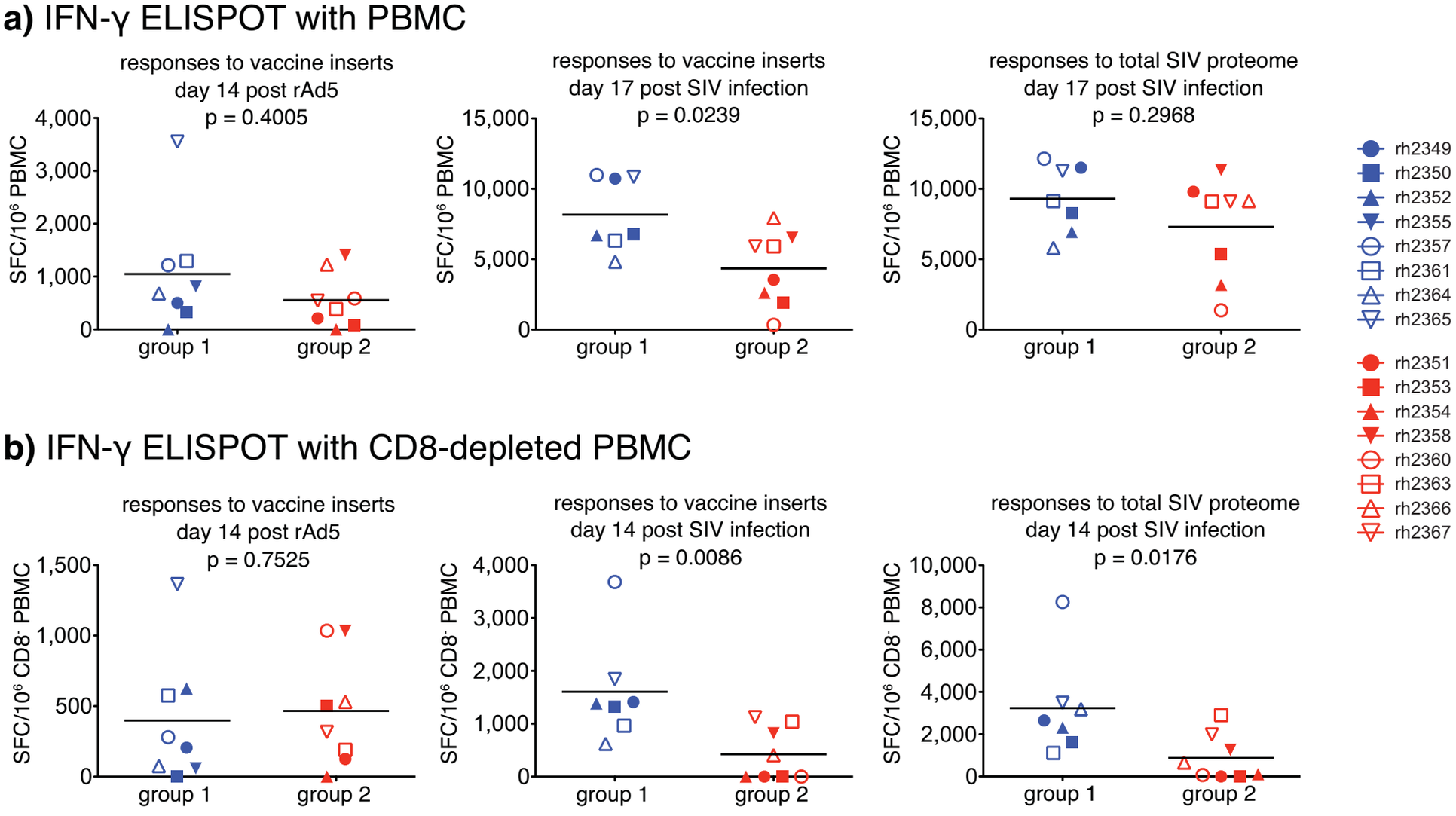
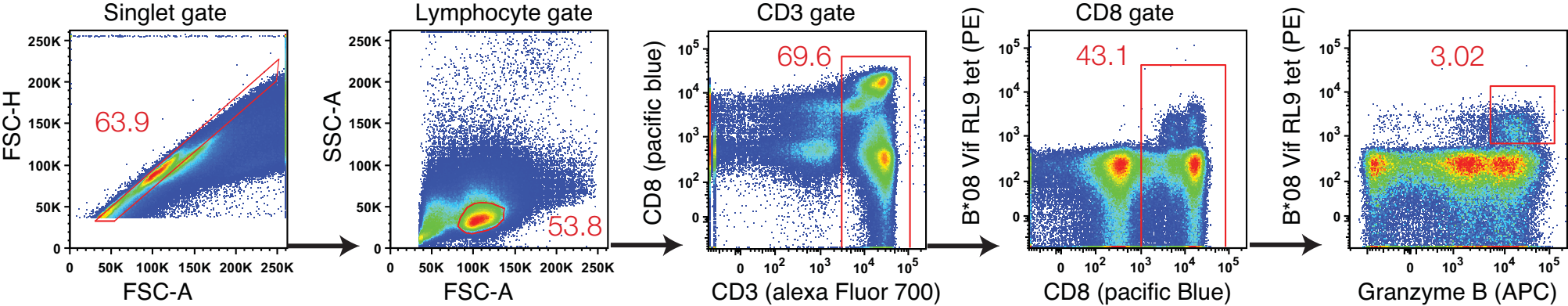


