

## **High Abundance of genus *Prevotella* in the gut of perinatally HIV-infected children is associated with IP-10 levels despite therapy**

Urvinder Kaur S<sup>1</sup>, Anita Shet<sup>2</sup>, Niharika Rajnala<sup>3</sup>, Bindu Parachalil Gopalan<sup>3</sup>, Preeti Moar<sup>1</sup>, Himanshu D<sup>4</sup>, Balendra Pratap Singh<sup>5</sup>, Rupesh Chaturvedi<sup>6</sup>, Ravi Tandon<sup>1#</sup>

<sup>1</sup>Laboratory of AIDS Research and Immunology, School of Biotechnology, Jawaharlal Nehru University, New Delhi, India; <sup>2</sup>International Vaccine Access Center, Johns Hopkins School of Public Health, Baltimore, USA; <sup>3</sup>Division of Infectious Diseases, St. John's Research Institute, St. John's National Academy of health Sciences, Bangalore, India; <sup>4</sup>Department of Medicine, King Georges Medical University, Lucknow, India; <sup>5</sup>Department of Prosthodontics, King Georges Medical University, Lucknow, India; <sup>6</sup>Host Pathogen Interaction Laboratory, School of Biotechnology, Jawaharlal Nehru University, New Delhi, India

# Corresponding Author:

Ravi Tandon  
School of Biotechnology  
Jawaharlal Nehru University  
New Delhi-110067, India  
Phone: +91-11-26738894  
Fax: +91-11-26717586  
E-mail: [ravitandon@jnu.ac.in](mailto:ravitandon@jnu.ac.in)

Figure S1

○ HIV negative  
● Untreated HIV positive  
▲ HIV positive on ART

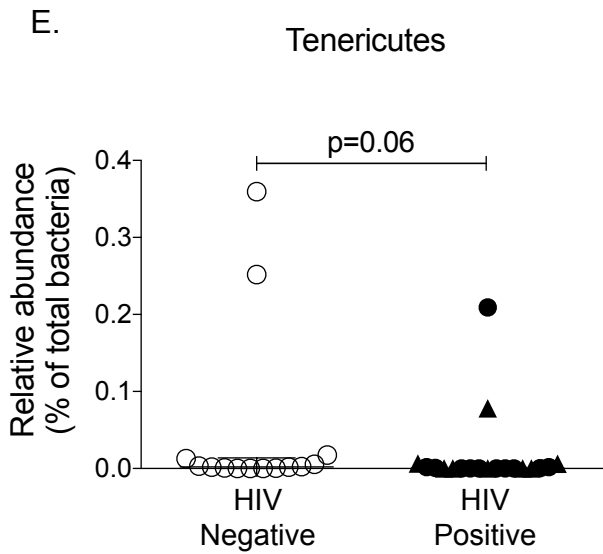
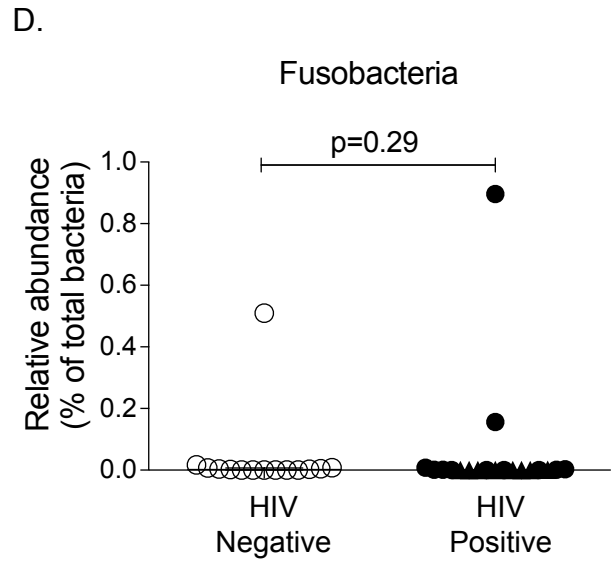
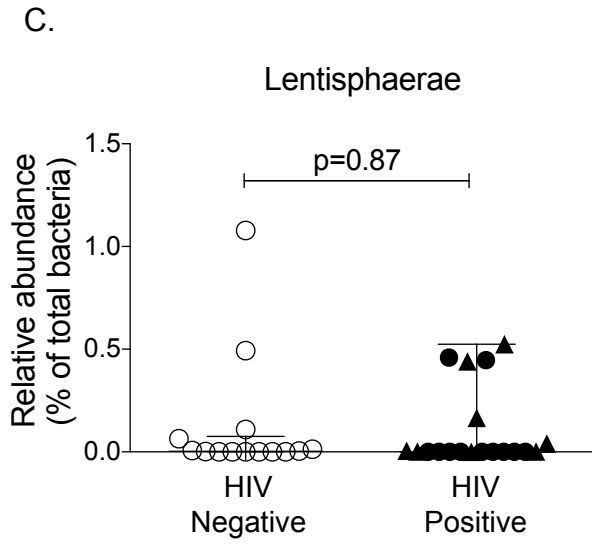
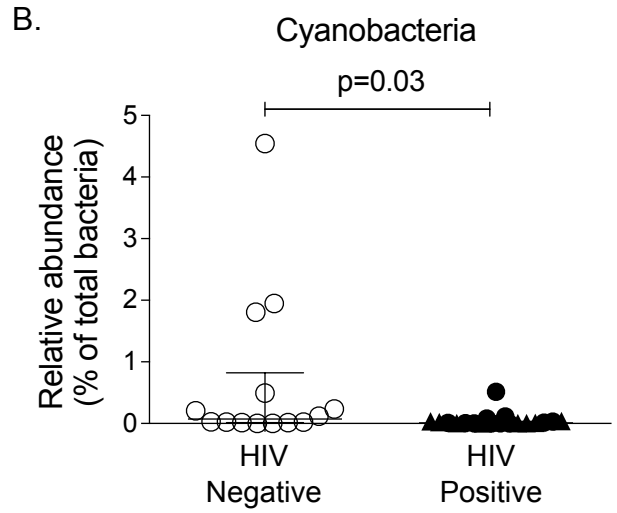
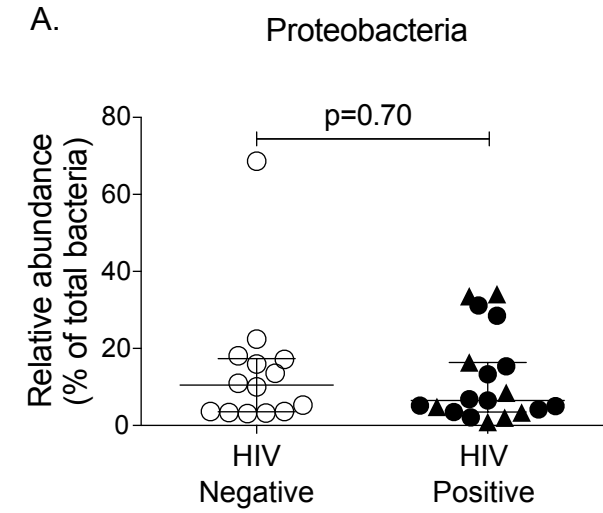


