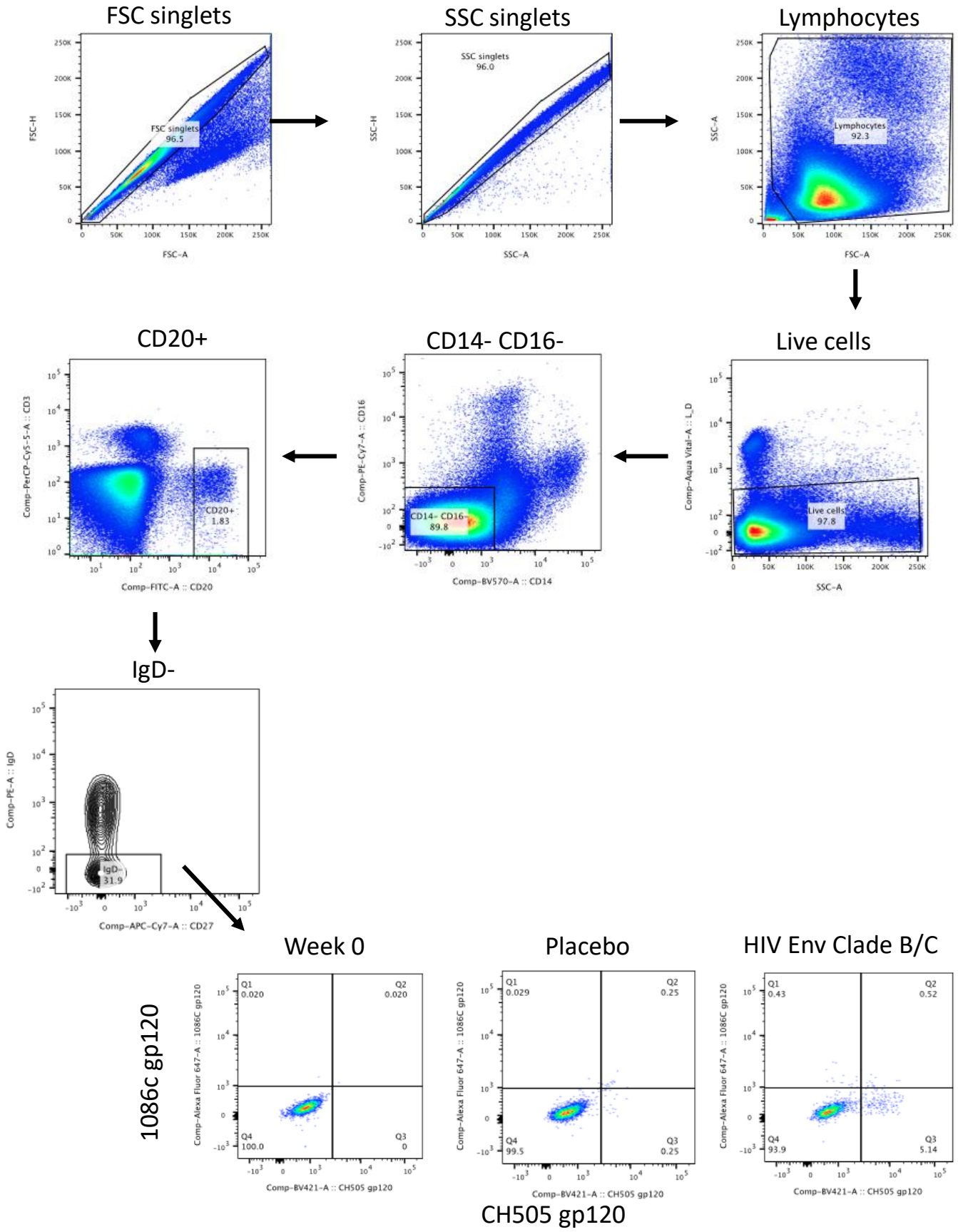
