Supplementary Table 1. Alignment of SIV Env Sequences.

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T/F_consensus      MGCLGNQLLIALLLVSALEIYCVQYVTVFYGVPAWKNATIPLFCTTRNRDTWGTTQCLPDNDDYSELAISITEAFDAWNNTVTEQAIEDVWNLFETSIKP  100
DNA_gp145          ...........I..L.VYG...TL...........R........A.K..............G....V.LNV..S.................Q........  100
rAd5_gp140         ...........I..L.VYG...TL...........R........A.K..............G....V.LNV..S.................Q........  100
DNA_mosaic1        ...........I..L..YG...T............R........A.K......................NV.......D.....................  100
DNA_mosaic2        ..W...........L...G.........................A.Q.......V......G.......NV..R.................Q........  100
E660.CP3C.FJ578816 .....................................................................N..............................  100
E660.CR54.FJ578939 ......................I.....................A.K......................N..............................  100
SME543.U72748      ................V...C.......................A.......................VN..............................  100

                    _________________________V1_loop__________________________ _____________________V2_loop->__________
T/F_consensus      CVKLTPLCIAMRCNKTETDRWGLT-R-NAGTTTTTTTT-TTAAT-PS-VAENVINESNPCIKNNSCAGLEQEPMIGCKFNMTGLKRDKRIEYNETWYSRD  195
DNA_gp145          ....S....T.....S........-K-SIT..A-S----..ST.-A.AK-VDMV..TSS..AQDN.T.....Q..S............KK........A.  191
rAd5_gp140         ....S....T.....S........-K-SIT..A-S----..ST.-A.AK-VDMV..TSS..AQDN.T.....Q..S............KK........A.  191
DNA_mosaic1        ...................K....-G-TPS...-AK---.PTT.-.TA..VE-...TSS.VR.DN.T.I.....VS............KK..........  192
DNA_mosaic2        .........T.K............-.-A.T..S-SP-S-SPTTAS..--G.E.V.DTMS.T...N.S.I........Q...........K........T.  193
E660.CP3C.FJ578816 ........................-.-.......S.-.-.....-..-................N...................................  194
E660.CR54.FJ578939 ........................-.-...........T.-...-..-....................................................  195
SME543.U72748      ........................G.AETT..AKS..S-.--T.-TT-.TPK....GDS.............................K...........  195

                   ____<-V2_loop__________________
T/F_consensus      LICEQ--S-A-NESESKCYMHHCNTSVIQESCDKHYWDAIRFRYCAPPGYALLRCNDSNYSGFAPNCSKVVVSSCTRMMETQTSTWFGFNGTRAENRTYI  291
DNA_gp145          .V...--GNN-TGN..R...N....................................T.....M.K..................................  288
rAd5_gp140         .V...--GNN-TGN..R...N....................................T.....M.K..................................  288
DNA_mosaic1        .V...NNGENSTAN..R...N....................................T..........................................  292
DNA_mosaic2        .V...-N.-NG..TD.....N.......R............Y.....................M....................................  291
E660.CP3C.FJ578816 .....--.-.-.........................................................................................  290
E660.CR54.FJ578939 .....--.-.-.........................................................................................  291
SME543.U72748      .....--P-.-.G.......Q............................................K..................................  291

                                          ___________V3_loop______________
T/F_consensus      YWHGKSNRTIISLNKYYNLTMRCRRPGNKTVLPVTIMSGLVFHSQPINERPKQAWCWFGGSWKEAIQEVKETLVKHPRYTG-TNDTKKINLTAPA-GGDP  389
DNA_gp145          ....RD...............K..........................D...........K..D..K...Q.I........-..N.D.......G-....  386
rAd5_gp140         ....RD...............K..........................D...........K..D..K...Q.I........-..N.D.......G-....  386
DNA_mosaic1        ....RD..............I..............................R...........K.................-....R.......G-....  390
DNA_mosaic2        .................................I..........................K..Q........I......K.G....R...F.E.GRKS..  391
E660.CP3C.FJ578816 .................................................................................-.............-....  388
E660.CR54.FJ578939 .................................................................................-....R........-....  389
SME543.U72748      ....N................K..................................R...N.S..................-....R........-....  389

