Supporting Information

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SI Materials and Methods

Isolation and Culturing of Myeloid DCs. Buffy coats of blood samples from healthy adult donors were purchased from Gulf Coast Blood Center (Houston, TX) and the peripheral blood mononuclear cells (PBMCs) were separated by the standard ficoll-centrifugation methodology to isolate the $CD11c^+$ myeloid DCs as previously described (19). In brief, the DC-enriched population (lineage$^-$cells) was obtained from PBMCs by negative selection using a mixture of monoclonal antibodies (mAbs) against the lineage markers CD3 (OKT3), CD14 (M5E2),CD16 (HB78), CD20 (L27), CD56 (B159), and CD235a (10F7MN) (BD Biosciences), followed by the use of goat anti–mouse IgG-coated magnetic beads (M-450; Dynal and Miltenyi Biotec). The $CD11c^+$, lineage$^-$ and $CD4^+$ cells were isolated by a FACS Aria sorter (BD Biosciences) using APC-labeled anti-CD11c (B-ly6), a mixture of FITC-labeled mAbs against lineage markers including CD3 (SK7), CD14 (MØP9),CD16 (3G8), CD19 (HIB19), and CD56 (NCAM16.2) (BD Biosciences), and pacific blue-labeled CD4 (OKT4; eBioscience) to reach >99% purity. The $CD11c^+$ DC were cultured in RPMI containing 10% FBS by seeding at a density of 1–3 $\times$ 10^5 cells/well in 200-μL volume in a flat-bottom 96-well plate in the presence of culture medium alone, 100 ng/mL rTSLP (prepared in-house using an adenovirus vector system as described previously (28), or supernatants collected from epithelial cells cultured without or with HIV (at an MOI of 0.5 or 600 $TCID_{50}$ based on MAGI assay and equivalent to 60,705 cpm of RT activity) and designated as HIV$^-$Sn and HIV$^+$Sn, respectively. The epithelial cells were incubated with HIV-1 overnight. The virus was removed by washing the cells 3$\times$ with PBS. Fresh medium was added and the cells were then incubated at 37 °C overnight and the resultant supernatant was assayed for reverse transcriptase activity to confirm removal of residual input virus. After the DCs were treated with neutralizing antibody against TSLP the cells were washed and then either recombinant TSLP or the HIV-1+ SN were added to the cells. The HIV$^+$Sn contained 100 ng/mL TSLP protein, as determined by ELISA. For determining TSLP-specificity of DC activation, a mixture of two neutralizing antibodies (including one TSLP monoclonal antibody and one rabbit anti-human TSLP polyclonal antibody) were add to mDCs for 30 min before culturing the cells with culture medium, rTSLP, HIV$^+$Sn, or HIV$^-$Sn to block the TSLP activity. The neutralizing monoclonal anti-TSLP antibody IgG1 (R&D Systems) and the polyclonal rabbit anti-TSLP IgG were used at concentrations of 10 and 20 μg/mL, respectively, along with the corresponding isotype control monoclonal and polyclonal antibodies at matching concentrations.

Isolation of Naïve $CD4^+$ T Cells. The naïve $CD4^+$ T cells were isolated from PBMC on the basis of $CD45RA^+$, $CD45RO^-$, and lineage$^-$ phenotype by using a human $CD4^+$ T-cell enrichment mixture (Stem Cell Technologies) followed by the use of a biotin labeled CD45RA, an APC-labeled anti-biotin antibody, a mixture of FITC-labeled mAbs against the lineage markers CD14(MØP9), CD16 (3G8), CD19 (HIB19), CD56 (NCAM16.2), CD11C (HL3), TCR$\gamma\delta$ (V65), PE-labeled mAbs against CD45RO (UCHL1) (all from BD Biosciences), FITC-labeled BDCA2 (AC144), PE-labeled BDCA4 (AD5–17F6), PE-labeled CD25(4E3) (all from Milteny Biotech), and pacific blue-labeled CD4 (OKT4) (eBioscience) in a FACS Aria sorter (BD Biosciences). The final $CD4^+CD45RA^+CD45RO^-CD25^-$ lineage$^-$ fraction isolated was >99% pure.

