Supplemental Material for:

First-in-Human Evaluation of a Hexon Chimeric Adenovirus Vector Expressing HIV-1 Env (IPCAVD 002)

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Methods

Vaccine

The Ad5HVR48.ENVA.01 vaccine product was produced in complementing HER96 cells by Crucell Holland BV, Leiden, Netherlands. A replication deficient (deletion in the early region 1/early region 3 [ΔE1/E3]) recombinant adenoviral vector type 5 (rAd5) with hexon hypervariable regions (HVR) from Ad48 was constructed to contain an HIV-1 Clade A Env gene encoding a modified envelope gp140 protein. The Ad5HVR48 vector is a modified rAd5 vector in which the seven short Ad5 hexon hypervariable regions have been exchanged with the corresponding regions from the rare serotype virus Ad48. The following Ad5 hexon hypervariable regions were modified: hexon amino acids 136-165 (HVR1), 188-194 (HVR2), 212-220 (HVR3), 248-258 (HVR4), 268-281 (HVR5), 305-310 (HVR6), and 418-451 (HVR7). To create the pWE.Ad5HVR48.AflII-rITR.dE3 cosmid required for the generation of Ad5HVR48 vectors, the modified hexon gene was produced synthetically (GeneART, Regensburg, Germany) and used to replace the corresponding region in the Ad5 cosmid pWE.Ad5.AflII-rITR.dE3. The basis for the generation of the Ad5HVR48.ENVA.01 vaccine vector is a gene encoding the HIV-1 clade A gp140 envelope protein that was derived from plasmid VRC5304 [1, 2]. The protein sequence of the envelope protein (gp160) from 92rw020 (R5-tropic, GenBank accession number U08794) was used to create a synthetic version of the gene (Clade A gp140delCFI) using codons altered for optimal expression in human cells. The HIV-1 Env A gp140 sequence was cloned into Crucell adapter pAdapt5 using restriction sites Xba I (blunted) and BamH I to place it in the Hind III (blunted) and BamH I digested pAdapt5 expression cassette under transcriptional control of the human cytomegalovirus

(CMV) promoter 37 and the SV-40 polyadenylation (pA) sequence. The pAdapt5 was derived from the commercially available pBR322 and modified to contain Ad5 specific sequences as well as the expression cassette. The pAdapt5 plasmid containing the EnvA insert was used to generate the Ad5HVR48.ENVA.01 vaccine in HER96 cells. The placebo was final formulation buffer.

Safety Assessments

To assess safety, subjects were provided a diary card on which they recorded local and systemic reactogenicity for 7 days post-vaccination. Viral cultures (throat, urine) for adenovirus were performed in the CLIA-approved clinical virology laboratory at Brigham and Women's Hospital for any subject with an illness suspected to be of viral origin. Safety laboratory studies were assessed on days 14, 42, 168, 182 and 365 and included: complete blood count, PT/PTT, chemistries and urine analysis. All female subjects had a negative pregnancy test prior to each vaccination. Reactogenicity and adverse events (AEs) were assessed as per the NIAID Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004 (Clarification August 2009), available on the RCC website at http://rcc.tech-res-intl.com.

Results

Supplemental Safety Data

No serious adverse events (SAEs) occurred. At least 1 adverse event (AE) occurred in 85% (34 of 40) of vaccinees and 63% (5 of 8) of placebo recipients. Five subjects had a greater than moderate AE and all were assessed as unrelated to vaccination: viral syndrome ($10^9 \times 3$ group), depression ($10^{10} \times 3$ group), kidney stone ($10^{11} \times 3$ group), deep venous thrombosis (placebo) and back pain (placebo). No significant laboratory abnormalities were observed. All of the grade 2 or higher AEs were deemed probably not or not related to study vaccination.

The systemic reactogenicity pattern noted with the first dose generally occurred within the first 24-48 hours following vaccination and typically resolved spontaneously within another day or two. The local reactogenicity symptoms were noted to occur within 1-2 days of vaccination and resolved over four to seven days. Other than the self-limited systemic reactogenicity associated with the initial dose of Ad5HVR48.ENVA.01 at 10^{11} viral particles (vp), no other pattern of clinical or laboratory AEs was identified. This reactogenicity pattern is similar to that reported for Ad5, Ad26, and Ad35 vectors at this same dose [1, 3-6].

Eighteen clinically based throat cultures for adenovirus were obtained, 12 were in vaccinees (10 subjects; range 1-2) and 6 in placebo recipients (4 subjects; range 1-2). All adenovirus cultures were negative.

