**Supplement materials**

**Supplementary tables**

**Table S1: Antibody responses at different time points in Y, M and O age groups in HIV+ and HIV**−

|  |  |
| --- | --- |
| **Geometric mean antibody titer** | **P Values** |
| **H1N1 antigen** | **T0** | **T1** | **T2** | **T3** | **T0 vs T1** | **T0 vs T2** | **T0 vs T3** | **T1 vsT2 T2** | **T1 vs T3** | **T2 vs T3** |
| HIV+ Y | 230.93 | 434.47 | 577.98 | 277.25 | **0.0084** | **0.0001** | NS | NS | NS | **0.0035** |
| HIV+ M | 191.22 | 423.12 | 457.05 | 276.25 | **0.0001** | **<0.0001** | NS | NS | NS | **0.0092** |
| HIV+ O | 122.95 | 237.76 | 324.47 | 234.25 | **0.0155** | **0.0003** | **0.0479** | NS | NS | NS |
| HIV− Y | 201.59 | 476.52 | 866.72 | 382.34 | **0.0008** | **<0.0001** | **0.0112** | **0.0199** | NS | **<0.0001** |
| HIV− M | 93.79 | 331.10 | 647.31 | 332.11 | **<0.0001** | **<0.0001** | **<0.0001** | **0.0006** | NS | **0.0009** |
| HIV− O | 109.36 | 269.09 | 356.76 | 201.59 | **<0.0001** | **<0.0001** | **0.0133** | NS | NS | **0.0204** |
| **B antigen** |
| HIV+ Y | 196.18 | 384.45 | 554.89 | 343.79 | **0.0012** | **<0.0001** | **0.0021** | NS | NS | NS |
| HIV+ M | 176.65 | 354.88 | 452.55 | 366.81 | **<0.0001** | **<0.0001** | **<0.0001** | NS | NS | NS |
| HIV+ O | 131.77 | 281.75 | 383.19 | 298.57 | **0.006** | **0.0001** | **0.0011** | NS | NS | NS |
| HIV− Y | 219.83 | 512.99 | 1045.71 | 746.58 | **<0.0001** | **<0.0001** | **<0.0001** | **0.0002** | **0.0485** | NS |
| HIV− M | 147.77 | 334.88 | 632.77 | 458.18 | **<0.0001** | **<0.0001** | **<0.0001** | **0.0002** | NS | NS |
| HIV− O | 155.71 | 320.00 | 455.63 | 241.15 | **0.0014** | **<0.0001** | **0.0063** | NS | NS | NS |
| **H3N2 antigen** |
| HIV+ Y | 135.92 | 217.23 | 288.99 | 195.67 | **0.0207** | **0.0042** | NS | NS | NS | **0.0361** |
| HIV+ M | 123.68 | 229.81 | 289.83 | 206.07 | **0.0091** | **0.0011** | **0.0490** | NS | NS | NS |
| HIV+ O | 98.49 | 176.65 | 274.74 | 205.87 | **0.0083** | **0.0001** | **0.0086** | NS | NS | NS |
| HIV− Y | 128.84 | 280.22 | 403.17 | 206.98 | **0.0026** | **<0.0001** | NS | **0.0465** | NS | **0.0008** |
| HIV− M | 85.64 | 191.90 | 282.40 | 133.58 | **<0.0001** | **<0.0001** | **0.0050** | NS | NS | **0.0039** |
| HIV− O | 126.99 | 293.44 | 431.53 | 262.51 | **0.0142** | **<0.0001** | NS | NS | NS | **0.0061** |

NS: not significant; Y=young, M=middle age, O=old.

