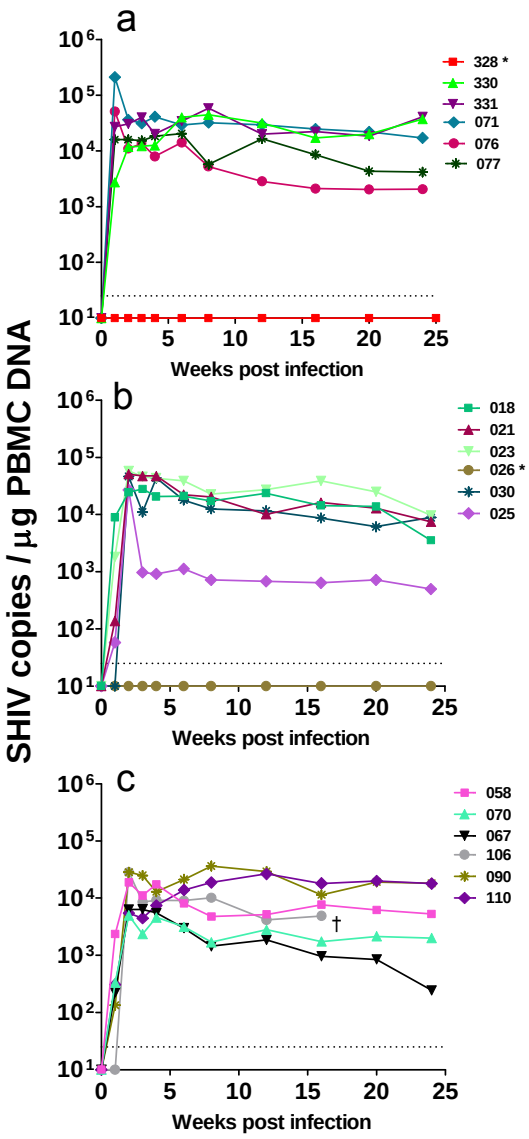
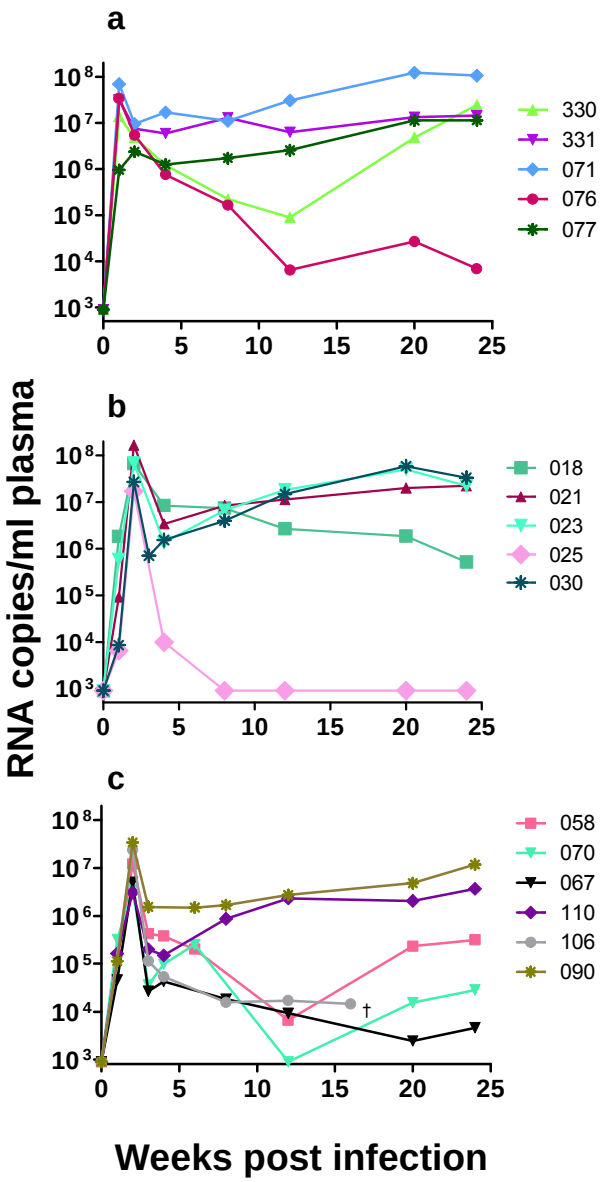
Passive neutralizing antibody controls SHIV viremia and enhances B cell responses in infant macaques