Figure S2

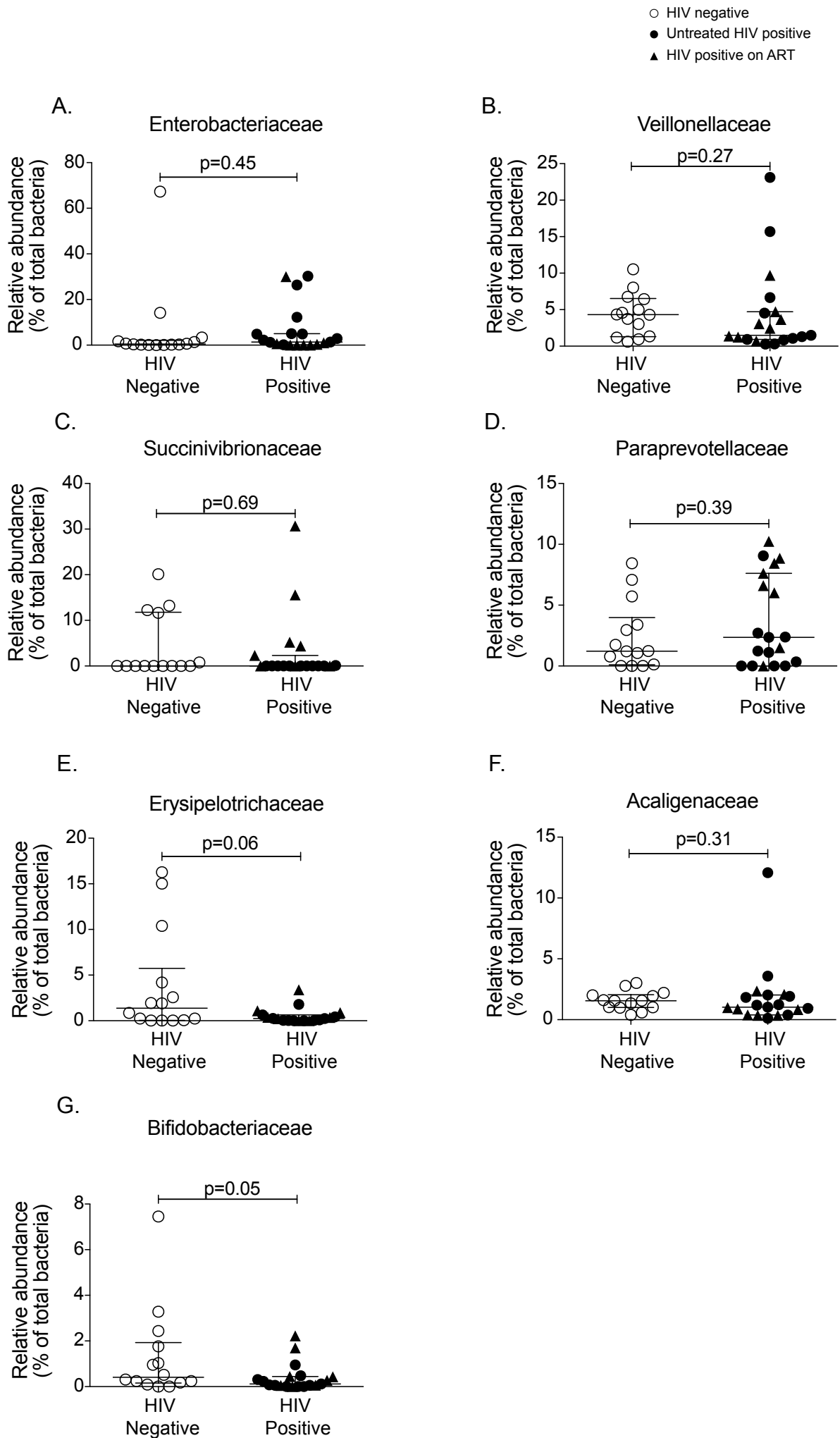
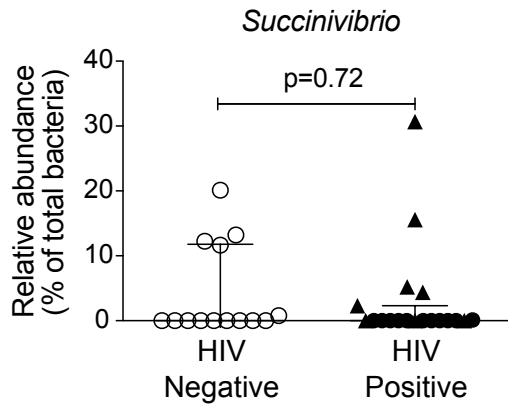


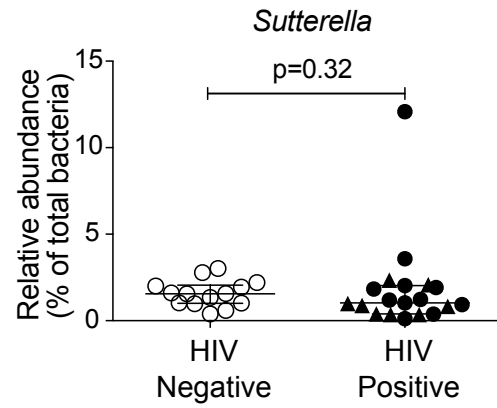
Figure S3

○ HIV negative  
● Untreated HIV positive  
▲ HIV positive on ART

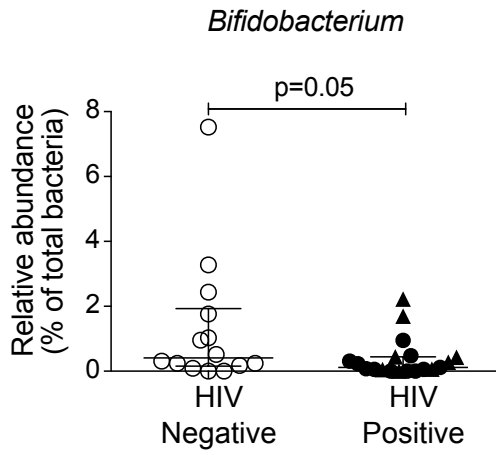
A.



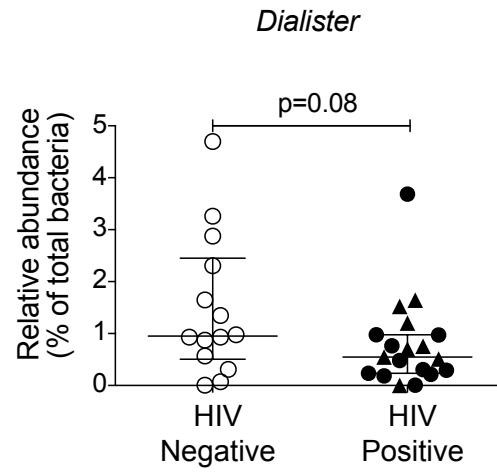
B.



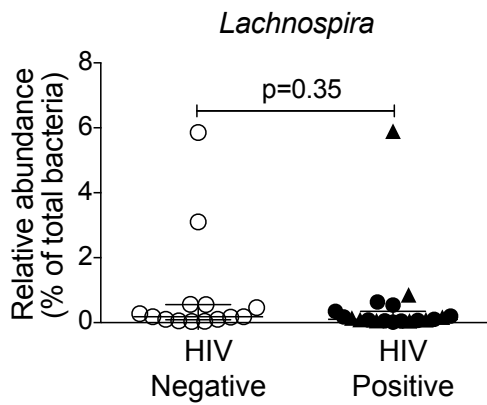
C.



D.



E.



F.

