1 Title:

2 Brief report: Mutations in SIV Nef that disrupt and restore tetherin downregulation

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15 Short title: Compensatory mutant in SIV Nef

Abstract

The H₁₉₆ residue in SIVmac239 Nef is conserved across nearly all HIV and SIV isolates, lies immediately adjacent to the AP-2 (adaptor protein 2) interacting domain (ExxxLM₁₉₅), and is critical for several described AP-2 dependent Nef functions, including the downregulation of tetherin (BST-2/CD317). Surprisingly, many stocks of the closely related SIVmac251 swarm virus harbor a *nef* allele encoding a Q₁₉₆, which is associated with loss of multiple AP-2 dependent functions in SIVmac239. Publicly available sequences for SIVmac251 stocks were mined for variants linked to Q₁₉₆ that might compensate for functional defects associated with this mutation. Variants were engineered into the SIVmac239 parental plasmid and mutant viruses were used to test tetherin downregulatory capacity in primary CD4 T cells using flow cytometry. SIVmac251 stocks that encode a Q₁₉₆ residue in Nef uniformly also encode an upstream R₁₉₁ residue. We show that R₁₉₁ restores the ability of Nef to downregulate tetherin in the presence of Q₁₉₆. However, a published report showed Q₁₉₆ commonly evolves to H₁₉₆ in vivo, suggesting a fitness cost. R₁₉₁ may represent compensatory evolution to restore the ability to downregulate tetherin lost in viruses harboring Q₁₉₆.

Introduction

The lentiviral Nef protein is a common target of CD8-T lymphocyte (CD8TL) responses in both HIV-1 infected persons and SIV infected rhesus macaques and readily evolves to evade these responses [1-6]. Nef is highly pleiotropic and mediates the downregulation of several cell surface molecules involved in innate and adaptive immune responses against virus infected cells such as TCR-CD3 (in most SIVs but not HIV-1) [7], CD4 [8-10], CD8 $\alpha\beta$ [11], CD28 [12], tetherin (BST2 or CD317; in most SIVs and in HIV-1 group O, but not HIV-1 group M) [13-15], MHC-I [16], MHC-II [17], CD1d [18], CD80/CD86 [19] and likely others as well as enhancing viral infectivity by preventing virion incorporation of host serine incorporator 3 (SERINC3) and SERINC5 proteins [20-23]. Nef-mediated modulation of several of these molecules, including CD4, CD8 $\alpha\beta$, CD28, tetherin, and SERINC3 and SERINC5 requires interactions between Nef and adaptor protein (AP)-2 complexes [11, 20, 24-28].

We used high throughput next generation sequencing to track evolution in SIV Nef [29, 30], with particular focus on viral escape from antiviral CD8TL responses, including CD8TL targeting the SIV Nef IW9 (IRYPKTFGW₁₇₃, with subscript numbers representing the position in the SIVmac239 Nef protein) and MW9 (MHPAQTSQW₂₀₃, hereafter referred to as MW9) epitopes in rhesus macaques that express Mamu-B*017:01. MW9 overlaps the well-defined “di-leucine” ExxxLM₁₉₅ motif and lies immediately upstream of the DD₂₀₅ di-acidic motif also important for AP-2 binding [31]. Though selection eventually favored changes of the first position in MW9, specifically M₁₉₅I or M₁₉₅V, an H₁₉₆Q (second position in MW9) substitution was initially favored in several animals. Since this variant was never fixed and generally lost soon after arising, we hypothesized it may have represented an effective escape mutation yet imparted a negative impact on Nef function. Specifically, we tested whether functions involving

interactions with AP-2 would most likely be impacted, given the close proximity of this epitope with the ExxxLM₁₉₅ AP-2 interaction domain. Not surprisingly, the H₁₉₆Q variant selectively disrupted Nef functions that rely on interactions with AP-2, such as downregulation of tetherin, CD4, and CD28 and disrupted Nef's ability to reduce SERINC5-mediated reductions of viral infectivity, while having no impact on MHC-I or CD3 downregulation, functions that do not rely on AP-2 interactions [32, 33]. In that study, we did not identify any potential compensatory mutations that allowed for regain of function in the presence of the H₁₉₆Q variant leading to this variant being only fleetingly detected and eventually replaced by escape mutations with less significant impacts on important Nef functions.