Supplementary Table 1. Summary of characteristics of infused IgG preparations and outcomes.

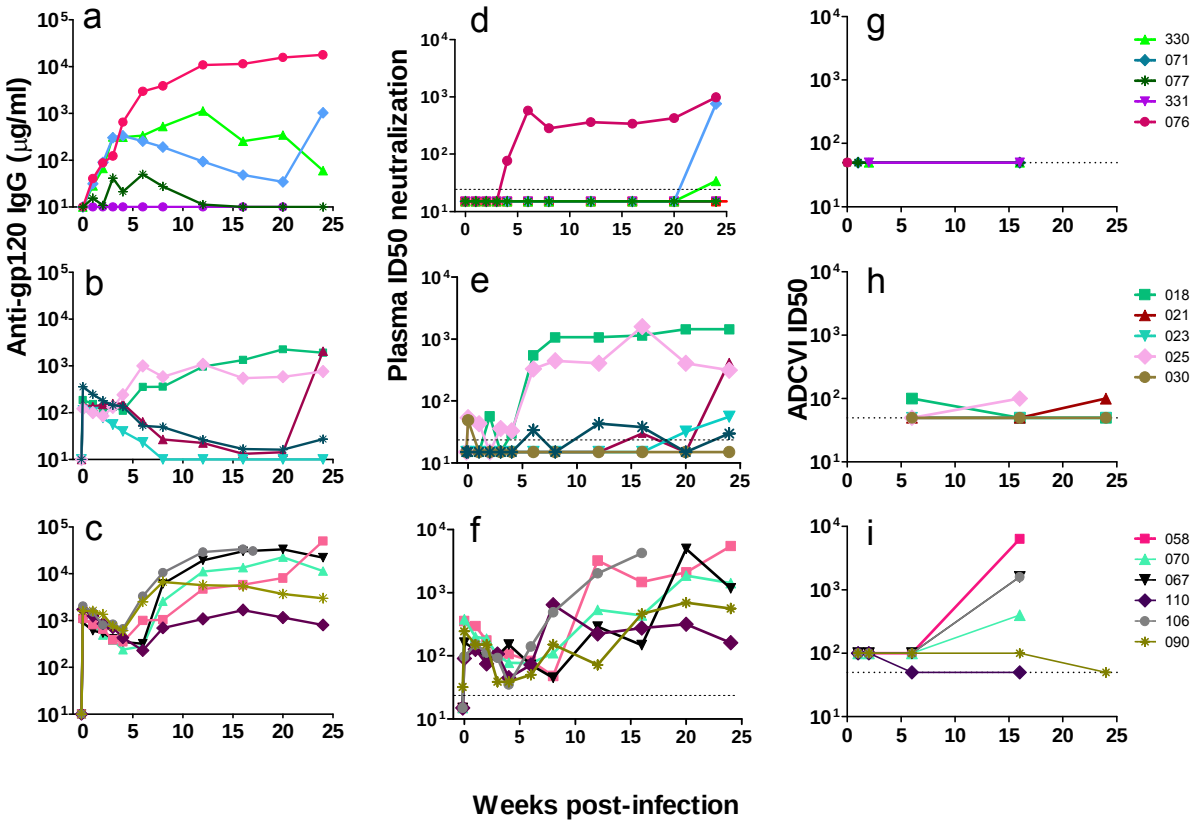
Group	Passive IgG treatment	IC_{80} (μg ml^{-1})		Passive IgG dose (mg kg^{-1})	No. infected
		$SHIV_{SF162P3}$	$SHIV_{89.6P}$		
Normal-IgG	IVIG	>3800	>3800	170	5/6
Matched-IgG	$SHIVIG_{SF162P3}$ + IgG1b12	788	>3800	200 0.2	6/6
Mismatched-IgG	SHIVIG-89.6P	>3800	109	200	5/6