 **Table S2: Comparisons of antibody responses between age groups and between HIV groups**

|  |
| --- |
| **Comparison between Y, M and O age groups for HIV+ or HIV**− **at each time point** |
|  | **T0** | **T1** | **T2** | **T3** |
| **Antigen : group** | **Y vs M** | **Y vs. O** | **M vs. O** | **Y vs M** | **Y vs. O** | **M vs. O** | **Y vs M** | **Y vs. O** | **M vs. O** | **Y vs M** | **Y vs. O** | **M vs. O** |
| H1N1: HIV+ | NS | **0.0162** | NS | NS | **0.0164** | **0.0166** | NS | **0.0104** | NS | NS | NS | NS |
| H1N1: HIV− | **0.0053** | **0.0303** | NS | NS | **0.0294** | NS | NS | **0.0004** | **0.0182** | NS | **0.0121** | **0.0167** |
| B: HIV+ | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| B: HIV− | **0.0342** | NS | NS | **0.0033** | **0.0183** | NS | **0.0011** | **<0.0001** | NS | **0.0050** | **0.0023** | NS |
| H3N2: HIV+ | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| H3N2: HIV− | **0.0145** | NS | **0.0250** | NS | NS | **0.0114** | **0.0330** | NS | **0.0084** | NS | NS | NS |
| **Comparison between HIV+ vs. HIV− in different age groups at each time point** |
| **Age groups** | **T0** | **T1** | **T2** | **T3** |
|  | **H1N1** | **B** | **H3N2** | **H1N1** | **B** | **H3N2** | **H1N1** | **B** | **H3N2** | **H1N1** | **B** | **H3N2** |
| HIV+ vs. HIV−: Y | NS | NS | NS | NS | NS | NS | NS | **0.013** | **0.038** | **0.0463** | **0.0342** | NS |
| HIV+ vs. HIV−: M | **0.0002** | NS | **0.0411** | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| HIV+ vs. HIV−: O | NS | NS | NS | NS | NS | **0.0280** | NS | NS | **0.001** | NS | NS | NS |
| HIV+Yvs. HIV−: M | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| HIV+Yvs. HIV−: O | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| HIV+Mvs. HIV−: Y | NS | NS | NS | NS | NS | NS | NS | **0.0026** | NS | NS | NS | NS |
| HIV+Mvs. HIV−: O | NS | NS | NS | NS | NS | **0.0498** | NS | NS | **0.004** | NS | NS | NS |
| HIV+Ovs. HIV−: Y | NS | NS | NS | **0.0179** | NS | NS | **0.0103** | **0.0091** | NS | NS | **0.0072** | NS |
| HIV+Ovs. HIV−: M | NS | NS | NS | NS | NS | NS | **0.0437** | NS | NS | NS | **0.0009** | NS |

