MATERIALS AND METHODS

ARROW randomized trial

Participant recruitment

Children and adolescents were enrolled at medical centers in Uganda (Joint Clinical Research Centre, Kampala; Baylor College of Medicine Children's Foundation, Mulago Hospital, Kampala; MRC/UVRI Uganda Research Unit on AIDS, Entebbe) and Zimbabwe (University of Zimbabwe, Harare) and follow-up clinics throughout the study was conducted by clinical staff to a common trial end date (16th March 2012)(27, 59). Children were excluded from the ARROW trial (for which ART was initiated at enrolment) if they did not meet the 2006 WHO criteria for ART initiation(64), had acute infections, had previously received ART or were perinatally exposed to ART (children <6 months old), were pregnant or breastfeeding, or were taking medications or had laboratory abnormalities that contraindicated ART(59). 98% of children enrolled during the last 6 months were also included in an immunology sub-study, as previously described(5)

Systemic inflammation

CRP, TNFα, IL-6, and sCD14 were quantified via ELISA (Quantikine kits; R&D Systems Inc.) in children with available baseline plasma samples who were enrolled into the immunology sub-study of ARROW in Uganda and Zimbabwe, and in all other children randomized to stop versus continue cotrimoxazole in Zimbabwe. Randomized groups were compared at 12, 24, 48, 72 and 96 weeks post-randomization using the closest available measurement to each time-point in equally-spaced windows (stop n=149, continue n=144). Albumin and total protein were quantified in one 48-week serum sample (+/- 24 weeks; equivalent to 3 years after ARROW enrollment and ART initiation) from children known to

have fasted for >6 hours prior to sample collection (stop n=151, continue n=159) using a Beckman CX5 Delta Chemistry Analyzer(*65*).

Clinical symptoms

Caregiver-reported clinical symptoms since the last study visit (6-week recall of incidents of cough, fever, abdominal pain/aching, nausea/vomiting, persistent, bloody or moderate-to-severe diarrhea, difficult/fast breathing and weight loss), weight and height were recorded every 6 weeks. CD4 counts and viral loads were quantified in blood samples collected every 12 weeks, as previously described(*59*). Clinical read-outs were compared between the stop (baseline n=150) versus continue (baseline n=145) groups for children who were also included in the plasma biomarker quantification assays.

Immunophenotyping of blood leukocytes

T cell immunophenotyping was conducted via flow cytometry in Ugandan participants enrolled into the immunology sub-study of ARROW who provided sufficient blood volume (stop n=48, continue n=47)(*5*). Leukocytes were labelled with the following fluorophore-conjugated antibodies: CD4-PerCP (BD Biosciences), CD31-PE (eBiosciences), CD45RA-APC (Caltag Medsystems), and Ki67-FITC (BD Biosciences; staining performed after membrane permeabilization). Immunophenotyping was conducted on a BD FACSCalibur flow cytometer and analyzed using CellQuest software (BD Biosciences; gating strategy is shown in **fig. S1A**).

Intestinal inflammation

Stool samples were collected at week-84 and week-96 from children enrolled in the ARROW

immunology sub-study in Zimbabwe (stop n=38, continue n=37). Myeloperoxidase,

neopterin, α1-antitrypsin, and REG1β were quantified using ELISA kits from Immun

diagnostik AG, Genway Biotech Inc, Immuchrom GmbH, and Techlab Inc, respectively.

Fecal DNA preparation and sequencing

Total DNA was extracted from 150 mg of stool sample collected at week-84 and week-96

from 72 children enrolled in the ARROW immunology sub-study in Zimbabwe (Stop n=36,

Continue n=36) using the MoBio DNA Extraction Kit with bead-beating modified for

simultaneous RNA isolation. Paired-end DNA libraries were prepared using Illumina TruSeq

Nano DNA Library Prep kits. As a quality control, the libraries were analyzed on a

TapeStation 2200 before pooling. Whole metagenome sequencing was performed with 125-

nucleotide paired-end read lengths using the Illumina HiSeq 2500 platform at Canada's

Michael Smith Genome Sciences Centre, Vancouver, Canada. 23-24 libraries were pooled

per sequencing lane. Sequenced reads were trimmed of adapters and filtered to remove low-

quality, short (<60 base-pairs), and duplicate reads, as well as those of human, other animal

or plant origin using *KneadData* with default settings. Species composition was determined

by identifying clade-specific markers from reads using *MetaPhlan2* with default settings(*66*).

Functional gene and metabolic pathway composition was determined using *HUMANn2* with

default settings against the UniRef90 database(*67*). Functionally annotated reads were further

classified into Pfam protein families(*68*) and level-4 enzyme commission (EC) categories

using provided scripts. Microbiome species, gene and pathway abundance were normalized

by the total read count in each stool sample (relative abundance). Median 10,507,352 versus

11,068,280 (p=0.23) reads were obtained at week-84, and 11,074,046 versus 10,875,436

(p=0.60) at week-96 from continue and stop groups, respectively.

***In vitro* cotrimoxazole treatment of blood leukocytes**

Participant recruitment

HIV-negative adults were recruited via email circular to research staff at the Blizard Institute, Queen Mary University of London, UK (n=8). HIV-positive adults on ART for >2 years (n=6) or who were ART-naïve (n=10) were identified from outpatient records by clinicians and nurses at the Grahame Hayton Unit of the Royal London Hospital, UK and invited to participate during routine clinical appointments (characteristics summarized in **table S1**).

Blood sample processing

A 50 mL venous blood sample was collected from each U.K. adult participant into sterile heparinized blood collection tubes (BD Biosciences) via venipuncture. 10 mL of blood was used for immunophenotyping and whole blood culture. PBMC and undiluted plasma were isolated from 40 mL of whole blood using Ficoll Plaque Plus (GE Healthcare) density separation. Plasma samples were aliquoted and stored in endotoxin-free cryovials (Greiner) at -80°C. Freshly isolated PBMC were washed twice in sterile PBS/1% v/v Fetal Bovine Serum (FBS; Gibco) prior to staining and/or culture.

Baseline inflammatory characteristics

To characterize the baseline inflammatory milieu and immune cell activation phenotype between HIV-uninfected and HIV-positive ART-treated and ART-naïve U.K. adult participants, circulating inflammatory mediators were quantified in plasma (**fig. S8A**) and monocyte and T cell activation phenotype were characterized in uncultured PBMC (**fig. S8B-E**). CRP, sCD14, TNFα (Duoset kits from R&D Systems), and IL-6 (OptEIA kit from BD Biosciences) were quantified by ELISA. For immunophenotyping, 1×10^6 uncultured PBMC

were incubated for 30 min with fluorophore-conjugated antibodies specific for T cell and monocyte surface markers for 30 min (**table S2**), washed once in PBS/1% FBS and fixed for 30 min in Fixation buffer (eBiosciences). Labelling with fluorescence-minus-one (FMO) and isotype control antibodies for each surface marker and a membrane impermeable viability dye (Zombie aqua Fixable Viability dye; Biolegend; **fig. S7D**) were conducted in parallel to determine hierarchical gating and cell viability, respectively (gating strategy shown in **fig. S9B**). Immunophenotyping was conducted on a BD LSR II flow cytometer and analyzed using FlowJo LLC version 10.

Antigens

Lyophilized heat-killed *Salmonella typhimurium* (HKST), ultrapure lipopolysaccharide from *Escherichia coli* 0111:B4 strain (LPS), *Saccharomyces cerevisiae* cell wall (zymosan), and *Staphylococcus aureus* enterotoxin B (SEB; Sigma) were reconstituted according to the manufacturer's instructions.

