Supplemental Digital Content - Methods:

SIVsmB7 and CEMx174 Proteomic Analysis

SDS-PAGE Gels:

20 µl of B7 and CEM each were loaded to a 12% NuPage 1-mm-thick gel. The gel was stained with Invitrogen Colloidal Coomassie Blue stain.

Protein Digestion:

Selected bands were excised from the gel and subsequently destained using 200 µl of 200 mM ammonium bicarbonate and 50% acetonitrile for 30 min with shaking at 37°C and then dried in a SpeedVac. This was followed by reduction/alkylation of the protein band by adding 100 µl 20 mM TCEP in 25 mM ammonium bicarbonate at pH 8.0, incubated for 15 min at 37°C with shaking. The supernatant was discarded and 100 µl of 40 mM iodoacetamide in 25 mM ammonium bicarbonate at pH 8.0 was added and incubated for 30 min at 37°C with shaking. The supernatant was again discarded and two subsequent washes of 200 µl 25 mM ammonium bicarbonate were done for 15 min each with shaking. A final wash was done under the same conditions, except 50% acetonitrile, 25 mM ammonium bicarbonate was used for the wash. The bands were dried in the SpeedVac and were then rehydrated with 20 µl of 0.02 µg/µl modified Trypsin (Promega) in 40 mM ammonium bicarbonate overnight with shaking at 37°C. The next morning, the supernatant was removed to a clean tube on ice and 20 µl of 40 mM ammonium bicarbonate was added to the gel piece for

30 min with shaking at 37°C. The supernatants were combined and 4 µl of neat acetic acid were added to stop the digest.

Protein Identification:

4 µls of the combined digest were injected onto a nanocapillary reverse-phase column (self-packed, New Objective 75 um column terminating in a nanospray 15 um tip) directly coupled to a ThermoElectron Orbitrap mass spectrometer. A top six method was used to obtain MS and MS/MS data. A customized database was created using a Simian database with common contaminants and bovine (tagged) added. The database was indexed for partial tryptic searching and the resulting masses and MS/MS spectra from the Orbitrap were searched against this database using the SEQUEST search engine [1, 2]. Subsequent data were filtered using 5 ppm, delta cN of 0.07. Results were reported as spectral counts.

Hormone Measurements:

Blood was taken every other day between days 7-23 of the menstrual cycle. Estradiol and progesterone concentrations were measured by enzyme-amplified chemiluminescence (Immulite 1000, Siemens). The analytical limits of sensitivity of the estradiol and progesterone assays were 15 pg/mL (references range of 20-2,000 pg/mL) and 0.1 ng/mL (reference range of 0.2-40 ng/mL), respectively.

Immuno-histochemistry:

Tissues were deparaffinized and rehydrated in deionized water. Heat-induced epitope retrieval was performed using the water-bath method (95–98°C for 10–

20 min) in 10 mM sodium citrate, pH6.0 for CD123 detection (Sc-681, 1:2000, Santa Cruz Biotechnology Inc.), and Mx1 (1:3000, ProteinTech) and CD68 (KP1, 1:200, Dako). For CD4 detection (IF6, 1:60, Leica Microsystems), epitope retrieval was performed using high-pressure Decloaking Chamber (121°C for 35 sec, Biocare Medical) in 1 mM EDTA, pH 8.0, followed by cooling to room temperature. Tissue sections were blocked with SNIPER Blocking Reagent 5% Non-fat milk (Biocare Medical) for 1 hr at room temperature. Endogenous peroxidase was blocked with 3% (v/v) H2O2 in methanol TBS (pH7.4) 3% [v/v] for CD68, Mx1 and CD123, and 0.9% for CD4 detection). Primary antibodies were diluted in 10% SNIPER Blocking Reagent in TNB blocking buffer (Tris-HCl, pH7.5, 0.15M NaCl, 0.05% Tween 20 with 0.5 Dupont blocking reagent buffer) and incubated overnight at 4°C. After the primary antibody incubation, sections were washed and then incubated with mouse, goat, or rabbit polymer system reagents conjugated with either horseradish peroxidase or alkaline phosphatase (ENVISON kit; Dako) according to the manufacturer's instructions, and developed with 3,3′-diaminobenzidine (Vector Laboratories). Sections were hematoxylin counterstained, mounted in Permount (Fisher Scientific), and examined by light microscopy. Non-specific, IgG was used as isotypic control.

Whole sections of each stained slide were digitized using Scanscope (Aperio), the image was opened in ImageScope, and endocervical areas were selected with ImageScope drawing tools for analysis. CD4+, CD68+, Mx1+ or CD123+ cells were quantified by using a positive pixel count algorithm in the Spectrum

Plus analysis program (Version 9.1, Aperio). The parameters of the algorithm were manually tuned to match the specific staining markup image accurately over background DAB stain. Once the parameters were set, the algorithm was applied automatically to all digital slides to measure the number of cells of interest. Data were reported as positive staining cells per square millimeter.

DNA Isolation and MHC Genotyping:

Genomic DNA was isolated from a maximum of 3.0 × 106 peripheral blood mononuclear cells using the MagNA Pure LC system (Roche Applied Science) and the MagNA Pure LC DNA Isolation–Large Volume protocol (version 3.0) according to manufacturer's guidelines. The elution volume of extracted DNA was 200 µl of MagNA Pure LC DNA Isolation–Large Volume elution buffer. DNA concentrations (ng/µl) and Abs 260 nm/Abs 280 nm ratios were determined using a NanoDrop UV Spectrophotometer (NanoDrop Technologies). Genotyping for Mamu-A*01, A*02, A*08, A*11, B*01, B* 03, B*04, B*17, B*08, and DRBw*201 was done as previously described [3-5].

Viral Load:

Plasma samples were spiked with armored RNA (aRNA; Asurgen) and centrifuged at 25,000 x g for 1 h. Viral RNA (vRNA) was extracted from the pellet with Proteinase K (2.5 µg/µl; Life Tech) and the High Pure Viral RNA kit (Roche). Eluted vRNA (100 µl) was then subjected to the RNA Clean and Concentrator kit (ZYMO Research) and eluted in 50 µl, from which 15 µl were reverse transcribed

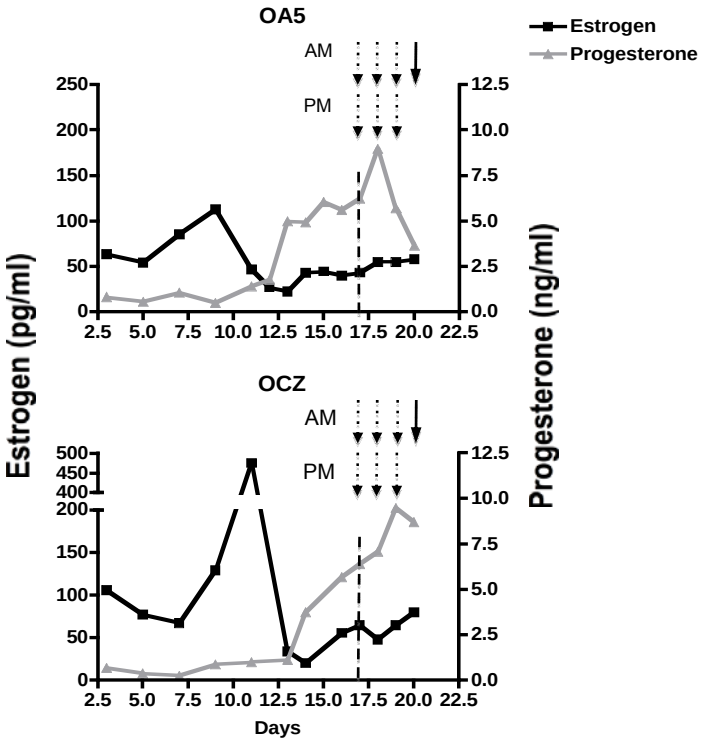
using MultiScribe™ Reverse Transcriptase (Life Tech) in a 50-µL gene-specific reaction. Fourteen microliters of cDNA were added to TaqMan gene expression master mix (Life Tech), along with primers and a probe targeting the gag region of SIVmac251, and subjected to 40 cycles of qPCR analyses. Fluorescence signals were detected with an Applied Biosystems 7900HT Sequence Detector. Data were captured and analyzed with Sequence Detector Software (Life Tech). Viral copy numbers were calculated by plotting CT values obtained from samples against a standard curve generated with in vitro-transcribed RNA representing known viral copy numbers. The limit of detection of the assay was five copies per reaction volume or 40 copies per ml of plasma.

