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Antigenic competition in CD4+ T cell responses in a randomized, multicenter, double-blind clinical HIV vaccine trial§

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Abstract

T cell responses have been implicated in reduced risk of HIV acquisition in uninfected persons and control of viral replication in HIV-infected individuals. HIV Gag-specific T cells have been predominantly associated with post-infection control, whereas Env antigens are the target for

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protective antibodies; therefore inclusion of both antigens is common in HIV vaccine design. Yet, inclusion of multiple antigens may provoke antigenic competition, reducing the potential effectiveness of the vaccine. HVTN084 was a randomized, multicenter, double-blind phase 1 trial to investigate whether adding Env to a Gag/Pol vaccine decreases the magnitude or breadth of Gag/Pol-specific T cell responses. Fifty volunteers each received one intramuscular injection of 1×10^{10} particle units (PU) rAd5 Gag-Pol, EnvA/B/C (3:1:1:1 mixture) or 5×10^{9} PU rAd5 Gag-Pol. $CD4^+$ T cell responses to Gag/Pol measured 4 weeks post vaccination by cytokine expression were significantly higher in the group vaccinated without Env, whereas $CD8^+$ T cell responses did not differ significantly between the two groups. Mapping of individual epitopes revealed greater breadth of the Gag/Pol-specific T cell response in the absence of Env compared to Env co-immunization. Addition of an Env component to a Gag/Pol vaccine led to reduced Gag/Pol $CD4^+$ T cell response rate and magnitude as well as reduced epitope breadth, confirming the presence of antigenic competition. Therefore, T cell based vaccine strategies should aim at choosing a minimalist set of antigens to reduce interference of individual vaccine components with the induction of the maximally achievable immune response.

One Sentence Summary

Antigenic competition of CD4+ T cell responses occurs in HIV vaccine recipients

Introduction

A highly effective HIV vaccine is one of the main goals in the fight against the HIV/AIDS epidemic. Env-specific broadly neutralizing antibodies (1) or Env V2-specific antibodies able to effectively promote Fc receptor-mediated functions (2, 3) are highly desirable, and most of the current vaccine concepts include an Env component to allow for their elicitation. Nevertheless, the induction of T cell responses remains an important goal for several vaccine candidates [reviewed in (4)], specifically those targeting Gag (5), based on numerous studies suggesting that T cells targeting epitopes within Gag are particularly important in the host defense against HIV-1 (6–10).

Several challenges remain for the induction of a protective cellular immune response (11), as highlighted by the lack of efficacy of the Step Study and HVTN 505 (12–14). One of the proposed reasons for the lack of efficacy in the Step Study was the inability of the MRKAd5 HIV vaccine to induce T cell responses of appropriate epitope breadth to provide recognition of potential infecting virus strains. With just one epitope targeted on average across vaccine recipients, the vaccine likely fell short of inducing the breadth necessary to at least mediate post-infection viral control (6). One hypothesis for why such low numbers of protective epitopes were recognized is that the inclusion of multiple antigens (Gag, Pol and Nef in the MRKAd5 HIV vaccine) may have prevented the generation of Gag-specific T cells targeting multiple epitopes within this protective antigen, consistent with the phenomenon of antigenic competition. Antigenic competition, the inhibition of an antibody response to one antigen when co-delivered with another rather than individually (15–17), was first described in 1904 (18), yet data on antigenic competition for T cell responses is sparse (19), mainly focusing on competition of naïve T cells for APC (20–23). Specific inhibition of Gag-specific cellular responses induced by vaccination in the presence of increasing doses of Env

has been shown in a non-human primate (NHP) vaccine model (24), in line with a previous observation in mice showing epitope-specific competition (25).

In this study, we present the results from a randomized, double-blind clinical study designed to address whether antigenic competition interferes with cellular immune responses after adenovirus-based HIV vaccination. We hypothesized that T cell responses to Gag and Pol would be diminished in rate, magnitude and epitope breadth when the vaccine also contained an Env component, suggesting that antigenic competition has the potential to restrain vaccine-induced T cell immunogenicity in candidate HIV vaccines.

Results

Participant demographics and vaccine schedule

One hundred volunteers were enrolled in HVTN 084 (). Fifty individuals in Group 1 were vaccinated with 5×10^9 particle units (PU) of the recombinant adenovirus serotype 5 (rAd5) Gag-Pol vector plus 5×10^9 PU of a 1:1:1 mixture of three rAd5 Env vectors (EnvA, EnvB, and EnvC). Fifty individuals in Group 2 were vaccinated with 5×10^9 PU of the rAd5 Gag-Pol vector. Enrollment and follow-up are described in Fig. 1.

The dose of Gag-Pol was identical in both groups. Participants enrolled between March 2011 and December 2012. Both groups were comparable regarding sex, race, and age distribution (Table 1), and all recipients had Ad5 neutralizing antibody titers <18.

As shown in previously published studies using VRC rAd5 vaccines, no safety concerns were identified (14, 26, 27)].

Gag/Pol-specific $CD4^+$ T cell responses are reduced after co-immunization with Env

The primary objective for HVTN 084 was to determine whether the magnitude and breadth of Gag– and/or Pol-specific T cell responses were higher in participants vaccinated in the absence of Env (Group 2) compared to those who were co-immunized with Env (Group 1). We assessed response rates and magnitudes of Gag/Pol-specific $CD4^+$ and $CD8^+$ T cells using intracellular cytokine staining (ICS) by flow cytometry. In the absence of Env, 85.7% of Group 2 participants showed positive $CD4^+$ T cell responses to Gag/Pol, whereas only 66.7% showed detectable responses in Group 1 co-immunized with Env (Fig. 2A). Positive responses were also of higher magnitude (median = 0.41% vs. 0.24% $CD4^+$ T cells expressing IFN-γ and/or IL-2) in the absence of Env ($p = 0.0005$, Lachenbruch test). The difference in the overall Gag/Pol-specific $CD4^+$ T cell responses was driven both by responses to Gag (Fig. S2A, $p = 0.0009$) and Pol (Fig. S2B, $p = 0.0001$).

In the absence of Env, response rates (89.9% vs. 87.8%) and magnitudes (median 0.54% vs 0.44% $CD8^+$ T cells expressing IFN-γ and/or IL-2) were not significantly higher for Gag/Pol-specific $CD8^+$ T cells ($p = 0.4$, Fig. 2B). Positive Gag-specific $CD8^+$ T cell responses were of significantly higher magnitude in the group not receiving Env ($p = 0.02$, Wilcoxon rank sum test, Fig. S2C).

