The Gut as a Site of Immunepathogenesis

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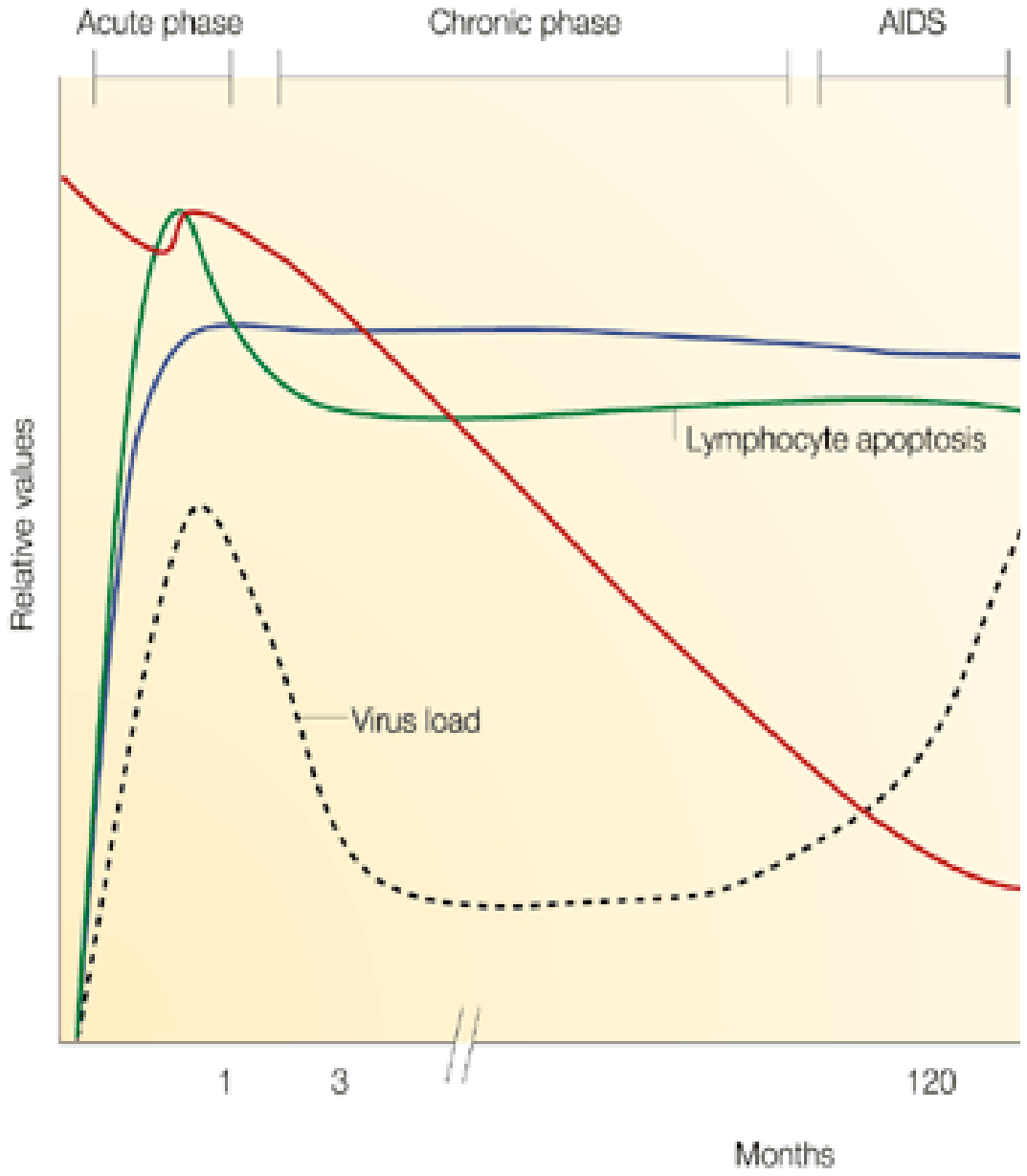
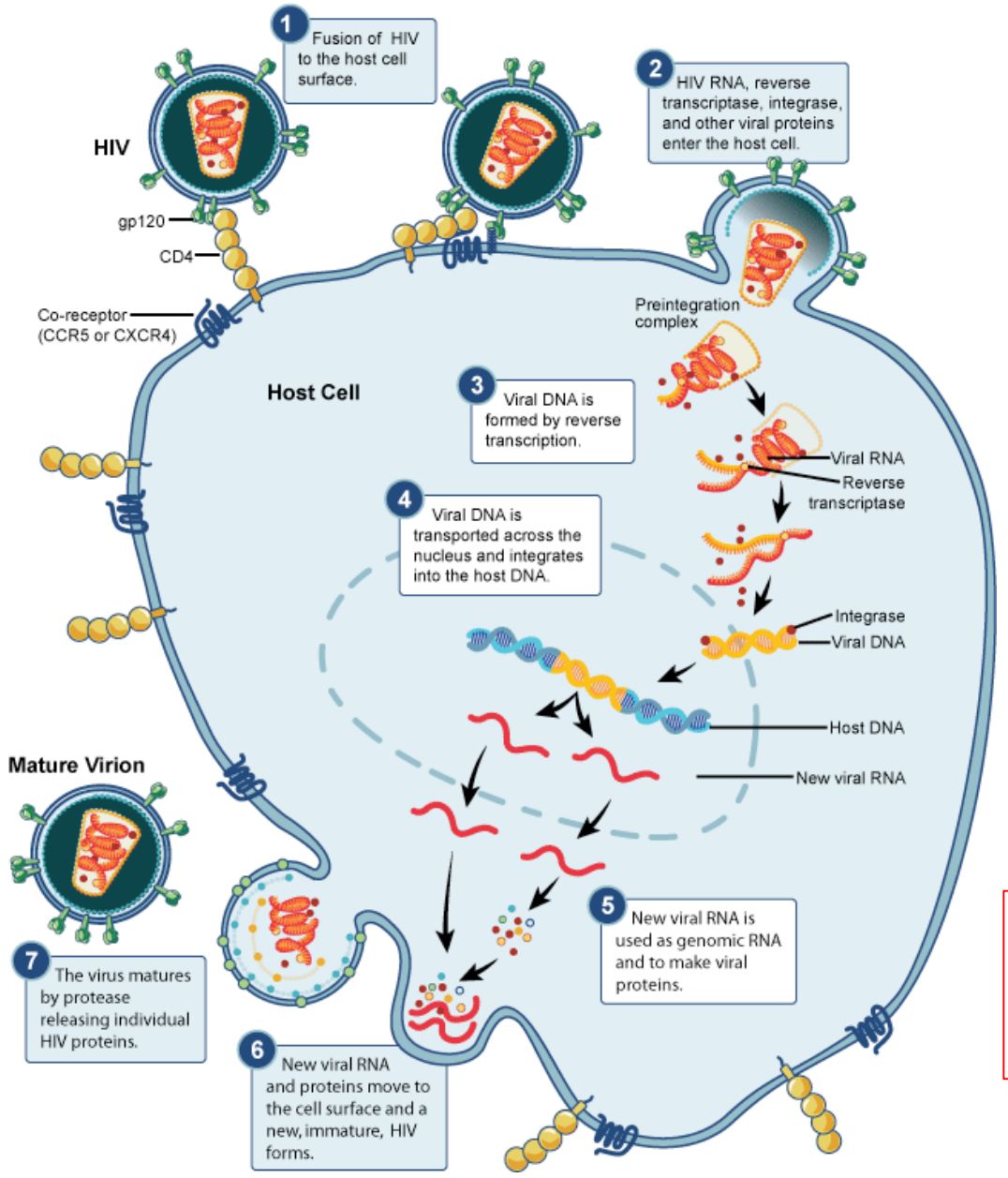
Clinical Progression of HIV



Nature Reviews | Immunology

What are the mechanisms underlying CD4+ T-cell loss in HIV infection?



http://www.niaid.nih.gov

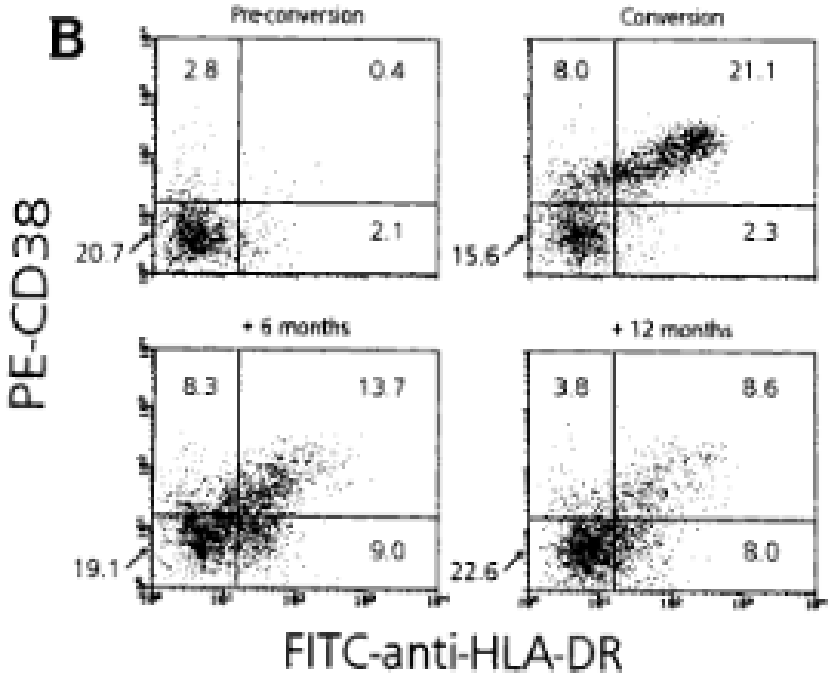
Direct attack by HIV

- **Tap** and **drain** hypothesis explains the slow depletion of CD4+ T-cells
- increased apoptosis
- increased proliferation and thymus output

Yet, also **uninfected cells** are also lost during HIV infection

Vijayan et al. Front. Immunol 2017

Immune activation (CD8+CD38+HLADR+) is a feature of HIV infection and is linked to disease progression



Giorgi, JID, 1994

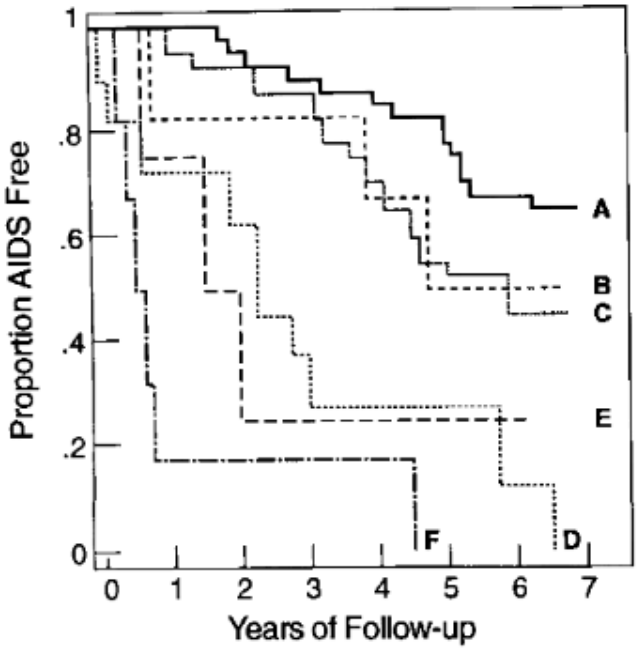


FIG. 3. Kaplan-Meier curves for the development of AIDS for the six groups of study participants stratified by $CD4^+$ cell number per mm^3 (CD4) and the mean fluorescence intensity of CD38 antigen expression on $CD8^+$ cells (RFI-CD38||$CD8^+$): group A, CD4>500, RFI-CD38||$CD8^+$ <6.1; group B, CD4 >500, RFI-CD38||$CD8^+$ ≥6.1; group C, CD4 = 200–500, RFI-CD38||$CD8^+$<6.1; group D, CD4 = 200–500, RFI-CD38||$CD8^+$ ≥6.1; group E, CD4 <200, RFI-CD38||$CD8^+$ <6.1; and group F, CD4 <200, RFI-CD38||$CD8^+$ ≥6.1.