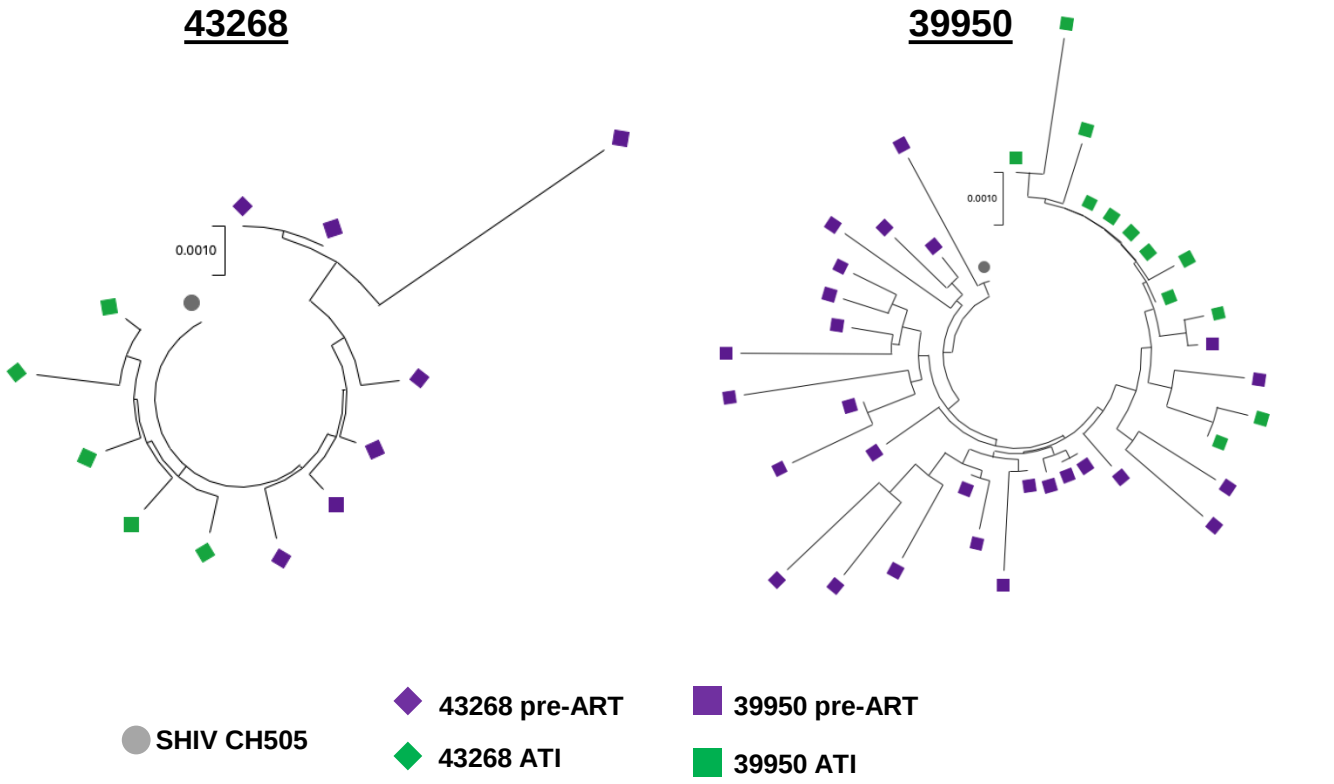