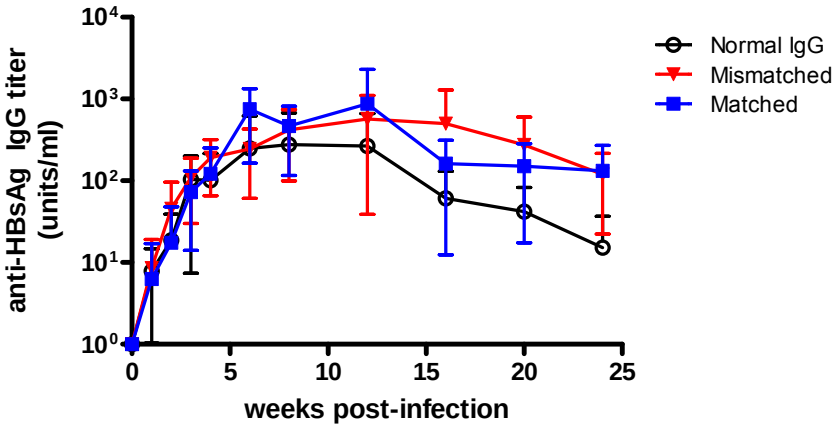
Supplementary Fig. 1: PBMC proviral load in $SHIV_{SF162P3}$-infected infant macaques treated with polyclonal IgG. Individual PBMC proviral loads are shown for macaque infants treated with Normal-IgG (*n* = 6, **a**), Mismatched-IgG (*n* = 6, **b**), and Matched-IgG (*n* = 6, **c**) prior to challenge with $SHIV_{SF162P3}$. Blood samples were collected at the indicated times after viral exposure and cell-associated infection was measured by quantitative PCR. *denotes uninfected macaque removed from all other analyses. Note: macaque 328 was subsequently intravenously exposed to $SHIV_{SF163P3}$ at a dose of 0.001 oral AID and became infected immediately. † denotes euthanasia due to AIDS.