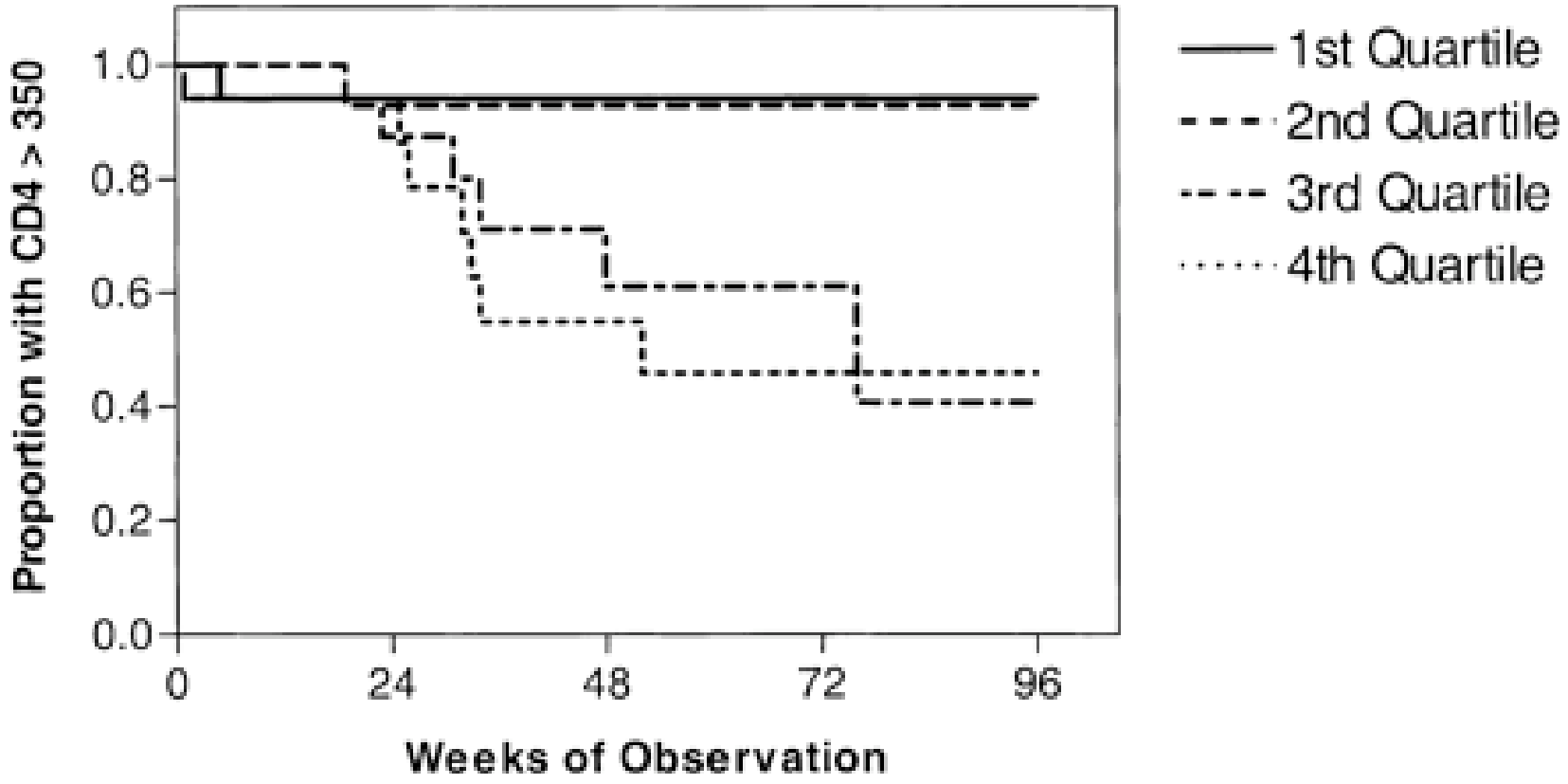
Liu, Cytometry, 1996

Group A:
highest CD4+ (< 500/Ul)
lowest CD8+CD38+

Group F:
lowest CD4+ (< 200/ul)
highest CD8+CD38+

Immune activation is a cause of CD4+ T-cell loss in untreated HIV

Individuals with higher baseline quartiles of CD8+ T-cell activation (P = .002 by the log-rank test) and viremia (P = .004) but not CD4+ T-cell activation (P = .21) **were more likely to experience subsequent CD4+ T-cell decline.**

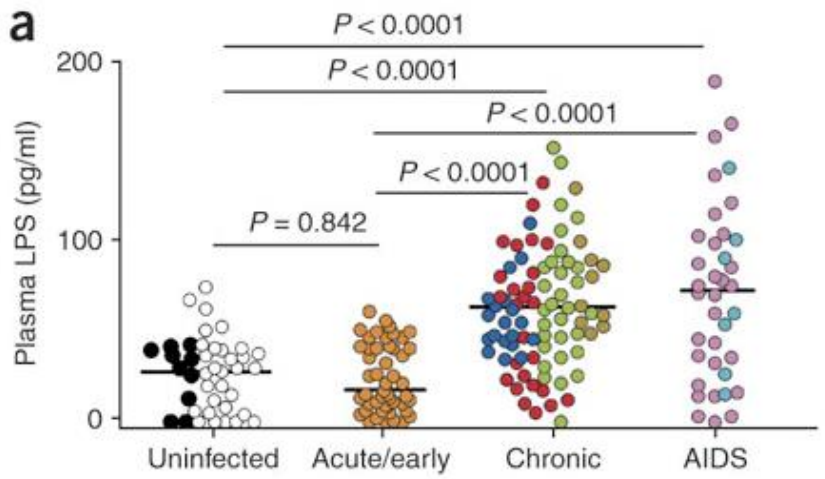


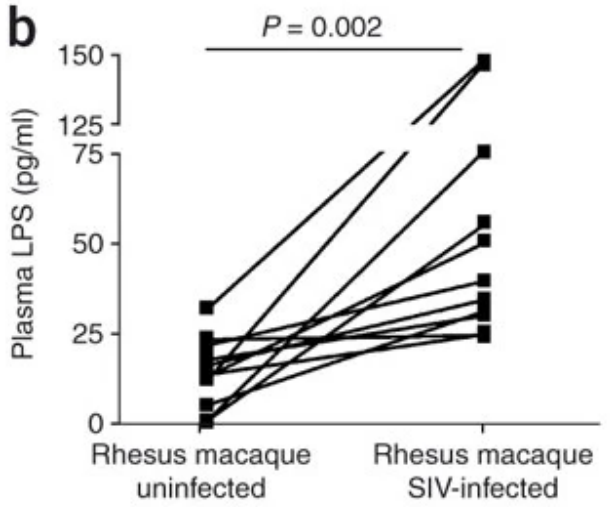
Deeks, Blood, 2004

What causes immune activation in HIV infection?

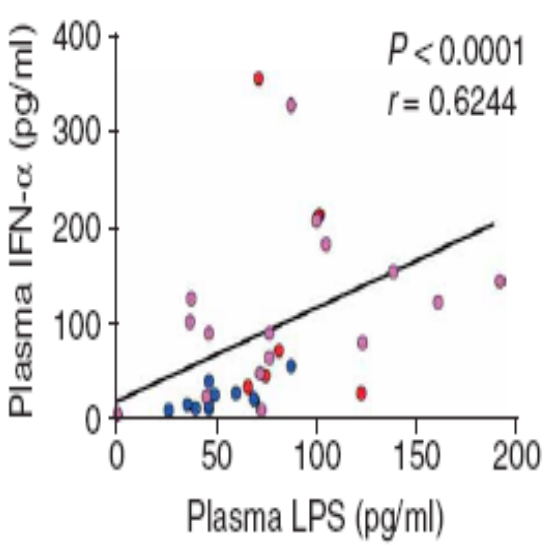
Multifactorial: co-infections (CMV, HCV), low-level viremia

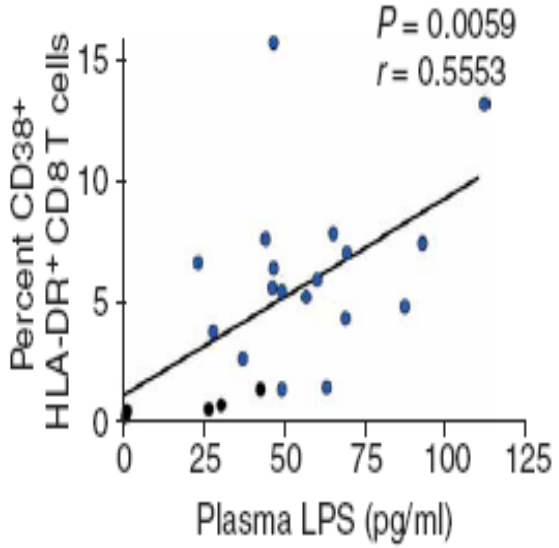
Microbial translocation features HIV/SIV and causes immune activation

