Delayed GALT Reconstitution in Duodenum Compared to Rectum in HIV-infected Patients Initiating Antiretroviral Therapy

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SUPPLEMENTAL MATERIALS

TABLE S1. T-cell subsets across compartments and timepoints.

		HIV$^-$ CONTROLS	HIV+ subjects		P value		
			Month 0	Month 9	HIV- vs. HIV+ Month 0	HIV- vs. HIV+ Month 9	Month 0 vs. 9 in HIV+
CD4+ T cells/mm^3	**blood**	-	436 (283-572)	693 (452-848)	-	-	**<0.001**
CD4+ T cells/mm^2	**rectum**	395 (340-478)	51 (29-88)	135 (54-167)	**<0.001**	**<0.001**	**0,013**
CD4+ T cells/mm^2	**duodenum**	566 (509-688)	48 (31-184)	158 (116-254)	**<0.001**	**<0.001**	**0,011**
%CD4+ T cells	**blood**	58.2 (52.7-69.8)	28.3 (21.5-35.5)	40.5 (35.4-36.1)	**<0.001**	**<0.001**	**<0.001**
%CD4+ T cells	**rectum**	63.1 (51.7-67.1)	18.9 (13.4-24.1)	38.7 (34.8-41.3)	**<0.001**	**<0.001**	**<0.001**
%CD4+ T cells	**duodenum**	44.4 (38.4-55.3)	6.3 (4.0-11.6)	17.3 (11.6-27.8)	**<0.001**	**<0.001**	**<0.001**
CD8+ T cells/mm3	**blood**						

CD8+ T cells/mm²	**rectum**	188 (133, 258)	698 (518, 792)	403 (328, 635)	**<0.001**	**<0.001**	**0,0019**
CD8+ T cells/mm²	**duodenum**	543 (360-713)	1345 (1084-1776)	843 (686-955)	**<0.001**	**0,003**	**<0.001**
%CD8+ T cells	**blood**	36.7 (26.6-42.6)	63.4 (56.8-69.1)	52.6 (45.6-58.5)	**<0.001**	**<0.001**	**<0.001**
%CD8+ T cells	**rectum**	30.9 (22.5-32.9)	72.2 (66.0-81.0)	51.4 (44.9-57.4)	**<0.001**	**<0.001**	**<0.001**
%CD8+ T cells	**duodenum**	44.2 (38-54.6)	88.7 (79.6-92.0)	73.0 (66.2-82.4)	**<0.001**	**<0.001**	**<0.001**
CD4/CD8 ratio	**blood**	1.59 (1.23-2.72)	0.43 (0.33-0.60)	0.76 (0.60-1.0)	**<0.001**	**<0.001**	**<0.001**
CD4/CD8 ratio	**rectum**	2.04 (1.57-2.98)	0.26 (0.17-0.35)	0.76 (0.59-0.88)	**<0.001**	**<0.001**	**<0.001**
CD4/CD8 ratio	**duodenum**	0.99 (0.70-1.45)	0.07 (0.04-0.13)	0.22 (0.14-0.42)	**<0.001**	**<0.001**	**<0.001**
CD4+/CCR5+ T cells	**blood**	35.8 (26.2-43.2)	35.5 (21.4-53.6)	25.2 (16-33)	0,89	0,054	**0,006**
CD4+/CCR5+ T cells	**rectum**	95 (92.7-98)	89.8 (81.5-93.8)	85.1 (71.7-91.9)	**0,017**	**0,001**	0,287
CD4+/CCR5+ T cells	**duodenum**	99 (96.7-99.3)	93.4 (88-94.7)	91.9 (83.5-96)	**0,001**	**<0.001**	0,53
CD4+/HLA-DR+/CD38+	**blood**	2.5 (1.9-4.8)	8.0 (5.0-16.8)	4.8 (2.6-19.8)	**<0.001**	**0,028**	**0,003**
CD4+/HLA-DR+/CD38+	**rectum**	19.1 (12.8-29.6)	37.7 (25.7-46.0)	30.1 (21.1-35.5)	**0,002**	0,159	**0,012**
CD4+/HLA-DR+/CD38+	**duodenum**	58.6 (54.8-65.5)	52.2 (44.7-61.6)	38.6 (33.1-57.5)	0,162	**0,025**	**0,029**
CD8+/HLA-DR+/CD38+	**blood**	7.3 (5.4-16)	52.1 (34.9-60.9)	24.0 (14.8-36.8)	**<0.001**	**<0.001**	**<0.001**
CD8+/HLA-DR+/CD38+	**rectum**	43.3 (33.2-48.3)	74.2 (68.6-83.8)	58.3 (41.7-67.3)	**<0.001**	**0,019**	**0,004**
CD8+/HLA-DR+/CD38+	**duodenum**	57.0 (51.9-63.3)	64.0 (55.1-75.1)	58.3 (44.4-66.7)	0,192	0,659	**0,028**