$CD4^+$ T-Cell Expansion. Purified mDC were activated, in the presence or absence of anti-TSLP antibody (w/antibody and w/o antibody, respectively) with supernatants collected from C33A cells cultured with or without $HIV\text{-}1_{IIIB}$ (HIV$^+$Sn/DCs and HIV$^-$Sn/DCs, respectively), and co-cultured with 2.5 to 5.0 $\times$ 10^4 purified autologous naïve $CD4^+$ T cells (DC:T cell ratio, 1:1 or 1:2) in a round bottomed 96-well culture plates for 7 days. On day 6, the co-cultures were pulsed for 16–18 h with 1 μCi [^{3}H]thymidine and then the cells were collected to assess for proliferation in terms of [^{3}H]thymidine incorporation. In some cases, activated mDCs were co-cultured with carboxyfluorescein diacetate succinimidyl diester (CFSE)-labeled $CD4^+$ T cells. The co-cultures were resuspended in RPMI medium containing 10% FBS. The mixture was then incubated for 5 min in 37 °C in 10% FBS-RPMI 1640 and the cells were pelleted by centrifugation. The cells were subsequently washed three times with 10% FBS-RPMI 1640. On day 7, the CFSE co-cultured cells were stained with CD11c APC and CD3 PE-Cy7 to separate $CD4^+$ T cells from mDCs and were analyzed with a FACSCalibur to determine proliferation.

HIV Infection Assay. Virus production in HIV-infected cells was analyzed by determining the reverse transcriptase (RT) activity as reported earlier (31). Viral particles from the infected culture supernatants were precipitated with 30% polyethylene glycol (50% volume/volume) and pelleted by centrifugation at 3,000 rpm for 30 min at 4 °C. The viral pellets were resuspended in 100 μL RT buffer (glycerol, 25 mM Tris-HCl, pH 7.5, 5 mM DTT, 150 mM KCl, and 0.025% Triton X-100) followed by lysing with the addition of 100 μL of 0.9% Triton X-100 in 1.5 M KCl. A 10-μL aliquot of this preparation was mixed with 90 μL RT mixture (50 mM Tris-HCl, pH 7.8, 63 mM KCl, 4.2 mM $MgCl_2$, 0.08% Nonidet P-40, and 0.85 mM EDTA), 4.2 μg/mL poly(A), 0.13 μg/mL of oligo(dT), 8 μL of 0.5 M DTT, and 10 μL of [Methyl-^{3}H] (ICN Pharmaceuticals Inc.) and incubated at 37 °C for 1.5 h. The reaction mix was precipitated with 10% trichloroacetic acid (TCA) and 1% sodium pyrophosphate. The TCA precipitable raw cpm (cpm) were determined on a Wallace 1409 liquid beta-scintillation counter (Wallace). The mean radioactivity for the duplicate infections were determined and the mean background cpm values from duplicate samples of uninfected control cultures run in parallel in each assay were subtracted to calculate the specific RT activity in the infected cell cultures.

Animals. Vaginal tissue samples were obtained from rhesus macaques (*Macaca mulatta*) infected with SIV_{mac251} by the vaginal route in a previous study at the California Primate Center (20). The animal facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and the study was conducted according to National Institute of Health Guidelines on Care and Use of Laboratory Animals.

Tissue Collection and RNA Isolation. Collection of vaginal tissues from animals euthanized at different days post-infection with SIV_{mac251} were described earlier (20). All of the samples for RNA analyses were collected and stored in RNAlater at −20 °C. The tissue samples were homogenized, and then the total RNA was isolated by using the RNeasy Qiagen kit (Qiagen). The purified RNA was used to determine tissue TSLP RNA levels by real-time quantitative RT-PCR using the rhesus macaque-

specific TSLP primers: 5′-CGACAG CATGGTTCTTCTCA-3′ and 5′-CGATTTGCTCGAACTTAGCC-3′.

Real-Time Quantitative RT-PCR. T cells cultured with TSLP activated mDC were lysed and mRNA was extracted with an RNeasy kit (Qiagen). Reverse transcription was done with SuperScript II (Invitrogen), and the cDNA samples were analyzed by real-time quantitative PCR assay with an ABI Prism 7500 Sequence Detection system (Applied Biosystems). Reactions were incubated for 2 min at 50 °C, denatured for 10 min at 95 °C, and subjected to 40 two-step amplification cycles with annealing-extension for 60 °C for 1 min followed by denaturation at 95 °C for 15 s. For the analysis of human IL-4, IL-5, IL-10, IL-13, TNF-α, and INF-γ, the primer sequences were as follows: IL-4: 5′-CACCGAGTTGACCGTAACAG-3′ and 5′-GCCCTGCAGAAGGTTTCC-3′; IL-5: 5′-AGCTGCCTACGTGTATGCCA-3′ and 5′-GCAGTGCCAAGGTCTCTTTCA-3′; IL-10: 5′-GAGATCTCCGAGATGCCTTCA-3′ and 5′-CAAGGACTCCTTTAACAACAAGTTGT-3′; IL-13: 5′-ACAGCCCTCAGGGAGCTCAT-3′ and 5′-TCAGGTTGATGCTCCATACCAT-3′ and INF-γ: 5′-GTTTTGGGTTCTCTTGGCTGTTA-3′ and 5′-AAAAGAGTTCCATTATCCGCTACATC-3′. The human TNFα real time PCR probe was purchased directly from the manufacturer (SuperArray).

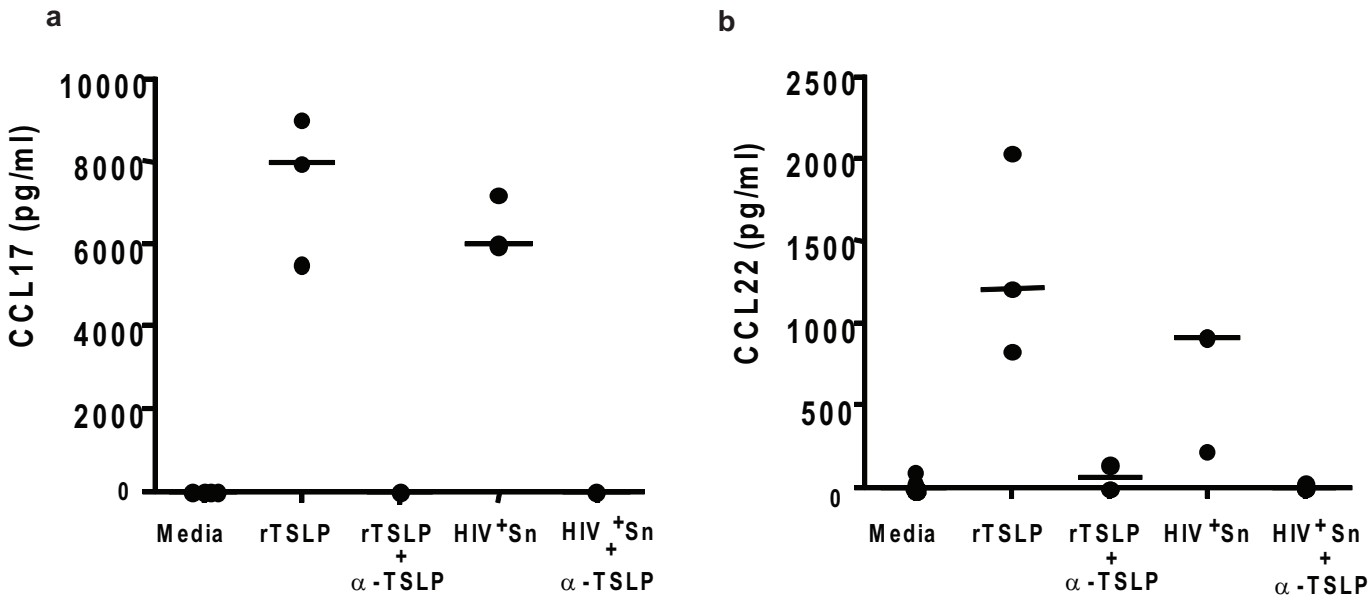

Fig. S1. TSLP within the supernatant from epithelial cells cultured with HIV potently activates human CD11c$^+$ myeloid dendritic cells (mDCs) and induces production of CCL17 and CCL22. Treatment of mDC isolated by fluorescence sorting from the PBMC samples of multiple donors with rTSLP or supernatants collected from C33A cells cultured with HIV-1_{IIIB} (HIV$^+$Sn) resulted in the expression of high amounts of the chemokines CCL17 and CCL22 as determined by protein analysis by ELISA. The mDC isolated from multiple donors were pretreated with TSLP-specific antibody (α-TSLP) before stimulation with culture medium, rTSLP, or supernatants collected from C33A cells cultured with or without HIV-1_{IIIB} (HIV$^+$Sn and HIV$^-$Sn, respectively), and the expression levels of the chemokines CCL17 (*A*) and CCL22 (*B*) were determined.

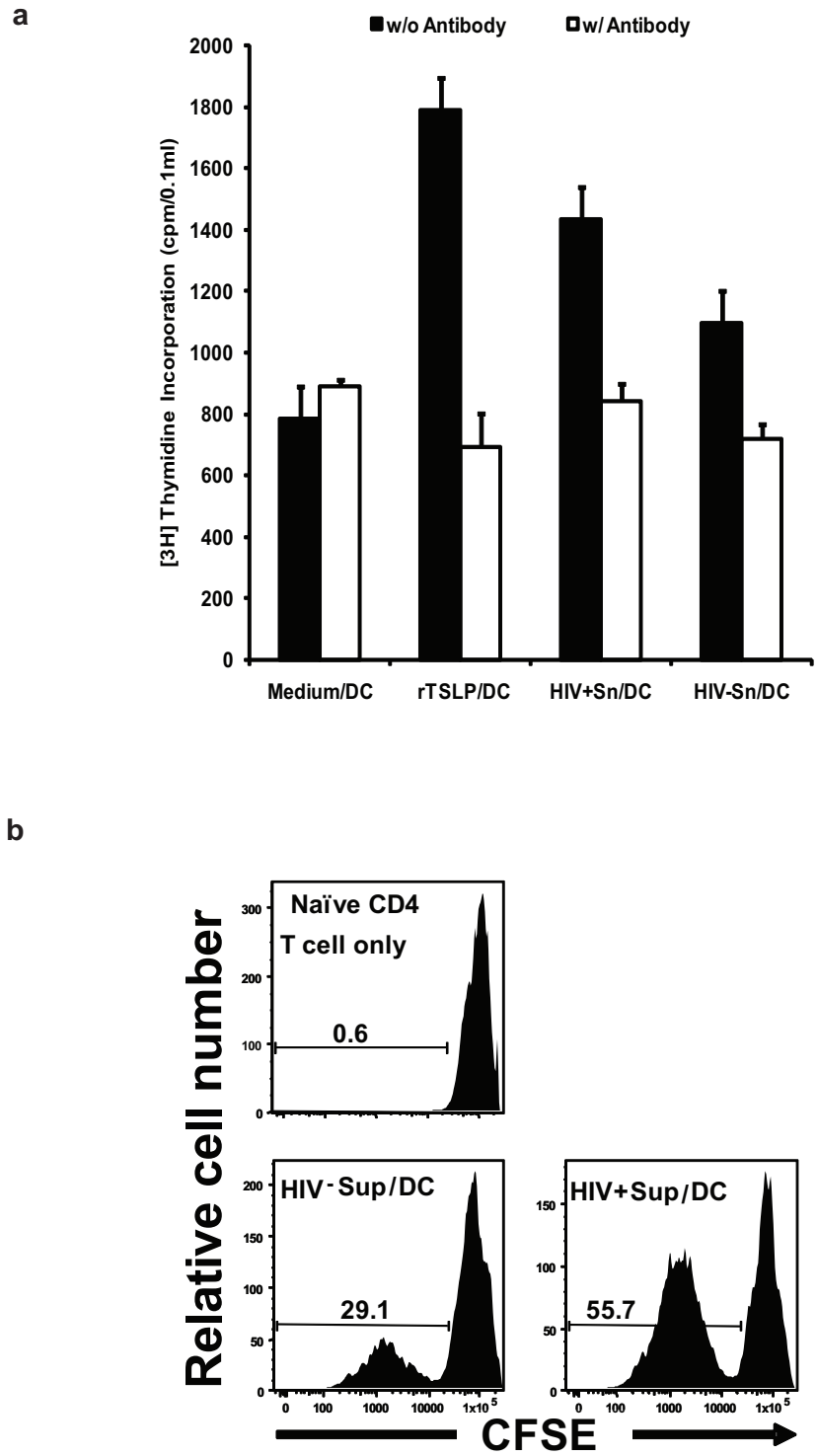

Fig. S2. mDCs activated by HIV-induced TSLP from epithelial cells promote naïve autologous $CD4^+$ T-cell proliferation. Naïve autologous $CD4^+$ T cells were co-cultured with mDCs that were activated in the presence or absence of anti-TSLP antibody (w/antibody and w/o antibody, respectively) by preincubating with the culture medium (Medium/DCs), rTSLP (TSLP/DCs), or supernatants collected from C33A cells cultured with or without HIV-1_{IIIB} (HIV^+Sn/DCs and HIV^-Sn/DCs, respectively). On day 7, cells were pulsed with [^{3}H]thymidine (*A*). Error bars, standard deviation values for triplicate cultures, and data shown are average values of three independent experiments. Proliferation of CFSE labeled naïve $CD4^+$ T cells was also assessed by culturing in medium alone or along with HIV^+Sn/DCs and HIV^-Sn/DCs (*B*). Data shown is representative of three independent experiments.

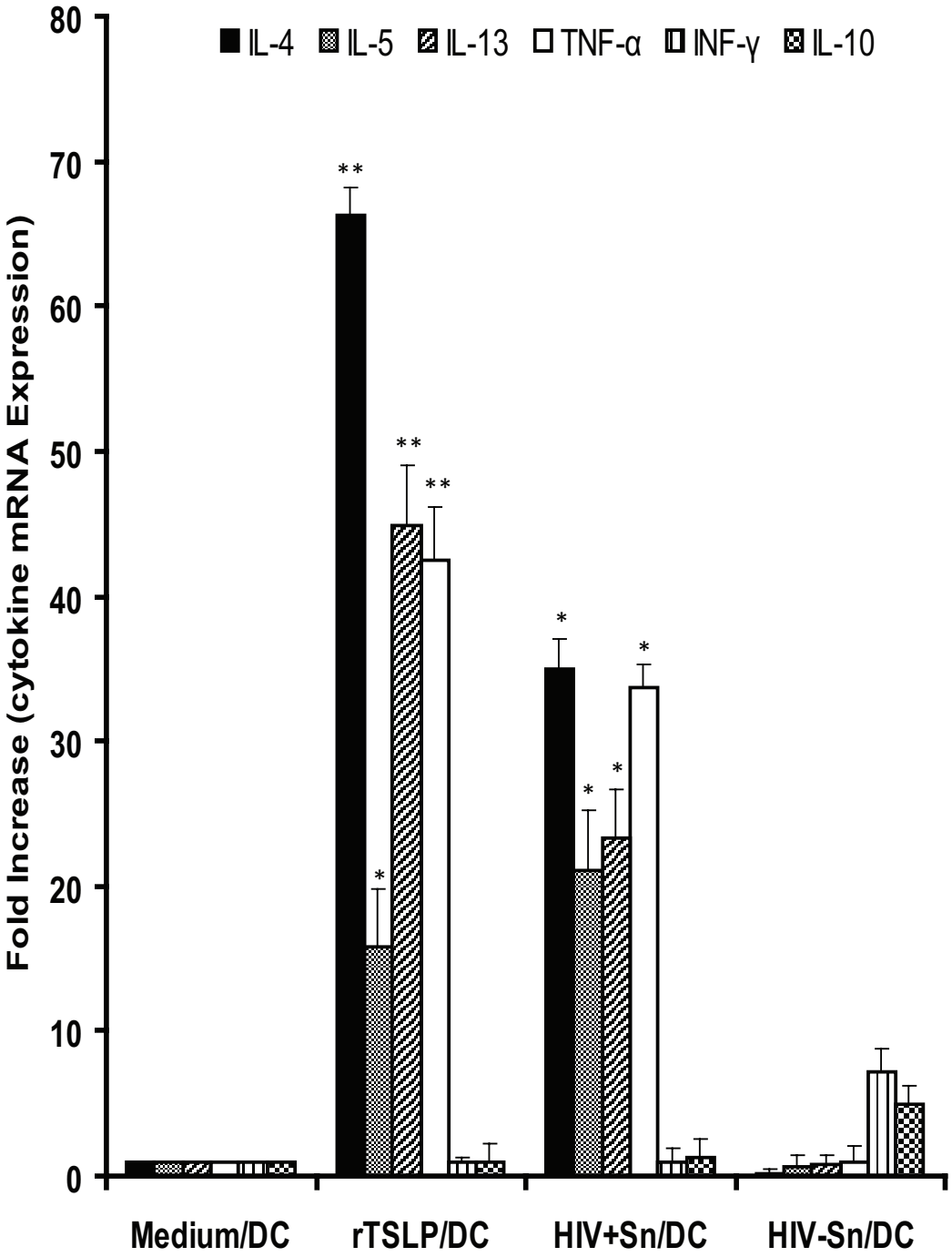

Fig. S3. mDCs activated by HIV-induced TSLP from epithelial cells promotes the generation of Th2 cells. Naïve $CD4^+$ T cells were co-cultured with mDCs that were activated by preincubating with the culture medium (Medium/DCs), rTSLP (TSLP/DCs), or supernatants collected from C33A cells cultured with or without HIV-1_{IIIB} (HIV^+Sn/DCs and HIV^-Sn/DCs, respectively). After 7 days, the T cells were analyzed for the induction of different cytokines shown by real-time quantitative RT-PCR. **, $P < 0.01$ comparing cytokine mRNA levels in rTSLP/DCs and HIV^+Sn/DCs to medium/DCs and HIV^-Sn/DCs, respectively. *, $P < 0.05$ comparing cytokine mRNA levels in rTSLP/DCs and HIV^+Sn/DCs to medium/DCs and HIV^-Sn/DCs, respectively.