**Supplementary Figure 1.** Gating strategy used to identify CH505 and 1086c gp120-specific memory B cells. Plots are representative of one RM from each cohort.

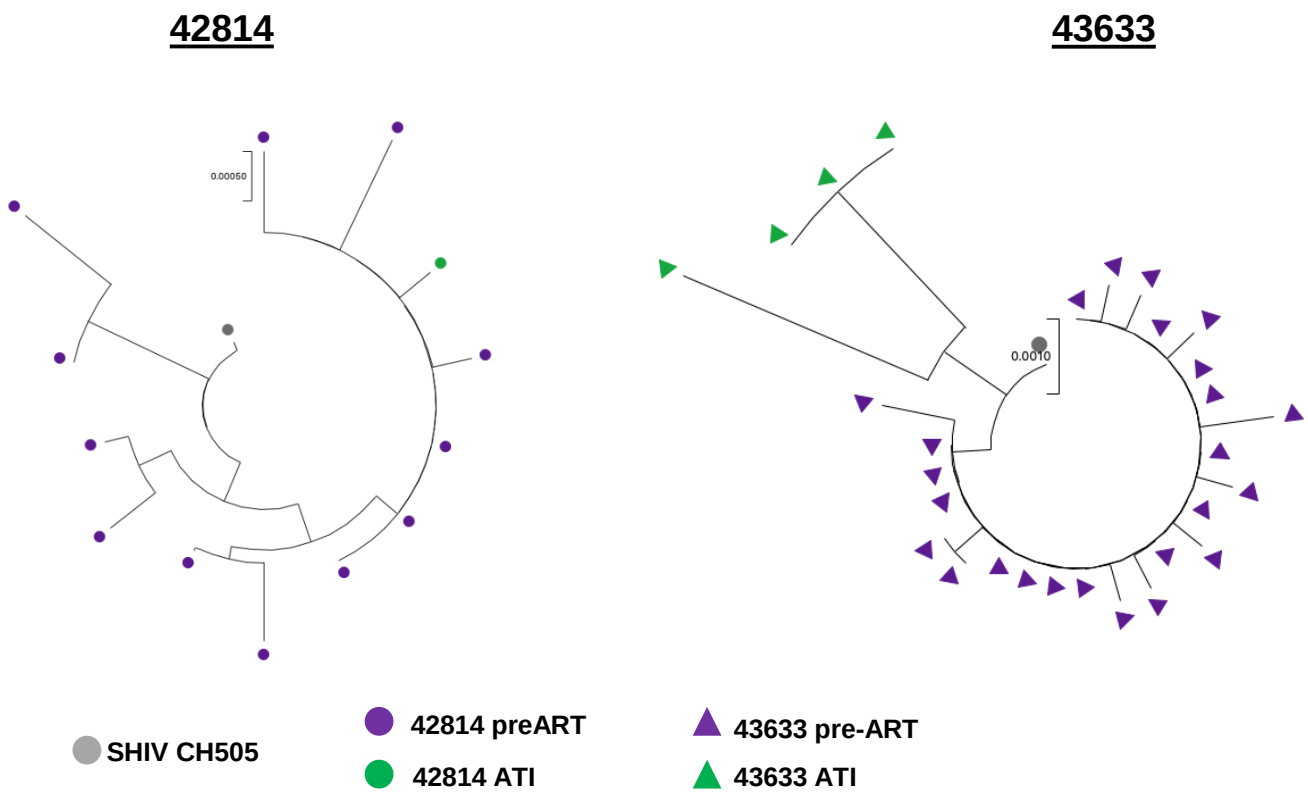
**Supplementary Table 1.** SGA summary table. Time points and viral loads at which pre- and +t-ART/immunization samples for each animal were analyzed are shown along with the number of SGAs that have been successfully isolated.

| Pre-ART/Immunization | PTID | Weeks Post-Infection | Viral Load (vRNA copies/mL) | SGAs |
|---|---|---|---|---|
| HIV Clade B/C | 42814 | 10 | 2900 | 12 |
| | 43633 | 8 | 1100 | 25 |
| Placebo | 43268 | 8 | 52000 | 9 |
| | 39950 | 12 | 28000 | 29 |
| **Rebound** | | **Weeks Post-ART** | **Viral Load (vRNA copies/mL)** | **SGAs** |
| HIV Clade B/C | 42814 | 3 (27wpi) | 3700 | 1 |
| | 43633 | 4 (28 wpi) | 1800 | 4 |
| Placebo | 43268 | 3 (27wpi) | 1500 | 6 |
| | 39950 | 2 (27 wpi) | 13000 | 14 |