Drugs

Trimethoprim and sulfamethoxazole were prepared in DMSO at a stock concentration of 100 mg/mL (all from Sigma). Drugs were titrated and assessed for antibiotic activity against bacterial isolates (**fig. S7A**), toxicity to cultured cells (**fig. S7B-D**; **Fig. 7B**), optimal culture duration (**fig. S7E**) and timing of treatment relative to antigen stimulus (**fig. S7F**).

Whole blood culture

Whole blood culture conditions for *in vitro* cotrimoxazole treatment were optimized using samples from HIV-negative adults (n=6; **fig. S7B, C, E** and **F**). Drug and antigen conditions

for whole blood culture with for U.K. adult participants were batch-prepared in RPMI 1640 GlutaMAX (Gibco) supplemented with 1% v/v Penicillin-streptomycin (P-S; Gibco) and stored at -80°C. Single-use aliquots of drug and antigen conditions were thawed for each donor and combined with 500 µL/well of 1:3 diluted blood (final concentrations in culture: blood at 1:6, HKST at 10^8 cells/mL, LPS at 5 EU/mL, and Zymosan at 5 µg/mL; low-dose cotrimoxazole (CTX$_{[Low]}$): 2 µg/mL trimethoprim and 50 µg/mL sulfamethoxazole, high-dose cotrimoxazole (CTX$_{[High]}$): 8 µg/mL trimethoprim and 200 µg/mL sulfamethoxazole). Controls for each cotrimoxazole treatment dose were prepared using DMSO without drug at the same total volume (i.e. volume of trimethoprim + volume of sulfamethoxazole). Final DMSO content in cultures was DMSO culture content was 0.05% v/v for CTX$_{[Low]}$ and DMSO$_{[Low]}$ and 0.2% v/v for CTX$_{[High]}$ and DMSO$_{[High]}$. Whole blood cultures were incubated for 24h at 37°C. 5% CO_2 after which cell-free supernatants were harvested and stored at -80°C until cytokine quantification by ELISA.

6h PBMC culture

Drug and antigen conditions for U.K. adult PBMC culture were batch-prepared in RPMI 1640 GlutaMAX/1% P-S/10% FBS (cRPMI) and stored at -80°C. Single-use aliquots of drug and antigen conditions were thawed for each donor and combined with 1×10^6 PBMC in 50µL cRPMI with 100 µL of pre-prepared drug condition (High dose CTX$_{[High]}$ or volume-matched DMSO control, DMSO$_{[High]}$), 50 µL of pre-prepared antigen and 50 µL cRPMI in sterile round-bottomed tubes (BD Biosciences). Final antigen concentrations in culture were: HKST at 10^8 cells/ mL and SEB at 1 µg/mL. PBMC cultures were incubated at 37°C, 5% CO_2. Brefeldin A (Sigma) was added to all cultures at a final concentration of 25 µg/mL after 1h incubation.

After a total of 6h culture, PBMC were washed in sterile PBS/1% FBS, labelled with fluorophore-conjugated antibodies specific for cell surface markers (**table S2**) for 30 min, washed in PBS/1% FBS and fixed overnight in fixation buffer (eBiosciences) at 4°C. Fixed cells were permeabilized in 1x Permeabilization buffer (eBiosciences) and labelled with fluorophore-conjugated antibodies specific for intracellular cytokines (**table S2**) for 40 min, washed once with Permeabilization buffer and once with PBS/1% FBS prior to analysis on a BD LSR II flow cytometer alongside FMO and isotype control-labelled samples (gating strategy shown in **fig. S9**). Parallel cultures were conducted for viability assessment prior to fixation using Zombie aqua fixable viability dye (BioLegend). Flow cytometry analysis was conducted using FlowJo LLC version 10.

In vitro cotrimoxazole treatment of gut epithelial cells

Caco-2 maintenance

The human colonic epithelial cell-line Caco-2 derived from colorectal adenocarcinoma (ATCC HTB-37) was maintained in DMEM (Lonza) supplemented with 1% v/v P-S, 1%v/v L-glutamine, 1% v/v non-essential amino acids, and 10% v/v FBS (cDMEM; all supplements from Gibco) in 75 cm^2 culture flasks at 37°C, 5% CO_2. Cells were passaged at 80-90% confluency using Trypsin/EDTA (Gibco). Caco-2 were used for experiments between passage 35 and 40. Caco-2 monolayers were washed with 1 mL/well sterile PBS pre-warmed to 37°C between media changes. All media changes, drug treatments and stimuli were pre-warmed to 37°C prior to addition to Caco-2 monolayers.

Caco-2 transwell culture

1x10^4 Caco-2 were seeded into Millicell Hanging Cell Culture Inserts (PET 0.4 μm; Merck-

Millipore) and cultured at 37°C, 5% CO_2 with 1 mg/mL cotrimoxazole (commercial

preparation pre-formulated in DMSO; 100 mg/mL; Sigma), volume-matched DMSO or

cDMEM without drug. Drug treatments were added apically and replenished at each media

change (day 3 and then every 2 days post-seeding). TEER across the growing monolayers

was monitored daily using an STX04 test electrode with a Millicell ERS-2-ohm meter

(Merck-Millipore). Once the plate mean TEER was >800Ω (day 7-10 post-seeding),

recombinant human IL-1β (1, 10 or 100 μg/mL; BioVision Inc) or cDMEM without stimulus

was added apically for 24h. Individual wells with TEER<600Ω (i.e. sub-confluent) were

excluded from analysis.

After stimulus, change in TEER was calculated (ΔTEER = 24h TEER - pre-treatment TEER)

and apical supernatants were harvested. Caco-2 monolayers were then washed once with pre-

warmed PBS and treated apically with pre-warmed 100 μg/mL Lucifer Yellow Biocytin

(Molecular Probes) for 1h on a plate shaker. Fluorescence intensity (FI) was quantified in

apical and basal culture supernatants relative to a Lucifer Yellow standard curve (100–0.1

μg/mL) using a BioTek plate reader (excitation/emission: 480/530nm); Lucifer Yellow

apical-to-basal passage = $(FI_{Basal} - FI_{PBS}) / (FI_{100μg/mL} - FI_{PBS})$. LDH activity and IL-8 were

measured in apical supernatants using LDH Cytotoxicity Assay (Pierce) and Human IL-

8/CXCL8 DuoSet ELISA (R&D Systems). Supernatants from parallel Caco-2 cultures treated

with cell lysis buffer (LDH_{Max}; Pierce) or volume-matched sterile endotoxin-free water

($LDH_{Spontaneous}$) were used to calculate LDH activity in cotrimoxazole-treated cultures:

$\%LDH = (LDH_{CTX} - LDH_{Spontaneous})/ (LDH_{Max} - LDH_{Spontaneous}) \times 100$.
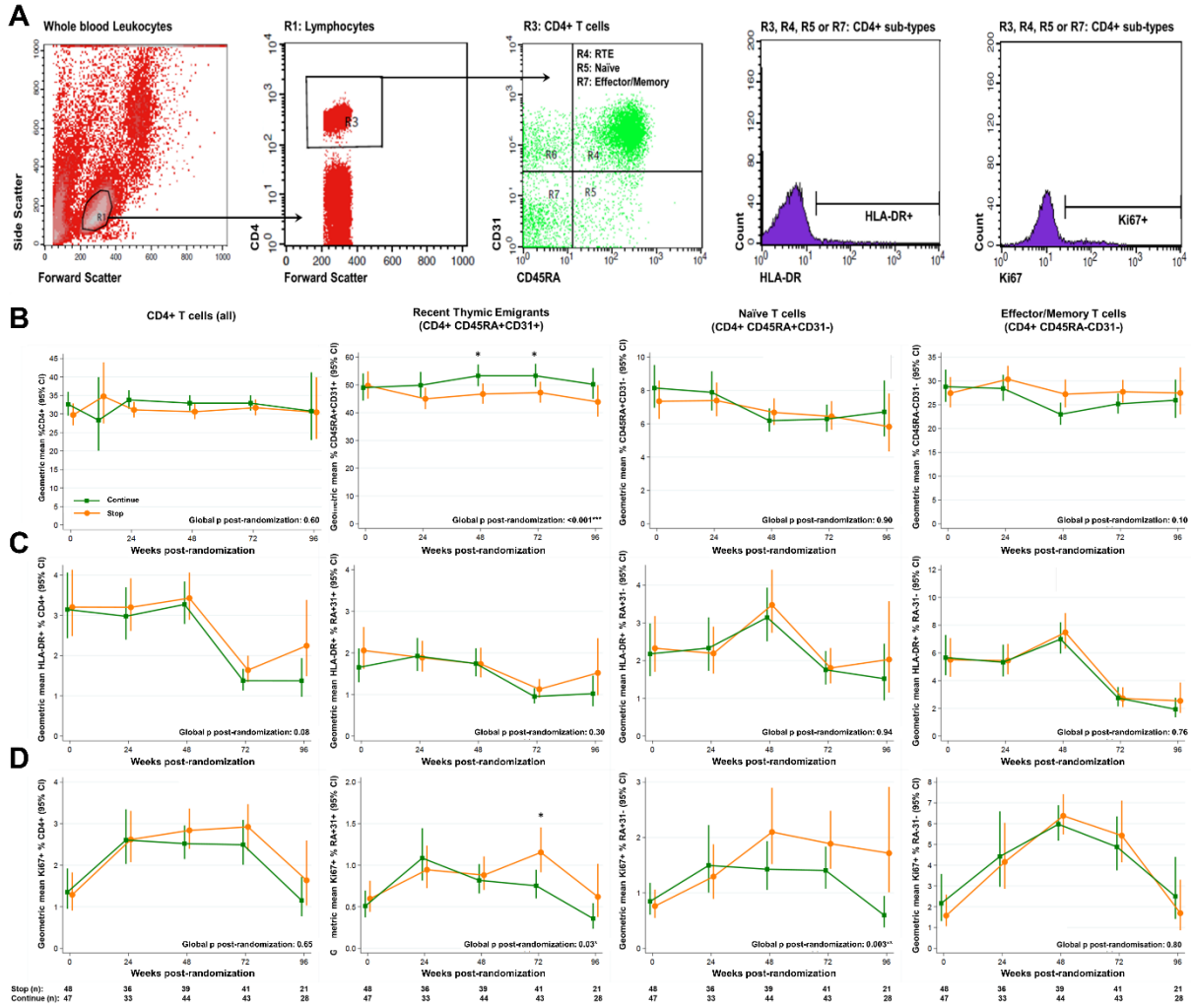
figure S1. Cotrimoxazole alters circulating CD4+ T cell phenotype in HIV infection. (**A**) Flow cytometry gating of uncultured blood leukocytes from HIV-positive ART-treated children randomized to stop (orange circles) versus continue (green squares) cotrimoxazole in ARROW. Lymphocytes (R1) were gated on CD4 expression (R3) and sub-divided according to CD31 and CD45RA expression: recent thymic emigrant-like (RTE; CD45RA+CD31+; R4), naïve (CD45RA+CD31-; R5) or effector-memory (CD45RA-CD31-; R7) CD4+ T cells. Graphs show proportions of each subset (**B**) and proportions (±95% confidence interval, CI) expressing HLA-DR (**C**) or Ki67 (**D**). Randomized groups were compared using Generalized Estimating Equations (global p) and standard regression models for individual timepoints, all adjusted for center and baseline percentages; *p<0.05, **p<0.01 ***p<0.001
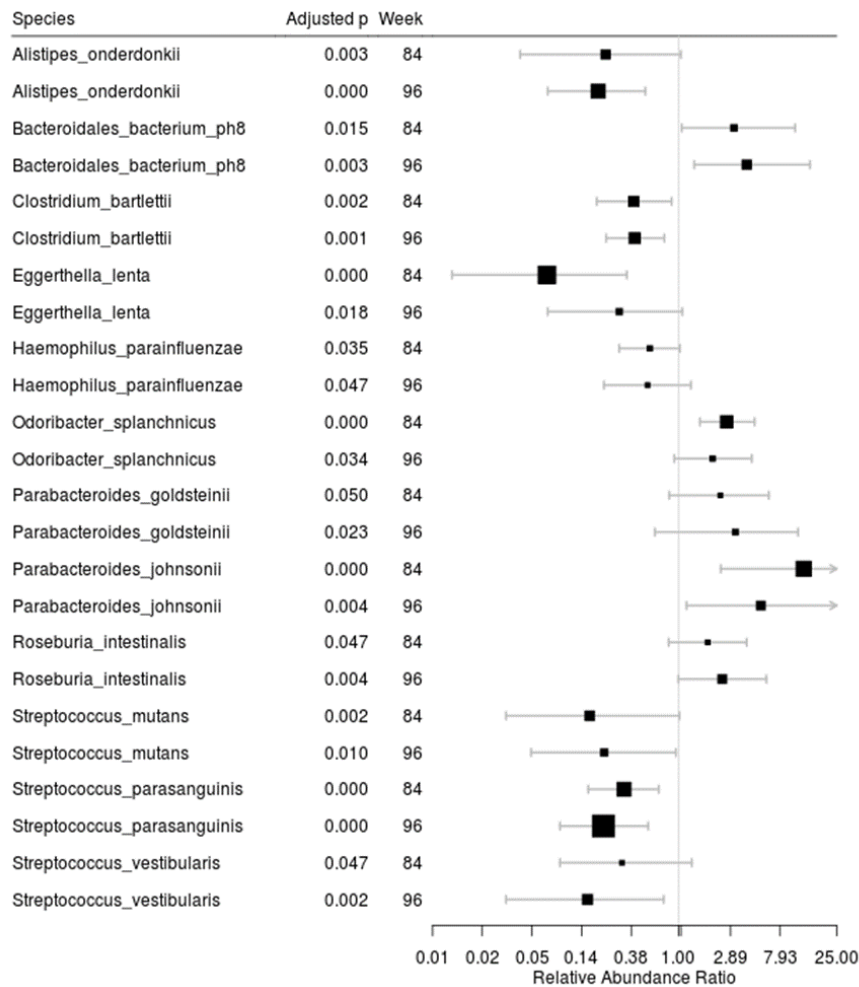
figure S2. Fecal bacterial species that differ between HIV-positive ART-treated Zimbabwean children randomized to continue versus stop cotrimoxazole prophylaxis. Effect size plots (relative abundance ratios ±95% confidence interval) of bacterial species that had a consistent statistically significant difference in relative abundance at both week-84 and week-96 post-randomization to continue (n=36) versus stop (n=36) cotrimoxazole after FDR adjustment for multiple hypothesis testing (adjusted p<0.05). Relative abundance ratio<1.0 indicates a decrease in relative abundance in children randomized to continue versus stop cotrimoxazole. Size of square is inversely proportional to p-value. Vertical line indicates null value. Comparison between randomized groups was made by zero-inflated beta regression. **Fig. 3C** presents a condensed version of this analysis for *Streptococcal* spp.