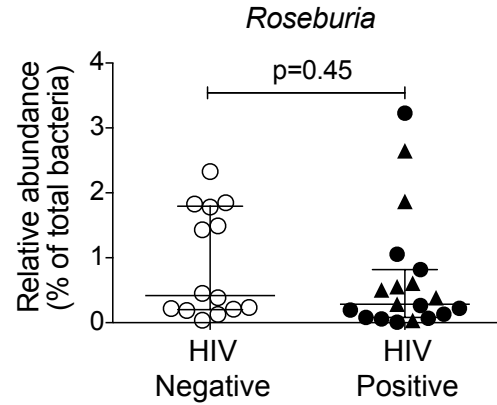


Figure S4

○ HIV negative  
 ● Untreated HIV positive  
 ▲ HIV positive on ART

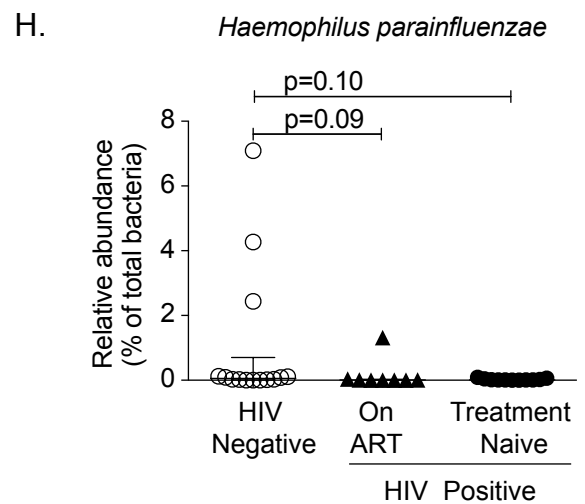
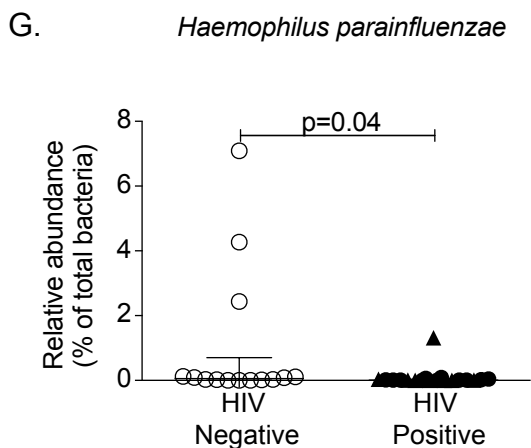
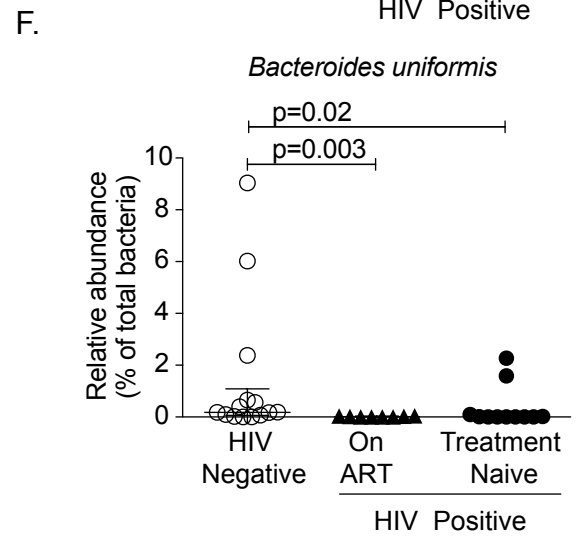
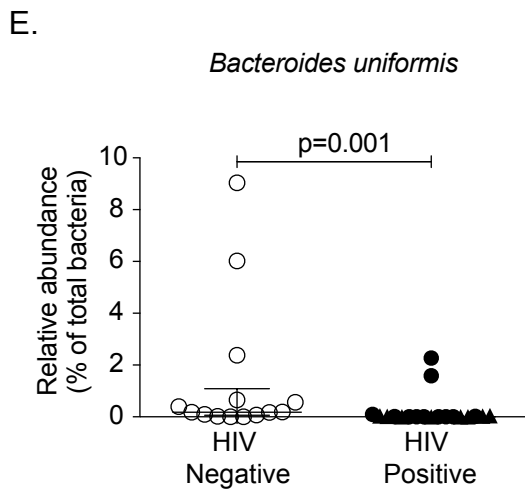
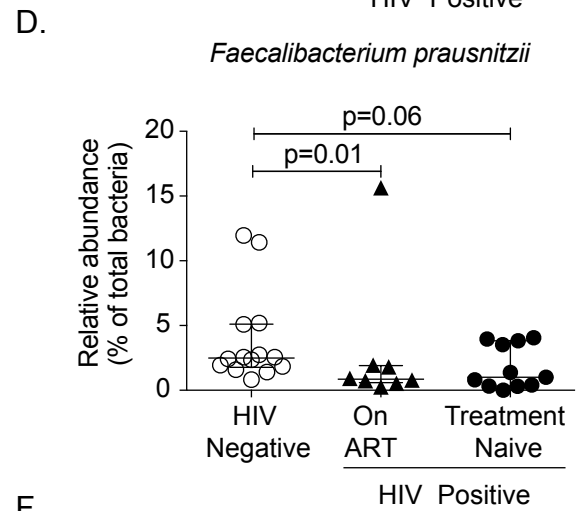
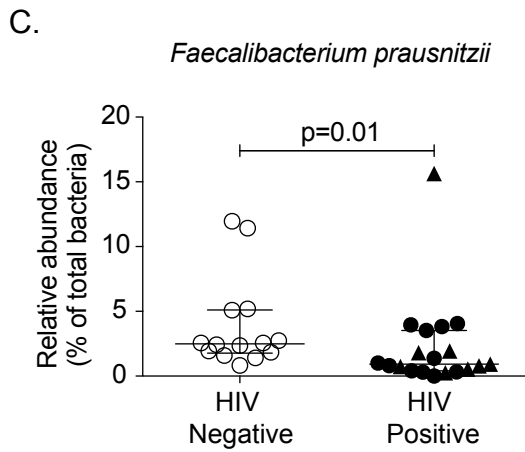
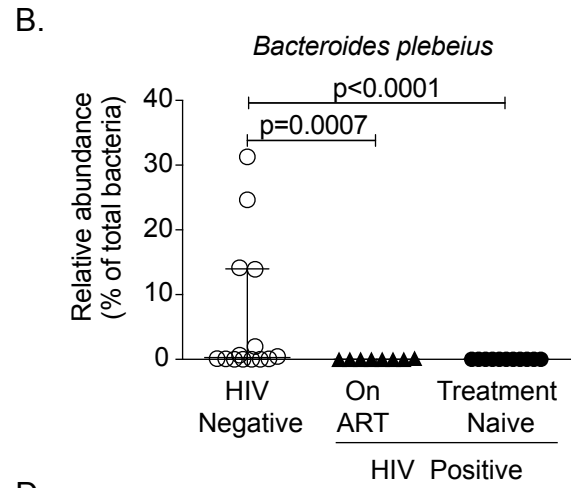
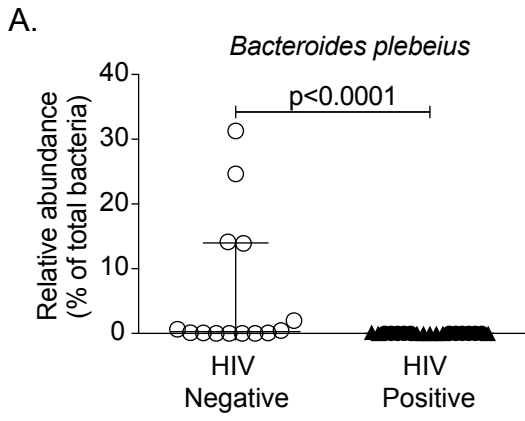
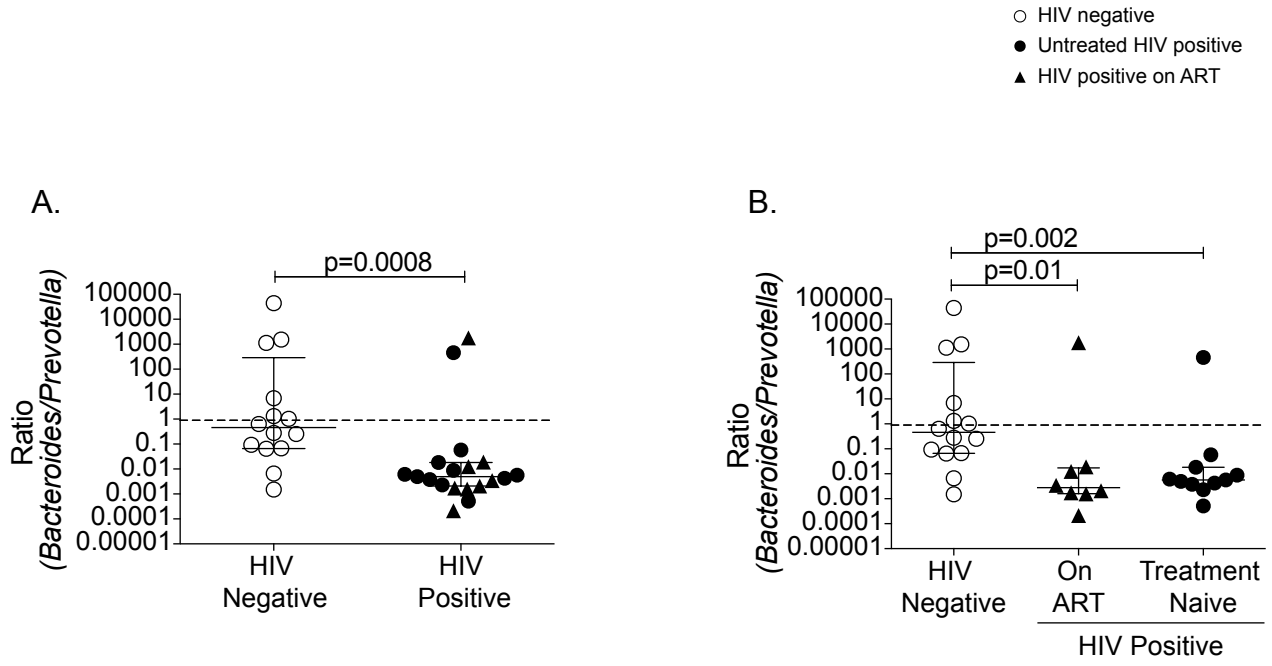