Many isolates of SIVmac251, a commonly used strain in SIV studies, harbor a Q₁₉₆ in the viral Nef protein. In this study, we sought mutations linked to Q₁₉₆ that might compensate for loss of function associated with this residue. We then used publicly available viral sequences to determine if Q₁₉₆ was stable in vivo. We identified an upstream variant, R₁₉₁ (E₁₉₁ in SIVmac239) that compensates for the loss of tetherin downregulation associated with Q₁₉₆. However, we also found that Q₁₉₆ routinely mutated to H₁₉₆ in vivo, suggesting reduced fitness despite the maintenance of tetherin downregulation associated with the combination of Q₁₉₆ and R₁₉₁ residues.

Materials and Methods

Ethics Statement. Cells used in this study were taken from blood from six Indian-origin rhesus macaques (*Macaca mulatta*) that are part of the breeding colony at the Tulane National Primate Research Center. Animals were anesthetized as part of their routine semi-annual health assessment (SAHA) and additional blood was drawn for this study. Thus, animals were not anesthetized specifically for the studies described herein. All animals were housed in compliance with the NRC Guide for the Care and Use of

Laboratory Animals and the Animal Welfare Act. Blood draws were approved by the Institutional Animal Care and Use Committee of Tulane University (OLAW assurance #A4499-01) under protocol P0191. The Tulane National Primate Research Center (TNPRC) is fully accredited by AAALAC International [Association for the Assessment and Accreditation of Laboratory Animal Care(AAALAC#000594)], Animal Welfare Assurance No. A3180-01. Breeding colony animals at the TNPRC are housed outdoors in social groups and frequently monitored by veterinarians and behavioral scientists. The animals were fed commercially prepared monkey chow and supplemental foods were provided in the form of fruit, vegetables, and foraging treats as part of the TNPRC environmental enrichment program. Water was available at all times through an automatic watering system. The TNPRC environmental enrichment program is reviewed and approved by the IACUC semiannually. Veterinarians at the TNPRC Division of Veterinary Medicine have established procedures to minimize pain and distress through several means. Monkeys were anesthetized with ketamine-HCl (10 mg/kg) or tiletamine/zolazepam (6 mg/kg) prior to all procedures. The above listed anesthetics were used in accordance with the recommendations of the Weatherall Report.

Primary cell isolation, culture and infection. Primary CD4 T cells were magnetically isolated from PBMC from healthy rhesus macaques using nonhuman primate CD4 microbeads (Miltenyi) according to the manufacturer's protocol. Isolated cells were stimulated with concanavalin A for two days and cultured thereafter with R15/50 media, comprised of RPMI media with 15% FBS and 50U/ml IL-2. SIV infections were conducted using the spinoculation technique [34] with each 1ml aliquot of virus (approximately 10^8 viral RNA copies per milliliter) layered on 100ul of 20% sucrose solution and centrifuged for 1 hour at 4°C at 20,000xg. After removal of the supernatant, the concentrated virus was resuspended in 100ul of R15/50 media and gently dripped

onto one million CD4 T cells plated at 1 million cells/ml in 48 well plates. Plates were then spun at 2000rpm for 2 hours at room temperature. After centrifugation, plates were placed in 37C humidified incubators with 5% CO₂. Cells were cultured for 36 hours before harvest for flow cytometry assays.

Mutant virus production. Mutants of the SIVmac239 virus were generated using site directed mutagenesis of the SIVmac239 3' hemiplasmid, using mutagenesis primers designed using web-based software (PrimerX from bioinformatics.org). To generate the H₁₉₆Q mutation alone, we used the following mutagenesis primers using the QuikChange II Site-Directed Mutagenesis Kit (Agilent); F: GCA TTA TTT AAT GCA GCC AGC TCA AAC TTC CC, and R: GGG AAG TTT GAG CTG GCT GCA TTA AAT AAT GC and to generate the E₁₉₁R mutation on the backbone that already contained the H₁₉₆Q variant, we used the following mutagenesis primers; F: GGC ACA GGA GGA TGA GAG GCA TTA TTT AAT GCA GC, and R: GCT GCA TTA AAT AAT GCC TCT CAT CCT CCT GTG CC. After mutagenesis, plasmids were treated with DpnI to remove non-mutated parental plasmids and cloned into Stbl2 cells (Life Technologies) using the manufacturer's protocol. Mutations were sequence-verified, and successfully mutated plasmids were used for follow-up studies. Mutated 3' plasmids were ligated with the 5' hemiplasmid, transfected into Vero cells followed by harvest of the virus-containing supernatant. Of note, the Nef region of interest overlaps with the 3' long terminal repeat (LTR). Attempts to mutate the full-length SIV plasmid resulted in mutations in both the 5' and 3' LTR regions, which rendered the viruses replication incompetent, necessitating the need for mutating only the 3' hemiplasmid followed by ligation to the wild type 5' plasmid. Some viruses were further expanded in CEMx174 cells. Viral RNA was extracted from all viral stocks and sequenced to ensure the presence of desired mutations.

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137 Flow cytometry. Thirty-six hours after infection, primary cells were stained with labeled
138 antibodies to CD4 (BV421, clone L200, BD Biosciences) and tetherin (PE, clone RS38E,
139 Biolegend), followed by fixation, permeabilization, and intracellular labeling with a FITC
140 labeled antibody against the Gag p27 protein (clone 55-2F12). Data was acquired on a
141 BD LSRII instrument and analyzed using Flowjo v10 software.

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143 Structural analysis. Structures showing interactions between SIVsm Nef, Tetherin, and
144 AP-2 subunits were recently published [35]. We used UCSF Chimera software [36] to
145 probe potential interactions between our Nef residues of interest at positions 191 and
146 196 and host AP-2 and tetherin proteins. The Rotamers function in UCSF Chimera was
147 used to predict impacts of mutations and the Matchmaker function was used to assess
148 positioning of Nef amino acids in SIV relative to HIV-1.

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150 Sequence analysis and alignments. Nef sequences from a broad array of SIV isolates
151 were identified from a published report [37] and downloaded from NCBI for amino acid
152 alignments using Geneious Prime 2019.1.3 using the built-in Geneious Alignment
153 algorithm with default settings. SIVmac251 sequences available from published reports
154 [38, 39] were downloaded from NCBI into Geneious Prime 2019.1.3 and mapped to
155 SIVmac239, used as the reference genome, followed by identification and quantification
156 of variations using the Find Variations/SNPs function. Sequences published in the
157 Lamers et al report [39], were first divided into those extracted from the inoculum and
158 from individual tissues, which were analyzed separately.

159

160 **Results**

Conservation of the Nef H₁₉₆ residue among primate lentiviruses. To assess conservation of the H₁₉₆ residue, we performed alignments of the flexible loop region of Nef from a diverse array of SIV isolates from Africa. Although the flexible loop is, in general, far more variable than the core, we found the H₁₉₆ residue to be almost completely conserved among all isolates sequenced to date (Figure 1A), even more conserved than important residues in the adjacent “di-leucine” ExxxLM₁₉₅ motif, E₁₉₀ and L₁₉₄.

Figure 1. Assessment of the conservation of residue 196 in SIV Nef. Alignment of the Nef core and flexible loop from several strains of SIV (A). Sequences were derived from a recent study [37]. The ExxxLM AP-2 binding motif is boxed and the H₁₉₆ residue (based on SIVmac239 numbering) is highlighted and noted by the arrow. SIVmac251 stock sequences from a published study [38] show a variant amino acid upstream of Q₁₉₆ (R₁₉₁) that was always associated with Q₁₉₆ (B). Alignments were performed using Geneious Prime 2019.1.3.

We next scanned publicly available sequences from a recent study that used single genome amplification to extensively examine SIVmac251 challenge stocks [38]. In this report, a Q₁₉₆ residue was detected in a large fraction of sequences from SIVmac251 stock viruses from several labs. Interestingly, there was a perfect linkage between the Q₁₉₆ residue and an upstream R₁₉₁ residue, which is E₁₉₁ in SIVmac239 (Figure 1B). Of 38 total sequences that contained the region of interest, derived from three different challenge stocks, 25 sequences contained both R₁₉₁ and Q₁₉₆ while Q₁₉₆ was never found in the absence of R₁₉₁. Other nearby variants relative to SIVmac239 were detected but only R₁₉₁ co-occurred with Q₁₉₆ in all sequences.