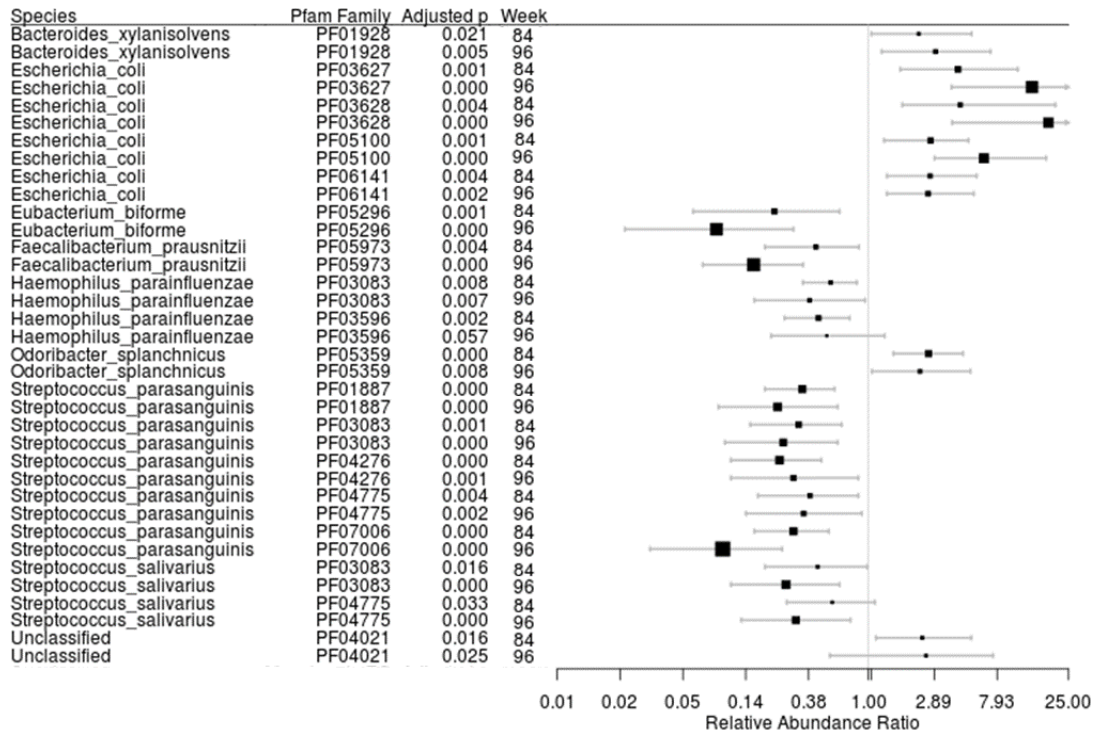| Species	Pfam Family	Adjusted p	Week
Bacteroides_xylanisolvens	PF01928	0.021	84
Bacteroides_xylanisolvens	PF01928	0.005	96
Escherichia_coli	PF03627	0.001	84
Escherichia_coli	PF03627	0.000	96
Escherichia_coli	PF03628	0.004	84
Escherichia_coli	PF03628	0.000	96
Escherichia_coli	PF05100	0.001	84
Escherichia_coli	PF05100	0.000	96
Escherichia_coli	PF06141	0.004	84
Escherichia_coli	PF06141	0.002	96
Eubacterium_biforme	PF05296	0.001	84
Eubacterium_biforme	PF05296	0.000	96
Faecalibacterium_prausnitzii	PF05973	0.004	84
Faecalibacterium_prausnitzii	PF05973	0.000	96
Haemophilus_parainfluenzae	PF03083	0.008	84
Haemophilus_parainfluenzae	PF03083	0.007	96
Haemophilus_parainfluenzae	PF03596	0.002	84
Haemophilus_parainfluenzae	PF03596	0.057	96
Odoribacter_splanchnicus	PF05359	0.000	84
Odoribacter_splanchnicus	PF05359	0.008	96
Streptococcus_parasanguinis	PF01887	0.000	84
Streptococcus_parasanguinis	PF01887	0.000	96
Streptococcus_parasanguinis	PF03083	0.001	84
Streptococcus_parasanguinis	PF03083	0.000	96
Streptococcus_parasanguinis	PF04276	0.000	84
Streptococcus_parasanguinis	PF04276	0.001	96
Streptococcus_parasanguinis	PF04775	0.004	84
Streptococcus_parasanguinis	PF04775	0.002	96
Streptococcus_parasanguinis	PF07006	0.000	84
Streptococcus_parasanguinis	PF07006	0.000	96
Streptococcus_salivarius	PF03083	0.016	84
Streptococcus_salivarius	PF03083	0.000	96
Streptococcus_salivarius	PF04775	0.033	84
Streptococcus_salivarius	PF04775	0.000	96
Unclassified	PF04021	0.016	84
Unclassified	PF04021	0.025	96

figure S3. Protein families that differ between stool samples from HIV-positive ART-treated Zimbabwean children randomized to continue versus stop cotrimoxazole prophylaxis. Effect size plots (relative abundance ratios ±95% confidence interval) of protein families (Pfam) that had a consistent statistically significant difference in relative abundance at both week-84 and week-96 post-randomization to continue (n=36) versus stop (n=36) cotrimoxazole after FDR adjustment for multiple hypothesis testing (adjusted p<0.05). Identities of bacterial species for each Pfam were established using *HUMANn2* with default settings against the UniRef90 database. Relative abundance ratio<1.0 indicates a decrease in relative abundance in children randomized to continue versus stop cotrimoxazole. Size of square is inversely proportional to p-value. Vertical line indicates null value. Comparison between randomized groups was made by zero-inflated beta regression. **Fig. 3C** presents a condensed version of this analysis for *Streptococcal* spp.
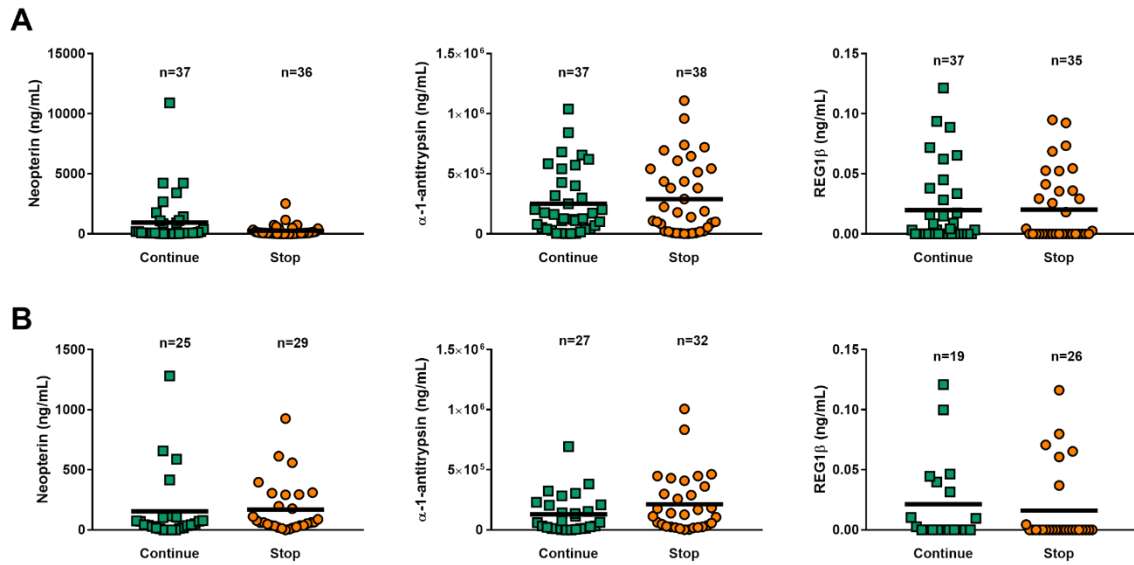
figure S4. Fecal biomarkers of enteropathy that were unaffected by continuing versus stopping cotrimoxazole prophylaxis. Concentrations of the enteropathy biomarkers neopterin, α-1-antitrypsin, and regenerating family member 1 beta (REG1β) in stool samples collected from HIV-positive ART-treated Zimbabwean children randomized to stop (orange circles) or continue (green squares) cotrimoxazole prophylaxis at (**A**) week-84 and (**B**) week-96 post-randomization within the ARROW trial. Horizontal lines indicate the median. Number of participant samples analyzed per timepoint are indicated above each graph. Comparisons were made between randomized groups by Mann-Whitney U test; p>0.05.