                                   ____________V4_loop_______________ ________V5_loop____________
T/F_consensus      EVTFMWTNCRGEFLYCKMNWFLNWVEDRDQKSSRWRQQNTKERQKKNYVPCHIRQIINTWHKVGKNVYLPPREGDLTCNSTVTSLIAEIDWTNNNETNIT  489
DNA_gp145          ............................NTAN-----.KP..QH.R.........................................N...IDG.Q....  481
rAd5_gp140         ............................NTAN-----.KP..QH.R.........................................N...IDG.Q....  481
DNA_mosaic1        ............................NTS.P..TT.TK..QH.R.........................................N...ID.......  490
DNA_mosaic2        ..A...............T.........NET.L..LK..P...K.R.........V.....R..........S.N.E..............YDK.Q....  491
E660.CP3C.FJ578816 ....................................................................................................  488
E660.CR54.FJ578939 ........................................R...........................................................  489
SME543.U72748      ..............................N.N..K..KKP.Q..R.............................................I........  489

                                                         gp120 ⟷ gp41
T/F_consensus      MSAEVAELYRLELGDYKLVEITPIGLAPTSVRRYTTTGASRNKRGVFVLGFLGFLATAGSAMGAASLTLSAQSRTLLAGIVQQQQQLLDVVKRQQELLRL  589
DNA_gp145          .............................D.K.--------------------------------....T..............................  549
rAd5_gp140         .............................D.K.--------------------------------....T..............................  549
DNA_mosaic1        ...............................K.....S...............................T..............................  590
DNA_mosaic2        .....G...................M.....K....S-T.K..................................................T........  590
E660.CP3C.FJ578816 ....................................................................................................  588
E660.CR54.FJ578939 ....................................................................................................  589
SME543.U72748      .............................D................................................................H.....  589

                                                                                                      ______MPER->_____
T/F_consensus      TVWGTKNLQTRVTAIEKYLKDQAQLNSWGCAFRQVCHTTVPWPNETLVPNWSNMTWQEWERQVDFLEANITQLLEEAQIQQEKNMYELQKLNSWDIFGNW  689
DNA_gp145          ..........................------------------AS.T.K.N.E.......K.....E...A.......................V....  631
rAd5_gp140         ..........................------------------AS.T.K.N.E.......K.....E...A.......................V....  631
DNA_mosaic1        ............................................D..I.D.N.........K.................................V....  690
DNA_mosaic2        A........A.............R................Q...DS...D.N...................AM...........L...............  690
E660.CP3C.FJ578816 ....................................................................................................  688
E660.CR54.FJ578939 ....................................................................................................  689
SME543.U72748      ............................................DS.....D........GK......................................  689

                   ______________TM________
T/F_consensus      FDLTSWIRYIQYGVLIVLGVVGLRIVIYVVQMLARLRQGYRPVFSSPPAYVQQIPIHKGQEPPTKEGEEGEGGDRGGNRSWPWQIEYIHFLIRQLIRLLT  789
DNA_gp145          ...A...K......Y..V..IL......I....                                                                   664
rAd5_gp140         ...A...K...                                                                                         642
DNA_mosaic1        .......K............I................K................................D.............................  790
DNA_mosaic2        ..............YL.V.IIL.......................P..T.I..........L...G........S..Y.........L...L...V....  790
E660.CP3C.FJ578816 .............................................................................S......................  788
E660.CR54.FJ578939 ....................................................................................................  789
SME543.U72748      .............................................P............D..................S......................  789