Figure S5



**Table S1**

|                         | <b>HIV Positive</b>       | <b>HIV Negative</b>      |                |
|-------------------------|---------------------------|--------------------------|----------------|
|                         | <b>Median<br/>(IQR)</b>   | <b>Median<br/>(IQR)</b>  | <b>p value</b> |
| <b>Phylum</b>           |                           |                          |                |
| Bacteroidetes           | 80.03<br>(56.53 – 87.19)  | 52.05<br>(40.66 – 57.70) | 0.003          |
| Firmicutes              | 9.406<br>(7.388 – 18.51)  | 36.36<br>(23.40 – 40.57) | 0.002          |
| Actinobacteria          | 0.195<br>(0.097 – 0.487)  | 1.165<br>(0.409 – 2.888) | 0.0007         |
| Spirochaetes            | 0.001<br>(0.0 – 0.002)    | 0.0<br>(0.0 – 0.0)       | 0.0007         |
| Cyanobacteria           | 0.009<br>(0.004 – 0.037)  | 0.074<br>(0.016 – 0.821) | 0.03           |
| <b>Family</b>           |                           |                          |                |
| Prevotellaceae          | 70.35<br>(44.84 – 82.96)  | 28.15<br>(1.235 – 39.7)  | 0.0007         |
| Bacteroidaceae          | 0.33<br>(0.166 – 0.56)    | 9.456<br>(2.151 – 26.02) | 0.0008         |
| Ruminococcaceae         | 3.034<br>(1.862 – 5.9)    | 11.31<br>(4.814 – 19.02) | 0.005          |
| Lachnospiraceae         | 1.374<br>(0.721 – 3.238)  | 4.429<br>(1.516 – 7.3)   | 0.01           |
| Rikenellaceae           | 0.003<br>(0.001 – 0.02)   | 0.33<br>(0.145 – 1.452)  | <0.0001        |
| <b>Genus</b>            |                           |                          |                |
| <i>Prevotella</i>       | 70.35<br>(44.84 – 82.96)  | 28.15<br>(1.235 – 39.7)  | 0.0007         |
| <i>Bacteroides</i>      | 0.33<br>(0.166 – 0.56)    | 9.456<br>(2.151 – 26.02) | 0.0008         |
| <i>Faecalibacterium</i> | 0.9185<br>(0.409 – 3.535) | 2.492<br>(1.776 – 5.116) | 0.01           |
| <i>Megasphaera</i>      | 0.57<br>(0.002 – 2.784)   | 0.001<br>(0.0 – 0.486)   | 0.02           |
| <i>Ruminococcus</i>     | 0.136<br>(0.022 – 0.64)   | 0.63<br>(0.244 – 6.088)  | 0.006          |
| <i>Haemophilus</i>      | 0.008<br>(0.003 – 0.03)   | 0.06<br>(0.012 – 0.736)  | 0.04           |
| <b>Species</b>          |                           |                          |                |
| <i>Prevotella copri</i> | 67.45<br>(40.52 – 76.72)  | 17.69<br>(1.121 – 37.44) | 0.001          |

**Table S2**

|                         | <b>Treatment naive</b>    | <b>On ART</b>             | <b>HIV Negative</b>      |
|-------------------------|---------------------------|---------------------------|--------------------------|
|                         | <b>Median<br/>(IQR)</b>   | <b>Median<br/>(IQR)</b>   | <b>Median<br/>(IQR)</b>  |
| <b>Phylum</b>           |                           |                           |                          |
| Bacteroidetes           | *84.25<br>(58.48 – 88.83) | 74.42<br>(40.08 – 86.93)  | 52.05<br>(40.66 – 57.70) |
| Firmicutes              | *8.56<br>(5.816 – 12.85)  | 11.08<br>(8.593 – 40.91)  | 36.36<br>(23.4 – 40.57)  |
| Actinobacteria          | *0.134<br>(0.099 – 0.357) | 0.395<br>(0.075 – 1.528)  | 1.165<br>(0.409 – 2.888) |
| Spirochaetes            | *0.0002<br>(0.0 – 0.002)  | * (0.0 – 0.002)           | 0.0<br>(0.0 – 0.0)       |
| <b>Family</b>           |                           |                           |                          |
| Prevotellaceae          | *81.6<br>(54.41 – 86.15)  | *69.69<br>(27.9 – 78.88)  | 28.15<br>(1.23 – 39.7)   |
| Bacteroidaceae          | *0.409<br>(0.234 – 1.08)  | *0.216<br>(0.122 – 0.526) | 9.456<br>(2.15 – 26.02)  |
| Ruminococcaceae         | *4.747<br>(1.14 – 5.545)  | 2.643<br>(1.989 – 18.07)  | 11.31<br>(4.814 – 19.02) |
| Lachnospiraceae         | *1.179<br>(0.524 – 1.678) | 1.976<br>(0.944 – 7.063)  | 4.429<br>(1.516 – 7.301) |
| Rikenellaceae           | *0.004<br>(0.002 – 0.012) | *0.002<br>(0.0 – 0.03)    | 0.33<br>(0.144 – 1.452)  |
| <b>Genus</b>            |                           |                           |                          |
| <i>Prevotella</i>       | *81.6<br>(54.41 – 86.15)  | *69.69<br>(27.9 – 78.88)  | 28.15<br>(1.23 – 39.7)   |
| <i>Bacteroides</i>      | *0.409<br>(0.234 – 1.08)  | *0.216<br>(0.122 – 0.526) | 9.456<br>(2.15 – 26.02)  |
| <i>Faecalibacterium</i> | 1.001<br>(0.318 – 3.829)  | *0.857<br>(0.592 – 1.906) | 2.492<br>(1.776 – 5.116) |
| <i>Megasphaera</i>      | *0.573<br>(0.004 – 5.106) | 0.548<br>(0.0009 – 2.682) | 0.001<br>(0.0 – 0.486)   |
| <i>Ruminococcus</i>     | *0.047<br>(0.014 – 0.136) | 0.343<br>(0.152 – 2.157)  | 0.633<br>(0.245 – 3.05)  |
| <i>Haemophilus</i>      | 0.012<br>(0.006 – 0.044)  | 0.008<br>(0.0 – 0.03)     | 0.063<br>(0.012 – 0.737) |
| <b>Species</b>          |                           |                           |                          |
| <i>Prevotella copri</i> | *68.21<br>(45.77 – 82.94) | *65.47<br>(20.01 – 72.85) | 17.69<br>(1.121 – 37.44) |

\*values that are significantly different from HIV negative controls (p<0.05)



### Supplementary figure and table legends:

**Table S1:** Relative abundance of significantly distinct fecal microbiota between perinatally HIV-infected children and HIV negative controls at phylum, family, genus and species levels.

**Table S2:** Relative abundance of significantly distinct fecal microbiota across treatment naïve, On ART perinatally HIV-infected children and HIV negative controls at phylum, family, genus and species levels.