Supplementary Figure 1. Magnitude of cellular immune responses to vaccine inserts and the entire SIV proteome. We performed IFN-γ ELISPOT in total PBMC (A) and CD8-depleted PBMC (B) at 14 days after the rAd5 boost, as well as days 14 and 17 after SIV infection. The frequency of vaccine-induced T-cell responses to the SIV inserts measured at day 14 after the rAd5 boost was not significantly different between Group 1 and Group 2 macaques. However, Group 1 animals had higher insert-specific responses early after infection in both PBMC and CD8-depleted PBMC. This difference remained statistically significant when we compared the magnitude of T-cell responses to the entire SIV proteome in CD8-depleted PBMC, but not in whole PBMC. These results show that our vaccine regimens were equally efficacious at engendering both $CD4^+$ and $CD8^+$ T cell responses in Group 1 and Group 2 animals. The lower frequency of anamnestic $CD4^+$ T-cell responses detected in group 2 macaques may be a consequence of the high levels of viral replication measured in this group. Bars represent mean values for each group. Statistical comparisons were made using two-tailed Mann-Whitney tests. Due to sample limitations, rh2355 was not included in the post-infection analyses.



Supplemental figure 2. Gating strategy used for the analysis of granzyme B expression among tetramer$^+$ CD8$^+$ T-cells. Numbers in each panel represent the percentage of the gated cell population.

a) acute phase

haplotype frequencies of Vif and Nef epitopes in Group 1 vaccinees and Group 2 controls

epitope	SIVmac239	rh2349	rh2355	rh2350	rh2352	rh2357	rh2361	rh2364	rh2365	rh2351	rh2353	rh2354	rh2358	rh2360	rh2363	rh2366	rh2367
Vif RL8	KQW RRDNRRGL RMA		100%	99.16%	96.92%	87.10%	100%	58.57%		21.53%	37.84%	91.88%	100%	50.32%	93.76%	100%	97.18%
acute	... ..N..... ...					6.61%		3.38%		38.87%	1.86%			25.89%	2.38%		
	... .D. ...					2.07%								3.81%			
	... ...Y.... ...					1.72%											
	... .G..... ...					1.40%		11.25%		2.06%	1.48%			6.64%	1.31%		
	... .F ...													4.48%			
	... .K... ...							2.56%			52.13%			1.73%			
	... G..... ...							2.04%						1.13%			
	... .G.. ...							20.90%		3.85%	1.46%				1.37%		
	... ..G..... ...									27.72%							
	... .P ...									3.31%		7.55%					
	... ..D....H ...									1.77%							
	... . .I.																1.72%
	Variants < 1%		0%	0.84%	3.08%	1.10%	0%	1.30%		0.89%	5.23%	0.57%	0%	6.00%	1.18%	0%	1.10%

epitope	SIVmac239	rh2349	rh2355	rh2350	rh2352	rh2357	rh2361	rh2364	rh2365	rh2351	rh2353	rh2354	rh2358	rh2360	rh2363	rh2366	rh2367
Vif RL9	GEV RRAIRGEQL LSC		100%	26.83%	46.74%	75.26%	100%	36.69%		32.83%	40.26%	91.99%	100%	64.28%	99.38%	69.16%	78.41%
acute	... ..V..... ...			71.82%		18.17%		28.20%		46.20%	55.41%			2.55%		28.57%	14.28%
	... ...V..... ...				51.57%												
	... .K..... ...					2.36%		10.62%		8.23%		7.03%					
	... .R. ...					2.30%								4.10%			
	... .R ...							20.11%									
	... .Q ...													12.96%			1.36%
	... K..... ...													7.83%			
	... .E... ...													4.20%			
	... .W.... ...									3.94%						2.26%	
	... .G.. ...							2.36%		2.99%							
	... .D.. ...									2.41%							
	... .K.... ...																5.12%
	Variants < 1%		0%	1.35%	1.69%	1.91%	0%	2.02%		3.40%	4.33%	0.98%	0%	4.08%	0.62%	0%	0.83%

