

Gut and Inflammation

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HIV, the gut & inflammation: (old) partners in crime

“my colleagues and I hypothesized that clinical symptoms and intestinal injury are directly related to the presence of HIV in the mucosa and that the intestinal lamina propria could be a site of accelerated infection and destruction of CD4 lymphocytes”

Conclusion

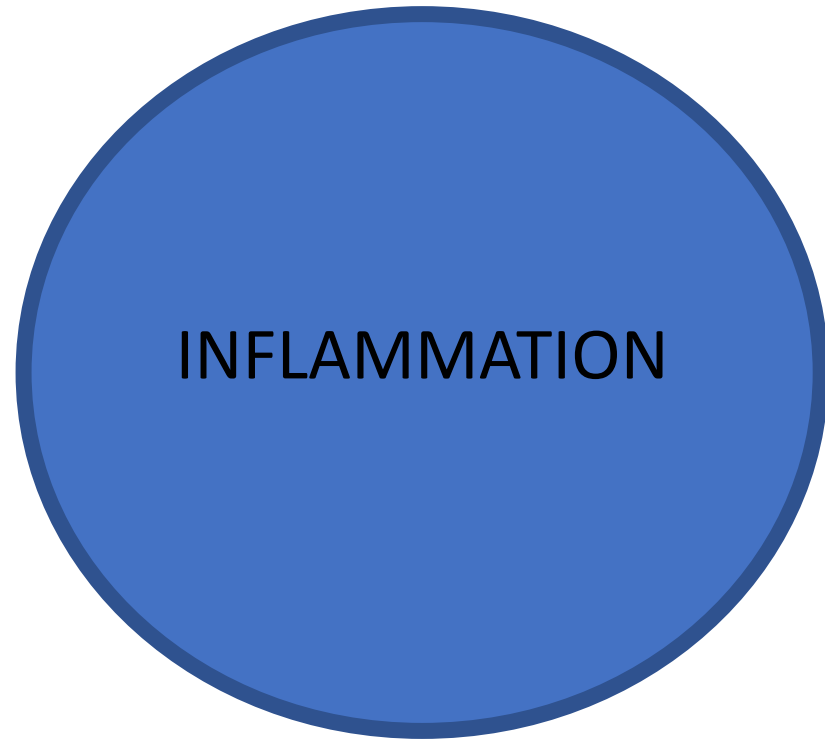
Further studies are required to define the precise mechanism for HIV-associated intestinal injury and its relationship to HIV replication. Evidence from this study and others [14] suggests

Kotler JID 1999;179 (suppl 3)

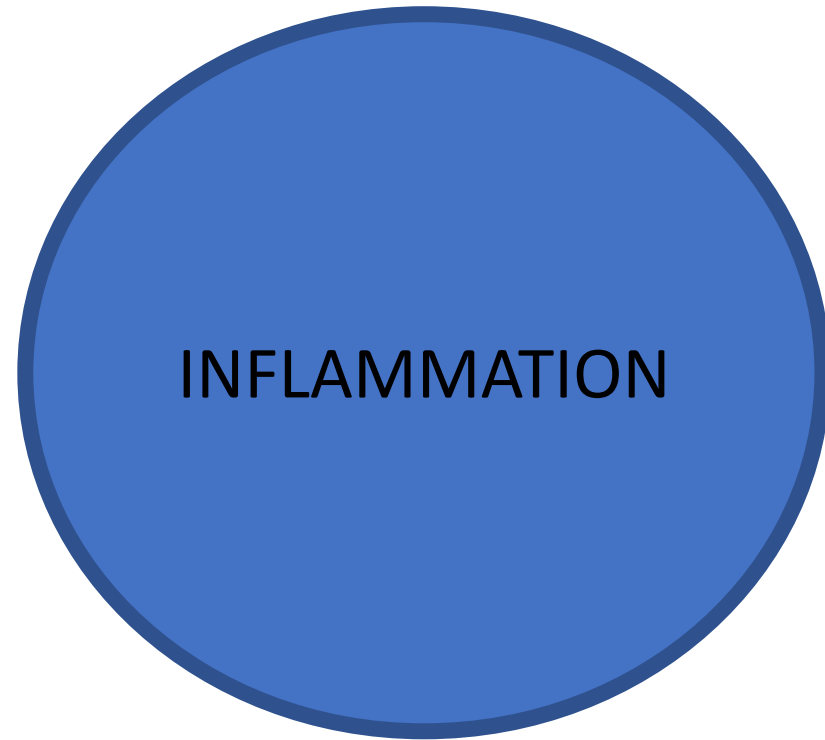
Subpopulation	CD8 ⁺ T Cell Subset	HIV ⁻ Controls (n = 10)	Asymptomatic HIV ⁺ (n = 8)	AIDS (n = 15)
1	DR ⁻ CD38 ⁻	434 ± 155	277 ± 130 (0.04)	175 ± 205 (0.003, NS)
2	DR ⁺ CD38 ⁻	34 ± 25	223 ± 115 (0.002)	93 ± 83 (0.02, 0.005)
3	DR ⁺ CD38 ⁺	7 ± 3	144 ± 132 (0.02)	253 ± 178 (0.0001, NS)
4	DR ⁻ CD38 ⁺	54 ± 26	71 ± 53 (NS)	178 ± 56 (0.0001, 0.0002)

Giorgi et al. J Immunol 1993

HIV: INFLAMMATION AND GUT DAMAGE



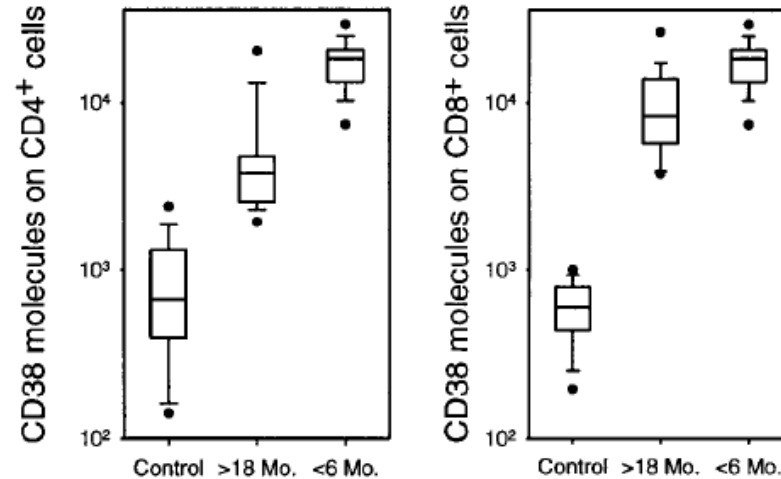
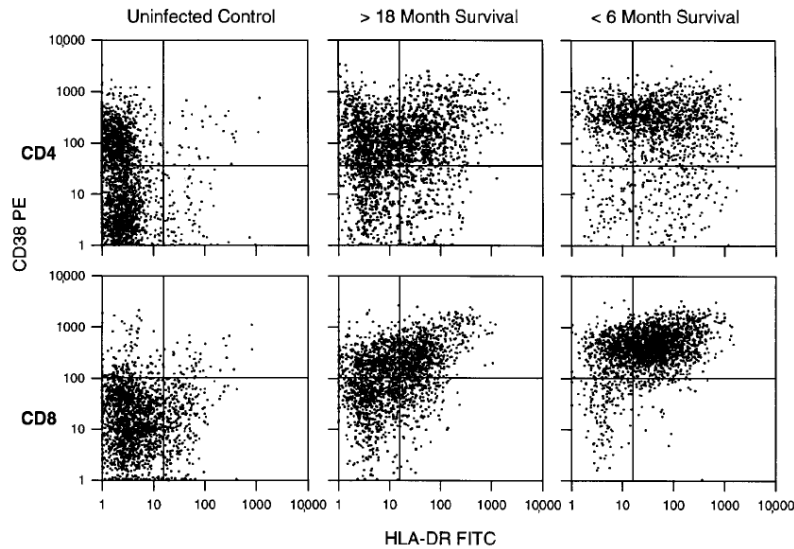
HIV: INFLAMMATION AND GUT DAMAGE



Parameter	Estimate	Standard error	<i>P</i>
Univariate model			
Plasma HIV RNA level, log ₁₀	−0.032	0.007	< .001
CD8 ⁺ T-cell activation, log ₁₀	−0.049	0.014	< .001
CD4 ⁺ T-cell activation, log ₁₀	−0.039	0.017	.021
Multivariate model			
Intercept	2.921	0.042	< .001
Plasma HIV RNA level, log ₁₀	−0.026	0.009	.005
CD8 ⁺ T-cell activation, log ₁₀	−0.033	0.015	.027
CD4 ⁺ T-cell activation, log ₁₀	−0.013	0.019	.474

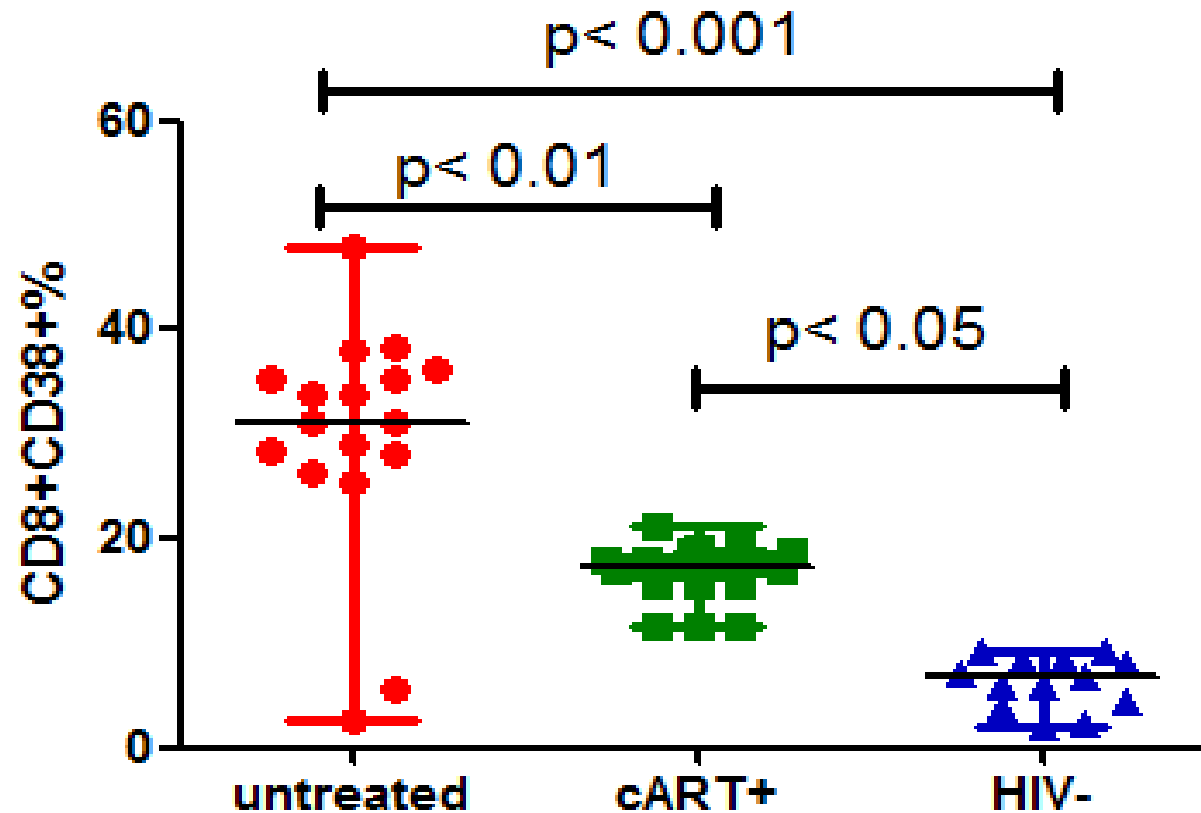
Untreated HIV:
T-cell activation
predicts CD4+
loss and survival

Deeks et al. Blood 2004

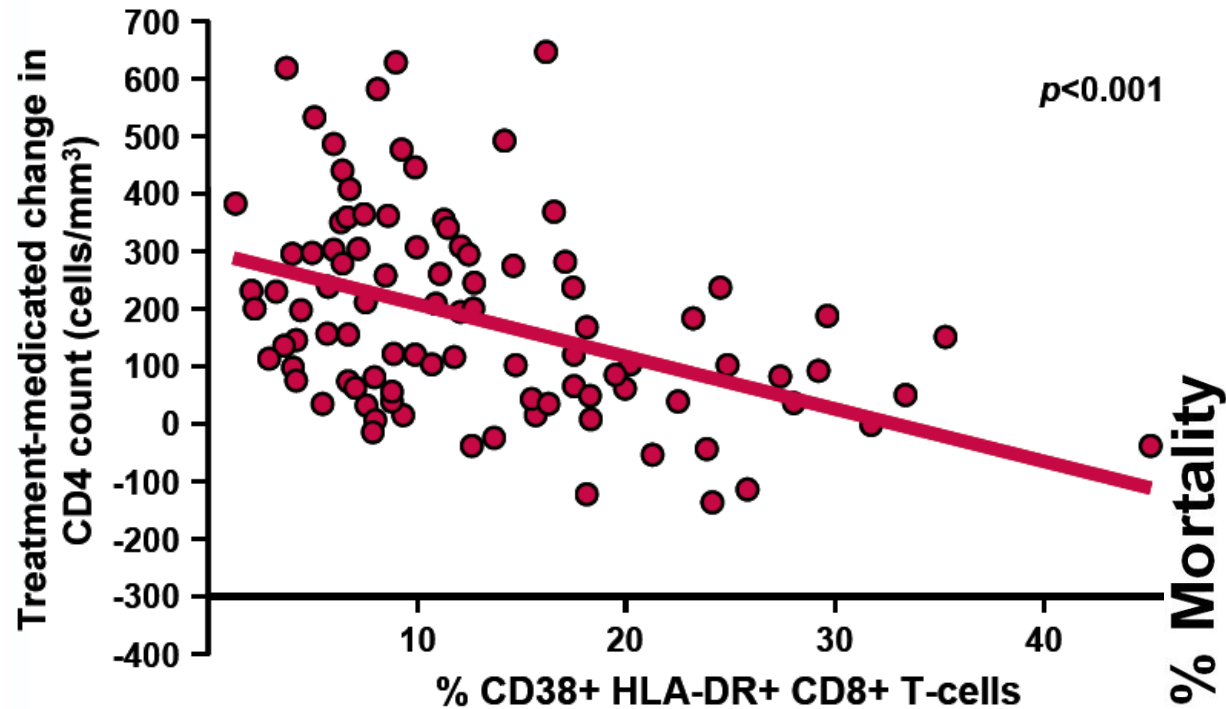


Giorgi, J et al. JID 1999

Immune (hyper)activation persists upon suppressive cART

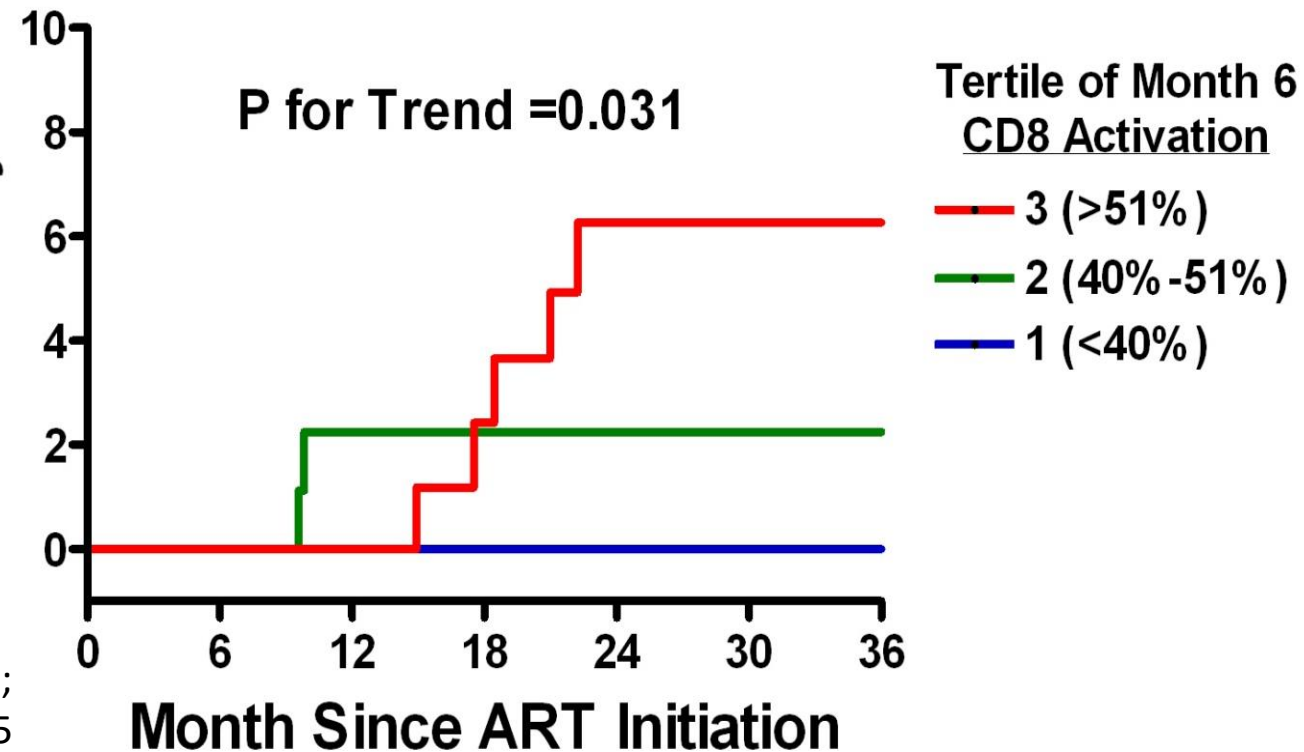


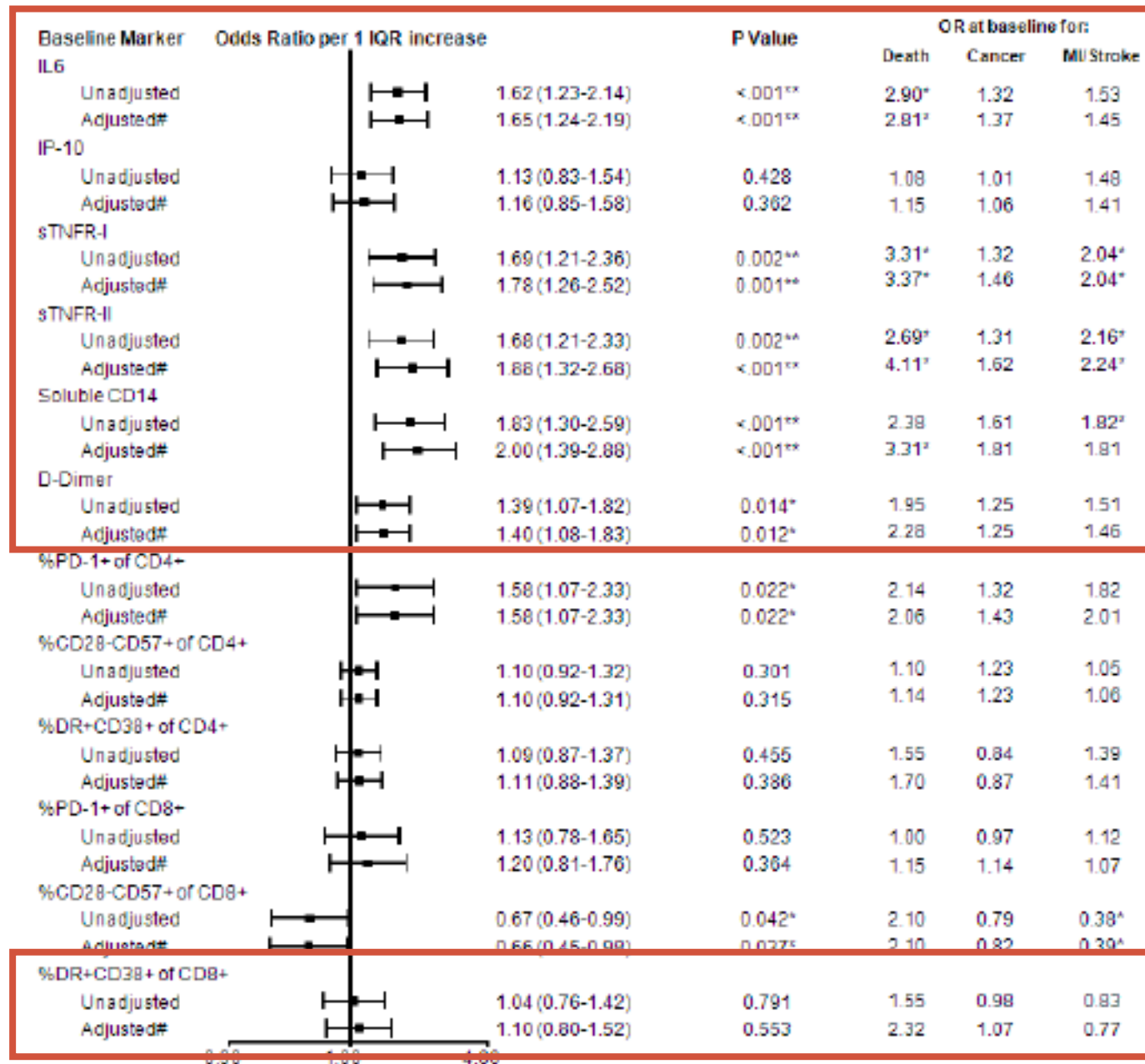
Immune activation affects immune reconstitution and disease progression on cART



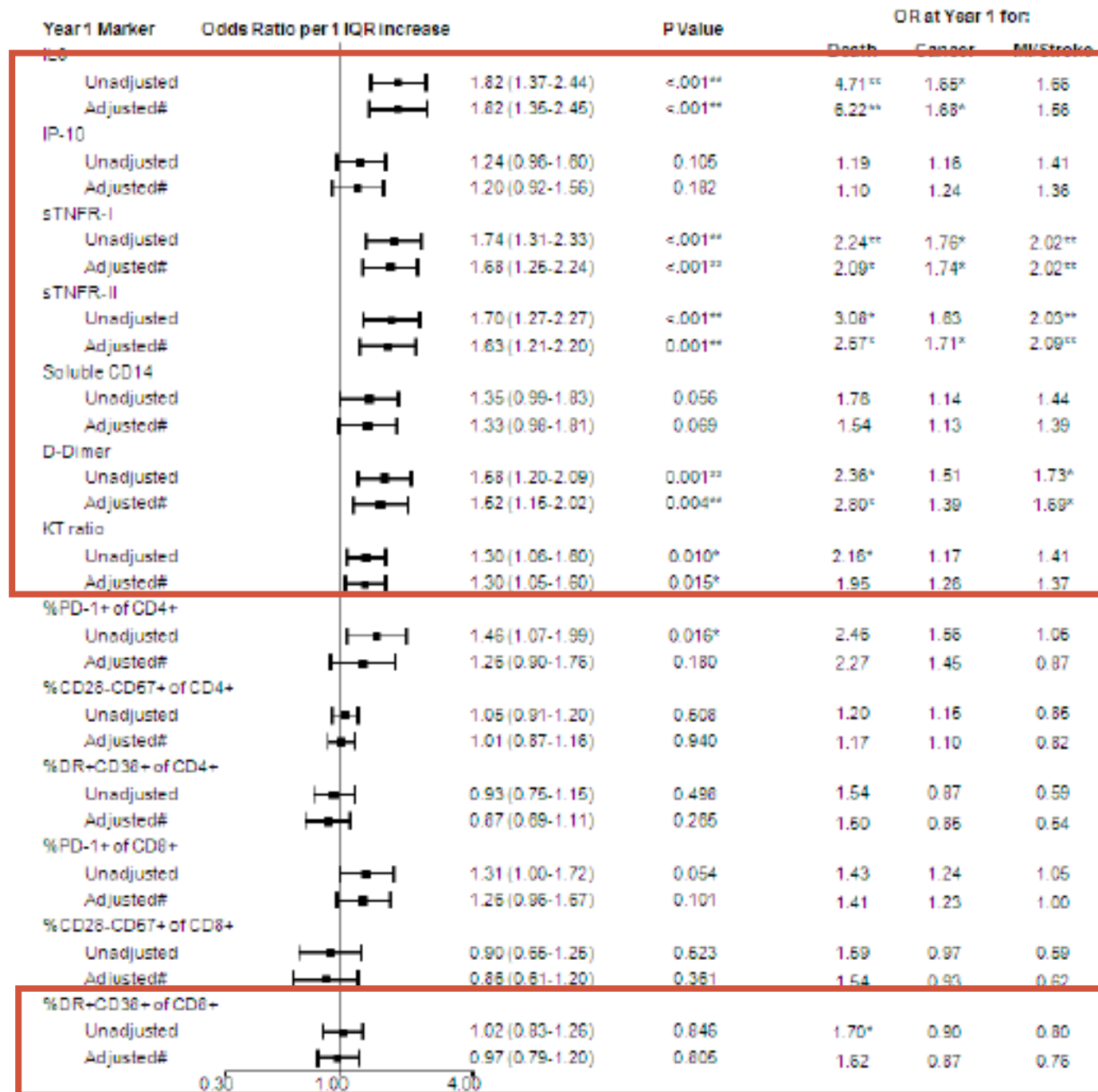
Hunt et al. JID 200

Hunt et al, AIDS 2011 25:2123;
also: Balagopal JAIDS 2015



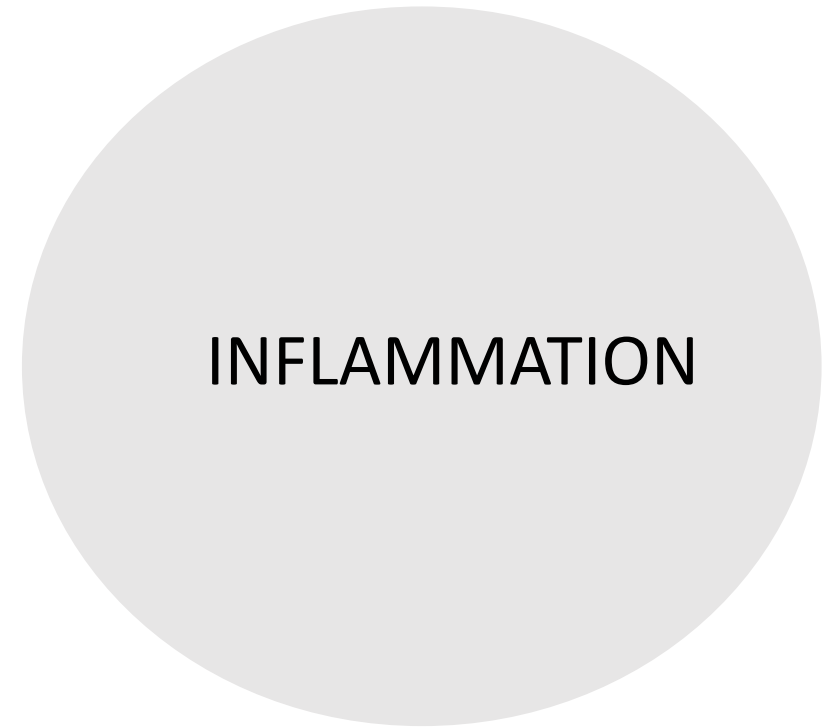


**Odds ratio of
non-AIDS
events
according to
baseline
marker**



**Odds ratio of
non-AIDS
events
according to
marker at
year 1 of
cART**

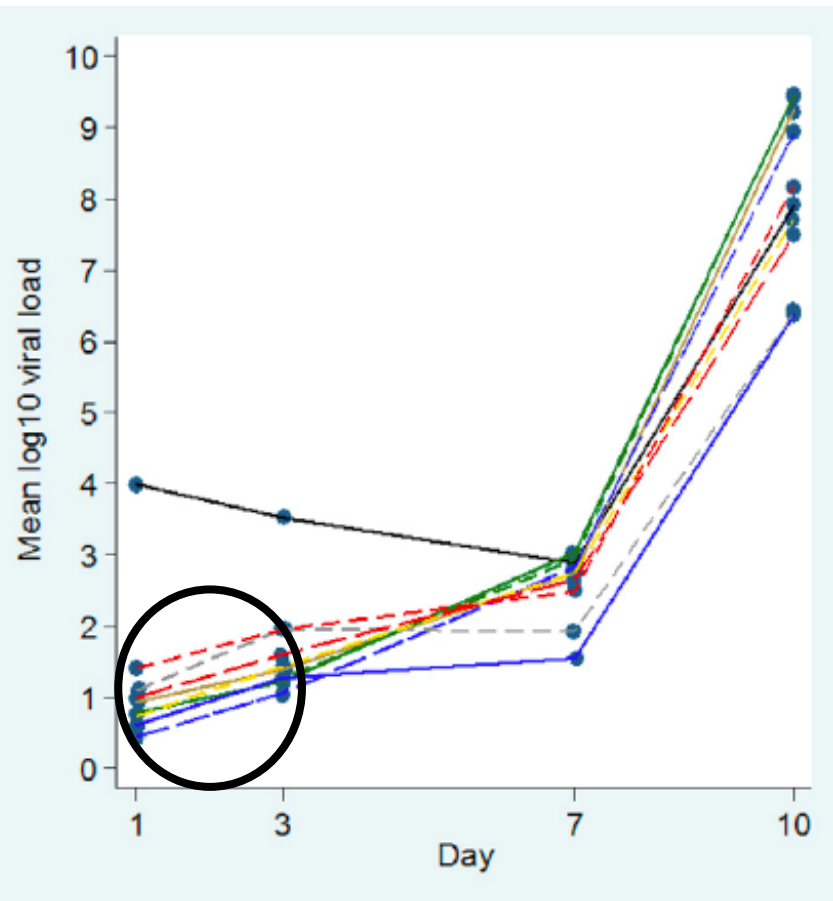
HIV: INFLAMMATION AND GUT DAMAGE



The gut is ostensibly susceptible to HIV (and SIV) infection

- First site of contact with virus
- Size: GALT comprises most (60%) of the secondary lymphoid organ system; 40-70% of GALT lymphocytes express CCR5
- Dense cell clustering in gut mucosa facilitating cell-cell virus transmission
- The majority of gut-residing lymphocytes are recently activated memory; state of “physiologic” inflammation

The GALT & HIV: one of the first sites infiltrated during acute HIV infection



Serial necropsies in 44 rhesus monkeys following intravaginal SIVmac251

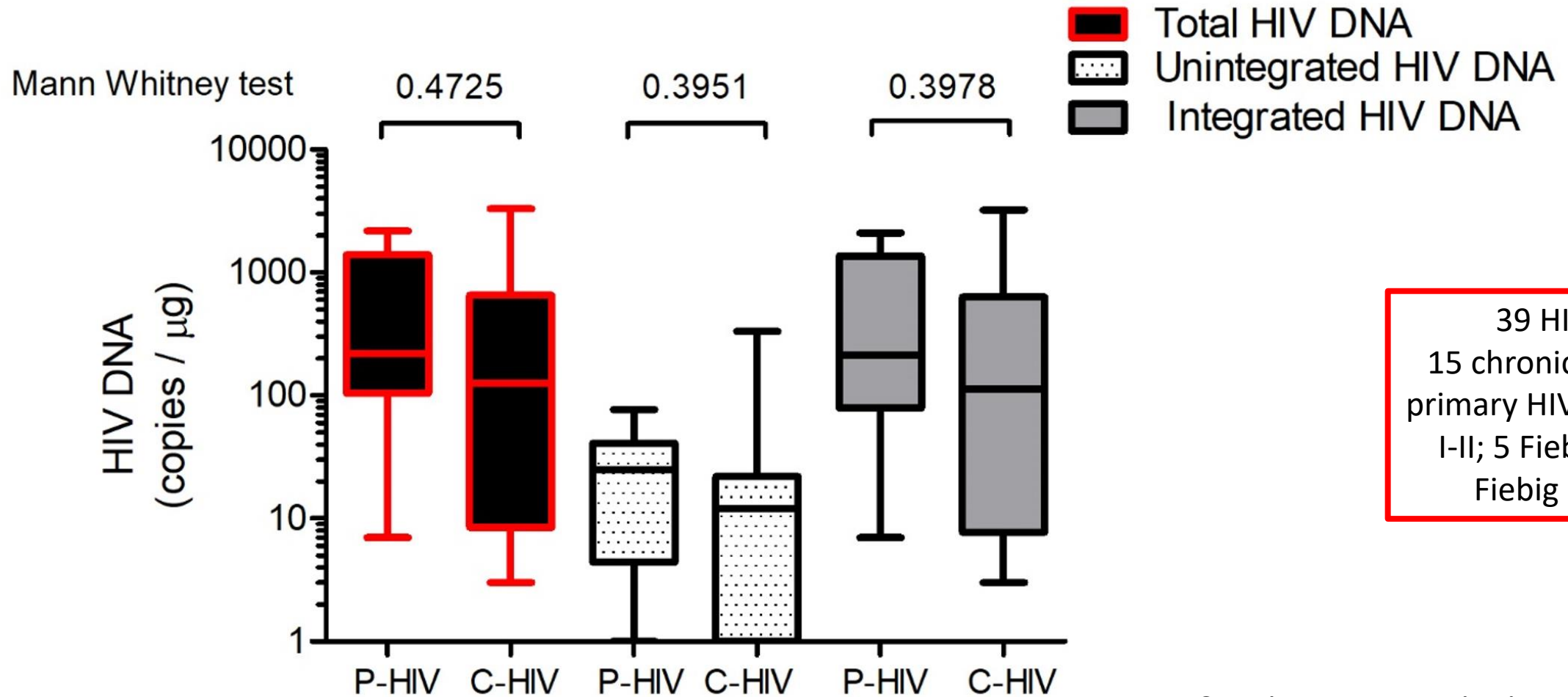
- Repr. tract
- GI Tract
- LNs-draining
- - LNs-other
- - Spleen
- Bone marrow
- - CNS
- - Thymus
- - Lungs
- - Liver

Viral dissemination within the gut occurs as early as during the eclipse phase of infection

Tissue Group	Linear Mixed Model (Tissue VL)					Fisher's Exact Test (% Positive)				
	Day 1 vs. Naïve	Day 3 vs. Naïve	Day 7 vs. Naïve	Day 10 vs. Naïve	AT-2 vs. Naïve	Day 1 vs. Naïve	Day 3 vs. Naïve	Day 7 vs. Naïve	Day 10 vs. Naïve	AT-2 vs. Naïve
Repr. tract	0.006	0.013	0.115	0.000	0.317	0.000	0.000	0.000	0.000	0.000
GI Tract	0.721	0.808	0.025	0.000	0.923	0.006	0.050	0.000	0.000	0.234
LNs-draining	0.989	0.568	0.002	0.000	0.931	1.000	0.130	0.003	0.000	0.238
LNs-other	0.741	0.596	0.003	0.000	0.775	0.444	1.000	0.000	0.000	1.000
Spleen	0.690	0.761	0.011	0.000	0.980	1.000	0.228	0.103	0.029	1.000
Bone marrow	0.605	0.816	0.555	0.000	0.671	1.000	1.000	0.517	0.029	1.000
Lungs	0.725	0.782	0.339	0.000	0.886	1.000	1.000	0.517	0.029	1.000
Liver	0.678	0.776	0.107	0.000	0.950	1.000	0.497	0.103	0.029	1.000

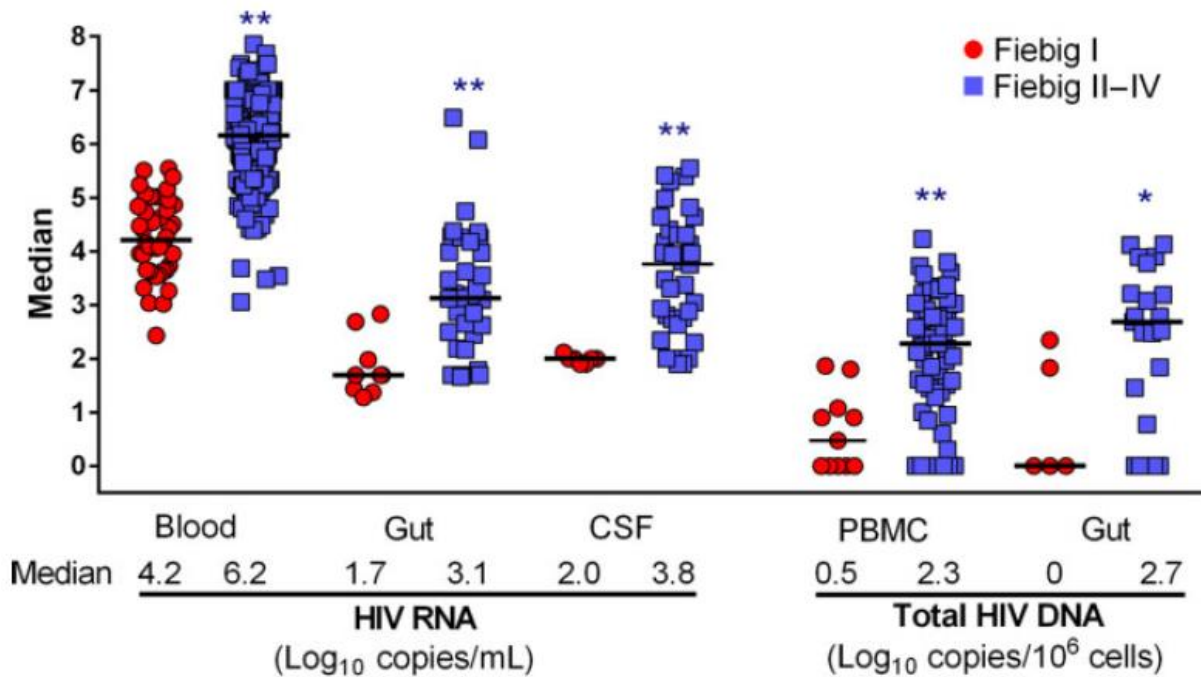
Day 1 post-infection: greater fraction of GI tissue HIV-RNA+ versus SIV uninfected control

Equal HIV-DNA in the gut of acute and chronic patients



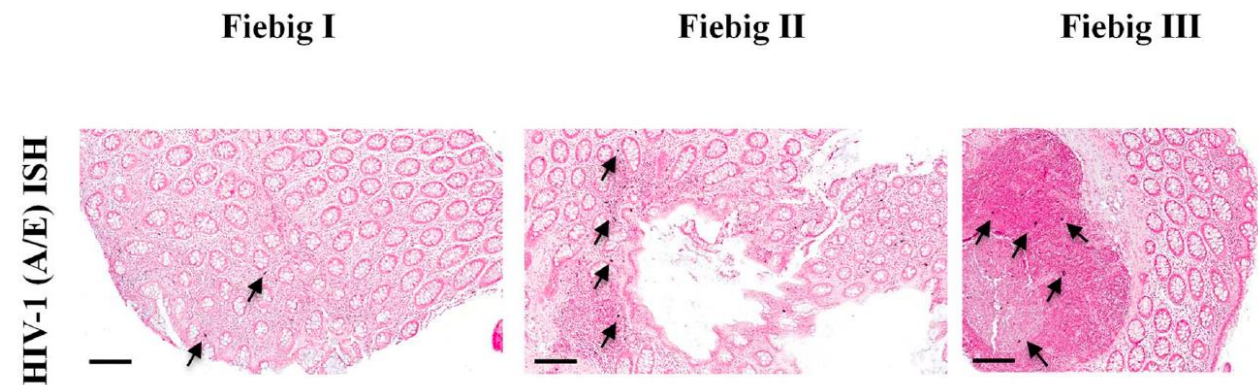
39 HIV+,
15 chronic HIV, 14
primary HIV (1 Fiebig
I-II; 5 Fiebig III; 8
Fiebig IV/V)

Cannizzo, manuscript in preparation



** $P < 0.001$, * $P < 0.05$

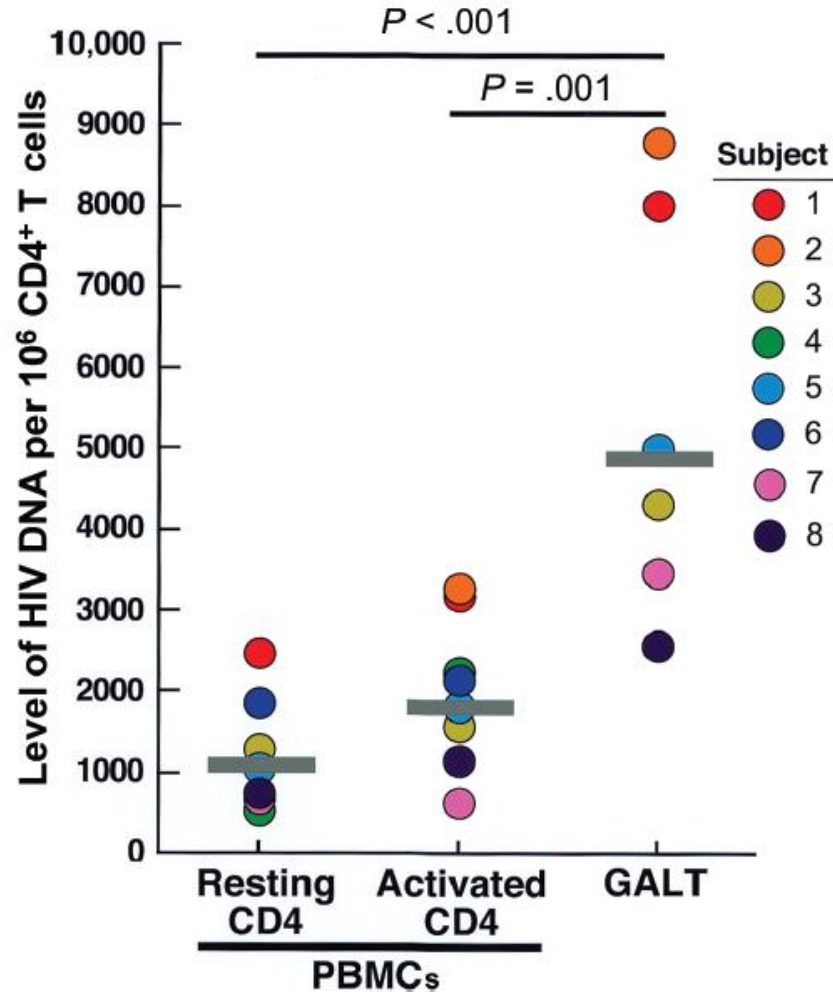
Ananworanich et al. J Viral Erad 2016



Shuetz et al. Plos Path 2014

Viral persistence despite cART

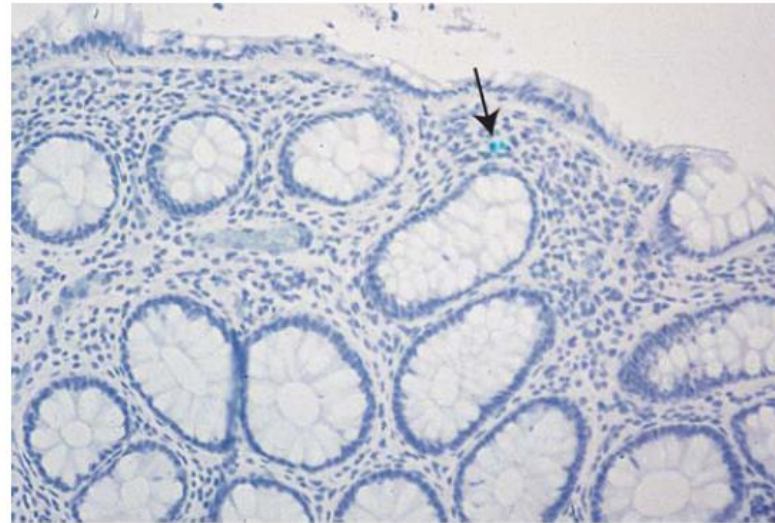
8 HIV+ patients; virologically-suppressive
cART average 8.4 years



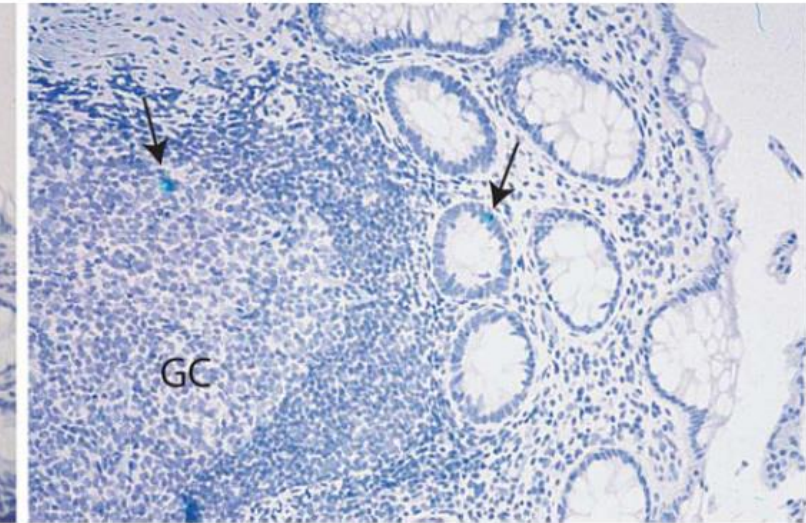
Chun T, JID 2008

54 HIV+ patients (acute)

Lamina propria



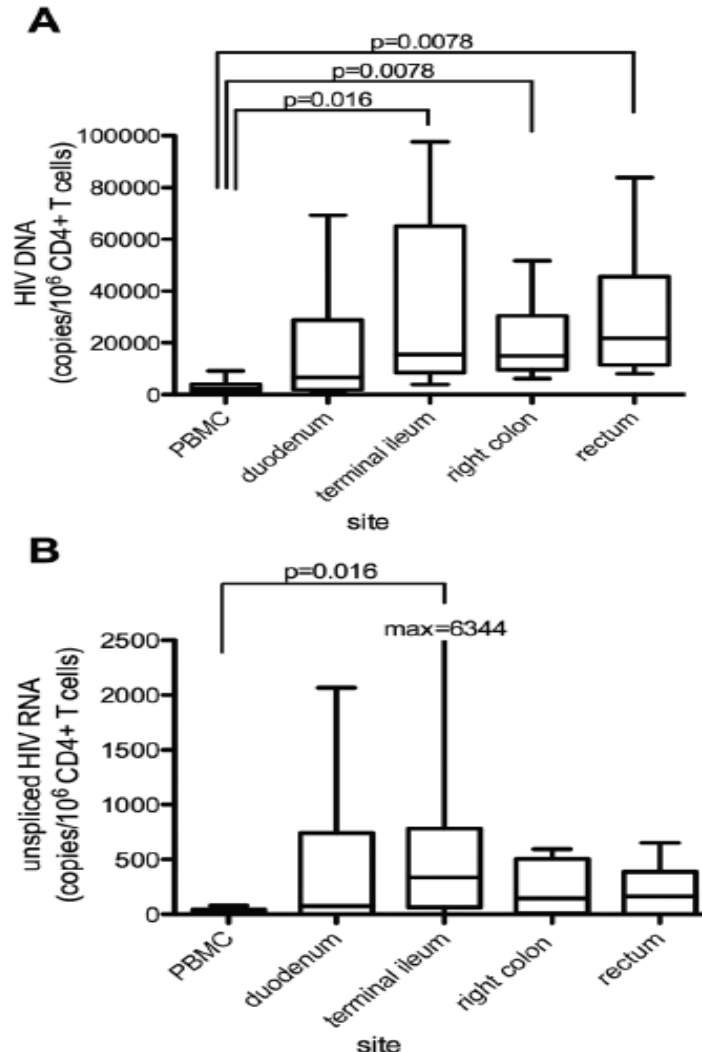
T-cell zones and
intraepithelial lymphocytes



RNA-expressing cells by in situ hybridization- 35S-labeled, single stranded anti-sense RNA probe of HIV-1, composed of fragments of 1.4–2.7 kb, representing approximately 90% of the HIV-1 genome

Mehandru S, Plos Med 2006

HIV DNA and unspliced HIV RNA is detectable in the gut, whereas multiply spliced HIV RNA is rarely detectable, suggesting infrequent productively infected cells



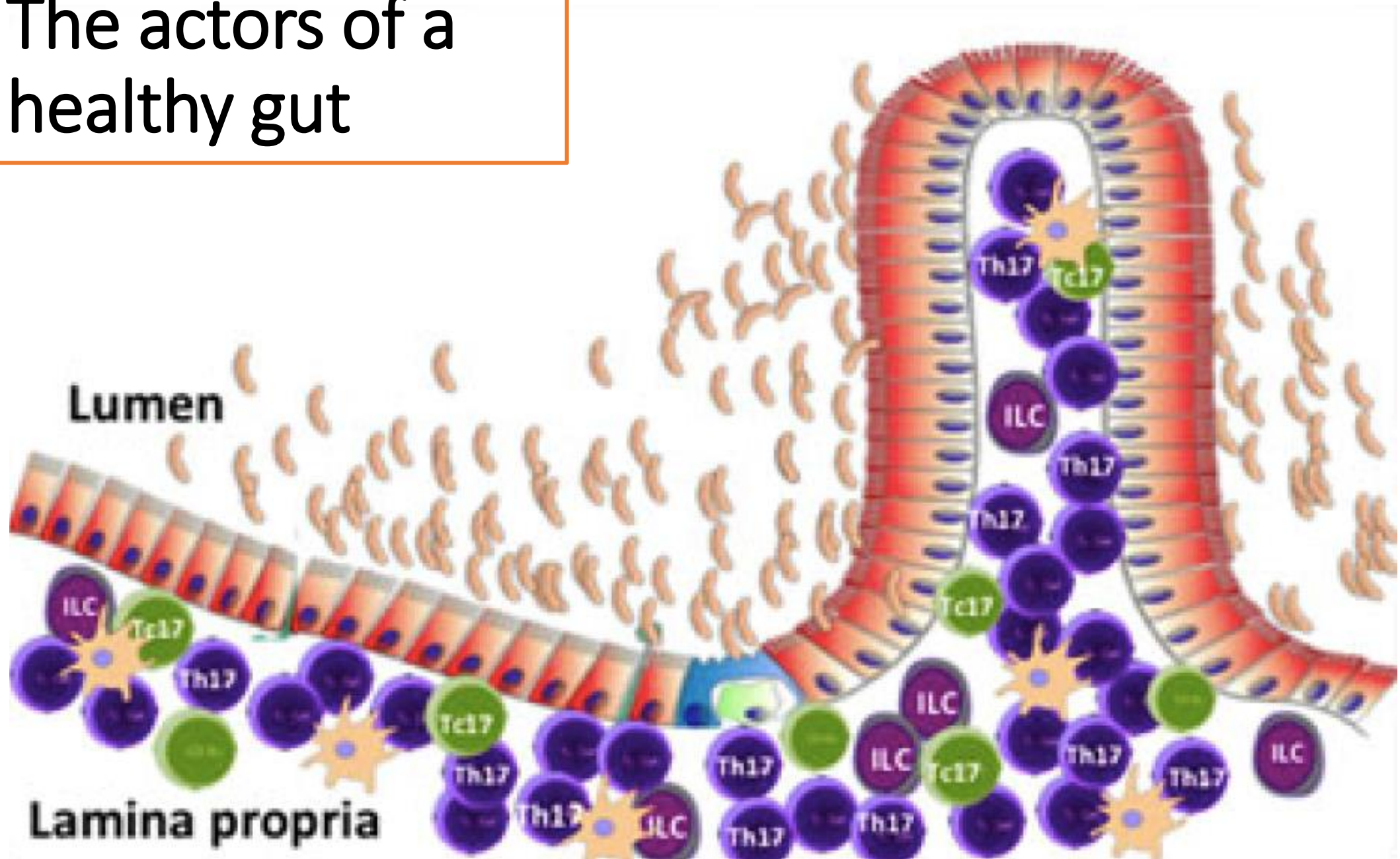
8 HIV+ patients; virologically-suppressive cART
 ≥ 1 year; CD4+ median 478/mm³

	No. (%) of patients with HIV detected					
	PBMCs (n = 8)	Blood CD4 ⁺ T cells (n = 8)	Duodenum (n = 8)	Terminal ileum (n = 8)	Ascending colon (n = 8)	Rectum (n = 8)
HIV DNA or RNA						
DNA	8 (100)	8 (100)	5 (62.5)	8 (100)	7 (87.5)	8 (100)
Unspliced RNA	8 (100)	8 (100)	5 (62.5)	7 (87.5)	5 (83.3) ^a	4 (66.7) ^a
Multiply spliced RNA encoding Tat and Rev	2 (25)	5 (62.5)	0 (0)	0 (0)	0 (0) ^a	0 (0) ^a
Total multiply spliced RNA	4 (50)	7 (87.5)	0 (0)	0 (0)	1 (16.7) ^a	1 (16.7) ^a

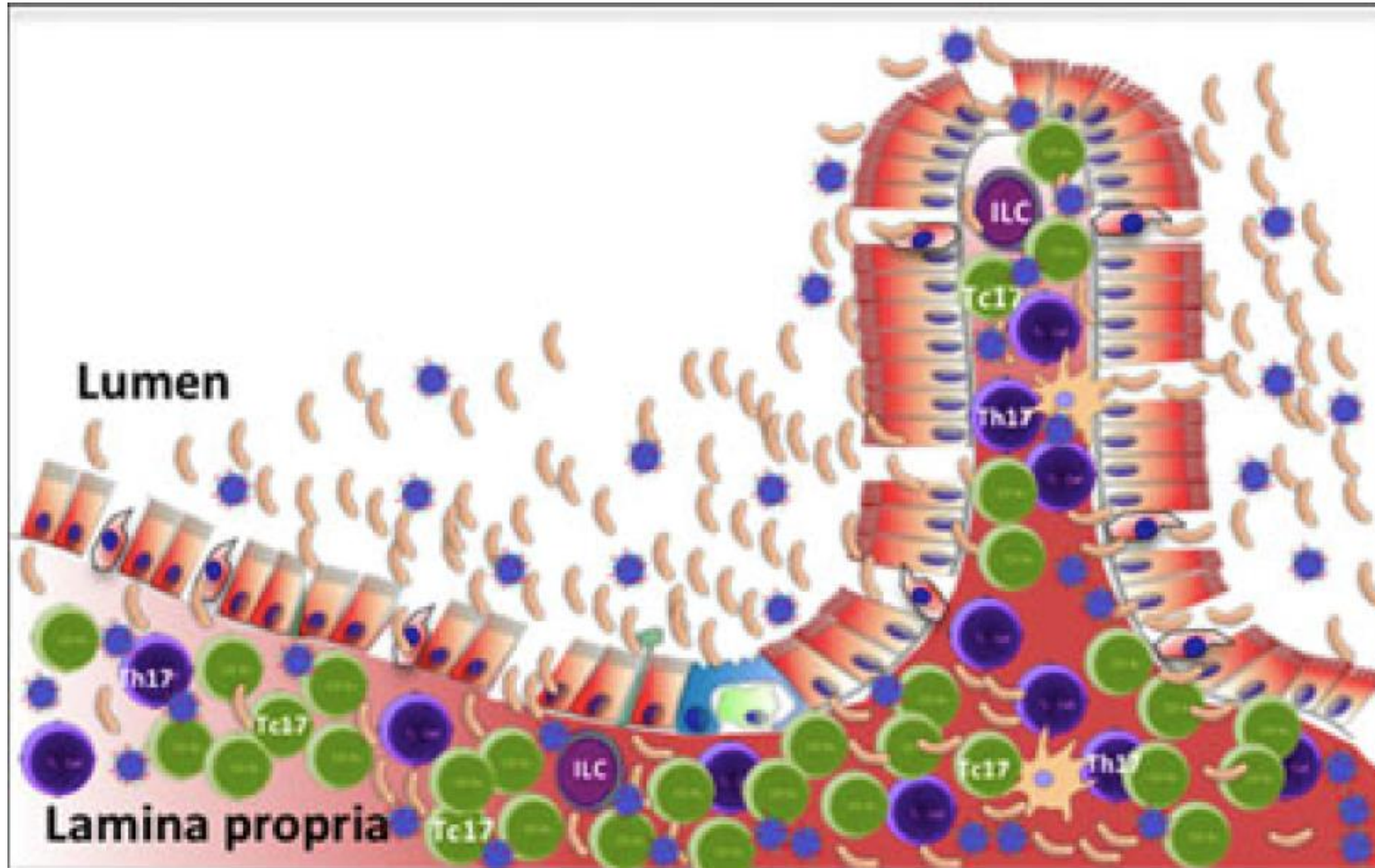
The GALT & HIV: a mucosal catastrophe

1. Local inflammation
2. Mucosal cell depletion
3. Loss of tight junctions

The actors of a healthy gut



The defeated actors of an HIV-infected gut



- HIV-1
- Commensal bacteria
- CD4+T cell
- CD8+T cell
- ILC
- CD103+ dendritic cell
- dying enterocyte
- Damaged/dying enterocyte

GI DAMAGE IN EARLIEST HIV

Table 3. Commonly Reported Signs, Symptoms, and Laboratory Findings in Patients with Acute HIV-1 Infection.*

Symptoms and signs

Fever†

Pharyngitis†

Lymphadenopathy†

Fatigue

Rash†

Diaphoresis or night sweats†

Headache†

Anorexia†

Nausea or vomiting†

Diarrhea

Myalgia and arthralgia†

Oral or genital ulcers

Nuchal rigidity, photophobia, or both (in patients with aseptic meningitis)†

Laboratory findings

Thrombocytopenia

Leukopenia

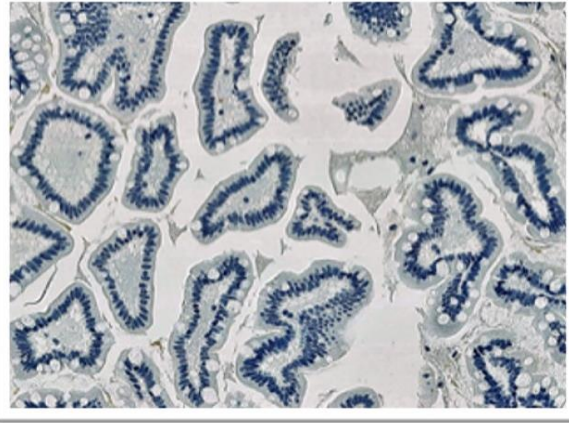
Lymphopenia†

Elevated aminotransferase levels

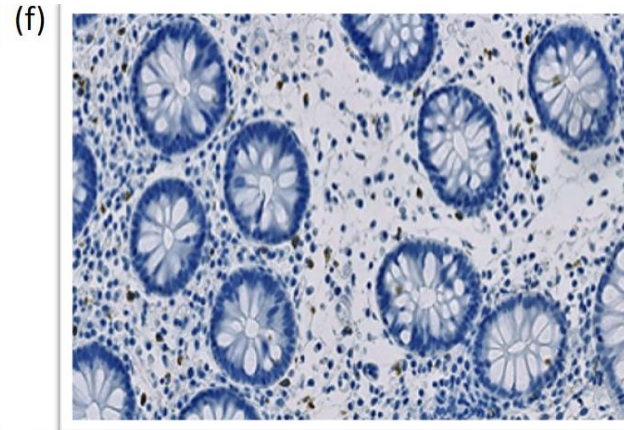
1. Highest local inflammation since early HIV infection.....

