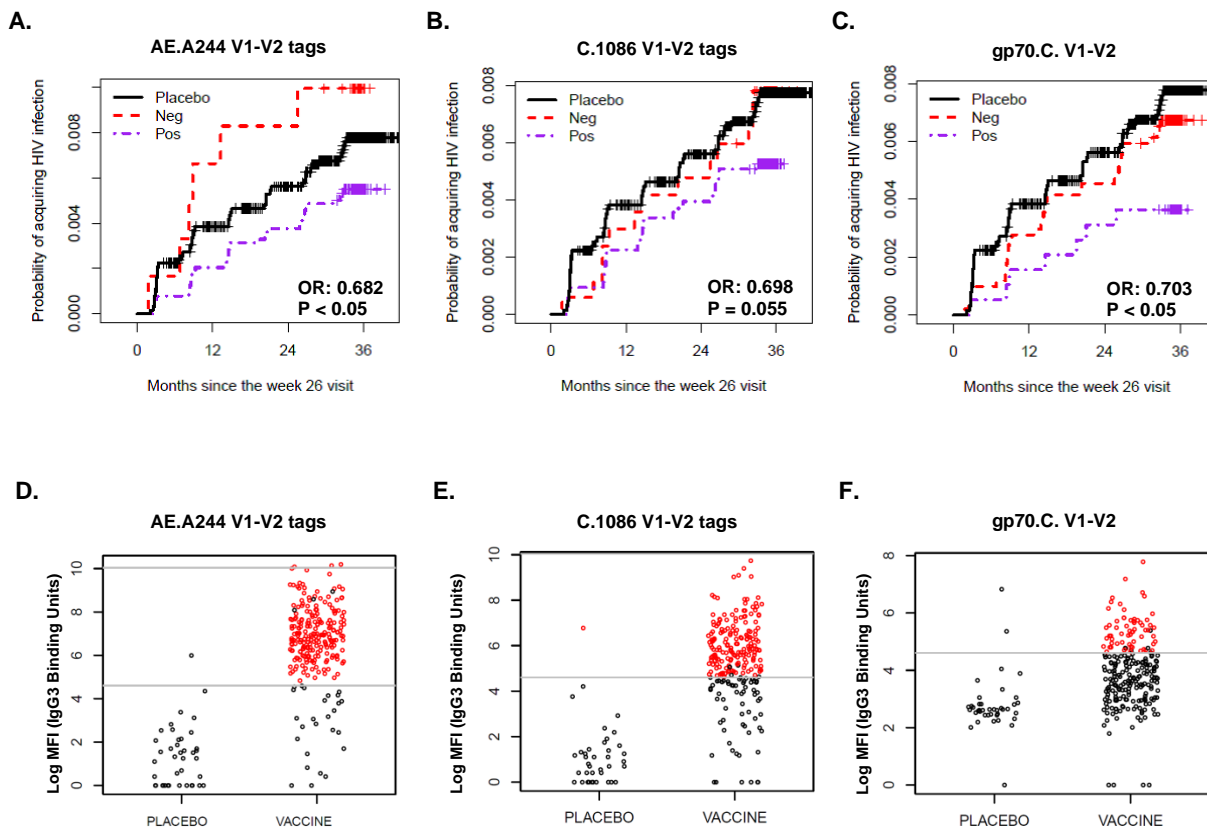
Fig. S4

A. AE.A244 V1-V2 tags

B. C.1086 V1-V2 tags

C. gp70.C. V1-V2

D. AE.A244 V1-V2 tags

E. C.1086 V1-V2 tags

F. gp70.C. V1-V2

Fig S4. **Cumulative Incidence Plots of IgG3 to V1-V2 Antigens in an RV144 Case-Control Analysis.** The magnitude of the IgG3 response to three different V1V2 antigens corresponded to a decreased infection risk (IgA Adjusted) (Odds ratio of less than 1) and two of these responses significantly correlated with decreased infection risk (<0.05, logisitic regression). In addition to the clade B V1V2 antigen shown in Figure 3 that significantly correlated with risk of infection, we show here that IgG3 responses to the V1-V2 of subtype AE and C also correlates with reduced risk thus indicating breadth of IgG3 V1V2 responses were induced by RV144. These data also enable direct comparison of antibody responses to clade C efficacy trials including HVTN 505 and planned South African trials. (**A**) Subtype AE.A244 V1-V2 Tags (Subtype of circulating strains in Thailand and same antigen in the RV144 immunogen), (**B**) Subtype C 1086 V1-V2 Tags (Same Clade C antigen in planned HIV-1 efficacy trials) and (**C**) subtype C gp70V1V2 (C.97ZA012) (same Clade C antigen in the HVTN 505 VRC vaccine regimen). **D-F.** Magnitude of IgG3 responses to V1/V2 Antigens in an RV144 case control analysis in placebos vs. vaccinees are shown for each antigen as log MFI binding units.
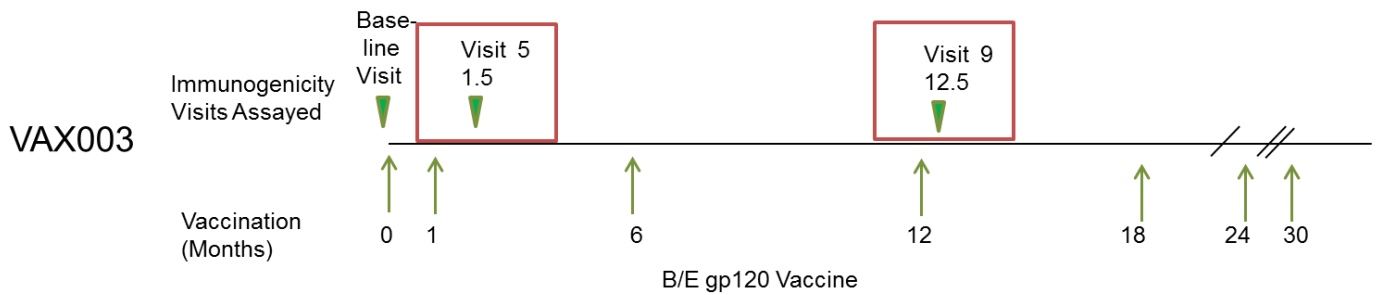
Fig. S5