Upstream compensatory variant restores tetherin downregulation. Given the strong linkage between the R₁₉₁ (E₁₉₁ in SIVmac239) variant and Q₁₉₆, we tested whether R₁₉₁ allowed tetherin downregulation in the presence of Q₁₉₆. The R₁₉₁ residue lies within the ExxxLM₁₉₅ motif (EEHYLM₁₉₅ in SIVmac239, ERHYLM₁₉₅ in many SIVmac251 isolates). We introduced the E₁₉₁R variant along with the H₁₉₆Q onto the SIVmac239 backbone to assess tetherin downregulation. Viruses harboring H₁₉₆Q alone were largely deficient in tetherin downregulation, as expected. When E₁₉₁R was introduced along with H₁₉₆Q, the resulting virus showed full competency in tetherin downregulation, similar to SIVmac239 (Figure 2A). N-fold analysis of tetherin downregulation in cells from multiple animals demonstrated significant loss of downregulation in the virus harboring only H₁₉₆Q, while the addition of E₁₉₁R restored this ability to wild type levels (Figure 2B).

Figure 2. SIVmac251 maintains tetherin downregulation despite a unique AP-2 binding motif. (a) Representative flow cytometric analysis of surface expression of tetherin on primary CD4 T cells infected with wild type SIVmac239 or SIVmac239 harboring the H₁₉₆Q variant alone or in combination with the E₁₉₁R variant. Cells were identified as infected via intracellular Gag p27 staining, top row, as we have described previously [29, 30]. Infected cells (p27+) are orange, while uninfected (p27-) are blue. Surface expression of tetherin compared between infected cells (orange line) and uninfected (blue line) are shown in the bottom panels. (b) N-fold analysis of tetherin downregulation from multiple experiments using cells derived from at least three different RM and compared by way of a two-tailed t-test.

Structural insights. The structure of SIVsm Nef bound to simian AP-2 was recently published [35]. We used UCSF Chimera structural analysis software [36] to assess how the residues at positions 191 and 196 interact with host AP-2 and tetherin molecules.

The critical residues in the dileucine motif [E₁₉₀, L₁₉₄, V₁₉₅ (M₁₉₅ in SIVmac239)] show clear interaction with AP-2, while H₁₉₆ is oriented in the opposite direction (Figure 3A), similar to H₁₆₆ in HIV-1 (Figure 3B) [28], which is homologous to H₁₉₆ in SIV. We next used the Rotamers function in UCSF Chimera to determine whether the H₁₉₆Q variant impacted interactions with AP-2. Replacement of the H with a Q at this position resulted in a large number of possible rotamers for Q₁₉₁, nearly all of which maintained a similar orientation as H₁₉₁, directed away from AP-2, suggesting no obvious impact on the interaction between Nef and AP-2. However, the H₁₉₆Q variant is predicted to disrupt a salt bridge between H₁₉₆ and tetherin residue D₁₅ as assessed using PISA (Proteins, Interfaces, Structures, and Assemblies) software [40], suggesting disruption of a direct interaction between Nef and tetherin may contribute to the selective disadvantage of this change.

Figure 3. Structural insights into Nef, AP-2, and tetherin interactions. (a) Although adjacent to the ExxxLM motif that directly binds Nef to AP-2, H196 is oriented away from this interaction. (b) Alignment between SIVsm and HIV-1 Nef (PDB: 4NEE) with H₁₆₆ (HIV-1) and H₁₉₆ (SIVsm) highlighted. (c) T₁₉₁ in SIVsm directly interacts with K₁₈ in the DIWK motif of tetherin.

Position 191 is a T in SIVsm, as opposed to E₁₉₁ in SIVmac239. While this residue does not contact AP-2, intriguingly, it does interact directly with the K₁₈ residue in the DIWK motif of the tetherin protein itself via a hydrogen bond (Figure 3C). Replacement of T₁₉₁ with an E (as in SIVmac239) maintained the predicted hydrogen bond with K₁₈, suggesting this interaction holds true between SIVmac239 and tetherin. Further, replacement of E₁₉₁ with an R resulted in only low probability orientations, preventing a meaningful analysis of this structural change.

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240 *In vivo stability of the Q₁₉₆ residue.* Finally, we wished to assess the in vivo stability of
 241 the Q₁₉₆ residue. We hypothesized that since the combination of Q₁₉₆ and R₁₉₁ residues
 242 allowed for efficient tetherin downregulation in vitro, that Q₁₉₆ would be stable in vivo
 243 when it exists in combination with R₁₉₁. We used publicly available sequences from a
 244 recent study wherein macaques were infected with an stock of SIVmac251 that harbored
 245 virus with nearly 90% containing the combination of Q₁₉₆ and R₁₉₁ [39] based on our
 246 analysis of their deposited sequences. That study used a modified Single Genome
 247 Amplification (SGA) method to quantify viral variation in plasma throughout infection and
 248 multiple neurological tissues at necropsy. After infection, the Q₁₉₆ residue was detectable
 249 primarily at three weeks post infection, with the exception of a small number of reads
 250 that contained Q₁₉₆ at 3 months. This residue was thereafter lost in all three animals and
 251 was not detected in any neurological sites in any animals at necropsy (meninges,
 252 parietal lobe, temporal cortex) (data available in the cited manuscript and in their
 253 deposited sequences).

