Supplemental Figures

Figure S1: **Ecological dynamics of fecal microbiota composition across SIV infection of rhesus macaques and comparisons to HIV-infected humans, related to Figure 1. A)** Difference between baseline (pre-infection) and post-SIV infection (p.i.) fecal microbiota significantly increases at 2 weeks p.i. and returns by 8 weeks p.i., as assessed by Unweighted Unifrac beta diversity metric (Wilcoxon matched-pairs signed rank test *P < 0.05, **P < 0.005). **B)** Comparison of fecal microbiota of SIV-infected rhesus macaques to those of HIV-infected humans. The phylogenetic similarity of the macaque fecal microbiota to HIV-infected human fecal microbiota does not change significantly after as compared to before SIV infection (unweighted UniFrac metric; P=ns by one-way repeated measures ANOVA within SIV-infected cohorts)

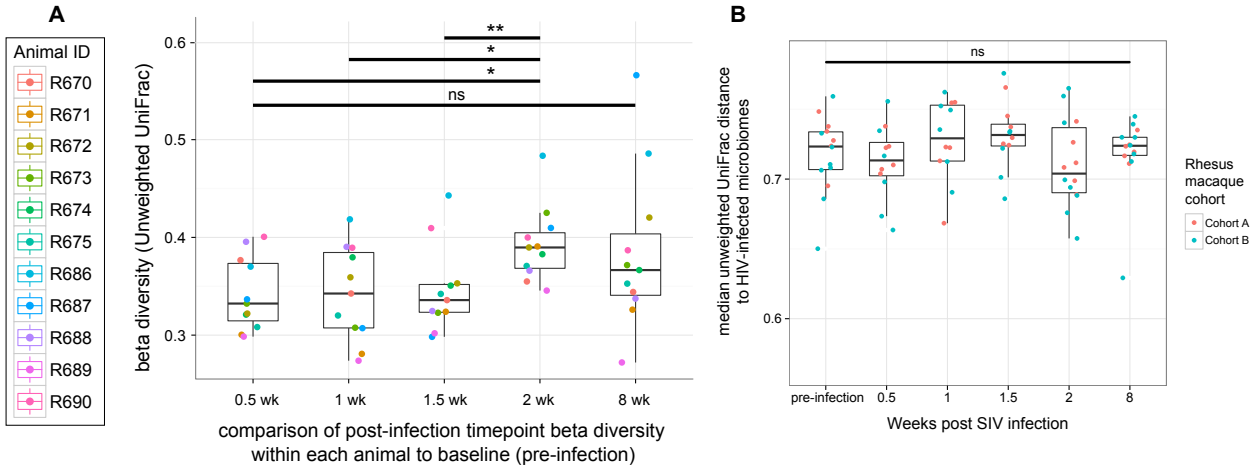


Figure S2: Fecal Lactobacillus abundance dynamics in the SIV-infected African green monkey model of non-pathogenic SIV infection, related to Figure 2. The abundance of fecal *Lactobacillus* increases during chronic SIV infection of the African green monkey while IDO activity reverts to pre-infection levels during chronic infection. A cross-sectional analysis of separate animals at each time point was employed using an unpaired Student's T test, *P < 0.05.