At the time the clinical protocol was written, IFN-γ ELISpot was chosen as the primary endpoint to determine whether there was a difference between both treatment groups; as shown in Fig. S3, this assay was not able to differentiate between co-immunization with and without Env.

Increased functionality of Gag/Pol-specific CD4+ T cell responses induced in the absence of Env

The functional profile of vaccine-induced T cells is of major interest considering that polyfunctional T cells (*i.e.*, those expressing multiple cytokines simultaneously) have been associated with reduced risk of HIV infection in two proof-of-concept HIV vaccine trials (28, 29). Polyfunctionality in those studies was assessed by a Bayesian hierarchical framework that models all observed cell subsets and selects those most likely to have antigen-specific responses (28). We used this combinatorial polyfunctionality analysis of antigen-specific T cell subsets (COMPASS) to compare the functional profiles of Gag– and Pol-specific $CD4^+$ and $CD8^+$ T cell responses in participants co-immunized with Env or receiving Gag and Pol alone. We assessed expression of IL-2, IFN-γ, TNF-α, CD40L, IL-4, and Granzyme B (GzB) to provide a comprehensive analysis of T_{h1}, T_{h2}, B-cell help, and cytotoxic profiles.

The functional profiles for $CD4^+$ T cells induced by rAd5 vaccination were dominated by highly functional cells (expression of three or more functional markers), with a marked absence of $CD4^+$ T cells expressing only a single marker (Fig.3A and B). Contrary to that pattern, monofunctional IFN-γ and TNF-α-expressing $CD8^+$ T cells were readily detected, whereas dual and triple functional $CD8^+$ T cells predominated overall (Fig. 3C and D). Inclusion of Env in the vaccine led to reduced percentages of 6-functional Gag- and Pol-specific $CD4^+$ T cells (Fig. 3A and B), and reduced co-expression of IFN-γ/TNF-α/GzB for Gag-specific $CD8^+$ T cells ($p = 0.04$, Fig. 3C).

In line with these observed increases in highly functional subsets, summary functionality scores (defined as the proportion of Ag-specific subsets detected among all possible functional subsets) were significantly higher in the group not receiving Env for $CD4^+$ T cells ($p < 0.05$, Fig. 4 A and B) overall and for Gag-specific $CD8^+$ T cells ($p = 0.07$, Fig. 4C).

Co-immunization with Env leads to reduced breadth of Gag/Pol-specific T cells

We performed detailed epitope mapping (i.e., we determined responses to individual 15-mer peptides) to determine the breadth of the response since targeting of multiple HIV epitopes is relevant for vaccine protection (6). Breadth was defined as described in the Materials and Methods. Fig. 5 shows the distribution of epitopes within Gag and Pol for participants who were immunized in the presence or absence of Env. There are clear hotspots of recognition as previously described for other studies (30), with no major differences between groups in the distribution of epitopes. Fig. S4 lists the HLA types and epitopes targeted for each individual.

Fig. 6 shows the number of epitopes targeted in Gag/Pol in participants vaccinated in the presence or absence of Env. Although the main distribution of responses is similar between both groups, exceptional breadth of 12 or more epitopes (7/50, 14%) was only seen in the

group that did not receive Env co-immunization, leading to a significantly higher mean breadth in Group 2 [p = 0.037, mean 4.5 epitopes in Group 2 vs. 2.9 epitopes in Group 1, breadth ratio = 1.56, 95% CI 1.03–2.36, Poisson regression with sandwich (Eicker-Huber-White) standard errors]. This effect is also demonstrated in the Reverse Cumulative Distribution Curve (RCDF) plot in Fig. 6B, in which the two groups are overlapping for the low responders and then separate above 4 targeted epitopes. This effect was mainly driven by epitopes in Pol (Fig. S5).

Discussion

Since the failure of two Ad5-vectored T cell based vaccine to provide protection in the Step and HVTN 505 vaccine trials (12–14), focus in the HIV vaccine field has shifted back to antibody-inducing strategies. Yet because the Step and HVTN 505 vaccines generated T cell responses of narrow breadth, these trials did not test whether induction of much broader T cell responses could confer protection, an untested possibility that remains important. Moreover, T cell based concepts are receiving increased attention based on promising NHP challenge data showing consistent protection in ~50% of non-human primates after vaccination with a cytomegalovirus (CMV)-vectored vaccine (31, 32), as well as protection in monkeys by an Ad26/protein combination vaccine for which T cell responses were identified as one of the correlates of risk of infection (33).

HVTN 084 was designed to test the hypothesis that antigenic competition – a phenomenon known since the early 20th century, though predominantly described for antibody responses (15–17) – would lead to reduced T cell responses to one antigen if a participant was co-immunized with a second antigen. We here confirmed that in the presence of Env, CD4$^+$ T cell responses to Gag and Pol were observed in fewer participants and were of lower magnitude than in the absence of Env, suggesting that antigenic competition does indeed occur for T cell responses. One possible explanation suggested previously is the competition of naïve antigen-specific T cells for access to cognate peptide/MHC complexes as well as costimulatory signals for activation and expansion (20, 22, 34), which could be overcome by increasing the number of APCs available during the priming stage (23). Although more commonly observed for T cells of the same specificity, this type of competition has been described for T cells targeting different antigens, as long as the antigens are presented by the same APC (21). It is therefore unlikely that this mechanism is at play in our study since the antigens are delivered by separate vectors which are probably delivered to different APCs. Alternatively, the differential effect of co-immunization with Env on T cell subsets, with highly substantial antigenic competition for CD4$^+$ T cells but no considerable consequence for CD8$^+$ T cells, suggests the possibility that binding of HIV Env to its primary receptor CD4 may be at least in part responsible for the observed outcome. Binding of gp120 to CD4 has been described to induce profound changes in CD4$^+$ T cell signaling that are implicated in the pathogenesis of HIV infection (35). Signal transduction events induced as a result of interaction of gp120 with the CD4 molecule include upregulation of the immunosuppressive cytokine TGF-β (35), which could have deleterious effects on the nascent vaccine-induced CD4$^+$ T cell response. This mechanism is different from that described in a mouse vaccine study, in which the effect was epitope-specific and observed for Gag-specific CD8$^+$ T cells rather than CD4$^+$ T cells, in line with the inability of HIV Env to bind to mouse CD4 (25).