Species	Adjusted p	Week
Alistipes_onderdonkii	0.597	84
Alistipes_onderdonkii	0.597	96
Bacteroidales_bacterium_ph8	0.453	84
Bacteroidales_bacterium_ph8	0.551	96
Clostridium_bartlettii	0.820	84
Clostridium_bartlettii	0.165	96
Eggerthella_lenta	0.756	84
Eggerthella_lenta	0.551	96
Haemophilus_parainfluenzae	0.597	84
Haemophilus_parainfluenzae	0.036	96
Odoribacter_splanchnicus	0.453	84
Odoribacter_splanchnicus	0.497	96
Parabacteroides_goldsteinii	0.453	84
Parabacteroides_goldsteinii	0.551	96
Parabacteroides_johnsonii	0.453	84
Parabacteroides_johnsonii	0.551	96
Roseburia_intestinalis	0.453	84
Roseburia_intestinalis	0.497	96
Streptococcus_mutans	0.696	84
Streptococcus_mutans	0.000	96
Streptococcus_parasanguinis	0.453	84
Streptococcus_parasanguinis	0.000	96
Streptococcus_vestibularis	0.453	84
Streptococcus_vestibularis	0.000	96

-200 -150 -100 -50 0 25 50 75 100 150 200
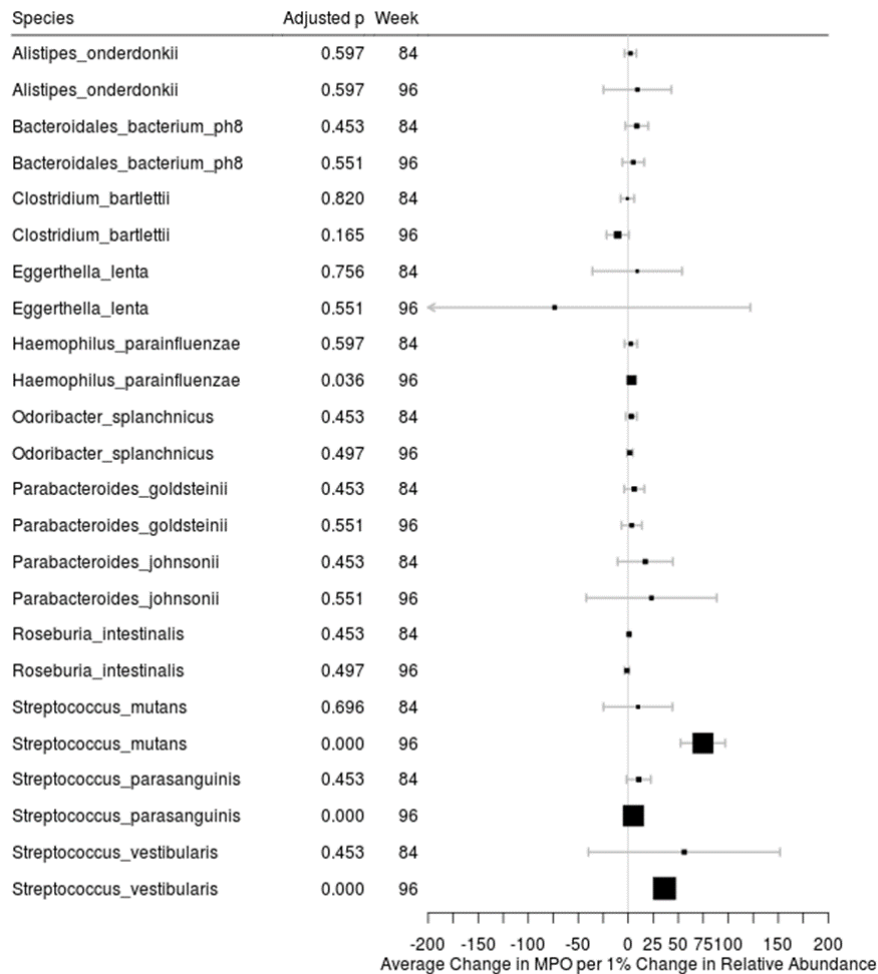Average Change in MPO per 1% Change in Relative Abundance