Gut neutrophil infiltration

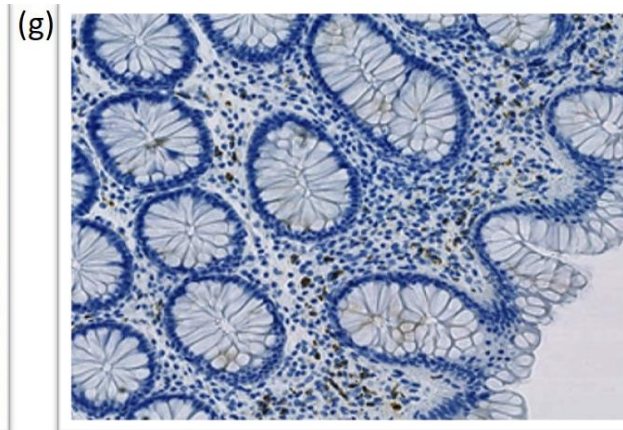
HC



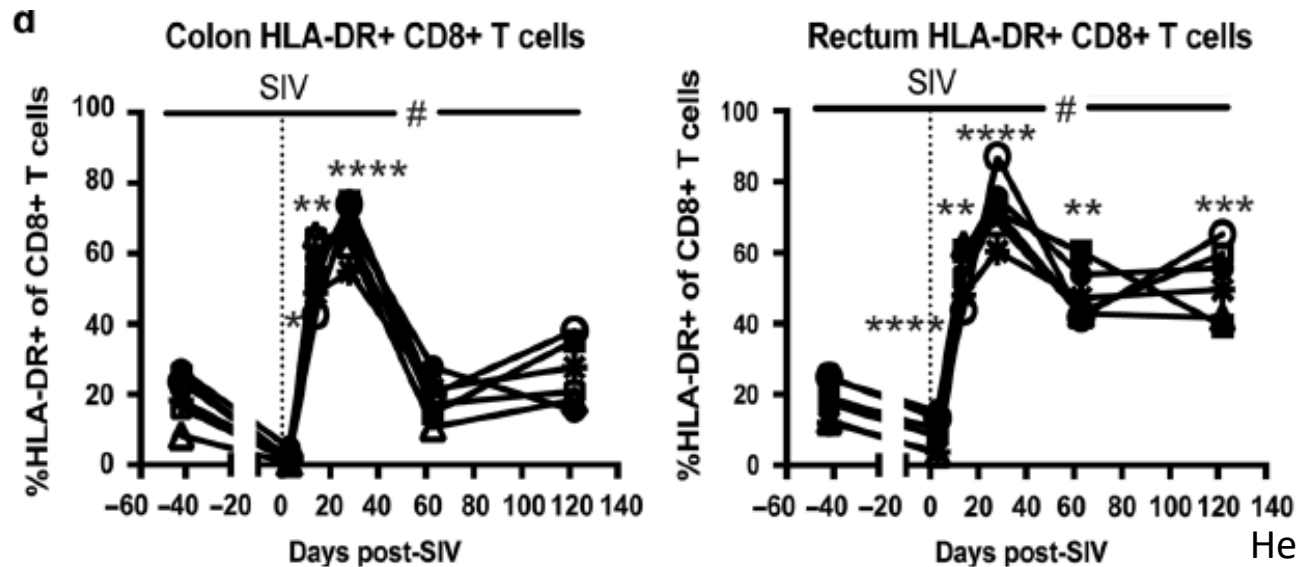
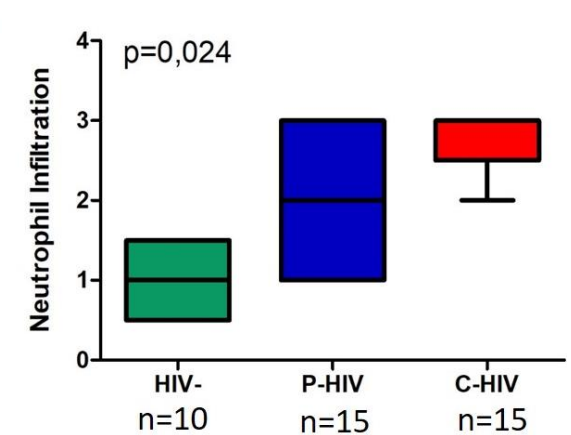
P-HIV



C-HIV



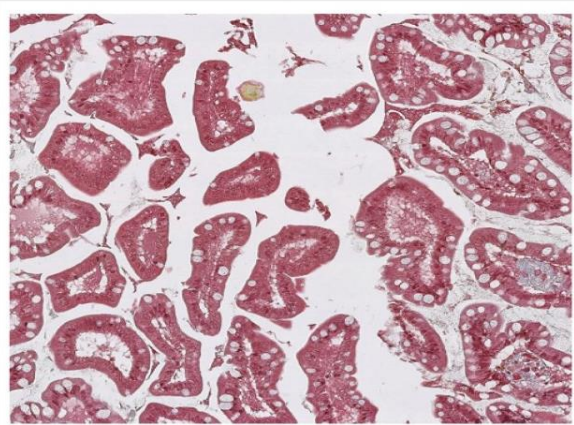
Cannizzo et al. CROI 2018



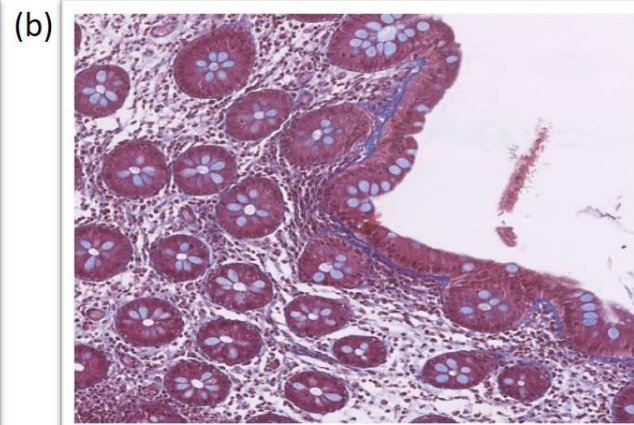
Hensley-McBain et al. Mucosal immunol 2018

....and collagen deposition

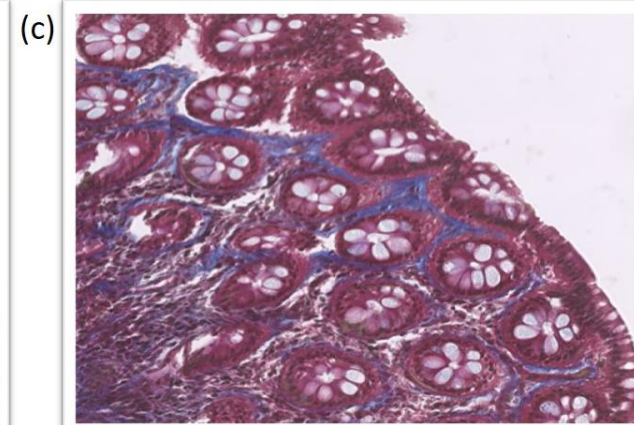
HC



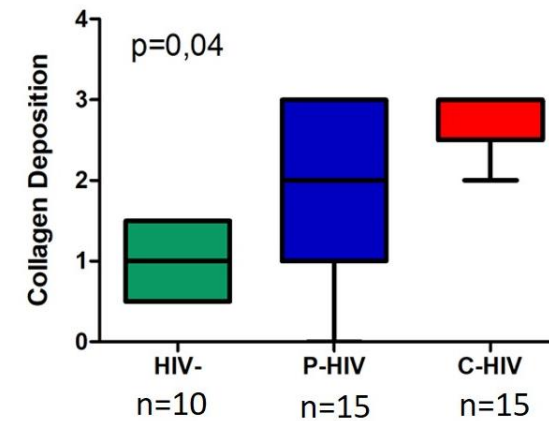
P-HIV



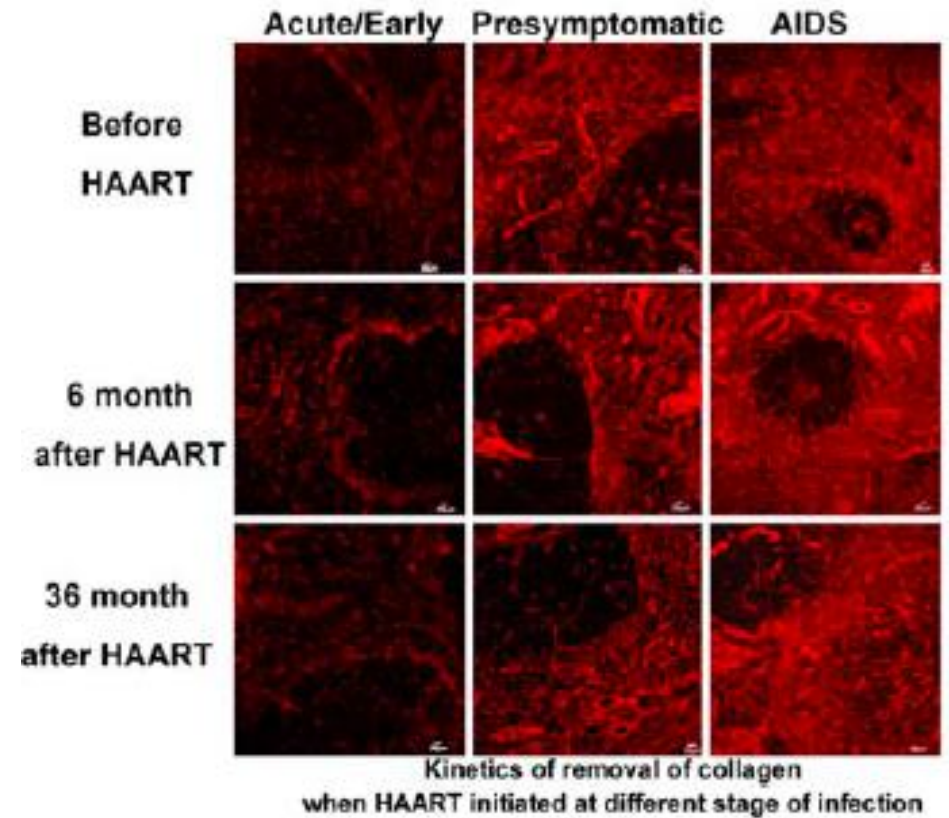
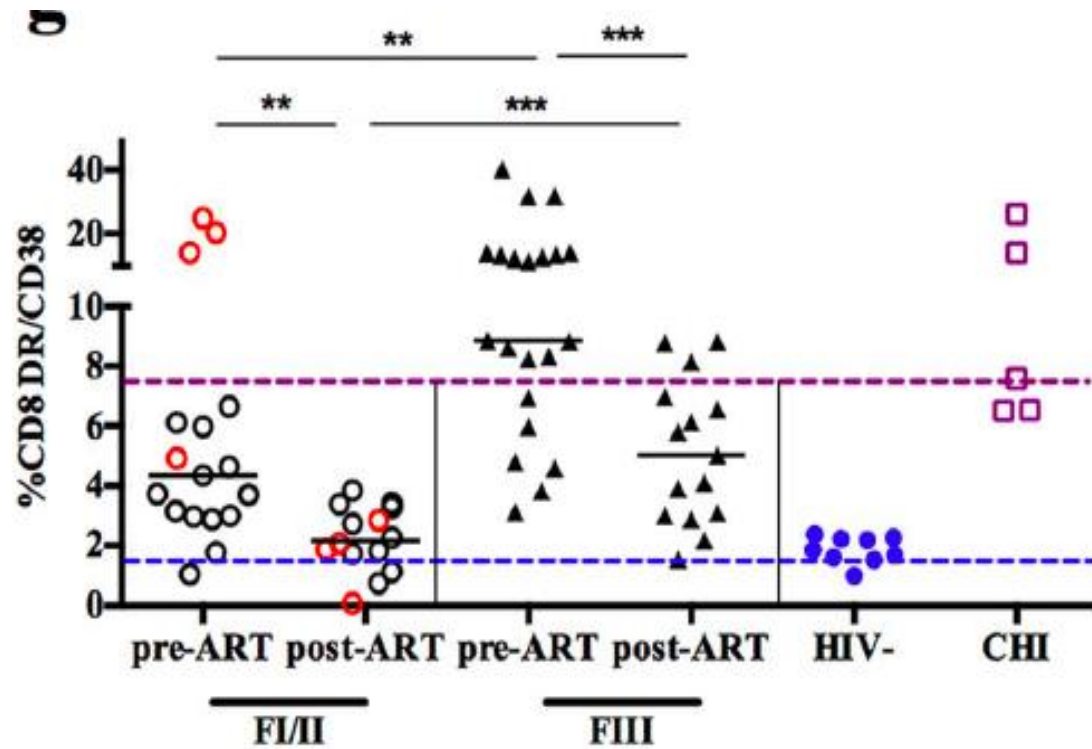
C-HIV



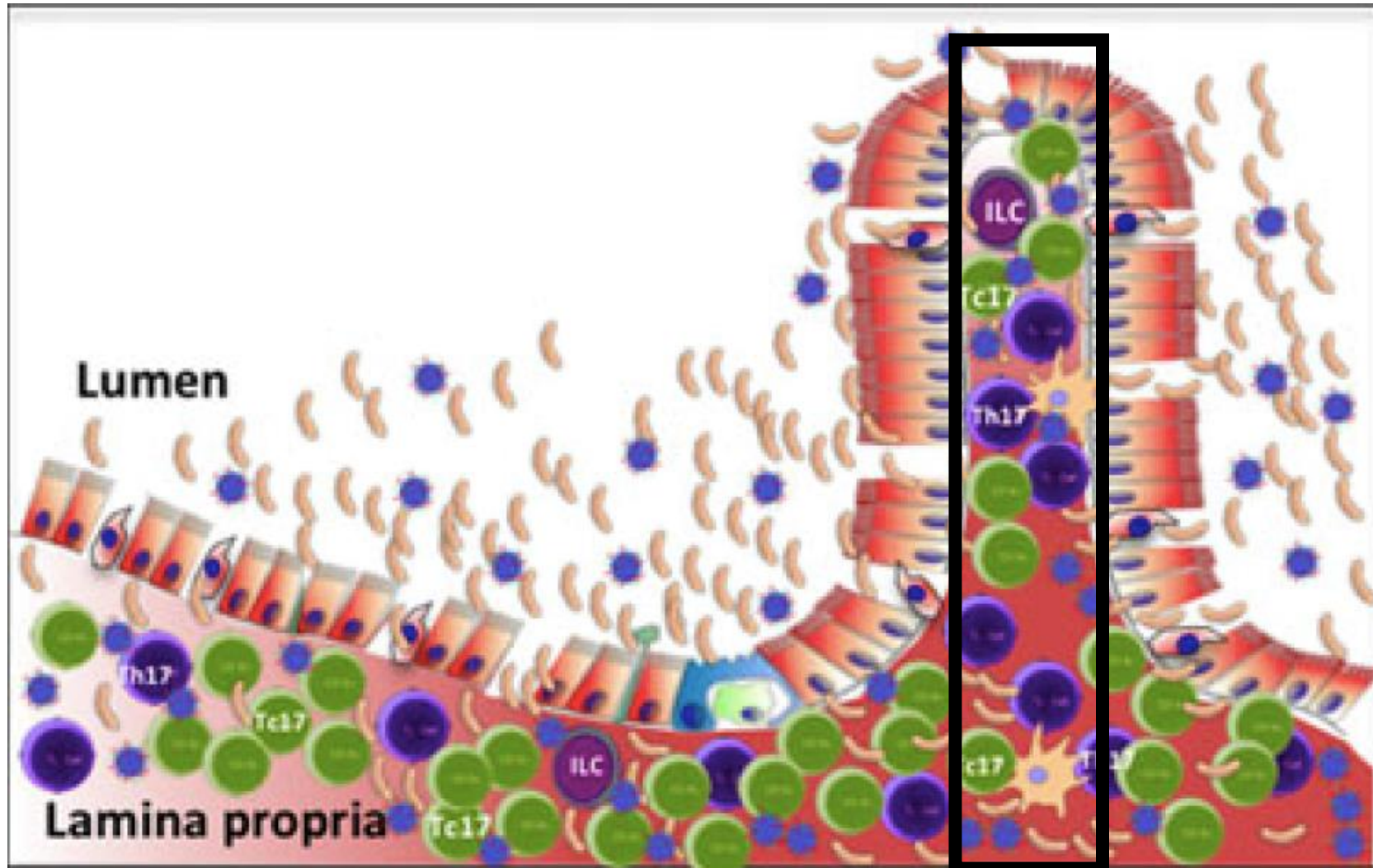
(d)



Early cART (FI/FII/FIII) contains gut CD8+ activation but fails to revert gut fibrosis

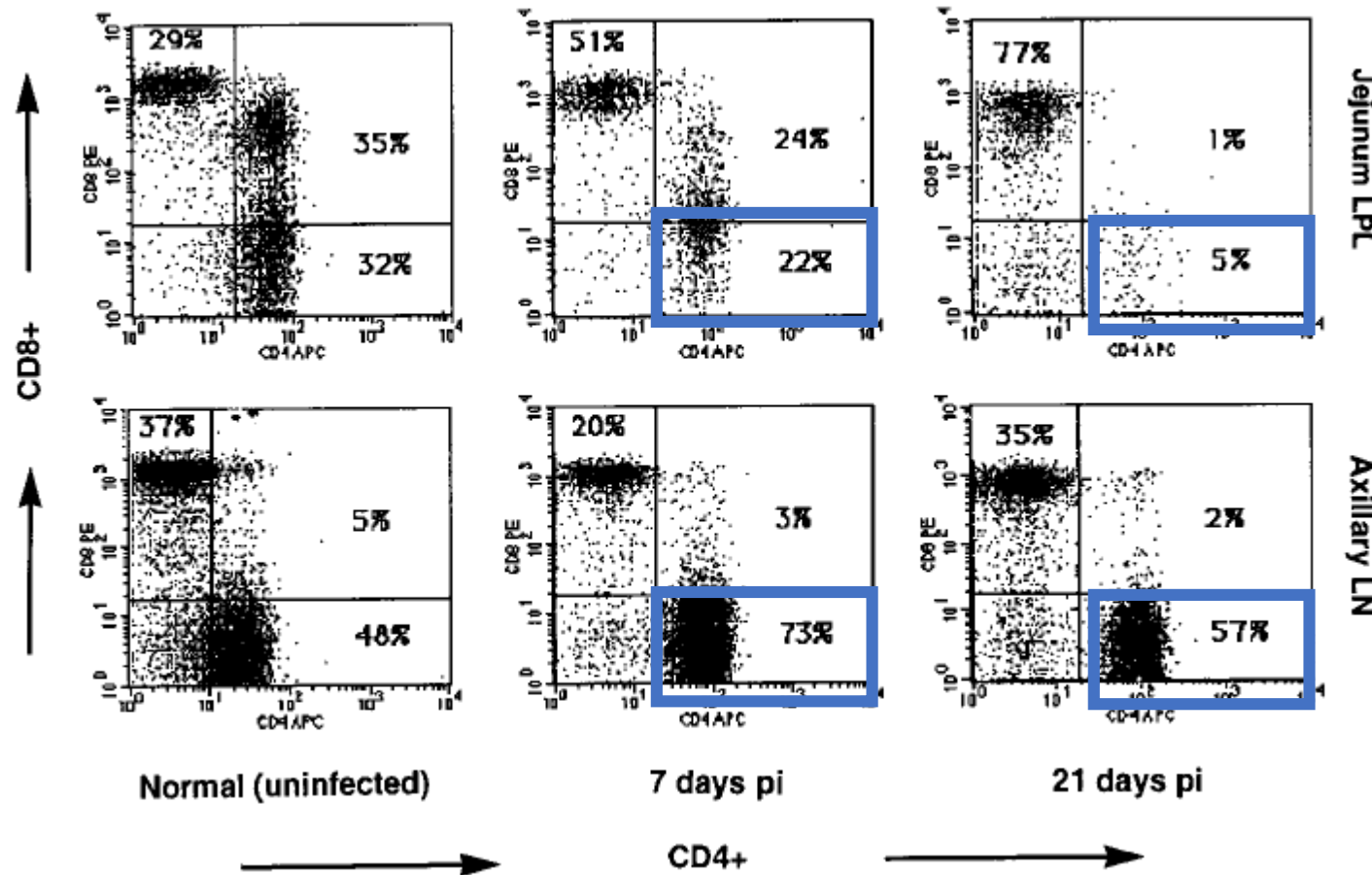


The defeated actors of an HIV-infected gut – 2: massive mucosal cell depletion

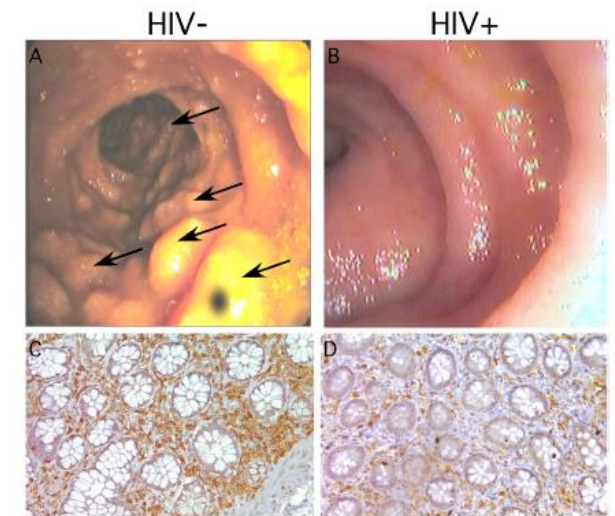
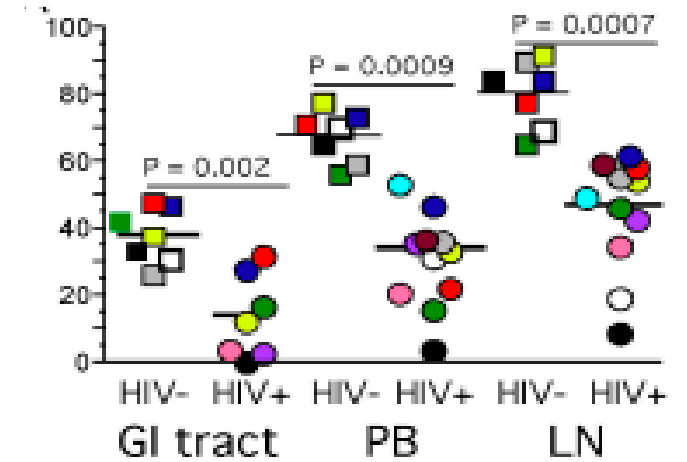


- HIV-1
- Commensal bacteria
- CD4+ T cell
- CD8+ T cell
- ILC
- CD103+ dendritic cell
- dying enterocyte
- Damaged/dying enterocyte