T/F_consensus      WLFSSCRDWLLRIYQVLQPVLQSLSTTSQRVREVIRIGIAYLQYGWRYFQEAVQAWWKFARETLASAWRDIWETLGRVGRGILAIPRRVRQGLELTLL  887
DNA_gp145                                                                                                            664
rAd5_gp140                                                                                                           642
DNA_mosaic1        ...NN..TC......I....F.R.FAAL...Q....TE.......C.W.......A.RA...........L.................I.........  888
DNA_mosaic2        ...N...T.....C.S....F.R..E.V..I.....LEVT......SW.K.....A.RS.G....G.GDYL..S.R......G.....I...F...M.  888
E660.CP3C.FJ578816 ............T..I...........LR.....................................................................  886
E660.CR54.FJ578939 ............T..I........................................................................I...F..A..  887
SME543.U72748      ...............I......R..R.L.........E.T......S.....A..........................................A..  887
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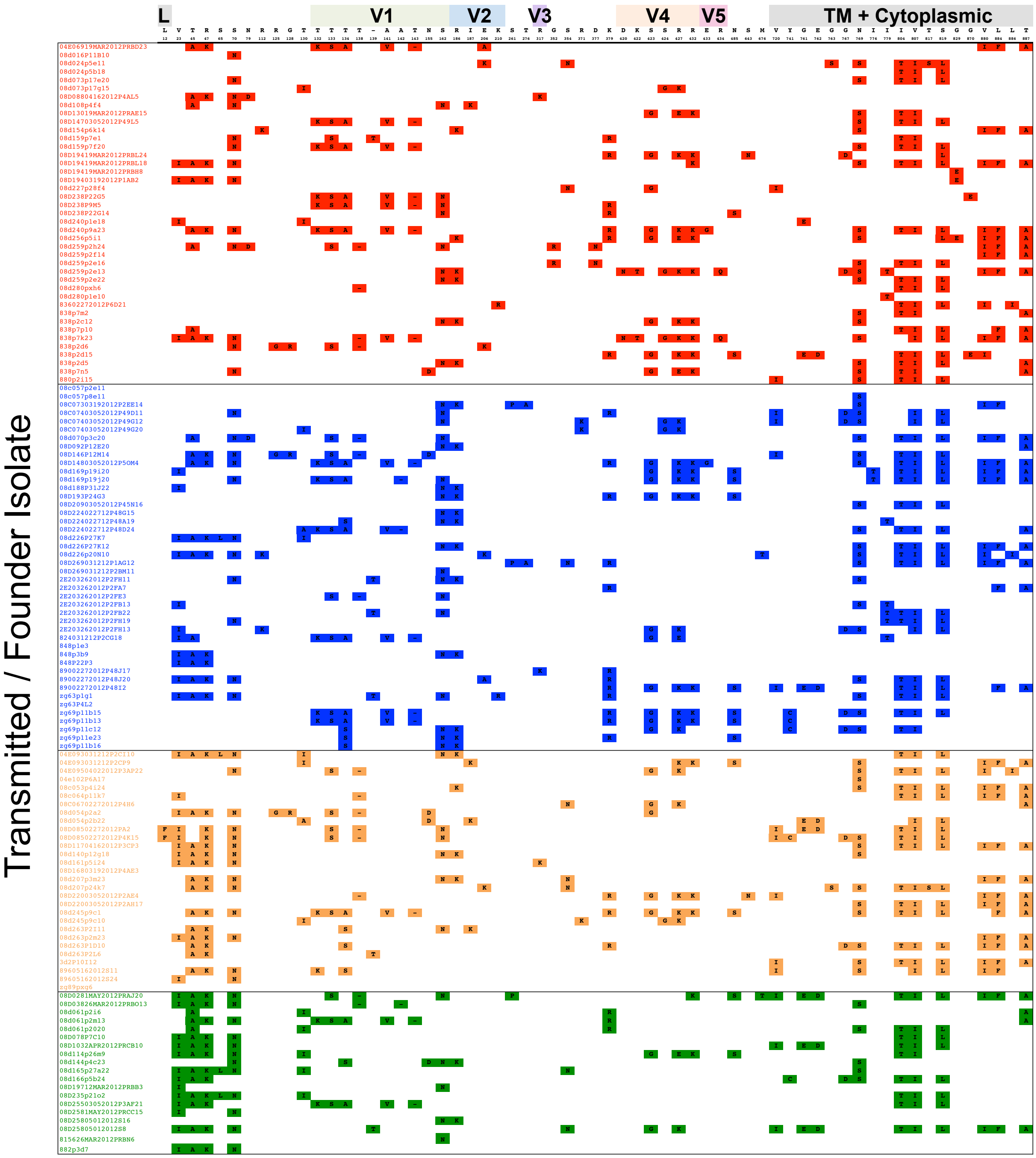
Notes. An amino acid alignment of sequences relevant to this manuscript, compared to the consensus of the transmitted/founder viruses. Comparisons are made to: (1) the DNA gp145 mac239 Env immunogen; (2) the rAd5 gp140 mac239 Env immunogen; (3, 4) the two gp160 Env mosaics, identical for both DNA and rAd5 immunogens; (5, 6) the sequences of the E660 clones, CP3C and CR54; and (7) the sequence of smE543, used for synthesizing overlapping peptides for T cell and B cell assays. Highlights: amino acids 23, 45, 47, and 70 in cyan; peptide 119+120 in yellow; and the approximate bounds of the five variable loops, the membrane-proximal extracellular region (MPER), and the transmembrane domain (TM). See also Supplementary Table 2.