SIVmac251 Titration:

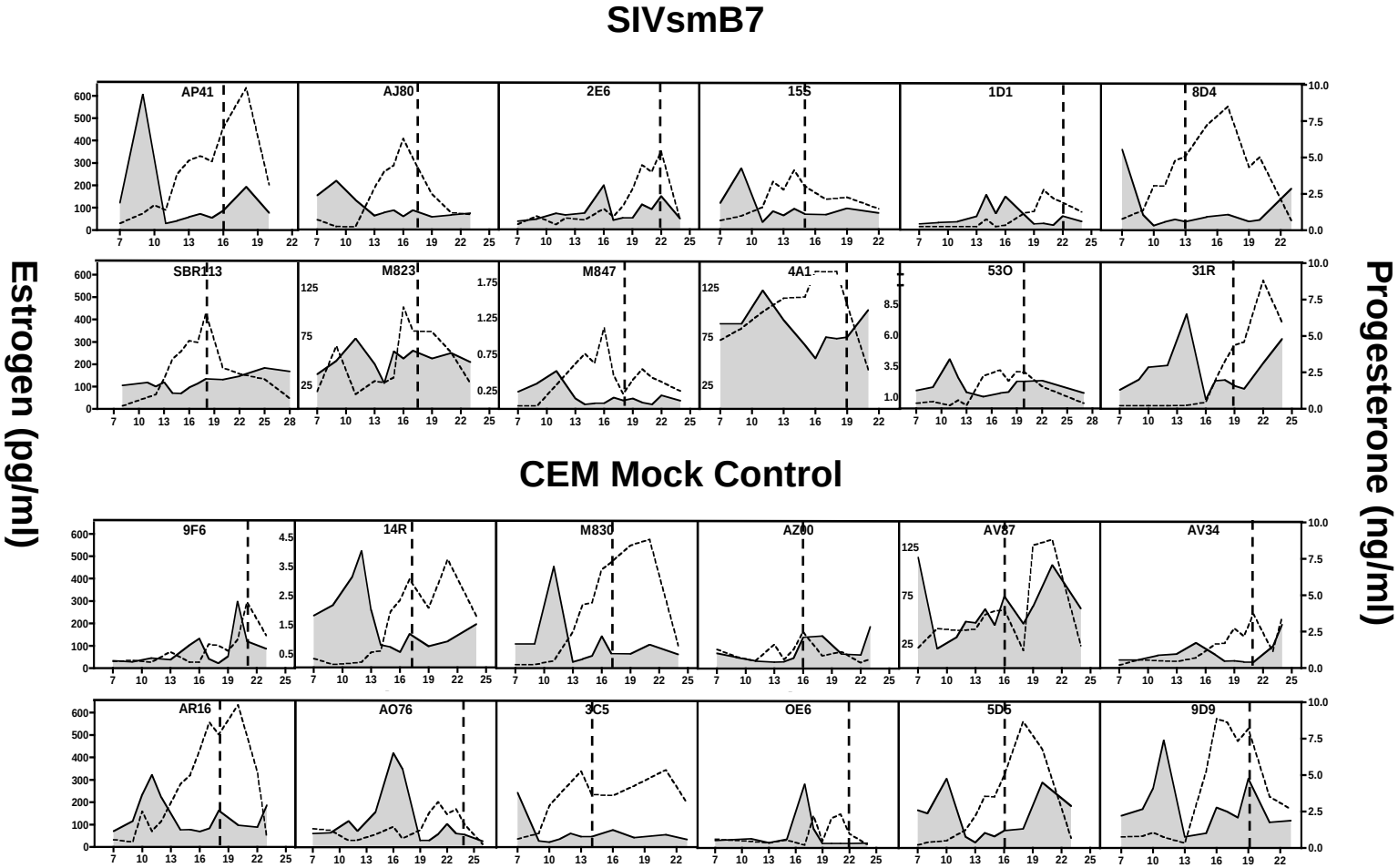
Infectious challenge was carried out with SIVmac251. The infectious dose via intravaginal delivery was independently determined in a titration study on 15 female rhesus macaques upon vaginal challenge preceding our study. Macaques were intravaginally inoculated with either a single dose of undiluted virus or a 1:5 or 1:10 dilution. All macaques given undiluted virus became infected. Thirty-eight percent of macaques given a 1:5 dilution became infected. To achieve a dose to infect all control animals a 1:2 dilution was chosen for the challenge dose.

References

1. Chittum HS, Lane WS, Carlson BA, Roller PP, Lung FD, Lee BJ, et al. Rabbit beta-globin is extended beyond its UGA stop codon by multiple suppressions and translational reading gaps. Biochemistry 1998,37:10866-10870.
2. Yates JR, 3rd, Eng JK, McCormack AL, Schieltz D. Method to correlate tandem mass spectra of modified peptides to amino acid sequences in the protein database. Analytical chemistry 1995,67:1426-1436.
3. Loffredo JT, Maxwell J, Qi Y, Glidden CE, Borchardt GJ, Soma T, et al. Mamu-B*08-positive macaques control simian immunodeficiency virus replication. Journal of Virology 2007,81:8827-8832.
4. Kaizu M, Borchardt GJ, Glidden CE, Fisk DL, Loffredo JT, Watkins DI, et al. Molecular typing of major histocompatibility complex class I alleles in the Indian rhesus macaque which restrict SIV CD8+ T cell epitopes. Immunogenetics 2007,59:693-703.
5. Kuroda MJ, Schmitz JE, Lekutis C, Nickerson CE, Lifton MA, Franchini G, et al. Human immunodeficiency virus type 1 envelope epitope-specific CD4(+) T lymphocytes in simian/human immunodeficiency virus-infected and vaccinated rhesus monkeys detected using a peptide-major histocompatibility complex class II tetramer. Journal of Virology 2000,74:8751-8756.



Supplemental Digital Content - Figure S1: Phase I hormone profiles for luteal phase inoculated macaques. [Top] OA5 was inoculated with CEM mock control, and [Bottom] OCZ was inoculated with SIVsmB7. Animal menstrual cycling was tracked for at least one month prior to inoculations to map out hormone profiles (data not shown). Dashed lines indicate start of twice daily, a.m. and p.m., inoculations. Dashed arrows represent individual inoculations as described in Methods. Inoculations were confirmed to take place approximately three days after the start of the luteal phase. Animals were euthanized (solid black arrow) and cervicovaginal tissue sections taken after the final estrogen/progesterone sample was measured. Axis scales were altered to highlight inflection of hormone profiles from follicular to luteal phases. Estrogen (Black Line/Left Axis) Progesterone (Grey Line/Right Axis).



Supplemental Digital Content - Figure S2: Estrogen/progesterone profiles for Phase II macaques. Estrogen (Grey/Left Axis) Progesterone (Dashed line/Right Axis). Horizontal dashed line indicates SIVmac251 challenge as described in Methods. Macaques were targeted for challenge during the estrogen low, progesterone high luteal phase to avoid potential reduced SIV infectivity during the follicular phase. Axes for M823, 4A1, 9F6, Av87 were modified to show change of menstrual stage.

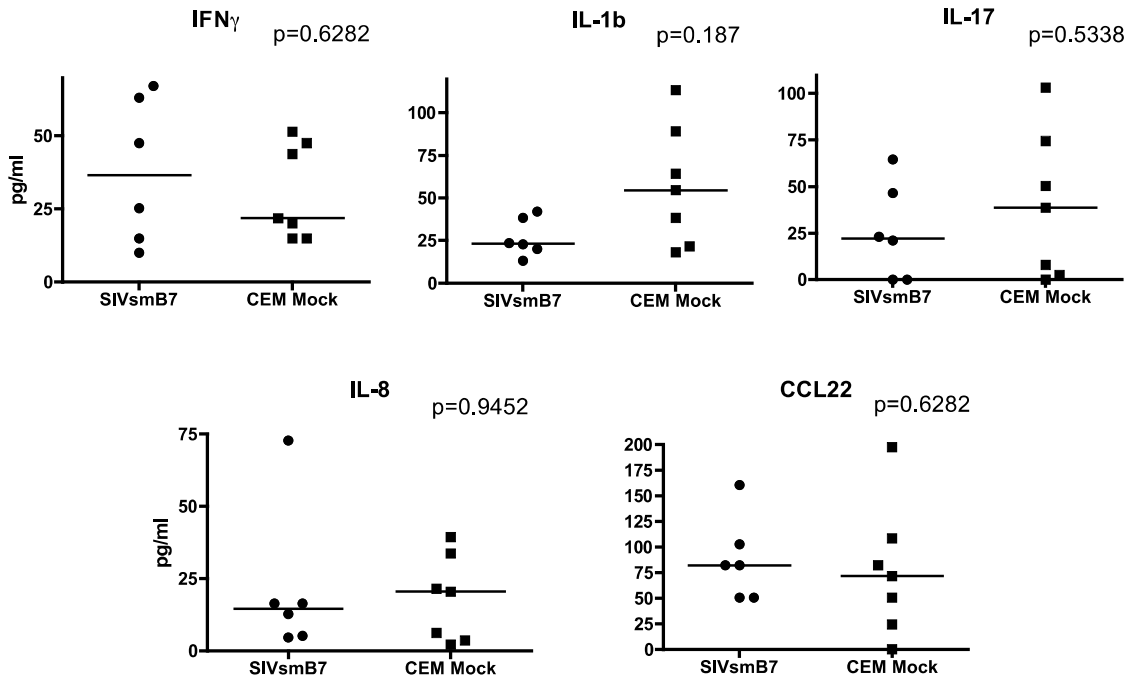
Supplemental Digital Content – Table S1: Target MHC I Genotypes for All Phase II Macaques*

Macaque ID	Study Arm	MHC I**
14R	SIVsmB7	A01/B17
3C5	SIVsmB7	A08
5D5	SIVsmB7	A01/A02
9D9	SIVsmB7	-
9F6	SIVsmB7	A01
AO76	SIVsmB7	-
AR16	SIVsmB7	A01
AV34	SIVsmB7	B01
AV87	SIVsmB7	-
AZ00	SIVsmB7	-
M830	SIVsmB7	A02
OE6	SIVsmB7	-
15S	CEM Mock Control	
1D1	CEM Mock Control	A08/B01
2E6	CEM Mock Control	A08
31R	CEM Mock Control	-
4A1	CEM Mock Control	A08
53O	CEM Mock Control	A08
8D4	CEM Mock Control	B01
AJ80	CEM Mock Control	A08
AP41	CEM Mock Control	A01
M823	CEM Mock Control	A01
M847	CEM Mock Control	A08
SBR113	CEM Mock Control	A08/B17

* MHC I alleles tested for include A*01, A*02, A*08, A*11, B*01, B* 03, B*04, B*17, and B*08.

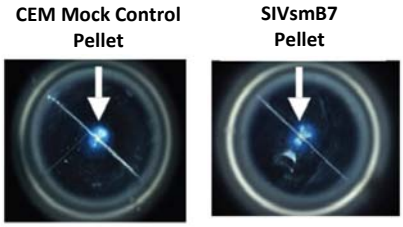
A*01, B*08 and B*17 have been associated with control of SIV infection in Rhesus macaques [33-35].

** Dashes indicate that macaque had a genotype not assessed within our panel



Supplemental Digital Content - Figure S3: Results from selected cytokines tested in CVL Cytokine Multiplex Assay. Luminex 28-plex Assay (Invitrogen) was done according to manufacturer protocol on PBS lavage samples obtained within 1-week prior to macaque manipulation. Nearly half of the inflammatory cytokines tested were below the level of detection. Of those with detectable levels of cytokine there was no significant differences between macaques, including inflammatory mediators IL-1b, IFNγ and IL-17 and chemokines IL-8 and CCL22. P-values were generated by two-sided Wilcoxon rank sum tests.