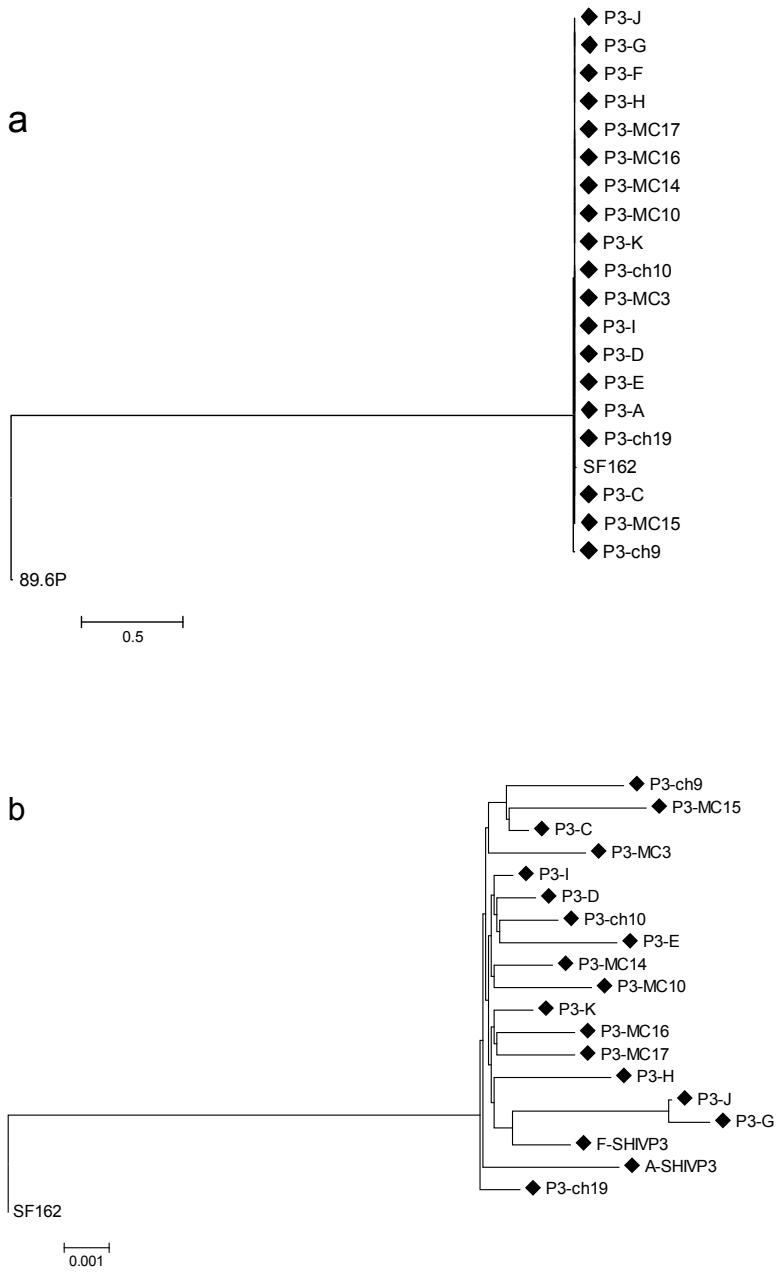
Supplementary Fig. 2: Effect of passive IgG treatment on plasma viremia in SHIV$_{SF162P3}$-infected infant macaques. RNA was isolated from plasma and viral RNA was quantified by real-time PCR. Curves represent individual animals that were treated with Normal-IgG (**a**), Mismatched-IgG (**b**), and Matched-IgG (**c**) prior to challenge with SHIV$_{SF162P3}$. Dotted line indicates limit of detection, while † denotes euthanasia due to AIDS.



Supplementary Fig. 3: Humoral responses during infection with SHIV$_{SF162P3}$. HIV-1$_{SF162}$ gp120-specific IgG concentration was determined by kinetic ELISA for animals treated with Normal IgG (**a**), Mismatched-IgG (**b**), and Matched-IgG (**c**). Neutralizing activity in plasma was evaluated against SHIV$_{SF162P3}$ clone MC17 in all four groups (**d**, Normal; **e**, Mismatched; **f**, Matched). ID$_{50}$ is the plasma dilution necessary to inhibit infection by 50%. ADCVI levels were evaluated to examine sources of virus inhibition other than neutralization (**g**, Normal; **h**, Mismatched; **i**, Matched). † denotes euthanasia and dotted line indicates limit of detection for the assay.

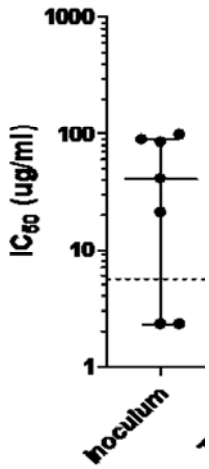


Supplementary Fig. 4: HbsAg-specific IgG development for $SHIV_{SF162P3}$-infected infant macaques in different treatment groups. Comparison of mean anti-HBsAg IgG levels (±SD) with among infants that received Normal IgG (*n* = 9, black line), that received Mismatched IgG (*n* = 5, red line), or that received Matched IgG (*n* = 6, blue line) against the infecting virus. HbsAg IgG levels were measured by kinetic ELISA and titers are expressed relative to control plasma positive for HbsAg IgG. Kinetics and titers of HbsAb IgG development did not differ significantly by treatment group.