Supplementary Table 2. Amino-acid distances between Env from vaccines, transmitted/founder sequences, and peptides used for immunological response testing

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	**T/F_consensus**		15.7%	15.5%	11.4%	16.1%	0.9%	1.2%	6.8%
2	**DNA_gp145**	104		0.0%	10.0%	15.6%	15.3%	15.4%	13.8%
3	**rAd5_gp140**	99	0		10.0%	15.6%	15.3%	15.4%	13.8%
4	**DNA_mosaic1**	101	66	62		16.4%	11.3%	11.1%	11.9%
5	**DNA_mosaic2**	142	103	100	145		16.0%	15.9%	16.9%
6	**E660.CP3C.FJ5788**	8	101	96	100	141		1.5%	6.6%
7	**E660.CR54.FJ5789**	11	102	97	98	140	13		6.8%
8	**SME543.U72748**	60	91	86	105	149	58	60	

Notes: Values below the diagonal show total number of amino acid differences between each pair of sequences. Values above the diagonals (percentages) show the fractional differences, adjusted for sequence length and missing data; matching amino acid gaps are not counted.

Supplementary Table 3. Transmitted/Founder (T/F) variants in Env.



Variable Amino Acid in Envelope

Transmitted / Founder Isolate

	L										V1										V2						V3						V4						V5					TM + Cytoplasmic																		
	L	V	T	R	S	S	N	R	R	G	T	T	T	T	T	-	A	A	T	N	S	R	I	E	K	S	T	R	G	S	R	D	K	D	K	S	S	R	R	E	R	N	S	M	V	Y	G	E	G	G	N	I	I	I	V	T	S	G	G	V	L	L	T
	12	23	45	47	65	70	79	112	125	128	130	132	133	134	138	139	141	142	143	155	162	186	187	206	210	261	276	317	352	354	371	377	379	420	422	423	424	427	432	433	434	485	643	676	720	741	761	762	763	767	769	776	779	804	807	817	819	829	870	880	884	886	887
04E06919MAR2012PRBD23			A	K								K	S	A			V		-					A																																				I	F		A
08d016P11B10						N																																																										
08d024p5e11																							K						N																			S		S			T	I	S	L						
08d024p5b18																																																							T	I		L						
08d073p17e20						N																																													S			T	I		L						
08d073p17g15											I																										G	K																										
08D08804162012P4AL5			A	K		N	D																					K																																				
08d108p4f4			A			N														N		K																																								
08D13019MAR2012PRAE15																																				G		E	K												S			T	I								
08D14703052012P49L5											K	S	A			V		-																																				S			T	I		L						
08d154p6k14								K													K																														S									I	F		A
08d159p7e1						N							S			T																			R																							T	I								
08d159p7f20						N					K	S	A			V		-																																				S			T	I		L						
08D19419MAR2012PRBL24																																	R			G		K	K				N							D							L						
08D19419MAR2012PRBL18		I	A	K		N																																K												S			T	I		L			I	F		A
08D19419MAR2012PRBH8																																																											E					
08D19403192012P1AB2		I	A	K		N																																																						E					
08d227p28f4																														N						G									I																				
08D238P22G5											K	S	A			V		-		N																																								E			
08D238P9M5											K	S	A			V		-		N											R																															
08D238P22G14																					N											R									S																							
08d240p1e18		I									I																																			E																		
08d240p9a23			A	K		N					K	S	A			V		-															R			G		K	K	G												S			T	I		L			I	F		A
08d256p5i1																						K										R			G		E	K												S						L	E		I	F		A
08d259p2h24			A			N	D						S		-						N							R			N																													I	F		A
08d259p2f14																																																													I	F		A
08d259p2e16																													R			N																				S			T	I		L						
08d259p2e13																					N	K												N	T		G	K	K		Q									D	S		T							I	F		A
08d259p2e22																					N	K																													S			T	I		L						
08d280pxh6															-																																							T	I		L						
08d280p1e10																																																					T										
83602272012P6D21																									R																													T	I		L			I		I	
838p7m2																																																				S			T	I										A
838p2c12																					N	K														G		K	K												S												
838p7p10			A																																																			T	I		L				F		A
838p7k23		I	A	K		N									-		V		-														N	T		G	K	K		Q											S				I		L			I	F		A
838p2d6						N			G	R			S		-								K																																						
838p2d15																																	R			G		K	K			S					E	D							T	I		L		E	I			
838p2d5																					N	K																													S			T	I		L						A
838p7n5						N														D																G		E	K												S			T	I								A
880p2i15																																														I						S			T	I		L						
08c057p2e11																																																															
08c057p8e11																																																				S												
08C07303192012P2EE14																					N	K				P	A																										S									I	F		
08C07403052012P49D11						N														N											R												I					D	S				I		L						
08C07403052012P49G12																					N										K						G	K							I					D	S				I		L						
08C07403052012P49G20											I																				K						G	K																								
08d070p3c20			A			N	D						S		-					N																															S			T	I		L			I	F		A
08D092P12E20																					N	K																																									A
08D146P12M14			A	K		N			G	R			S		-					D																											I					S			T	I		L						
08D14803052012P50M4			A	K		N					K	S	A			V		-															R			G		K	K	G												S			T	I		L			I	F		A
08d169p19i20		I																																		G		K	K			S											T		T	I		L			I	F		A