**Figure S1:** Plots representing relative abundance of **A.** Proteobacteria **B.** Cyanobacteria **C.** Lentisphaerae **D.** Fusobacteria and **E.** Tenericutes phyla in HIV negative and HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney-U-test was performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S2:** Plots representing relative abundance of **A.** Enterobacteriaceae **B.** Veillonellaceae **C.** Succinivibrionaceae **D.** Paraprevotellaceae **E.** Erysipelotrichaceae **F.** Acaligenaceae and **G.** Bifidobacteriaceae families in HIV negative and HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney-U-test was performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S3:** Plots representing relative abundance of **A.** *Succinivibrio* **B.** *Sutterella* **C.** *Bifidobacterium* **D.** *Dialister* **E.** *Lachnospira* and **F.** *Roseburia* genera in HIV negative and HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney-U-test was performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S4:** Plots representing relative abundance of **A.** *Bacteroides plebeius* **C.** *Faecalibacterium prausnitzii* **E.** *Bacteroides uniformis* and **G.** *Haemophilus parainfluenzae* species in HIV negative and HIV positive children. Plots representing relative abundance of **B.** *Bacteroides plebeius* **D.** *Faecalibacterium prausnitzii* **F.** *Bacteroides uniformis* and **H.** *Haemophilus parainfluenzae* species across HIV negative controls, On ART and treatment naive HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney *U*-test and Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison were performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S5:** Plot showing the ratio of the relative abundance of *Bacteroides* to *Prevotella* **A.** between HIV negative and HIV positive children **B.** across HIV negative controls, On ART and treatment naive HIV positive children. Dotted line shows a ratio of 1.0 (relative abundance of *Bacteroides* = relative abundance of *Prevotella*). The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney *U*-test and Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison were performed for statistical analysis

Figure S1

○ HIV negative  
● Untreated HIV positive  
▲ HIV positive on ART

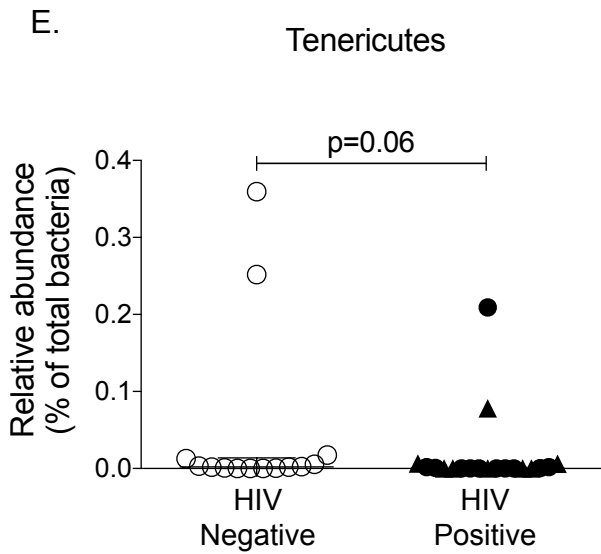
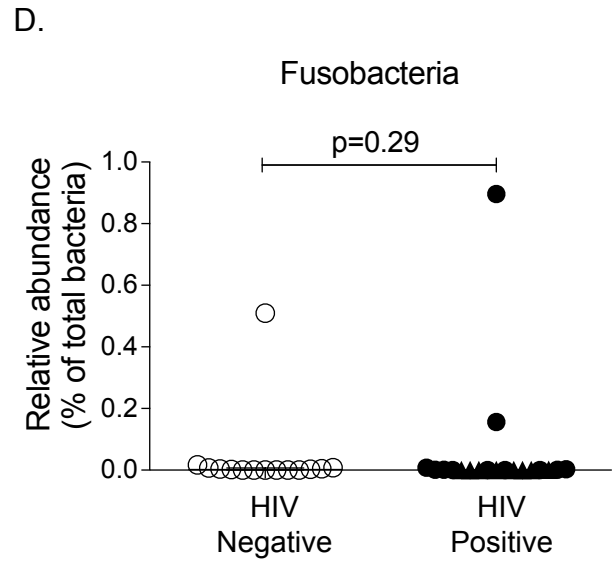
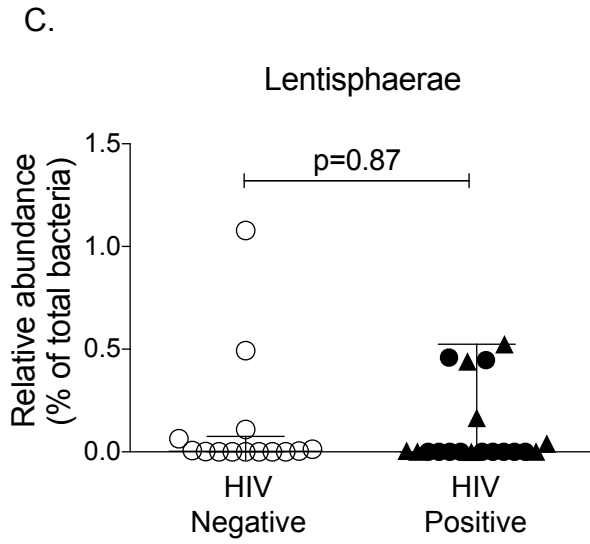
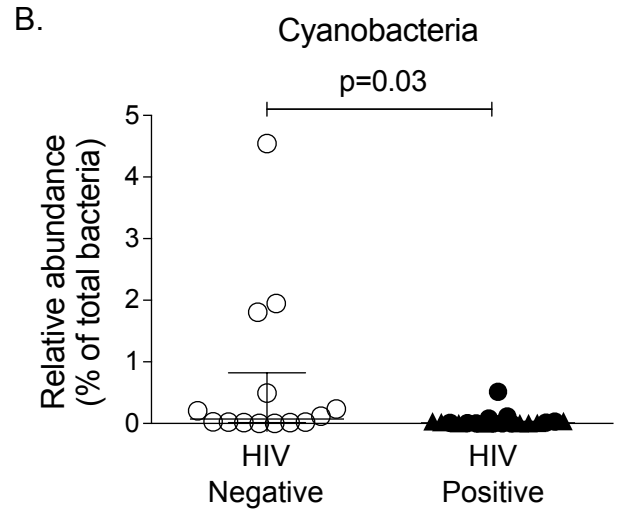
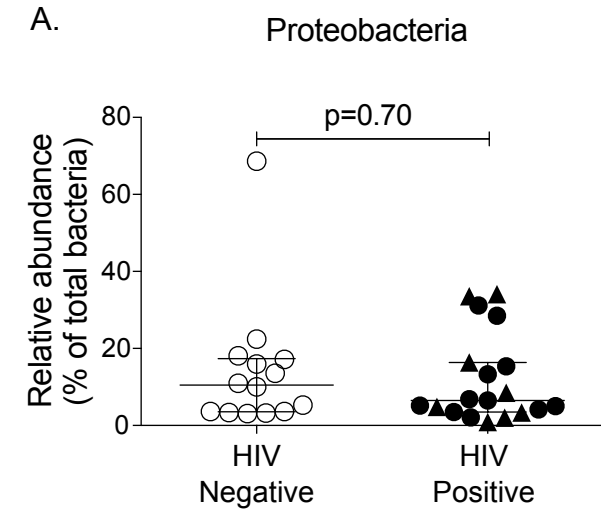