A



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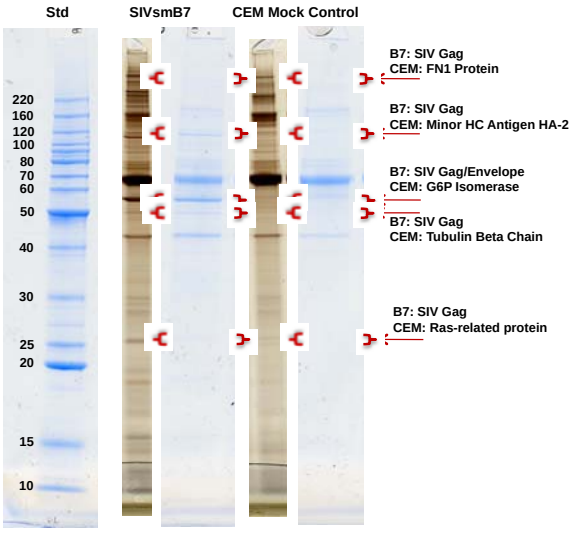

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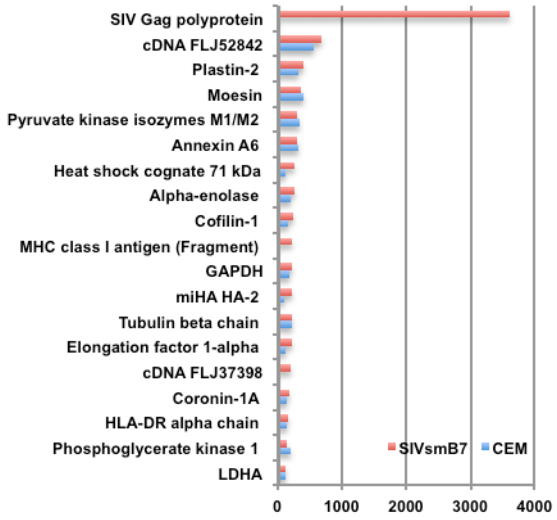

D.

Combined Spectral Counts for MHC Proteins

Prep	MHC Class I	MHC Class II
CEM Mock	108	306
SIVsmB7	954	556

Supplemental Digital Content - Figure S4: Proteomic analysis and comparison of SIVsmB7 and CEM control. (A) Picture of pellets after ultra-centrifugation. Note the similarities in size and appearance of the pellets. (B) Silver staining of protein gels for CEM mock control and SIVsmB7 showed a high degree of similarity outside of five distinct bands in SIVsmB7 not found in CEM mock control. A colloidal stain was done to confirm the band differences. Once confirmed, those bands were excised for proteomic analysis. In each of these five bands, SIV gag polyprotein or envelope was identified. (C) Top 15 proteins for SIVsmB7 and CEM mock control organized by SIVsmB7 abundance. The list only contains 19 proteins, showing the high degree of protein composition similarity between the two. The only truly notable protein not found in CEM mock control is SIV gag polyprotein, which is the most abundant protein found in SIVsmB7. (D) Table showing the abundances of MHC protein fragments detected in SIVsmB7 or CEM Mock control. Individual spectral counts were grouped according to MHC Class I or Class II for clarity.

Method:

SIVsmB7 is a virus-like particle (VLP) derived from a clone of a CEMx174 cell line infected with SIVsmH3 . Clone B7 cells have a single provirus integrated into their 20th chromosome. SIVsmB7 is noninfectious due to a 1.6 kbp deletion,

including integrase, vif, vpr, and vpx genes [29]. To produce cell-free SIVsmB7 and CEM mock control, 200,000 cells/ml of Clone B7 and uninfected CEMx174 cells were cultured in RPMI 1640 (Mediatech, Inc.) supplemented with FBS. VLPs was purified on 20% (w/v) sucrose gradient by ultra-centrifugation. Figure 1B illustrates individual pellets from CEM mock control and SIVsmB7 after supernatants are removed. The pellet was resuspended in RPMI 1640 without supplementation. ELISA was done to measure P27 content. SIVsmB7 was diluted to a concentration of 500 µg P27 SIVsmB7 per 1 ml dose. CEMx174 dose was prepared using identical techniques.