# a. Placebo Phylogenetic Trees



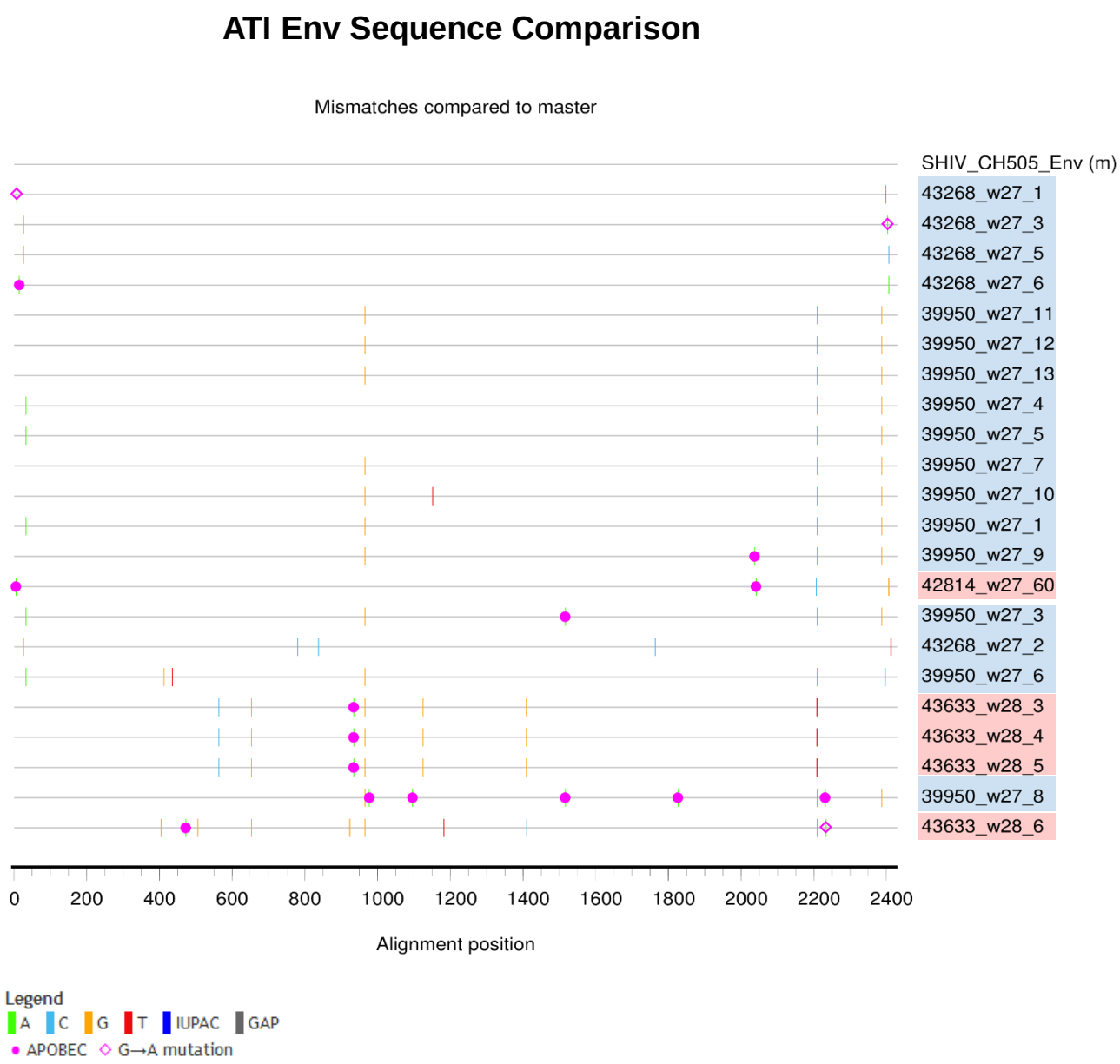

# b. HIV Env Clade B/C Phylogenetic Trees



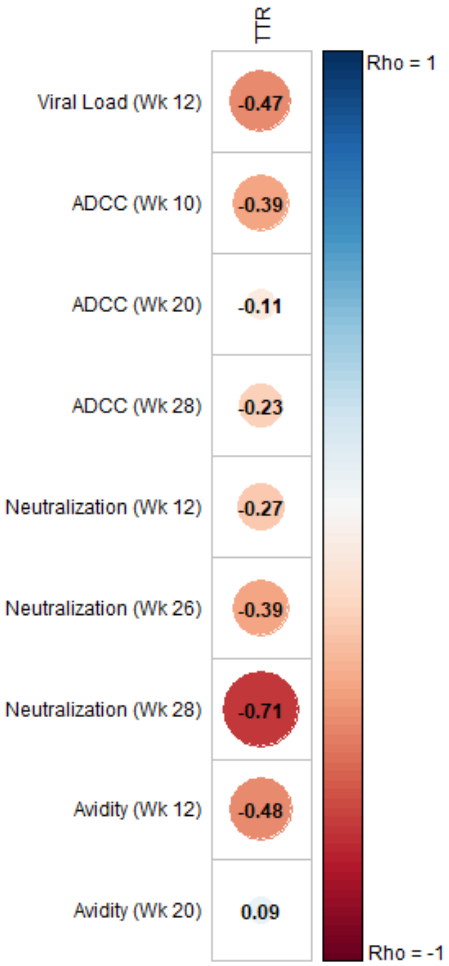
**Supplementary Figure 2**. **Neighbor-joining phylogenetic tree of pre-ART and ATI Env sequences from vaccinated and placebo RMs.** Phylogenetic trees were constructed for Placebo (A) and HIV Env vaccinated (B) RMs for nucleotide sequences of *env* gene prior to treatment initiation (purple), and during ATI (green) using the time-point of peak viral load during rebound . Each tree is rooted to the SHIV.C.CH505 challenge virus (gray).

# Pre-ART/Immunization Env Sequence Comparison

**Supplementary Figure 3. Highlighter plot of HIV Env diversity pre-ART from vaccinated and placebo RMs.** Highlighter plot comparing pre-ART/immunization (A) *env* sequences to the SHIV.C.CH505 challenge virus master *env* sequence +itioned at the top of the plot. Each colored tick mark is a mutation compared to the master sequence. HIV Env vaccinated animals are highlighted in red, and placebo recipients are highlighted in blue.




**Supplementary Figure 4. Highlighter plot of diversity among ATI *env* sequences from vaccinated and placebo RMs.** Highlighter plot comparing rebound *env* sequences to the SHIV.C.CH505 challenge virus master *env* sequence positioned at the top of the plot. Each colored tick mark is a mutation compared to the master sequence. HIV Env vaccinated animals are highlighted in red, and placebo recipients are highlighted in blue.




