Supporting Information

Walker et al. 10.1073/pnas.1117531108

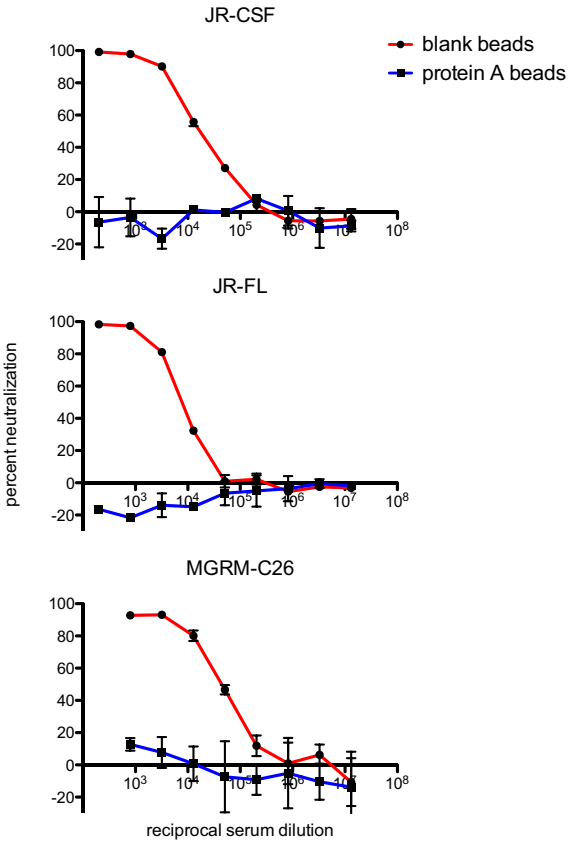

Fig. S1. The broad neutralizing activity in macaque CE8J plasma is mediated by IgG. Plasma was adsorbed with Protein A-Sepharose beads or blank control beads, and the depleted fractions were tested for neutralizing activity using a single round of replication pseudovirus assay.

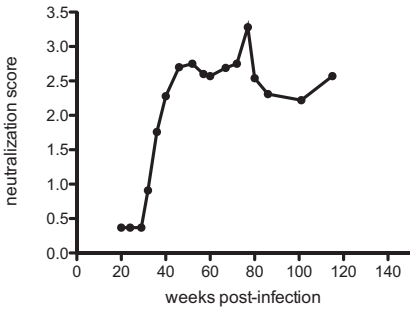

Fig. S2. Neutralization score of macaque CE8J plasma taken at serial time points. Neutralization scores were calculated as described previously (1). Data were plotted using Graphpad Prism© software.

1. Simek MD, et al. (2009) Human immunodeficiency virus type 1 elite neutralizers: Individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J Virol* 83:7337–7348.

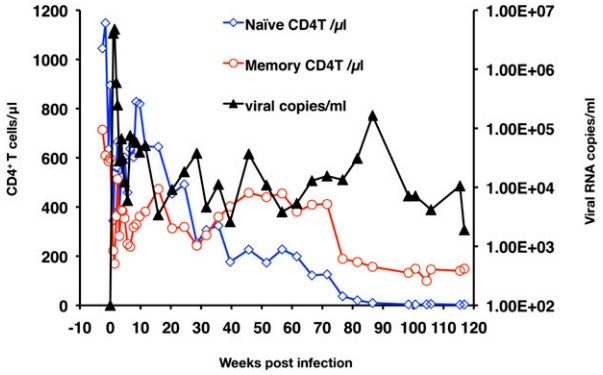

Fig. S3. Plasma virus loads and naïve and memory CD4+ T-cell numbers during the course of the infection of macaque CE8J. Plasma viral loads and absolute numbers of naïve and memory CD4+ T cells in macaque CE8J during 120 wk of $SHIV_{AD8}$ infection.

JR-FL gp120 construct	% neutralization adsorbed on the indicated construct			
	JR-FL	92RW020	ADA	MGRM-C26
WT	96	98	69	94
ΔV3	28	1	1	21
ΔV1/V2	80	71	70	1

Fig. S4. Neutralizing activity in macaque CE8J plasma can be adsorbed on select gp120 molecules. Macaque CE8J plasma was tested for neutralizing activity against a cross-clade pseudovirus panel after adsorption with WT gp120-, gp120 ΔV3-, or gp120 ΔV1/V2-coupled beads or blank control beads. Percent neutralizing activity adsorbed on each construct was calculated using the equation [1 − (IC_{50} blank beads/IC_{50} antigen-coated beads)] × 100. Boxes are color coded as follows: gray, 0–45%, yellow, 45–65%; orange, 65–85%; red, 85–100%.