%ϒδ T cells	**blood**	0.9 (0.4-2.4)	3.4 (2.4-5.5)	2.8 (1.7-6.1)	**<0.001**	**0.003**	0.443
%ϒδ T cells	**rectum**	5.4 (2.0-9.5)	4.6 (3.3-8.4)	6.5 (4.9-10.0)	0.803	0.264	0.072
%ϒδ T cells	**duodenum**	3.0 (2.1-3.5)	2.4 (1.4-4.6)	2.8 (1.7-6.0)	0.207	0.888	0.054
%ϒδ low T cells	**blood**	71.5 (0.12-88.1)	44.8 (36-54.4)	43.9 (15.3-56.9)	0.118	0.116	0.499
%ϒδ low T cells	**rectum**	71.9 (36-86.6)	59.7 (23.1-76.3)	60.3 (30.7-79.1)	0.289	0.567	0.314
%ϒδ low T cells	**duodenum**	72.3 (67.3-93.2)	67 (35.8-79.1)	64.1 (48.2-84.6)	0.090	0.451	0.879
%ϒδ high T cells	**blood**	30.5 (12.9-99.6)	55.8 (46.2-64.2)	60.5 (47.7-89.7)	0.118	**0.045**	0.276
%ϒδ high T cells	**rectum**	27.4 (10-67)	42.7 (24.3-78.7)	39.4 (21.7-71.4)	0.162	0.403	0.398
%ϒδ high T cells	**duodenum**	28.6 (7.6-34.4)	30.3 (18.2-59.9)	38.3 (15.7-53.6)	0.402	0.423	0.170
ϒδ high/low ratio T cells	**blood**	0.38 (0.14-0.76)	1.24 (0.84-1.76)	1.25 (0.86-3.37)	**0.005**	**0.003**	0.619
ϒδ high/low ratio T cells	**rectum**	0.36 (0.11-1.86)	0.65 (0.29-2.98)	0.56 (0.16-2.03)	0.272	0.491	0.352
%ϒδ high/low ratio T cells	**duodenum**	0.41 (0.08-0.51)	0.44 (0.27-0.66)	0.56(0.16-1.04)	0.378	0.539	0.149
%T regulatory cells	**blood**	8.6 (6.0-10.7)	9.1 (7.9-11.8)	7.5 (5.2-10.5)	0.725	0.207	**0.041**
%T regulatory	**rectum**	13.0 (10.9-18.8)	19.1 (15.8-24.1)	20.3 (16.5-27.2)	0.052	**0.021**	0.071
%T regulatory	**duodenum**	5.1 (3.8-8.4)	21.6 (11.6-28.9)	16.4 (11.6-25.8)	**<0.001**	**<0.001**	0.544

P values between HIV- and HIV+ groups were calculated using Mann Whitney's U test.

P values between month 0 and month 9 time-points in the HIV+ group were calculated using Wilcoxon signed-rank test.