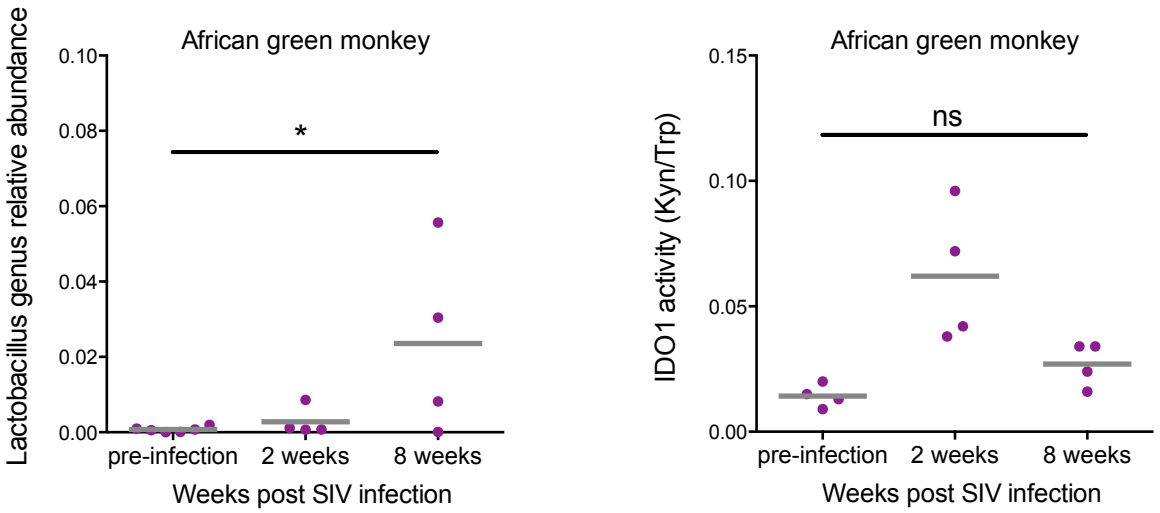


Figure S3: **Microbiota relationships to IDO1 pathway enzymes and primary *Lactobacillus* isolate growth dynamics with IDO1 products, related to Figure 3. A)** Bacterial families that encode kynurenine (IDO) pathway enzymatic machinery do not preferentially correlate with peripheral blood Kyn:Trp ratios of SIV-infected rhesus macaques. In contrast, those families that had members which bore genomically encoded kynurenine pathway enzyme homologs appeared to have weaker correlations to peripheral blood Kyn:Trp ratios than families which had no genomic evidence of possessing kynurenine pathway machinery, as assessed by querying the UniProt database (UniProt Consortium, 2012). **B)** Composition of *Lactobacillus spp*. isolated from RM stool specimens. Isolations were performed anaerobically on MRS selective agar. **C)** Kynurenine does not impact *Lactobacillus* primary isolate growth. Exogenous kynurenine was added to cultures of *L. animalis* and *L. reuteri* in MRS medium, and OD_{600} was measured across time to determine growth kinetics. Results shown are from representative primary isolates collated from sextuplicate cultures.

