Supporting Information

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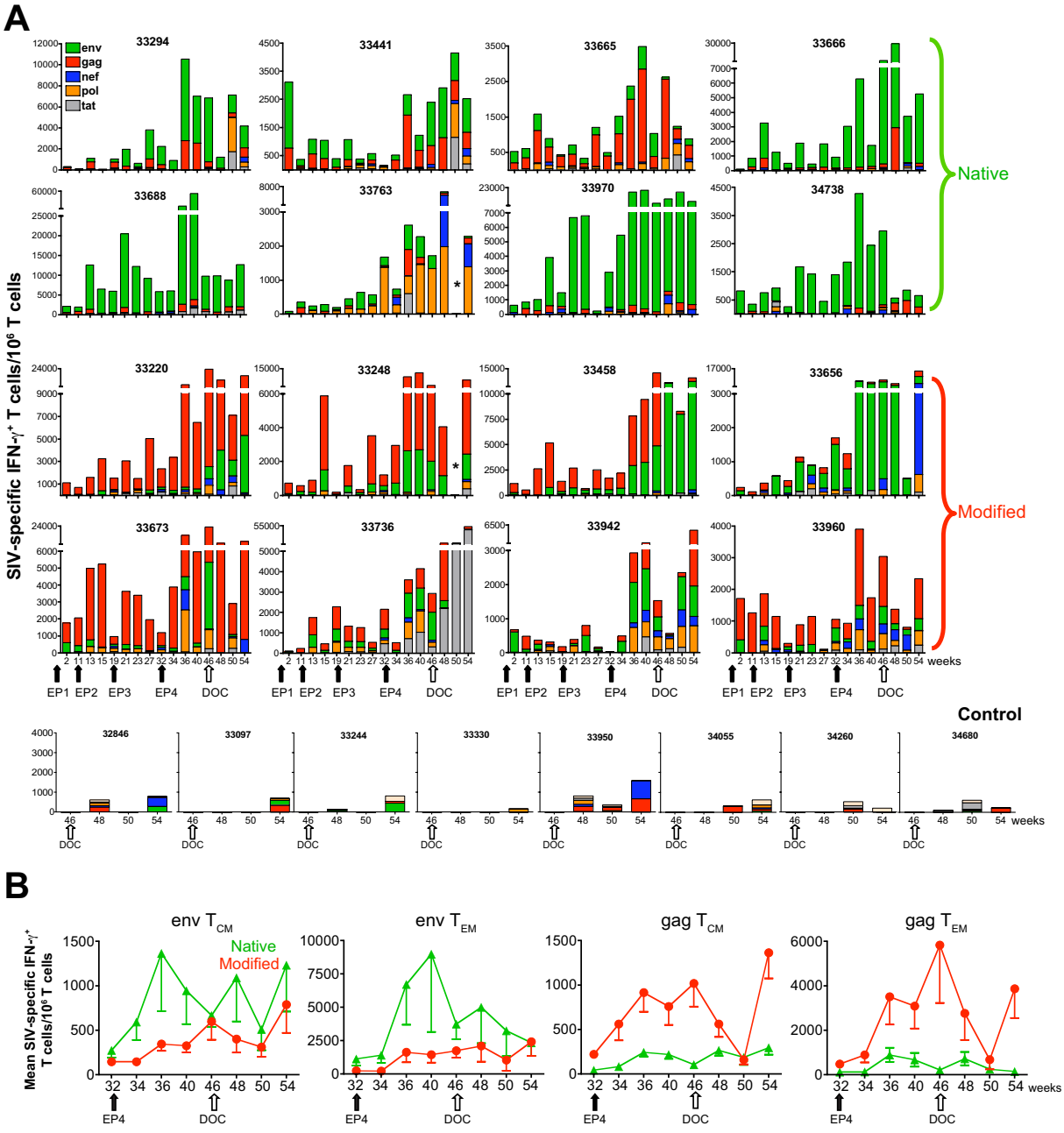