**Table S3: Regression analysis: Contribution to WV titer by individual vaccine antigen titers in young, middle aged and old HIV+ groups at T0, T1, T2 and T3.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Measures** | **β Coefficient** | **P value** | **Product Measure** |
| **T0: HIV+ Y** |
| Intercept | 4.51E-16 | 1 | Total: 0.5374 |
| H1N1\_T0 | 0.4466 | **0.00404** | 0.287 (53.5%) |
| B\_T0 | 0.3353 | **0.02586** | 0.1956 (36.3%) |
| H3N2\_T0 | 0.1746 | 0.17874 | 0.0547 (10%) |
| **T0: HIV+ M** |
| Intercept | -2.06E-18 | 1 | Total: 0.4777 |
| H1N1\_T0 | 0.4349 | **<0.0001** | 0.2672 (55.9%) |
| B\_T0 | 0.2891 | **0.0048** | 0.1497 (31.2%) |
| H3N2\_T0 | 0.1432 | 0.1544 | 0.0608 (12.7%) |
| **T0: HIV+ O** |
| Intercept | -4.98E-17 | 1 | Total: 0.7097 |
| H1N1\_T0 | 0.3154 | **0.0040** | 0.2185 (30.7%) |
| B\_T0 | 0.4293 | **0.0002** | 0.3213(45.2%) |
| H3N2\_T0 | 0.2407 | **0.0389** | 0.1699 (23.8%) |
| **T1: HIV+ Y** |
| Intercept | -3.04E-16 | 1 | Total: 0.6388 |
| H1N1\_T1 | 0.1526 | 0.2435 | 0.0790 (12.3%) |
| B\_T1 | 0.6880 | **<0.0001** | 0.5346 (83.6%) |
| H3N2\_T1 | 0.1129 | 0.3214 | 0.0252 (3.9%) |
| **T1: HIV+ M** |
| Intercept | -3.37E-17 | 1 | Total: 0.4955 |
| H1N1\_T1 | 0.4767 | **<0.0001** | 0.3086 (62.2%) |
| B\_T1 | 0.2311 | **0.0271** | 0.1156 (23.2%) |
| H3N2\_T1 | 0.1522 | 0.1477 | 0.0713 (14.3%) |
| **T1: HIV+ O** |
| Intercept | 3.79E-16 | 1 | Total: 0.5954 |
| H1N1\_T1 | 0.2183 | 0.0581 | 0.1216 (21.1%) |
| B\_T1 | 0.4103 | **0.0020** | 0.2856 (47.8%) |
| H3N2\_T1 | 0.2912 | **0.0235** | 0.1883 (37.9%) |
| **T2: HIV+ Y** |
| Intercept | -1.76E-16 | 1 | Total: 0.6616 |
| H1N1\_T2 | 0.3676 | **0.0045** | 0.2300 (34.7%) |
| B\_T2 | 0.5350 | **0.0001** | 0.3919 (59.1%) |
| H3N2\_T2 | 0.1407 | 0.2045 | 0.0397 (5.9%) |
| **T2: HIV+ M** |
| Intercept | -3.34E-16 | 1 | Total: 0.3885 |
| H1N1\_T2 | 0.5076 | **<0.0001** | 0.3056 (78.6%) |
| B\_T2 | 0.1451 | 0.2053 | 0.0607 (15.4%) |
| H3N2\_T2 | 0.0770 | 0.4647 | 0.0223 (57.2%) |
| **T2: HIV+ O** |
| Intercept | 2.46E-16 | 1 | Total: 0.6305 |
| H1N1\_T2 | 0.2663 | **0.0106** | 0.1460 (23.2%) |
| B\_T2 | 0.3853 | **0.0016** | 0.2664 (42.2%) |
| H3N2\_T2 | 0.3337 | **0.0044** | 0.2181(34.6%) |
| **T3: HIV+ Y** |
| Intercept | 5.78E-16 | 1 | Total: 0.4736 |
| H1N1\_T3 | 0.3795 | **0.0230** | 0.2081 (43.9%) |
| B\_T3 | 0.3148 | 0.0745 | 0.1709 (35.9%) |
| H3N2\_T3 | 0.2066 | 0.2223 | 0.0946 (19.8%) |
| **T3: HIV+ M** |
| Intercept | -9.63E-17 | 1 | Total: 0.3837 |
| H1N1\_T3 | 0.4027 | **0.0006** | 0.2080 (54.3%) |
| B\_T3 | 0.3744 | **0.0013** | 0.1866 (48.5) |
| H3N2\_T3 | -0.0402 | 0.7262 | 0.0109 (2.6%) |
| **T3: HIV+ O** |
| Intercept | -2.76E-16 | 1 | Total: 0.4225 |
| H1N1\_T3 | 0.3669 | **0.0141** | 0.1846 (43.6%) |
| B\_T3 | 0.3648 | **0.0139** | 0.1823 (43.1%) |
| H3N2\_T3 | 0.1237 | 0.4280 | 0.0556 (13%) |

Product measuresfrom standardized coefficient (β coefficient) and Pearson correlation coefficient were calculated to identify the proportion of response to each antigen (H1N1, B, H3N2) contributing to variance of WV response. A p value <0.05 considered significant. Values shown in parenthesis indicate the percent product measure for a particular antigen contributing to the total product measure for the whole vaccine.