Supplemental Digital Content - Table S2: Proteomic Analysis of Individual Differential Bands from SIVsmB7 and CEM mock control samples

CEM Band Protein Name	Coverage	Spectral Count
Band 1		
FN1 protein	14.80%	29
Oleoyl-[acyl-carrier-protein] hydrolase	3.80%	6
Uncharacterized protein	2.20%	5
Dermcidin isoform 2	18.20%	3
Hornerin	4.50%	3
Type I keratin 16; K16	8.50%	2
Band 2		
Minor Histocompatibility Antigen HA-2	23.00%	27
Alpha Actin 4	27.60%	26
Kinesin-like protein KIF23	17.20%	15
Intercellular adhesion molecule 3	8.40%	7
cDNA FLJ56016, highly similar to C-1-tetrahydrofolate synthase, cytoplasmic	8.20%	9
Ubiquitin-like modifier-activating enzyme 1	8.10%	7
Band 3		
Glucose-6-phosphate isomerase	19.30%	14
Pyruvate kinase isozymes M1/M2	30.10%	19
cDNA FLJ61188, highly similar to Basigin	37.80%	13
Uncharaterized protein	31.10%	14
cDNA FLJ37935 fis, clone CTONG2005290, highly similar to FASCIN	28.20%	14
Plastin-2	23.10%	13
Band 4		
Tubulin beta chain	41.20%	29
Alpha-enolase	39.60%	28
Coronin-1A	25.60%	16
Elongation factor 1-alpha	7.60%	5
APT synthast subunit beta, mitochondrial	15.10%	8
Tubulin beta-2C chain	9.40%	5
Band 5		
Ras-related protein Rab-35	15.90%	4
RAB11B protein	19.30%	4
60S ribosomal protein L18	13.80%	3
Uncharacterized protein	13.60%	3
B-lymphocyte antigen CD20	12.80%	3
S-9 (Fragment)	19.00%	3

SIVsmB7 Band Protein Name	Coverage	Spectral Count
Band 1		
Gag Polyprotein	60%	71
Filamin-A	15%	27
Desmoplakin	5.90%	17
Junction plakoglobin	11.90%	7
Desmoglein-1	9.10%	6
Ubiquitin/40S ribosomal protein S27a	44.40%	6
Band 2		
Gag Polyprotein	59.80%	130
Minor histocompatibility HA-2	34.60%	51
Kinesin-like protein KIF23	28.10%	26
Alpha-actinin	26.50%	26
Major vault protein	23.60%	18
Putative helicase MOV-10	16.10%	17
Band 3		
Gag Polyprotein	75.70%	397
Tubulin beta chain	55.20%	26
cDNA FLJ37935 fis, clone CTONG2005290, highly similar to FASCIN	44.30%	23
Coronin-1A	41.40%	18
cDNA FLJ61188, highly similar to Basigin	37.80%	9
cDNA FLJ32131 fis, highly similar to Tubulin alpha-ubiquitous chain	29.60%	6
Band 4		
Gag Polyprotein	44.40%	41
Alpha-Enolase	41.70%	39
Tubulin beta chain	45.90%	35
Elongation factor 1-alpha	31.80%	26
cDNA FLJ60299, highly similar to Rab GDP dissociation inhibitor beta	43.90%	23
Coronin-1A	20.00%	23
Band 5		
Gag Polyprotein	37.70%	52
cDNA FLJ75480, highly similar to Human lactoferrin	22.50%	15
Ras-related protein Rab-35	24.80%	8
Ras-related protein Rab-11A	34.70%	6
Elongation factor 1-alpha	9.70%	5
40S ribosomal protein S9	19.6%	4

Supplemental Digital Content- Table S2: Results of individual band proteomic analysis for SIVsmB7 and CEM control. Top six proteins from each of the excised bands shown in Figure 1 from CEM mock control [Left] and SIVsmB7 [Right]. As noted previously, SIV gag polyprotein was the dominant protein in each of those excised bands from SIVsmB7. The remaining proteins found in those bands were comparable to proteins identified from bands excised from CEM mock control taken from the same position in the CEM mock control lanes. Total coverage of the given protein is shown along with total spectral counts.