Figure S2

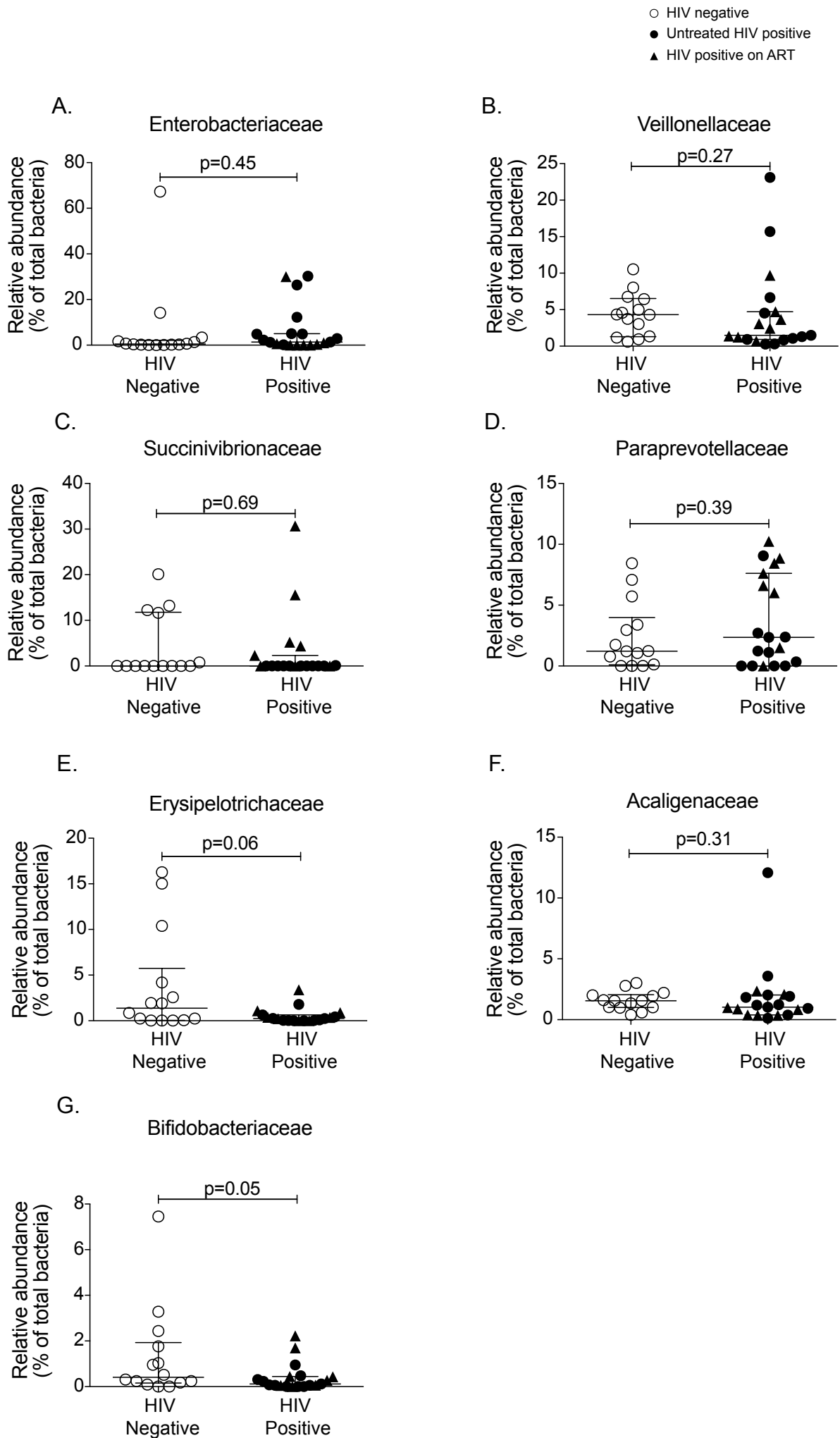
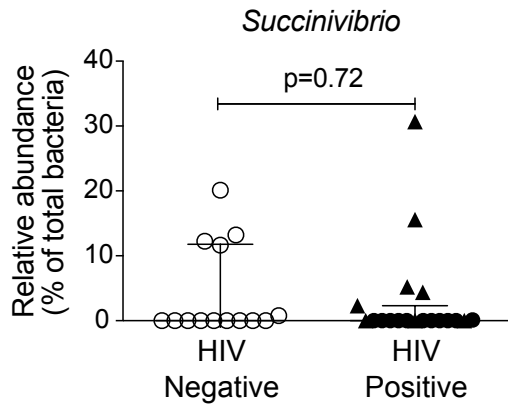


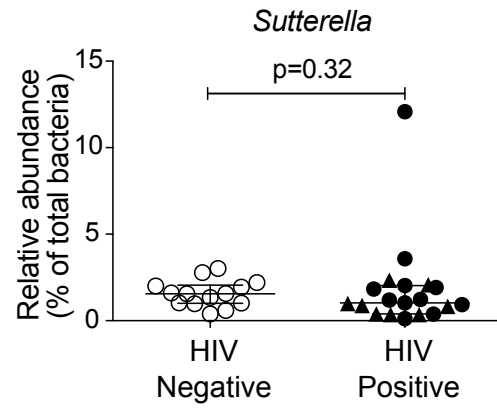
Figure S3

○ HIV negative  
● Untreated HIV positive  
▲ HIV positive on ART

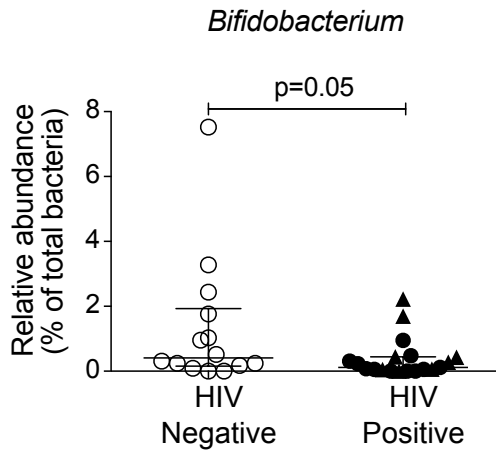
A.



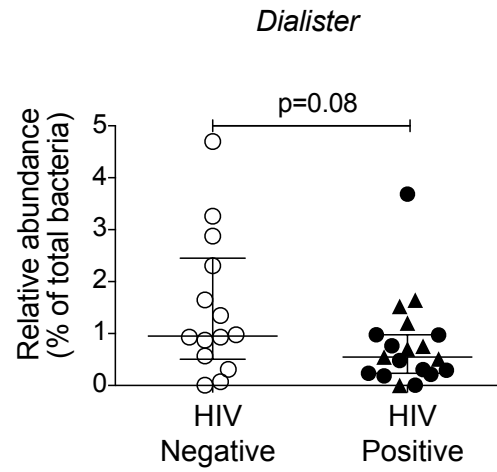
B.



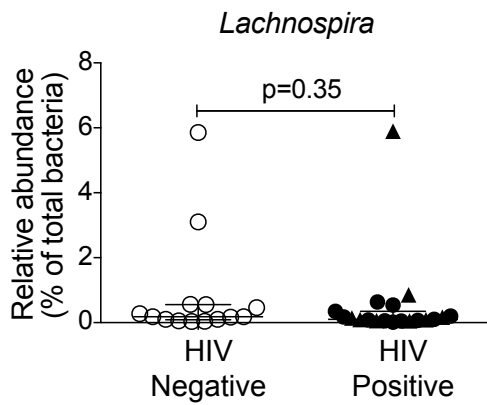
C.



D.



E.



F.