08d169p19j20						N					K	S	A				-			N															G		K	K			S											T		T	I		L			I	F		A
08d188P31J22		I																			N	K																																								
08D193P24G3																					N	K											R			G		K	K			S																					
08D20903052012P45N16																																																			S			T	I		L						
08D224022712P48G15																					N	K																																								
08D224022712P48A19														S							N	K																															T									
08D224022712P48D24											A	K	S	A			V	-																																		S			T	I		L						A
08d226P27K7		I	A	K	L	N					I																																																			
08d226P27K12																					N	K																													S			T	I		L			I	F		A
08d226p20N10		I	A	K		N		K															K																					T							S			T	I		L			I		I	
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2E203262012P2FH11						N										T					N	K																													S												
2E203262012P2FA7																																	R																														F		A
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2E203262012P2FB13		I																																																		S		T											
2E203262012P2FB22																T					N																															T	T	I		L						
2E203262012P2FH19						N																																														T	T	I		L						
2E203262012P2FH13		I						K																												G		K												D	S				I		L						
824031212P2CG18		I	A									K	S	A			V		-																			G		E															T									
848p1e3																																																															
848p3b9		I	A	K																	N	K																																								
848P22P3		I	A	K																																																											
89002272012P48J17																												K					R																													
89002272012P48J20		I	A	K		N																	A									R																				S			T	I		L						
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zg63p1g1		I	A	K		N										T					N				R								R																				S			T	I		L						
zg63P4L2																																																															
zg69p11b15											K	S	A			V		-														R			G		K	K			S				C				D	S			T	I		L						
zg69p11b13											K	S	A			V		-														R			G		K	K			S				C																
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08c053p4i24																						K																													S			T	I		L			I	F		A
08c064p11k7		I													-																																							T	I		L			I	F		A
08C06702272012P4H6																														N						G		K																										A
08d054p2a2		I	A	K		N			G	R			S		-					D																G																											
08d054p2b22											A									D			K																									E	D							I		L						
08D08502272012PA2	F	I		K		N							S		-						N																							I		E	D							T	I		L						
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08d161p5i24		I	A	K		N																					K																																		
08D16803192012P4AE3																																																															
08d207p3m23			A	K		N														N	K								N																													I	F		A
08d207p24k7			A	K		N																	K						N																			S		S			T	I	S	L						
08D22003052012P2AE4															-																		R			G		K	K				N		I															I	F		A
08D22003052012P2AH17																																																			S			T	I		L			I	F		A
08d245p9c1			A	K		N					K	S	A			V		-															R			G		K	K			S									S			T	I		L				F		A
08d245p9c10											I																				K						G	K																										
08d263P2I11			A	K										S							N		K																																							
08d263p2m23		I	A	K		N																																																				I	F		A
08d263P1D10			A	K										S																			R																D	S			T	I		L			I	F		A
08d263P2L6			A	K												T																																														
3d2P10I12																																														I						S			T	I		L			I	F		A
89605162012S11			A	K		N					K		S																																I						S				I		L			I	F		
89605162012S24		I				N																																																									
zg89pxg6																																																															
08D0281MAY2012PRAJ20		I	A	K		N							S		-						N					P													K			S		T	I		E	D							T	I		L			I	F		A
08D03826MAR2012PRBO13		I	A	K		N									-			-																																			S												
08d061p2i6			A								I																						R																														A
08d061p2m13			A	K		N					K	S	A			V		-															R																														A
08d061p2020			A								I																						R																		S			T	I		L						
08D078P7C10		I	A	K		N																																															T	I		L						
08D1032APR2012PRCB10		I	A	K		N																																					I		E	D							T	I		L						
08d114p26m9		I	A	K		N					I																										G		E	K			S													T	I								
08d144p4c23						N									S						D	N	K																													S												
08d165p27a22		I	A	K	L	N					I																			N																							S												
08d166p5b24		I	A	K																																										C				D	S			T	I		L						
08D19712MAR2012PRBB3		I																			N																																										
08D235p21o2		I	A	K	L	N					I																																											T	I		L						
08D25503052012P3AF21		I	A	K									K	S	A			V		-																																					T	I		L						
08D2581MAY2012PRCC15		I				N																																																									
08D25805012012S16																					N	K																																								
08D25805012012S8		I	A	K		N										T													N						G		K							I		E	D							T	I		L			I	F		A
815626MAR2012PRBN6																					N																																										
882p3d7		I	A	K		N																																																								