2. Massive CD4+ T-cell depletion in the gut...

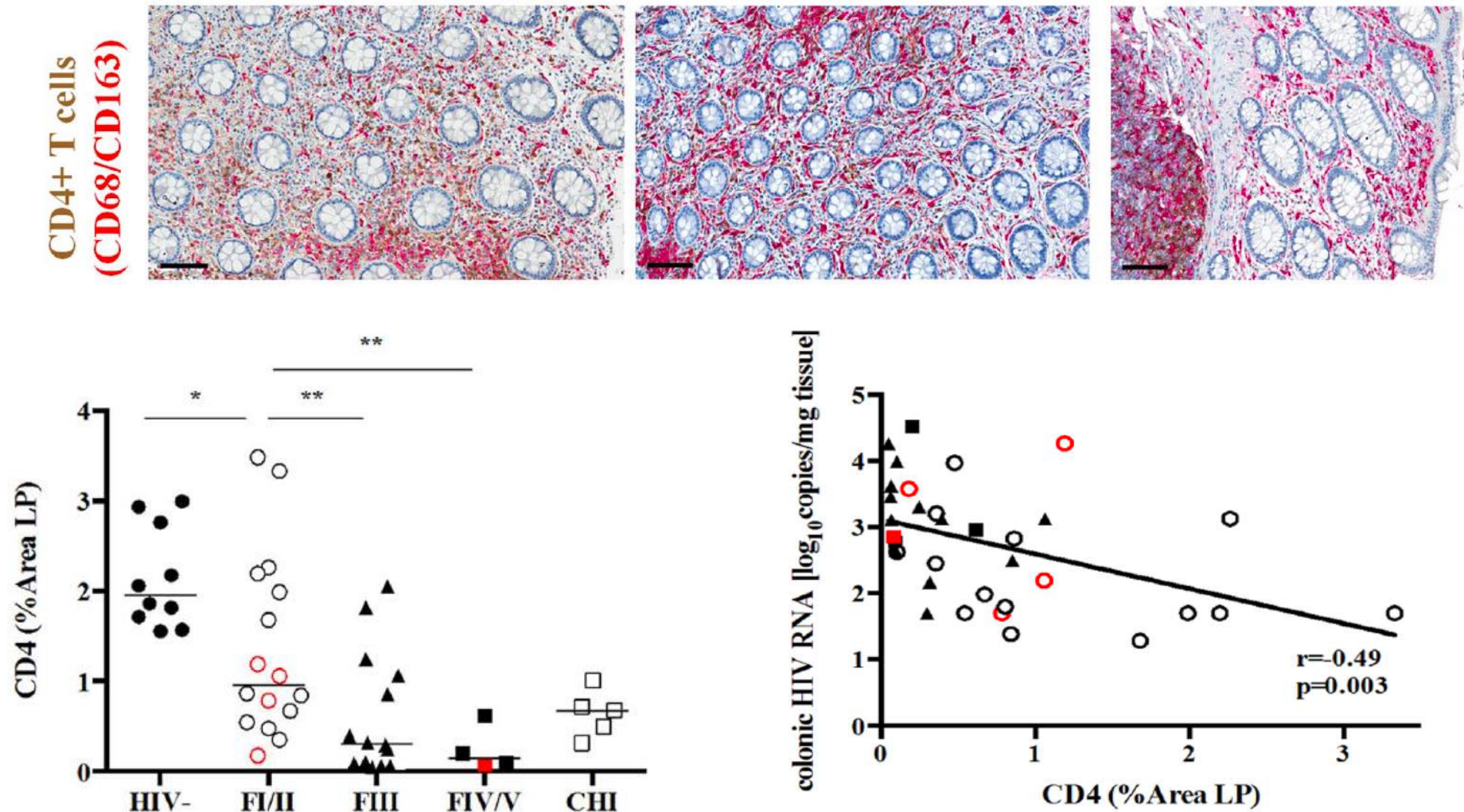


Veazey RS et al. Science 1998

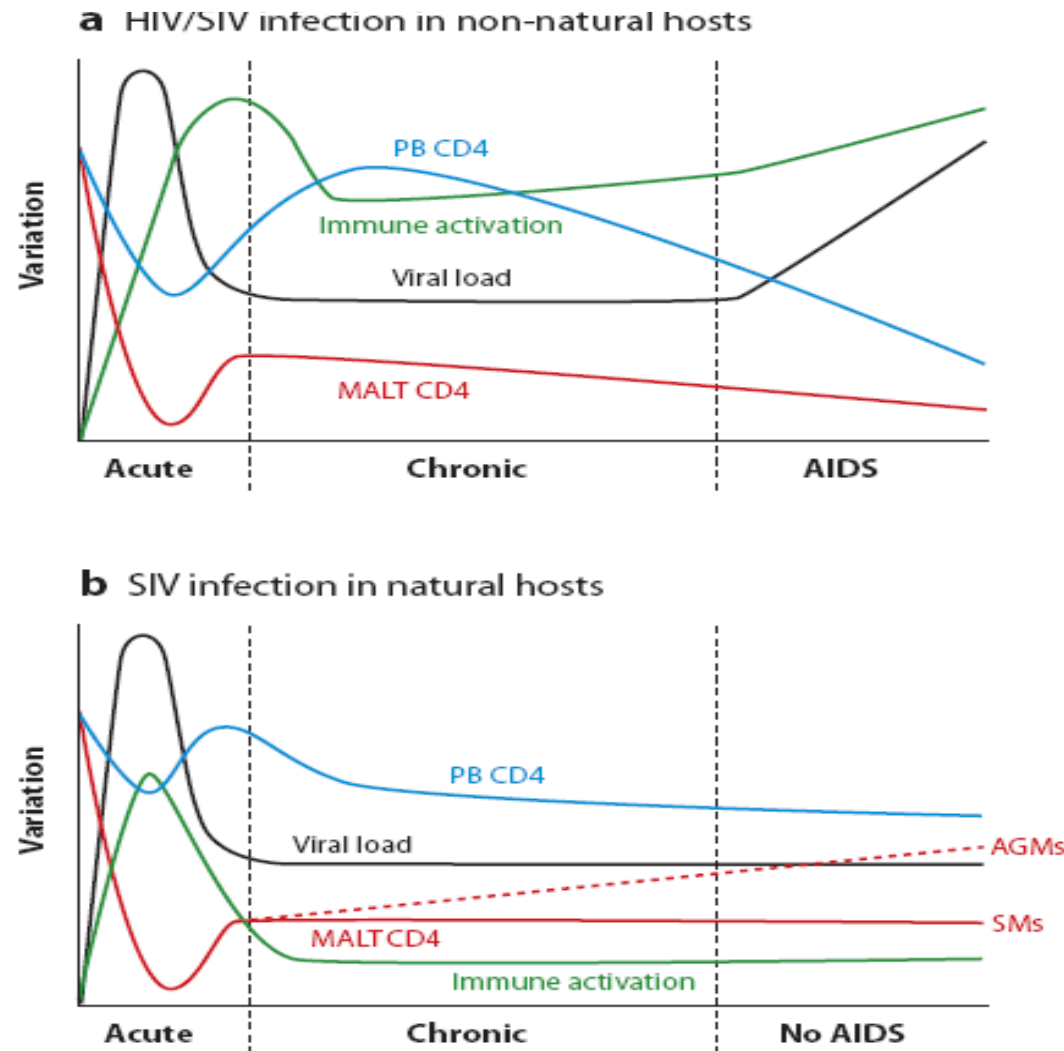


Brenchley J et al. J Exp Med 2004

.....starting in earliest infection



Moderate CD4+ depletion also in non-progressive SIV infection



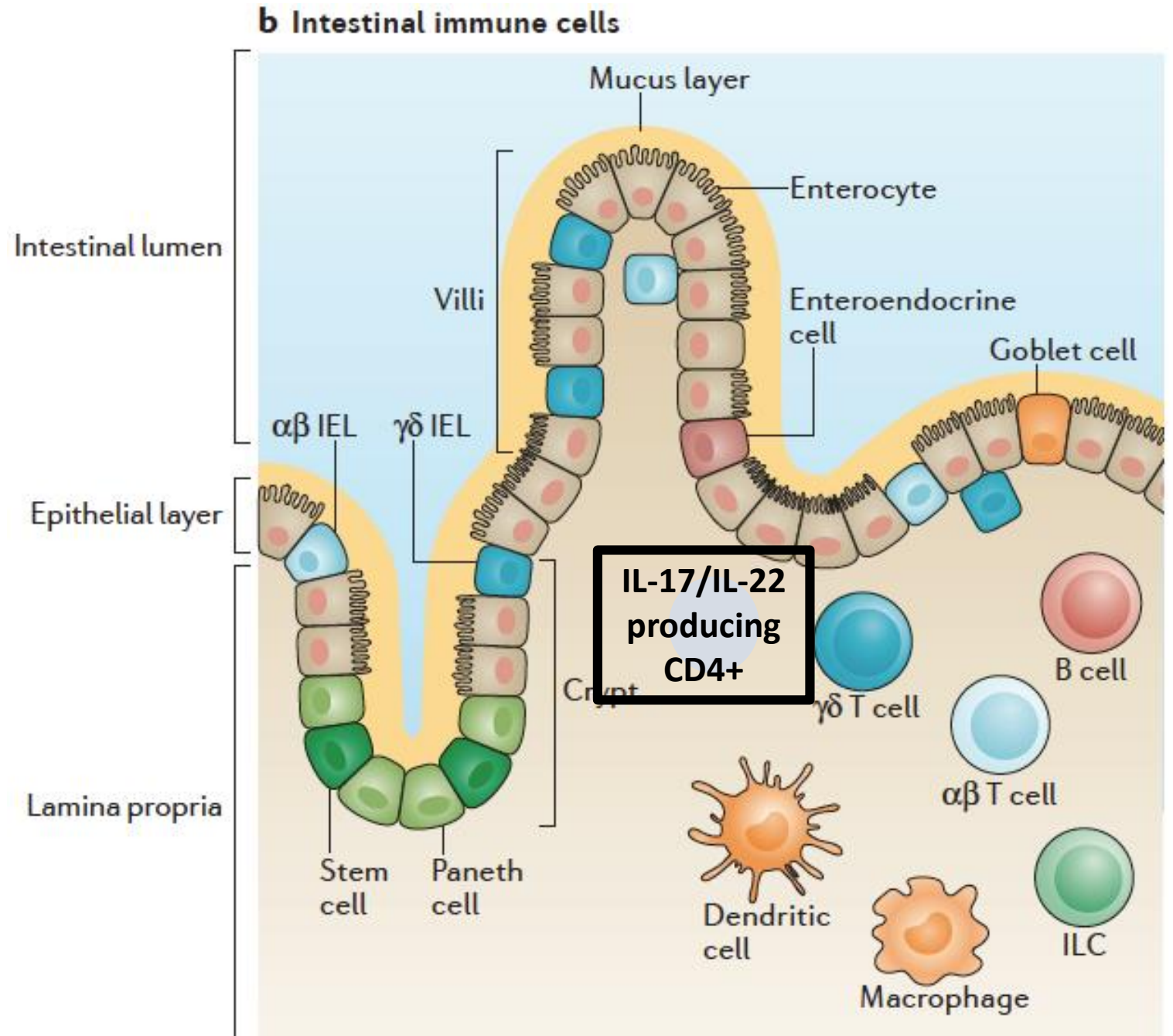
Rhesus macaques -
RM



Sooty mangabeys -
SM

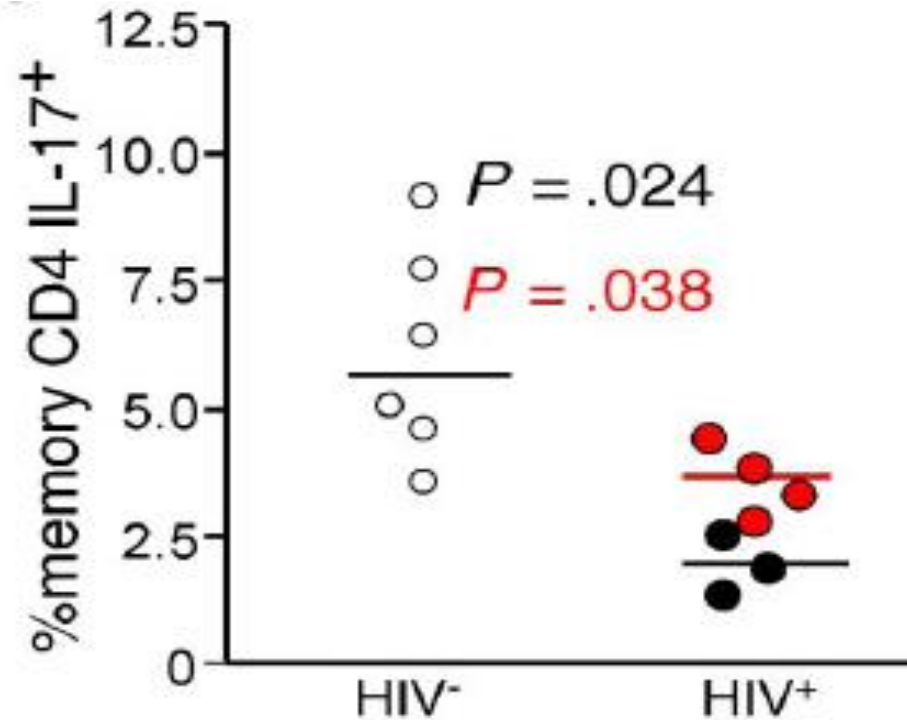


Alterations of gut- resident cells

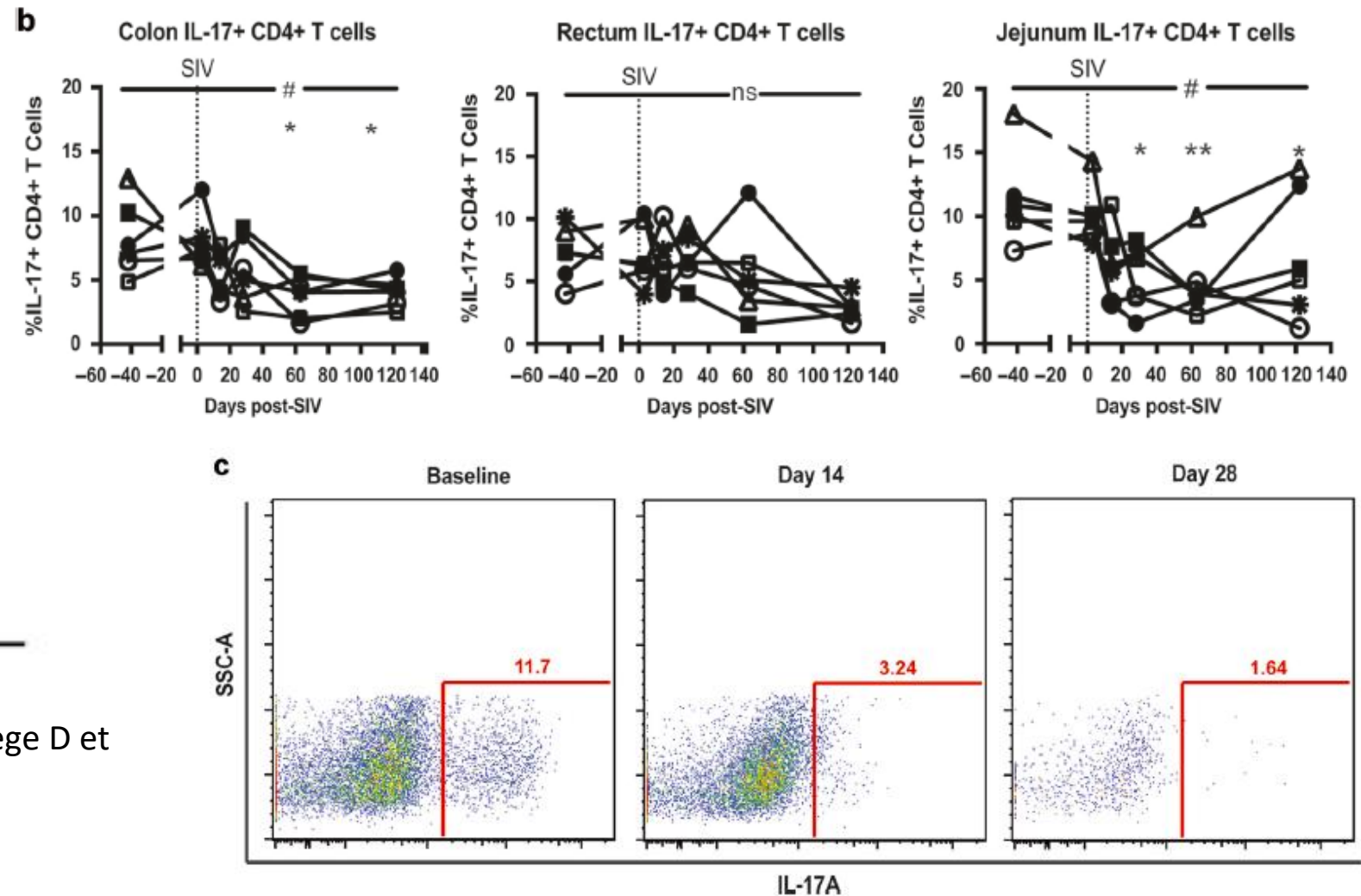


Preferential depletion of Th17 cells in the GI tract of HIV-infected patients since early(est) infection

6 RM experimentally infected

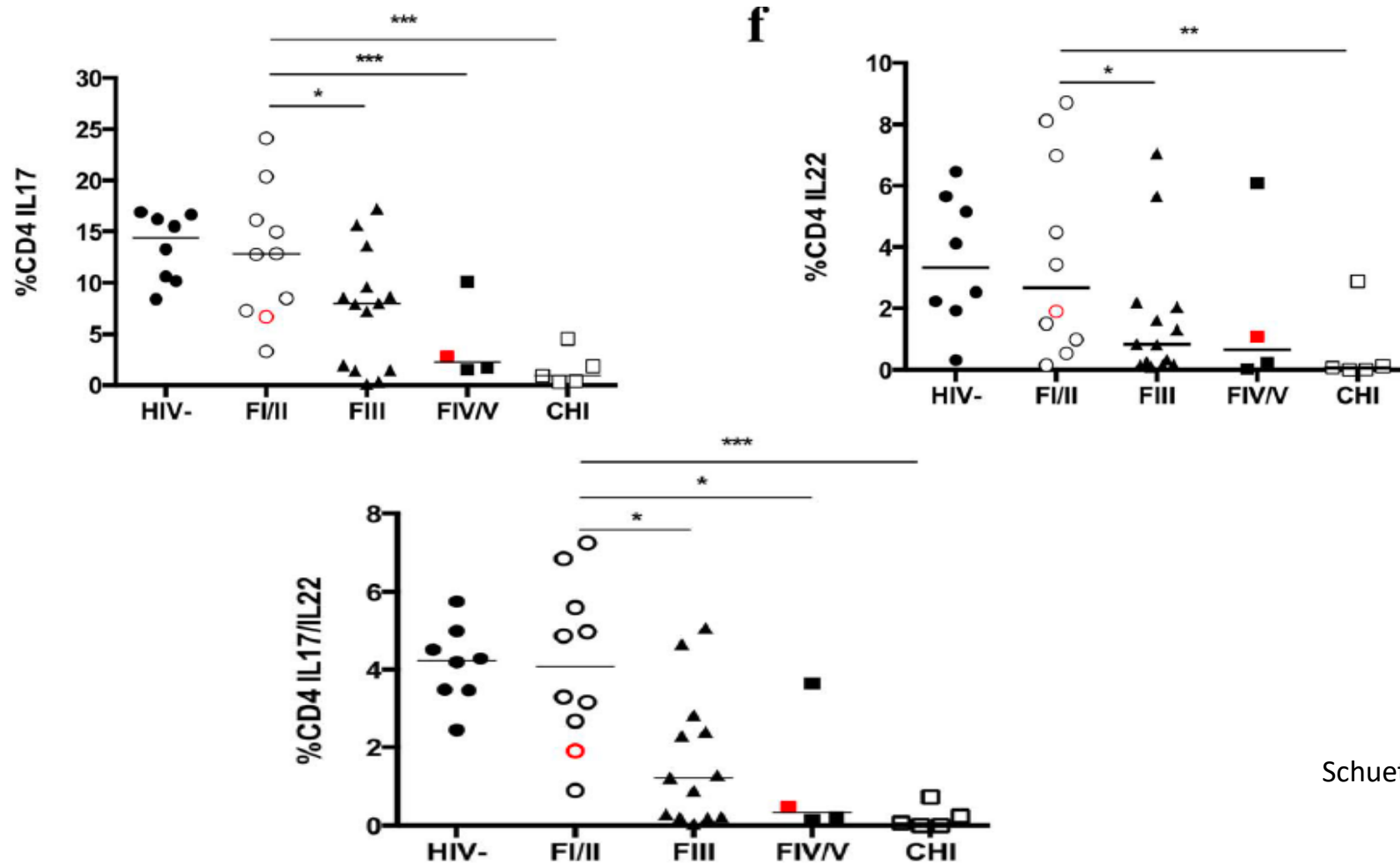


Brenchley J, Paiardini M et al. Blood 2008, also Chege D et al. AIDS 2011

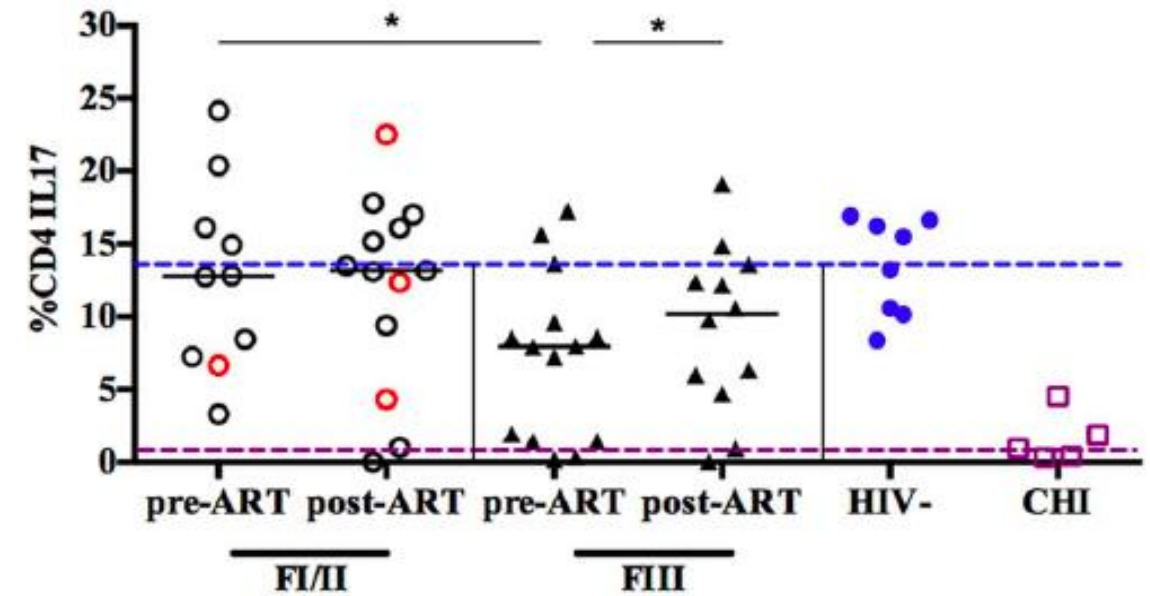
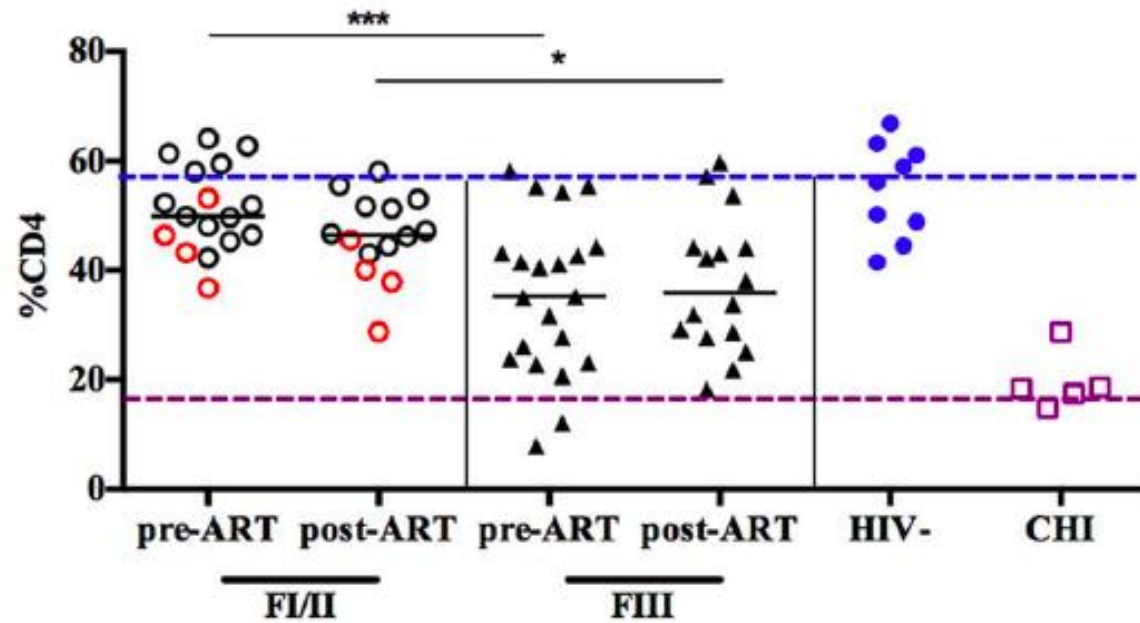


Hensley-McBain et al. Mucosal immunol 2018

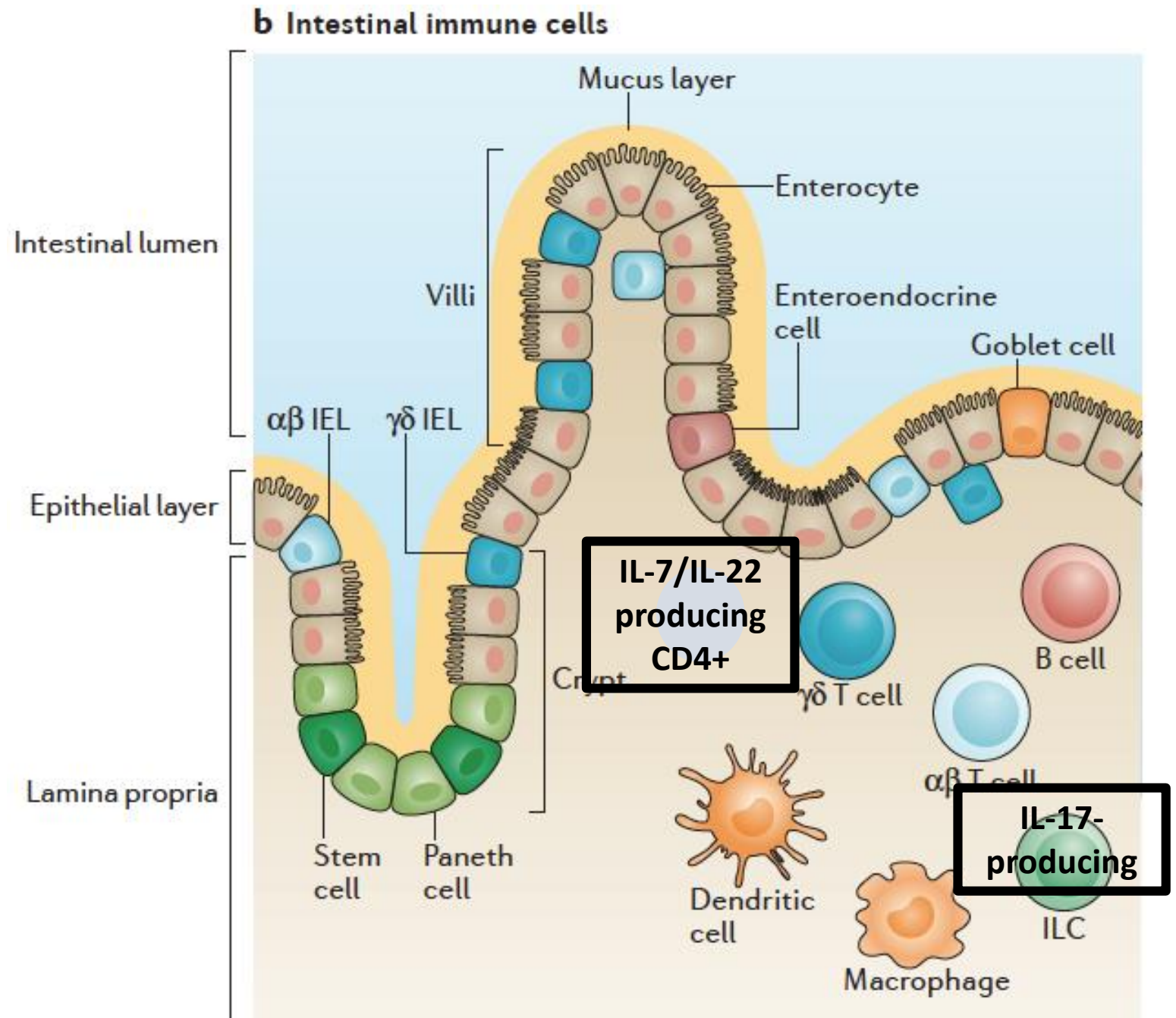
The frequency of IL-17/IL-22 mucosal CD4⁺ decreases with progression of Fiebig stage



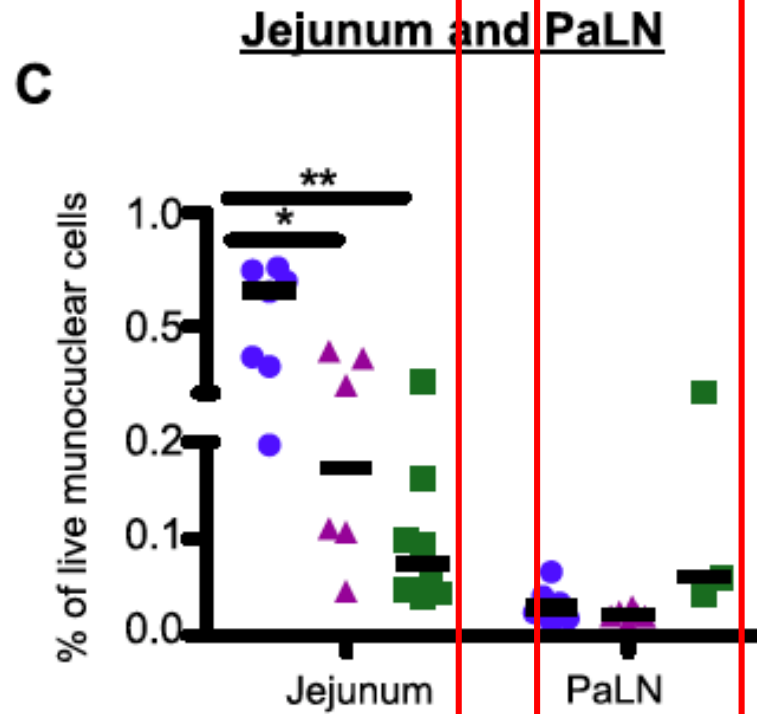
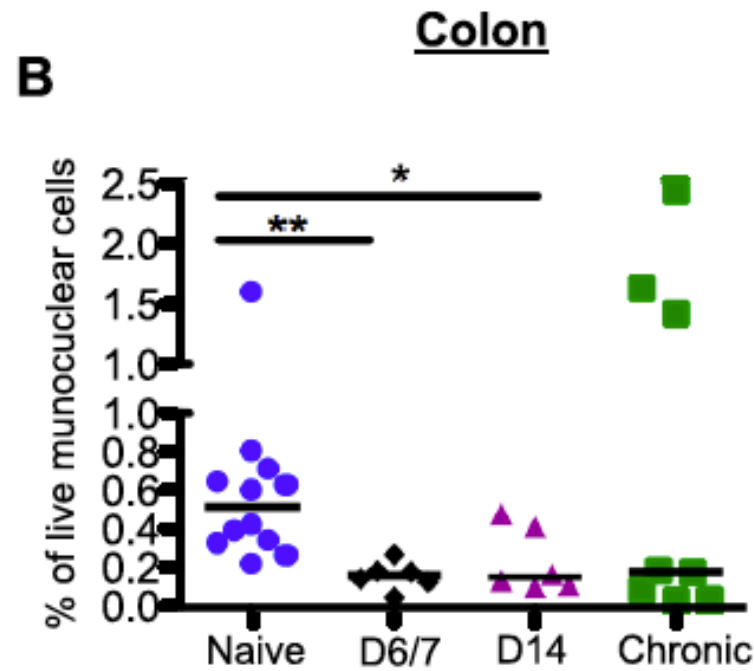
Early cART (FI/FII) preserves mucosal CD4+ and IL-17+CD4+