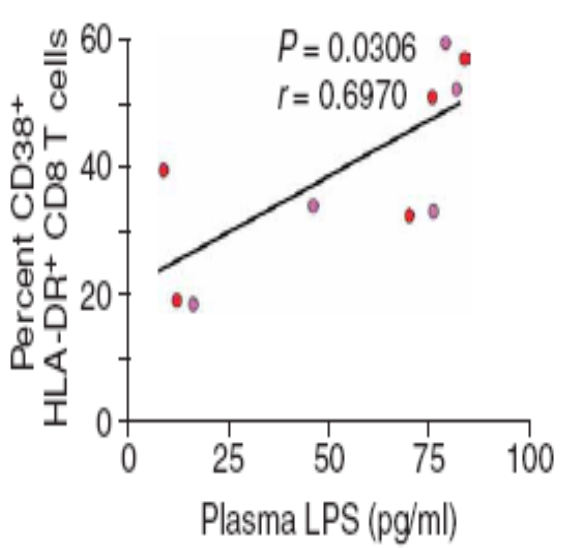




Markers of microbial transolcation:
- LPS
- sCD14
- EndocAb



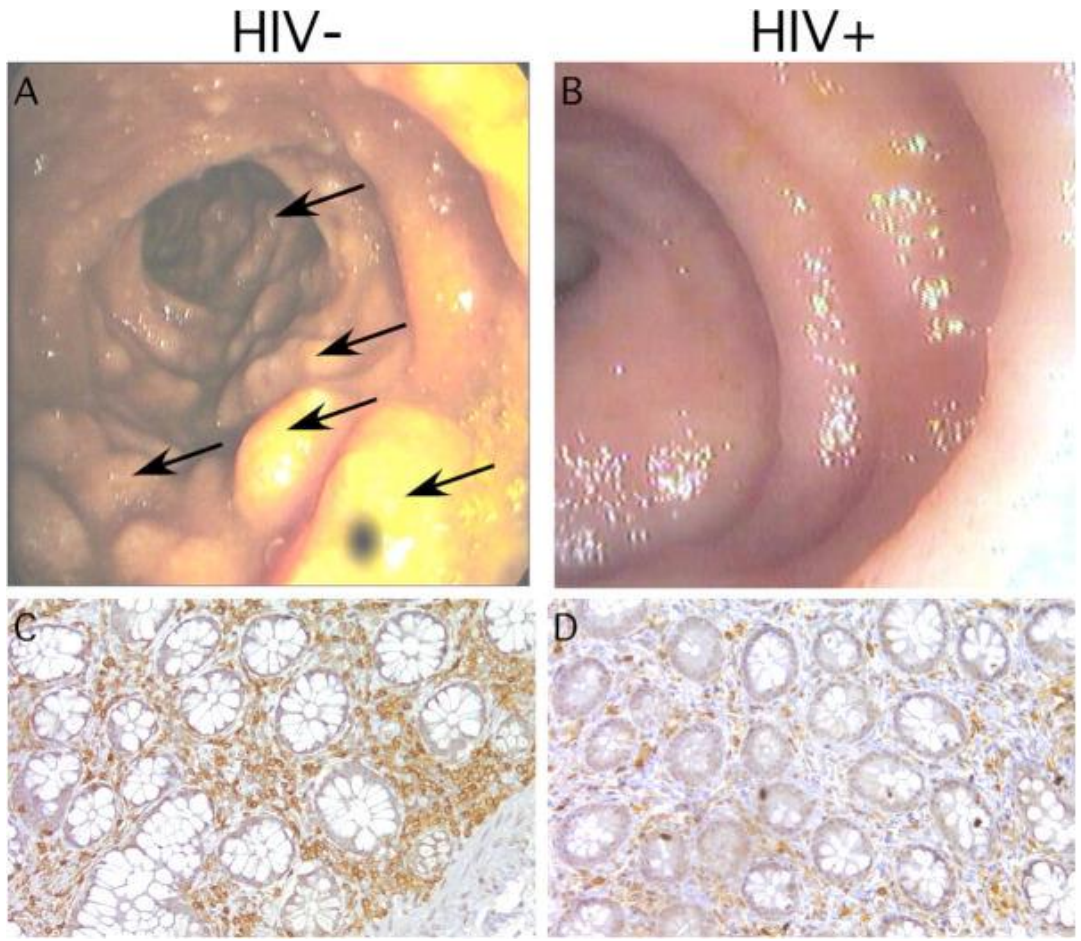




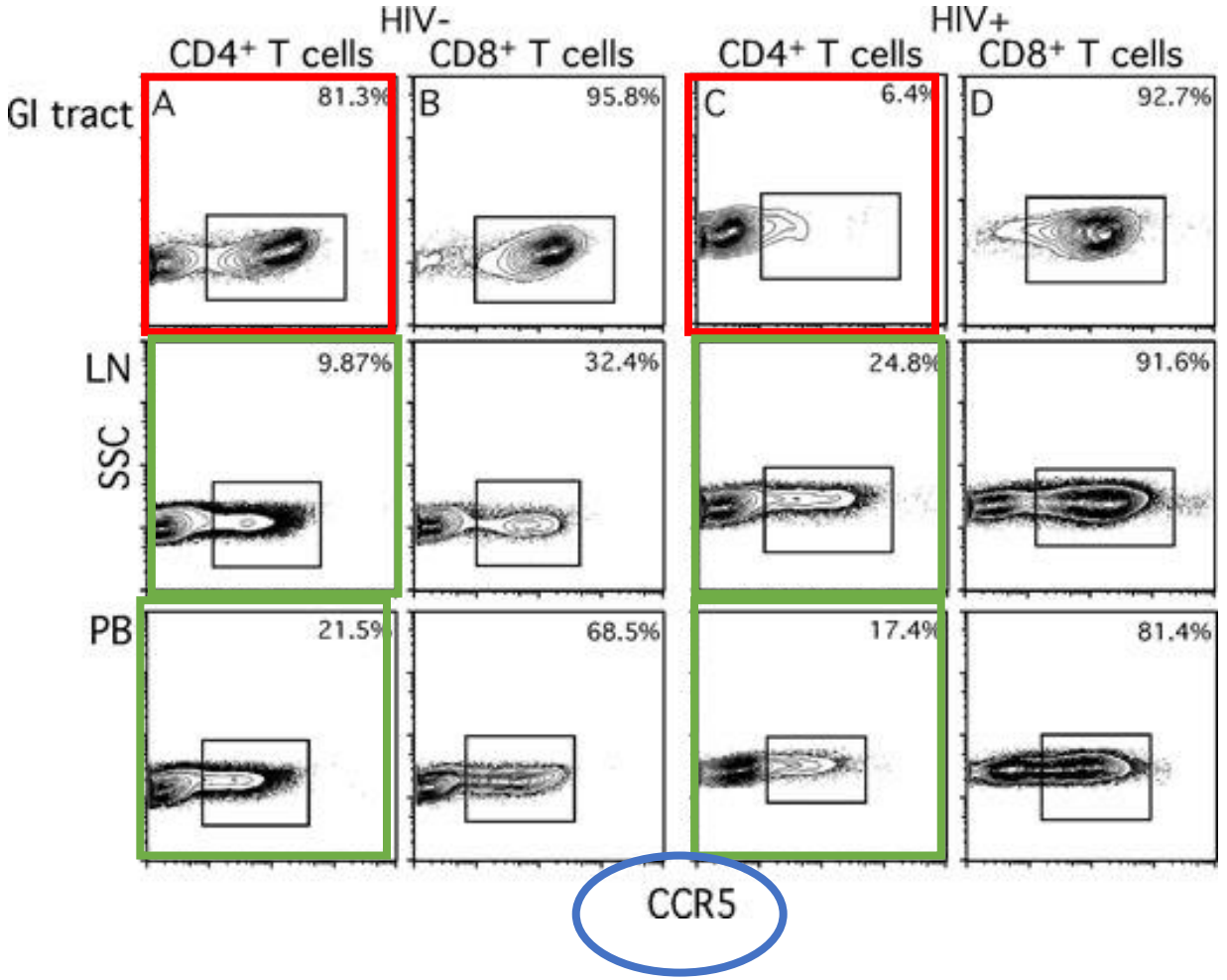
Brenchley et al., Nat Med 2006

Why does microbial translocation occur in SIV/HIV?

CD4+ T-cell are preferentially lost in the gut during HIV infection



IHC staining for CD4+

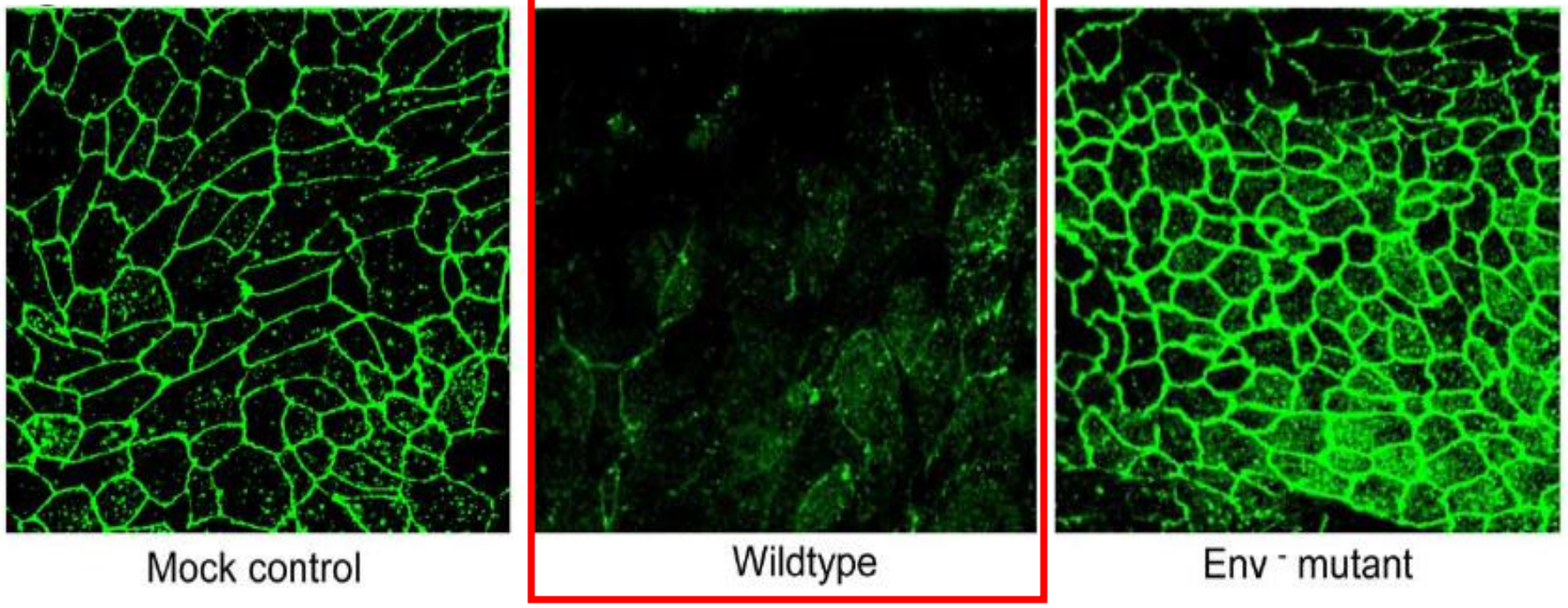


Brenchley et al., J Exp Med 2004

HIV causes direct damage of the gut barrier

gp120 neutralization

Env defective mutant:
lack of Env polyprotein→
lack of gp120 and gp41



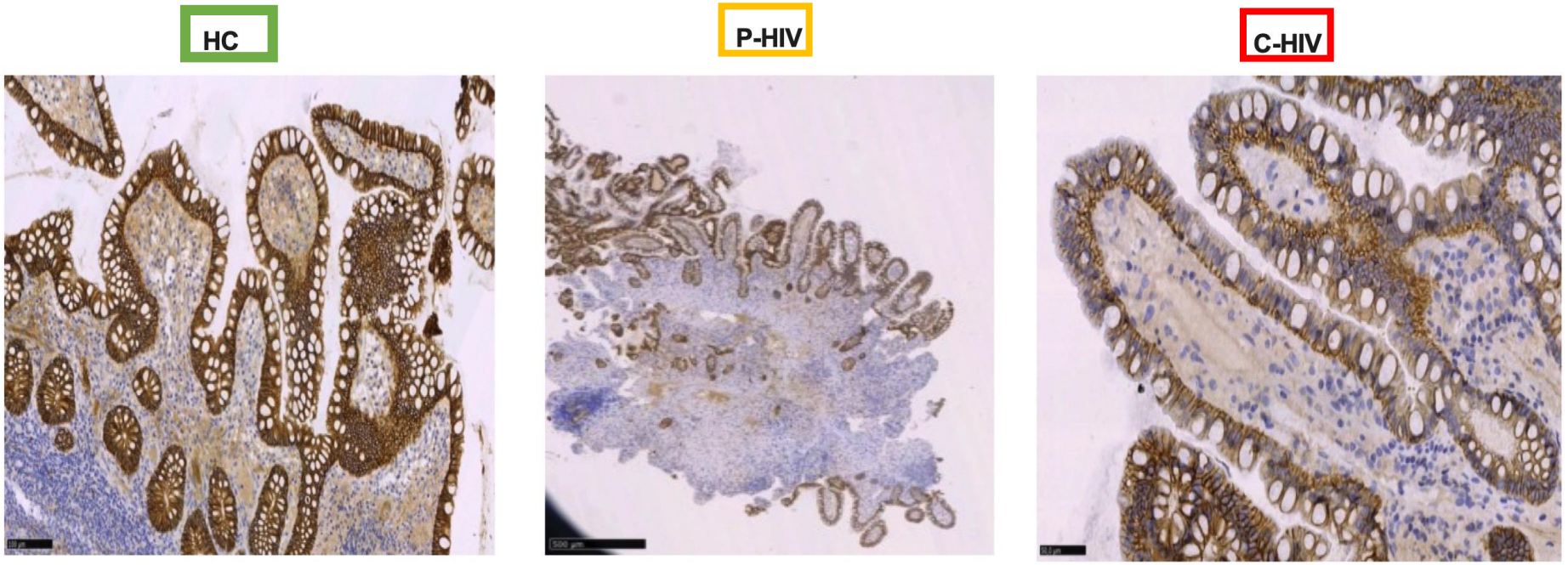
TER: transepitehlial resistance, measure of monolayer integrity

HIV-1 induced barrier permeability is dependent on virus envelope glycoprotein

Nazli et al., Plos Pathogens, 2010

The Loss of Gut Junctional Protein Expression Occurs in Early HIV

A.

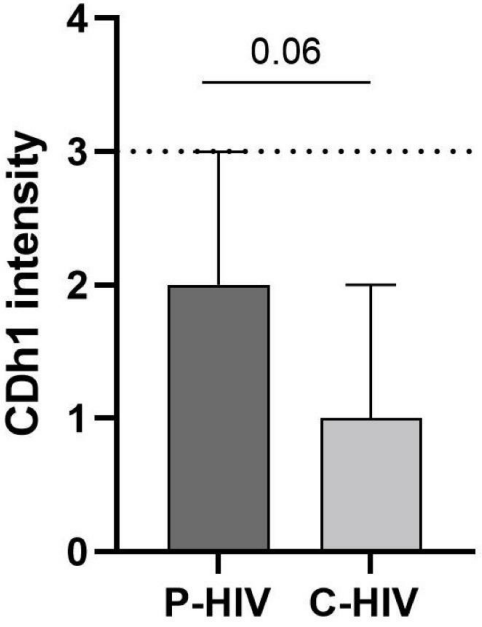


Healthy Control

Primary HIV (untreated)

Chronic HIV (untreated)

B.



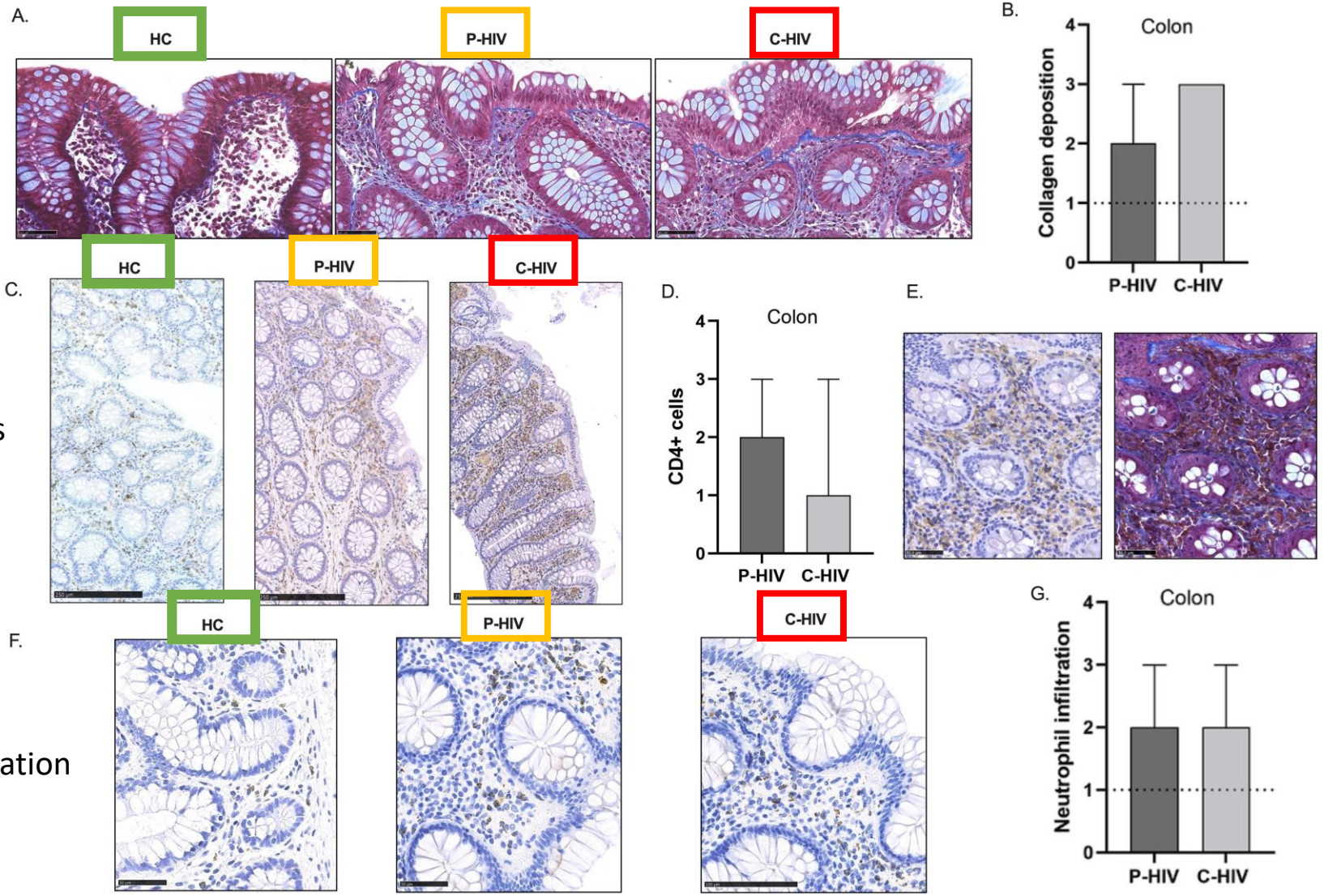
Tincati et al, AIDS, submitted

Gut Fibrosis and Inflammation Occur Early in HIV

Collagen

CD4+ T-cells

Neutrophil infiltration



Tincati et al, AIDS, submitted

Th17 and Th22 mantain intestinal barrier integrity

- Th17 cells:

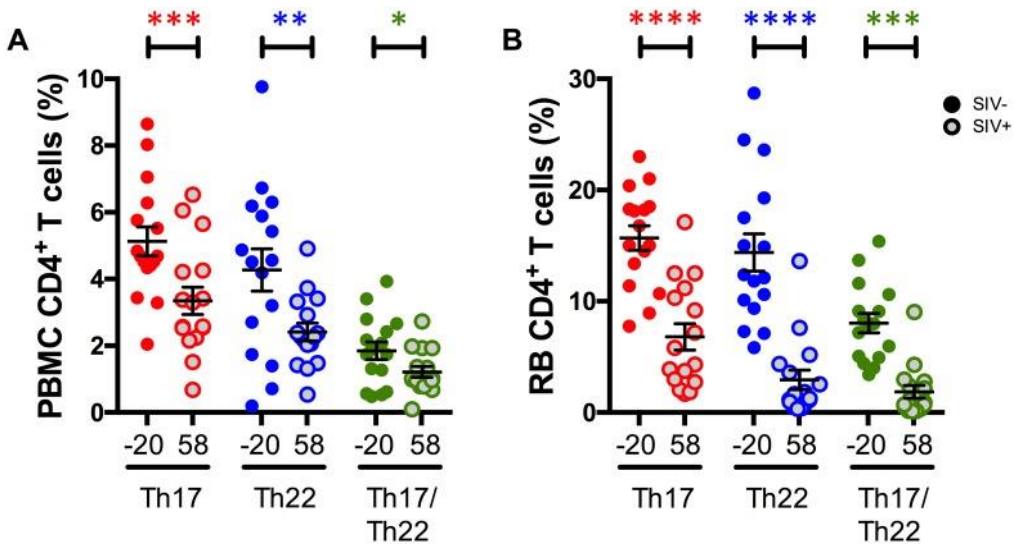
- produce IL-17, (IL-22), but not IL-4 or IFN-γ → critical functions for **maintaining mucosal antimicrobial immune defenses**; balance Th1-Th17(inflammatory)/Th3-Treg (suppressive) for normal homeostasis
- **abundant in mucosal tissues**, including the female reproductive tract and throughout the intestine
- IL-17A blockade: **increased intestinal permability, increased susceptibility to gram negative bacteria**
- **produces and stabilizes/positions tight junction proteins** that maintain mucosal epithelial integrity

- Th22 cells: produce (only) IL-22

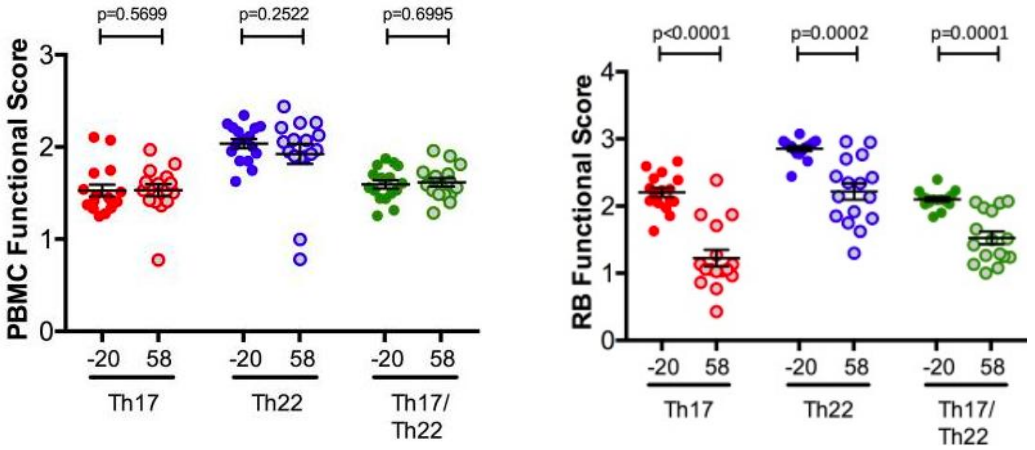
- **host resistance to extracellular pathogens (restrict commensal bacteria from the systemic circulation in mouse models)**

Veazey, Curr Immunol Rev, 2019

Loss of Th17 and Th22 at mucosal sites in HIV/SIV



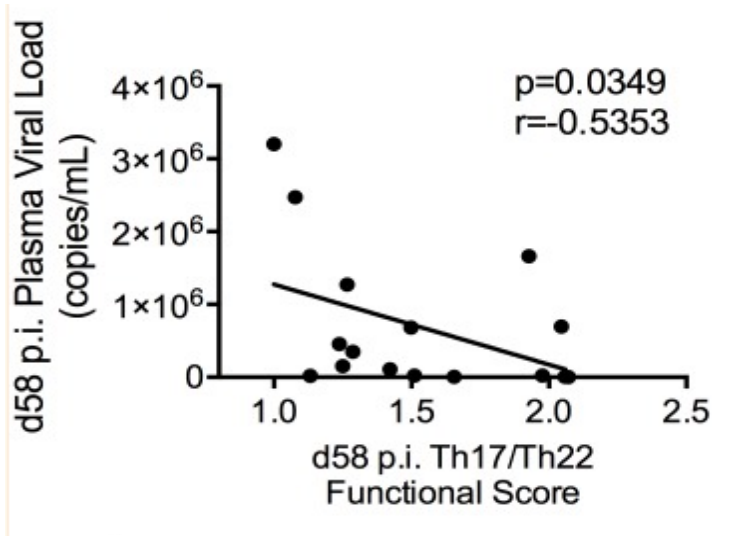
Levels of Th17, Th22, and Th17/Th22 are more drastically depleted by SIV infection in RB than PBMC

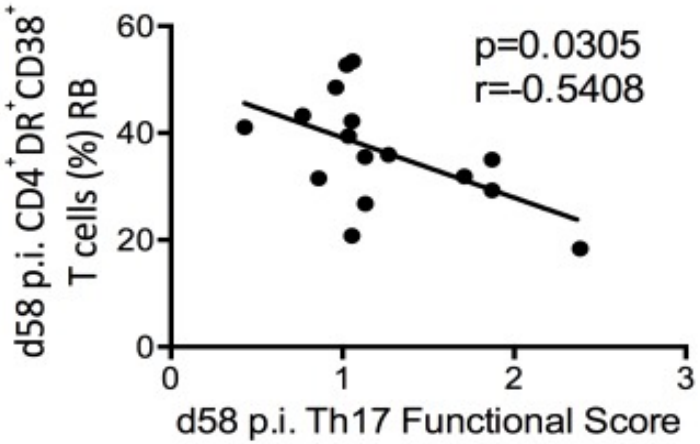


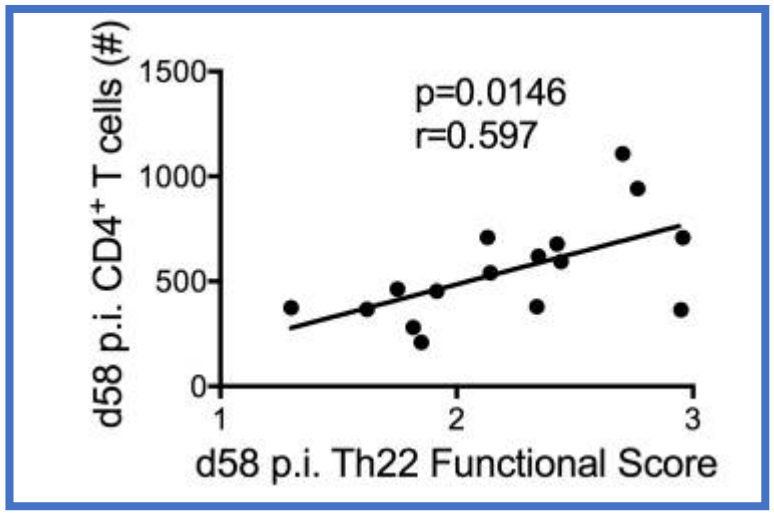
SIV infection severely ablates intestinal IL-17 and IL-22 producing cell function and levels