figure S5. Associations between all fecal bacterial species that differed between HIV-positive children randomized to continue versus stop cotrimoxazole prophylaxis and fecal myeloperoxidase. Effect size plots showing the average change in fecal myeloperoxidase per 1% change in relative abundance (±95% confidence interval) for all bacterial species that significantly differed in abundance between stool samples from children randomized to continue versus stop cotrimoxazole at both week-84 and week-96 post-randomization in zero-inflated beta regression analysis after FDR adjustment for multiple hypothesis testing (**fig S2**). Size of square is inversely proportional to p-value. Vertical line indicates the null value. **Fig. 4C** presents a condensed version of this analysis for *Streptococcal* spp.
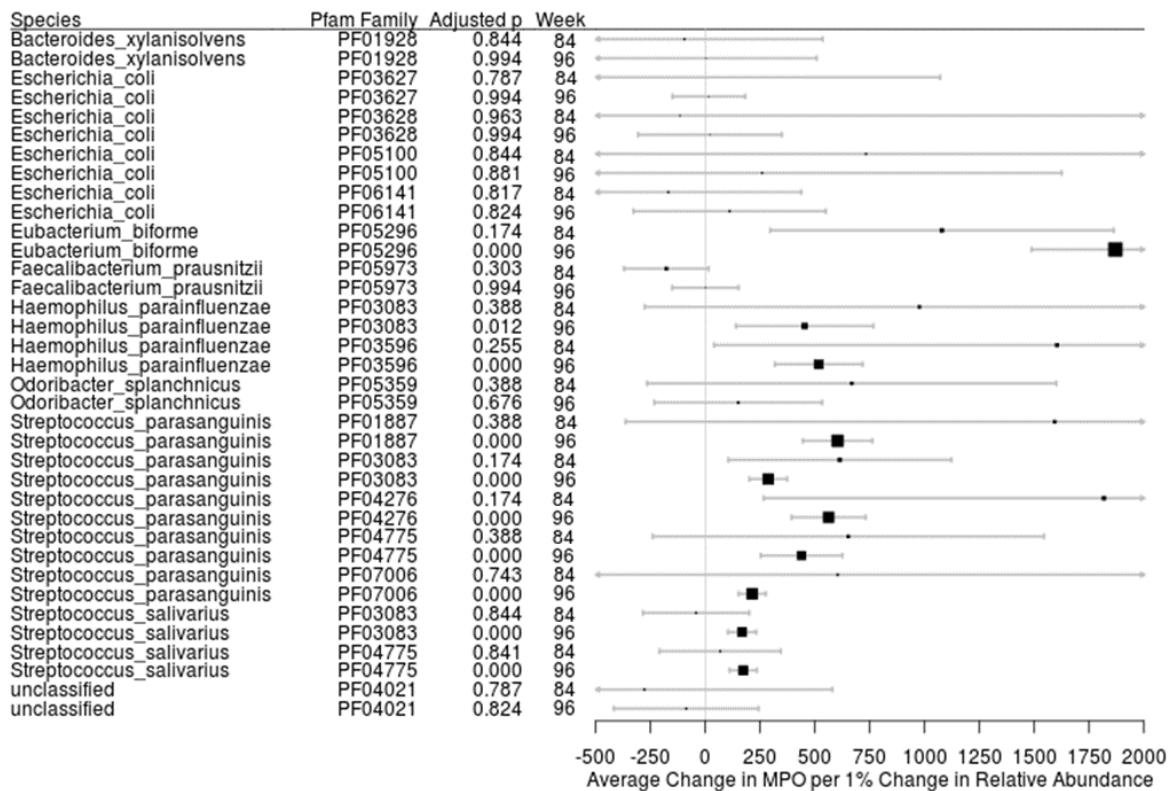
Species	Pfam Family	Adjusted p	Week
Bacteroides_xylanisolvens	PF01928	0.844	84
Bacteroides_xylanisolvens	PF01928	0.994	96
Escherichia_coli	PF03627	0.787	84
Escherichia_coli	PF03627	0.994	96
Escherichia_coli	PF03628	0.963	84
Escherichia_coli	PF03628	0.994	96
Escherichia_coli	PF05100	0.844	84
Escherichia_coli	PF05100	0.881	96
Escherichia_coli	PF06141	0.817	84
Escherichia_coli	PF06141	0.824	96
Eubacterium_biforme	PF05296	0.174	84
Eubacterium_biforme	PF05296	0.000	96
Faecalibacterium_prausnitzii	PF05973	0.303	84
Faecalibacterium_prausnitzii	PF05973	0.994	96
Haemophilus_parainfluenzae	PF03083	0.388	84
Haemophilus_parainfluenzae	PF03083	0.012	96
Haemophilus_parainfluenzae	PF03596	0.255	84
Haemophilus_parainfluenzae	PF03596	0.000	96
Odoribacter_splanchnicus	PF05359	0.388	84
Odoribacter_splanchnicus	PF05359	0.676	96
Streptococcus_parasanguinis	PF01887	0.388	84
Streptococcus_parasanguinis	PF01887	0.000	96
Streptococcus_parasanguinis	PF03083	0.174	84
Streptococcus_parasanguinis	PF03083	0.000	96
Streptococcus_parasanguinis	PF04276	0.174	84
Streptococcus_parasanguinis	PF04276	0.000	96
Streptococcus_parasanguinis	PF04775	0.388	84
Streptococcus_parasanguinis	PF04775	0.000	96
Streptococcus_parasanguinis	PF07006	0.743	84
Streptococcus_parasanguinis	PF07006	0.000	96
Streptococcus_salivarius	PF03083	0.844	84
Streptococcus_salivarius	PF03083	0.000	96
Streptococcus_salivarius	PF04775	0.841	84
Streptococcus_salivarius	PF04775	0.000	96
unclassified	PF04021	0.787	84
unclassified	PF04021	0.824	96

Average Change in MPO per 1% Change in Relative Abundance

figure S6. Associations between all fecal Pfam that differed between HIV-positive children randomized to continue versus stop cotrimoxazole prophylaxis and fecal myeloperoxidase. Effect size plots showing the average change in fecal myeloperoxidase per 1% change in relative abundance (±95% confidence interval) for all Pfam that significantly differed in abundance between stool samples children randomized to continue versus stop cotrimoxazole at both week-84 and week-96 post-randomization in zero-inflated beta regression analysis after FDR adjustment for multiple hypothesis testing (**fig S3**). Identities of bacterial species for each Pfam were established using *HUMANn2* with default settings against the UniRef90 database. Size of square is inversely proportional to p-value. Vertical line indicates the null value. **Fig. 4C** presents a condensed version of this analysis for Pfam with identify to *Streptococcal* spp.
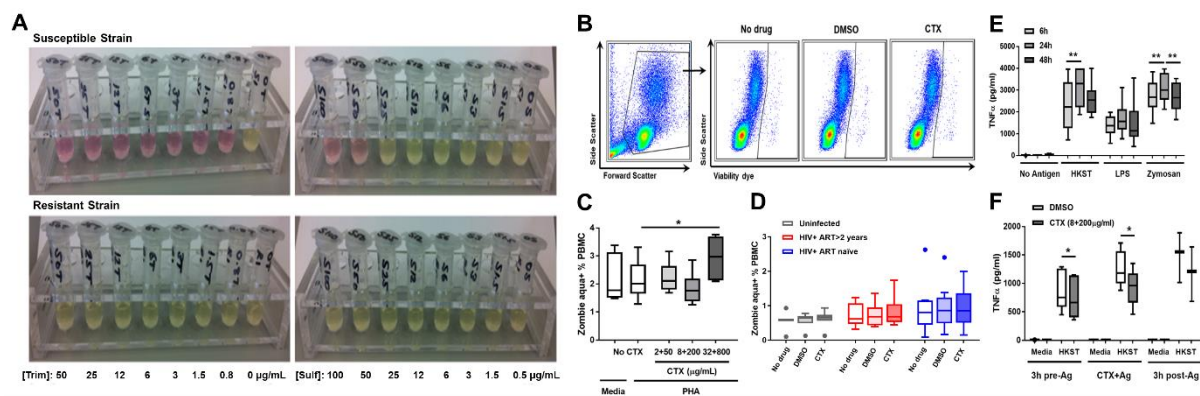
figure S7. Optimization of *in vitro* blood leukocyte activation and cotrimoxazole treatment conditions. (**A**) Photographs of 24h cultures of cotrimoxazole-susceptible (isolate ID: 15A076507S; top) and cotrimoxazole-resistant (isolate ID: 15A076598R; bottom) bacterial isolates from human urinary tract infections treated with titrated concentrations of trimethoprim (Trim, left) and sulfamethoxazole (Sulf, right) prepared in DMSO diluent; representative of 2 experimental repeats. Pink transparent media indicates an absence of bacterial growth, confirming cotrimoxazole antibiotic activity at minimum concentrations of 0.8 μg/mL trimethoprim and 50 μg/mL sulfamethoxazole. Yellow opaque media indicates bacterial growth, which was not inhibited in cultures of resistant bacteria. (**B**) Flow cytometry gating strategy showing Zombie aqua cell viability staining (positive staining identifies dead cells) of unstimulated PBMC cultured for 6h without drug treatment, with cotrimoxazole (CTX: 8 μg/mL trimethoprim and 200 μg/mL sulfamethoxazole) or volume-matched DMSO control; representative of 24 samples. (**C**) Tukey boxplots showing median proportions of Zombie aqua+ PBMC after 24h culture without antigen or with 1 μg/mL of phytohaemagglutinin (PHA) with titrated concentrations of cotrimoxazole. Freidman's test with post-hoc uncorrected Dunn's; n=6, *p<0.05. (**D**) Tukey boxplots showing median proportions of Zombie aqua+ cells in unstimulated 6h PBMC from HIV-negative (n=8, grey), HIV-positive ART-treated (n=8, red) and HIV-positive ART-naïve (n=10, blue) adults