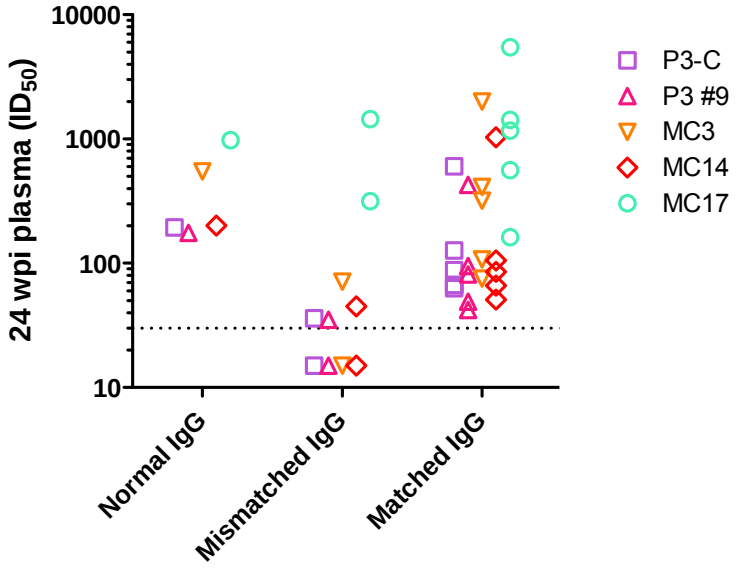


Supplementary Fig. 5: Maximum likelihood analysis of full-length envelope sequences isolated from $SHIV_{SF162P3}$. *Envelope* gene (*Env)* variants were cloned and sequenced from the RNA of the challenge virus. Those clones that were found to be free of truncations were aligned and analyzed for homology. (**a**) Maximum likelihood tree of *Env* rooted in $SHIV_{89.6P}$ which belongs to the same clade but is distantly related. The tree clearly shows that all $SHIV_{SF162P3}$ *Env* variants are highly homologous. (**b**) To better view the small percentage of divergence, the same sequences were rooted in HIV-1_{SF162} the virus from which $SHIV_{SF162P3}$ was derived. ♦ designates a single clone and the scale of divergence is noted below each tree.