**Supplementary Figure 5.** Correlogram showing spearman correlations among viral load at week 12, CH505-specific ADCC antibody titers, CH505TF virus neutralization, and CH505-specific avidity scores. The size of the circle and the color (blue is a positive correlation, red is negative) indicate the strength and direction of the association, and the estimated Spearman correlation coefficient is displayed in the center.

**Supplementary Table 2. Antibodies used in flow cytometry analysis.** Table shows the list of each antibody used in flow cytometry for HIV Env-specific B cell phenotyping including the clone of each mAb used and the manufacturer.

| Marker | Fluorophore | Clone | Manufacturer | Catalogue # | Experiment | Staining |
|---|---|---|---|---|---|---|
| CD20 | FITC | L27 | BD Biosciences | 347673 | Env-specific B cell phenotyping | surface |
| CD3 | PerCP Cy5.5 | SP34-2 | BD Biosciences | 552852 | Env-specific B cell phenotyping | surface |
| IgD | PE | polyclonal | Southern Biotech | 2030-09 | Env-specific B cell phenotyping | surface |
| CD8 | PE Texas Red | 3B5 | Invitrogen | MHCD0817 | Env-specific B cell phenotyping | surface |
| IgM | PE Cy5 | G20-127 | BD Biosciences | 551079 | Env-specific B cell phenotyping | surface |
| CD16 | PE Cy7 | 3G8 | BD Biosciences | 557744 | Env-specific B cell phenotyping | surface |
| CD14 | BV570 | M5E2 | Biolegend | 301832 | Env-specific B cell phenotyping | surface |
| CD27 | APC Cy7 | O323 | Biolegend | 302816 | Env-specific B cell phenotyping | surface |