cultured for 6h without drug (ND) or with CTX (8+200 μg/mL) or volume-matched DMSO. (**E**) Tukey boxplots showing median TNFα concentrations in whole blood culture supernatants after 6, 24, or 48h culture with bacterial and fungal TLR ligands; Kruskall-Wallis with post-hoc uncorrected Dunn's test; n=6, **p<0.01. (**F**) Tukey boxplots showing median TNFα concentrations in whole blood culture supernatants after 24h culture with HKST and addition of CTX (8+200 μg/mL) for 3h before (3h pre-Ag), at the same time as (CTX+Ag) or 3h after (3h-post Ag) addition of HKST; Mann-Whitney U test; n=6, *p<0.05.
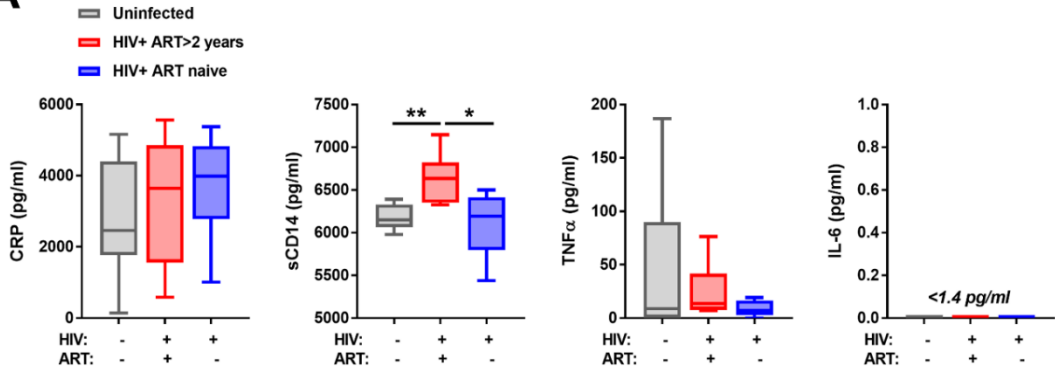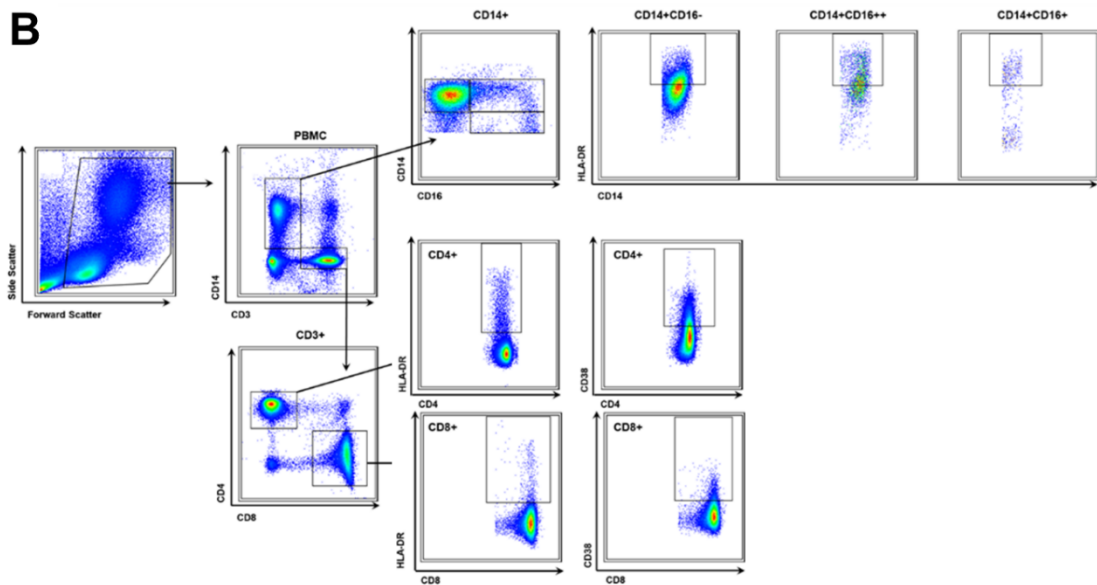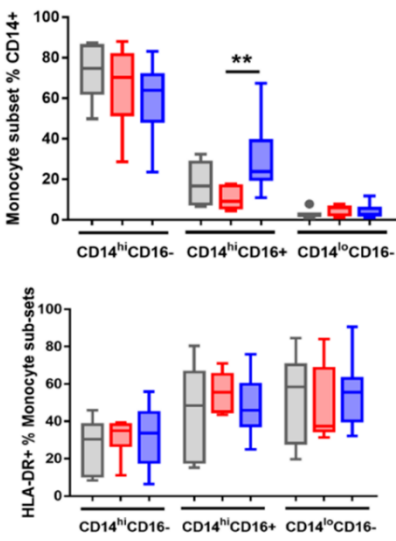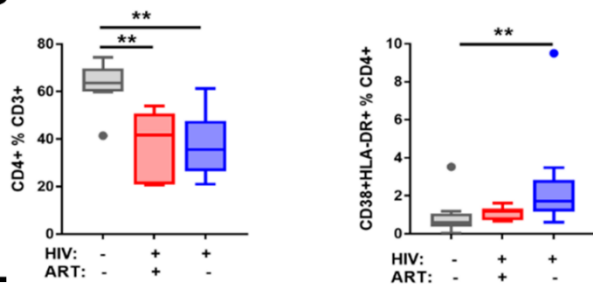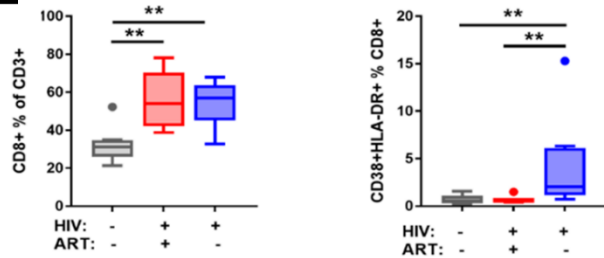
figure S8. HIV-positive adults have greater systemic inflammation, monocyte and T cell activation than HIV-negative adults. Background inflammation and circulating immune cell activation was assessed in blood samples from HIV-negative (grey; n=8), HIV-positive ART-treated (red; n=6) and HIV-positive ART-naïve adults (blue; n=10) recruited in the U.K. (**A**) Concentrations of systemic inflammatory mediators in plasma samples. IL-6 ELISA limit of detection was 1.4 pg/mL. Statistical comparisons between groups were made using Kruskall-Wallis test with post-hoc pair-wise Dunn's test; *p<0.05, **p<0.01. (**B**) Representative flow cytometry gating strategy for monocyte and T cell phenotyping in freshly isolated PBMC; representative of 24 samples. (**C**) Monocyte activation phenotype: proportions of monocyte subtypes segregated according to CD14 and CD16 expression (classical CD14^{hi}CD16$^-$; intermediate CD14^{hi}CD16$^+$; and non-classical CD14^{lo}CD16$^+$; above), and HLA-DR expression by monocyte subtypes (below). Proportions of (**D**) CD4+ and (**E**) CD8+ T cells within the CD3+ T cell pool (left) and proportions of T cells co-expressing HLA-DR and CD38 markers associated with activation (right). Statistical comparisons between groups were made using Kruskall-Wallis test with post-hoc pair-wise Dunn's test; *p<0.05, **p<0.01.
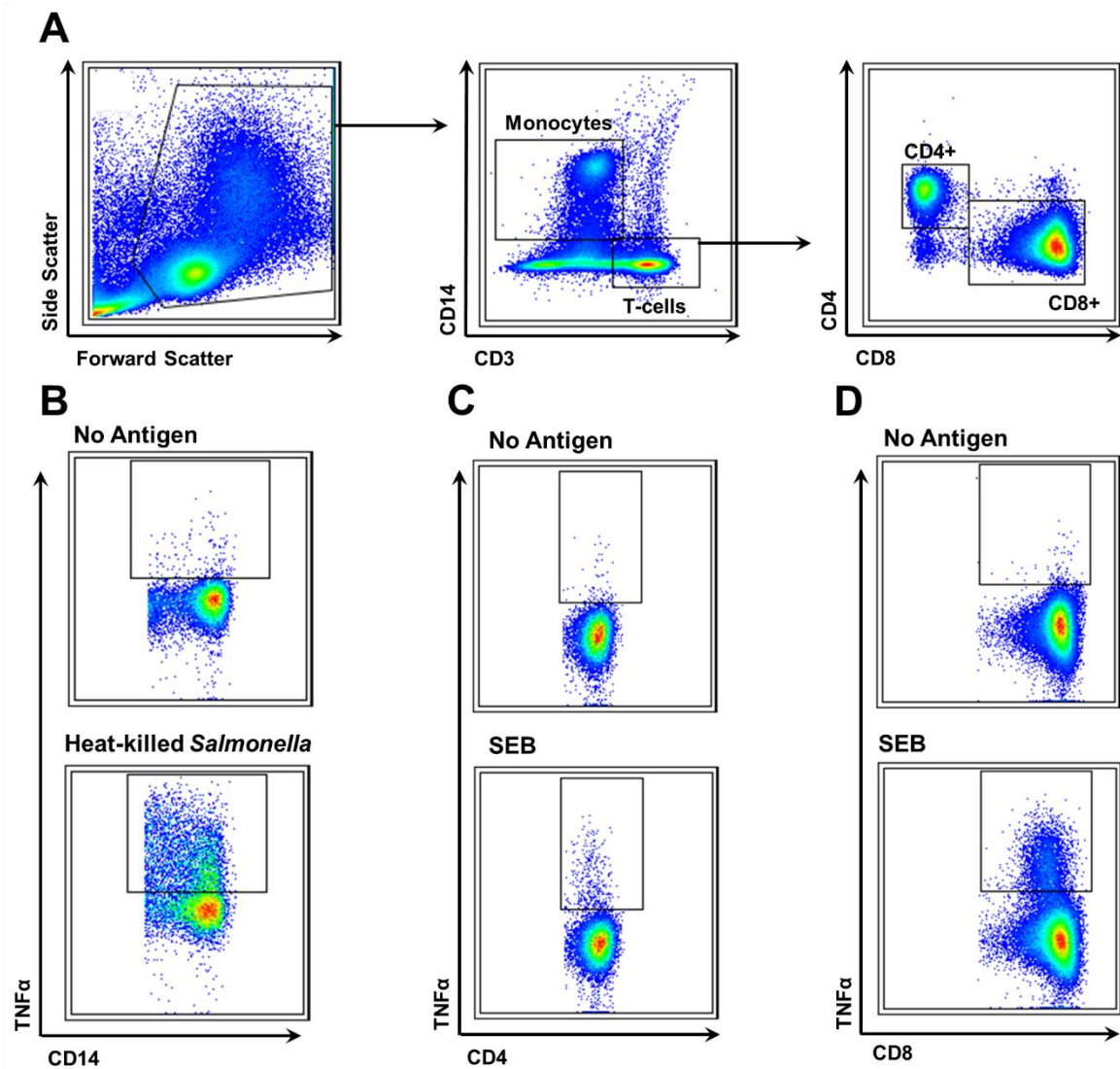
figure S9. Flow cytometry gating strategy for analysis of monocyte and T cell intracellular cytokine responses. (**A**) Flow cytometry gating strategy for identification of monocytes (CD14+) and T cells (CD3+, sub-divided by CD4 and CD8 expression) in PBMC cultured for 6h. (**B**) Flow cytometry gating of TNFα-expressing monocytes after 6h culture without antigen or with 10^8 cells/mL of heat-killed *Salmonella typhimurium*. Flow cytometry gating of TNFα-expressing (**C**) CD4+ and (**D**) CD8+ T cells after 6h culture without antigen or with 1 μg/mL Staphylococcal enterotoxin B (SEB). Flow cytometry plots are representative of 24 PBMC cultures conducted without drug treatment.