**Table S4; Regression analysis: Contribution to WV titer by individual vaccine antigen titers in young, middle aged and old HIV**− **groups at T0, T1, T2 and T3.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Measures** | **β Coefficient** | **P value** | **Product Measure** |
| **T0: HIV− Y** |
| Intercept | 2.29E-17 | 1 | Total: 0.5787 |
| H1N1\_T0 | 0.3935 | **0.0011** | 0.2510 (43.4%) |
| B\_T0 | 0.3746 | **0.0022** | 0.2417 (41.6%) |
| H3N2\_T0 | 0.2111 | **0.0412** | 0.0861 (14.8%) |
| **T0: HIV− M** |
| Intercept | -6.99E-05 | 0.9995 | Total: 0.3678 |
| H1N1\_T0 | 0.2026 | 0.0783 | 0.0815 (22%) |
| B\_T0 | 0.4725 | **<0.0001** | 0.2670 (72.7%) |
| H3N2\_T0 | 0.1181 | 0.2766 | 0.0192 (5.1%) |
| **T0: HIV− O** |
| Intercept | -7.69E-17 | 1 | Total: 0.6622 |
| H1N1\_T0 | 0.4071 | **<0.0001** | 0.2447 (36.8%) |
| B\_T0 | 0.3309 | **0.0009** | 0.1941(29.3%) |
| H3N2\_T0 | 0.3824 | **0.0001** | 0.2234 (33.6%) |
| **T1: HIV− Y** |
| Intercept | 3.21E-16 | 1 | Total: 0.6877 |
| H1N1\_T1 | 0.4534 | **<0.0001** | 0.3161 (45.9%) |
| B\_T1 | 0.4081 | **0.0003** | 0.2913 (42.3%) |
| H3N2\_T1 | 0.1787 | 0.0591 | 0.0804 (11.6%) |
| **T1: HIV− M** |
| Intercept | 0.0156 | 0.871 | Total: 0.3834 |
| H1N1\_T1 | 0.2004 | **0.0995** | 0.0865 (20.8%) |
| B\_T1 | 0.4065 | **0.0005** | 0.2078 (54%) |
| H3N2\_T1 | 0.2317 | **0.0493** | 0.0890 (23.2%) |
| **T1: HIV− O** |
| Intercept | 1.71E-18 | 1 | Total: 0.6446 |
| H1N1\_T1 | 0.5207 | **<0.0001** | 0.3436 (53.2%) |
| B\_T1 | 0.3975 | **0.0002** | 0.2232 (34.6%) |
| H3N2\_T1 | 0.1576 | 0.1292 | 0.0777 (11.9%) |
| **T2: HIV− Y** |
| Intercept | 2.31E-16 | 1 | Total: 0.5204 |
| H1N1\_T2 | 0.4603 | **0.0001** | 0.2706 (51.9%) |
| B\_T2 | 0.3728 | **0.0018** | 0.1960 (37.6%) |
| H3N2\_T2 | 0.1357 | 0.2406 | 0.0539 (10%) |
| **T2: HIV− M** |
| Intercept | 0.0367 | 0.7028 | Total: 0.5133 |
| H1N1\_T2 | 0.2437 | **0.0198** | 0.0975 (18.9%) |
| B\_T2 | 0.4934 | **<0.0001** | 0.2751(53.6%) |
| H3N2\_T2 | 0.2870 | **0.0088** | 0.1407 (27.2%) |
| **T2: HIV− O** |
| Intercept | -4.36E-16 | 1 | Total: 0.6374 |
| H1N1\_T2 | 0.5190 | **<0.0001** | 0.3579 (56%) |
| B\_T2 | 0.2846 | **0.0053** | 0.1512 (23.5%) |
| H3N2\_T2 | 0.2365 | **0.0224** | 0.1282 (20%) |
| **T3: HIV− Y** |
| Intercept | -2.58E-16 | 1 | Total: 0.4781 |
| H1N1\_T3 | 0.2672 | 0.0693 | 0.1338 (27.8%) |
| B\_T3 | 0.4837 | **0.0016** | 0.2886 (60%) |
| H3N2\_T3 | 0.2087 | 0.1182 | 0.0557 (11.5%) |
| **T3: HIV− M** |
| Intercept | 0.0732 | 0.4792 | Total: 0.3843 |
| H1N1\_T3 | 0.1763 | 0.1054 | 0.0615 (15.6%) |
| B\_T3 | 0.3501 | **0.0047** | 0.1230 (32%) |
| H3N2\_T3 | 0.4019 | **0.0004** | 0.1998 (49.4%) |
| **T3: HIV− O** |
| Intercept | 4.20E-16 | 1 | Total: 0.4816 |
| H1N1\_T3 | 0.5070 | **<0.0001** | 0.2694 (55.9%) |
| B\_T3 | 0.4119 | **0.0004** | 0.1872 (38.8%) |
| H3N2\_T3 | 0.1763 | 0.1081 | 0.0250 (5.2%) |

