Supplementary information

Simian immunodeficiency virus infection evades vaccine-elicited antibody responses to V2 region

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Table S1.
Probability of antibody response against SIVmac239 antigenic region and domain (AUC).

Table S2.
Multivariate bootstrap linear regression analysis of the role of various HIV-specific immune responses in controlling viremia in vaccinated monkeys

Figure S1
Yeast surface display of a combinational antigen library of the full length SIVmac239-Env.

Figure S2
Comparison of vaccine-elicited antibody responses in G1 and G2 macaques.

Figure S3. Anti-V1V2 binding antibody responses by the YSD-based FACS titration assay

Supplemental information for Methods
Enzyme-linked immunosorbent assay
SIV neutralization assay

Supplemental References
Table S1. Probability of antibody response against SIV<sub>mac239</sub> antigenic region and domain (AUC)

<table>
<thead>
<tr>
<th>Regions or Domains</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
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<tbody>
<tr>
<td>gp120</td>
<td>60</td>
<td>46</td>
<td>55</td>
<td>49</td>
<td>53</td>
<td>24</td>
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<tr>
<td>gp41</td>
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<td>54</td>
<td>45</td>
<td>51</td>
<td>47</td>
<td>76</td>
</tr>
<tr>
<td>Long fragments (&gt;100 residues)</td>
<td>54</td>
<td>55</td>
<td>59</td>
<td>53</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>Short fragments (&lt;100 residues)</td>
<td>46</td>
<td>45</td>
<td>41</td>
<td>47</td>
<td>40</td>
<td>37</td>
</tr>
</tbody>
</table>

* The stretch of residues indicating the MADs of specific antigenic domains is shown.

Table S2. Multivariate bootstrap linear regression analysis of the role of various HIV-specific immune responses in controlling viremia in vaccinated monkeys

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Set-point viral load</th>
<th>Peak viral load</th>
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<tbody>
<tr>
<td></td>
<td>β coefficient</td>
<td>p value</td>
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<tr>
<td>Polyfunctional CD8&lt;sup&gt;+&lt;/sup&gt; Tem</td>
<td>0.2324</td>
<td>0.607</td>
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<td>Elispot response to Gag</td>
<td>-3.5304</td>
<td>0.1441</td>
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<tr>
<td>Elispot response to Pol</td>
<td>1.3534</td>
<td>0.2213</td>
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<tr>
<td>Anti-V2 antibody titer</td>
<td>0.1562</td>
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<tr>
<td>Anti-V1V2 antibody titer</td>
<td>0.2883</td>
<td>0.485</td>
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</table>
Figure S1. Yeast surface display of a combinational antigen library of the full length SIV$_{mac239}$-Env.  

(A) Generation of SIV$_{mac239}$-env gene fragments for library construction. The procedure includes PCR amplification of full-length SIV-env gene (lane 1), DNase I digestion of env gene into small fragments less than 100bp (lane 2), reassembling the digested products by PCR into larger fragments (lane 3, 100-300bp; lane 4, 200-1000bp). (B) Confirmation of the frequency and coverage of SIV$_{mac239}$-env library. Individual yeast clones were selected for sequence analysis. Overlapping nucleotide sequences indicate the complete coverage of the full-length SIV$_{mac239}$-env sequence by the randomly constructed antigen library. (C) Evaluation of FACS-sorted positive yeast clones under the fluorescence microscope. (D) FACS verification of individual antibody reactive yeast clones.
Figure S2. Comparison of vaccine-elicited antibody responses in G1 and G2 macaques. (A) The level of neutralizing antibodies against tier 1 virus SIV$_{mac1A11}$. (B) The level of binding antibodies against whole SIV$_{mac239}$ virus. (C) The binding antibody titers against V1V2 peptide.

Figure S3. Anti-V1V2 binding antibody responses by the YSD-based FACS titration assay. Monkey sera II-MVTT/Ad5 were collected from vaccine study II (week 2 post boost vaccination).
Supplemental information for Methods:

Enzyme-linked immunosorbent assay

The serum-binding antibodies against SIV_{mac239} were determined by an enzyme-linked immunosorbent assay (ELISA) targeting lysed SIV_{mac239} particles as described previously \(^1\). The SIV_{mac239} V1V2-specific binding antibodies in monkey serum were also measured by ELISA. Streptavidin (Sigma) was diluted into 5μg/ml (in ddH2O) and coated in 96-well Elisa plate (Corning, NY) with 100μl/well. The plate was then exposed at 37°C to dry (overnight). The plate was blocked by protein-free blocking buffer (Thermo, pierce, +T20) with 200μl/well, incubate at 20°C for 1 hour, and then washed with PBS containing 0.05% Tween 20 (PBST) for 4 times. Synthesized biotinylated V1V2 peptide CIAQDNCTGLEQEQMISCKFNMTGLKRDKKKEYNETWYSADLVCEQGNNTGNESRC (aa151-206, containing full V2 and partial V1 aa151-167) was diluted and added as 400ng/well with 100μl PBST, incubated at room temperature (RT) for 1 hour and washed with PBST 4 times. Serial diluted monkey sera were then added (100μl/well in PBST) and incubated at RT for 1 hour, then washed with PBST for 4 times. The plate was then added with HRP-conjugated goat-anti-monkey IgG secondary antibody (50,000-fold dilution) and incubated at RT for another 1 hour. After three times washes with PBST and one time wash with PBS, the plate was added with 50μl/well 3,3’,5,5’-Tetramethylbenzidine (Sigma, MO) for 10 min and stopped with 10μl/well 2M H₂SO₄. The 450nm absorbance value was immediately measured by Victor3 Multilable Reader (PerkinElmer, MA) and the data was analyzed by Excel. The endpoint binding titer
was defined as the dilution with a value that two times higher than the background value.

**SIV neutralization assay**

A neutralization assay based on pseudotyped SIV virus was used to determine neutralizing antibody responses induced by vaccination as previously described. Single-cycle luciferase reporter virus SIV\textsubscript{mac1A11} (tier 1), were generated by co-transfection of 293T cells with the proviral plasmid SIV\textsubscript{mac239-Luc-E R} and envelope-containing plasmids pcDNA-1A11\textsuperscript{3}. Heat-inactivated sera (56°C, 30min) were serially diluted and incubated with equal amount of pseudotyped virus at 37°C for an hour. Then the mixtures were added to TZM-bl cells and luciferase activity was measured 48-72 hours after infection. The serum dilution that reduced SIV infection by 50% (half maximal inhibition concentration, IC\textsubscript{50}) was determined using the GraphPad Prism software.
Supplemental references:

