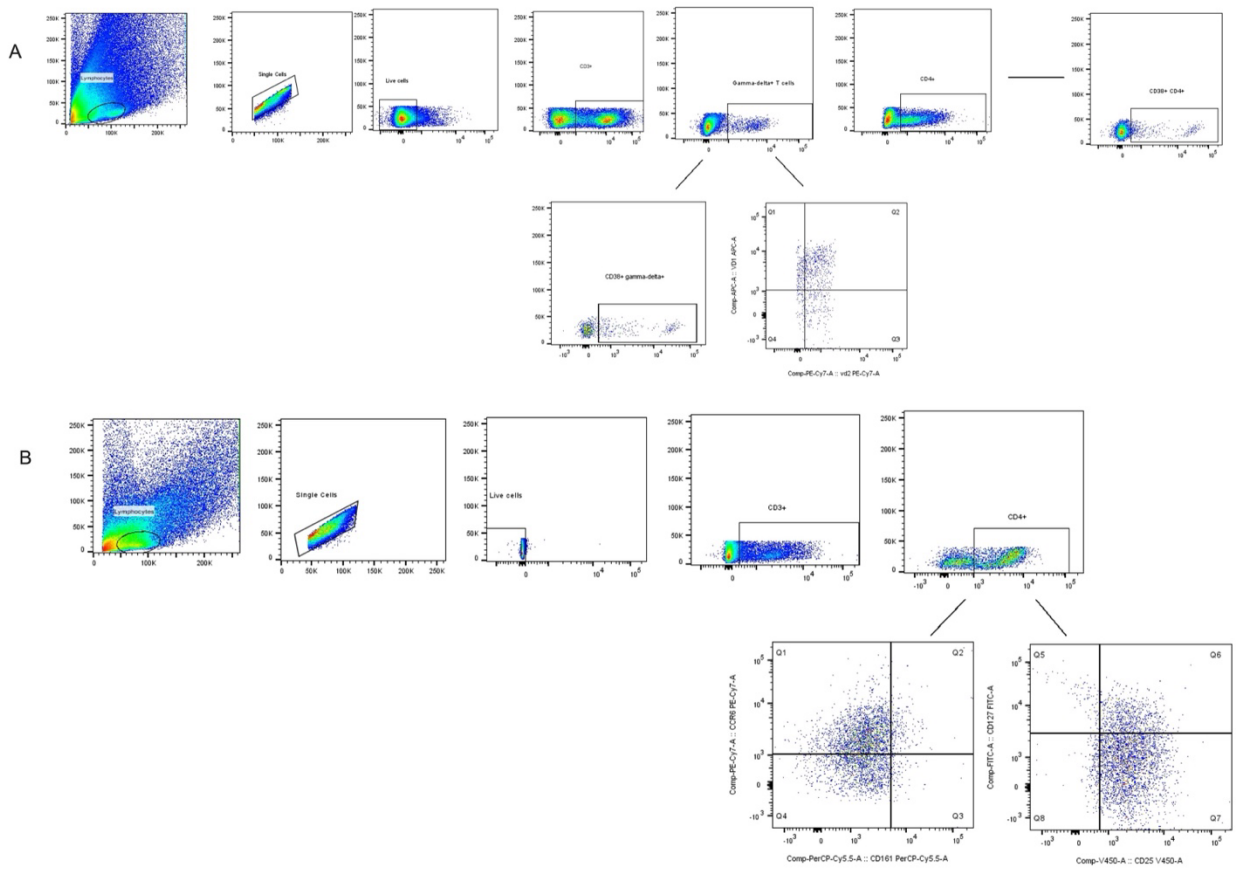
PATHOGENS AND IMMUNITY
IN ASSOCIATION WITH CASE WESTERN RESERVE UNIVERSITY AND THE RICHARD J. FASENMYER FOUNDATION

## Supplementary Figures and Table




**Supplementary Figure 1. Representative plots to illustrate the gating strategy.**
Lymphocytes, single cells, live cells, CD3+ and CD4+ T-cells are identified. Th17 (CCR6+ CD161+) and Treg (CD25+ CD127-) cells are identified in the CD4+ T cells and are expressed within the CD3+ T cells (A). Lymphocytes, single cells, live cells, CD3+, TCR-γδ, activated γδ T cells (CD38+ TCR-γδ+), CD4+, activated CD4+ T cells (CD38+ CD4+), Vδ1 and Vδ2 cells are identified. Vδ1 and Vδ2 cells are identified in the γδ T cells (B).

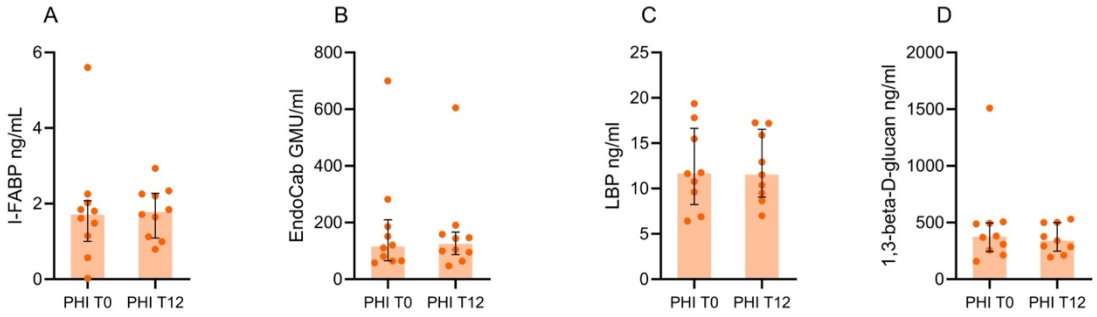
Tincati C, BonoV, Nozza S, Bandera A, Tosi D, Sala V, Ancona G, Calcagno A, Muscatello A, Rusconi S, Augello M, Rovito R, Gianelli U, Pescia C, Santoro A, Falleni M, Gori A, Marchetti G. Three-Month cART Initiated During Primary HIV Does Not Correct the Structural, Immune and Microbial Abnormalities within the Gastrointestinal Tract. *Pathogens and Immunity*. 2025;10(2): 263-285. doi: 10.20411/pai.v10i2.864

PATHOGENS AND IMMUNITY
IN ASSOCIATION WITH CASE WESTERN RESERVE UNIVERSITY AND THE RICHARD J. FASENMYER FOUNDATION


**Supplementary Figure 2. Distribution of monocytic/macrophagic populations along the glandular axis according to study groups.** CD14 (A); CD68 (B); CD163 (C). Data are presented as mean percentage values.

Tincati C, BonoV, Nozza S, Bandera A, Tosi D, Sala V, Ancona G, Calcagno A, Muscatello A, Rusconi S, Augello M, Rovito R, Gianelli U, Pescia C, Santoro A, Falleni M, Gori A, Marchetti G. Three-Month cART Initiated During Primary HIV Does Not Correct the Structural, Immune and Microbial Abnormalities within the Gastrointestinal Tract. *Pathogens and Immunity*. 2025;10(2): 263-285. doi: 10.20411/pai.v10i2.864

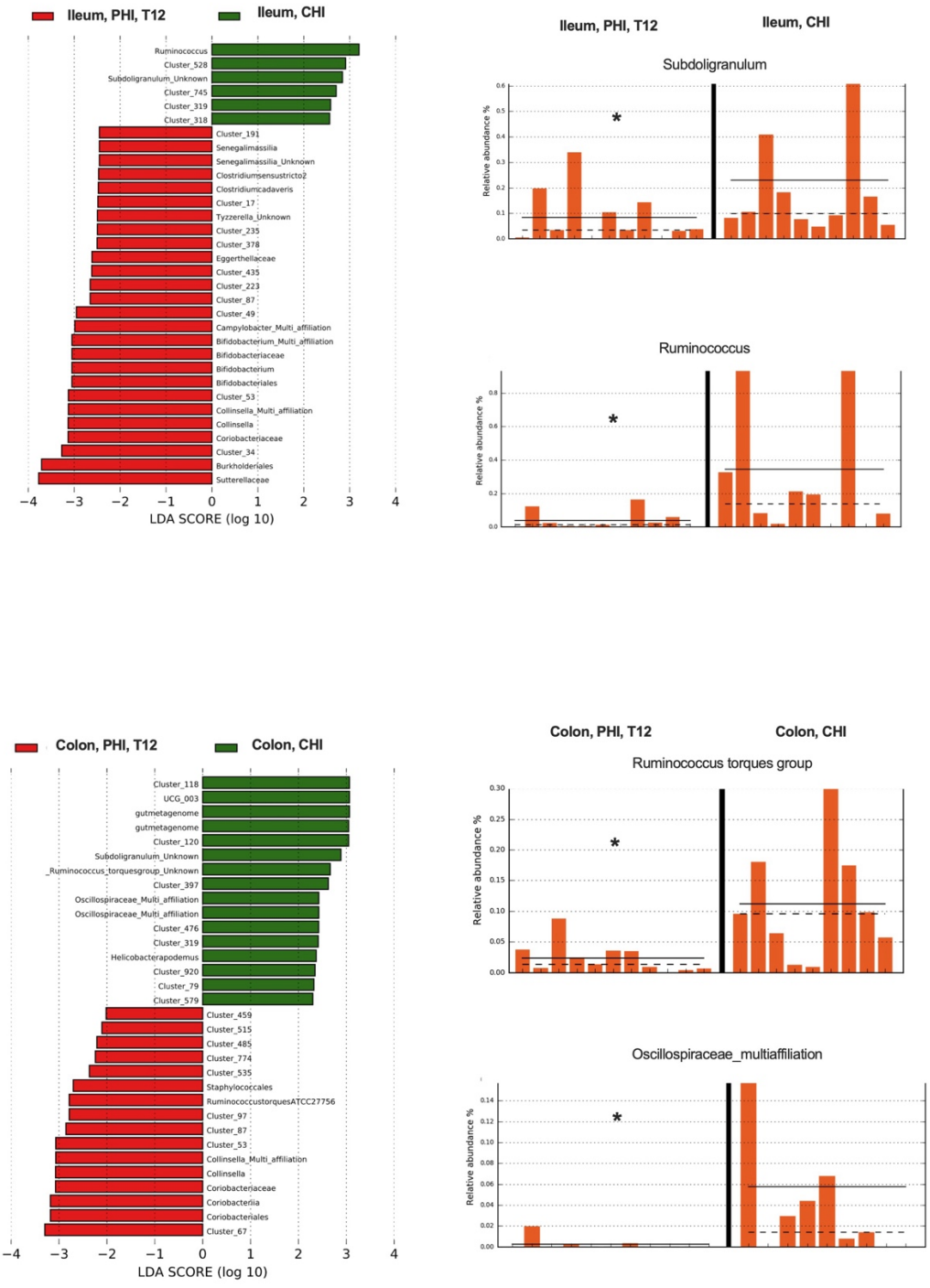
PATHOGENS AND IMMUNITY
IN ASSOCIATION WITH CASE WESTERN RESERVE UNIVERSITY AND THE RICHARD J. FASENMYER FOUNDATION




**Supplementary Figure 3. Additional markers of gut barrier damage and microbial translocation in cART- treated PHI.** I-FABP (A), EndoCab (B), LBP (3) and 1,3-beta-D-glucan (4) levels remained stable at 12 weeks from cART introduction in individuals with primary HIV infection.

Tincati C, BonoV, Nozza S, Bandera A, Tosi D, Sala V, Ancona G, Calcagno A, Muscatello A, Rusconi S, Augello M, Rovito R, Gianelli U, Pescia C, Santoro A, Falleni M, Gori A, Marchetti G. Three-Month cART Initiated During Primary HIV Does Not Correct the Structural, Immune and Microbial Abnormalities within the Gastrointestinal Tract. *Pathogens and Immunity*. 2025;10(2): 263-285. doi: 10.20411/pai.v10i2.864

PATHOGENS AND IMMUNITY
IN ASSOCIATION WITH CASE WESTERN RESERVE UNIVERSITY AND THE RICHARD J. FASENMYER FOUNDATION




**Supplementary Figure 4. Mucosal microbiome composition in PHI at T12 and CHI.** Linear discriminant analysis Effect Size (LEfSe)-analysis and representative plots of the taxonomic differences between groups are shown.

Tincati C, BonoV, Nozza S, Bandera A, Tosi D, Sala V, Ancona G, Calcagno A, Muscatello A, Rusconi S, Augello M, Rovito R, Gianelli U, Pescia C, Santoro A, Falleni M, Gori A, Marchetti G. Three-Month cART Initiated During Primary HIV Does Not Correct the Structural, Immune and Microbial Abnormalities within the Gastrointestinal Tract. *Pathogens and Immunity*. 2025;10(2): 263-285. doi: 10.20411/pai.v10i2.864

PATHOGENS AND IMMUNITY
In Association with Case Western Reserve University and The Richard J. Fasenmyer Foundation

**Supplementary Table 1. Pathological Data**

**A. Enterocyte CDH1 Expression by Immunohistochemistry in Gut Biopsies**

| PHI, T0 | PHI, T12 | CHI | Uninfected controls |
|---|---|---|---|
| 1 | 1 | 3 | 1 |
| 1 | 3 | 2 | 1 |
| 2 | 2 | 3 | 1 |
| 2 | 3 | 4 | 1 |
| 2 | 2 | 2 | 1 |
| 3 | 3 | 3 | |
| 2 | 2 | 4 | |
| 2 | 3 | 4 | |
| 1 | 3 | 2 | |
| 3 | 2 | 3 | |
| 3 | 2 | | |

Legend: 1 indicates complete expression; 2 indicates baso-lateral expression; 3 indicates lateral expression; 4 indicates basal expression. Each row represents a study participant.

**B. Masson's Trichrome Staining of Gut Biopsies**

| PHI, T0 | PHI, T12 | CHI | Uninfected controls |
|---|---|---|---|
| 3 | 21 | 26 | 7 |
| 5 | 19 | 32 | 6 |
| 5 | 16 | 29 | 7 |
| 5 | 20 | 28 | 2 |
| 4 | 16 | 28 | 2 |
| 4 | 15 | 27 | |
| 4 | 17 | 30 | |
| 3 | 17 | 27 | |
| 4 | 18 | 29 | |
| 5 | 20 | 28 | |
| 4 | 21 | | |

Legend: data are presented as % of fibrotic area. Each row represents a patient.

Tincati C, BonoV, Nozza S, Bandera A, Tosi D, Sala V, Ancona G, Calcagno A, Muscatello A, Rusconi S, Augello M, Rovito R, Gianelli U, Pescia C, Santoro A, Falleni M, Gori A, Marchetti G. Three-Month cART Initiated During Primary HIV Does Not Correct the Structural, Immune and Microbial Abnormalities within the Gastrointestinal Tract. *Pathogens and Immunity*. 2025;10(2): 263-285. doi: 10.20411/pai.v10i2.864

PATHOGENS AND IMMUNITY
IN ASSOCIATION WITH CASE WESTERN RESERVE UNIVERSITY AND THE RICHARD J. FASENMYER FOUNDATION

## C. Quantification of CD14$^+$, CD68$^+$, and CD163$^+$ Cells by Immunohistochemistry in Gut Biopsies

| PHI, T0 | | | | | | | | | PHI, T12 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CD14 | | | CD68 | | | CD163 | | | CD14 | | | CD68 | | | CD163 | | |
| Upper | Middle | Lower | Upper | Middle | Lower | Upper | Middle | Lower | Upper | Middle | Lower | Upper | Middle | Lower | Upper | Middle | Lower |
| 28 | 57 | 43 | 53 | 28 | 27 | 57 | 36 | 41 | 55 | 58 | 55 | 44 | 30 | 23 | 59 | 45 | 47 |
| 31 | 58 | 44 | 60 | 35 | 32 | 65 | 54 | 43 | 57 | 64 | 58 | 52 | 27 | 32 | 63 | 44 | 48 |
| 33 | 61 | 48 | 46 | 32 | 26 | 68 | 37 | 51 | 59 | 64 | 60 | 48 | 25 | 28 | 71 | 42 | 48 |
| 28 | 59 | 47 | 51 | 37 | 32 | 68 | 44 | 42 | 58 | 63 | 57 | 50 | 28 | 29 | 65 | 32 | 39 |
| 31 | 59 | 51 | 55 | 28 | 31 | 71 | 51 | 46 | 60 | 62 | 59 | 51 | 28 | 30 | 66 | 38 | 47 |
| 25 | 57 | 46 | 52 | 29 | 29 | 72 | 39 | 52 | 56 | 60 | 58 | 46 | 26 | 27 | 62 | 44 | 51 |
| 34 | 62 | 45 | 52 | 31 | 28 | 69 | 49 | 50 | 61 | 64 | 60 | 48 | 28 | 29 | 68 | 41 | 46 |
| 33 | 61 | 45 | 49 | 31 | 30 | 69 | 43 | 48 | 60 | 63 | 62 | 47 | 26 | 25 | 65 | 43 | 42 |
| 34 | 63 | 49 | 61 | 34 | 26 | 66 | 39 | 44 | 59 | 66 | 57 | 49 | 24 | 29 | 68 | 45 | 50 |
| 30 | 57 | 43 | 54 | 29 | 27 | 67 | 42 | 46 | 59 | 65 | 56 | 50 | 28 | 27 | 62 | 39 | 48 |
| 28 | 61 | 42 | 50 | 33 | 25 | 64 | 45 | 48 | 56 | 59 | 51 | 47 | 26 | 26 | 63 | 43 | 48 |

| CHI | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CD14 | | | CD68 | | | CD163 | | |
| Upper | Middle | Lower | Upper | Middle | Lower | Upper | Middle | Lower |
| 53 | 55 | 55 | 43 | 20 | 20 | 55 | 38 | 29 |
| 32 | 44 | 39 | 41 | 20 | 19 | 52 | 34 | 28 |
| 38 | 42 | 39 | 48 | 20 | 19 | 53 | 41 | 37 |
| 44 | 41 | 42 | 57 | 26 | 22 | 54 | 43 | 37 |
| 42 | 46 | 43 | 55 | 25 | 21 | 55 | 36 | 33 |
| 30 | 33 | 38 | 56 | 24 | 22 | 49 | 35 | 31 |
| 38 | 39 | 41 | 53 | 22 | 20 | 53 | 40 | 31 |
| 41 | 43 | 40 | 55 | 25 | 23 | 56 | 42 | 30 |
| 36 | 45 | 44 | 54 | 26 | 23 | 56 | 37 | 36 |
| 43 | 47 | 45 | 56 | 24 | 22 | 52 | 36 | 35 |

Tincati C, BonoV, Nozza S, Bandera A, Tosi D, Sala V, Ancona G, Calcagno A, Muscatello A, Rusconi S, Augello M, Rovito R, Gianelli U, Pescia C, Santoro A, Falleni M, Gori A, Marchetti G. Three-Month cART Initiated During Primary HIV Does Not Correct the Structural, Immune and Microbial Abnormalities within the Gastrointestinal Tract. *Pathogens and Immunity*. 2025;10(2): 263-285. doi: 10.20411/pai.v10i2.864

PATHOGENS AND IMMUNITY
IN ASSOCIATION WITH CASE WESTERN RESERVE UNIVERSITY AND THE RICHARD J. FASENMYER FOUNDATION

| Uninfected controls | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CD14 | | | CD68 | | | CD163 | | |
| Upper | Middle | Lower | Upper | Middle | Lower | Upper | Middle | Lower |
| 34 | 38 | 29 | 38 | 20 | 19 | 56 | 30 | 30 |
| 33 | 45 | 36 | 39 | 18 | 23 | 50 | 23 | 20 |
| 36 | 36 | 42 | 41 | 17 | 21 | 45 | 28 | 25 |
| 35 | 37 | 35 | 39 | 16 | 17 | 51 | 29 | 24 |
| 36 | 42 | 37 | 40 | 16 | 23 | 49 | 27 | 24 |

Legend: data represent counts (%) of monocyte/macrophage lineage cells in tissue section according to their distribution along the glandular axis. Each row represents a study participant.

Tincati C, BonoV, Nozza S, Bandera A, Tosi D, Sala V, Ancona G, Calcagno A, Muscatello A, Rusconi S, Augello M, Rovito R, Gianelli U, Pescia C, Santoro A, Falleni M, Gori A, Marchetti G. Three-Month cART Initiated During Primary HIV Does Not Correct the Structural, Immune and Microbial Abnormalities within the Gastrointestinal Tract. *Pathogens and Immunity*. 2025;10(2): 263-285. doi: 10.20411/pai.v10i2.864