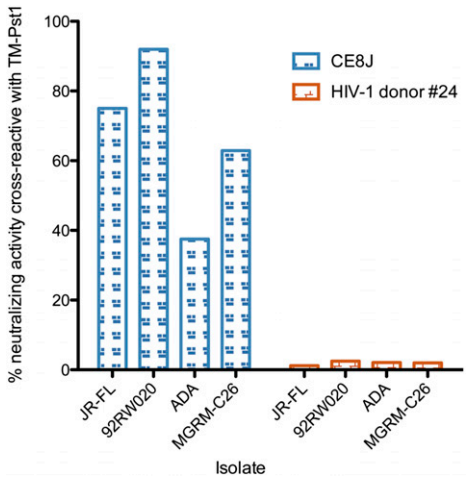

Fig. S5. Glycan-specific antibodies mediate broad plasma neutralization in macaque CE8J. Macaque CE8J plasma was tested for neutralizing activity after adsorption with TM-Pst1–coupled beads or blank control beads. A broadly neutralizing HIV-1 serum that has previously been shown to be insensitive to the N332A mutation (donor 24) was included as a negative control (1). Percent neutralizing activity cross-reactive with TM-Pst1 was calculated using the equation [1 − (IC_{50} blank bead-adsorbed plasma/IC_{50} Pst1-adsorbed plasma)] × 100.

1. Hubbard SC, Ivatt RJ (1981) Synthesis and processing of asparagine-linked oligosaccharides. *Annu Rev Biochem* 50:555−583.

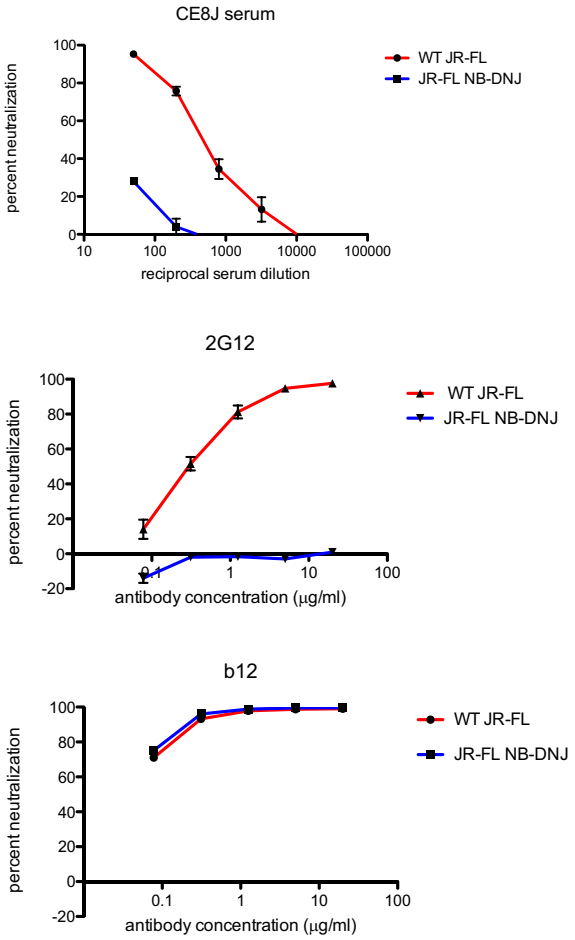

Fig. S6. Neutralizing activity of macaque CE8J plasma against JR-FL pseudoviruses treated with *N*-butyldeoxynojirimycin (NJ-DNJ). Macaque CE8J plasma was tested for neutralizing activity against HIV-1 JR-FL in the presence and absence of the glucosidase inhibitor NJ-DNJ using a single round of replication pseudovirus assay. The bNAbs 2G12 and b12 were included as controls.