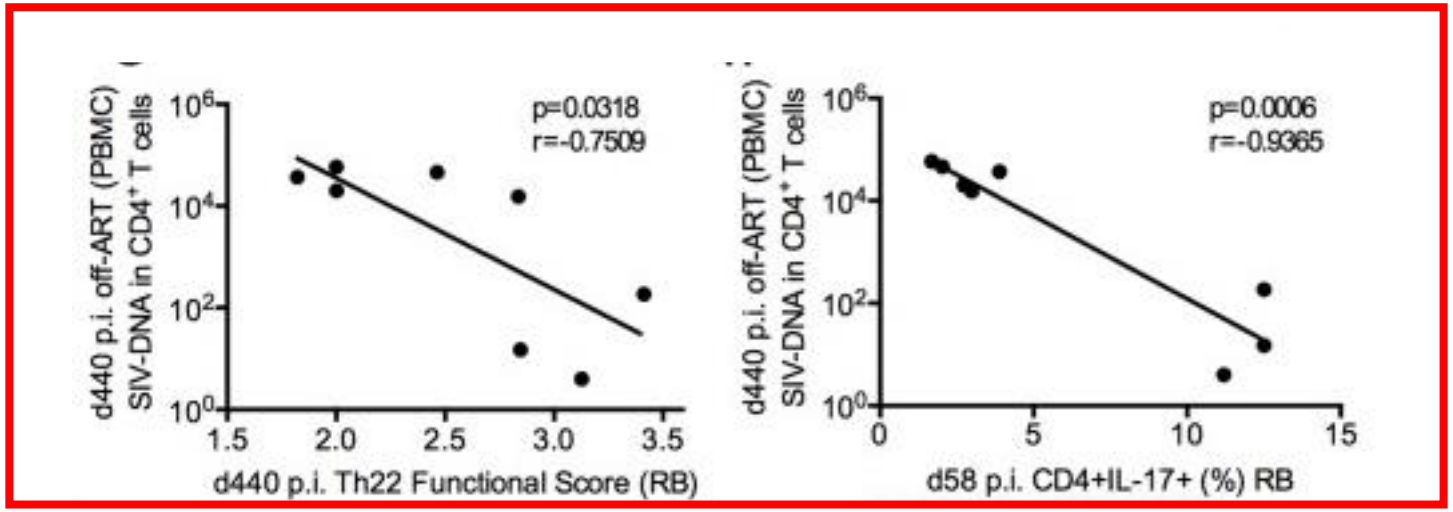
Micci et al,. Plos Path, 2016

Loss of gut Th17 and Th22 is key in HIV pathogenesis



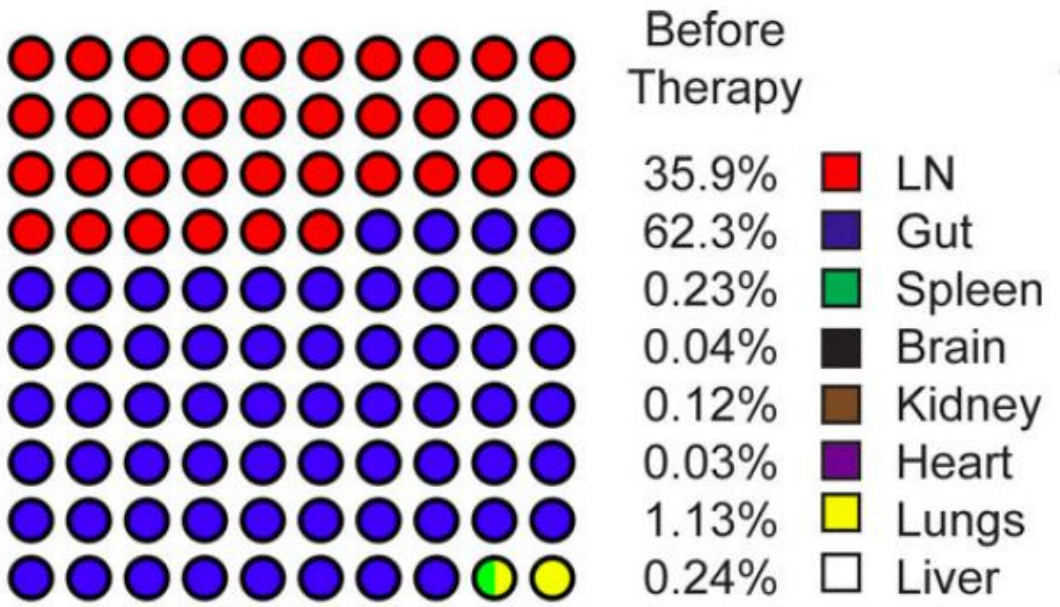


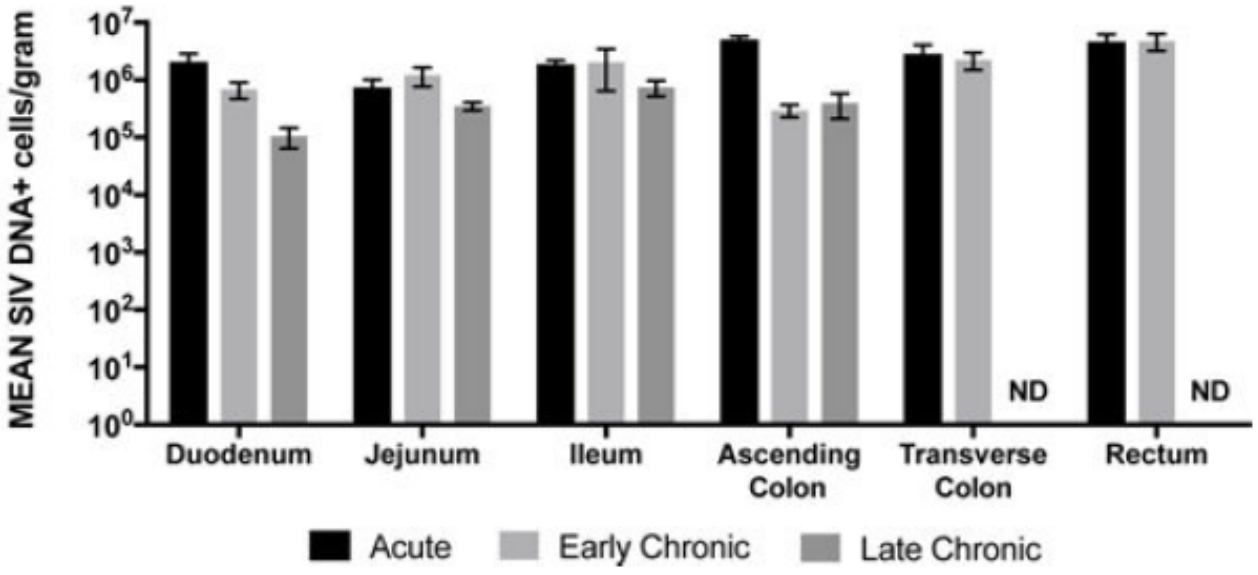




Micci et al,. Plos Path, 2016

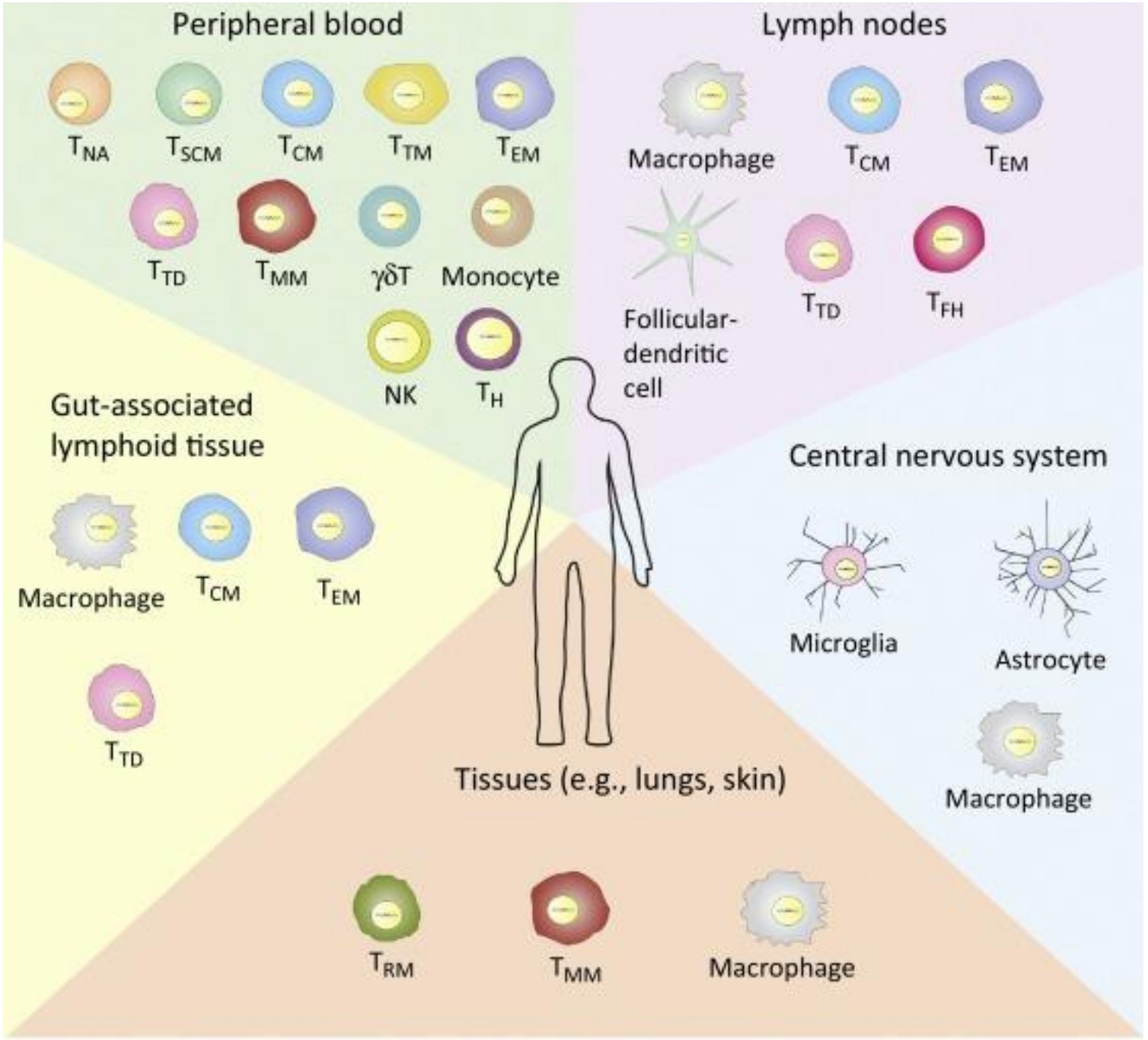
The gut is a major site of viral replication/tissutal reservoir





Estes et al., Nat Med, 2017

Figure 1.
Key Figure: Latent HIV Is a Multifactorial Challenge
In addition to **the CD4⁺ memory T cell subsets found in the peripheral blood**, **the lymphoid tissue, gut-associated lymphoid tissue, and the central nervous system are significant anatomic focuses of latent HIV infection**. Furthermore, additional tissues, such as the lungs and skin, may also contain cells harboring latent HIV. Additionally, unknown potential reservoirs remain to be defined. Each of these tissues contains unique cell types that contribute to the persistence of HIV during effective ART. Abbreviations: T_H, CD4⁺ helper T cells; NK, natural killer cells.

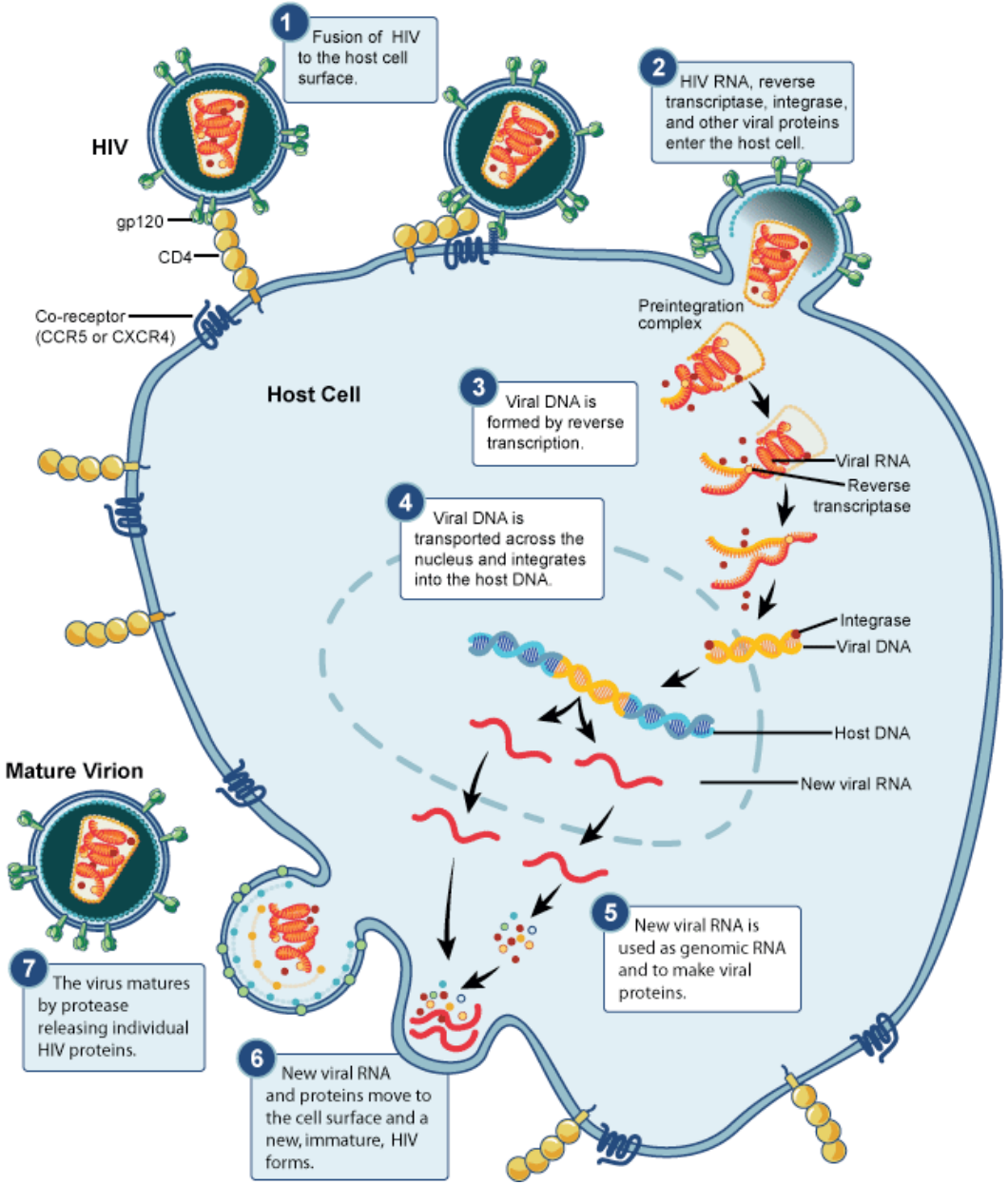


Glossary

Cellular reservoir: cells that are infected with latent HIV-1.

Latent HIV-1: transcriptionally silent, but replication-competent HIV proviruses that persist during treatment with ART and are capable of resuming replication upon discontinuation of ART.

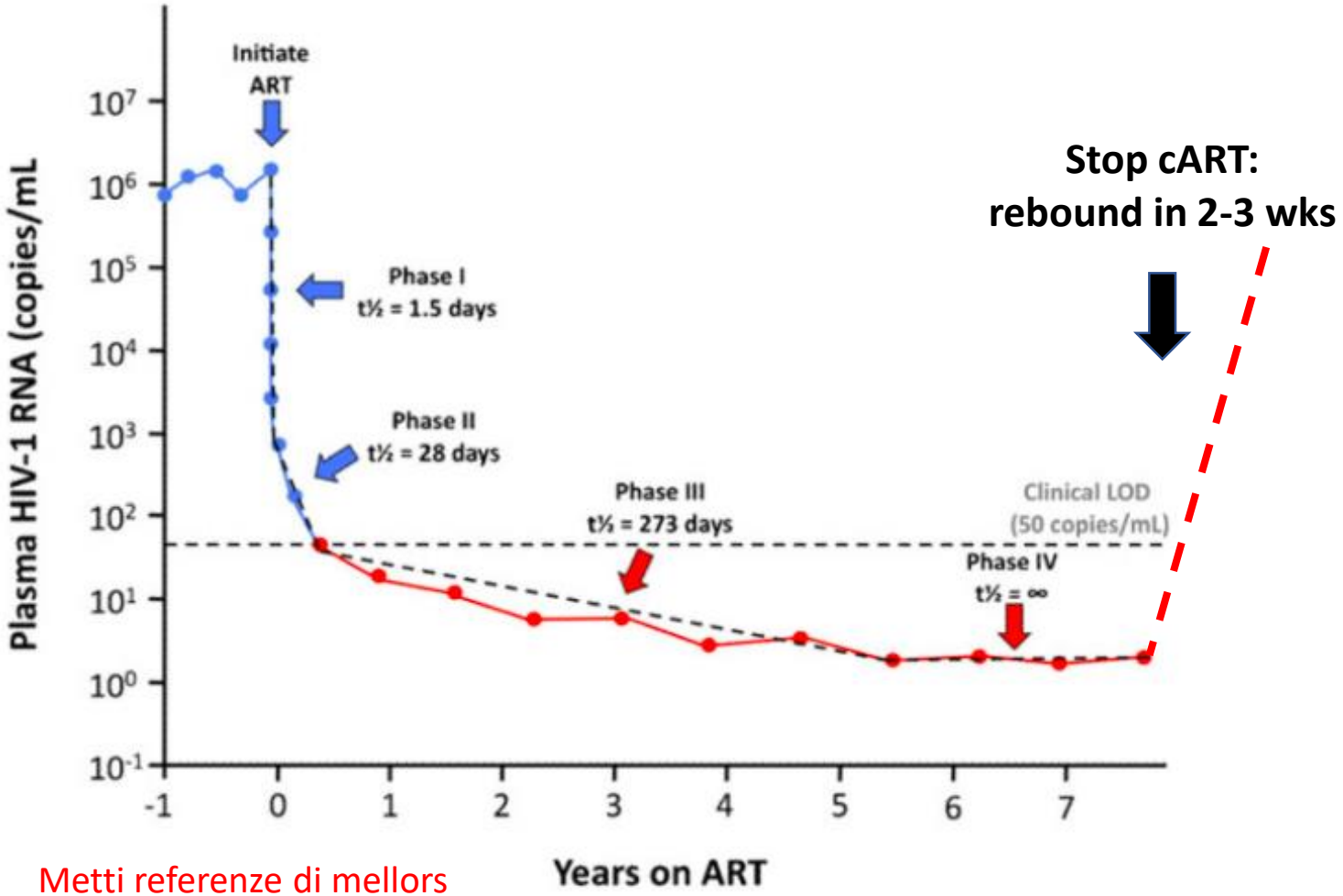
Tissue reservoir: anatomically defined structures that contain cells that harbor latent HIV-1.



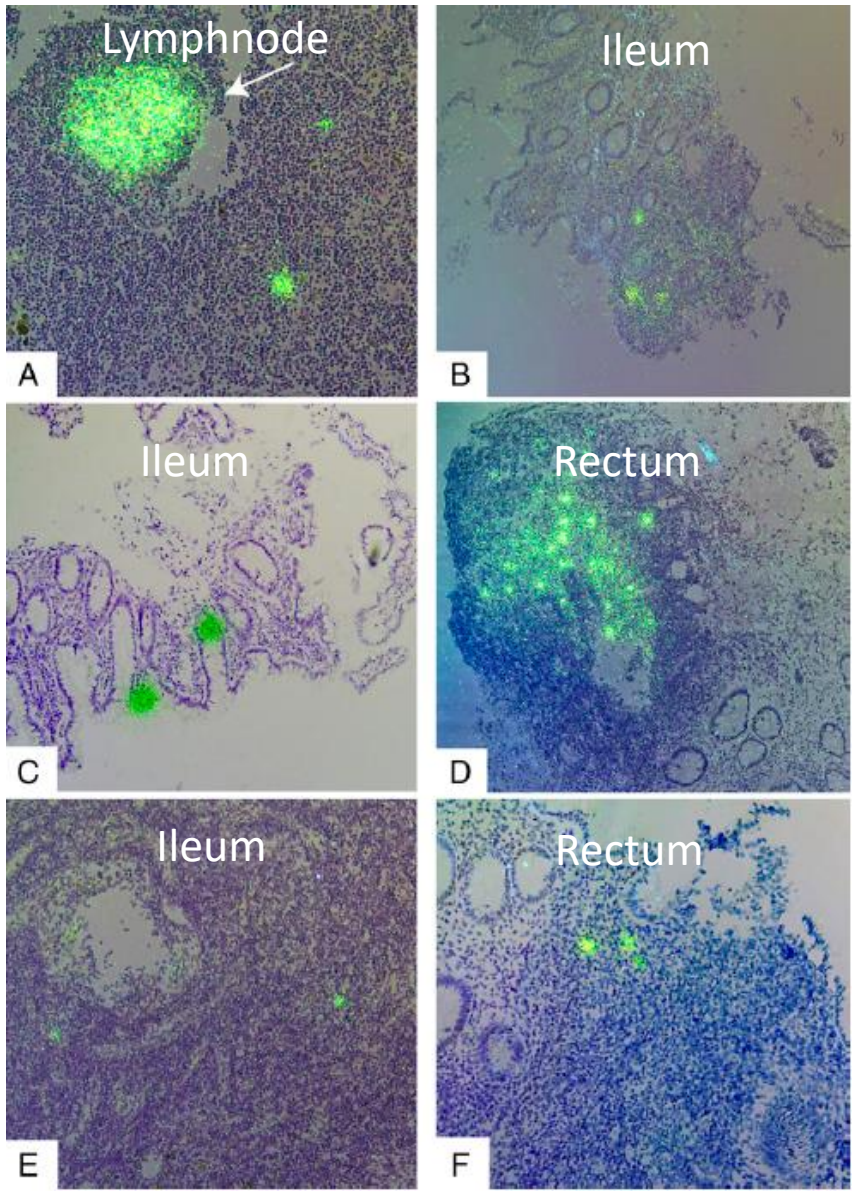
http://www.niaid.nih.gov

Reservoir=what causes rebound=obstacle to a cure

The HIV reservoir causes rebound

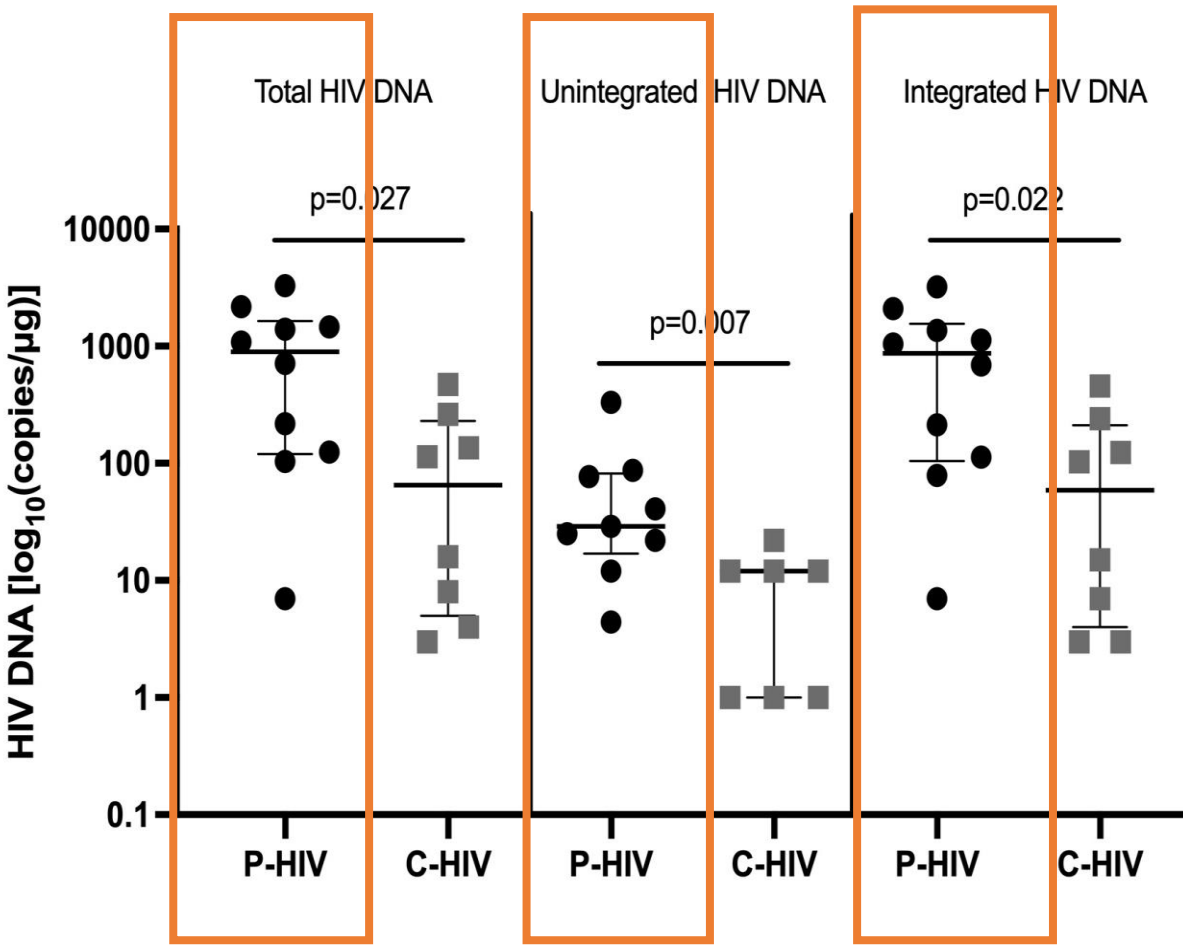


Metti referenze di mellors

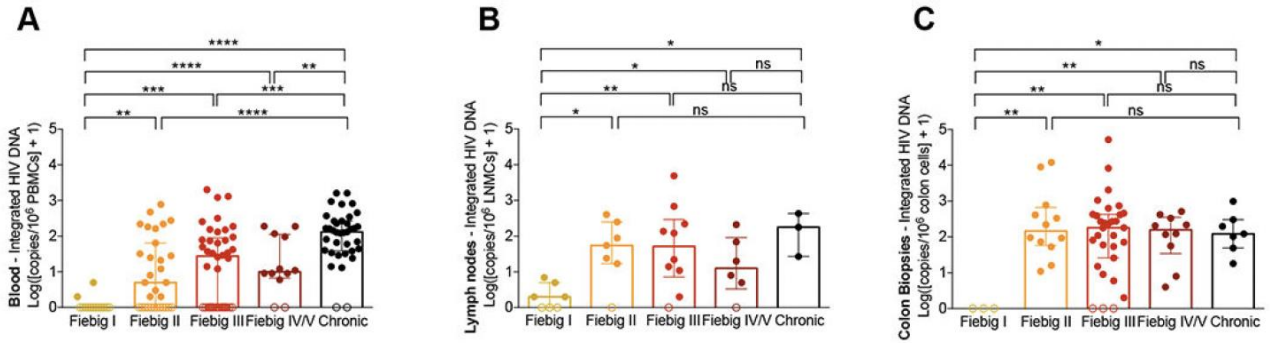


Rothenberger, PNAS 2015

Increased Gut Reservoirs in Early HIV (according to Fiebig stage)



Tincati, et al, AIDS, submitted



Leyre, Sci Translational Med, 2020

Gut Dysbiosis and the Translocating Microbiome in HIV

Disruption of gut microbiota early in the course of SIV/HIV disease

		Macaque #575			Macaque #588		
		Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Gram–	*Escherichia coli*	++++	–	–	++++	–	–
	Kluyvera sp.	++++	–	–	++	–	–
	Pseudomonas sp.	+++	–	–	++++	–	–
	Klebsiella pneumoniae	+++	–	–	++++	–	–
	Citrobacter freundii	+++	++++	++++	++++	++++	++++
	Klebsiella oxyloca	+++	–	–	–	–	–
	Enterobacter sp.	–	–	–	+++	–	++++
	S. maltophilia	–	–	+	–	–	–
	Campylobacter sp.	–	–	–	++++	–	–
	Salmonella sp.	–	–	–	–	–	–
	Yersinia sp.	–	–	–	–	–	–
	Shigella sp.	–	–	–	–	–	–
Gram+	*Staphylococcus* sp.	++	++	+++	++	–	–
	Bacillus sp.	+++	++++	–	+++	–	++++
	Lactobacillus sp.	+++	–	+++	++++	–	–
	Enterococcus sp.	–	++++	–	–	++++	–

'+' signs signify relative amounts of bacterial species cultured

Brenchley et al., Nat Med 2006

fluorescence in situ hybridization or quantitative real-time PCR (Q-PCR)

↑ *P. aeruginosa*
C. albicans

↓ *lattobacilli*

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0095-1137/08/$08.00+0 doi:10.1128/JCM.01729-07
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Vol. 46, No. 2

Early Impairment of Gut Function and Gut Flora Supporting a Role for Alteration of Gastrointestinal Mucosa in Human Immunodeficiency Virus Pathogenesis[▽]

Andrea Gori,[1,2*] Camilla Tincati,[2] Giuliano Rizzardini,[3] Carlo Torti,[4] Tiziana Quirino,[5] Monique Haarman,[6] Kaouther Ben Amor,[6] Jacqueline van Schaik,[6] Aldwin Vriesema,[6] Jan Knol,[6] Giulia Marchetti,[7] Gjalt Welling,[8] and Mario Clerici[9]