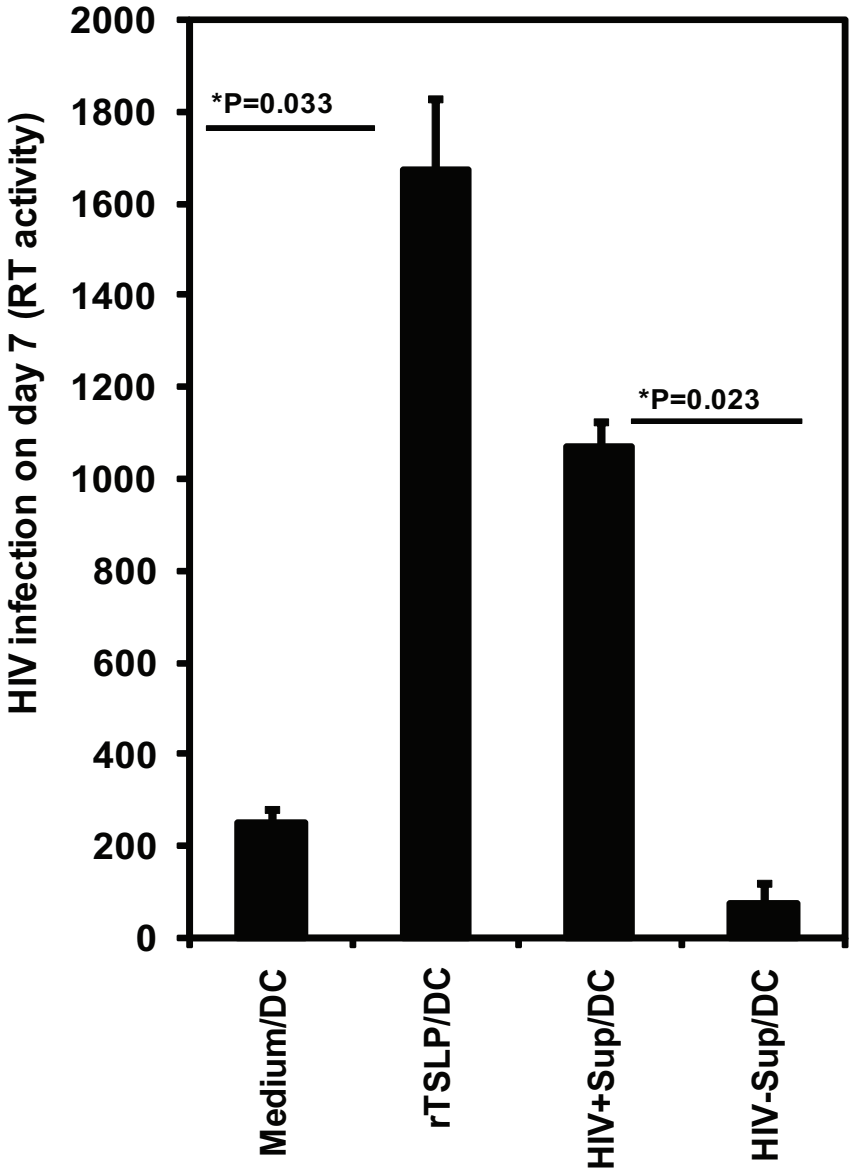

Fig. S4. mDCs activated by HIV-induced TSLP from epithelial cells promote increased HIV infection. Naïve $CD4^+$ T cells were co-cultured with mDCs that were activated by preincubating with the culture medium (Medium/DCs), rTSLP (TSLP/DCs), or supernatants collected from C33A cells cultured with or without $HIV\text{-}1_{IIIB}$ (HIV^+Sn/DCs and HIV^-Sn/DCs, respectively) and infected with a CCR5-tropic R5 strain $HIV\text{-}1_{YU2}$ for 7 days and the amount of virus produced in the supernatants was measured by estimating the RT activity on day 7. Error bars, standard deviation values for triplicate cultures, and data shown are average values of three independent experiments.