HIV-1 UG37 Env-Specific Antibody Responses

Compared with the homologous EnvA protein, ELISA response rates to a heterologous clade A envelope protein, UG37, were slightly lower. **Supplemental Figure 2** shows the kinetics of the response by dose group. At week 4 the response rates were 40, 90, 90, and 60% and at week 8 they were 80, 100, 100, and 70% respectively in the $10^9 \times 3$, $10^{10} \times 3$, $10^{11} \times 3$ and $10^{10} \times 1$ groups. At week 52 responses persisted in 60, 100, 90, and 50% of subjects in the $10^9 \times 3$, $10^{10} \times 3$, $10^{11} \times 3$ and $10^{10} \times 1$ groups respectively as well.

Furthermore, ELISA titers were also lower when assayed with the heterologous clade A envelope UG37 protein compared to the homologous EnvA protein. The geometric mean UG37 EnvA ELISA titers elicited at week 4 were 49, 138, 868, and 70 and increased slightly at week 8 after the second vaccination in the $10^9 \times 3$ and $10^{10} \times 3$ groups to 109 and 401, but was little changed in the $10^{11} \times 3$ group (GMT 868). The geometric mean UG37 EnvA ELISA titer at week 24 decreased to 39, 155, and 98 in the $10^9 \times 3$, $10^{10} \times 3$, and $10^{11} \times 3$ groups respectively indicating that many subjects had seroreverted (titer ≤ 16). These responses then increased substantially after the third vaccination to 347, 1116, and 990 in the $10^9 \times 3$, $10^{10} \times 3$, $10^{11} \times 3$ groups respectively. At week 52 modest titers persisted in all 4 groups with GMTs of 77, 351, 306, and 50 respectively.

Comparison of anti-vector NAb responses with anti-EnvA responses

We explored in detail the relationship between anti-vector Ad5 and Ad48 NAb responses and binding antibody and T cell responses directed against the HIV-1-derived EnvA immunogen to determine if nascent anti-vector responses interfered with the development of insert-specific immune responses. Only the $10^9 \times 3$, $10^{10} \times 3$, and $10^{11} \times 3$ groups were analyzed. With

respect to anti-Ad5 NAbs, there was only a modestly significant, but positive, correlation at week 28 (following the third inoculation) between anti-Ad5 NAb titer and anti-EnvA ELISA titer (**Supplemental Figure 3A**). With respect to anti-Ad48 NAbs, there were only modestly significant, but again positive, correlations at both week 8 (following the second inoculation, **Supplemental Figure 3B**) and week 28 (following the third inoculation, **Supplemental Figure 3C**) between anti-Ad48 NAb titers and cellular immune responses measured by EnvA-specific ELISPOT. When the vaccinees were subdivided by dose group and analyzed at specific time points, the only significant correlation was for Ad5 NAb titer and EnvA ELISA titer in the $10^{11} \times 3$ group at week 28 ($r=0.7416$, $p=0.0141$). No other significant differences were found.

Overall, these limited associations suggest that vector-specific humoral responses elicited by Ad5HVR48.ENVA.01 do not interfere with development of immune responses directed against the HIV-1-derived EnvA insert. In fact, most interactions observed are in the opposite direction; there is some evidence of a modest, but positive, association between anti-vector neutralization activity and immune responses elicited by the insert. Similar modest, but positive, correlations have previously been noted with a distinct vector-insert pair [7].