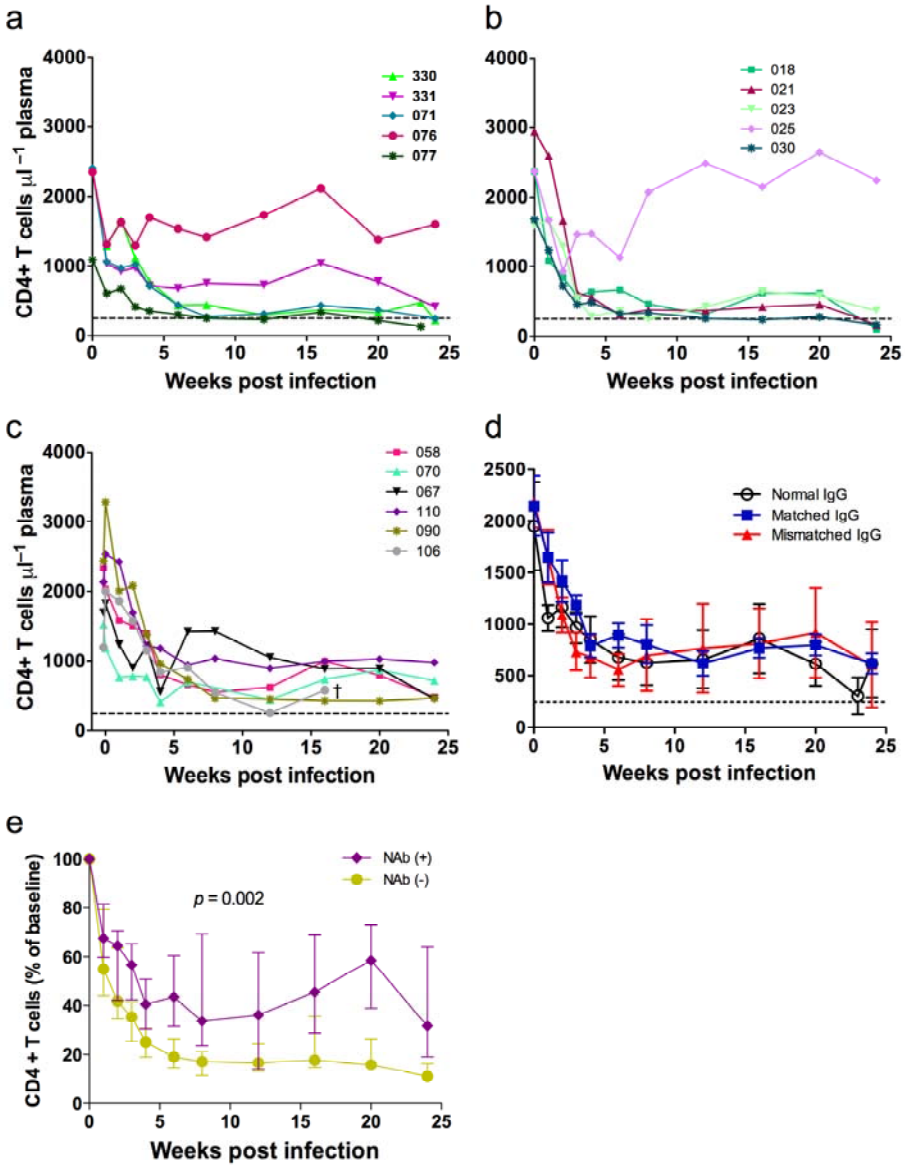
a



b



Supplementary Fig. 6: Neutralization of SHIV$_{SF162P3}$ *Envelope* clones. (**a**) Seven gp160 clones isolated from the challenge virus were examined for sensitivity to Matched IgG. Clones demonstrated a range of sensitivity where IC_{50} is the concentration of Matched IgG necessary to inhibit infection by 50%. Dotted line indicates the lowest concentration tested (5.6 μg ml^{-1}) and clones with values below this level were plotted as half the lowest concentration. Horizontal lines indicate the median value and interquartile ranges (upper and lower) (**b**) Neutralizing antibodies against two resistant and two moderately resistant *Env* clones from the challenge virus was measured in plasma collected at 24 weeks post-infection from infants that developed consistent NAb against the sensitive *Env* clone, MC17. 50% inhibitory dilutions are plotted by treatment group. Titers against MC17 are included in the plot for comparison. Dotted line denotes lowest dilution tested (1:30); samples that were below this level are plotted as half the lowest dilution.



Supplementary Fig. 7: Temporal analysis of CD4$^+$ T lymphocyte levels in SHIV$_{SF162P3}$-infected infant macaques treated with polyclonal IgG with differing neutralizing activity against SHIV$_{SF162P3}$. Peripheral CD4$^+$ T cell levels were measured by flow cytometry are for animals treated with Normal IgG (**a**), Mismatched IgG (**b**), and Matched IgG (**c**) prior to challenge with SHIV$_{SF162P3}$. Each curve represents an individual animal. The dotted line indicates the CD4 level of 250 cells µl^{-1} blood. † denotes euthanasia. (**d**) Mean peripheral CD4$^+$ T cell levels (±SD) are depicted for animals treated with Normal-IgG (*n* = 5), Mismatched-IgG (*n* = 5), and Matched-IgG (*n* = 6). Levels were measured by flow cytometry at the indicated timepoints. (**e**) Mean CD4 counts ((±SD) of infants that did (purple line) or did not (yellow line) develop NAb by 8 wpi, regardless of treatment, to analyze the relationship between CD4 count and NAbs. Macaques that developed NAb had significantly higher CD4 levels (* *P* = 0.002, Mann-Whitney U test).