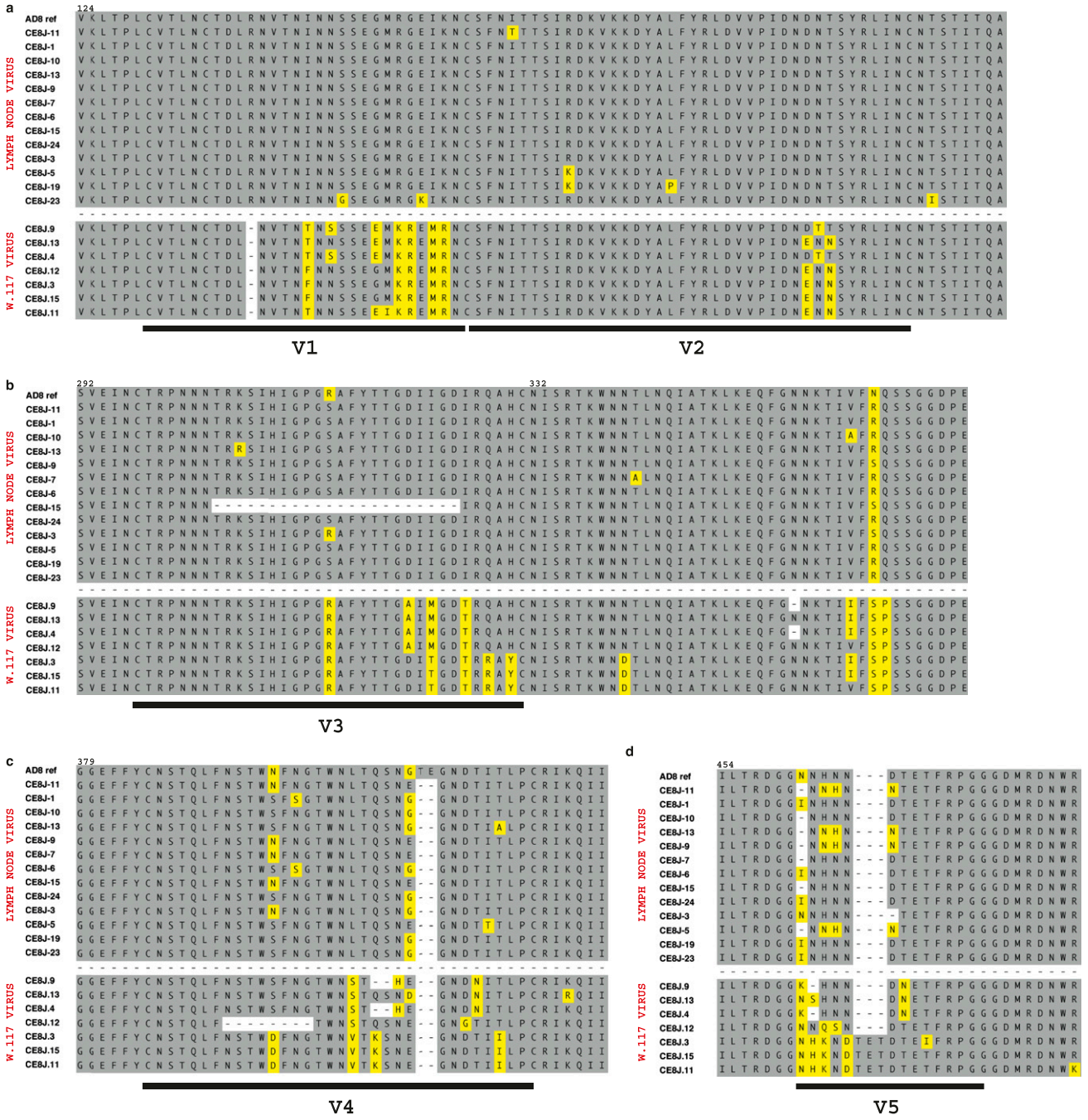

Fig. S7. A comparison of variable-region gp120 sequences of the starting $SHIV_{AD8}$ lymph node virus inoculum with the virus (W.117) recovered at the time of euthanasia. The deduced amino acid sequences of (*A*) V1 and V2, (*B*) V3 and C3, (*C*) V4, and (*D*) V5 regions of gp120 were aligned with the HIV-1_{AD8} reference sequence at the top. Amino acid changes are highlighted in yellow.