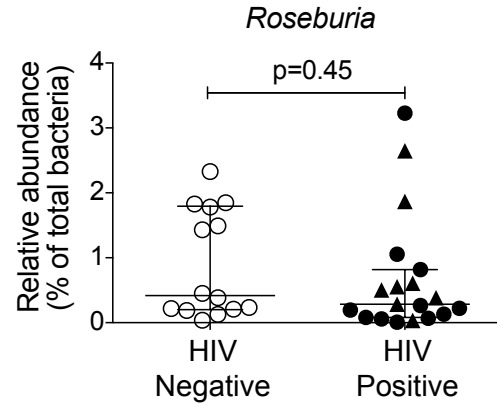


Figure S4

○ HIV negative  
 ● Untreated HIV positive  
 ▲ HIV positive on ART

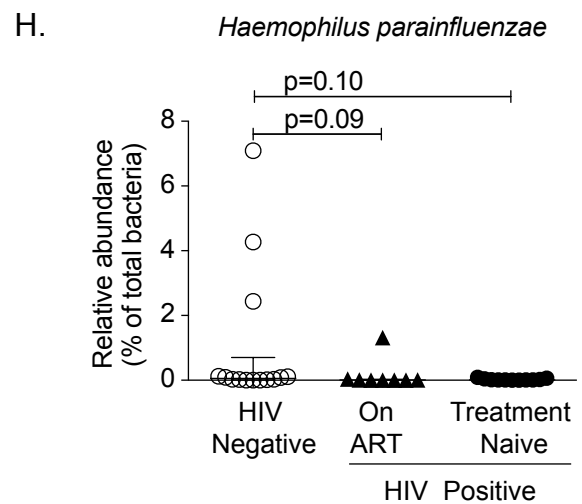
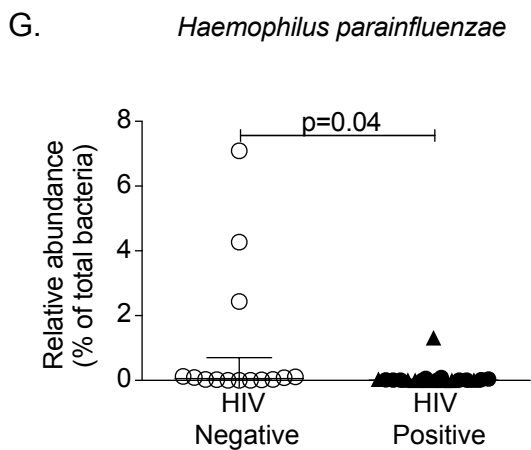
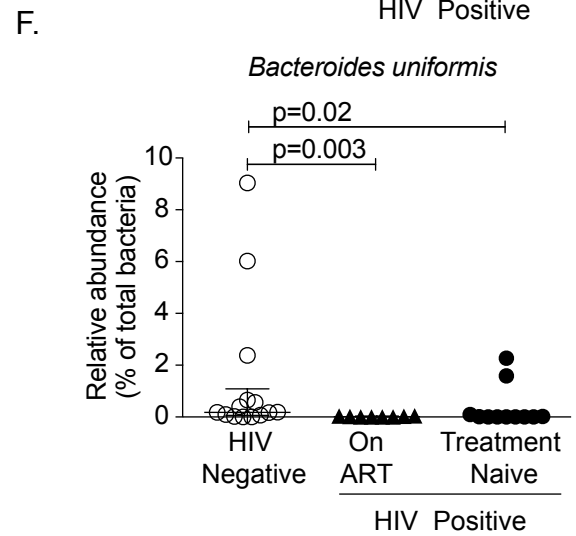
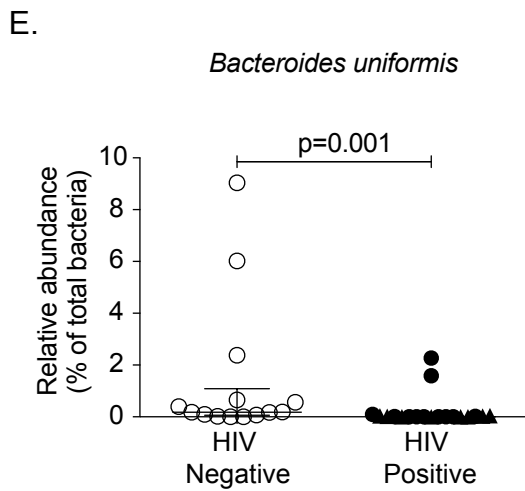
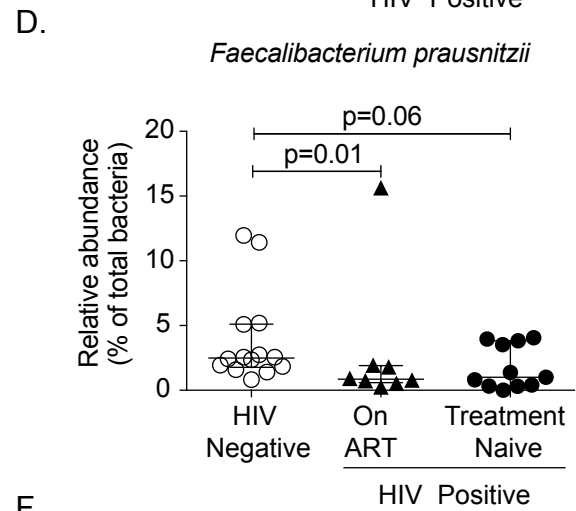
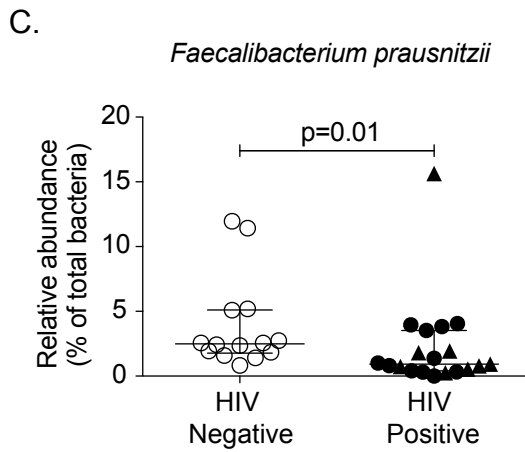
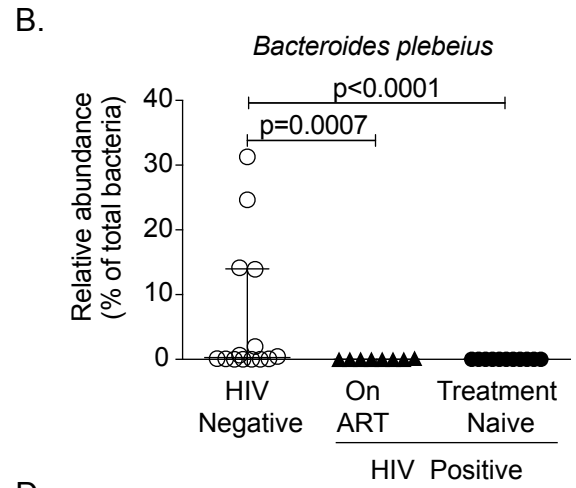
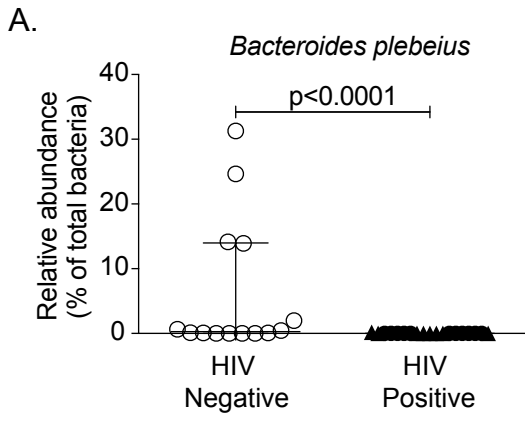
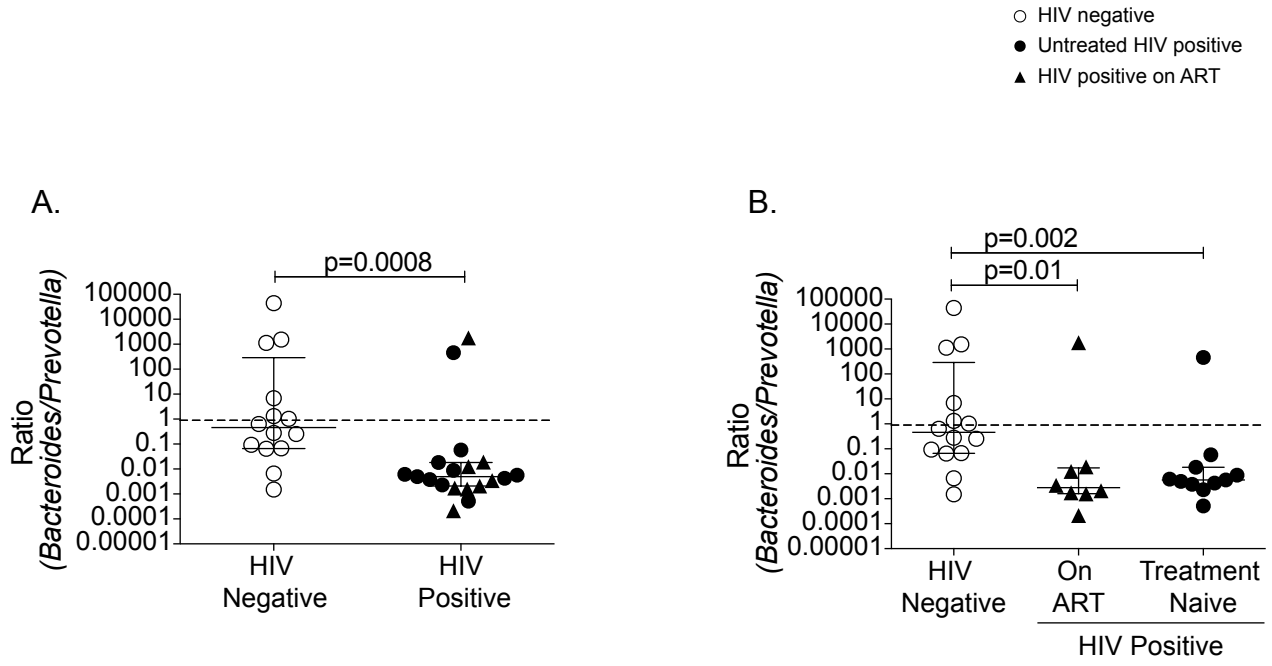


