

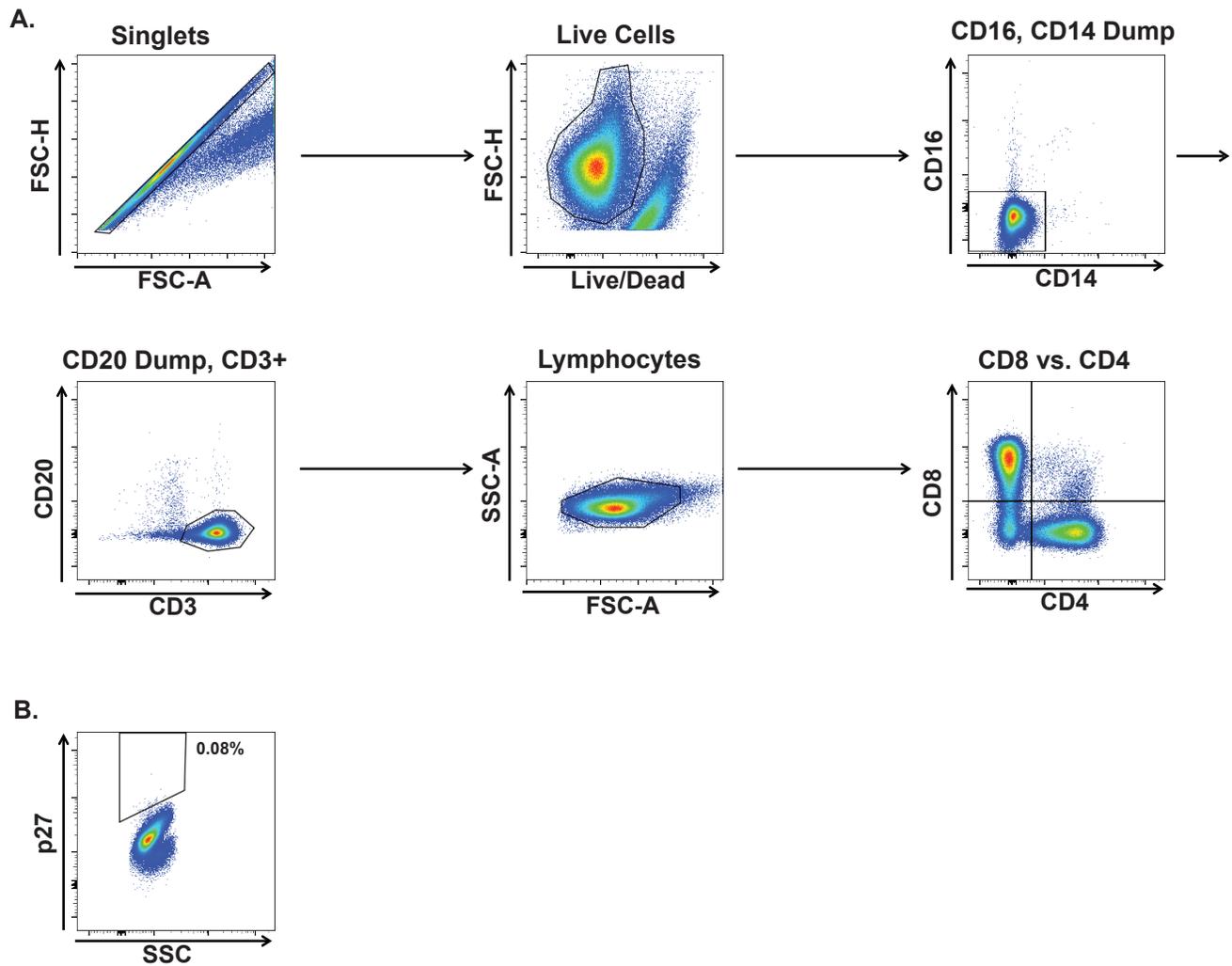
368/385

HIV-1	HIV-1 (HXB) Clade-B	S	G	G	D	P	E	I	V	T	H	S	F	N	C
	HIV-1 (Plum) Clade-A	S	G	G	D	P	E	V	T	T	H	S	F	N	C
	HIV-1 (EI55748) Clade-C	S	G	G	D	L	E	V	T	T	H	S	F	N	C
	HIV-1 (DGOB) Clade-D	S	G	G	D	P	E	I	T	T	H	M	F	N	C
	HIV-1 (AAB06205) Clade-E	S	G	G	D	L	E	I	T	M	H	H	F	N	C
HIV-2	HIV-1 (PCMO2.3) Group O	S	G	G	D	P	E	I	T	Q	L	H	F	N	C
	HIV-2- ROD	K	G	S	D	P	E	V	A	Y	M	W	T	N	C
SIVcpz	SIVcpz- TAN1	R	D	G	D	P	E	V	T	S	F	W	F	N	C
	SIVcpz- ANT	Q	D	G	D	P	E	V	K	V	H	W	F	N	C
	SIVcpz- GAB1	S	G	G	D	P	E	V	T	H	H	M	F	N	C
	SIVcpz- CAM3	P	G	G	D	P	E	V	T	N	M	M	F	N	C
SIV	SIVmac- 239	G	G	G	D	P	E	V	T	F	M	W	T	N	C
	SIVsm- 543	A	G	G	D	P	E	V	T	F	M	W	T	N	C
	SIVagm- VER3	L	F	G	D	P	E	A	A	N	L	W	F	N	C
	SIVagm- TYO	Q	W	G	D	P	E	A	S	N	L	W	F	N	C
	SIVagm- TAN1	Q	W	G	D	P	E	A	A	N	I	W	L	N	C
	SIVagm- SAB1	Q	W	G	D	P	E	S	E	F	F	F	F	N	C
	SIVrcm- GB1	Y	G	G	D	D	E	A	R	Y	F	W	L	N	C
	SIVdrl- FAO	T	K	G	D	L	A	S	E	N	L	M	M	L	C