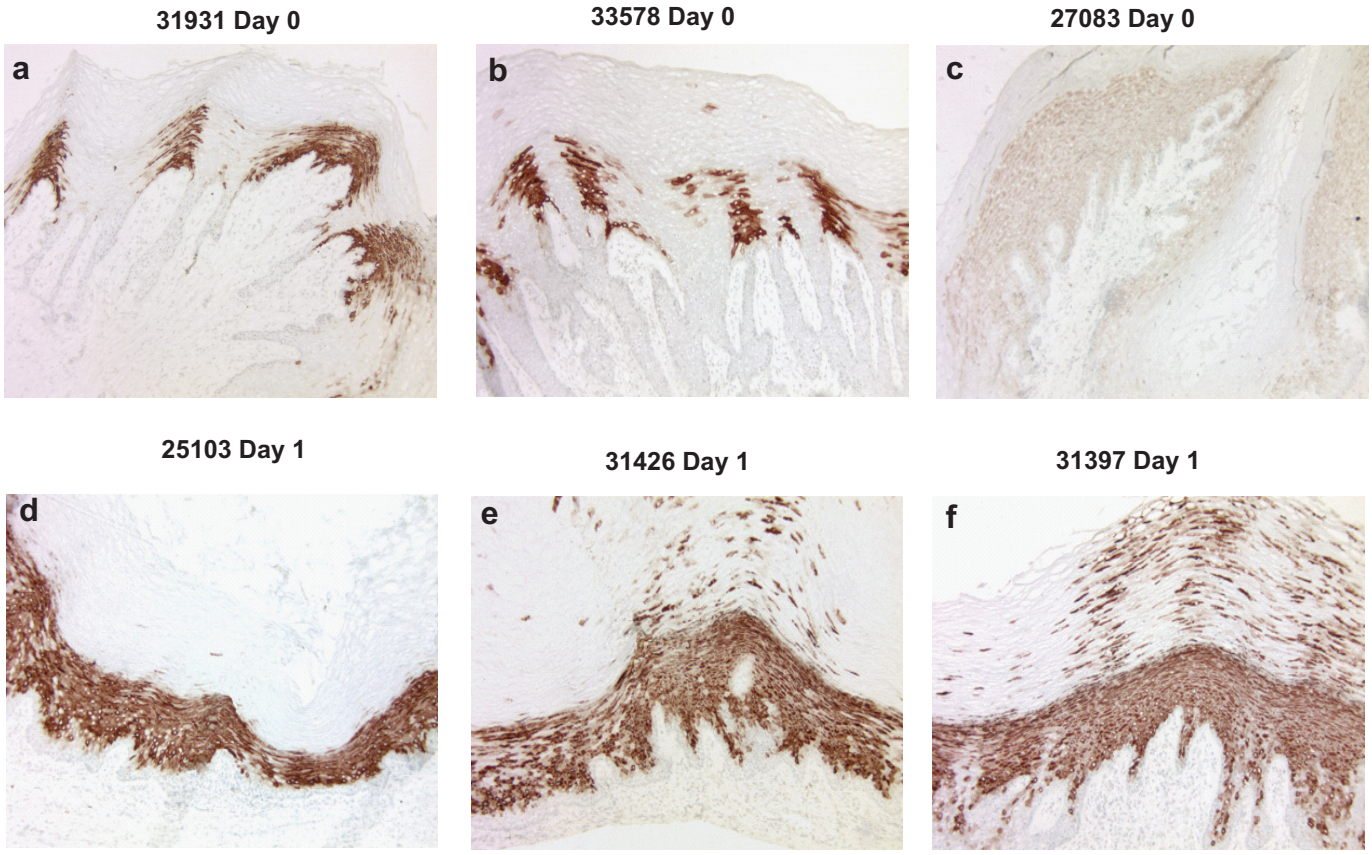

Fig. S5. Increased levels of TSLP in vaginal mucosal tissues from rhesus macaques after vaginal SIV infection. Immunohistochemical staining for TSLP expression in the vaginal tissues of monkeys 31931 (*A*), 33578 (*B*), and 27083 (*C*) before infection with SIV_{mac251} (day 0) and increased levels in monkeys 25103 (*D*), 31426 (*E*), and 31397 (*F*) day 1 postinfection.

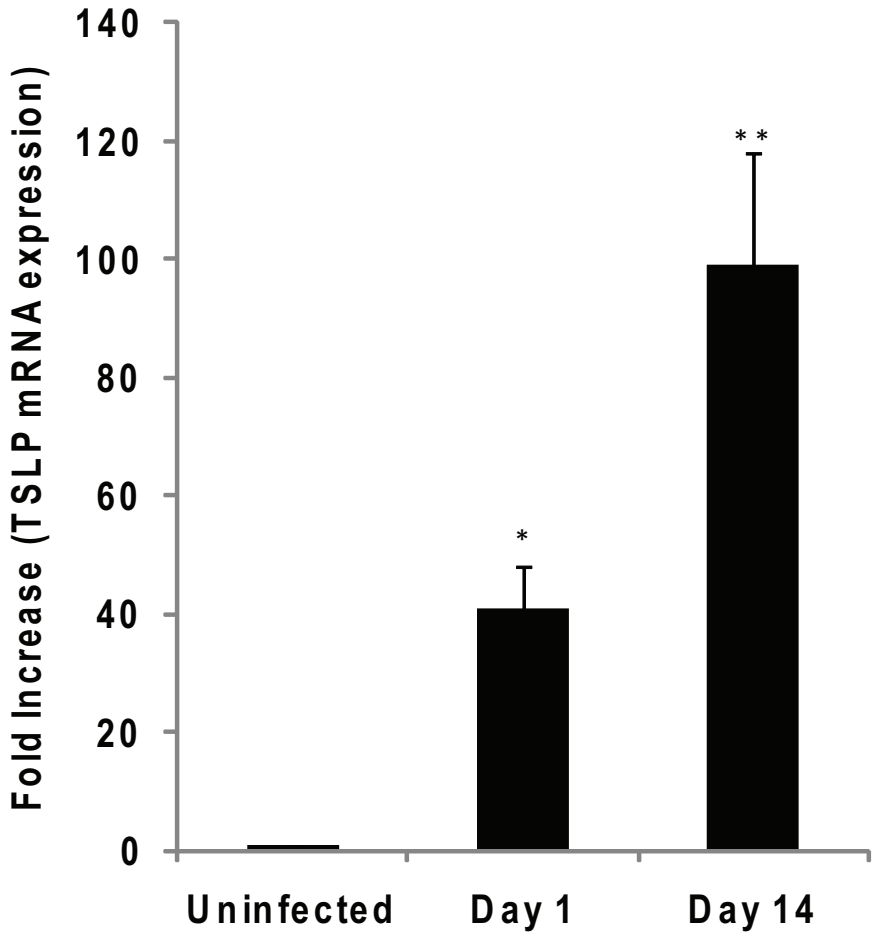

Fig. S6. Increased expression of TSLP in vaginal mucosal tissues from rhesus macaques after vaginal SIV infection. TSLP expression was determined by real time PCR analyses of mRNA isolated from the vaginal tissues samples of monkeys uninfected or at days 1 and 14 postinfection with SIV_{mac251}. *, $P < 0.05$ comparing tissue collected day 1 post infection to uninfected vaginal tissue. **, $P < 0.01$ comparing tissue collected at day 14 post infection to uninfected vaginal tissues.