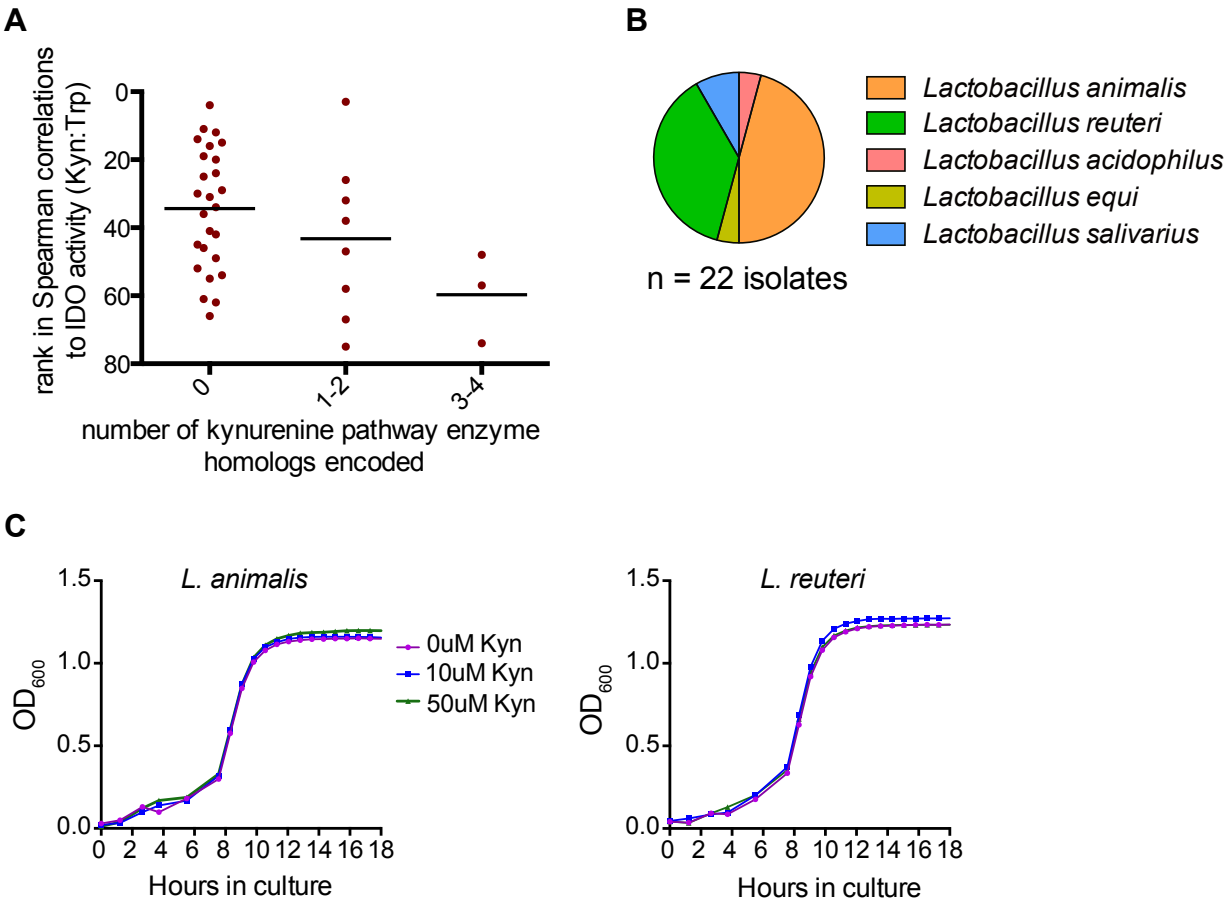
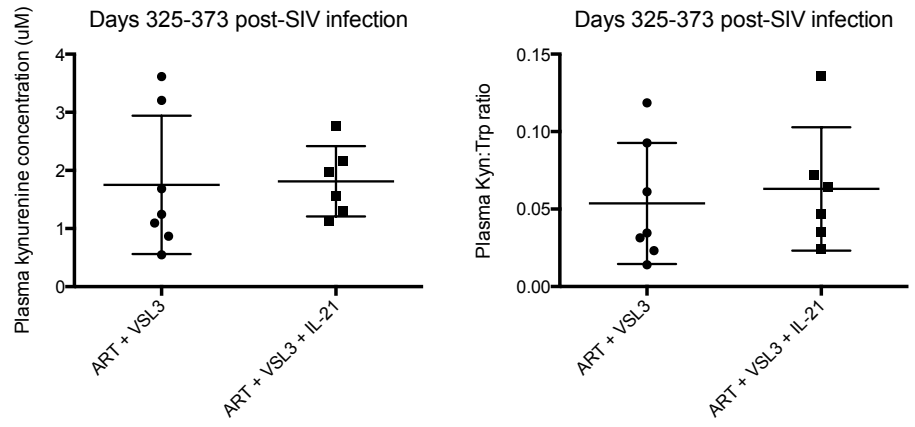


Figure S4: Cohort differences in pigtail macaques treated with VSL#3, related to Figure 4. No significant differences in plasma Kyn or Kyn:Trp were observed between animals supplemented with VSL#3 with or without IL-21 administration.



Supplemental Tables

Table S1: Statistical results of longitudinal SIV infection fecal microbiota comparisons and HIV-infected human cohort characteristics and *Lactobacillus* dynamics, related to Figure 2. **A)** Statistical comparisons of the abundances of all OTUs detected in fecal microbiomes of rhesus macaques cohorts A and B at pre-infection time points, as compared to 0.5, 1, 1.5, 2, and 8 weeks p.i. **B)** Statistical comparisons of the abundance of all detected bacterial genera in rhesus cohorts A and B at pre-infection time points as compared to 2 weeks post-infection. **C)** Cohort characteristics of human subjects for whom fecal microbiome profiling was performed. **D)** Comparisons of *Lactobacillus* OTU abundances between early HIV-infected human and uninfected human fecal microbiomes, profiled using the same techniques as performed for the rhesus study animals and analyzed using non-parametric Wilcoxon tests. Benjamini-Hochberg false discovery rate Q values were calculated for all statistical comparisons.