	SIVgsn- 99CM166	P	K	G	D	L	E	V	Q	T	H	W	F	Q	C
	SIVden	V	G	G	D	P	E	T	S	S	A	R	F	Q	C
	SIVdeb- CM5	P	G	G	D	R	E	V	Q	N	T	W	F	Q	C
	SIVdeb- CM40	P	K	G	D	R	E	V	Q	S	V	W	F	Q	C
	SIVsyk- KE5	P	Q	G	D	L	E	V	R	T	H	W	F	Q	C
	SIVsyk -173	P	G	G	D	L	E	V	R	T	H	W	F	Q	C
	SIVmon	P	Q	G	D	K	E	V	Q	T	H	W	F	N	C
	SIVmus- 1085	P	K	G	D	Q	E	V	Q	T	H	W	F	N	C
	SIVcol- CGU	R	T	S	D	P	E	A	T	F	T	F	V	I	C
	SIVsun- L14	H	G	A	D	A	A	T	E	M	L	M	M	T	C
	SIVl'hst- 7	H	G	A	D	L	A	T	E	M	L	M	H	T	C
	SIVmnd- GB1	T	S	G	D	R	A	A	E	M	M	M	M	T	C
SIVmnd- 14	T	I	G	E	P	G	A	E	T	I	M	I	L	C	
SIVmnd- 2	T	K	G	E	P	G	A	E	T	I	M	L	L	C	

**Supplemental Figure 1: Amino acid sequence alignment of HIV-1, HIV-2, and SIV gp120.** Shown is a region that for HIV-1 contributes to the CD4 binding site. Aspartic acid-368 for HIV-1, which is critical for CD4 binding (i.e., D-385 for SIVmac239), is highlighted and is highly conserved with the only exception being two SIVmnd isolates.