table S1. Characteristics of HIV-negative and HIV-positive U.K. adult volunteers

	HIV-	HIV+ ART >2 years	HIV+ ART naïve[1]
n	8	6	10
Gender (M, F)	7, 1	6, 0	8, 2
Age (range)	38 (27-59)	59 (41 -81)	40 (27 - 61)
HIV viral load (copies/mL)	-	<40	17,225 (<40 - 78,318)
CD4 count (cells/mm^3)	-	545 (211-826)	585 (278 - 997)
Years on ART (range)	-	10 (4-17)	-

Mean values are shown.

[1]One participant in group 2 was an 'elite controller' (viral load <40 copies/mL, CD4 count: 723 cells/mm^3, ART-naïve); minimum viral load of non-elite controller participants in the HIV+ ART naïve group was 615 copies/mL.

table S2. Details of fluorophore-conjugated antibody combinations used for flow cytometry analysis of PBMC from HIV-negative and HIV-positive adults.

Target	Clone	Isotype	Flurophore	Manufacturer	Catalogue#
Uncultured PBMC Phenotyping Panel					
CD16	3G8	IgG1	Pacific Blue	BioLegend	302032
CD38	HB7	IgG1	BV 510	BioLegend	356612
CD14	M5E2	IgG2a, κ	BV 605	BioLegend	301834
CD3	UCHT1	IgG1, κ	FITC	BioLegend	300406
CD4	OKT4	IgG2b, κ	PE	eBiosciences	12-0048-42
CD8a	SK1	IgG1, κ	PE-Cy7	eBiosciences	25-0087-42
HLA-DR	L243	IgG2a, κ	APC-Cy7	BioLegend	307618
Cultured PBMC Intracellular Cytokine Panel					
CD3	UCHT1	IgG1, κ	Pacific Blue	BioLegend	300431
CD4	OKT4	IgG2b, κ	BV 510	BioLegend	317444
CD14	M5E2	IgG2a, κ	BV 605	BioLegend	301834
TNFα	MAb11	IgG1, κ	PE	eBiosciences	12-7349-81
CD8a	SK1	IgG1, κ	PE-Cy7	eBiosciences	25-0087-42
HLA-DR	L243	IgG2a, κ	APC-Cy7	BioLegend	307618

BV - Brilliant Violet; EF - eBiosciences Fluor; AF - Alexa Fluor