Fig. S1. Frequency of SIV-specific IFN-γ^+ T cells. (*A*) Antigen-specific IFN-γ production was determined by flow cytometric analysis of the frequency of IFN-γ^+ T cells obtained upon incubation with each of the five peptide pools (Env, Gag, Nef, Pol, Tat). Analysis during the entire vaccination period and at three time points after challenge is shown. The bottom panel shows the frequency of IFN-γ^+ T cells in the control animals at the same times after challenge. At EP1wk2, #33665 was only tested for Env and Gag responses. *, #33763 and #33248, no cells were available for analysis at wk 50. (*B*) Mean frequencies of SIV-specific IFN-γ^+ T cells with different memory phenotypic markers. The frequencies of the Gag- and Env-specific T cells for the two vaccine groups shown as CM and EM subsets are shown for the period of the last EP (EP4) and up to 8 weeks PC. Note the different scales of the panels.

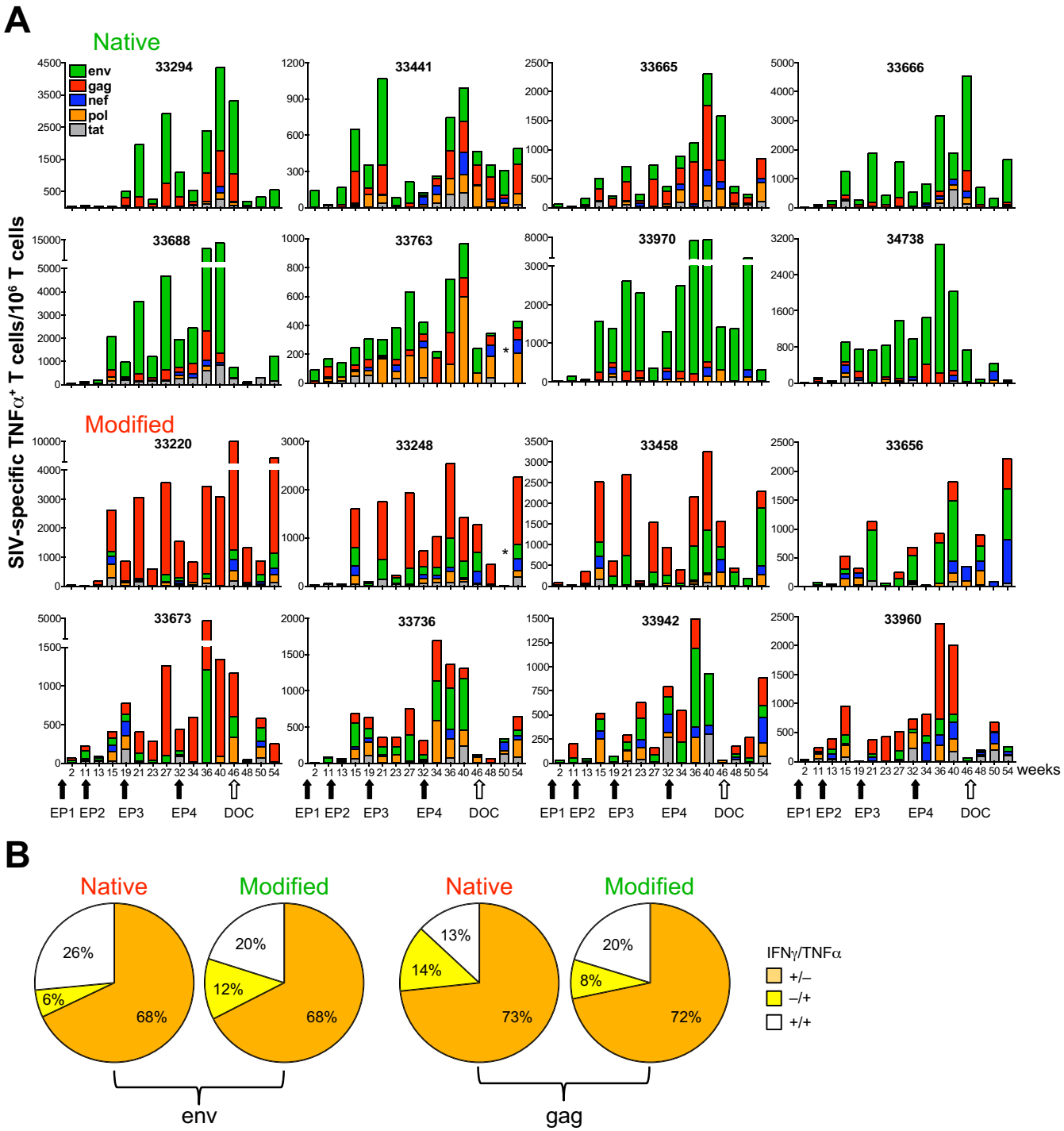

Fig. S2. (*A*) Frequency of SIV-specific TNFα^+ T cells in individual vaccinated animals. Antigen-specific TNFα^+ T cells obtained upon incubation with the five peptide pools (Env, Gag, Nef, Pol, Tat) in the individual vaccinated animals. (*B*) Induction of dual functional antigen-specific T cells. The mean (%) of the single-positive IFN-γ and TNFα and double-positive (IFN-γ^+TNFα^+) T cells of animals vaccinated with native and modified Gag and Env are shown at EP4 week 4.