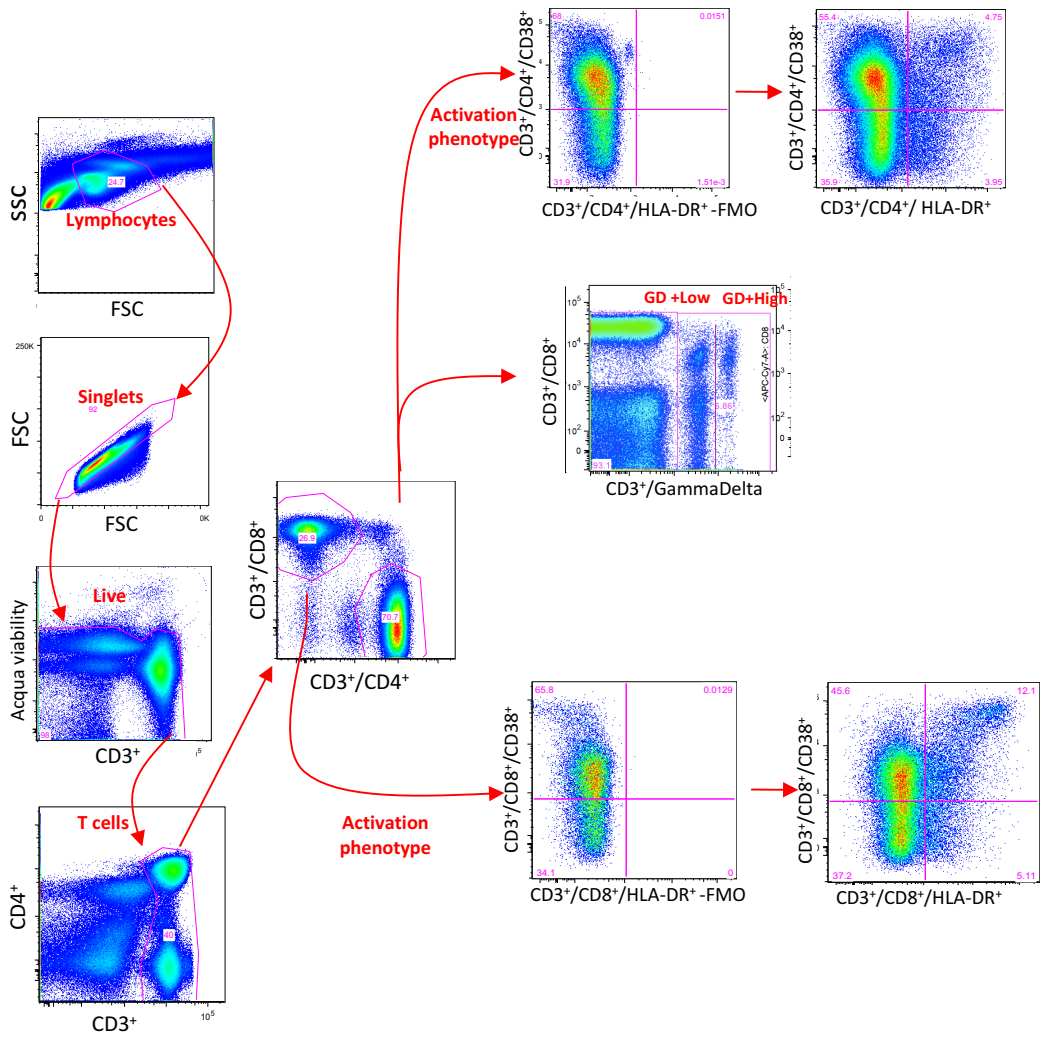

Figure S1. Gating strategy.
The single-cell suspensions were stained with Aqua-viability dye and QuantumDot655 anti-CD45RA (clone MEM-56) from Invitrogen (Carlsbad, California, USA); PacBlue-anti-CD3 (clone UCHT1) and fluorescein isothiocyanate-anti-human leukocyte antigen-DR (clone L243) from Biolegend (San Diego, California, USA); ECD-anti-CD4 (clone SFCI12T4D11) from Beckman- Coulter (Brea, California, USA); and PE-anti-CD38 (clone HB7), and APC-H7-anti-CD8 (clone SK1) from Becton-Dickinson (San Jose, California, USA) according to manufacturers' recommendations. PBMCs were processed with the intestinal cells in identical fashion. Fluorescence-activated cell sorter (FACS) analysis was performed on a custom Becton-Dickinson LSR II flow cytometer used for data acquisition and analyzed with FlowJo (TreeStar, Ashland, Oregon, USA). Gating strategy included using an FMO (fluorescence-minus-one) to determine the cut-off for positive cells