epitope	SIVmac239	rh2349	rh2355	rh2350	rh2352	rh2357	rh2361	rh2364	rh2365	rh2351	rh2353	rh2354	rh2358	rh2360	rh2363	rh2366	rh2367
Nef RL10	YSA RRHRILDIYL EKE		100%			97.46%	100%	91.67%		96.94%	95.17%	100%	99.37%	99.11	95.04%	99.27%	100%
acute	... .T.. ...					2.54%		5.02%							1.60%		
	... .M.. ...							1.69%									
	..V									1.95%							
	... .V.. ...									1.11%							
	Variants < 1%		0%			0%	0%	1.62%		0%	4.83%	0%	0.63%	0.89%	3.36%	0.73%	0%

b) chronic phase

haplotype frequencies of Vif and Nef epitopes in Group 1 vaccinees

epitope	SIVmac239	rh2349	rh2355	rh2350	rh2352	rh2357	rh2361	rh2364	rh2365
Vif RL8	KQW RRDNRRGL RMA	3.69%	0%		96.76%		98.78%		
chronic	... .G.. ...	39.21%	3.91%						
	... ...D.... .V.	27.63%							
	... . .V.	14.21%							
	... ...D.... ...	10.49%							
	... .G..... ...		31.37%						
	... GG..... ...		56.96%						
	... G..... ...		3.01%						
	Variants < 1%	4.78%	4.75%		3.24%		1.22%		

epitope	SIVmac239	rh2349	rh2355	rh2350	rh2352	rh2357	rh2361	rh2364	rh2365
Vif RL9	GEV RRAIRGEQL LSC	3.92%	0%		17.04%		68.47%		
chronic	... ..V..... ...	72.30%	7.28%				29.86%		
	... ..V.K.... ...	18.50%							
	... ..V....R. ...	1.44%	10.81%		7.08%				
	... .K..... ...		2.41%						
	... ..VL..... ...	1.37%	72.52%						
	... .Q ...		2.48%						
	... .W.... ...				31.32%				
	... ...V..... ...				42.34%				
	Variants < 1%	2.47%	4.50%		2.22		1.67%		

epitope	SIVmac239	rh2349	rh2355	rh2350	rh2352	rh2357	rh2361	rh2364	rh2365
Nef RL10	YSA RRHRILDIYL EKE	7.41%	0%	90.90%	68.53%	99.36%	98.86%		
chronic	..P	87.52%	96.38%	9.10%					
	..V ....M.. ...	1.51%							
	... .M.. ...				28.88%				
	... .T.. ...				2.59%				
	..E						1.14%		
	Variants < 1%	3.56%	3.62%	0%	0%	0.64%	0%		

nd = not determined

breakthrough vaccinees

Supplemental figure 3. 454 deep sequencing of the three Mamu-B*08-restricted Vif RL8, Vif RL9, and Nef RL10 CD8+ T-cell epitopes. Percentages indicate haplotype frequencies of each epitope at either acute (A) or chronic (B) phases of infection. Sequencing analysis in the acute phase was performed using plasma samples collected at week 6 post SIV infection. The time points for the chronic phase sequencing analysis ranged from week 35 to week 48 post SIV infection, as indicated in Table 1. We utilized the ReadClean 454 (RC454) and V-Phaser algorithms as previously described[21,22] to call variants from the 454 datasets with manual inspection and correction. Briefly, RC454 was used to align reads to SIVmac239 and reads were corrected for sequencing related artifacts such as InDels resulting from overcalls and undercalls in homopolymeric regions and Carry forward and Incomplete Extension (CAFIE) errors. Furthermore, RC454 optimizes read alignments using coding frame information. The V-Phaser algorithm was then used to distinguish an observed variant as a true variant from an amplification or sequencing artifact. Average read depth for the Vif RL8, Vif RL9 and Nef RL10 epitopes was 8,285 reads, 8,383 reads, and 7,169 reads, respectively. Breakthrough animals in Group 1 are highlighted in yellow. nd = not determined.