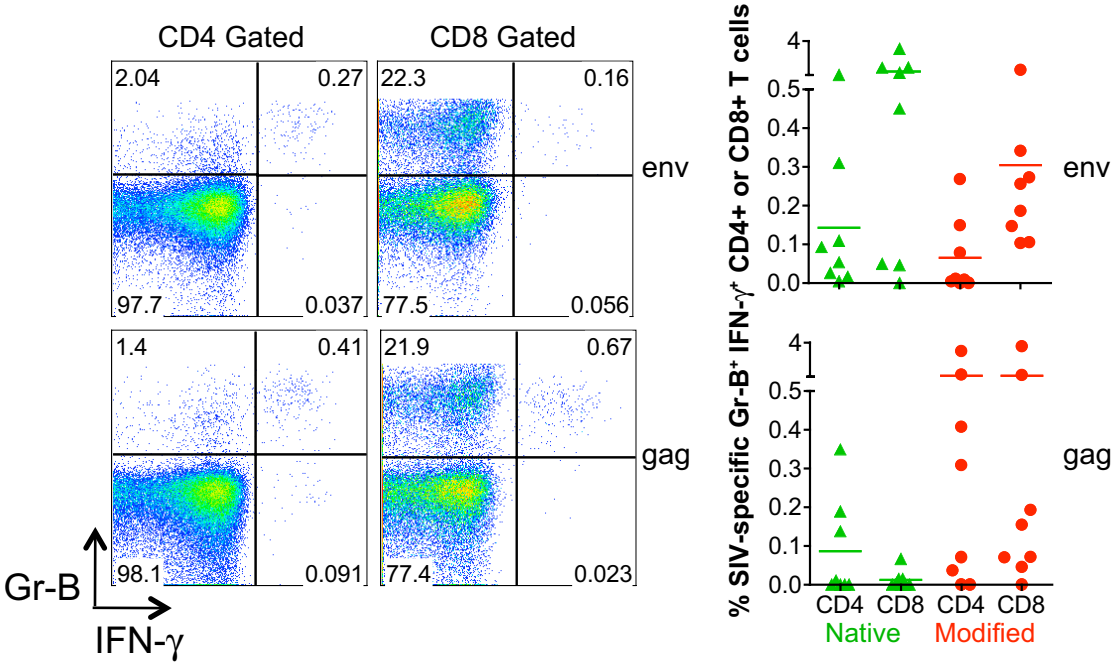

Fig. S3. Induction of functional cytotoxic antigen-specific T cells. Gag and Env-specific $CD4^+$ and $CD8^+$ T cells were analyzed for their ability to produce IFN-γ and Granzyme B (Gr-B) at DOC. The flow cytometric analysis of a representative animal (#33248) is shown in the left panel. The plots on the right show the frequencies of Env- and Gag-specific Gr-B^+ IFN-γ^+ $CD4^+$ or $CD8^+$ T cells of the individual animals in the two vaccine groups. Values shown as percent of T cells.