The absence of antigenic competition measured using IFN-γ ELISpot is likely due to the preferred detection of the dominant $CD8^+$ T cell responses using this assay as well as limiting the readout to a single cytokine (36), for which the difference between groups is much more muted (Fig. 2B).

Gag/Pol-specific $CD4^+$ and $CD8^+$ T cell responses showed reduced functionality in participants co-immunized with an Env vaccine, and the epitope breadth of responses to Gag/Pol was lower in that group than in those who did not receive the Env-containing vaccine regimen. Reduced breadth of vaccine-specific T cells has also been observed following immunization with a nanoparticle neoantigen cancer vaccine when multiple antigens were given in the same injection site compared to separation of antigens across multiple sites (19).

Overall, co-immunization with Env in HVTN 084 led to a substantial reduction in cellular immune responses associated with partial protection in the Step and HVTN 505 trials (6, 29), suggesting that in the absence of antigenic competition, cellular vaccines may provide efficacy not seen in clinical trials to date.

Vector selection for HIV vaccines has experienced far greater priority than insert design to determine the most promising antigens included in those vectors. Protein vaccines have mainly focused on Env, driven by the propensity of this strategy to mainly induce antibody and $CD4^+$ T cell responses (37), but recombinant viral vectors (including poxviruses, adenoviruses, and CMV) frequently aim at including as many inserts as possible. While inclusion of multiple antigens may lead to greater overall epitope breadth when aggregating over all inserts, it may come at the cost of targeting regions that are less frequently associated with virus control (7), rather than those most likely to confer benefit (6–10). Identifying a parsimonious set of antigens that induces potentially protective antibody as well as T cell responses is of utmost importance for vaccine design.

There are a number of limitations in our study: therefore, the generalizability of our findings will require confirmation in follow up studies using different vaccine strategies (such as poxvirus vectors) and different combinations of inserts. HIV Env has been shown to suppress $CD4^+$ T cell activation, likely due to its binding to the CD4 molecule (38); thus the observed effect may be limited to vaccines including Env inserts. In addition, we only present T-cell response data at peak immunogenicity 4 weeks after immunization, and have not confirmed the longevity of the observed effect since the study only included a single large blood draw to allow for epitope mapping. Lastly, vaccination with Ad vectors leads to induction of Ad-specific T-cell responses (39), opening the possibility that the observed antigenic competition is driven by the higher dose of the Ad5 vector in Group 1 including Env rather than by the insert itself. Our data from a previously published phase 1 study, HVTN 054 (26), makes this interpretation of our data unlikely since in that study, a 10fold increased dose of the Ad5 vector (10^{11} PU) did not deleteriously affect immune responses, and the lower vector dose in HVTN 084 was chosen based on the increased reactogenicity observed with the higher dose. Nevertheless, our results can directly inform vaccine design. Because Env is the only antigen on HIV available for neutralization of the virus by antibodies, its inclusion in prophylactic vaccine regimens seems imperative, but strategies

can be considered to separate vaccination of Env from other antigens (specifically Gag) in time and/or space to avoid unwarranted interaction of the vaccine components. In addition, strategies for therapeutic immunization may fare better if Env is excluded from those vaccines.

Taken together, our data obtained in HVTN 084 are consistent with antigenic competition between different HIV vaccine components for the induction of $CD4^+$ T cell responses, and have direct implications for vaccine design aiming at the induction of protective T cells.

Materials and Methods

Study design

HVTN 084 was a randomized, double-blind phase 1b trial, designed to examine the influence of antigenic competition on the immunogenicity of HIV-1 Gag/Pol vaccine either administered alone or in combination with rAd5 Env A/B/C (ClinicalTrials.gov identifier:). The study enrolled 100 participants between March 2011 and March 2012 in eight sites in the United States [Brigham and Women's Hospital, Boston, MA; Columbia University and New York Blood Center (Bronx and Union Square), New York, NY], Brazil (São Paulo, SP), Peru (Lima and Iquitos), and Switzerland (Lausanne). The protocol was approved by the ethics review committee of every site, and the study was undertaken in conformance with applicable local and country requirements.

Participants

This study enrolled 18–50 year-old HIV-uninfected, adenovirus serotype 5 (Ad5) seronegative (neutralizing antibody titer <18) female and circumcised male volunteers in good health. Volunteers were required to have a history of low risk for HIV infection in order to participate in the study. Participants underwent a thorough written informed consent process.

Vaccine Products

The primary vaccine product used in this study is VRC-HIVADV014–00-VP, composed of a mixture of adenovirus serotype 5 vectors each expressing 1 of the 4 HIV antigens gp140(A), gp140(B)dv12, gp140(C) and Gag-Pol(B). The comparison group used only 1 component of this combination product, consisting of the Gag-Pol(B) antigen expressed by the same rAd5 vector (VRC-HIVADV054–00-VP). All vaccines were delivered intramuscularly (IM).

Randomization and masking

One hundred volunteers were randomized to receive one dose containing 1×10^{10} PU rAd5 Gag-Pol, EnvA/B/C (3:1:1:1 mixture) (Group 1) or 5×10^{9} PU rAd5 Gag-Pol (Group 2), at a 1:1 randomization ratio. The randomization sequence was obtained by computer-generated random numbers and provided to each HVTN Clinical Research Site (CRS) through the Statistical and Data Management Center's (SDMC) web-based randomization system. The randomization was done in blocks to ensure balance across groups. Participants and site staff (except for site pharmacists) were blinded as to participant treatment group. Study product assignments were accessible to those HVTN CRS pharmacists, DAIDS protocol pharmacists

and contract monitors, and SDMC staff who were required to know this information in order to ensure proper trial conduct.

Procedures

The vaccines were delivered via 1 mL IM injection in the deltoid and peripheral blood mononuclear cell (PBMC) samples were obtained at baseline and after one month to assess HIV-specific T cell immune responses.

IFN-γ ELISpot assay

Ex vivo HIV-specific T cell responses were assessed with a validated MabTech/Millipore IFN-γ ELISpot assay using cryopreserved PBMC stimulated overnight with synthetic peptides. HIV-1 peptides representing the HIV inserts, clade B Gag and Pol as well as clade A, B, and C Env were used for this study. Peptides were validated prior to use in these assays. The following wells were tested for each specimen: 6 negative control wells without antigen, 3 positive control wells containing phytohemagglutinin (PHA) and 3 experimental wells containing each peptide pool or individual peptide (for epitope mapping). The assay was run with 100,000 PBMC per well with 2μg/ml of each peptide; results were reported as the average number of spot forming cells (SFC) per million PBMC in the experimental wells minus the average SFC/million PBMC in the negative control wells. Results deemed unreliable by the lab were excluded from statistical analysis.