254

255 **Discussion**

256 The SIVmac251 viral swarm is pathogenic in rhesus macaques and has been
 257 used in hundreds of studies to date. However, this swarm has been independently
 258 grown in many labs using multiple cell types and under a variety of conditions [38]. It
 259 stands to reason that there may be genetic differences between SIVmac251 viral stocks
 260 leading to unique biological differences, but few of these differences have been
 261 characterized for how they impact specific virologic properties, including the
 262 downregulation of host tetherin.

263 The ability to downregulate host tetherin is a feature of a wide variety of
 264 enveloped viruses ranging from Ebola to HIV [41, 42]. Most SIV isolates use the viral Nef

protein to perform this task [13, 43, 44] but several isolates use alternate pathways, suggesting strong selection to maintain this function. Surprisingly, Nef encoded by SIVcpz cannot downregulate human tetherin and studies suggest that evolution of HIV-1 Vpu to gain the ability to downregulate tetherin was a critical event in the HIV-1 epidemic [43, 45]. Thus, countering tetherin likely is an important feature of all or nearly all SIV and HIV isolates.

In addition to interactions with host AP-2 proteins, SIV Nef is known to interact directly with the tetherin protein [46] and a subset of those interactions were recently verified structurally [35]. These structures show that H₁₉₆ does not directly interact with host AP-2 but is predicted to form a salt bridge with tetherin, which is predicted to be disrupted in the H₁₉₆Q variant using PISA software [40]. However, our previous report showed that the H₁₉₆Q variant disrupted multiple Nef functions that rely on AP-2 interactions suggesting that disruption of a direct interaction with tetherin likely does not fully explain the functional deficits identified in this variant. Here, we show that evolution of the E₁₉₁R variant restored tetherin downregulation in the presence of Q₁₉₆. Intriguingly, T₁₉₁ in SIVsm interacts directly with the lysine in the DIWK motif in the tetherin protein [35], suggesting variation at this residue may impact tetherin downregulation via a direct effect on this interaction. E₁₉₁ in SIVmac may also interact with this K₁₈ residue as these two amino acids are well known to form hydrogen bonds, although we cannot confirm without structural data.

Many strains of SIVmac251 encode a Nef protein with a Q₁₉₆ residue, which is always linked to an upstream R₁₉₁ residue. Here we show that the presence of R₁₉₁ fully restores competency in downregulation of tetherin in the presence of Q₁₉₆. Nonetheless, our data also suggest that Q₁₉₆ is not stable in vivo and evolves to H₁₉₆, the residue present in nearly all SIV isolates. These data beg the question of how the Q₁₉₆ residue arose in the first place. It's possible it arose during replication in cultured cells where

selection pressures are undoubtedly different than those the virus experiences in vivo.

Given our data suggesting the H₁₉₆Q variant can arise in vivo in SIVmac239 infected macaques as a result of escape from CD8TL responses [30], these data may suggest that SIVmac251 was isolated from an animal that targeted this region with CD8TL, leading to viral escape, and prior to other escape variants becoming dominant, as happened in our previous study [30].

Taken together, our mutational and functional data combined with published structural and sequence data suggest the possibility that the E₁₉₁R variant in SIVmac might enhance an interaction between Nef and tetherin thus restoring the ability of Nef to downregulate tetherin in the presence of the H₁₉₆Q variant, but that this variant may not restore other functions, thus leading to selection to restore H₁₉₆ in vivo. The existence of compensatory variation in viral proteins has been described in SIV and HIV-1 [47-50] but those descriptions are restricted to viral structural proteins, primarily Gag. Our data suggest R₁₉₁ in SIVmac251 may exist to compensate for loss of function associated with Q₁₉₆. If so, this may be the first report of compensatory variation in the viral Nef protein or any nonstructural viral protein. However, our analysis of published in vivo data clearly demonstrate that Q₁₉₆ evolves to H₁₉₆ in vivo. Finally, our data do not suggest that stocks of SIVmac251 that harbor a Q₁₉₆ residue are in any way less useful than stocks that do not. Instead, our data underscore the need to understand the evolutionary pressures that give rise to particular viral variants, which may be relevant in the choice of virus stock for animal model experiments.