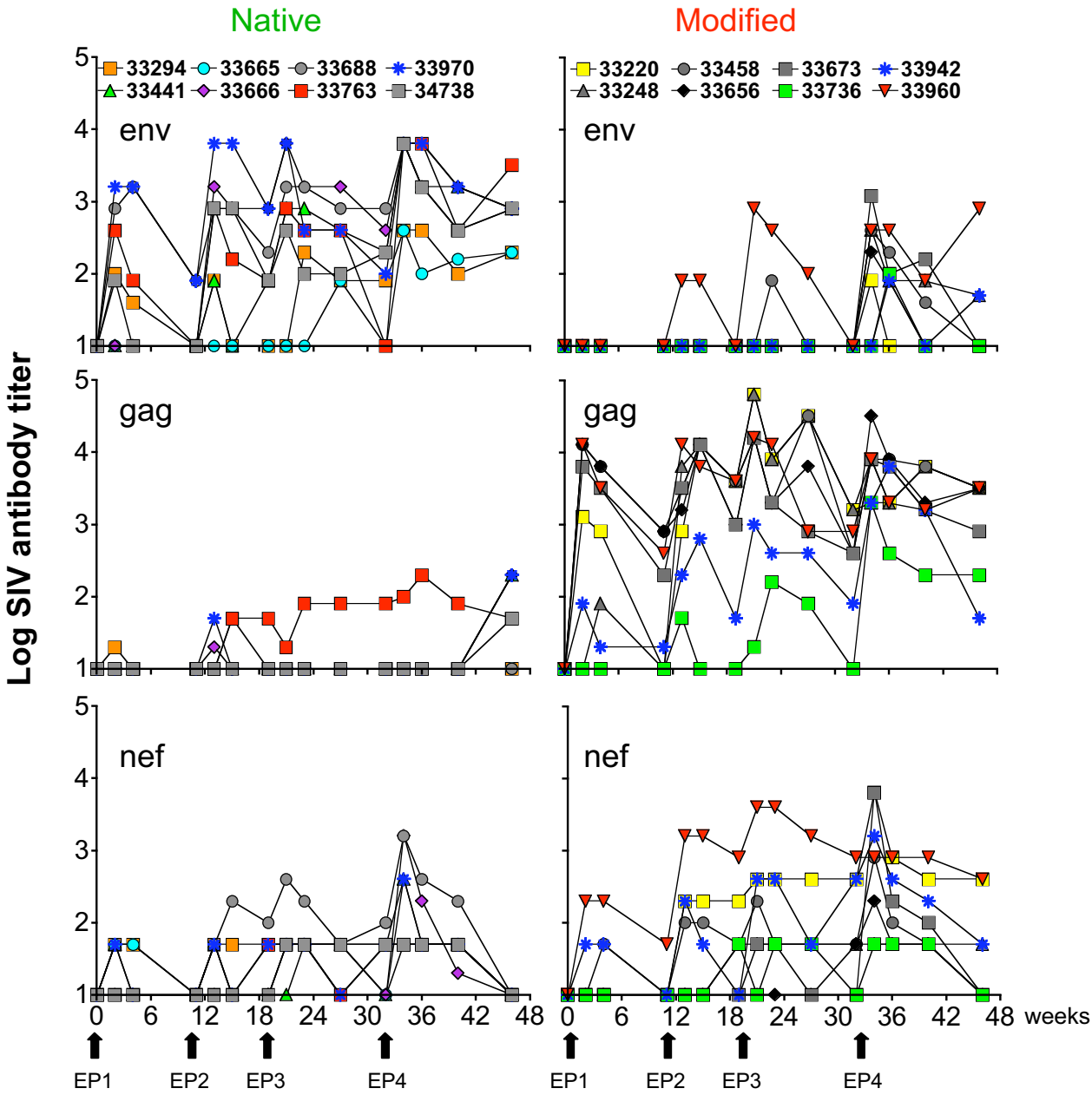

Fig. S4. SIV-specific humoral immune responses in individual animals during the vaccination period. Log reciprocal titers for Env, Gag, and Nef binding antibodies of all animals in the Native and Modified groups.