Previously cryopreserved PBMC were first stimulated with master-pools of 122 to 159 individual 15-mer peptides encompassing each protein (Gag: 122 peptides, Pol-1: 124 peptides, Pol-2: 122 peptides, EnvA: 152 peptides, EnvB: 158 peptides, EnvC: 149 peptides), and 12 mini-pools per Gag and Pol master-pool with 10 to 14 15-mers each. For pools eliciting positive responses, additional PBMCs were tested with each 15mer contained within the pool.

Intracellular cytokine staining (ICS)

A validated intracellular cytokine staining (ICS) assay (40) was performed on cryopreserved PBMC by flow cytometry to examine HIV-1-specific vaccine-induced $CD4^+$ and $CD8^+$ T cell responses one month after vaccination. Cytokine production was assessed after stimulation with 15-mer peptides representing the HIV inserts, clade B Gag and Pol, at 1 μg/ml as previously described (40, 41). Briefly, the six-hour stimulation included brefeldin A (10 μg/ml; Sigma-Aldrich) and anti-CD28/anti-CD49d (each at 1 μg/ml; BD Biosciences). PHA (Remel) was used as a positive control, and peptide diluent (DMSO at a final concentration of 1%) was used as a negative control. Cells were stained with Aqua Live/Dead Fixable Dead Cell Stain (Invitrogen), then fixed, permeabilized, and stained intracellularly with fluorescently labeled antibodies to CD14 (exclusion marker), CD3, CD4, CD8, IFN-γ, IL-2, IL-4, TNF-α, CD40L, and granzyme B (41). Data were acquired on an LSRII and analyzed using FlowJo. A gating tree is presented in Fig. S6. Data was filtered if background responses (DMSO control) were >0.1% cytokine secretion, or if <5,000 events were acquired within the $CD4^+$ or $CD8^+$ T cell subpopulations. Data are reported as the percentage of T cells secreting IFN-γ and/or IL-2 in the antigen-stimulated sample minus the percentage of T cells secreting IFN-γ and/or IL-2 in the DMSO control. Data are

available for 98 participants (49 from each group); data for $CD4^+$ T cells for one participant in each group were filtered for high background.

Statistical analyses

IFN-γ ELISpot—The Mixture Models for Single-Cell Assays (MIMOSA) method (42) was used to determine a positive response to a pool or 15-mer peptide. The MIMOSA test compares cell counts between antigen-stimulated and unstimulated samples from a participant to identify significant differences. Cell counts are modeled by a binomial distribution and information is shared across participants by means of a prior distribution placed on the unknown proportion of the binomial likelihood. For peptide pools, aggregate responses (to Gag/Pol or to any protein) are considered positive if responses to any of the individual peptide pools are positive. The aggregate response magnitude is the sum of response magnitude to each individual peptide pool with the exception of the aggregate response to Env (any Env), which is defined as the maximum response of EnvA, EnvB, and EnvC due to the overlapping nature of those sequences.

After obtaining response calls via MIMOSA, the number of reactive epitopes was assessed. In general, each positive response was counted as one epitope. However, if two 15-mers with a positive call for a given participant overlapped by at least eight amino acids, this was counted as one epitope. Participants without 15-mer data were those without positive responses at the mini-pool level and were thus considered to have zero epitopes.

For comparisons between treatment groups, Poisson regression with sandwich (Eicker-Huber-White) standard errors was used to compare the mean number of epitopes per participant for Gag and Pol combined and separately. As a secondary analysis, the Wilcoxon rank-sum test was used to test the null hypothesis of equal distributions of breadth by treatment group. Statistical significance was declared if two-sided p-values were < 0.05.

Reverse cumulative distribution function (RCDF) curves were used to display the distribution of the number of epitopes (breadth) by treatment group. Each point on the RCDF curve displays the proportion of participants with breadth $\geq x$ for a given breadth x noted on the x-axis.

Intracellular cytokine staining (ICS)—One-sided Fisher's exact tests were used to define the positivity of ICS assay responses to a specific peptide pool. A response was defined as positive if the proportion of $CD4^+$ or $CD8^+$ T cells secreting IFN-γ or IL-2 from the stimulated well was significantly greater than that for the negative control well. A multiplicity adjustment across the antigens was made using the Holm-Bonferroni method. An adjusted p-value $<10^{-5}$ was deemed significant (40). A permutation-based Lachenbruch's Binomial + Wilcoxon test (43) using 10,000 random permutations was used to test the composite null hypothesis of equal response rates and equal distributions among positive responders. For these calculations and for graphing, magnitudes less than 0.025% were set to 0.025%. Responses to Gag/Pol were considered positive if the response to at least one of the individual pools was positive; the aggregate magnitude is the sum of the magnitudes of the individual pools.