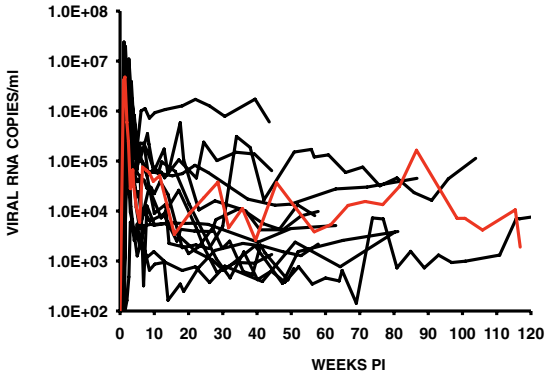

Fig. S8. Plasma viremia in the $SHIV_{AD8}$-infected macaques. Plasma viral loads in the 14 $SHIV_{AD8}$-infected macaques were measured over the course of infection. Plasma viral loads of macaque CE8J and the other 13 SHIV-infected macaques are shown in red and black, respectively.

Table S1. Development of autologous NAbs in 14 $SHIV_{AD8}$ infected macaques

		TZM-bl assay (RC virus)				TZM-bl assay [pseudotyped virus (CK15 Env clone)]			
Animal	Virus inoculum (swarm)	Plasma collection, wk	Target virus	% Neut, 1:20	Titer	Plasma collection, wk	Target virus, clone	% Neut, 1:20	Titer
DA24	CE8J swarm*	22	CE8J (Sw)	**26.0**		41	CK15	**65.4**	<1:100
DBX9	CE8J swarm					28	CK15		<1:100
CZH	CE8J swarm					29	CK15		<1:100
DBZL	CE8J swarm	23	CE8J (Sw)	5.6		41	CK15		<1:100
CL5E	CE8J swarm	36	CE8J (Sw)	ND		49	CK15		<1:100
A3EO50	CK15 swarm					50	CK15		<1:100
DBJE	CE8J swarm	23	CE8J (Sw)	**34.7**		42	CK15	**90.0**	<1:100
DB1A	CE8J swarm					72	CK15		<1:100
CK5T	CJ58 swarm					84	CK15		<1:100
A3EO51	CJ58 swarm					87	CK15		<1:100
CJ7D	CK15 swarm					86	CK15		<1:100
CJ58	AD8#2 (blood from CJ8B at week 60)	26	AD8#2	**94.2**	**1:102**				
		90	AD8#2	**29.2**		156	CK15		<1:100
CJ8B	AD8#2	85	AD8#2	**97.8**	**1:159**	180	CK15		<1:100
CE8J	AD8#2LN	30	AD8#2	**25.7**		32	CK15		**1:450**
		40	AD8#2	**93.7**		40	CK15		**1:3,282**
		80	AD8#2	**95.2**		80	CK15		**1:1,181**

The replication competent SHIVAD8#2-related swarm inocula were prepared as described previously (1). Pseudotyped virus was prepared from an infectious SHIVAD8 molecular clone, carrying an *env* gene present in the SHIVCK15 swarm, as described previously (2). ND, not determined; Neut, neutralization; RC, replication competent. Bold indicates those animals developing detectable autologous anti-HIV-1 NAbs.
*The four different swarm inocula were prepared from the indicated animals (1) at the time of euthanasia from AIDS.

1. Nishimura Y, et al. (2010) Generation of the pathogenic R5-tropic simian/human immunodeficiency virus SHIVAD8 by serial passaging in rhesus macaques. *J Virol* 84:4769–4781.
2. Shingai M, Yoshida T, Martin MA, Strebel K (2011) Some human immunodeficiency virus type 1 Vpu proteins are able to antagonize macaque BST-2 in vitro and in vivo: Vpu-negative simian-human immunodeficiency viruses are attenuated in vivo. *J Virol* 85:9708–9715.

Table S2. Nine of a cohort of 40 $SHIV_{AD8}$ infected macaques develop autologous NAbs