for CD38, and HLA-DR for each run. Combinations of markers were calculated in FlowJo, using the Boolean gate function. T-cells with an activation phenotype are defined as co-expression of HLA-DR and CD38 on respective lymphocyte population. Rainbow beads (Spherotec, Lake Forest, Illinois, USA) from a single lot were used to calibrate each photomultiplier tube to a uniform gain to insure stability in signal between samples. An additional control standard peripheral blood sample aliquoted and cryopreserved at the beginning of the clinical trial was run in parallel at the time each sample was analyzed to further assess run to run variability.

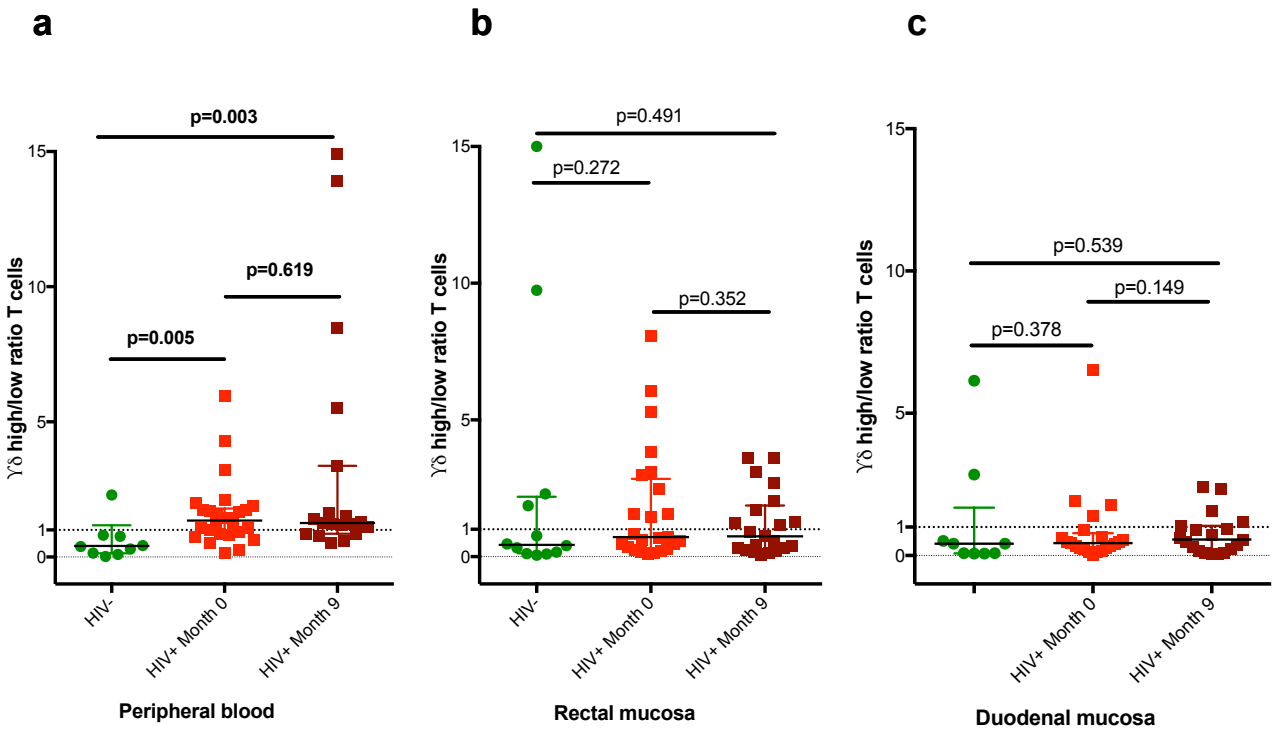

Figure S2. Percentage of γδ T-cells, Vδ1+ (low MFI) and Vδ2+ (high MFI) subpopulations. P values between HIV- and HIV+ groups were calculated using the Mann Whitney's U test. P values between month 0 and month 9 time-points in the HIV+ group were calculated using the Wilcoxon signed-rank test.