T cell functionality analyses—COMPASS was used to analyze polyfunctionality of ICS responses. COMPASS is a computational framework for unbiased polyfunctionality analysis of antigen-specific T cell subsets (28). COMPASS uses a Bayesian hierarchical framework to model all observed functional cell subsets and select those most likely to exhibit antigen-specific responses. Cell subset responses are quantified by posterior probabilities, while participant-level responses are quantified by two summary statistics ("scores") that can be correlated directly with outcomes of interest, and describe the quality of an individual's (poly)functional response. The functionality score is defined as the proportion of Ag-specific subsets detected among all possible ones. The polyfunctionality score is similar, but it weighs the different subsets by their degree of functionality, favoring subsets with higher degrees of functions, motivated by the observation that higher degree function has been correlated with good outcomes in certain vaccine studies. For this analysis, expression of IFN-γ, IL-2, TNF-α, IL-4, CD40L and GzB were included. Functionality scores were compared between groups using Wilcoxon rank sum test statistics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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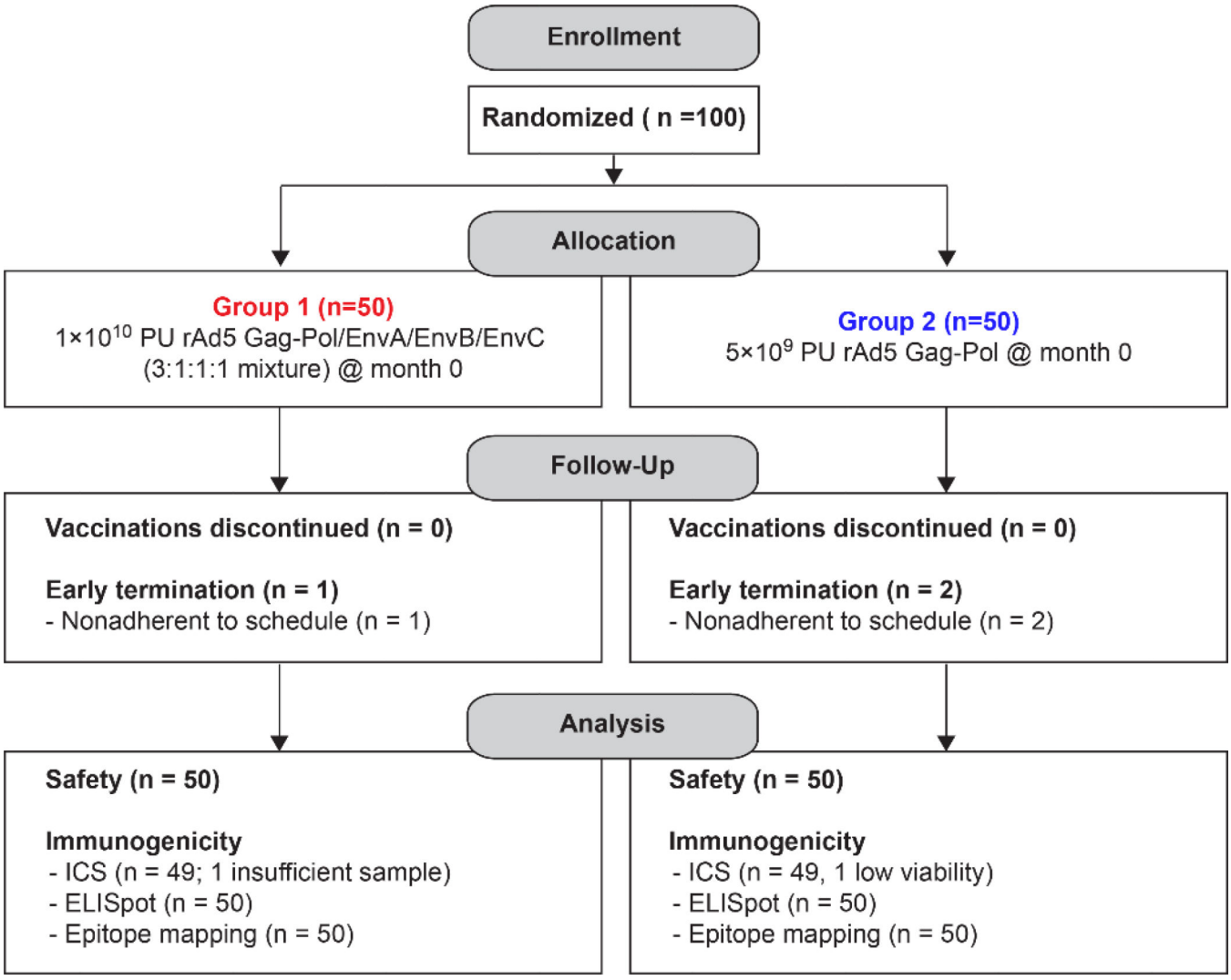

Figure 1:
Consort Diagram for HVTN 084.

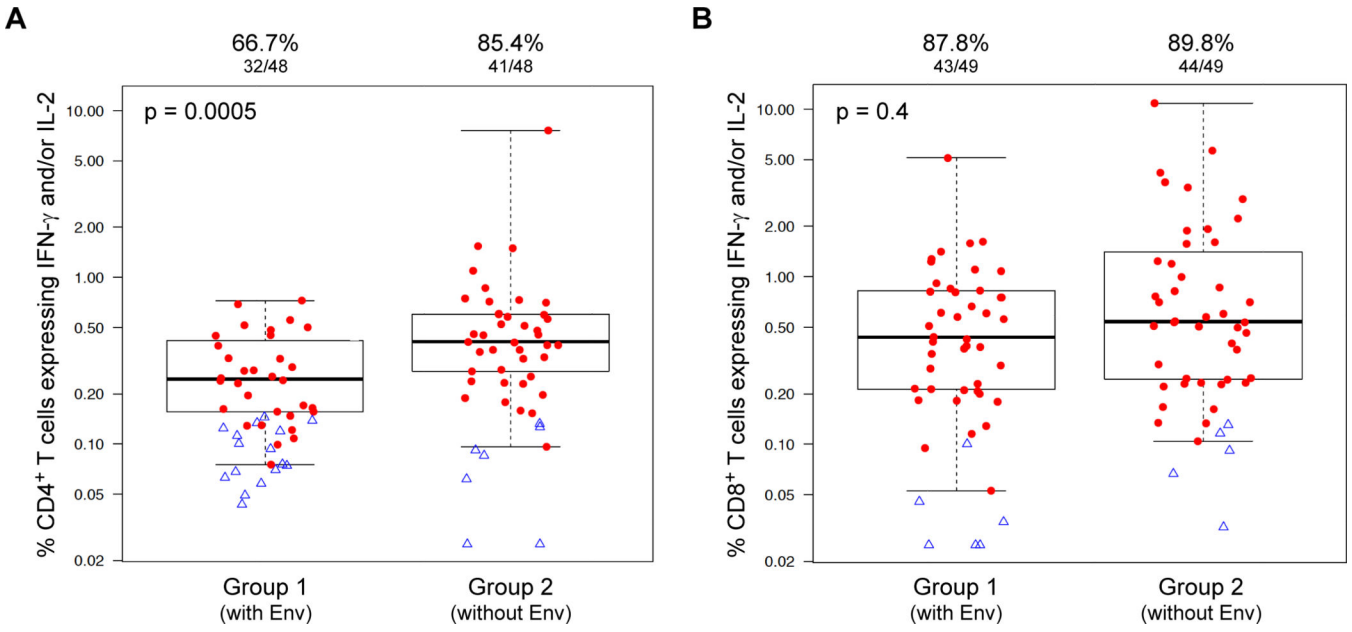

Figure 2: Gag/Pol-specific T cell responses in HVTN 084 one month after vaccination in participants receiving rAd5 expressing Gag/Pol/Env (with Env) or Gag/Pol (without Env).