A.
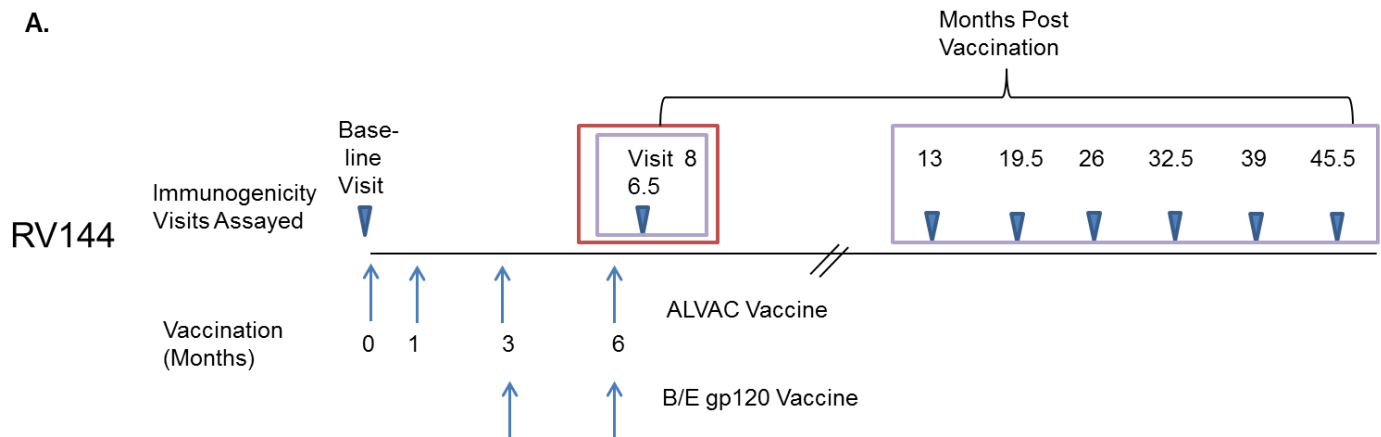
B.

Fig S5. Vaccination scheme for RV144 (top) and VAX003 (bottom) vaccine regimens. Blue or green arrows indicate the times of vaccination. The inverted triangles indicate the sampling time points. Red boxes are around the immunogenicity visits examined in the RV144/VAX003 comparison study and lavendar boxes are around the immunogenicity visits examined in the RV144 longitudinal follow-up study.

Table S1

Correlation of IgG3 Response Rate With Risk of Infection - RV144 Case Control

Type of Env Protein	HIV-1 V1V2 Antigen	Response Rate				Response Magnitude			
		Univariate		IgA adj.		Univariate		IgA adj.	
		OR	p-val	OR	p-val	OR	p-val	OR	p-val
V1V2 from RV144 vaccine boost (A244 gp120)	AE.A244 V1-V2 tags	0.494	0.162	0.391	0.075	0.791	0.185	0.682	**0.045**
V1V2 from Clade C	C.1086 V1-V2 tags	0.564	0.142	0.511	0.093	0.785	0.168	0.698	0.055
V1V2 scaffold utilized in^2 with mutation (169K)	gp70.B.CaseA2 V1-V2 169K	0.354	**0.006**	0.317	**0.003**	0.804	0.208	0.744	0.095
V1V2 from VRC Clade A gp120	Gp70 V1-V2 A	0.424	0.129	0.379	0.091	0.919	0.630	0.839	0.343
V1V2 scaffold utilized in^2	Gp70 V1-V2 CaseA2	0.531	0.191	0.476	0.130	0.749	0.088	0.689	**0.034**
V1V2 from Clade C gp120	gp70.C. V1-V2	0.493	0.121	0.446	0.081	0.753	0.104	0.703	**0.049**

Table S1. ORs for HIV-1 infection risk in univariate analyses of IgG3 measurements. IgG3 responses were measured in the plasma of 246 case control subjects and examined for correlation of HIV-1 risk. In the IgA adjusted model, IgA score is added to the model as a continuous variable. Significant p-values are shown in bold.

Table S2

Response Rates of IgG4 Binding Antibodies – RV144

Type of Env Protein	Envelope Antigen	Response Rate (%)	
		Week 26	Week 52
RV144 Vaccine Boost (monomer form)	A244 gp120	11	21
RV144 Vaccine Boost	MN gp120	11	16
Clade B	GNE8 gp120	8	34

Table S2. HIV-1 Env IgG4 response rates in RV144 vaccinees do not decline between peak immunogenicity (week 26) and week 52. Binding antibody levels were determined and the percent responders for each antigen at each time point post vaccination are shown.