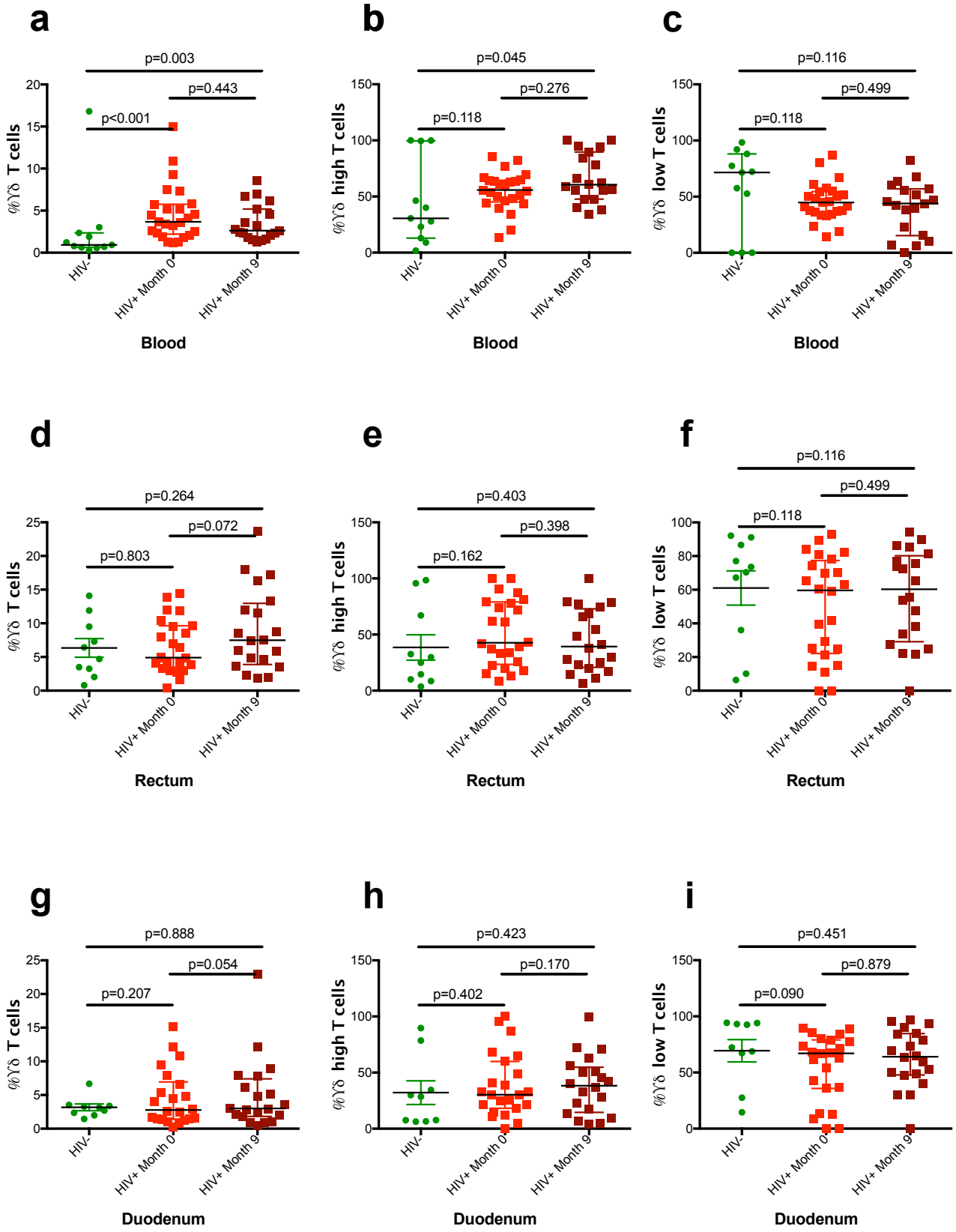

Figure S3. Vδ1+ (low MFI)/Vδ2+ (high MFI) ratio subpopulations across compartments. P values between HIV- and HIV+ groups were calculated using the Mann Whitney's U test. P values between month 0 and month 9 time-points in the HIV+ group were calculated using the Wilcoxon signed-rank test.