Alterations of gut- resident cells



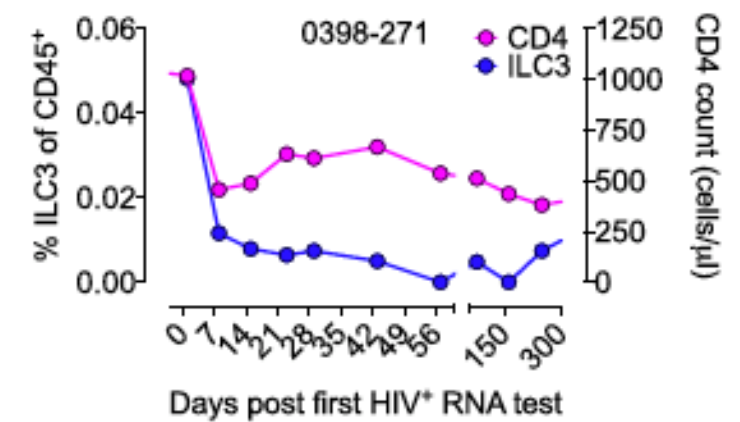
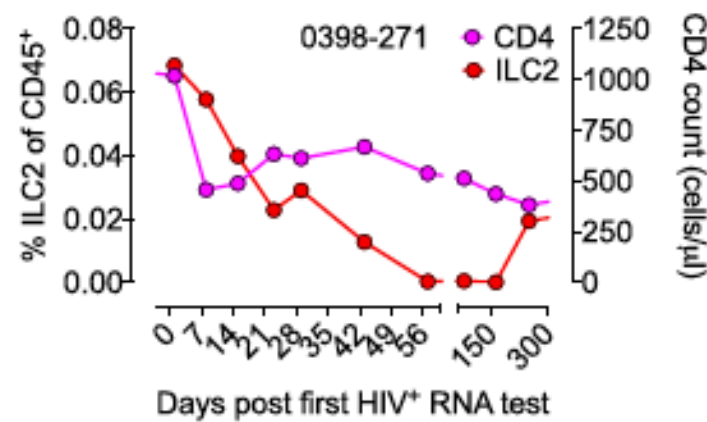
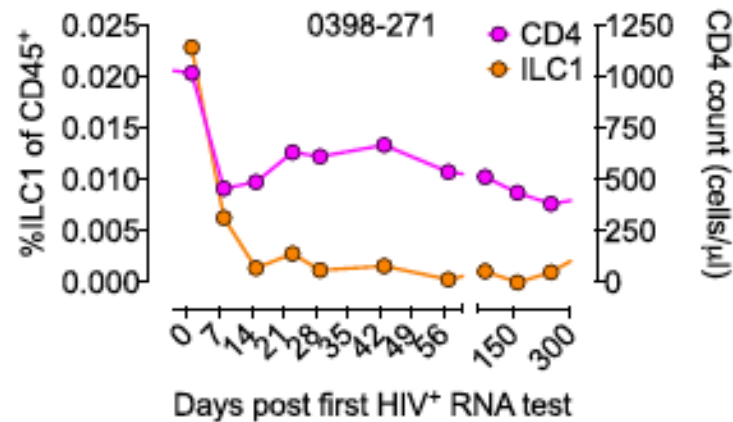
NKp44+ ILCs



Loss of gut Innate lymphoid cells (ILCs) in 12 naive macaques, 6 sacrificed at D6 and 6 sacrificed at D14

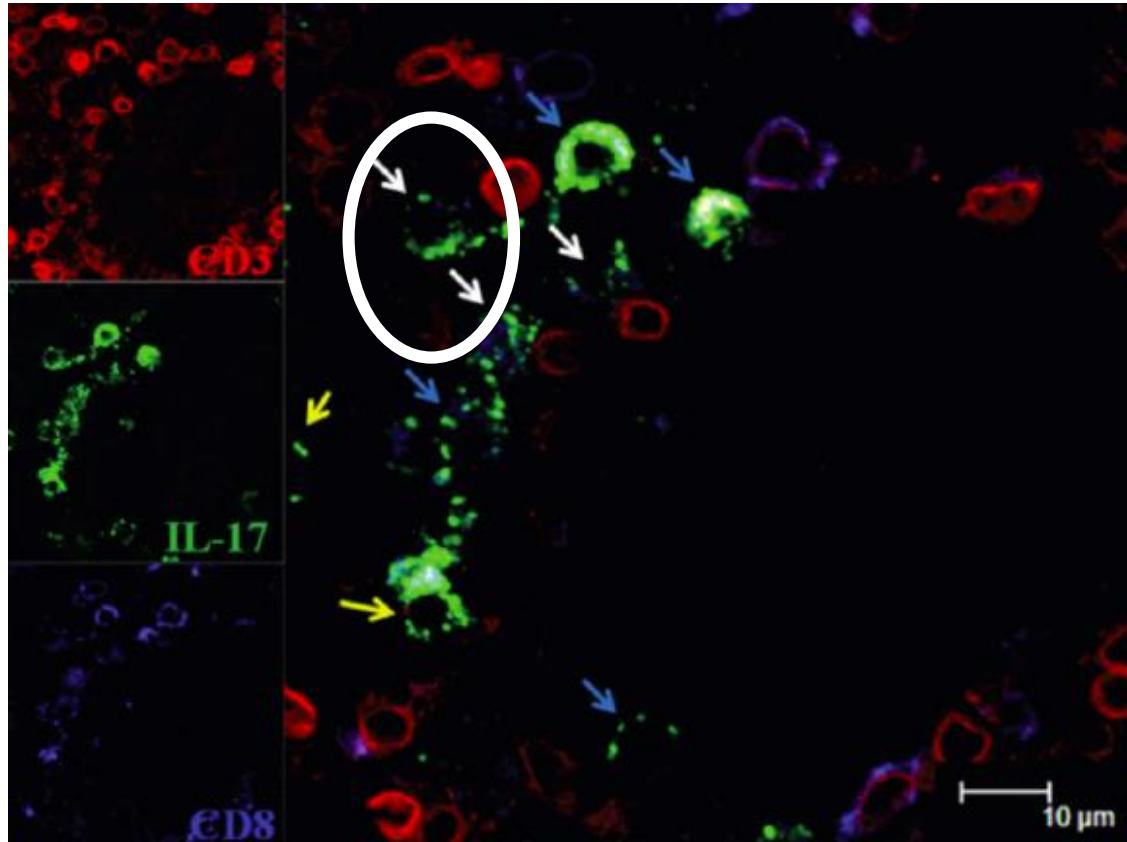
Li Plos Path 2014

Depletion of circulating ILCs in acute HIV



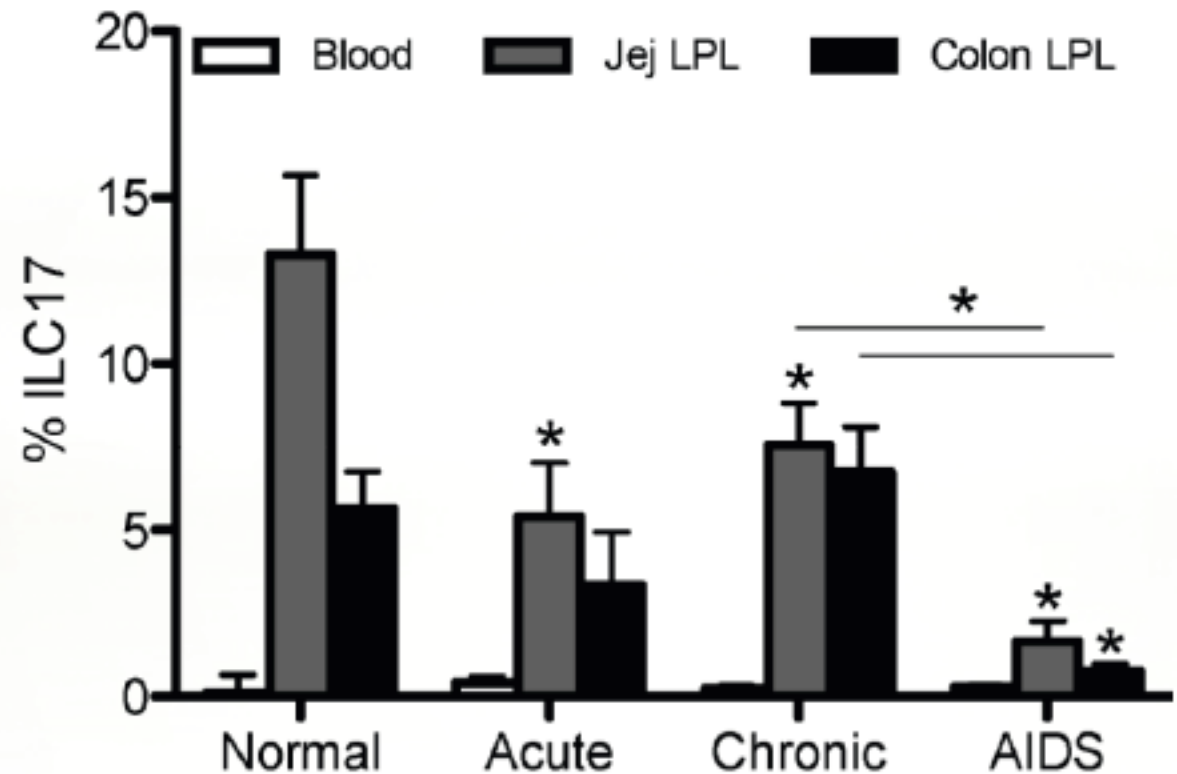
Kloverpris Immunity 2016

Loss of gut IL-17-producing ILCs

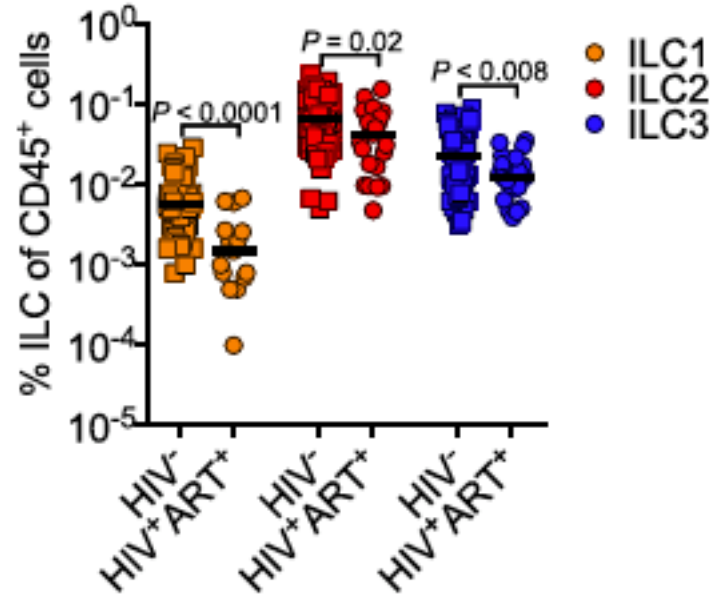


Xu, Mucosal Immunol 2012

Jejunum- RM

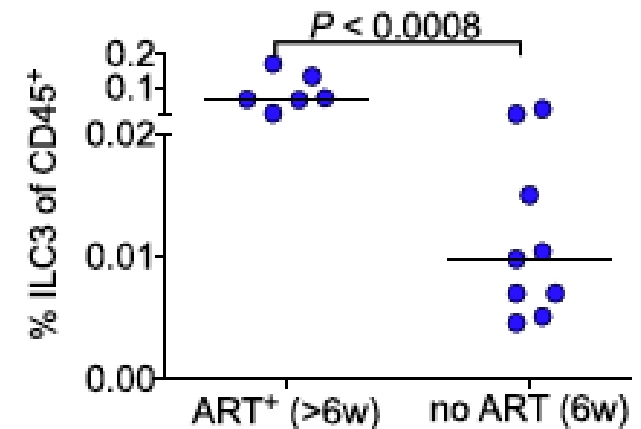
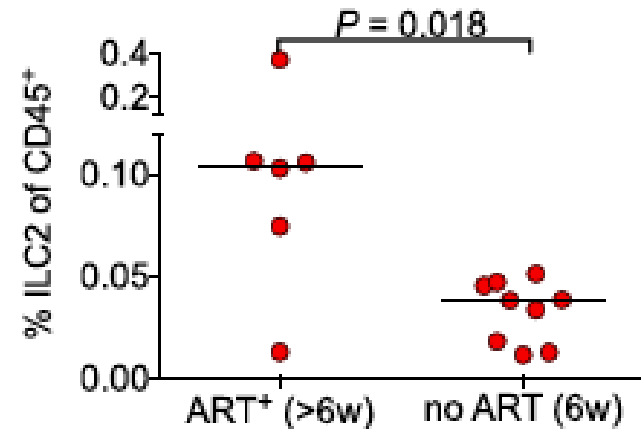
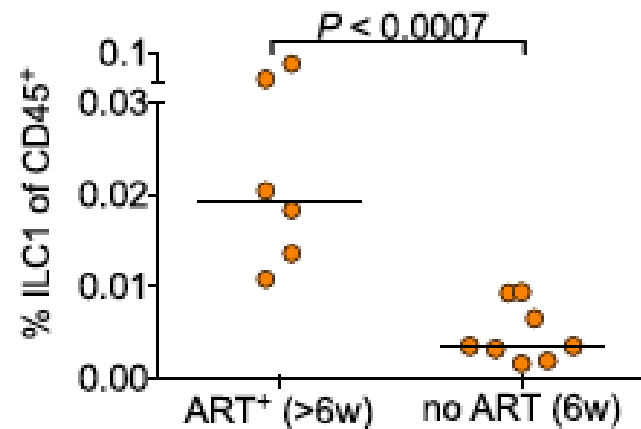


Early cART may preserve the ILCs compartment

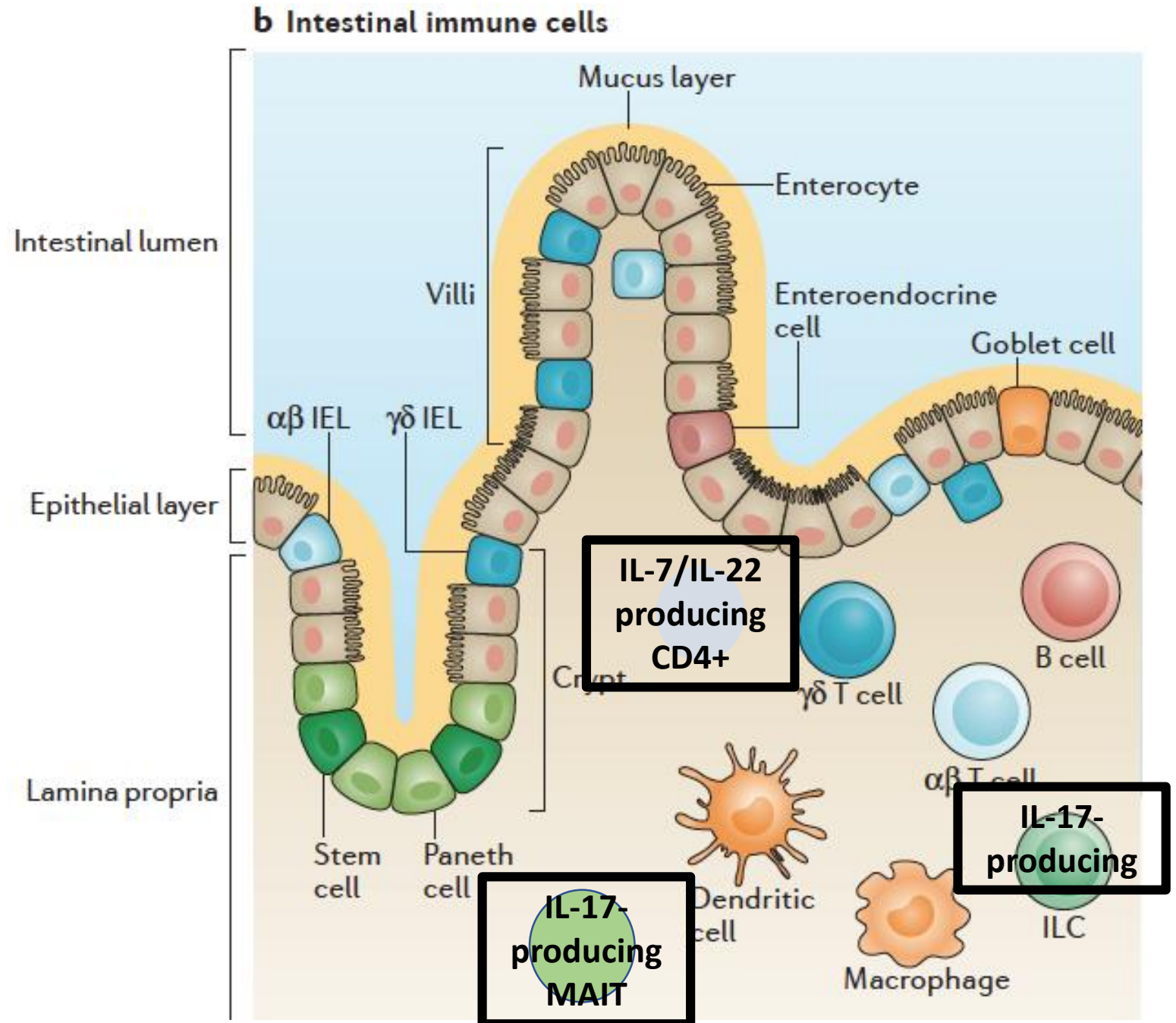


No ILC recovery when cART started in chronic infection

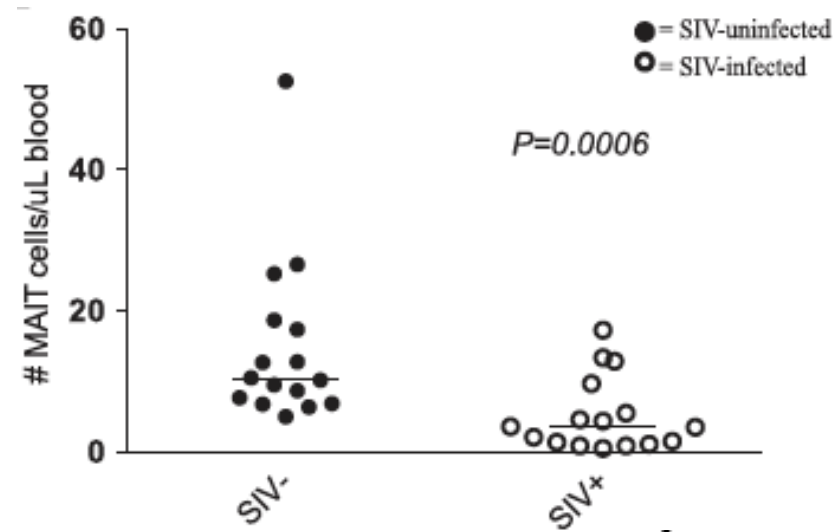
Acute HIV: ILC recovery when cART (vs no cART start)



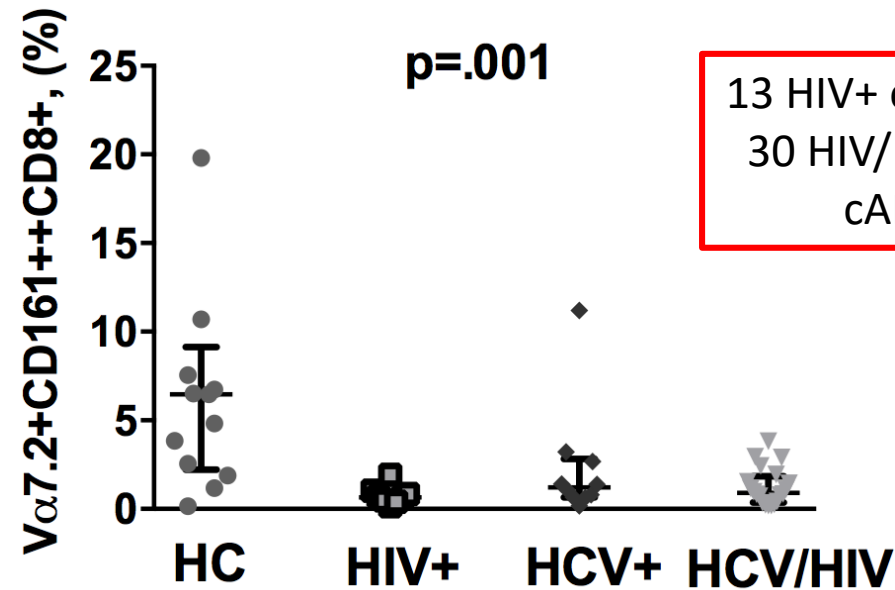
Alterations of gut- resident cells



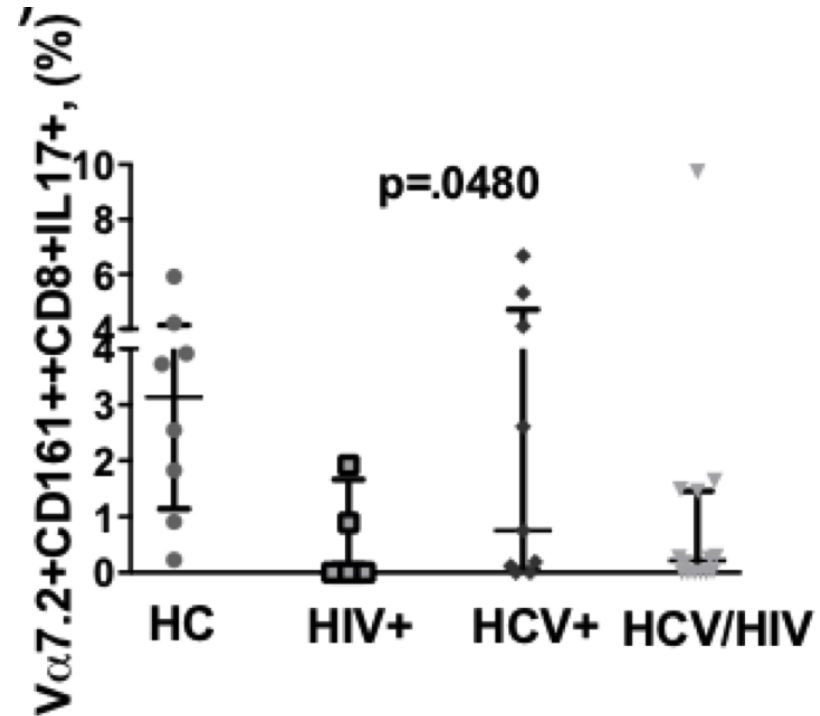
Loss of IL-17-producing MAIT that is not recovered by cART