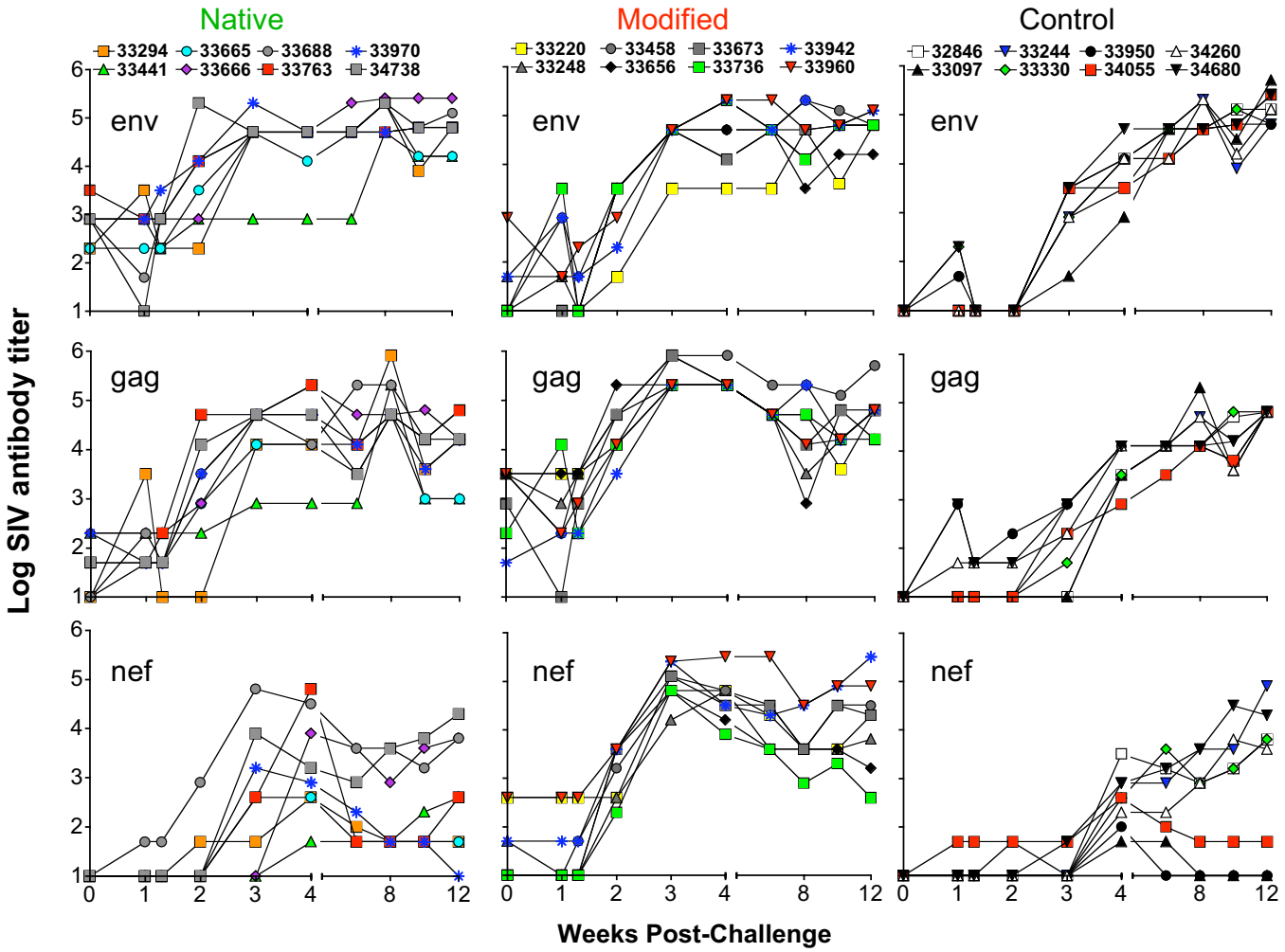

Fig. S5. SIV-specific humoral immune responses in individual animals after challenge. Log reciprocal titers for Env, Gag, and Nef binding antibodies of all animals in the Native, Modified, and controls groups after SIV challenge.

Table S1. Analysis of major histocompatibility complex (MHC) haplotypes

Group native ($N = 8$)		Group modified ($N = 8$)		Group control ($N = 11$)	
33294	negative	33220	negative	32846	A*08, B*17, B*29
33441	A*01, B*01	33248	negative	33097	A*11, B*01
33665	A*11	33458	negative	33244	A*02
33666	A*02	33656	A*02	33330	A*02, A*08
33688	A*01, A*08	33673	negative	35003	A*02
33763	A*11, B*08	33736	A*01	33390	B*01
33970	A*11, B*01	33942	B*17, B*29	33950	negative
34738	A*08, B*17, B*29	33960	A*01, B*01	34055	A*11, B*01
				34260	A*01, B*17, B*29
				34680	A*01
				35014	A*01

Animals were subjected to haplotype analysis for a set of 10 MHC alleles (Mamu-A*01, -A*02, -A*08, -A*11, -B*01, -B*03, -B*04, -B*08, -B*17, -B*29) and distributed to the 3 groups. ″Negative″, indicates animals scoring negative for all of the tested haplotypes. Animals 33390, 35003 and 35014 were not tested for Mamu-B*29. As described in Materials and Methods, 11 macaques with the 3 MHC haplotypes reported to affect viremia (Mamu-A*01, -B*08, -B*17) were assigned with a balanced distribution over the 3 groups. The median of the peak log