**Supplementary Table 3**. Secondary endpoint analysis comparing immune responses of primary interests between HIV Env vaccinated and placebo vaccinated RMs at specific time points. Wilcoxon rank-sum tests with exact p-values, adjusted to control the FDR at α = 0.05. FDR-adjusted p-values <0.05 are in bold.

| Assay | Outcome | Antigen/Pseudovirus | Weeks post-infection | Raw_P | fdr_p |
|---|---|---|---|---|---|
| Elisa | Concentration binding | ch505tf_gp120_293f | 12 | 0.937 | 1.000 |
| Elisa | Concentration binding | ch505tf_gp120_293f | 16 | 0.002 | **0.005** |
| Elisa | Concentration binding | ch505tf_gp120_293f | 20 | 0.002 | **0.005** |
| Elisa | Concentration binding | ch505tf_gp120_293f | 24 | 0.002 | **0.005** |
| Elisa | Concentration binding | 1086d7_gp120k160n | 12 | 0.699 | 0.961 |
| Elisa | Concentration binding | 1086d7_gp120k160n | 16 | 0.002 | **0.005** |
| Elisa | Concentration binding | 1086d7_gp120k160n | 20 | 0.002 | **0.005** |
| Elisa | Concentration binding | 1086d7_gp120k160n | 24 | 0.002 | **0.005** |
| Elisa | Concentration binding | b_63521_d11gp120 | 12 | 0.699 | 0.961 |
| Elisa | Concentration binding | b_63521_d11gp120 | 16 | 0.002 | **0.005** |
| Elisa | Concentration binding | b_63521_d11gp120 | 20 | 0.002 | **0.005** |
| Elisa | Concentration binding | b_63521_d11gp120 | 24 | 0.002 | **0.005** |
| Elisa | Concentration binding | CH505_tf_v3 | 12 | 0.937 | 1.000 |
| Elisa | Concentration binding | CH505_tf_v3 | 16 | 0.002 | **0.005** |
| Elisa | Concentration binding | CH505_tf_v3 | 20 | 0.002 | **0.005** |
| Elisa | Concentration binding | CH505_tf_v3 | 22 | 0.002 | **0.005** |
| Elisa | Concentration binding | 1086c_k160n_v3 | 12 | 0.589 | 0.863 |
| Elisa | Concentration binding | 1086c_k160n_v3 | 16 | 0.002 | **0.005** |
| Elisa | Concentration binding | 1086c_k160n_v3 | 20 | 0.002 | **0.005** |
| Elisa | Concentration binding | 1086c_k160n_v3 | 22 | 0.002 | **0.005** |
| Elisa | Concentration binding | b_63521_v3 | 12 | 0.937 | 1.000 |
| Elisa | Concentration binding | b_63521_v3 | 16 | 0.002 | **0.005** |
| Elisa | Concentration binding | b_63521_v3 | 20 | 0.002 | **0.005** |
| Elisa | Concentration binding | b_63521_v3 | 22 | 0.002 | **0.005** |
| ADCC | Endpoint titer_dilution | 1086d7_gp120k160n | 10 | 0.792 | 1.000 |
| ADCC | Endpoint titer_dilution | 1086d7_gp120k160n | 14 | 0.234 | 0.367 |
| ADCC | Endpoint titer_dilution | 1086d7_gp120k160n | 20 | 0.002 | **0.005** |
| ADCC | Endpoint titer_dilution | 1086d7_gp120k160n | 28 | 0.024 | **0.046** |
| ADCC | Endpoint titer_dilution | 1086d7_gp120k160n | 32 | 0.065 | 0.110 |
| ADCC | Endpoint titer_dilution | ch505tf_gp120_293f | 10 | 0.931 | 1.000 |
| ADCC | Endpoint titer_dilution | ch505tf_gp120_293f | 14 | 1.000 | 1.000 |
| ADCC | Endpoint titer_dilution | ch505tf_gp120_293f | 20 | 0.002 | **0.005** |
| ADCC | Endpoint titer_dilution | ch505tf_gp120_293f | 28 | 0.002 | **0.005** |
| ADCC | Endpoint titer_dilution | ch505tf_gp120_293f | 32 | 0.065 | 0.110 |
| Neutralization | ID50 | CH0505TF | 12 | 0.922 | 1.000 |
| Neutralization | ID50 | CH0505TF | 26 | 1.000 | 1.000 |
| Neutralization | ID50 | CH0505TF | 28 | 1.000 | 1.000 |
| Neutralization | ID50 | CH0505TF | 32 | 1.000 | 1.000 |
| Neutralization | ID50 | CH0505.w4.3 | 12 | 0.387 | 0.588 |
| Neutralization | ID50 | CH0505.w4.3 | 26 | 0.061 | 0.110 |
| Neutralization | ID50 | CH0505.w4.3 | 28 | 0.981 | 1.000 |
| Neutralization | ID50 | MW965.26.LucR.T2A.ecto | 12 | 0.937 | 1.000 |
| Neutralization | ID50 | MW965.26.LucR.T2A.ecto | 26 | 0.004 | **0.009** |
| Neutralization | ID50 | MW965.26.LucR.T2A.ecto | 28 | 0.180 | 0.293 |