Table S2: Linear mixed effects modeling to understand relationships between IDO1 activity (Kyn:Trp ratios), Th17 cell abundances, and fecal bacterial genus abundances, related to Figure 3. A) Correlations between IDO1 activity (Kyn:Trp ratios) and fecal bacterial genus abundances, related to Figure 3. The *Lactobacillus* genus, noted in bold, ranked as the 5th strongest correlate (and strongest overall inverse correlate) out of a total of 86 genera detected. Taxonomic classification abbreviations: k_, kingdom; p_, phylum; c_, class; o_, order; f_, family; g_, genus. **B)** Correlations between peripheral blood Th17 cell abundance and fecal bacterial genus abundances. The *Lactobacillus* genus, noted in bold, ranked as the 2nd strongest correlate out of a total of 86 genera detected. Taxonomic classification abbreviations: k_, kingdom; p_, phylum; c_, class; o_, order; f_, family; g_, genus.

Supplemental Experimental Procedures

Non-human primate cohort information. Rhesus macaque animals of cohorts A and B were housed at Advanced BioScience Laboratories, Inc (IACUC Animal Use Protocol #552). Animals were singly housed on stool collection days, and stool was collected either from the pan (if fresh) or digitally from the rectum prior to freezing at -80°C. Animals were fed High Protein Monkey Diet 5045 (LabDiet), with occasional enrichment using PRIMA-Treats or Monkey Dough (BioServ). Stool from Cohort A was preserved in RNAlater (Life Technologies) prior to cryopreservation while stool from Cohort B was immediately cryopreserved. Animals of cohort C were housed at the RIDC Park facility of University of Pittsburgh in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International Standards and with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animals were singly housed on stool collection days, and stool was collected fresh from the pan and immediately cryopreserved at -80°C. The University of Pittsburgh IACUC approved these studies (Protocol # 1008829). Plasma and mononuclear cells were separated from the blood and stored at -80°C until used. Peripheral blood plasma was collected in EDTA tubes for all studies.

Human cohort information. Human fecal samples from HIV-infected and uninfected subjects were obtained via the *Consortium for the Evaluation and Performance of HIV Incidence Assays* (CEPHIA) (Kassanjee et al., 2014). Subjects were men who have sex with men who were either HIV infected or were at risk for HIV infection based on self-report of high risk sexual activity, and were participants in the UCSF Options study. Subjects were regularly tested for HIV antibodies via a HIV viral lysate-based western blot assay performed on peripheral blood plasma, as previously described (Kassanjee et al., 2014), and for HIV RNA by PCR. Total days between sampling date and estimated dates of HIV infection were determined by both methods. Days between sampling date and estimated dates of seroconversion (defined as the first date that a full positive antibody result was obtained) for HIV-infected subjects of the subset studied ranged between 19 days and 97 days. Where EDSC were unavailable, samples were included for two subjects that were regularly tested for HIV RNA and that showed a first positive HIV RNA test within the 6 months prior to sampling (11 and 102 days), with last negative HIV RNA tests being 376 and 495 days, respectively.

Fecal sample processing, 16S rRNA amplification, and 16S rRNA sequencing. Fecal samples from rhesus macaques, African green monkeys, and humans were processed in the same fashion. DNA extracted using the QIAamp DNA Stool Mini Kit (QIAGEN), as per manufacturer instructions, in conjunction with Tallprep Lysing Matrix E tissue disruption tubes and the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedical). The V4 hypervariable region of the 16S rRNA gene was amplified using the 515F/806R primer pair with12 basepair golay barcodes affixed to the 806R primer and Illumina adapter oligonucleotides on both primers. Amplification was performed using Hot Start ExTaq (Takara) for 30 cycles (98°C for 20 seconds, 50°C for 30 seconds, 72°C for 45 seconds). Amplicon concentrations were quantified by comparison to Low Mass DNA Ladder (Life Technologies) on an agarose gel, pooled at equimolar concentrations, and purified by agarose gel extraction using the QIAquick Gel Extraction Kit (QIAGEN). Pooled amplicon libraries were quantified using the Qubit 2.0 Fluorometer (Life Technologies) and sequenced on a MiSeq Genome Sequencer (Illumina) using the V3 Reagent Kit (Illumina).