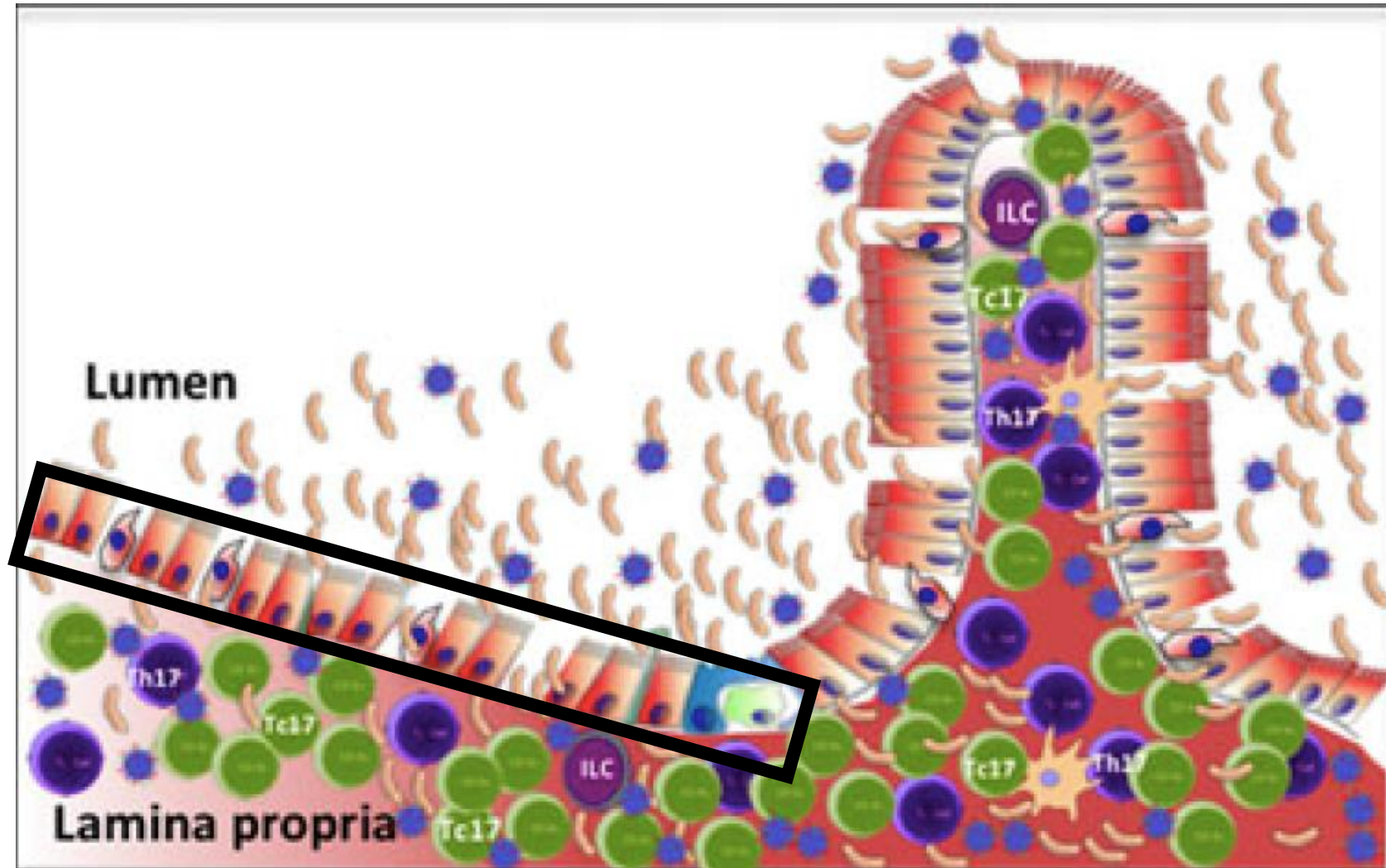
Vinton J Virol 2016



13 HIV+ on cART;
30 HIV/HCV on
cART

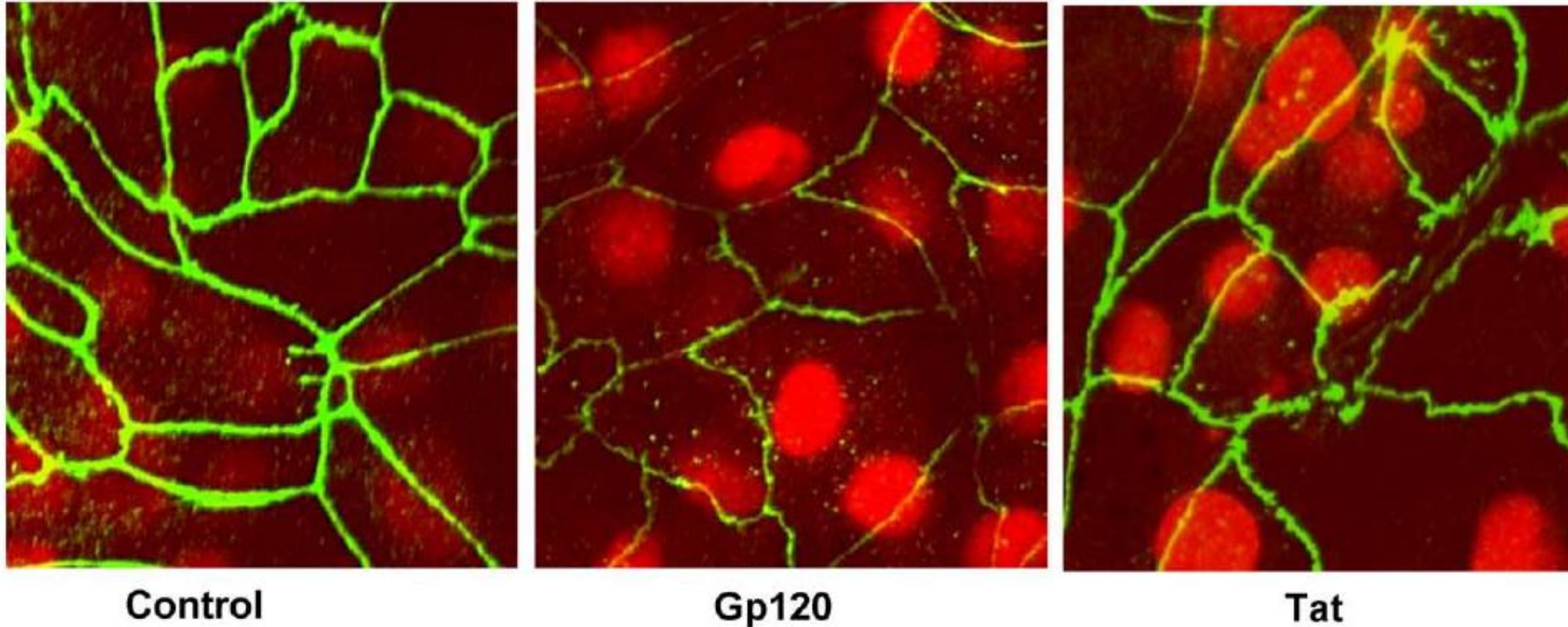


The defeated actors of an HIV-infected gut – 3: the structural damage

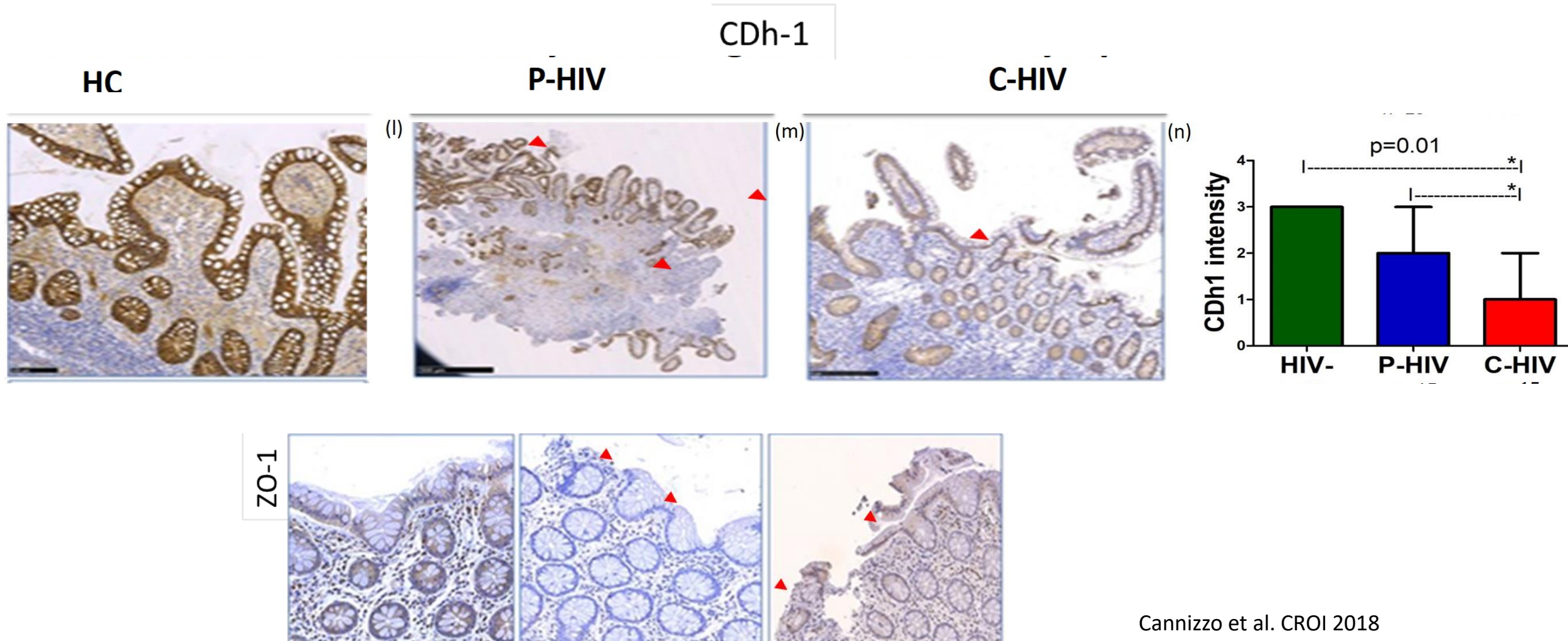


- HIV-1
- Commensal bacteria
- CD4+T cell
- CD8+T cell
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ZO-1 staining after gp120 treatment and Tat treatment



3. Loss of tight junctions.....



....that is not reverted by cART

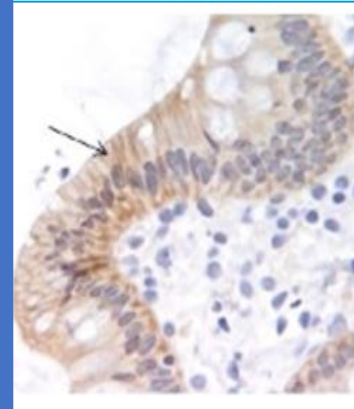
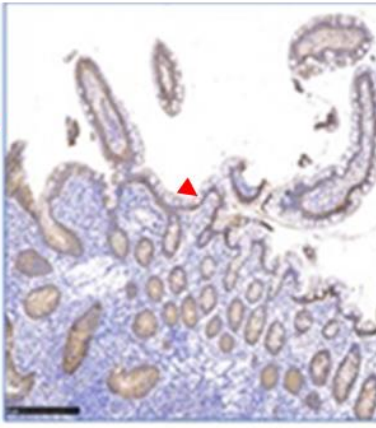
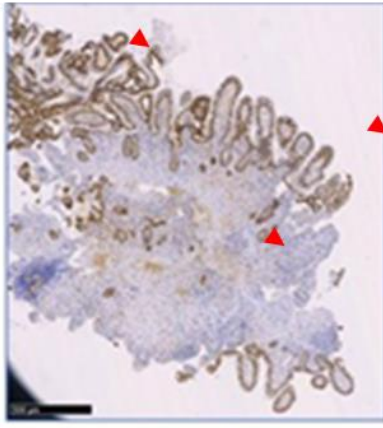
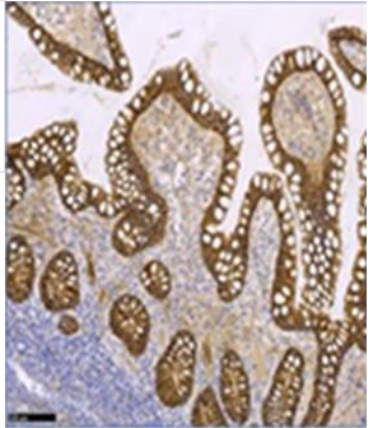
HC

P-HIV

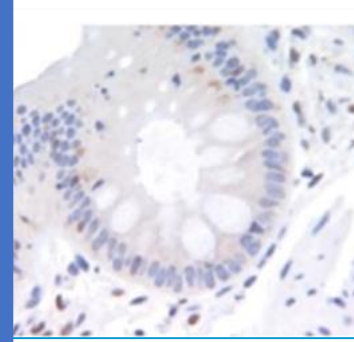
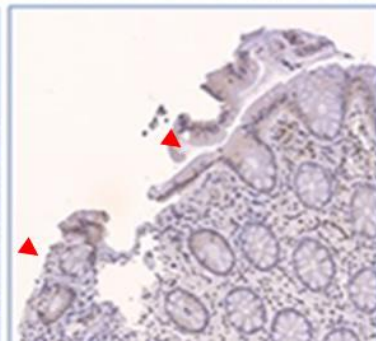
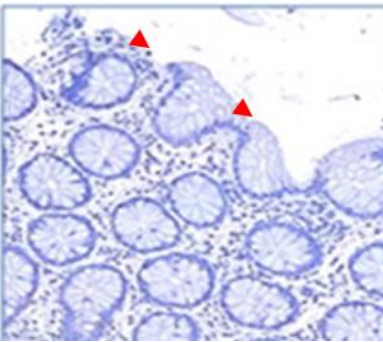
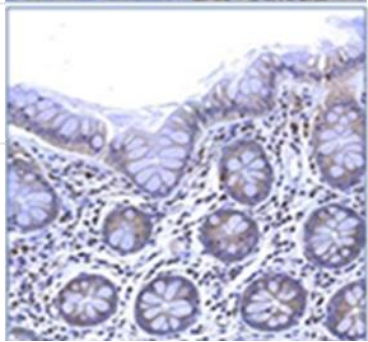
C-HIV

cART

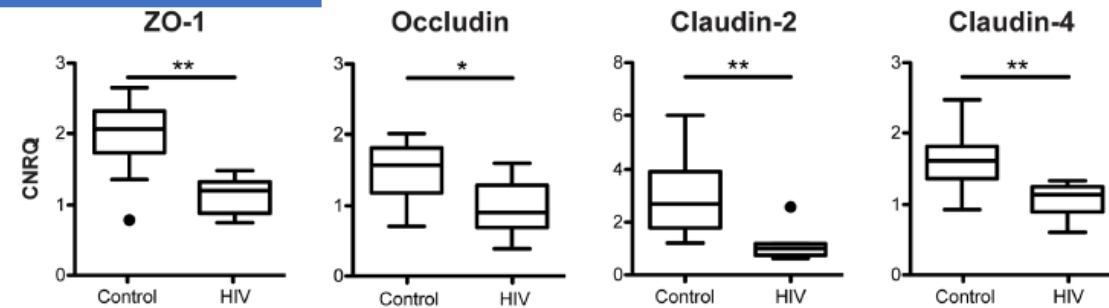
CDh-1



ZO-1

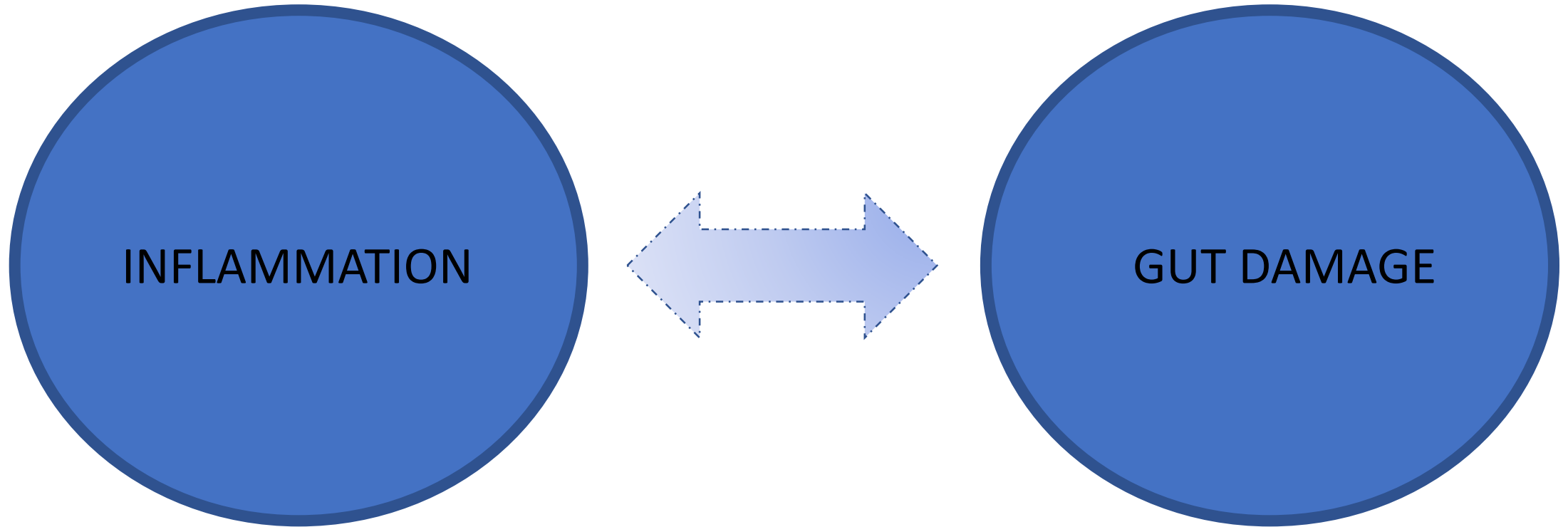


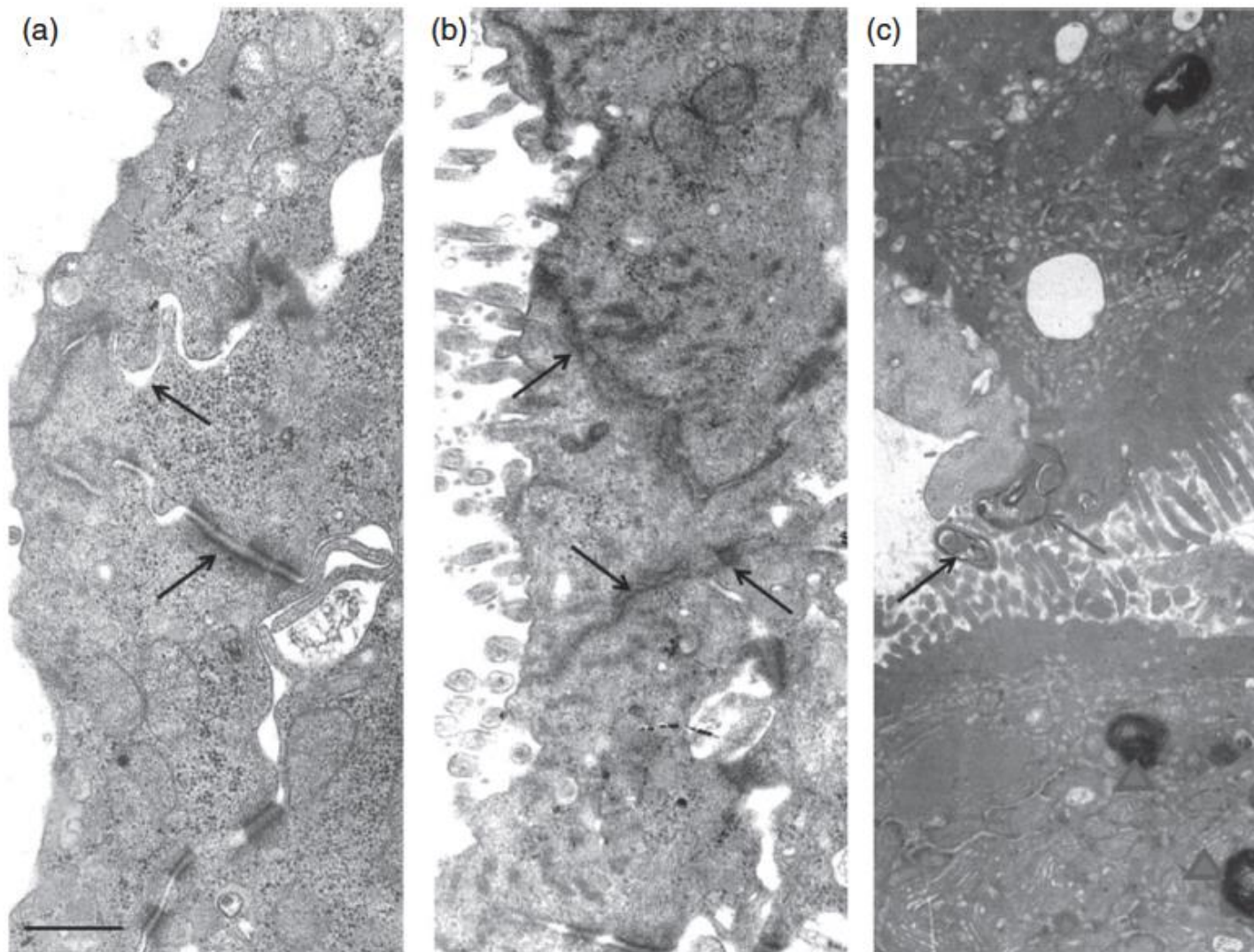
Tincati et al. AIDS 2016;
See also Somsouk et al, AIDS 2015



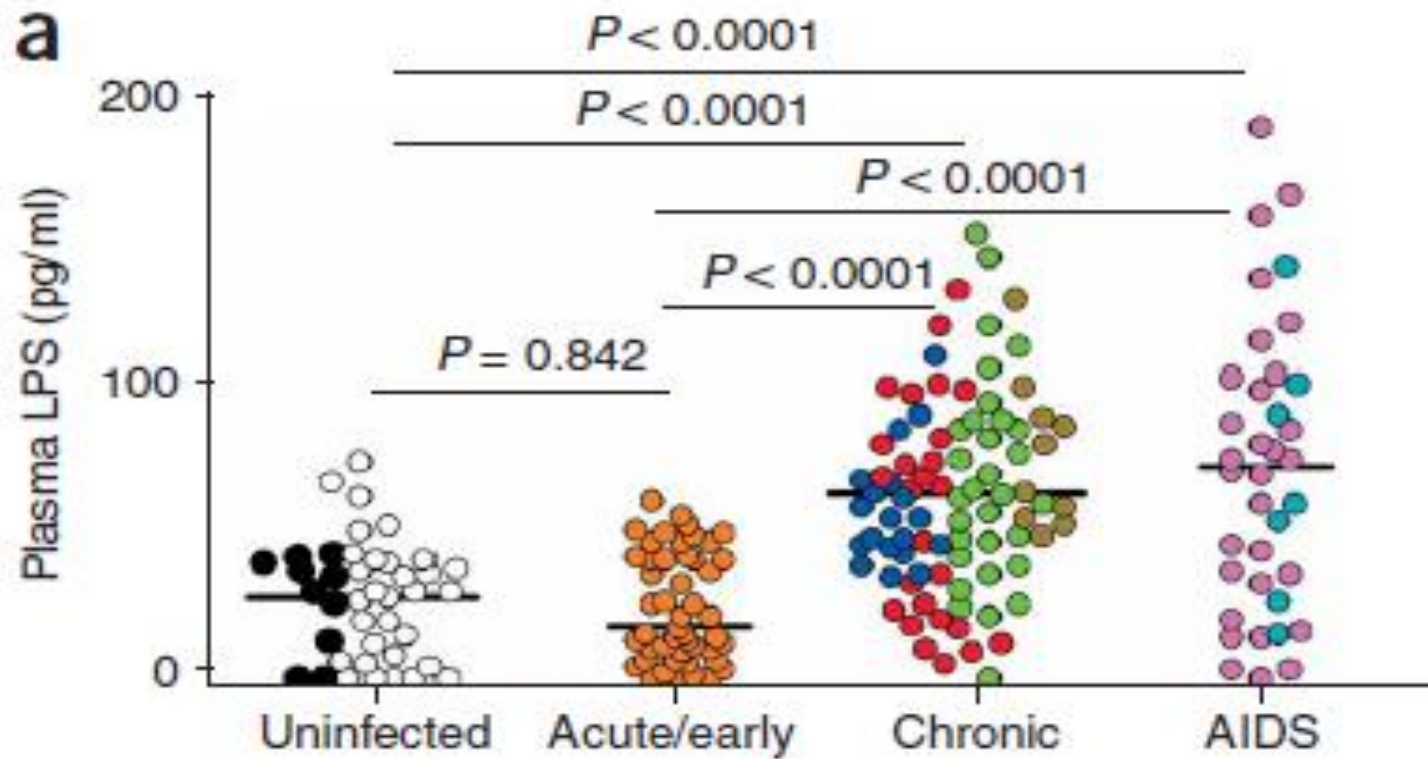
Chung et al., Plos Path , 2014

HIV: INFLAMMATION AND GUT DAMAGE



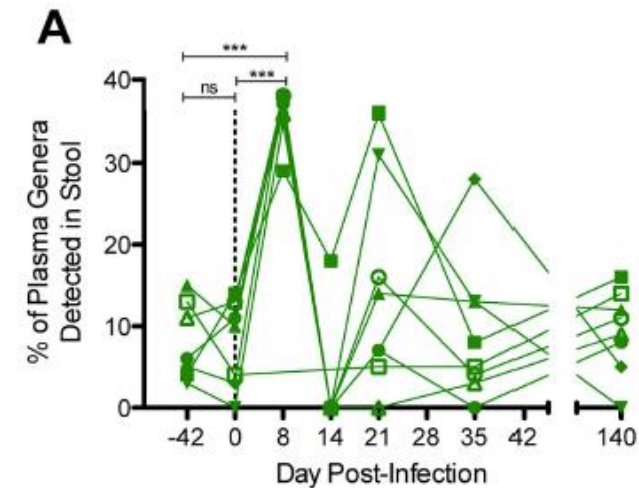
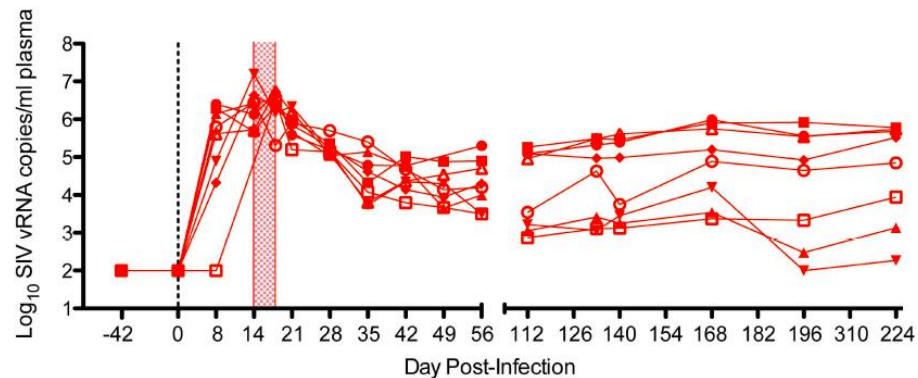
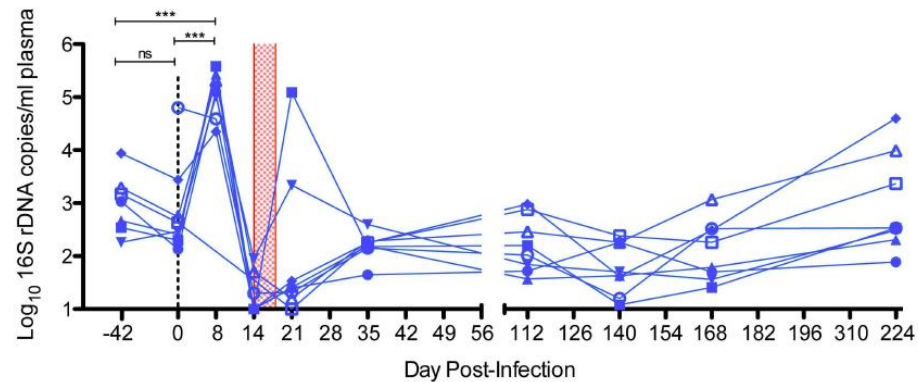
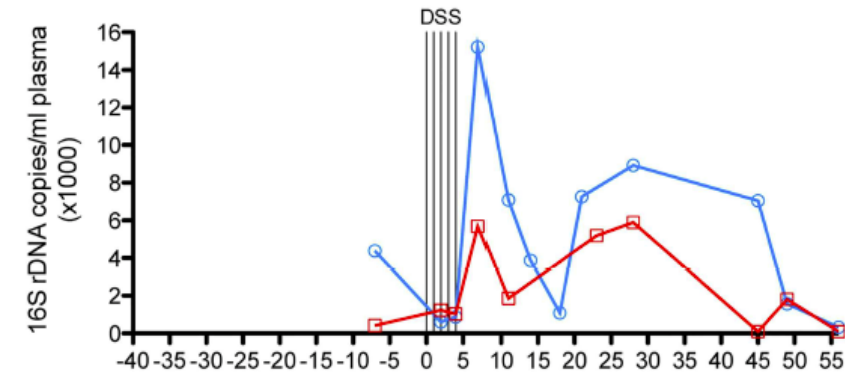
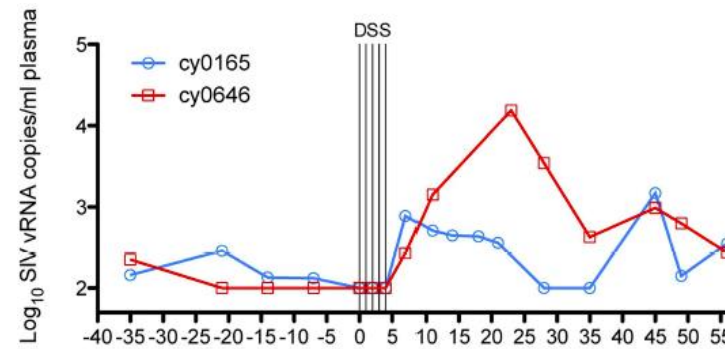


