

Figure S1. Representative sections of micrographs from negative staining electron microscopy. Shown are image details of micrographs acquired by negative staining electron microscopy. Scale bar represents 50 nm. Names of individual Env variants are given.

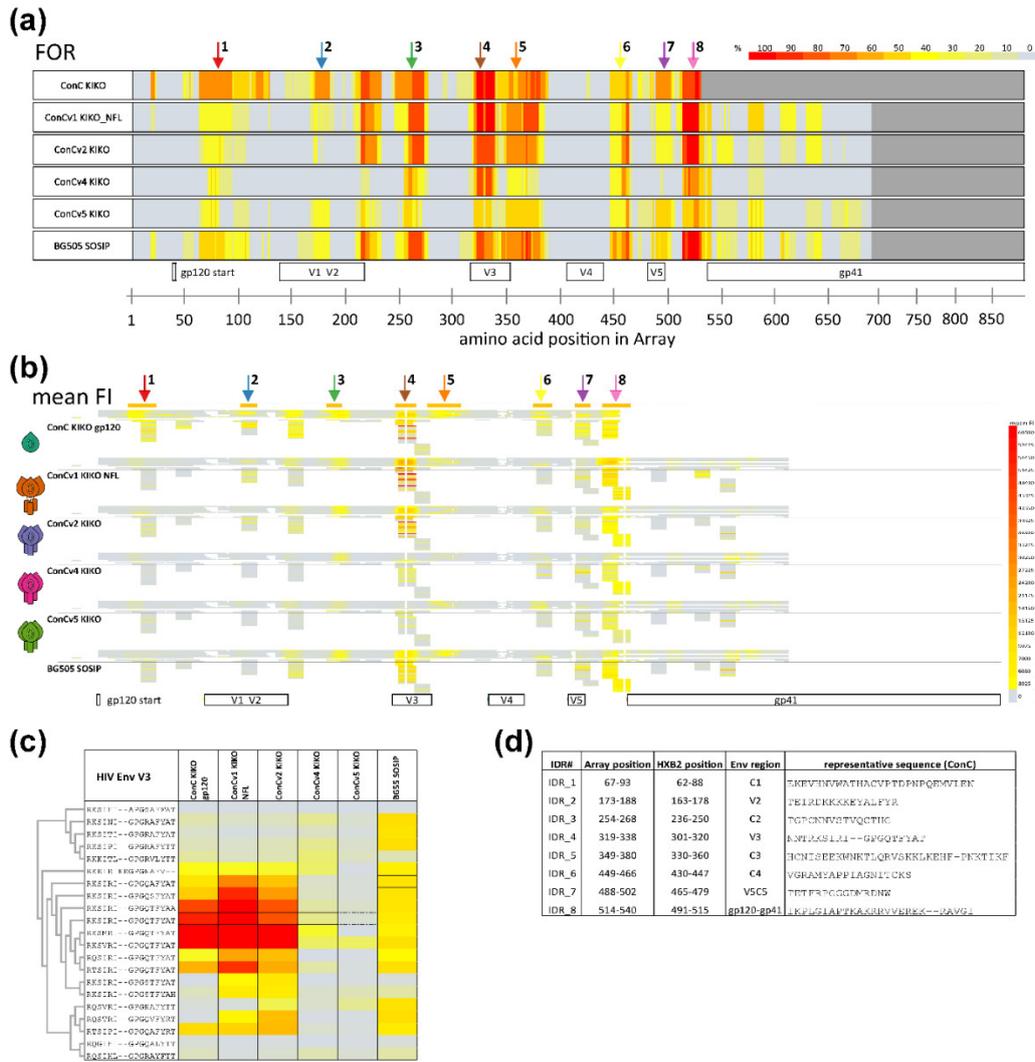


Figure S2. Immunogen conformation and sequence influence depth and breadth of IgG response to HIV-1 Env antigenic regions. (a) Heat map of frequency of responders (FOR) plotted against individual antigenic regions along the entire HIV-1 Env as included in the peptide microarray. Heat maps of antigenic regions targeted by Env-specific IgG responses are shown for all vaccination groups two weeks after the final vaccination. Each row represents one vaccination group comprised of 6 animals each. Responses above 2500 FI (assay background) after baseline subtraction (pre-vaccination response) were considered positive and the maximum response was selected per position. The frequency of positive responses (>2500 FI) was calculated per amino acid position and is given as frequency of responders (FOR). Immunodominant regions (IDRs) 1–8 are indicated by colored arrows. Dark grey areas demark the end of the immunogens. (b) Heat maps of antigenic regions targeted by Env-specific IgG responses are shown for all vaccination groups two weeks after the final vaccination. Each block represents one vaccination group comprised of 6 animals each. Each row represents the mean FI values per vaccination group corresponding to each amino acid mapped to the 10 full-length Env sequences included in the peptide array (above the black line) as well as all additional sequences at areas of special interest included in the array (rows below the black line). Responses above 2500 FI (assay background) after baseline subtraction were considered positive and the mean response for each position was calculated per vaccination group. Data for the length of the immunogen is shown per group. Immunodominant regions (IDRs) 1–8 are indicated by orange lines. (c) Heat map of signal intensities for each peptide variant included for HIV-1 Env V3 (IDR_4) and vaccination group. The mean FI for each variant is shown per vaccination group for all HXB2 aa304-320 peptides included in the array. Sequences are sorted by phylogenetic relationship using Fast Tree Full sorting (CLUSTAL 2.1 Multiple Sequence Alignments). Immunogen sequences included in the peptide microarray are boxed for each vaccination group, with the native sequence (T316) boxed for ConCv5 KIKO. (d) Summary of immunodominant antigenic regions (IDR). IDRs were defined as regions within the Env covered by 15mer peptides being recognized by at least 60% of mice per vaccination group and with a mean FI for individual amino acids within the region of >10512.17 (mean of all responses in all groups + 2xSD) occurring in one of the vaccination groups.