Background adjusted magnitude of A) CD4+ and B) CD8+ T cells producing IFN-γ and/or IL-2 measured by ICS. Box-plots represent the distribution for the positive responders only, where the mid-line of the box denotes the median and the ends of the box denote the 25th and 75th percentiles, with whiskers extended to the extreme data points that are no more than 1.5 times the inter-quartile range or, if no value meets this criterion, to the data extremes. The boxplots are overlaid with individual data points of both positive (in red circles) and negative responders (in blue triangles). Percentages above the boxes are response rates.

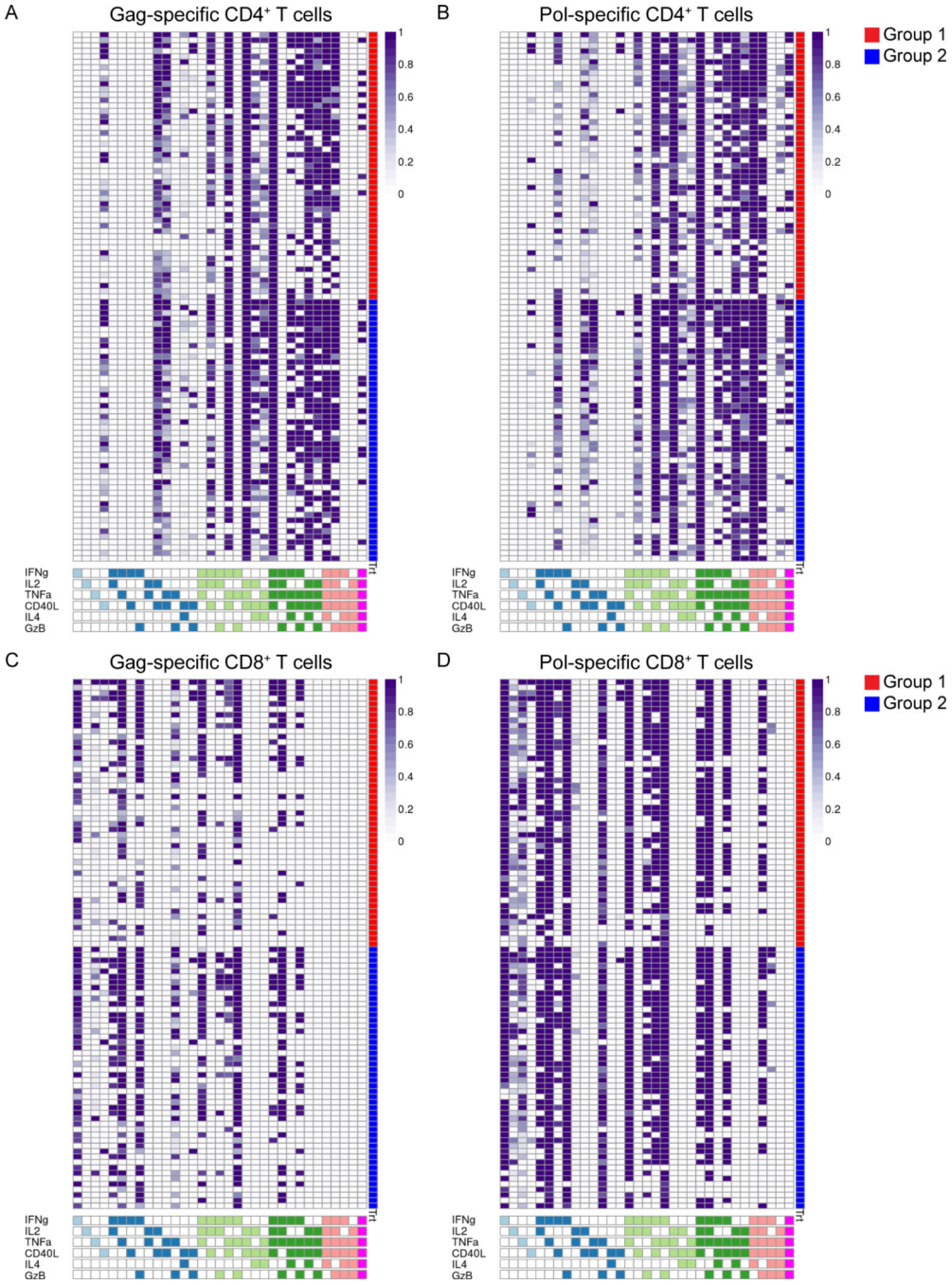

Figure 3. Expression of different combinations of functional markers by CD4$^+$ and CD8$^+$ T cells.

Heatmap of COMPASS posterior probabilities for Gag-specific CD4$^+$ (**A**) and CD8$^+$ T cells (**C**), and Pol-specific CD4$^+$ (**B**) and CD8$^+$ T cells (**D**). Columns correspond to the different cell subsets modeled by COMPASS, color-coded by the cytokines they express (white="off", shaded="on", grouped by color="degree of functionality"), and ordered by degree of functionality from one function on the left to six functions on the right. Subsets with maximum posterior probabilities less than 0.005 are removed from the heatmap. Rows correspond to participants in treatment (Trt) Group 1 (red) and Group 2 (blue). Each cell

shows the probability that the corresponding cell-subset (column) exhibits an Ag-specific response in the corresponding participant (row), where the probability is color-coded from white (zero) to purple (one). The participant-level posterior probabilities reflect the certainty from the COMPASS model that the subset exhibits an Ag-specific response in a participant (*i.e.* that the magnitude of the stimulated sample is above the magnitude of the (paired) non-stimulated sample).