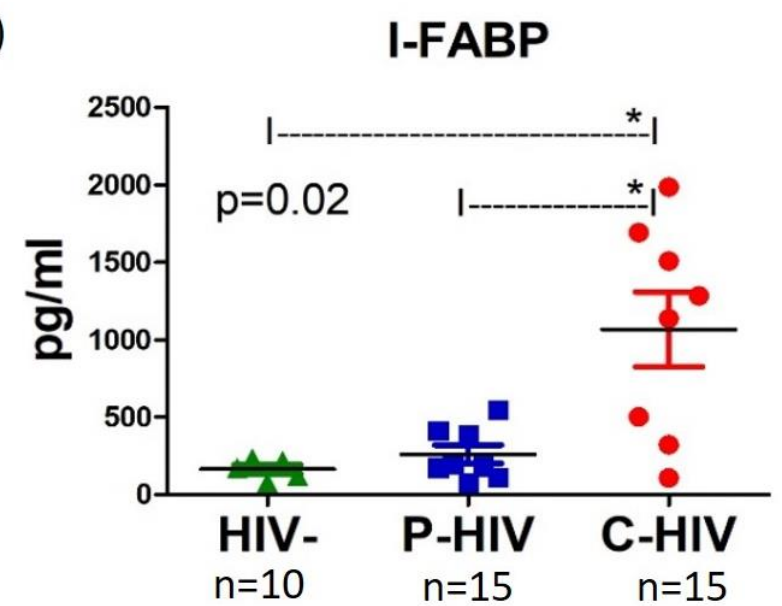
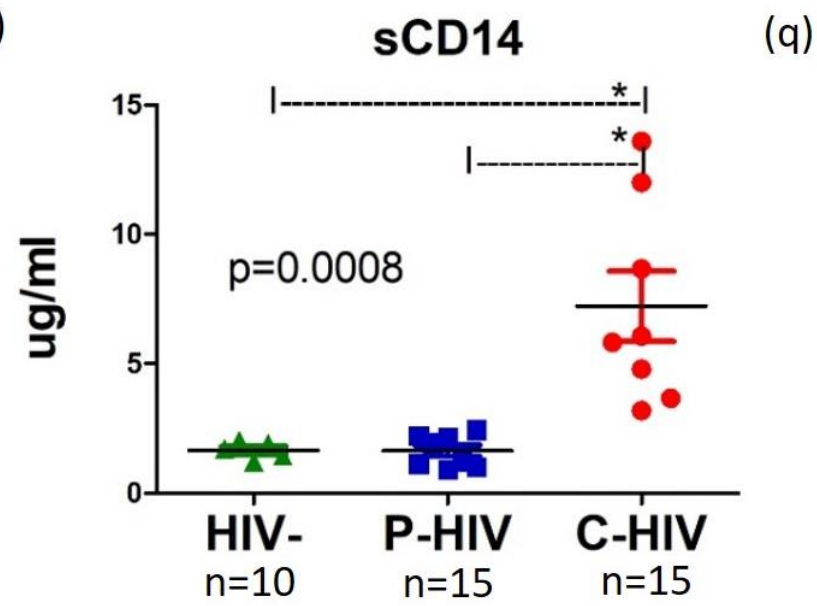
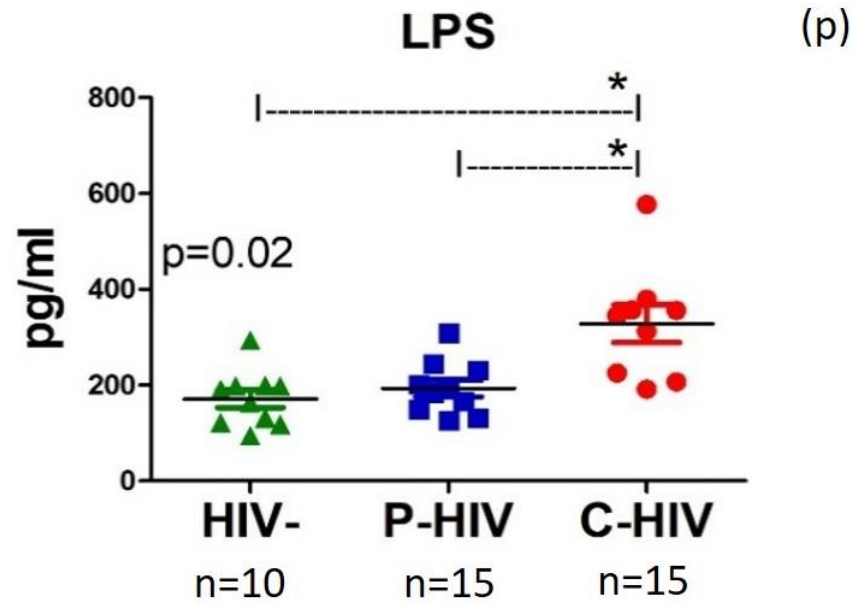
Elevated microbial translocation in all stages of HIV infection



Hyperacute microbial translocation precedes viremia

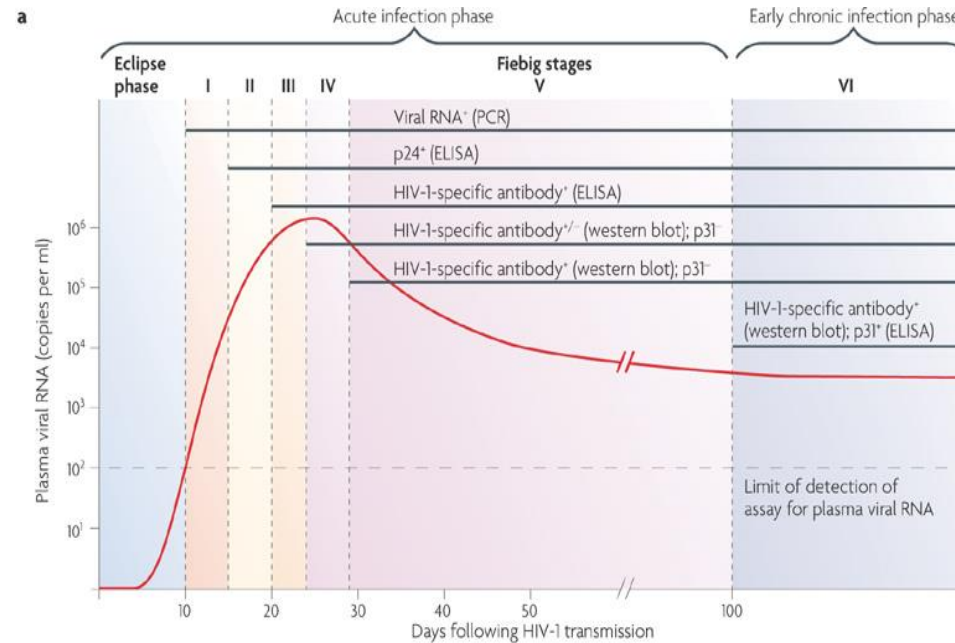
8 macaques infected with SIVmac239





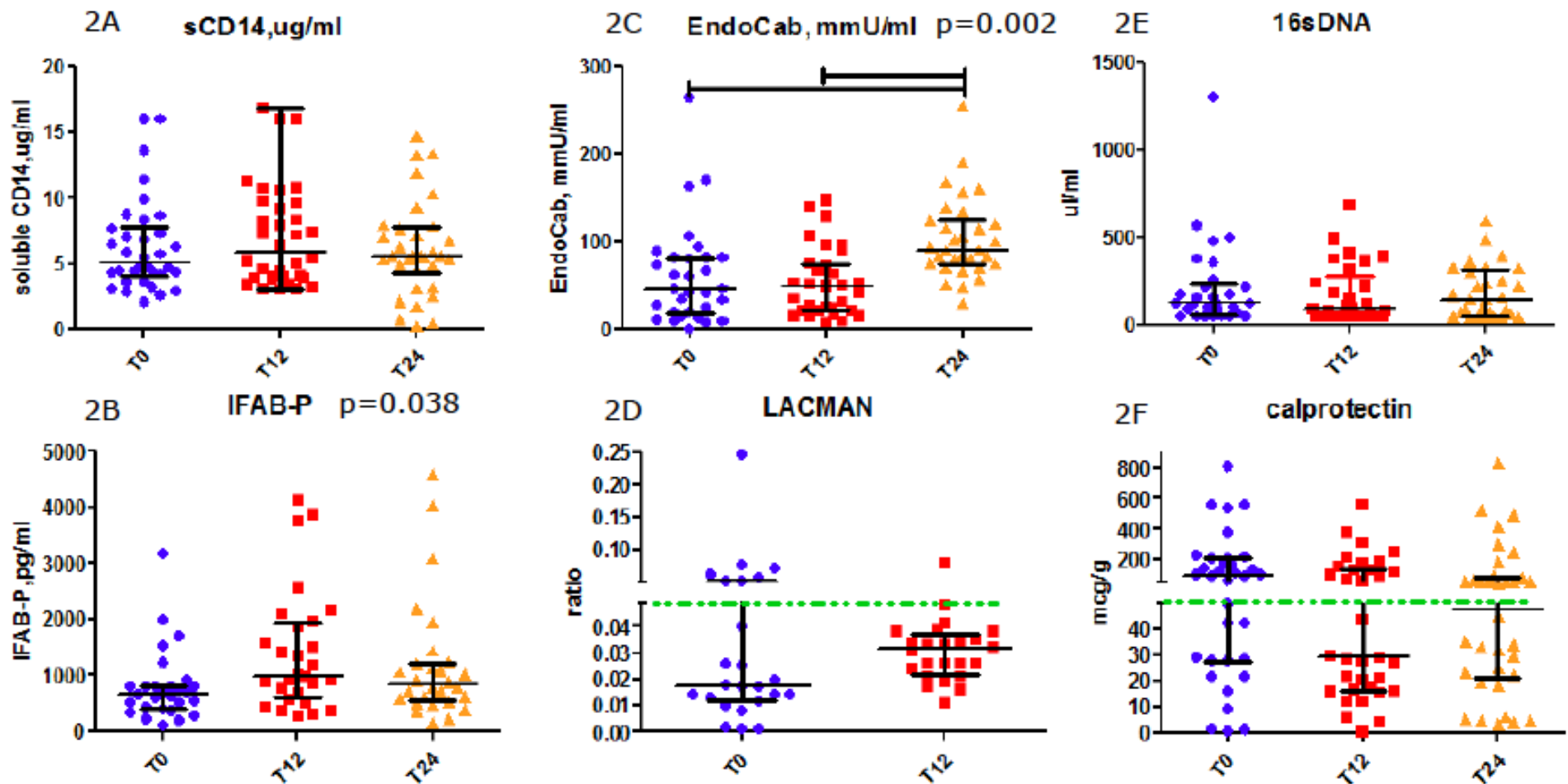
Fiebig Stage, n

I-II	2
III	5
IV-V	8 (3 IV; 5 V)

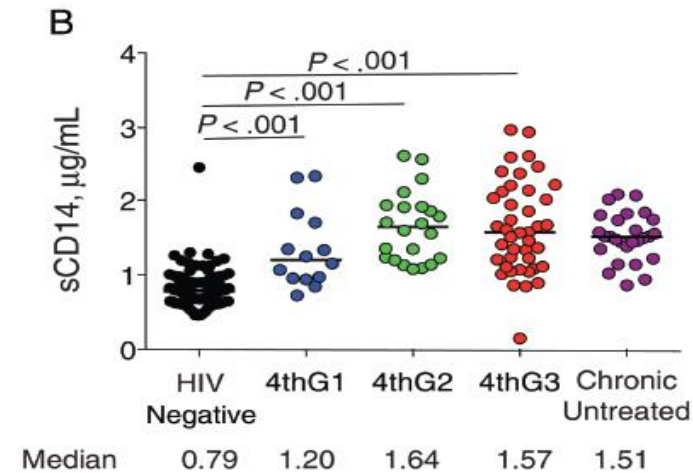
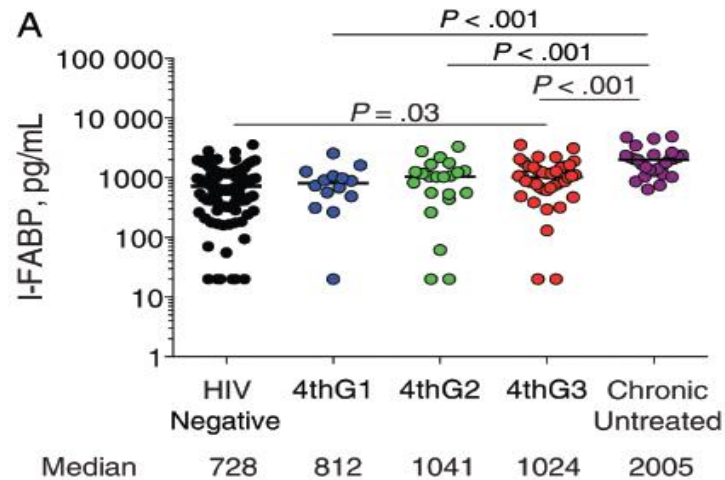


Persistent microbial translocation and gut damage on long-term cART started during chronic HIV

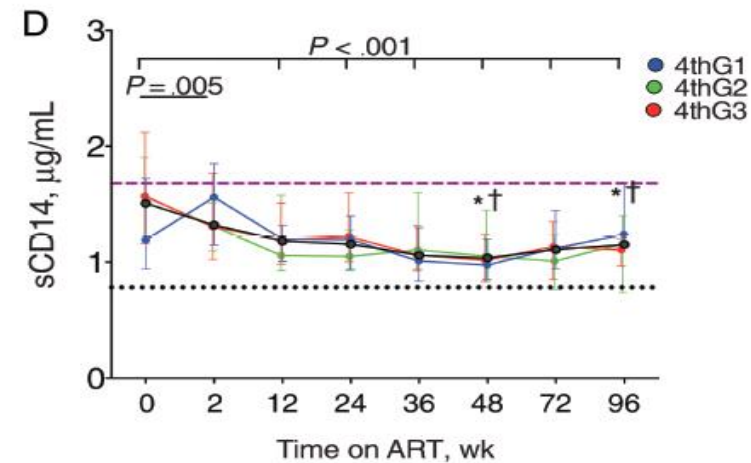
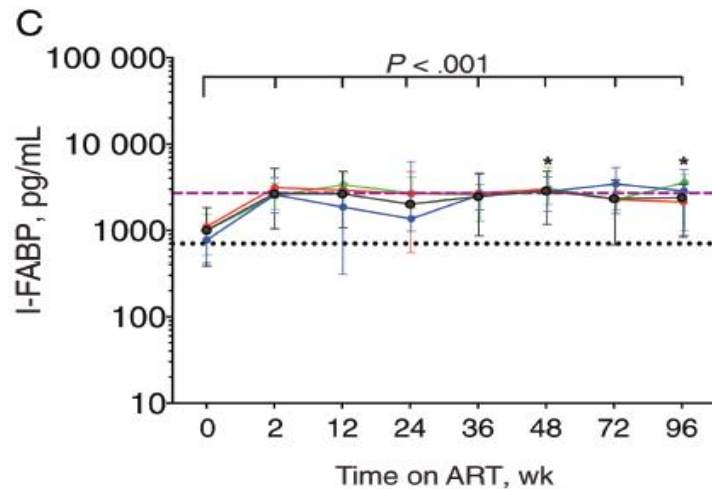
40 HIV+, nadir CD4 =300/ μ l



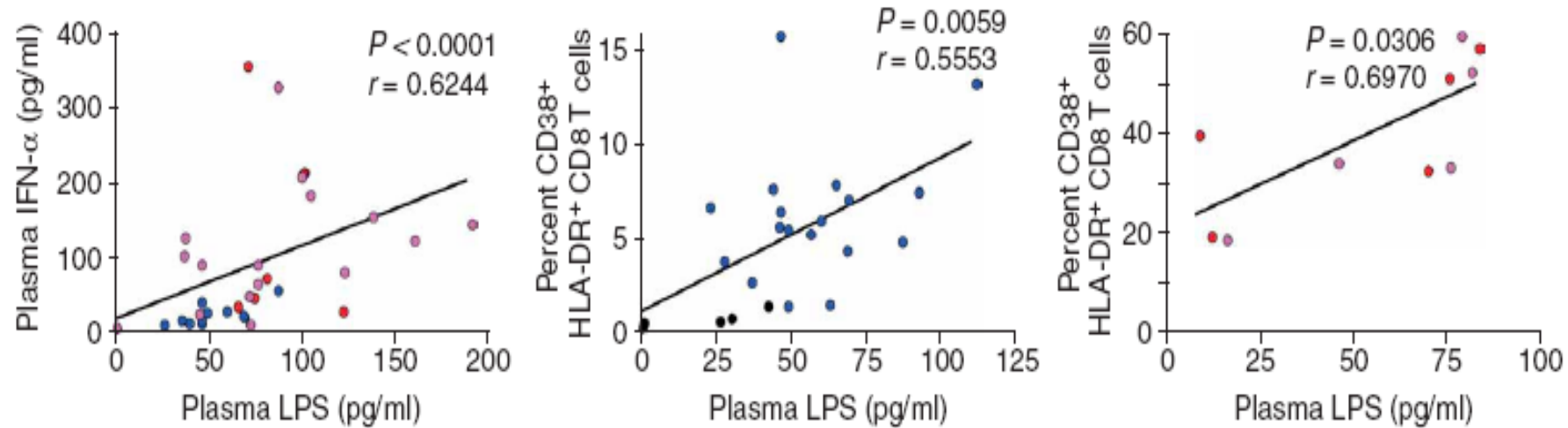
Persistent enterocyte turnover and partial reduction of monocyte activation in patients treated during acute HIV



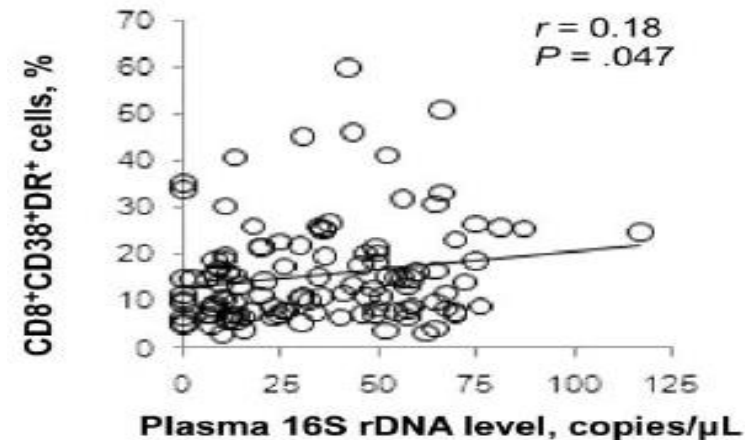
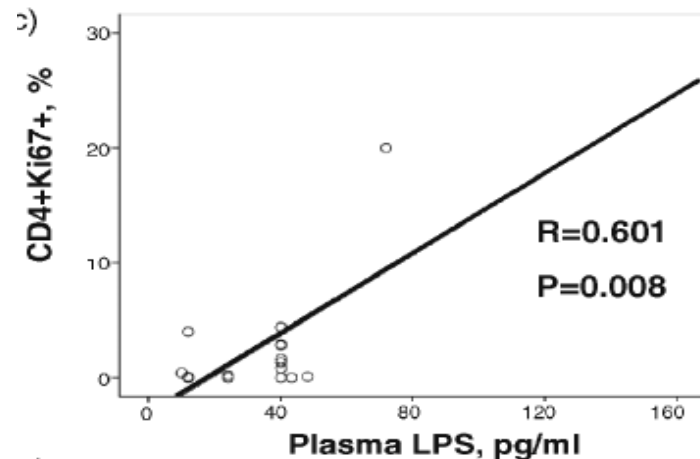
N=78



Microbial translocation drives immune activation

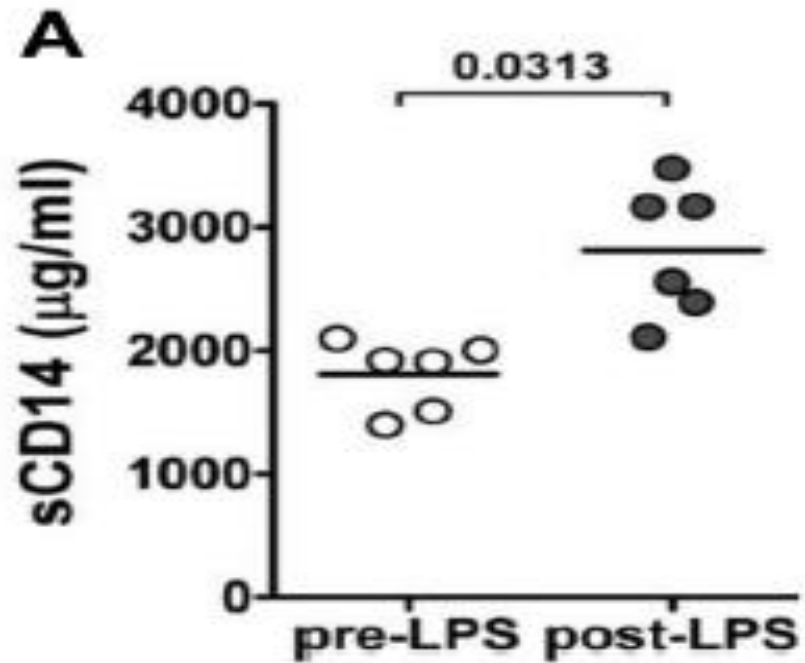


Brenchley J et al. Nat Med 2006



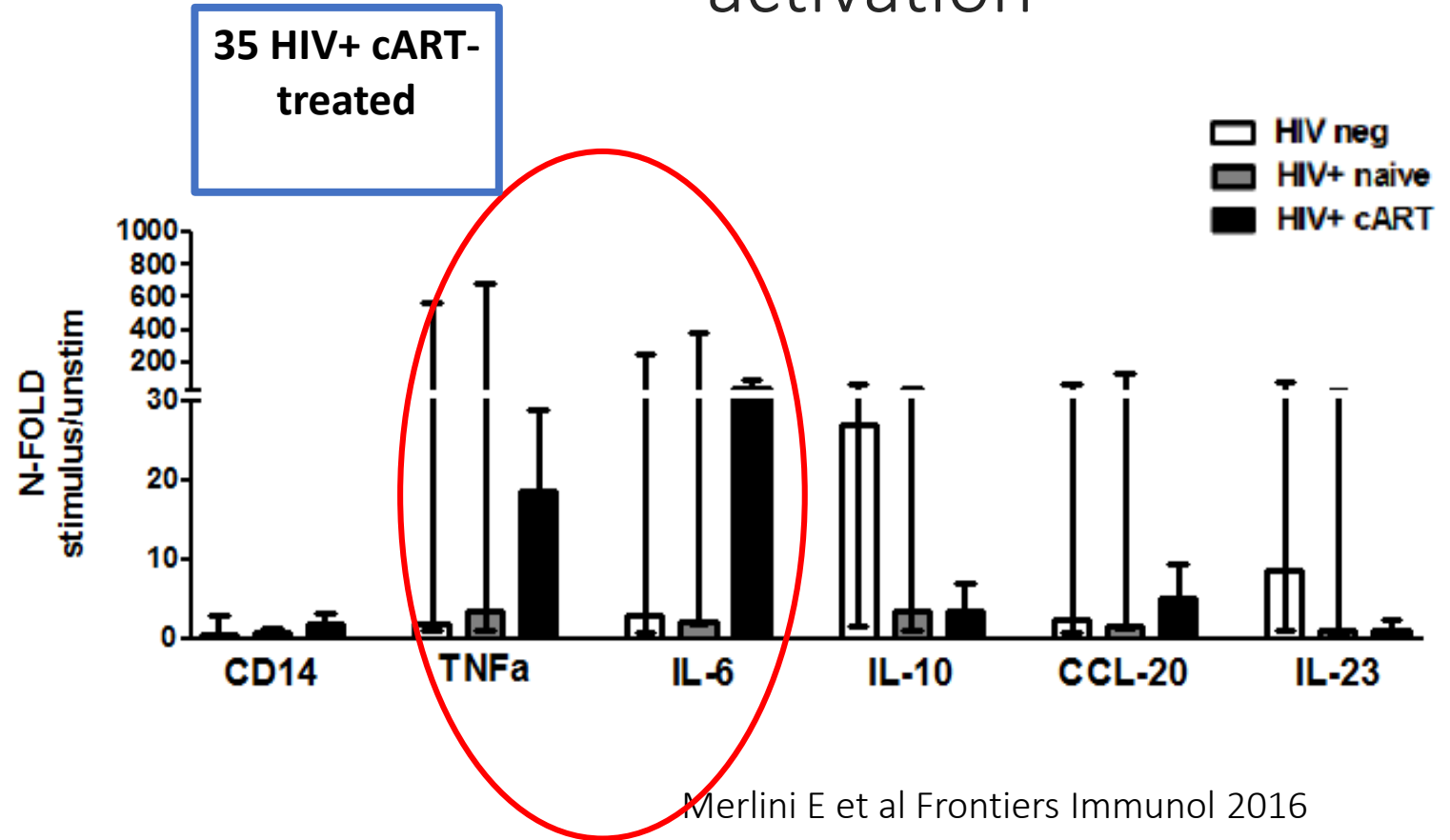
Marchetti G et al. AIDS 2008; Jiang et al. J Infect Dis 2009

In vivo LPS administration
enhances immune
activation



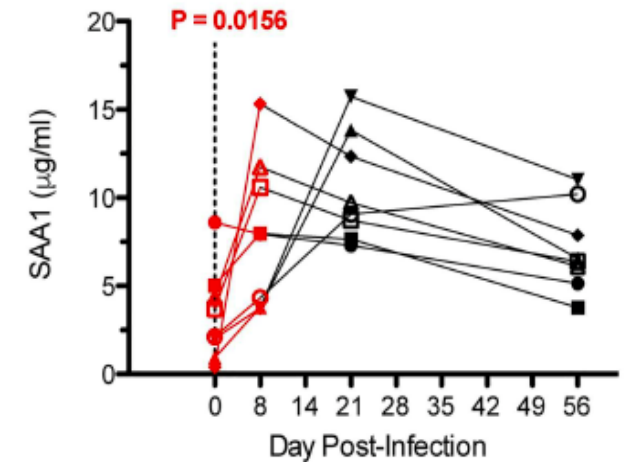
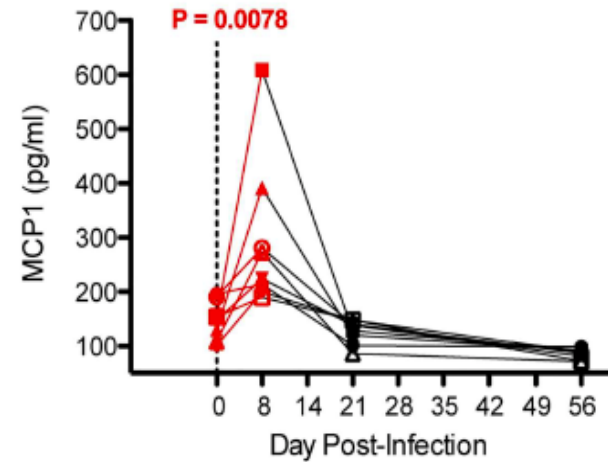
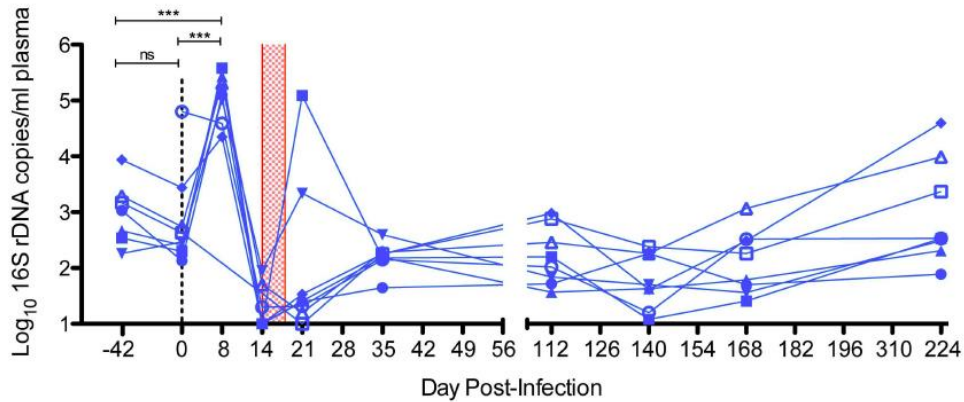
Pandrea et al Blood 2012

Ex vivo LPS stimulation
enhances monocyte
activation

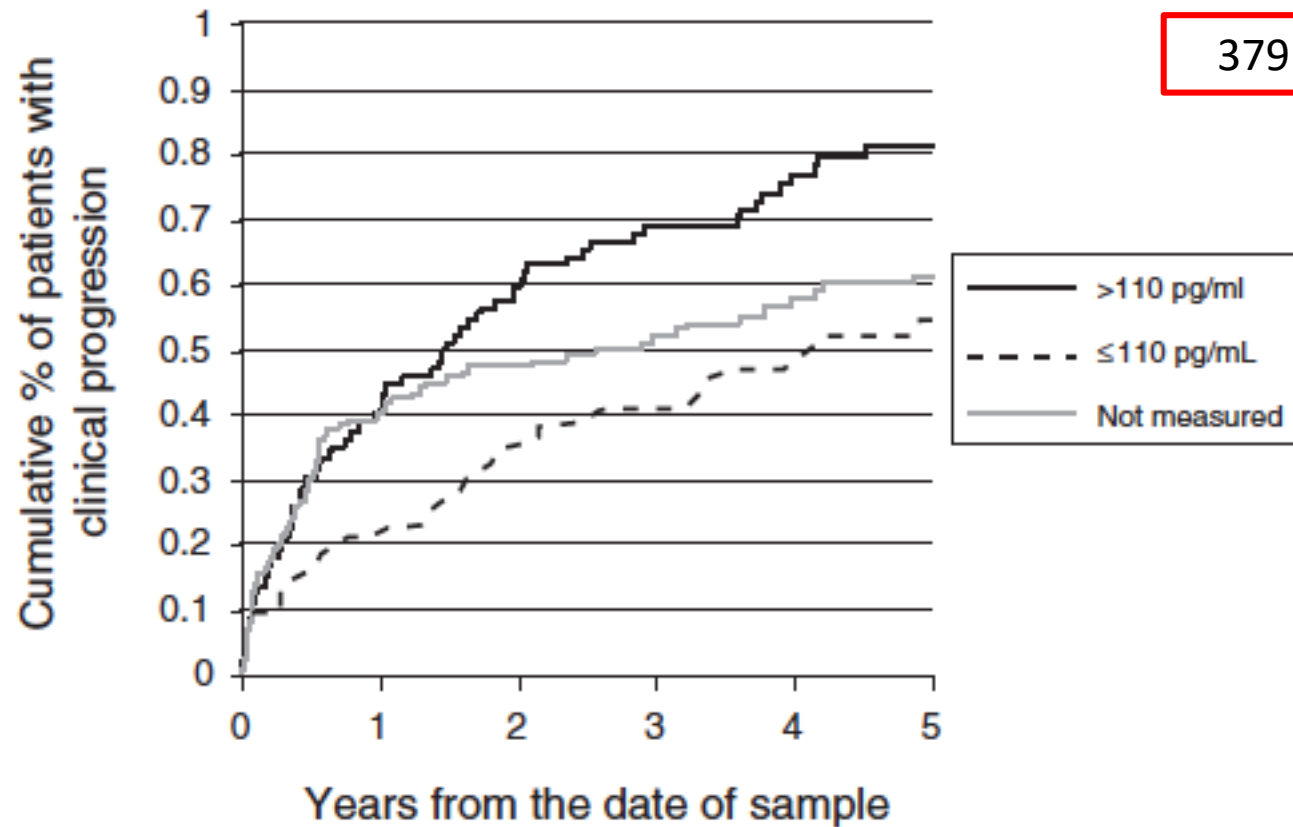


Hyperacute microbial translocation precedes HIV viremia

8 macaques infected with SIVmac239



Microbial translocation drives disease progression in untreated HIV



379 patients

Marchetti et al. AIDS 2010;
See also Sandler N et al. JID 2011

**Crude and adjusted relative hazards of AIDS/death or non-AIDS
Severe Events (SNAEs) in total population**

Crude RH (95% CI)	p- value	Adjusted* RH (95% CI)	p- value	Adjusted** RH (95% CI)	p- value
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(a) Biomarkers fitted as categorical variables