References

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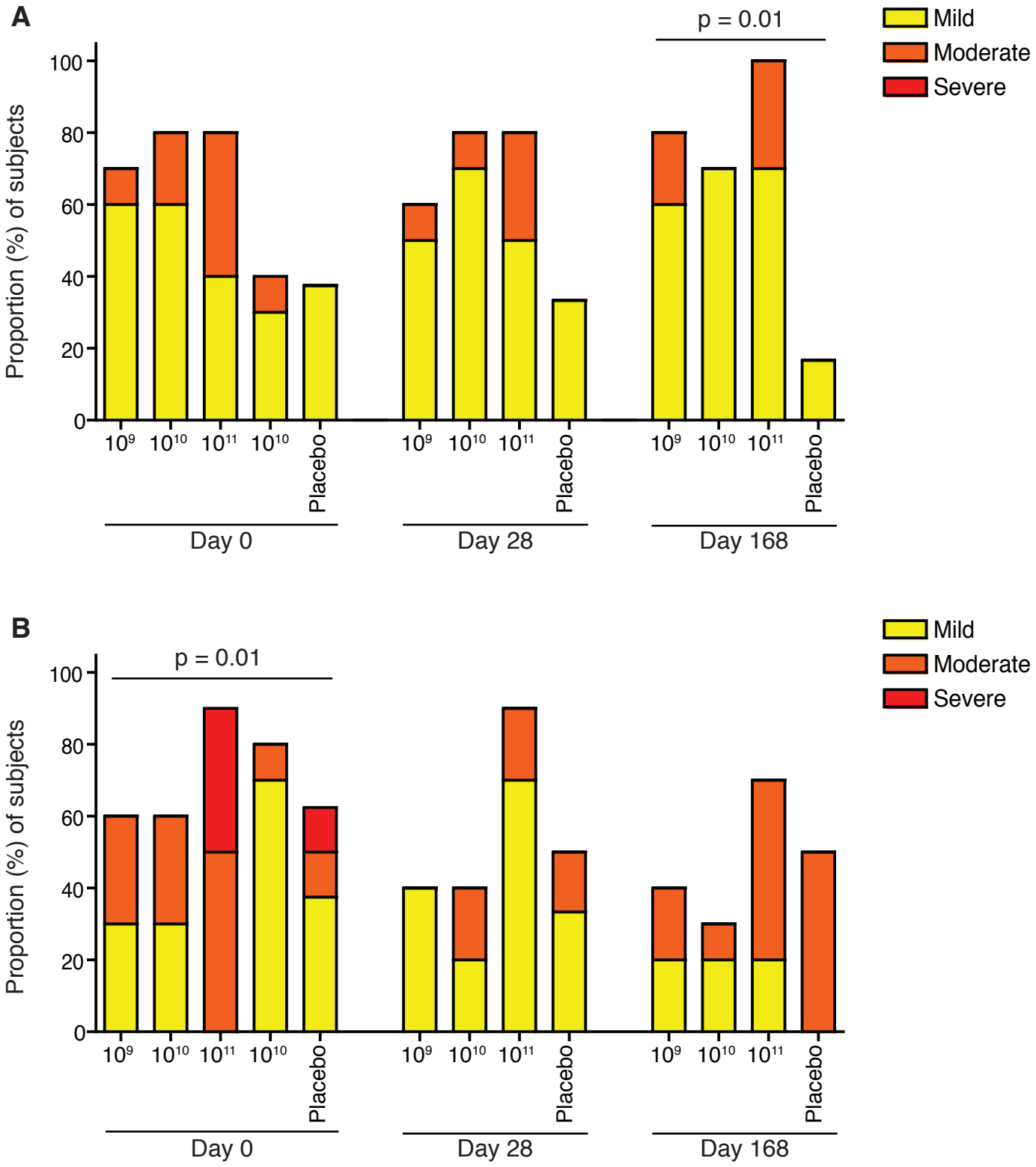
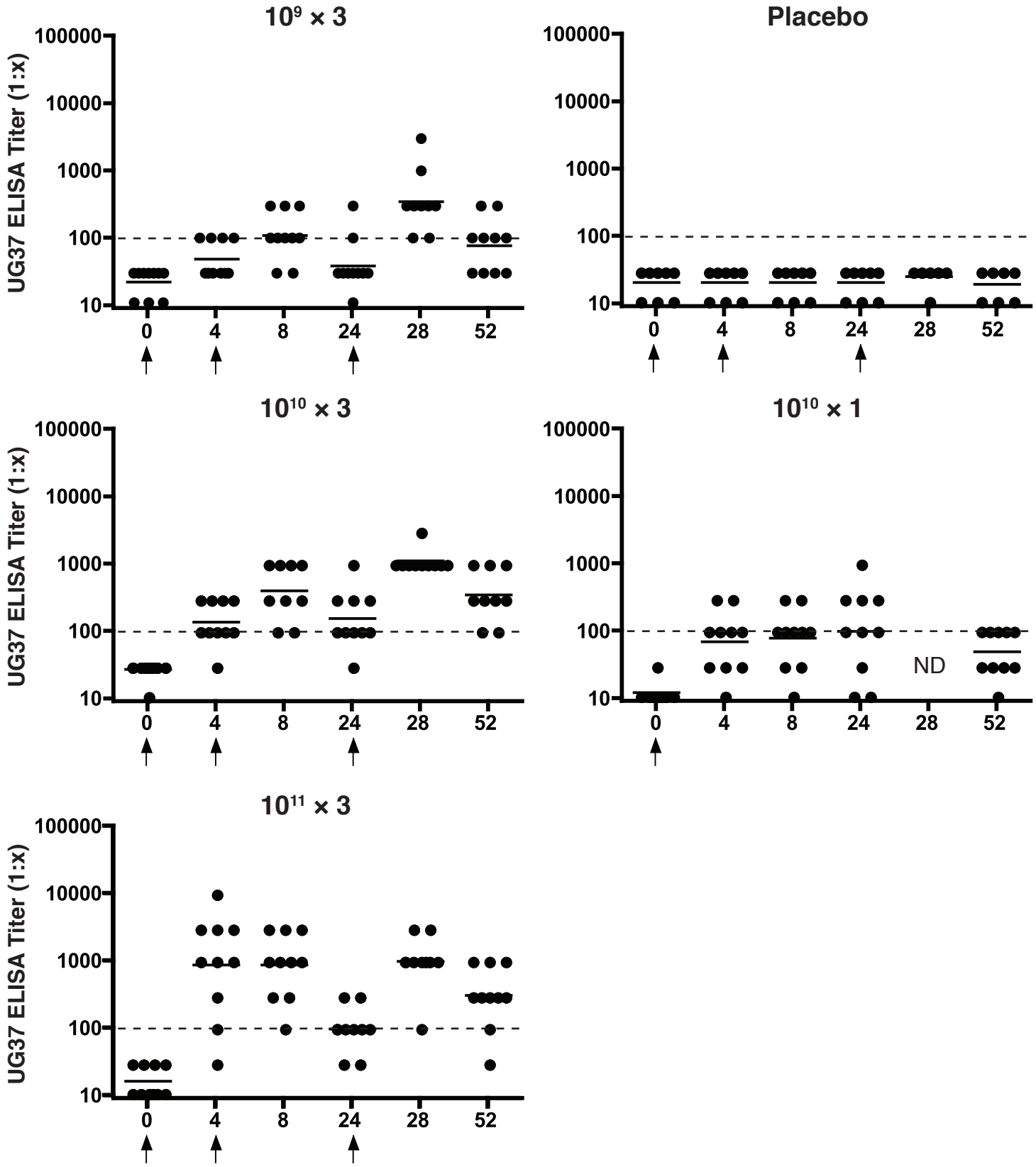
Supplemental Figure 1.