viral loads of these macaques was observed to be lower than the median of the other macaques in each of the groups (6.8 vs. 6.9 log in the Native group, 7.2 vs. 7.7 log in the Modified group, 7.7 vs. 7.9 log in the controls). With the 3 groups as the strata, these comparisons combine to suggest a small difference associated with the presence of one or more of the haplotypes, which did not reach statistical significance ($P = 0.090$, exact stratified Wilcoxon rank sum test). To account for any possible effect on the intergroup comparisons, we adjusted the comparisons between the 3 groups using the presence of any protective haplotype as a stratification factor. The results were similar to the unadjusted tests above: $P = 0.0001$ for control vs. Native, $P = 0.0059$ for control vs. Modified, and $P = 0.070$ for Native vs. Modified (exact stratified Wilcoxon rank sum test). Similarly, the subset of macaques with reportedly protective MHC haplotypes did not have significantly lower levels of chronic viral load ($P = 0.26$ for weeks 8–20, $P = 0.27$ for weeks 8–32, exact stratified Wilcoxon rank sum test). Nevertheless, we stratified the comparisons by the haplotype groups and saw results similar to those of the unstratified tests (for weeks 8–20, Native vs. controls, $P = 0.0053$, Modified vs. controls, $P = 0.012$; for weeks 8–32, $P = 0.012$ and $P = 0.053$, respectively).