Animal	$SHIV_{AD8}$ inoculum	Assay virus	Neut assay, wk postinfection	Neut response	% Neut at 1:20	Notes
DA24	**CE8J swarm***	**Pseudotyped CK15**	**41, 52**	**+**	**65.4**	**Undetectable at week 52**
DBX9	CE8J swarm	Pseudotyped CK15	44	–		
CZH	CE8J swarm	Pseudotyped CK15	44	–		
DBZL	CE8J swarm	Pseudotyped CK15	23, 36, 42, 47,53, 58	–		
CL5E	CE8J swarm	Pseudotyped CK15	51	–		
DB37	CE8J swarm	Pseudotyped CK15	77	–		
DBGL	CE8J swarm	Pseudotyped CK15	62	–		
DB93	CE8J swarm	Pseudotyped CK15	22	–		
DBJE	**CE8J swarm**	**Pseudotyped CK15**	**42, 47, 53**	**+**	**90.0**	**30% at week 53**
DB1A	CE8J swarm	Pseudotyped CK15	84	–		
A3E051	CL98 swarm	Pseudotyped CK15	87	–		
DA55	**CL98 swarm**	**Pseudotyped CK15**	**57**	**+**	**75.5**	
DB18	CL98 swarm	Pseudotyped CK15	47	–		
CL5B	CL98 swarm	Pseudotyped CK15	29	–		
DB0F	CL98 swarm	Pseudotyped CK15	33	–		
CJ7D	CK15 swarm	Pseudotyped CK15	86	–		
A3E050	CK15 swarm	Pseudotyped CK15	39	–		
DC1W	CK15 swarm	Pseudotyped CK15	58	–		
DC87	CK15 swarm	Pseudotyped CK15	58	–		
DBRL	CK15 swarm	Pseudotyped CK15	48	–		
DC65	CK15 swarm	Pseudotyped CK15	48	–		
A3E046	CJ58 swarm	Pseudotyped CK15	26	–		
DC0L	Molecular clone (CK15 *env* gene)†	Pseudotyped CK15	32, 40	–		
DCF1	**Molecular clone (CK15 *env* gene)**	**Pseudotyped CK15**	**40**	**+**	**79.5**	
DC8T	Molecular clone (CK15 *env* gene)	Pseudotyped CK15	32, 40	–		
DCV9	Molecular clone (CK15 *env* gene)	Pseudotyped CK15	32, 40	–		
DC6W	**Molecular clone (CK15 *env* gene)**	**Pseudotyped CK15**	**40**	**+**	**58.5**	
DC7W	Molecular clone (CK15 *env* gene)	Pseudotyped CK15	32, 40	–		
DA70	**Molecular clone (CK15 *env* gene)**	**Pseudotyped CK15**	**78**	**+**	**76.5**	
DA7F	Molecular clone (CK15 *env* gene)	Pseudotyped CK15	78	–		
DA7A	Molecular clone (CK15 *env* gene)	Pseudotyped CK15	70	–		
DBFN	Molecular clone (CK15 *env* gene)	Pseudotyped CK15	57	—		
DA4R	Molecular clone (CK15 *env* gene)	Pseudotyped CK15	78	–		
CJ8B	**AD8#2 swarm‡**	**RC AD8#2**	**85**	**+**	**97.8**	
CK15	Week 60 whole blood from CJ8B swarm	RC AD8#2PBMC	46, 67, 82	–		
CJ58	**Week 60 whole blood from CJ8B swarm**	**RC AD8#2PBMC**	**26, 86**	**+**	**93.2**	**24% at week 86**
CJ3V	AD8#2PBMC swarm	RC AD8#2PBMC	70, 104	–		
CK5G	AD8#2PBMC swarm	RC AD8#2PBMC	30, 50, 84	–		
CE8J	AD8#2LN swarm	RC AD8#2LN	40, 106	+	93.7	91.2% at week 106
CJ35	AD8#2LN swarm	RC AD8#2LN	32, 52, 71	–		

Neut, neutralization; RC, replication competent. Bold indicates those animals developing detectable autologous anti-HIV-1 NAbs.

*Swarm inocula were prepared from indicated animals (1) at the time of euthanasia from AIDS.

†These replication-competent SHIVAD8#2-related swarm inocula were prepared as described previously (1).

‡An infectious SHIVAD8 molecular clone carrying an *env* gene present in the SHIVCK15 swarm was prepared as described previously (2).

1. Nishimura Y, et al. (2010) Generation of the pathogenic R5-tropic simian/human immunodeficiency virus SHIVAD8 by serial passaging in rhesus macaques. *J Virol* 84:4769–4781.
2. Shingai M, Yoshida T, Martin MA, Strebel K, et al. (2011) Some human immunodeficiency virus type 1 Vpu proteins are able to antagonize macaque BST-2 in vitro and in vivo: Vpu-negative simian-human immunodeficiency viruses are attenuated in vivo. *J Virol* 85:9708–9715.