

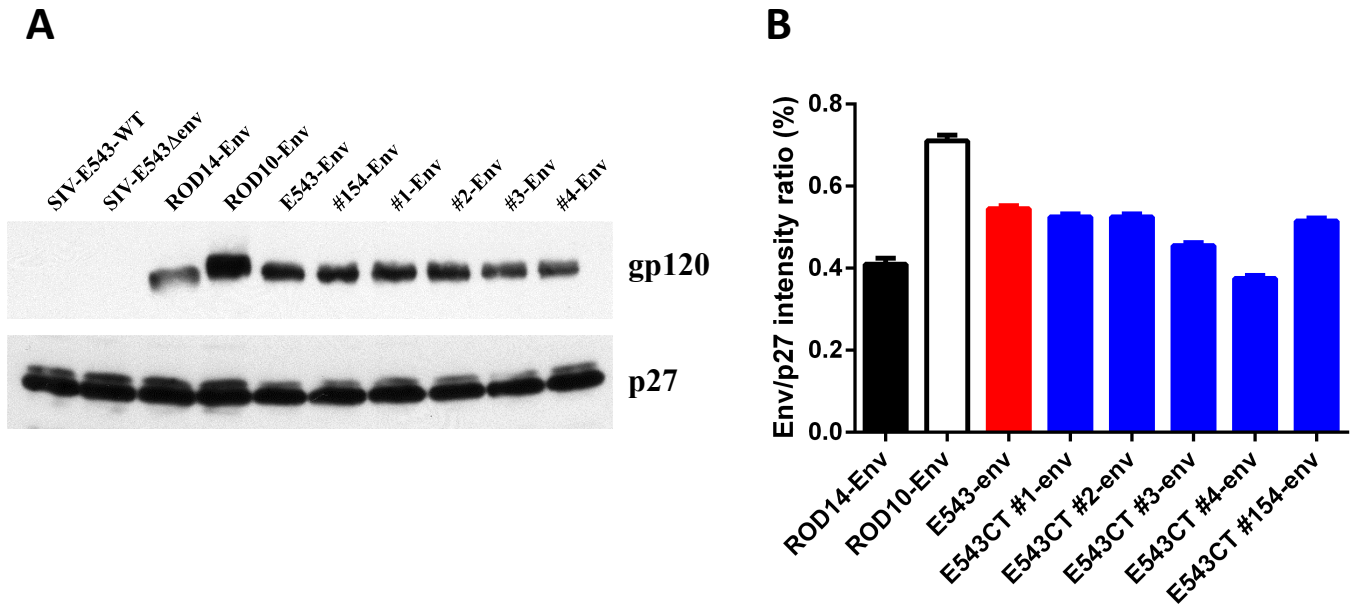
Primers

gag-R	TGA CGC AGA CAG TAT TAT AAA GGC TC
R-R	TGC TTA CTT CTA AAT GGC AGC TTT
R-F	CAGTCG CTC TGC GGA GAG GCT GG
Bgl-F	GGC AGC AGA TCT TGG CCT TGG C
nefend-R	GCC TCT TGC GGT TAG CCT TC
env10651-F	GAT GTG CTT TTA GGC AAG
nefstart-R	TAT ATT TCT GAG GCT CAC AAG AGA G
Hind-R	GAC CAT GAT TAC GCC AAG CTT
nefend-F	GAA GGC TAA CCG CAA GAG GC
nefstart-F	CTC TCT TGT GAG CCT CAG AAA TAT A
9341-R	CAT CAT CCA CAT CAT CCA TG
6463-F	GGT GTT GCT ATC ATT GTC AGC

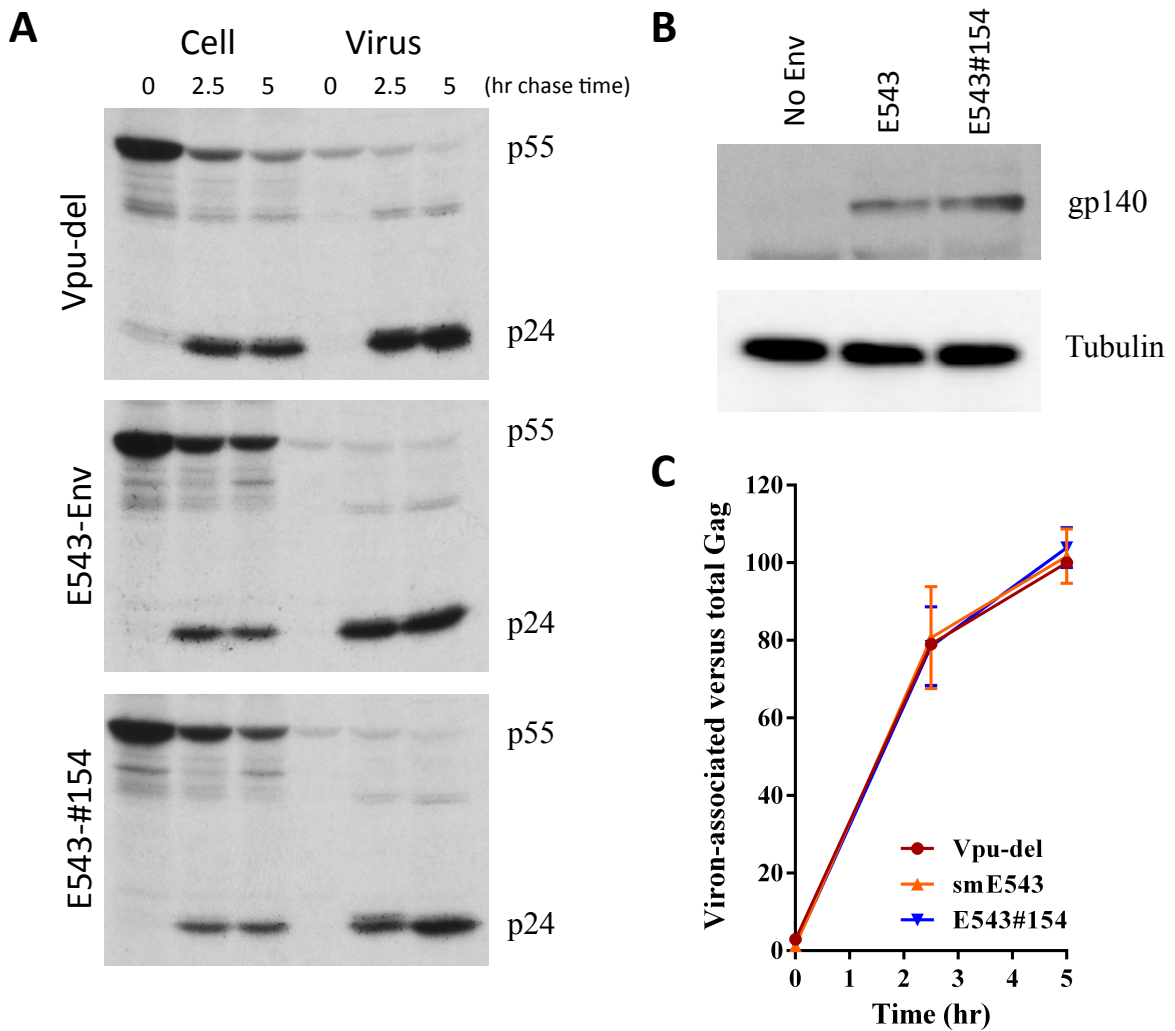
Supplemental Table 1: Primers used for construction of chimeric SIV and obtaining cDNA from viral stocks for sequencing.