Notes. A total of 63 amino acid positions in SIV Env showed heterogeneity among T/F viruses. All variants are graphically depicted; white space indicates consensus to the E660 challenge swarm. Color coding indicates immunization arm (red: control; blue: Gag; orange: mosaic Env; green: mac239 Env).

Supplementary Table 4. Sequence alignment for C1 region of engineered envelopes.

```
              1       10        20        30        40        50        60        70
                       |         |         |         |         |         |         |         |
CP3C-P-A8     MGCLGNQLLIALLLVSALEIYCVQYVTVFYGVPAWKNATIPLFCTTRNRDTWGTTQCLPDNDDYSELAINITEAFDAWNN
CP3C-VTRS     ......................V.....................T.R......................S..........
CP3C-ITRN     ......................I.....................T.R......................N..........
CP3C-VTKN     ......................V.....................T.K......................N..........
CP3C-IAKN     ......................I.....................A.K......................N..........
CP3C-VAKN     ......................V.....................A.K......................N..........
CP3C-VARN     ......................V.....................A.R......................N..........

CR54-PK-2A5   MGCLGNQLLIALLLVSALEIYCIQYVTVFYGVPAWKNATIPLFCATKNRDTWGTTQCLPDNDDYSELAINITEAFDAWNN
CR54-VTRN     ......................V.....................T.R.................................

22 CVQ: TGTGTTCAA
22 CIQ: TGTTATCAA

44 CTT: TGTACAACC
44 CAT: TGTGCAACC

46 TRN: ACCAGGAAT
46 TKN: ACCAAGAAT

69 INI: ATCAATATC
69 ISI: ATCAGTATC
```

Notes. Individual mutations were introduced into the C1 region of CP3C or CR54 to construct viruses with amino acid variants as listed in Fig 3. The upper rows shows an amino acid alignment for all viruses. The 9 nucleotides coding for each variant (and the previous and successive amino acid) are shown below, and are identical for each virus containing the variant.

Supplementary Table 5. SIV Env peptides targeted by antisera in animals showing greater protection from acquisition.

Peptides	Region	Position	Sequence (smE543)
12-13	C1	34	AWKNATIPLFCATKNRDT
73	C2	215	VIQESCDKHYWDAIR
119-120	C3	353	AIQEVKETLVKHPRYTGT
146-151	V5	441	WHKVGKNVYLPPREGDLTCNSTVTSLIAEI

Notes. The sequences of the peptides within the four regions shown in Fig 4g. Blue lines indicate the individual overlapping 15mer peptides assayed within each region.