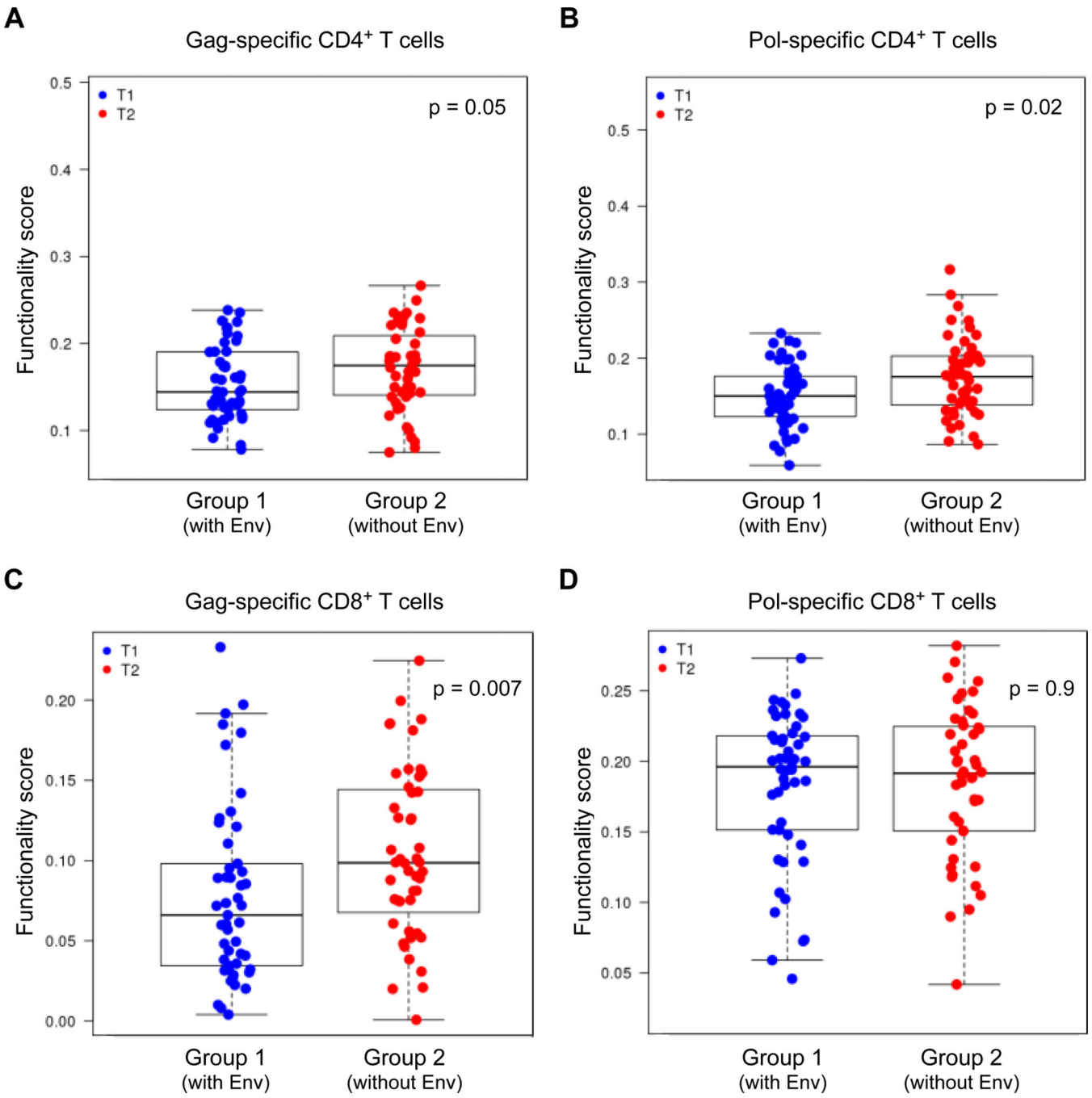

Figure 4: Functionality scores for Gag and Pol-specific T cell responses in HVTN 084 one month after vaccination in participants receiving rAd5 expressing Gag/Pol/Env (red) or Gag/Pol (blue).

Functionality scores from COMPASS are defined as the proportion of antigen-specific subsets detected among all possible ones based on expression of IFN-γ, IL-2, TNF-α, CD40L, IL-4, and granzyme B by CD4+ T cells (A and B) and CD8+ T cells (C and D) for Gag and Pol, respectively. p-values are based on Wilcoxon rank sum test.

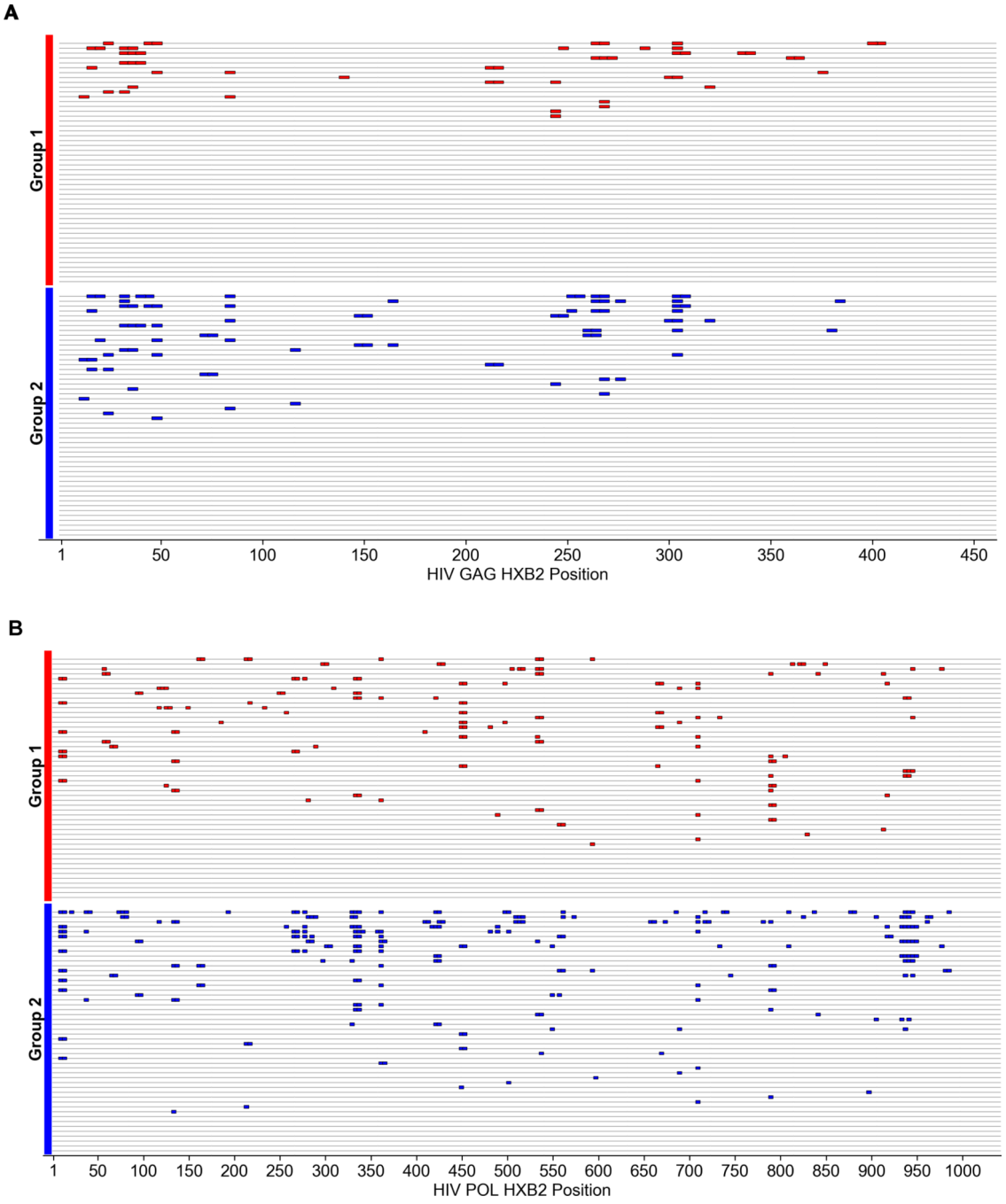

Figure 5: Distribution of epitopes for Gag and Pol-specific T cell responses in HVTN 084.