LPS, pg/ml						
≤250.8	1.0		1.0		1.0	
>250.8	0.85 (0.53, 1.36)	0.495	0.76 (0.46, 1.26)	0.292	0.88 (0.52, 1.49)	0.643
not measured	1.09 (0.72, 1.65)	0.681	1.11 (0.72, 1.73)	0.631	1.22 (0.77, 1.92)	0.400
sCD14, ug/ml						
≤2.83	1.0		1.0		1.0	
>2.83	1.10 (0.77, 1.58)	0.586	0.95 (0.65, 1.39)	0.805	0.88 (0.59, 1.29)	0.504
not measured	1.26 (0.40, 4.03)	0.695	0.87 (0.26, 2.86)	0.819	1.44 (0.41, 5.02)	0.570
EndoCAb, MMU/ml						
≤36.5	1.0		1.0		1.0	
>36.5	0.75 (0.52, 1.06)	0.107	0.82 (0.56, 1.19)	0.296	0.84 (0.57, 1.24)	0.378
not measured	0.22 (0.03, 1.56)	0.129	0.19 (0.03, 1.38)	0.100	0.18 (0.02, 1.40)	0.100
hs-CRP, mg/L						
≤1.51	1.0		1.0		1.0	
>1.51	1.52 (1.01, 2.29)	0.044	1.47 (0.96, 2.26)	0.077	1.54 (0.99, 2.39)	0.056
not measured	1.19 (0.74, 1.93)	0.478	1.26 (0.76, 2.07)	0.369	1.28 (0.77, 2.13)	0.345

(b) Biomarkers fitted as continuous variables in the log_e scale

LPS, pg/ml						
per log _e higher	0.99 (0.73, 1.35)	0.971	0.94 (0.68, 1.31)	0.729	0.72 (0.46, 1.12)	0.147
sCD14, ug/ml						
per log _e higher	1.17 (0.84, 1.63)	0.342	0.95 (0.64, 1.40)	0.798	0.79 (0.41, 1.51)	0.471
EndoCAb, MMU/ml						
per log _e higher	0.87 (0.70, 1.07)	0.195	0.94 (0.75, 1.17)	0.567	1.03 (0.70, 1.52)	0.874
hs-CRP, mg/L						
per log _e higher	1.12 (0.99, 1.27)	0.065	1.07 (0.94, 1.21)	0.334	1.06 (0.89, 1.25)	0.539



486 patients, 125
clinical events (>50%
SNAEs)

**Markers measured
pre-cART**

Marchetti et al. EACS 2015, manuscript
submitted

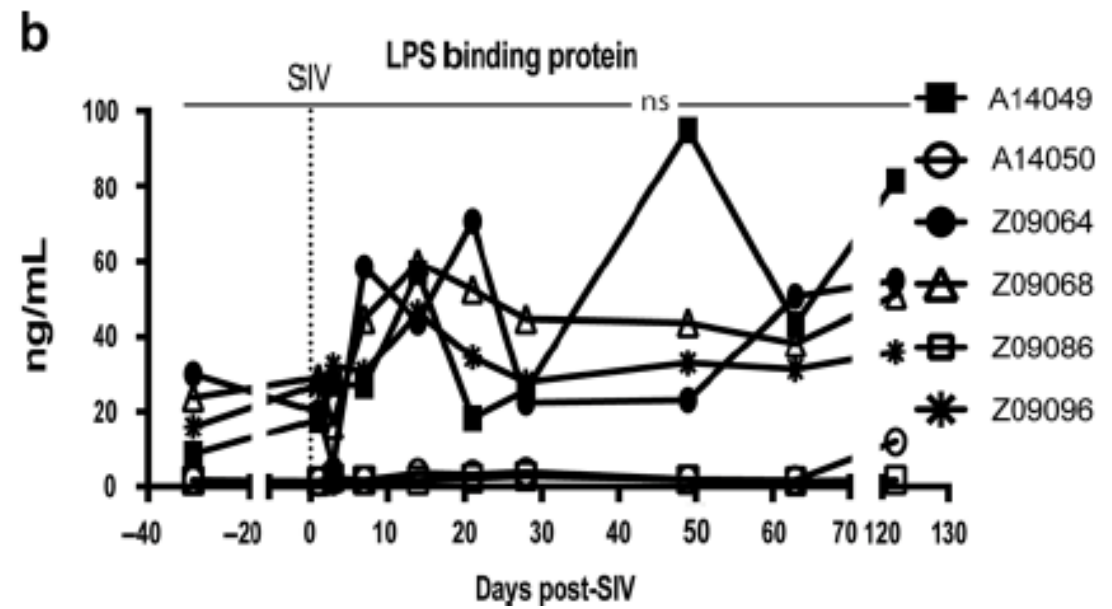
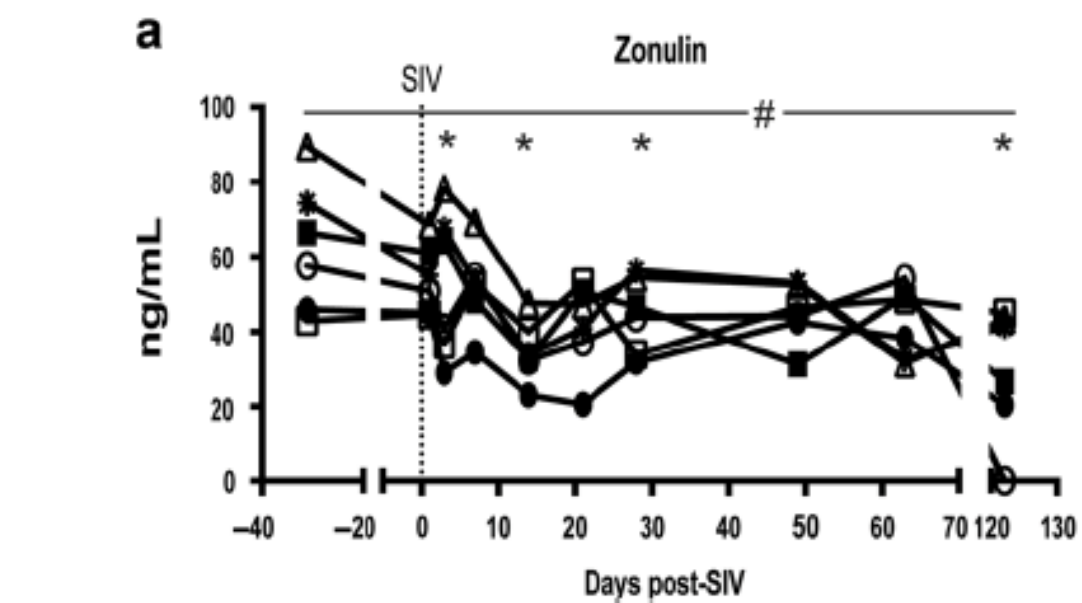
Table 2. Soluble Biomarker Predictors of Mortality Among 192 Participants in the Longitudinal Study of the Ocular Complications of AIDS Who Had Antiretroviral Therapy–Suppressed Human Immunodeficiency Virus Infection

Characteristic, Analysis ^a	OR (95% CI) for Death, by Quartile ^b						OR per IQR Increase (95% CI)	
	Second	P Value	Third	P Value	Fourth	P Value		P Value
Proximal CD4 ⁺ T-cell count, cells/mm ³								
Primary	0.50 (.22–1.1)	.099	0.41 (.17–.98)	.045	0.44 (.18–1.1)	.076	0.62 (.40–.95)	.030
I-FABP level, pg/mL								
Primary	1.76 (.61–5.1)	.30	4.5 (1.5–13.3)	.007	8.3 (2.8–25.1)	<.001	3.5 (2.0–6.1)	<.001
Adjusted	1.69 (.56–5.1)	.35	4.2 (1.4–12.8)	.011	8.6 (2.7–27.8)	<.001	3.5 (1.9–6.1)	<.001
Zonulin-1 level, ng/mL								
Primary	0.29 (.12–.69)	0.005	0.21 (.08–.53)	.001	0.24 (.09–.60)	.002	0.43 (.28–.64)	<.001
Adjusted	0.28 (.12–.69)	.005	0.20 (.08–.53)	.001	0.25 (.09–.64)	.004	0.43 (.28–.66)	<.001
sCD14 level, µg/mL								
Primary	2.7 (.82–9.1)	.10	7.7 (2.3–25.7)	.001	17.6 (4.4–55.1)	<.001	5.4 (2.8–10.4)	<.001
Adjusted	4.5 (1.09–18.6)	.038	11.4 (2.9–46)	.001	30.1 (6.2–145)	<.001	7.5 (3.4–16.5)	<.001
KT ratio, nM/µM								
Primary	1.48 (.51–4.3)	.47	2.3 (.82–6.5)	.11	4.6 (1.72–12.3)	.002	2.3 (1.45–3.5)	<.001
Adjusted	1.50 (.50–4.4)	.47	2.4 (.82–6.9)	.11	4.3 (1.51–12.4)	.006	2.3 (1.40–3.7)	.001
IL-6 level, pg/mL								
Primary	6.4 (1.33–30.6)	.020	9.8 (1.89–50.5)	.007	69.7 (12.4–392)	<.001	6.1 (2.9–12.9)	<.001
Adjusted	12.0 (1.42–102)	.023	17.8 (2.1–154)	.009	139 (14–1362)	<.001	6.6 (2.9–15.0)	<.001
sTNF-RI level, pg/mL								
Primary	1.42 (.44–4.5)	.55	3.8 (1.3–11.0)	.012	9.0 (3.1–26)	<.001	4.6 (2.5–8.5)	<.001
Adjusted	1.25 (.38–4.2)	.71	3.6 (1.23–10.8)	.02	10.2 (3.2–32)	<.001	5.2 (2.6–10.4)	<.001
hsCRP level, ng/mL								
Primary	1.61 (.57–4.6)	.37	2.1 (.78–5.6)	.14	10.9 (3.7–33)	<.001	3.7 (2.1–6.7)	<.001
Adjusted	1.58 (.53–4.7)	.42	2.3 (.81–6.6)	.12	10.9 (3.4–35)	<.001	3.8 (2.0–7.0)	<.001
D-dimer level, ng/mL								
Primary	1.33 (.35–5.1)	.68	5.9 (1.80–19.1)	.003	30.3 (7.2–128)	<.001	7.7 (3.6–16.7)	<.001
Adjusted	1.23 (.31–4.9)	.77	6.2 (1.8–21.3)	.003	29.4 (6.6–131)	<.001	7.7 (3.5–17.3)	<.001
CMV IgG index								
Primary	0.42 (.17–1.05)	.064	0.54 (.22–1.30)	.17	1.37 (.58–3.2)	0.47	0.91 (.80–1.05)	.19
Adjusted	0.38 (.15–.98)	.045	0.43 (.17–1.12)	.085	1.07 (.42–2.7)	.89	0.89 (.77–1.03)	.11

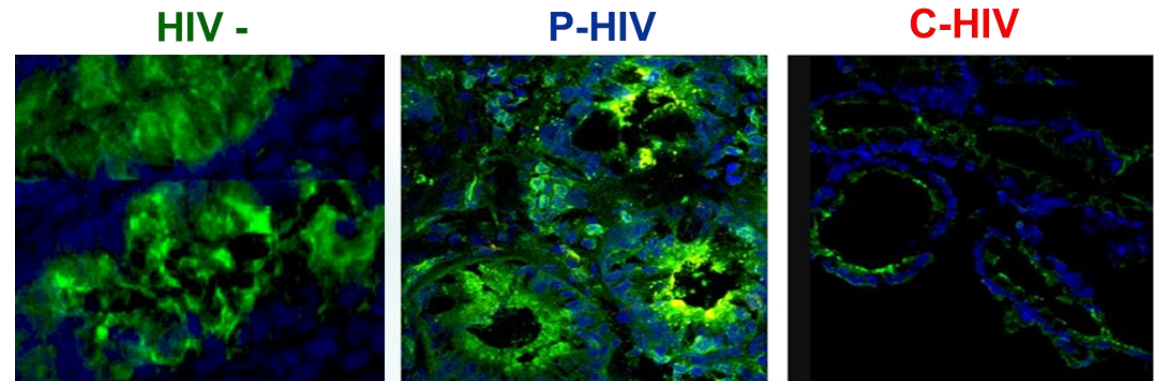
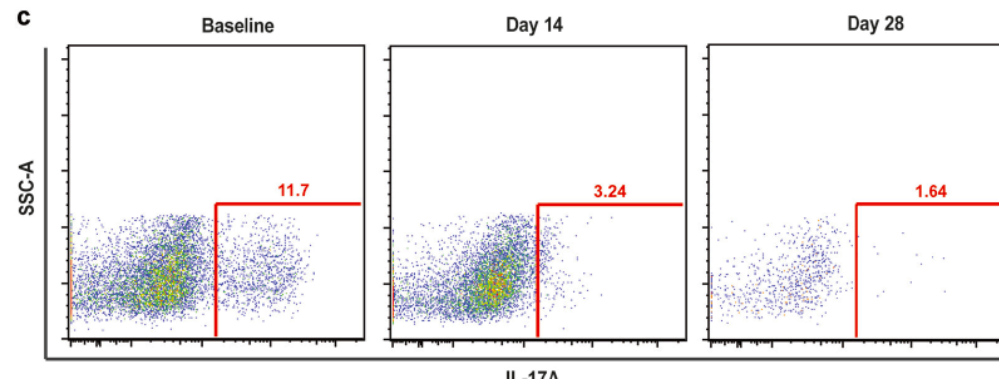
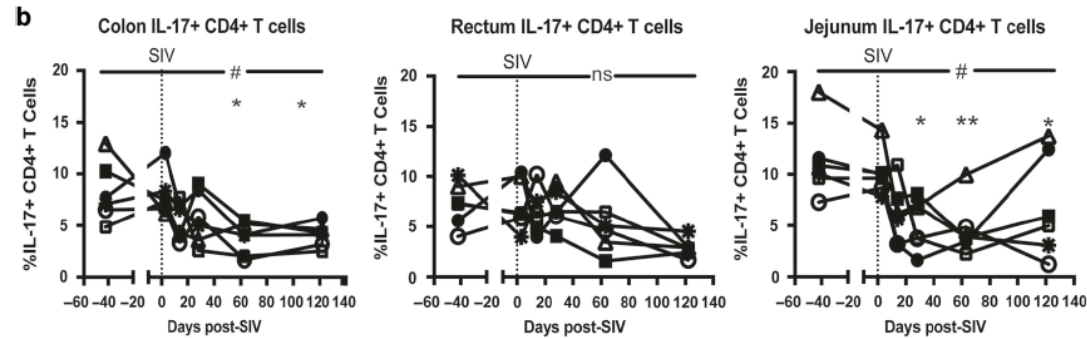
192 patients

**Markers measured
on cART**

**Immune and epithelial
damage: what comes first?**

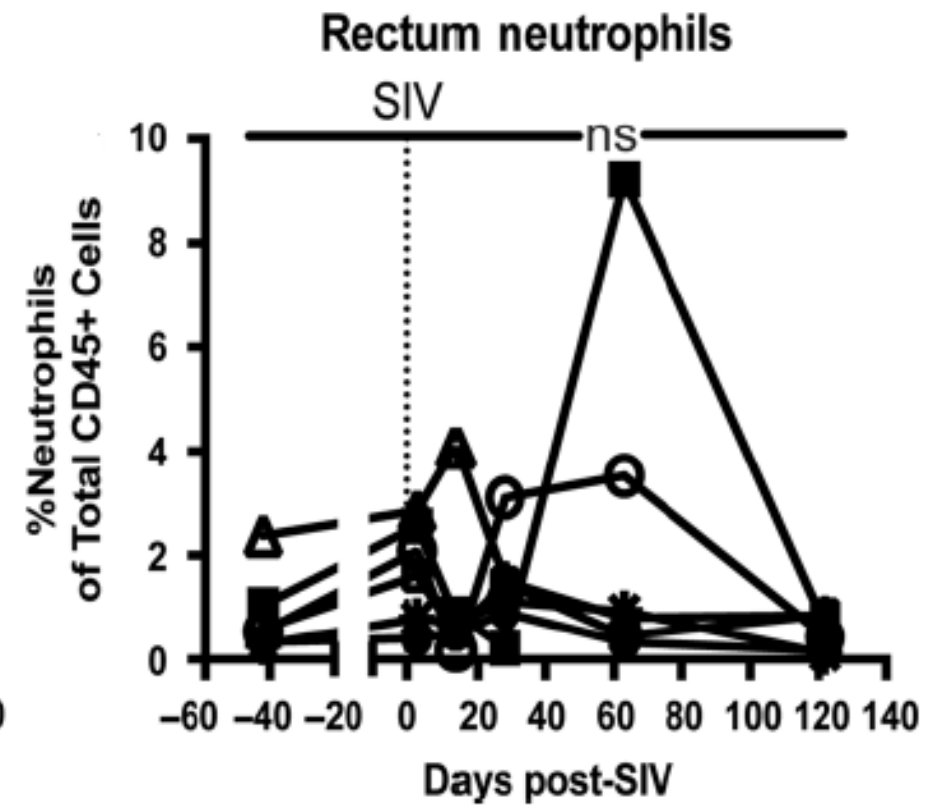
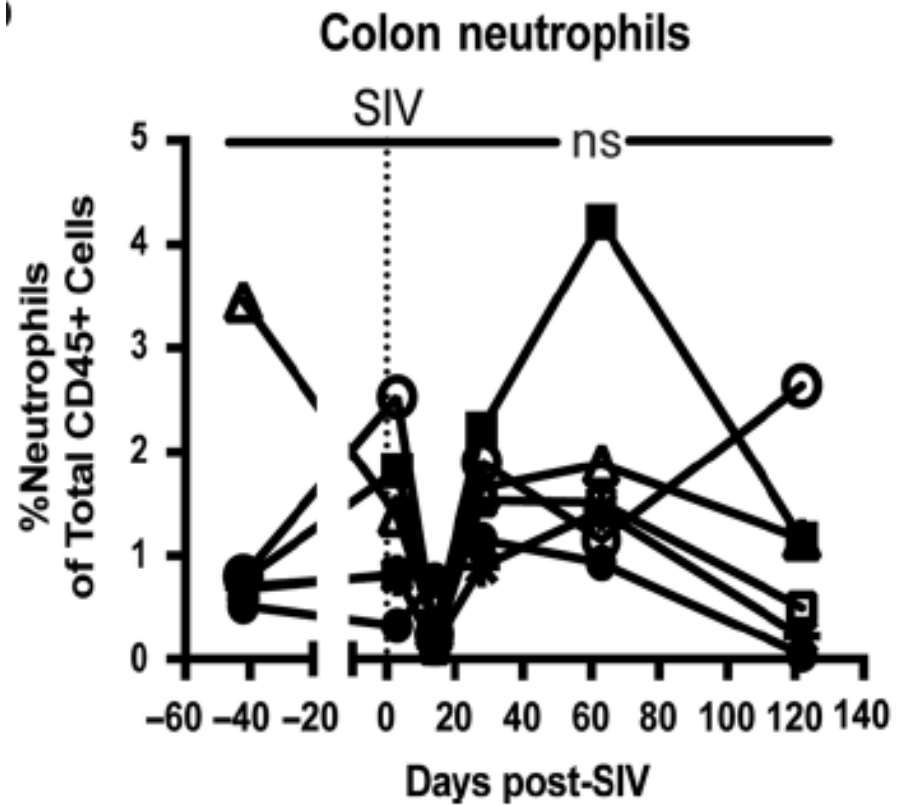


Hensley-McBain et al. Mucosal immunol 2018

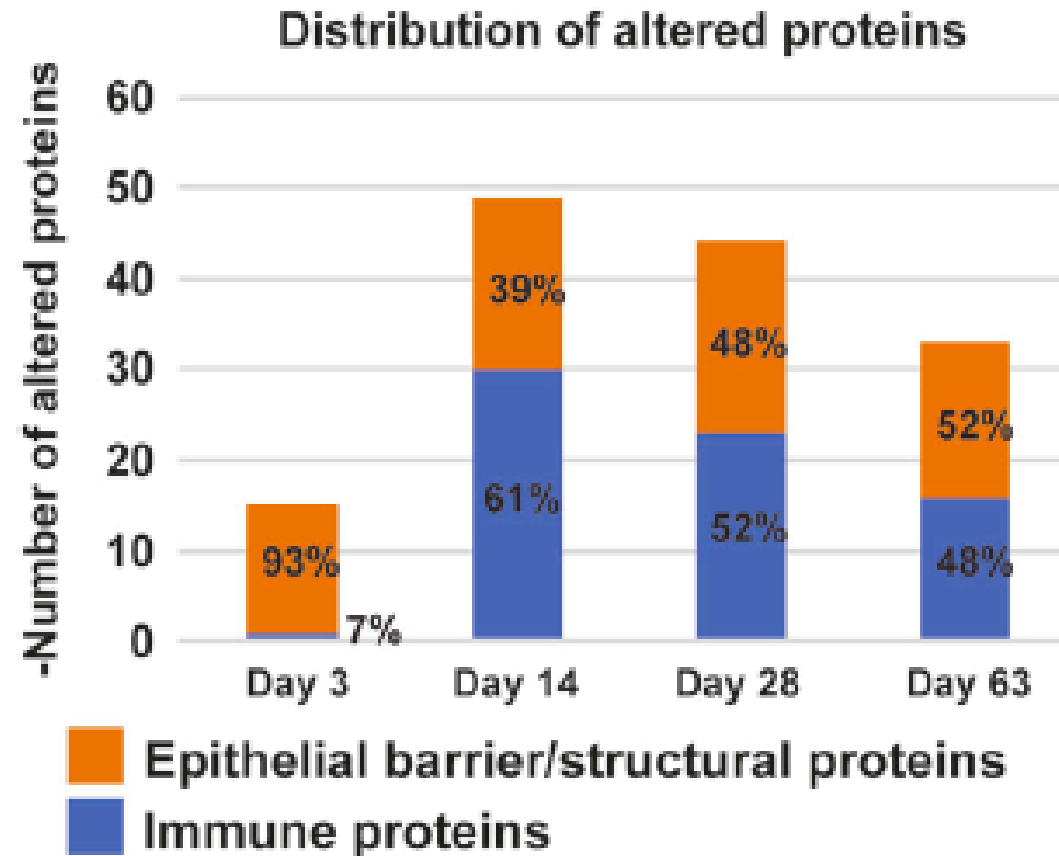


Confocal images
20x DAPI- $\gamma\delta$

Cannizzo CROI 2018, manuscript in preparation

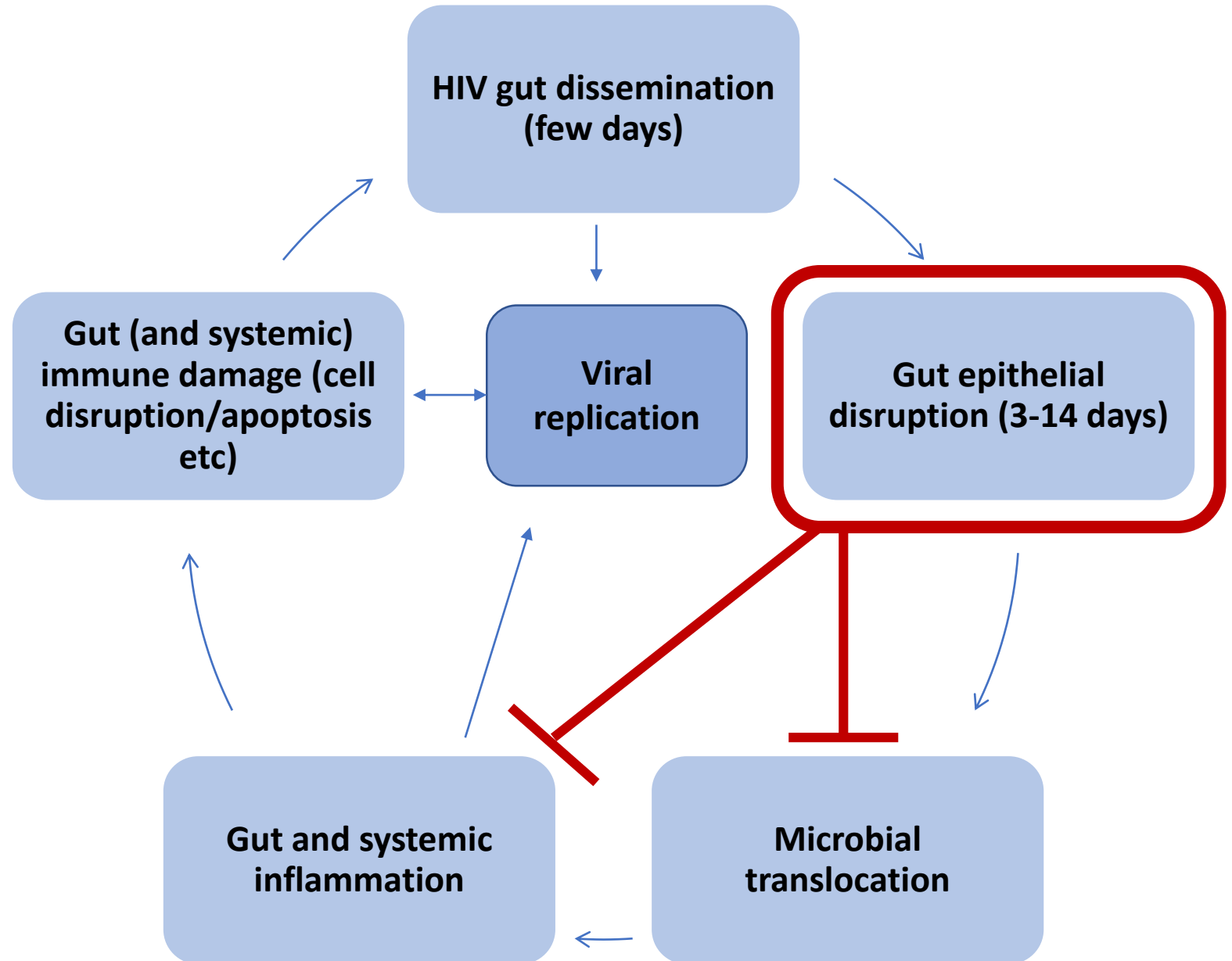


Proteins involved in epithelial structure are altered prior to proteins involved in immune responses (mass spectrometry to assess colon proteome)



The tempo of a mucosal disaster

Lead to future therapeutic strategies?



Thanks

*Dept of Health Sciences-
Clinic of Infectious Diseases-
Univ of Milan, San Paolo H

Elvira S Cannizzo
Esther Merlini
Giuseppe Ancona
Francesca Bai
Camilla Tincati
Antonella d'Arminio Monforte
*****all the patients and staff**



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Stefano Rusconi, Massimo Galli



Alessandro Cozzi-Lepri, Antonella d'Arminio Monforte