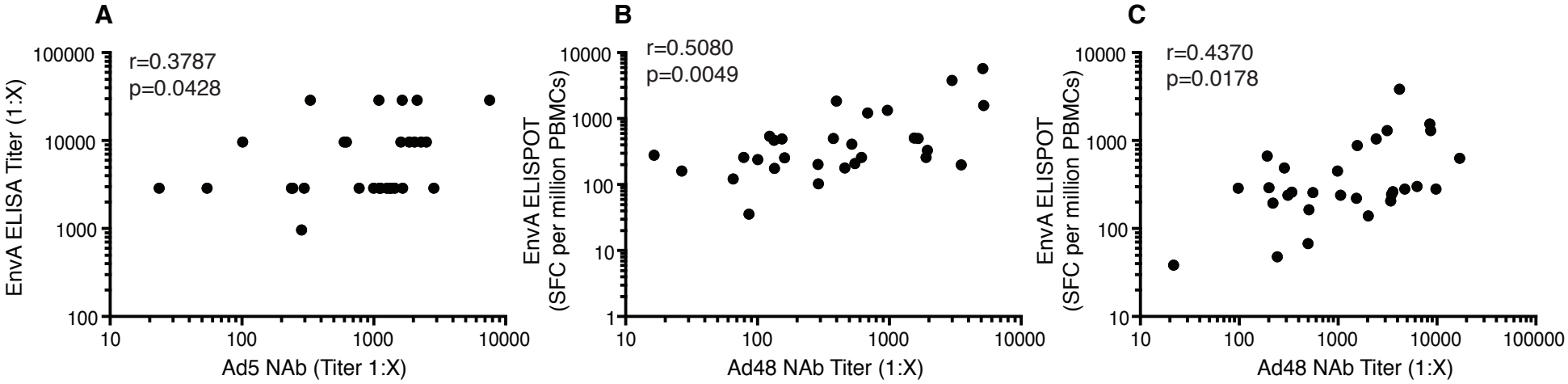
Supplemental Figure 1. Local and systemic reactogenicity. Local (Panel A) and systemic (Panel B) reactogenicity reported by subjects after each vaccination was assessed through day 7 after vaccination. Group 4 ($10^{10} \times 1$) did not have a vaccination at day 28 or day 168. The placebo recipients from all 4 study groups are pooled together (n=8 at Day 0; n=6 at Day 28 and Day 168). Groups were compared at each time point using Fisher's exact test.

Supplemental Figure 2



Supplemental Figure 2. UG37 ELISA responses by group. Individual UG37 clade A envelope ELISA responses from subjects by week and vaccine group are shown. Dots indicate individual responses at a given time point. The dashed lines indicate the cutoff for the assay. Horizontal lines indicate geometric mean titers at a given time point for the group. Arrows indicate times when vaccine or placebo were administered. ND, not done.

Supplemental Figure 3.



Supplemental Figure 3. Limited correlation between anti-vector and anti-insert immune responses. Anti-Ad5 (**A**) and anti-Ad48 neutralizing antibody (NAb) responses (**B**, **C**) were compared with EnvA-specific ELISA titers (**A**) and EnvA-specific ELISPOT responses (**B**, **C**). Week 8 (**B**) and week 28 (**A**, **C**) data are shown. Spearman correlations were used to assess the correlation between anti-vector NAb titers and the magnitude of insert-specific T cell responses or ELISA titers for vaccinees for the two assays being compared.