Epitope mapping was performed one month after vaccination by IFN-γ ELISpot by deconvoluting responses to pools down to single 15-mer peptides. Responses to peptides overlapping by 8 or more amino acids are shown as one epitope for A) Gag and B) Pol. Group 1 (immunized with Env) is shown in red, group 2 (immunized without Env) in blue. Each row represents a single individual, empty rows represent subjects for which no individual epitopes were identified.

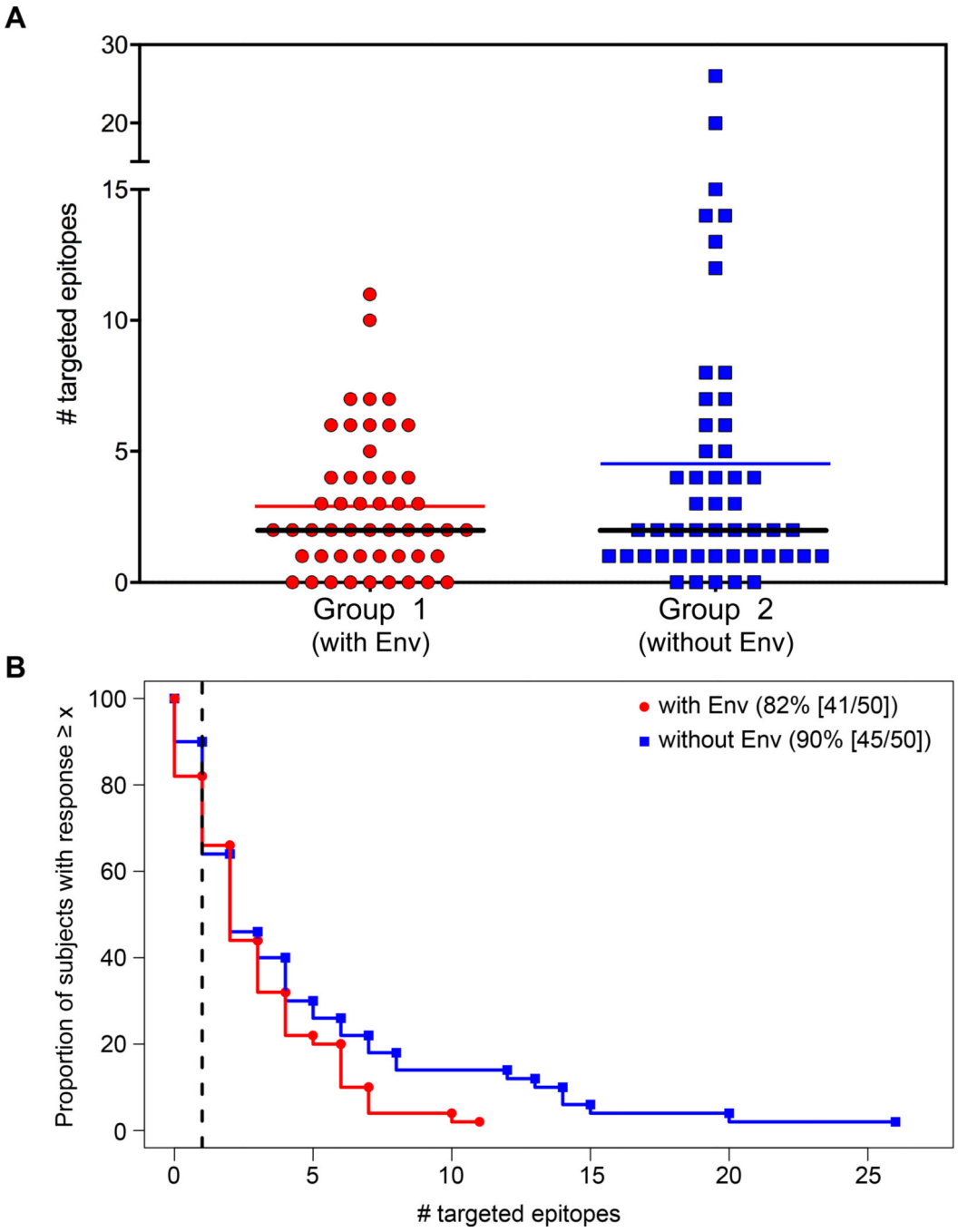

Figure 6: Epitope breadth of Gag/Pol-specific T cell responses in HVTN 084.

Epitope mapping was performed one month after vaccination by IFN-γ ELISpot by deconvoluting responses to pools down to single 15-mer peptides. Responses to peptides overlapping by 8 or more amino acids were counted as one epitope. Group 1 (immunized with Env) is shown in red, Group 2 (immunized without Env) in blue. A. The number of targeted epitopes to Gag/Pol induced by vaccination. Colored lines represent means, black lines represent medians. B. Reverse cumulative distribution function plot showing the proportion of participants on the y-axis with a response greater than or equal to the number

of epitopes shown on the x-axis. The dotted horizontal line separates non-responders to the left (breadth 0) from responders targeting at least one epitope. Percentages in the key denote the proportion of responders.

Table 1.

Baseline characteristics of the intent-to-treat population.

	Group 1: With Env (n=50)	Group 2: Without Env (n=50)	Total (n=100)
Sex			
Female	43 (86%)	39 (78%)	82 (82%)
Race/ethnicity			
White	27 (54%)	30 (60%)	57 (57%)
Black/African American	2 (4%)	2 (4%)	4 (4%)
Hispanic	17 (34%)	17 (34%)	34 (34%)
Asian	3 (6%)	0 (0%)	3 (3%)
Other*	1 (2%)	1 (2%)	2 (2%)
Age (years)	22 (18 – 48)	21 (18 – 43)	21 (18–48)

Data are n (%), median (range).