Clones		Forward Primer	Reverse Primer
SIVsm543CT #1		GGA TCT ACC AGA CCC TCC AAC CAG TG	CAC TGG TTG GAG GGT CTG GTA GAT CC
SIVsm543CT #2		CAG AAT TGA AAG AAC CTA CCT AC	GTA GGT AGG TTC TTT CAA TTC TG
SIVsm543CT #3		CAG AAT TGA AAT AGC CTA CCT AC	GTA GGT AGG CTA TTT CAA TTC TG
SIVsm543CT #4		CCC TAG GCG CAT CAG GCA AGG GC	GCC CTT GCC TGA TGC GCC TAG GG
SIVsm543CT #154	1 st PCR	CAG AAT TGA AAG AGC CTA CCT AC	GTA GGT AGG CTC TTT CAA TTC TG
	2 nd PCR	GGA TCT ACC AGA CCC TCC AAC CAG TG	CAC TGG TTG GAG GGT CTG GTA GAT CC
	3 rd PCR	CCC TAG GCG CAT CAG GCA AGG GC	GCC CTT GCC TGA TGC GCC TAG GG

Supplemental Table 2: Primers used for introducing point mutations to gp41 cytoplasmic tail of SIVsmE543.



Supplemental Figure 1: Assessment of Virion incorporation of Envelope. (A) 293T cells were transfected with 4 μ g of pSIV-E543 WT or pSIV-E543 Δ env DNA in the presence or absence of pHA-smE543-Env (SIVsmE543-3), pHA-E543#1 (SIVsmE543CT #1), pHA-E543#2 (SIVsmE543CT #2), pHA-E543#3 (SIVsmE543CT #3), pHA-E543#4 (SIVsmE543CT #4) and pHA-E543#154 (SIVsmE543CT #154) as indicated. Supernatants were collected 24 hour after transfection. A fraction of the cell-free virus (80%) was pelleted through a 20% sucrose cushion and suspended in sample buffer for immunoblot analysis (virus). Samples were separated by SDS-PAGE and analyzed with antibodies to HA (Envelope) or SIV Ig-p27 (Gag: Advanced Bioscience Laboratories, Rockville, MD (cat# 4323)) as indicated. A representative of two independent experiments is shown. (B) Relative amounts of Envelope and Gag (p27) ratio were calculated. Data represent means \pm SEM from two independent analyses.



Supplemental Figure 2: HIV-1 particle release in the absence of BST-2. (A) Kinetic analysis of viral particle release by the Vpu-deficient pNL4-3/Udel-1 in the presence of HIV-2 Env or SIVsm Env. 293T cells were transfected with pNL4-3/Udel-1 together with vectors for the expression of HA-tagged Envs from SIVsmE543 wt or mutant SIVsmE543#154. Samples were subjected to pulse-chase analysis and viral proteins recovered by immunoprecipitation were separated by SDS-PAGE. The HIV-1 major Gag proteins p55_{gag} and p24_{CA} are identified on the right. (B) Protein expression for Env was verified by western blot analysis using cellular α -tubulin as a loading control. (C) Bands corresponding to the precursor and mature Gag proteins in panel A were quantified and the efficiency of particle release at each time point was calculated and plotted as a function of time. Maximal release of Vpu-del virus at 5 hr chase was defined as 100%. All other data points were normalized accordingly. No statistical significances of differences between particle release kinetics in the presence of SIVsmE543 or mutant SIVsmE543#154 Env proteins were observed compared to the cells transfected with only pNL4-3/Udel-1 vector, as assessed by the repeated measures two-way ANOVA ($F(2, 3) = 0.004609$, $P = 0.9954$).