**Supplementary Table 4**. Exploratory endpoints comparing a subset of immune responses between the two vaccine groups. Wilcoxon rank-sum tests with exact p-values, adjusted to control the FDR at α = 0.05

| Assay | Outcome | Antigen | Weeks post-infection | Raw_P | fdr_p |
|---|---|---|---|---|---|
| B cell phenotyping | CH505_gp120_sp | CH505TF_D8gp120/293F | 24 | 0.008 | 0.061 |
| B cell phenotyping | 1086c_gp120_sp | C.1086_D7gp120K160N/293F | 24 | 0.016 | 0.074 |
| Avidity | ant1_avidity score | CH505TF_D8gp120/293F | 12 | 0.937 | 0.937 |
| Avidity | ant1_avidity score | CH505TF_D8gp120/293F | 20 | 0.065 | 0.130 |
| Avidity | ant2_avidity score | C.1086_D7gp120K160N/293F | 12 | 0.699 | 0.753 |
| Avidity | ant2_avidity score | C.1086_D7gp120K160N/293F | 20 | 0.026 | 0.091 |
| Avidity | ant3_avidity score | B.63521_D11gp120_mutC/293F | 12 | 0.662 | 0.753 |
| Avidity | ant3_avidity score | B.63521_D11gp120_mutC/293F | 20 | 0.065 | 0.130 |

**Supplementary Table 5.** Primary endpoint analysis comparing vaccine (1086c and b.63521) versus challenge virus antigen (CH505) specific responses among HIV Env vaccinated RMs. Wilcoxon signed-rank tests with exact p-values , adjustments to control the FDR at α = 0.05.

| Assay | Antigen 1 | Antigen 2 | Weeks post-infection | raw_p | fdr_p |
|---|---|---|---|---|---|
| Elisa | CH505 | 1086c | 12 | 0.031 | 0.058 |
| Elisa | CH505 | 1086c | 16 | 0.031 | 0.058 |
| Elisa | CH505 | 1086c | 20 | 0.031 | 0.058 |
| Elisa | CH505 | 1086c | 24 | 0.063 | 0.074 |
| Elisa | CH505 | B63521 | 12 | 0.031 | 0.058 |
| Elisa | CH505 | B63521 | 16 | 0.031 | 0.058 |
| Elisa | CH505 | B63521 | 20 | 0.031 | 0.058 |
| Elisa | CH505 | B63521 | 24 | 0.031 | 0.058 |
| Avidity | CH505 | 1086c | 12 | 0.063 | 0.074 |
| Avidity | CH505 | 1086c | 20 | 0.313 | 0.313 |
| Avidity | CH505 | B63521 | 12 | 0.063 | 0.074 |
| Avidity | CH505 | B63521 | 20 | 0.156 | 0.169 |
| B cell phenotyping | CH505 | 1086c | 24 | 0.063 | 0.074 |