Author contributions

NJM conceived the study and wrote the first draft. BS conducted the experiments and edited all drafts.

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Figure 1

A

SIVmac239	LVGVSVRPKVPLRTMSYKLAIDMSHFKEKGGLEGIIYSARRHRILDIYLEKEEGIIIPDWQDYTSGLGIRYPKTFGLWKLVPV	NVS--DEAQED EEHYLMHPAQTSQWDDPWGE
SIVsmm	EI...H.R.Q..A.T.....E.....N..K.....MY.....	D...T...T.C.V...V.N.....
HIV-2A	...T.R.Q...T...V...L...R...MF..E.....N..H.....MF.....	D.P--Q.GEDT.T.C.L...V...RH...TH..
SIVcpz	E..FP...Q...P.T..A.F.L...L.....LI..Q..QE...LWVYHTQ.FF...N..P...V...V...CF.....	DPTDVE..N.GDNNV.L...MCQHGE.EHR.
HIV-1A	E..FP...Q...T.P.T..A.V.L...L.....D.LIW.QK.QD...LWVYHTQ.YF...N..P...F.L...CF.....	EPEAVE..TGG.NNS.L...ICQHGM...ER.
HIV-1B	E..FP.T.Q...P.T..A.V.L...L.....LI..QK.QD...LWVYHTQ.YF...C.N..P...V.F.L...CF.....	EPEKVE..N.G.NNS.L...LSPHGME..EK.
HIV-1C	E..FP.T.Q...P.T..S.F.L.F.L.....LI..KK.QE...LWVYHTQ.FF...N..P...V...L...CF.....	DPREVE..N.G.DNC.L...VCQHGE.EHR.
SIVrcm	...FP.K.Q...P.....L.....W.I..Q...M...N.H.....N..P.....TL...Q...	D...R...S.L...E..GME.....
SIVagn	...FP...R...Q.T...V.F...L.....W.PK.EQ..NL.ALN.W...D...A.SP...T.K.RC..FCFE...	D...Q...R.C.L...IEWES...K.
SIVmnd-2	ET.FP.Y.QC.V.EPT..DLV..P..L.....WH.K..EE...L.AQN.W.F.T..S..D.....RF.F..C...	A.P-P.Q-ENN.CNK.LQSS.LGIQESL.R
SIVtan	...FP...Q...Q.T...V.F...L.....W.P..EQ..NL.ALN.W...D...V.SP...K..C..FCFE...	RL--EA.ATN.R.C.L...NYM.....
SIVsun	E..FP...QR..TQPT..NL..H.F.....LW..KT.EE..I...A.N.W...G..A.....P.....M..	TID-E.RGPNHPCQA.L.SS.QGVNE.S...
SIVdeb	E..FP.K.R..I.DPT...M..Y...L.....D.F.....A..ELHAQN.W...G.LQ..E.....Y..F.F.....	EIADP.YEN-..RNI.L.D.HQG.ME..YK.
SIVden	E..FP...RR..HAPT..DM.....L.....QD.F..P...A...L.AQN.H.F.TG..T..D.....LE..F.....	TIEE-EYDN-K..NC.L.DRYEG.QA...R.
SIVgsn	--SCP...Q...DPT...MV.L...L.....AMF.CED..QK.ES.CYY.W..V.G.LQW.P.....TMP.FC.C.R..	AMTEDS.PG-.DQYL.N...YQG.QE.HHR.
SIVmon	G..CP...R...DPTW..MM.L..YL.....GEMF.CED...KIEQ.AYL.W.L..G.LQ..E...V...TMP.F..C.R..	ATTEDS.EG-.DFL.T...YQGRME..HRQ
SIVmus	--SCP...QH.IQNPT..I.NL...L.....MF..EE..QK.ET.AYV.W.L..G.LM..E...V...LMP.V.FC.R..	HITENS.LG-.D..L.C...FIGREE..HK.
SIVtal	...FP.....CT...G..F.F.L.....K..F.NK...A..NL.AHN.W..L...N..E...T...LC..I...C...	EIHED.T--EDG.L.P...YDG.AE.....

Globular Core

Flexible Loop

B

SIVmac239	191 196
SIVmac251	EEHYLMH
	.R....Q

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Figure 2

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Figure 3