Gori et al., Journal of Clinical Microbiology,2008

	Vujkovic-Cvijin *et al.*[51]	Lozupone *et al.*[40]	Perez-Santiago *et al.*[47]	McHardy *et al.*[42]
HIV-1-infected (M/F)	23 (23/0)	25 (22/3)	13 (13/0)	40 (40/0)
HIV-1 status				
Chronic	22	22	–	40
Early	–	3	13	–
AIDS	–	–	–	–
Other	–	LTNP $N=1$	–	–
ART	16 (+2)[a]	14 (8, 6)[b]	13[c]	20
Controls (M/F)	9 (9/0)	13 (8/5)	–	20 (20/0)
Geographical location	The United States	The United States	The United States	The United States
Sample site	Rectosigmoid biopsies	Fecal	Fecal	Rectal mucosa (sponge)

	Mutlu *et al.*[44]	Dillon *et al.*[37,38]	Lozupone *et al.*[41]	Vázquez-Castellanos *et al.*[49]
HIV-1-infected (M/F)	21 (16/5)	18 (13/5)	40 (M/F not reported)	15 (12/3)
HIV-1 status				
Chronic	21	18	40	15
Early	–	–	–	–
AIDS	–	–	–	–
Other	–	–	–	–
ART	19	–	28 (17/11)[e]	15
Controls (M/F)	22 (17/5)	14 (9/5)	15 (M/F not reported)	15 (8/7)
Geographical location	The United States	The United States	The United States	Spain
Sample site	Multiple biopsy sites[d], fecal	Colon biopsies, fecal	Fecal	Fecal

	Volpe *et al.*[50]	Dinh *et al.*[39]	Nowak *et al.*[46]	Yang *et al.*[52]
HIV-1-infected (M/F)	15 (13/2)	21 (17/4)	31 (16/15)	8 (5/3)
HIV-1 status				
Chronic	15	21	31	8[g]
Early	–	–	–	–
AIDS	–	–	–	–
Other	–	–	Elite controller $N=3$	–
ART	15	21	19[f]	–
Controls (M/F)	17 (13/4)	16 (12/4)	9 (5/4)	8 (4/4)
Geographical location	The United States	The United States	Sweden	The United States
Sample site	Fecal	Fecal	Fecal	Duodenum (mucosal brushes)[h]

	Noguera-Julian *et al.*[i] [45]	Monaco *et al.*[43]	Sun *et al.*[48]
HIV-1-infected (M/F)	129 (101/28)	82 (31/51)	13 (9/4)
HIV-1 status			
Chronic	129	82[k]	13
Early	–	–	–
AIDS	11	25	–
Other	Elite controller $N=8$	–	–
ART	84	40	11
Controls (M/F)	27 (23/3/1[j])	40 (20/20)	7 (5/2)
Geographical location	Spain	Uganda	China
Sample site	Fecal	Fecal	Fecal

ART, antiretroviral therapy; LTNP, long-term nonprogressor.
[a]Two viremic untreated study participants returned after 9 months of effective ART.
[b]Of the 14 chronically HIV-1-infected study participants on ART, eight study participants were on long-term (≥ 12 months) ART and six study participants on short-term (≤12 months) ART with three of the short-term ART sampled before and after ART initiation.
[c]All HIV-1-infected study participants started ART within 1 week of enrollment and followed for longitudinally for 48 weeks.
[d]Samples collected from terminal ileum, right colon, left colon.
[e]Of the 28 chronically HIV-1-infected study participants on ART, 17 were on long-term (≥ 12 months) ART and 11 study participants were on short-term (≤12 months) ART.
[f]Of the 31 HIV-1-infected study participants s, 19 were followed for a median 10 months after initiating ART.
[g]Years since first HIV-seropositive test reported as median 2.1 years with a range of 0.16–7 years.
[h]Samples were also collected from esophagus and stomach.
[i]Study included an external validation cohort; data reported for test cohort.
[j]Of uninfected study participants, one study participant reported as transgender.
[k]Time since diagnosis not reported.

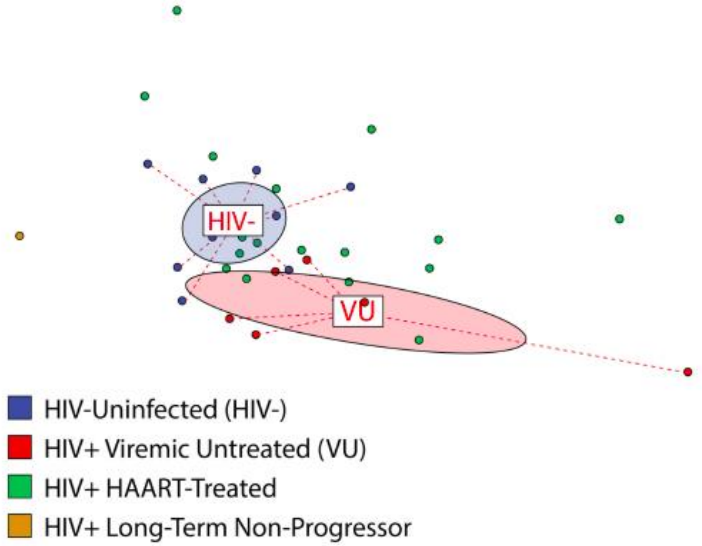
AIDS

	Mucosal tissue	References	Fecal samples	References
Phylum Proteobacteria	↑	[37,51,52]	↑	[39,48,50]
Family				
Enterobacteriaceae	↑	[44,51]	↑	[39]
Brucellaceae	↑	[37]		
Xanthomonadaceae	↑	[37]		
Rhodspirillaceae	↓	[37]		
Genus				
Escherichia	↑	[44,51]		
Serratia	↑	[51]		
Shigella	↑	[51]		
Klebsiella	↑	[51]		
Ralstonia	↑	[44]		
Actinobacter	↑	[37]		
Burkholderia	↑	[52]		
Desulfovibrio			↑	[40]
Thalossospira	↓	[37]		
Phylum Bacteroidetes				
Family				
Rikenellaceae	↓	[42,44]	↓	[39,40]
Bacteroidaceae	↓	[37]	↓	[37,40,48]
Prevotellaceae	↑	[37]	↑	[40]
Genus				
Bacteroides	↓	[37,44,51]	↓	[37,40,48,49]
Alistipes	↓	[37,42,51]	↓	[37,39,40,50]
Barnesiella	↓	[37]	↓	[37]
			↑	[39]
Prevotella	↑	[37,44]	↑	[40,49]
Phylum Firmicutes	↓	[37,42,52]		
Family				
Lachnospiraceae	↓	[37,42,44]		
Ruminococcaceae	↓	[37,42,44]	↓	[37]
Christensenellaceae	↓	[37]		
Erysipelotrichaceae	↑	[51]	↑	[39,40]
Veillonellaceae			↑	[40]
Genus				
Coprococcus	↓	[37,42,44]		
Faecalibacterium	↓	[44]	↓	[46,48,49]
Blautia	↓	[37,44]		
Rumminococcus	↓	[42,44]		
Dorea	↓	[44]		
Oscillospira	↓	[44]		
Lachnospira	↓	[42,44]	↓	[48]
Roseburia	↓	[42,44]	↓	[48,49]
Dialister	↓	[44]	↑	[40]
Eubacterium	↓	[42,44]		
Lactobacillus	↓	[52]	↑	[46]
Mitsuokella			↑	[40]
Catenibacterium	↑	[44]	↑	[40]
Mogibacterium	↑	[44]		
Bulleidia			↑	[40]

AIDS

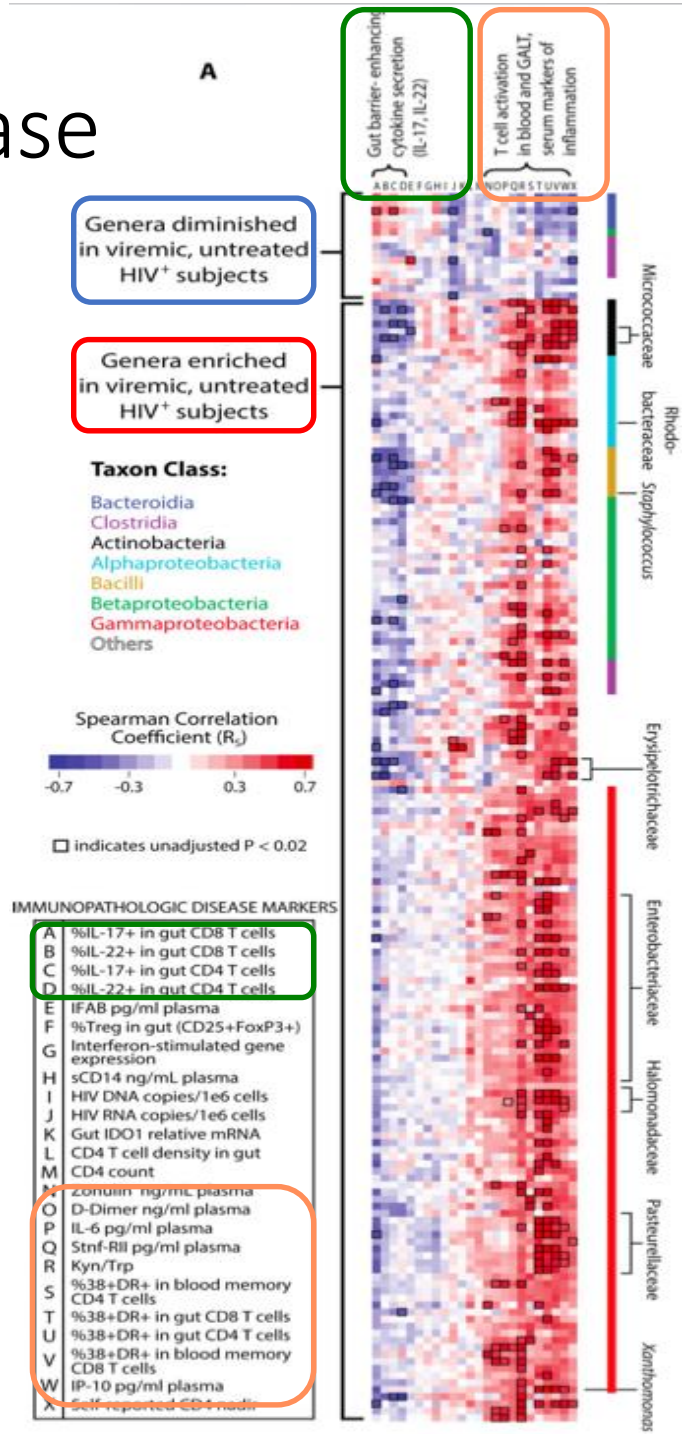
Dillon et al. AIDS 2016

Gut dysbiosis is linked to HIV disease progression

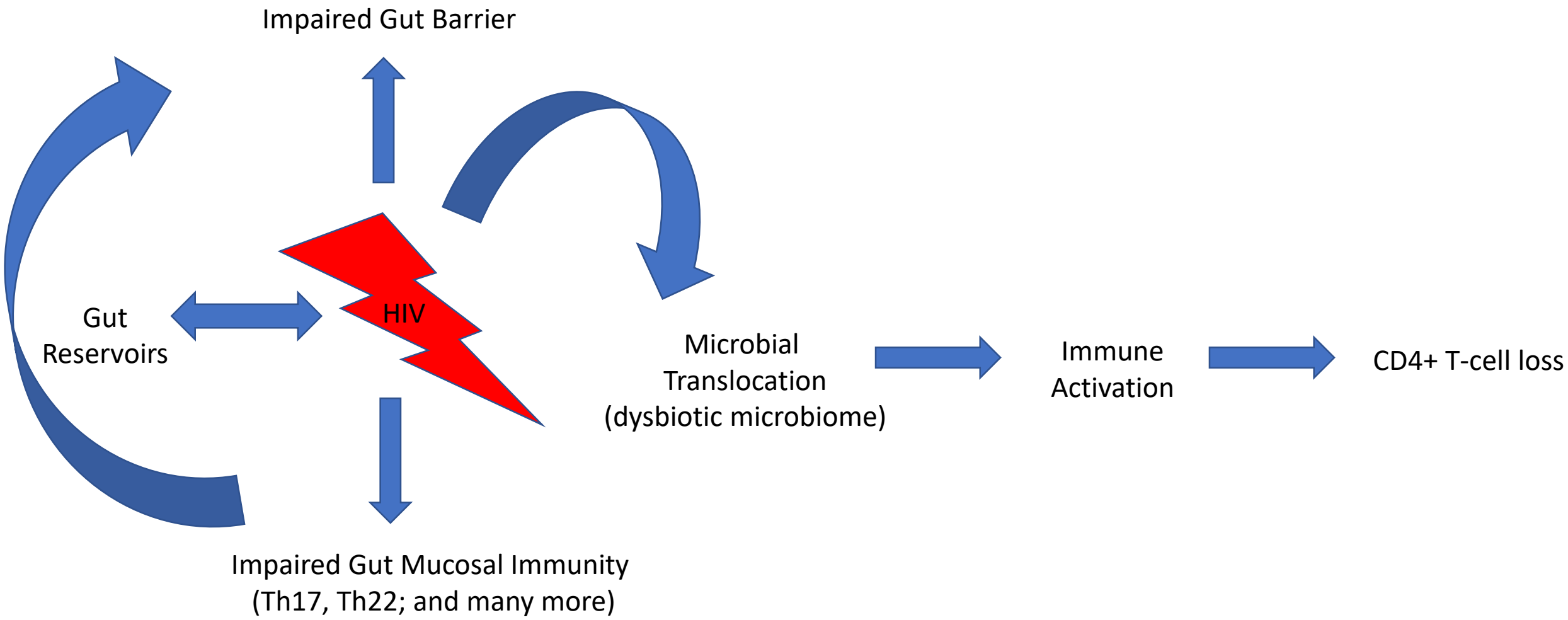


- Bacteria **enriched** in VU subjects correlate with markers of **disease progression**