16S rRNA sequence analysis and statistical methods. Sequencing read libraries were split by barcodes using a Phred q score threshold cutoff of 30 and collapsed into operational taxonomic units (OTUs) via an open-reference OTU picking scheme as implemented in QIIME (Caporaso et al., 2010). The 97% sequence identity May 2013 Greengenes database was used for the reference-based portion of open-reference OTU picking, while a 97% sequence identity threshold cutoff was retained for the open-reference portion. De novo OTUs were picked and reads were assigned using UCLUST, and taxonomy was likewise assigned using UCLUST and the Greengenes database as implemented in the QIIME package. OTUs with read abundance below 0.001% of total reads were removed. For identifying discriminating OTUs after SIV infection, ten rarefaction tables were generated at 34,000 reads each for rhesus macaque analyses, while a rarefaction at 97,000 was performed for acute HIV and uninfected human sample analyses. Wilcoxon tests (R package 'exactRankTests') were then serially performed on all OTUs at each time point post-infection compared to each respective animal's pre-infection time point sample, and statistical values were collated between the resultant ten output tables by calculating median P values and mean $\log_{10}$ fold changes for each OTU. At each time point, OTUs were removed which had zero abundance both at that time point and pre-infection. For visualization of data in Figure 2A, OTUs belonging to the ten most frequently significant bacterial families were shown. The linear mixed effects modeling implementation in the R package 'lme4' fitted by maximum likelihood was used to compare abundances of all bacterial families to peripheral blood IDO1 activity and Th17 cell abundances. For comparing rhesus macaque fecal microbiomes to human (HIV-infected and uninfected) fecal microbiomes, rarefaction to 6,000 reads was performed and an open OTU picking strategy used as implemented in QIIME. Visualizations of data were generated using Prism 6 (GraphPad Software, Inc.) and the R package 'ggplot2'. False discovery rate (FDR q value) calculations were performed using the Benjamini-Hochberg technique (Benjamini and Hochberg, 1995).

Flow cytometry and analysis. Peripheral blood mononuclear cells (PBMC) were isolated from rhesus macaque peripheral blood by Ficoll-Paque (Sigma) density gradient separation. PBMCs were washed twice with PBS containing 2% FBS and cryopreserved in 10% DMSO/90% fetal bovine serum (FBS). Cryopreserved PBMCs were thawed in batch, washed twice in RPMI containing 10% FBS and rested overnight. $1x10^6$ cells were added to individual wells in 96-well round bottom plates. Cells were stimulated with phorbol 12-myristate 13-acetate (20 ng/ml) and ionomycin (1 µg/ml) or left unstimulated. Plates were incubated at 37°C and 5% CO_2 for 2 hours, and then treated with a transport inhibitor (Golgi Plug, BD Biosciences), after which they were returned to 37°C and 5% CO_2 for another 16 hours. Cells were harvested by centrifugation, washed in PBS-2% FBS, and surface stained with the viability dye, Aqua Amine Reactive Dye (Invitrogen), as well as with monoclonal antibodies recognizing CD4 (NIH-NHP Reagent Resource Program) and CD8 (Clone 3B5, Invitrogen) for 20 minutes at room temperature. Cells were washed in PBS-2% FBS and permeabilized with BD Cytofix/Cytoperm reagents (BD Biosciences), as per the manufacturer's instructions. Permeabilized cells were then stained intracellularly for CD3 (Clone SP 34-2, BD Biosciences) and IL-17A (Clone eBio64DEC17, eBiosciences) for 30 minutes at 4°C. Cells were washed twice in Permeabilization Wash Buffer (BD Biosciences) and analyzed by flow cytometry. Events were acquired on a BD LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star, Inc.).

VSL#3 trial characteristics and analysis. Pigtail macaque animals in the first VSL#3 study initiated 6/25/2010 (Klatt et al., 2013) were treated with 20–30 mg/kg PMPA and 30 mg/kg FTC once daily, s.c., and 120 mg L812 with 50 mg L564 twice daily, orally beginning on day 160 post-infection. Pigtail macaque animals from the second study initiated on 10/9/12 were treated as above but omitting L564. All animals were housed and cared in accordance with the American Association for Accreditation of Laboratory Animal Care standards in American Association for Accreditation of Laboratory Animal Care-accredited facilities. All animal procedures were performed according to protocols approved by the Institutional Animal Care and Use Committees of the National Institute of Allergy and Infectious Diseases under protocol LMM12.

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