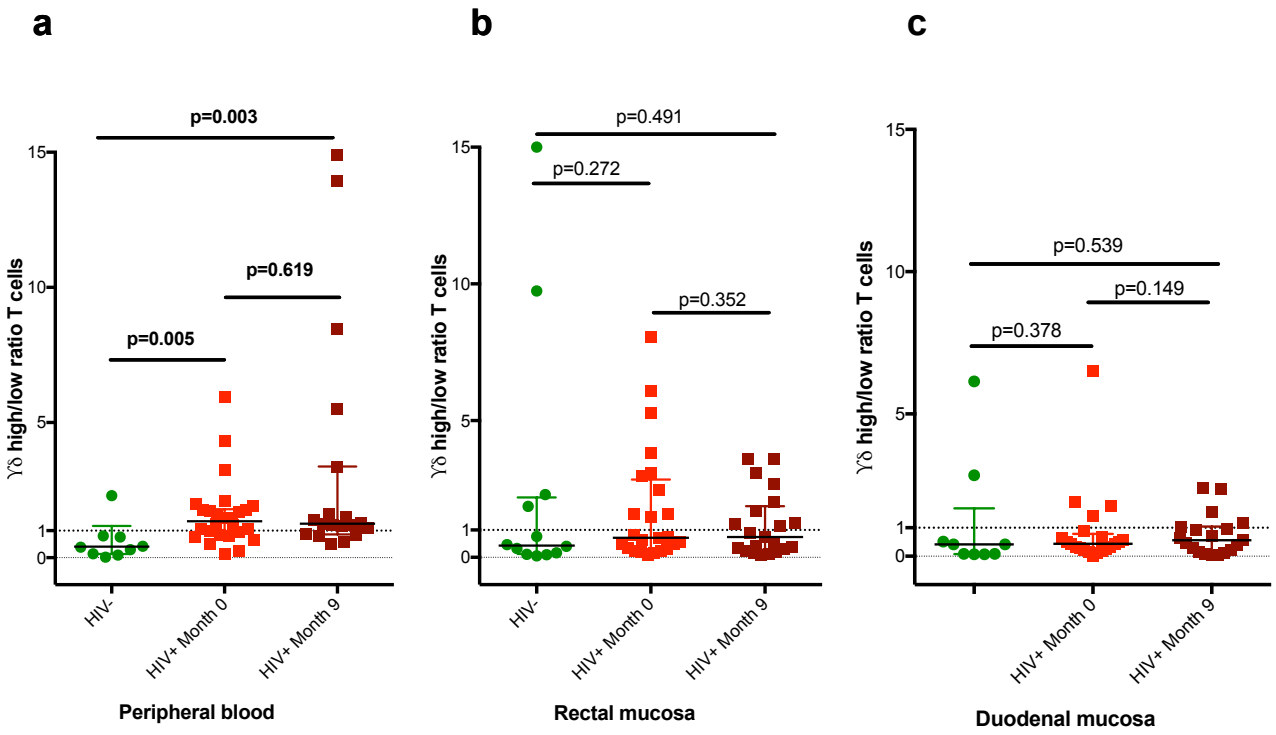

Figure S4. Vδ1+ (low MFI)/Vδ2+ (high MFI) ratio subpopulations across compartments. P values between HIV- and HIV+ groups were calculated using the Mann Whitney's U test. P values between month 0 and month 9 time-points in the HIV+ group were calculated using the Wilcoxon signed-rank test.