Product measuresfrom standardized coefficient (β coefficient) and Pearson correlation coefficient were calculated to identify the proportion of response to each antigen (H1N1, B, H3N2) contributing to variance of WV response. A p value <0.05 considered significant. Values shown in parenthesis indicate the percent product measure for a particular antigen contributing to the total product measure for the whole vaccine.

**Table S5: multiple univariant linear regression analysis between titer and age, T0 CD4 count, CD4/CD8 ratio**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Titer ~ Age + T0 CD4count + T0 CD4/CD8 ratio** | **HIV−**p value | **HIV−**R square (%) | **HIV+**p value | **HIV+**R square (%) |
| H1N1 T0 | **0.0066** | 9.1485 | 0.1786 | 1.7847 |
| H1N1 T1 | **<0.0001** | 20.8979 | **0.0251** | 6.0787 |
| H1N1 T2 | **<0.0001** | 25.0125 | **0.0069** | 8.3793 |
| H1N1 T3 | **0.0073** | 10.7498 | 0.7476 | -1.8458 |
| B T0 | 0.3909 | 0.0360 | 0.5858 | -0.9690 |
| B T1 | 0.3666 | 0.2168 | 0.1754 | 1.9319 |
| B T2 | **0.0001** | 16.9648 | **0.0280** | 5.6903 |
| B T3 | **0.0029** | 12.7429 | 0.5735 | -1.0256 |
| B T3/T0 | 0.0904 | 4.27615 | 0.7819 | -1.9981 |

Titer ~ Age + T0 CD4count + T0 CD4/CD8 ratio: Formula for linear regression

**Supplement figures**



**Figure S1: Pre-vaccination Ab responses for H1N1, B and H3N2 did not differ significantly between seasons:** Antibody titers at T0 for H1N1, B and H3N2 for each season (2013-2014, 2014-2015 and 2015-2016) were determined by hemagglutination inhibition young (Y), middle aged (M) and old (O) HIV+ and HIV− groups. Scatter plots indicate the GMT for HIV+ (A, C, E) and HIV− (B, D, F) with error bars showing mean ±SD. P values were calculated using Student’s *t*-test and significant (p<0.05) differences are indicated by black horizontal bars over 2 groups.



**Figure S2: Baseline HAI response inversely correlates with fold change response at T2 over T0 in HIV**− **and HIV+ for H1N1, B and H3N2 antigens:** Significant inverse correlation is evident between Log2 HAI responses at T0 with fold changre response at T2 over T0in HIV− and HIV+ for **A)**, H1N1; **B)**, B; **C)**, H3N2. All correlations were performed by Pearson correlation and a p value of <0.05 was considered significant. Closed circles indicate HIV+ and open circles indicate HIV-



**Figure S3: Higher CMV seropositivity in HIV+ groups:** CMV seropositivity was determined in plasma by ELISA. Proportion of CMV seropositive individuals within HIV+ and HIV− A); young, B) middle aged and C); older adults. Data analyzed by Chi-squared test with Yates' continuity correction. \* p<0.01; \*\* p<0.001; \*\*\*p<0.0001.