Figure S5



**Table S1**

|                         | <b>HIV Positive</b>       | <b>HIV Negative</b>      |                |
|-------------------------|---------------------------|--------------------------|----------------|
|                         | <b>Median<br/>(IQR)</b>   | <b>Median<br/>(IQR)</b>  | <b>p value</b> |
| <b>Phylum</b>           |                           |                          |                |
| Bacteroidetes           | 80.03<br>(56.53 – 87.19)  | 52.05<br>(40.66 – 57.70) | 0.003          |
| Firmicutes              | 9.406<br>(7.388 – 18.51)  | 36.36<br>(23.40 – 40.57) | 0.002          |
| Actinobacteria          | 0.195<br>(0.097 – 0.487)  | 1.165<br>(0.409 – 2.888) | 0.0007         |
| Spirochaetes            | 0.001<br>(0.0 – 0.002)    | 0.0<br>(0.0 – 0.0)       | 0.0007         |
| Cyanobacteria           | 0.009<br>(0.004 – 0.037)  | 0.074<br>(0.016 – 0.821) | 0.03           |
| <b>Family</b>           |                           |                          |                |
| Prevotellaceae          | 70.35<br>(44.84 – 82.96)  | 28.15<br>(1.235 – 39.7)  | 0.0007         |
| Bacteroidaceae          | 0.33<br>(0.166 – 0.56)    | 9.456<br>(2.151 – 26.02) | 0.0008         |
| Ruminococcaceae         | 3.034<br>(1.862 – 5.9)    | 11.31<br>(4.814 – 19.02) | 0.005          |
| Lachnospiraceae         | 1.374<br>(0.721 – 3.238)  | 4.429<br>(1.516 – 7.3)   | 0.01           |
| Rikenellaceae           | 0.003<br>(0.001 – 0.02)   | 0.33<br>(0.145 – 1.452)  | <0.0001        |
| <b>Genus</b>            |                           |                          |                |
| <i>Prevotella</i>       | 70.35<br>(44.84 – 82.96)  | 28.15<br>(1.235 – 39.7)  | 0.0007         |
| <i>Bacteroides</i>      | 0.33<br>(0.166 – 0.56)    | 9.456<br>(2.151 – 26.02) | 0.0008         |
| <i>Faecalibacterium</i> | 0.9185<br>(0.409 – 3.535) | 2.492<br>(1.776 – 5.116) | 0.01           |
| <i>Megasphaera</i>      | 0.57<br>(0.002 – 2.784)   | 0.001<br>(0.0 – 0.486)   | 0.02           |
| <i>Ruminococcus</i>     | 0.136<br>(0.022 – 0.64)   | 0.63<br>(0.244 – 6.088)  | 0.006          |
| <i>Haemophilus</i>      | 0.008<br>(0.003 – 0.03)   | 0.06<br>(0.012 – 0.736)  | 0.04           |
| <b>Species</b>          |                           |                          |                |
| <i>Prevotella copri</i> | 67.45<br>(40.52 – 76.72)  | 17.69<br>(1.121 – 37.44) | 0.001          |



**Table S2**

|                         | <b>Treatment naive</b>    | <b>On ART</b>             | <b>HIV Negative</b>      |
|-------------------------|---------------------------|---------------------------|--------------------------|
|                         | <b>Median<br/>(IQR)</b>   | <b>Median<br/>(IQR)</b>   | <b>Median<br/>(IQR)</b>  |
| <b>Phylum</b>           |                           |                           |                          |
| Bacteroidetes           | *84.25<br>(58.48 – 88.83) | 74.42<br>(40.08 – 86.93)  | 52.05<br>(40.66 – 57.70) |
| Firmicutes              | *8.56<br>(5.816 – 12.85)  | 11.08<br>(8.593 – 40.91)  | 36.36<br>(23.4 – 40.57)  |
| Actinobacteria          | *0.134<br>(0.099 – 0.357) | 0.395<br>(0.075 – 1.528)  | 1.165<br>(0.409 – 2.888) |
| Spirochaetes            | *0.0002<br>(0.0 – 0.002)  | * (0.0 – 0.002)           | 0.0<br>(0.0 – 0.0)       |
| <b>Family</b>           |                           |                           |                          |
| Prevotellaceae          | *81.6<br>(54.41 – 86.15)  | *69.69<br>(27.9 – 78.88)  | 28.15<br>(1.23 – 39.7)   |
| Bacteroidaceae          | *0.409<br>(0.234 – 1.08)  | *0.216<br>(0.122 – 0.526) | 9.456<br>(2.15 – 26.02)  |
| Ruminococcaceae         | *4.747<br>(1.14 – 5.545)  | 2.643<br>(1.989 – 18.07)  | 11.31<br>(4.814 – 19.02) |
| Lachnospiraceae         | *1.179<br>(0.524 – 1.678) | 1.976<br>(0.944 – 7.063)  | 4.429<br>(1.516 – 7.301) |
| Rikenellaceae           | *0.004<br>(0.002 – 0.012) | *0.002<br>(0.0 – 0.03)    | 0.33<br>(0.144 – 1.452)  |
| <b>Genus</b>            |                           |                           |                          |
| <i>Prevotella</i>       | *81.6<br>(54.41 – 86.15)  | *69.69<br>(27.9 – 78.88)  | 28.15<br>(1.23 – 39.7)   |
| <i>Bacteroides</i>      | *0.409<br>(0.234 – 1.08)  | *0.216<br>(0.122 – 0.526) | 9.456<br>(2.15 – 26.02)  |
| <i>Faecalibacterium</i> | 1.001<br>(0.318 – 3.829)  | *0.857<br>(0.592 – 1.906) | 2.492<br>(1.776 – 5.116) |
| <i>Megasphaera</i>      | *0.573<br>(0.004 – 5.106) | 0.548<br>(0.0009 – 2.682) | 0.001<br>(0.0 – 0.486)   |
| <i>Ruminococcus</i>     | *0.047<br>(0.014 – 0.136) | 0.343<br>(0.152 – 2.157)  | 0.633<br>(0.245 – 3.05)  |
| <i>Haemophilus</i>      | 0.012<br>(0.006 – 0.044)  | 0.008<br>(0.0 – 0.03)     | 0.063<br>(0.012 – 0.737) |
| <b>Species</b>          |                           |                           |                          |
| <i>Prevotella copri</i> | *68.21<br>(45.77 – 82.94) | *65.47<br>(20.01 – 72.85) | 17.69<br>(1.121 – 37.44) |

\*values that are significantly different from HIV negative controls (p<0.05)

### Supplementary figure and table legends:

**Table S1:** Relative abundance of significantly distinct fecal microbiota between perinatally HIV-infected children and HIV negative controls at phylum, family, genus and species levels.

**Table S2:** Relative abundance of significantly distinct fecal microbiota across treatment naïve, On ART perinatally HIV-infected children and HIV negative controls at phylum, family, genus and species levels.

**Figure S1:** Plots representing relative abundance of **A.** Proteobacteria **B.** Cyanobacteria **C.** Lentisphaerae **D.** Fusobacteria and **E.** Tenericutes phyla in HIV negative and HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney-U-test was performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S2:** Plots representing relative abundance of **A.** Enterobacteriaceae **B.** Veillonellaceae **C.** Succinivibrionaceae **D.** Paraprevotellaceae **E.** Erysipelotrichaceae **F.** Acaligenaceae and **G.** Bifidobacteriaceae families in HIV negative and HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney-U-test was performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S3:** Plots representing relative abundance of **A.** *Succinivibrio* **B.** *Sutterella* **C.** *Bifidobacterium* **D.** *Dialister* **E.** *Lachnospira* and **F.** *Roseburia* genera in HIV negative and HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney-U-test was performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S4:** Plots representing relative abundance of **A.** *Bacteroides plebeius* **C.** *Faecalibacterium prausnitzii* **E.** *Bacteroides uniformis* and **G.** *Haemophilus parainfluenzae* species in HIV negative and HIV positive children. Plots representing relative abundance of **B.** *Bacteroides plebeius* **D.** *Faecalibacterium prausnitzii* **F.** *Bacteroides uniformis* and **H.** *Haemophilus parainfluenzae* species across HIV negative controls, On ART and treatment naive HIV positive children. The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney *U*-test and Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison were performed for statistical analysis.  $p < 0.05$  was considered to be significant.

**Figure S5:** Plot showing the ratio of the relative abundance of *Bacteroides* to *Prevotella* **A.** between HIV negative and HIV positive children **B.** across HIV negative controls, On ART and treatment naive HIV positive children. Dotted line shows a ratio of 1.0 (relative abundance of *Bacteroides* = relative abundance of *Prevotella*). The horizontal line and deviation in the dot plot represents median and interquartile range (IQR), respectively. Mann-Whitney *U*-test and Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison were performed for statistical analysis