10 20 30 40 50 60 70 80 90 100  
**SIVmac239:** MGCLGNQLLIAILLLSVYGIYCTLYVTVFYGVPAWRNATIPLFCATKNRDTWGTQCLPDNGDYSEVALNVTESFDANNVTVEQAIEDVWQLFETSIKP  
**iMac239:** -----  
 |----- V1/V2 -----  
 110 120 130 140 150 160 170 180 190 200  
**SIVmac239:** CVKLSPLCITMRCNKSETDRWGLTKSITTTASTTSTTASAKVDMVNETSSCIAQDNCTGLEQEQMISCKFNMTGLKRDKKKEYNETWYSADLVCEQGNNT  
**iMac239:** -----G-----  
 |-----  
 210 220 230 240 250 260 270 280 290 300  
**SIVmac239:** GNESRCYMNHCNTRSVIQESCDKHYWDAIRFRYCAPPGYALLRCNDTNYSGFMPKCSKVVSSTRMMETQTSTWFGFNGTRAENRTYIYWHGRDNRTIIS  
**iMac239:** -----Q-----  
 |----- V3 -----  
 310 320 330 340 350 360 370 380 390 400  
**SIVmac239:** LNKYYNLTMKRRPKNKTVLPVTIMSGLVFHSQPINDRPKQAWCWFGGKWKDAI KEVKQTI V KHPRYTGTNNTDKINLTAPGGG<sup>Δ</sup>PEVTFMWTNCRGEFL  
**iMac239:** -----Y-----  
 |----- V4 -----  
 410 420 430 440 450 460 470 480 490 500  
**SIVmac239:** YCKMWNFLNWVEDRNTANQPKPEQHKRNYVPCHIRQIINTWHKVGKNVYLPREGDLTNCNSTVTSLIANIDWIDGNQTNITMSAEVAELYRLELGDYKLV  
**iMac239:** -----K-----  
 |----- gp120 gp41 -----  
 510 520 530 540 550 560 570 580 590 600  
**SIVmac239:** EITPIGLAPT<sup>←</sup> DVKRYTTGGTSRNKRGV<sup>|</sup> FVLGFLGFLATAGSAMGAASLTLTAQSRLLAGIVQQQQQLLDVVKRQEQELRLTVWGTKNLQTRVTAIEKYL  
**iMac239:** -----T-----  
 610 620 630 640 650 660 670 680 690 700  
**SIVmac239:** KDQAQLNAWGCAFRQVCHTTVPWPNASLTPKWNNETWQEWERKVDLFLEENITALLEEAQIQQEKMYELQKLN<sup>←</sup>SWDFGNWFDLASWIKYIQYGVYIVVG  
**iMac239:** -----I-----  
 710 720 730 740 750 760 770 780 790 800  
**SIVmac239:** VILLRIVYIVQMLAKLRQGYRPFVSSPPSYFQQTHIQQDPALPTREGKERDGGEGGNSWPWQIEYIHFLIRQLIRLLTWLFSNCRLLSRVYQILQP  
**iMac239:** -----\*-----  
 810 820 830 840 850 860 870 880  
**SIVmac239:** ILQRLSATLQRIREVLRTELTYLQYGWSYFHEAVQAVWRSATETLAGAWGLDWETLRRGGRWILAI<sup>←</sup>PRRIRQGLELTL\*  
**iMac239:** -----M-----

**Supplemental Figure 2: Alignment of Env sequences for SIVmac239 and iMac239 are shown.** Variable loops and the cleavage site for gp120 and gp41 are indicated. Stop codons are denoted by asterisks (\*). A stop codon at position 734 in the gp41 cytoplasmic tail, acquired as expected (76) during in vitro passaging of iMac239 in human T cell lines and present in the p8cl18 env clone, was repaired to create the iMac239 Env. The red delta ( $\Delta$ ) symbol at position 385 denotes the site of mutagenesis to remove D385 within the CD4 binding site.



**Supplemental Figure 3: The flow cytometry gating strategy is shown for analysis of SIV-infected PBMCs.** Monoclonal antibodies included those reactive with CD3, CD4, CD8, CD20, CD14, CD16 and SIV p27 Gag. **(A)** Gating strategy is shown for CD4+ and CD8+ T cells. Shown are cytograms for uninfected cells. **(B)** A representative cytogram of uninfected cells is shown to demonstrate negligible staining for p27 Gag.