- Bacteria **depleted** in VU subjects correlate positively with **protective immune markers** (low T cell activation in the blood and gut, higher gut **IL-17 secretion**)



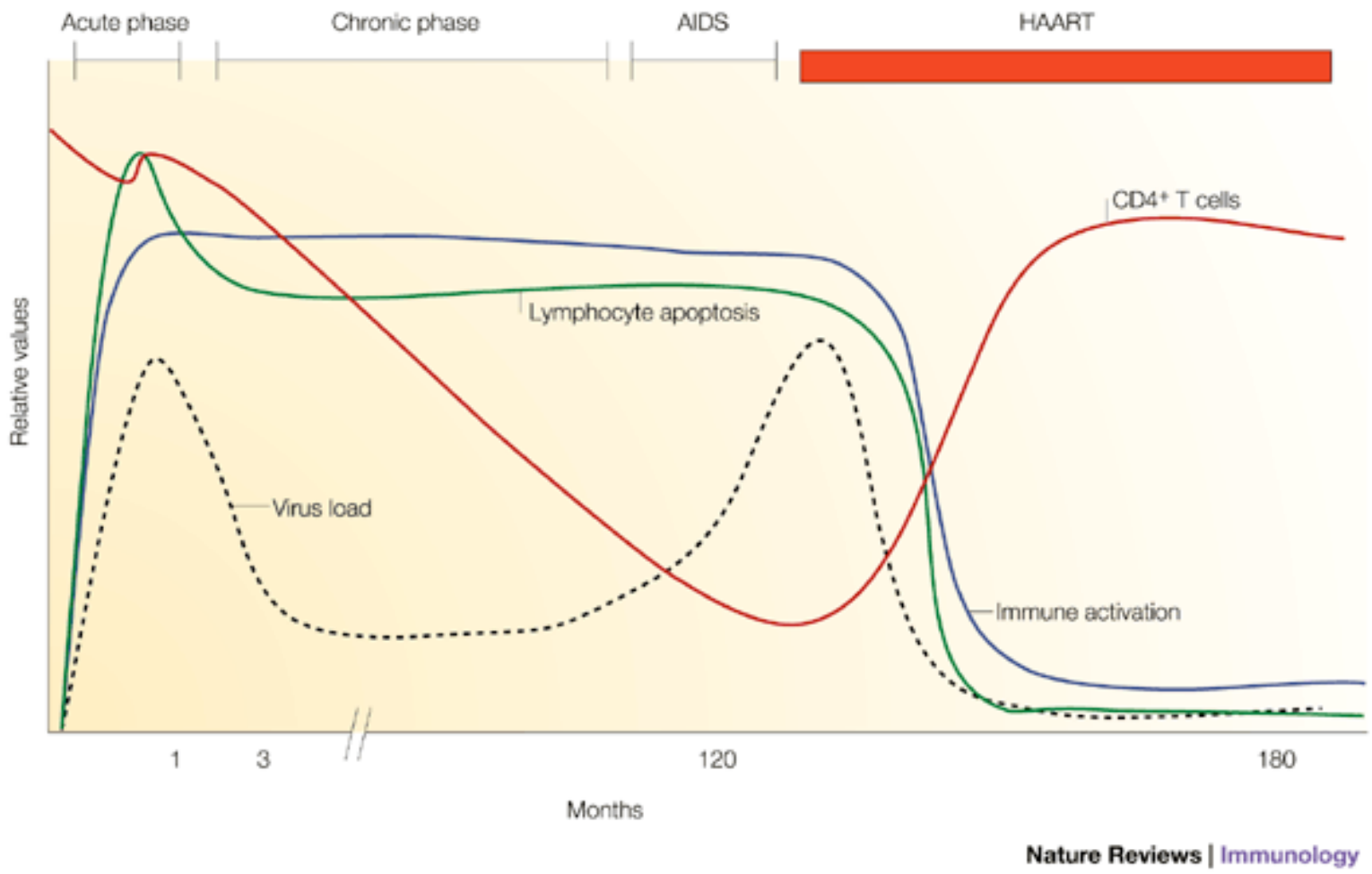
Vujkovic-Cvijin et al., Sci Transl Med, 2013



GUT

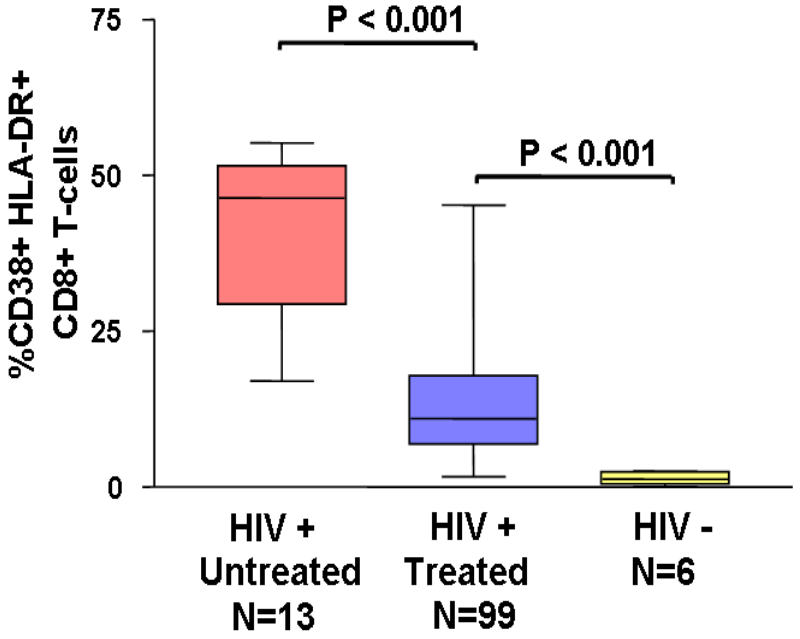
PERIPHERAL BLOOD

Clinical Progression of HIV

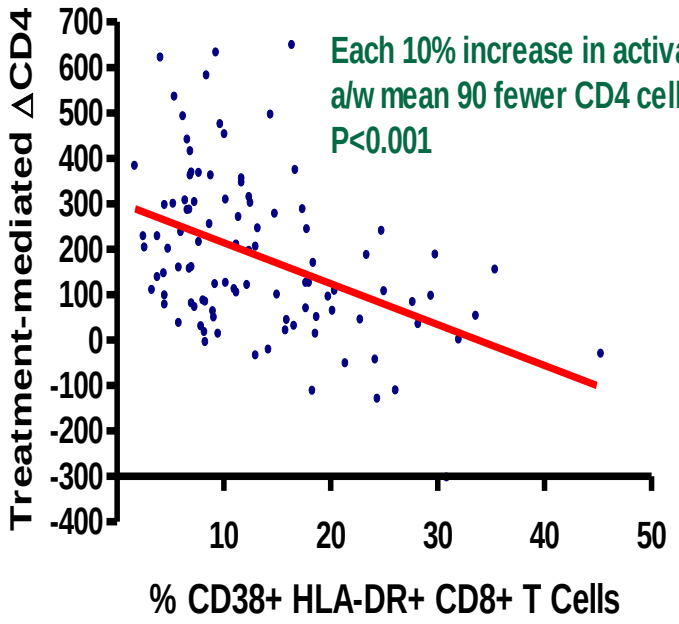


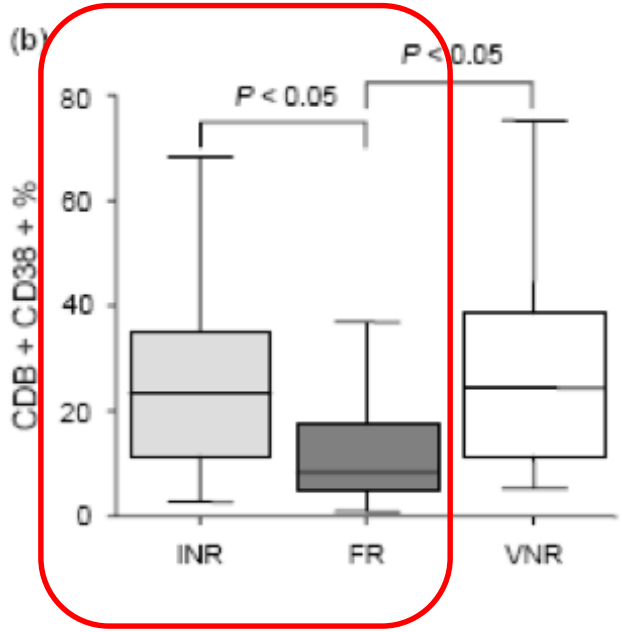
Does cART restore the structural, immune and microbiological imbances in the gut?

Higher CD8+ T cell activation persists on cART and is associated with lower treatment-mediated CD4+ T cell gains



Hunt et al. JID 2003

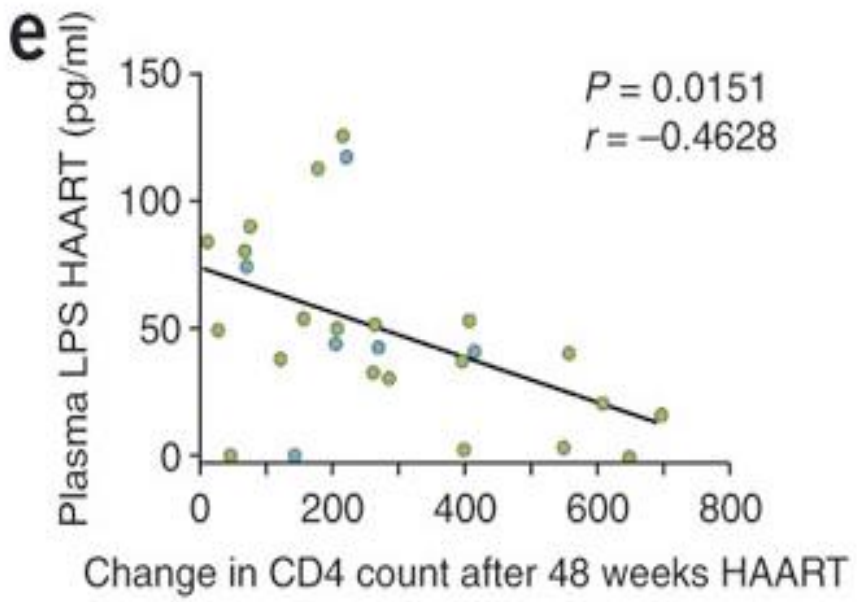




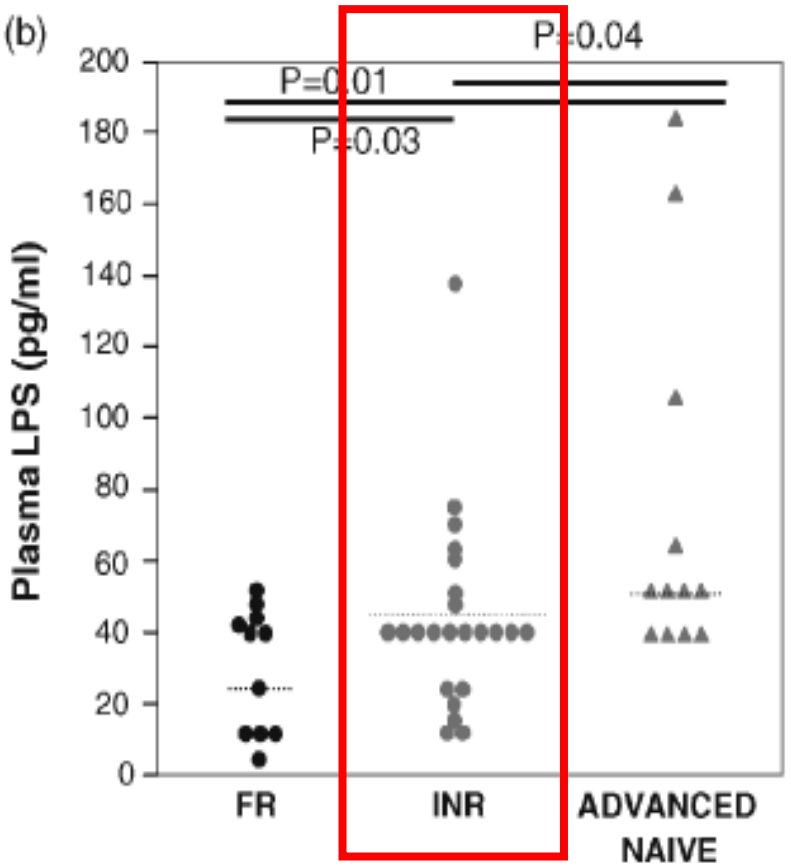
Marchetti et al., AIDS 2006

(see also Pitrak AIDS 2001)

Microbial translocation persists on cART and hampers CD4+ T-cell gain



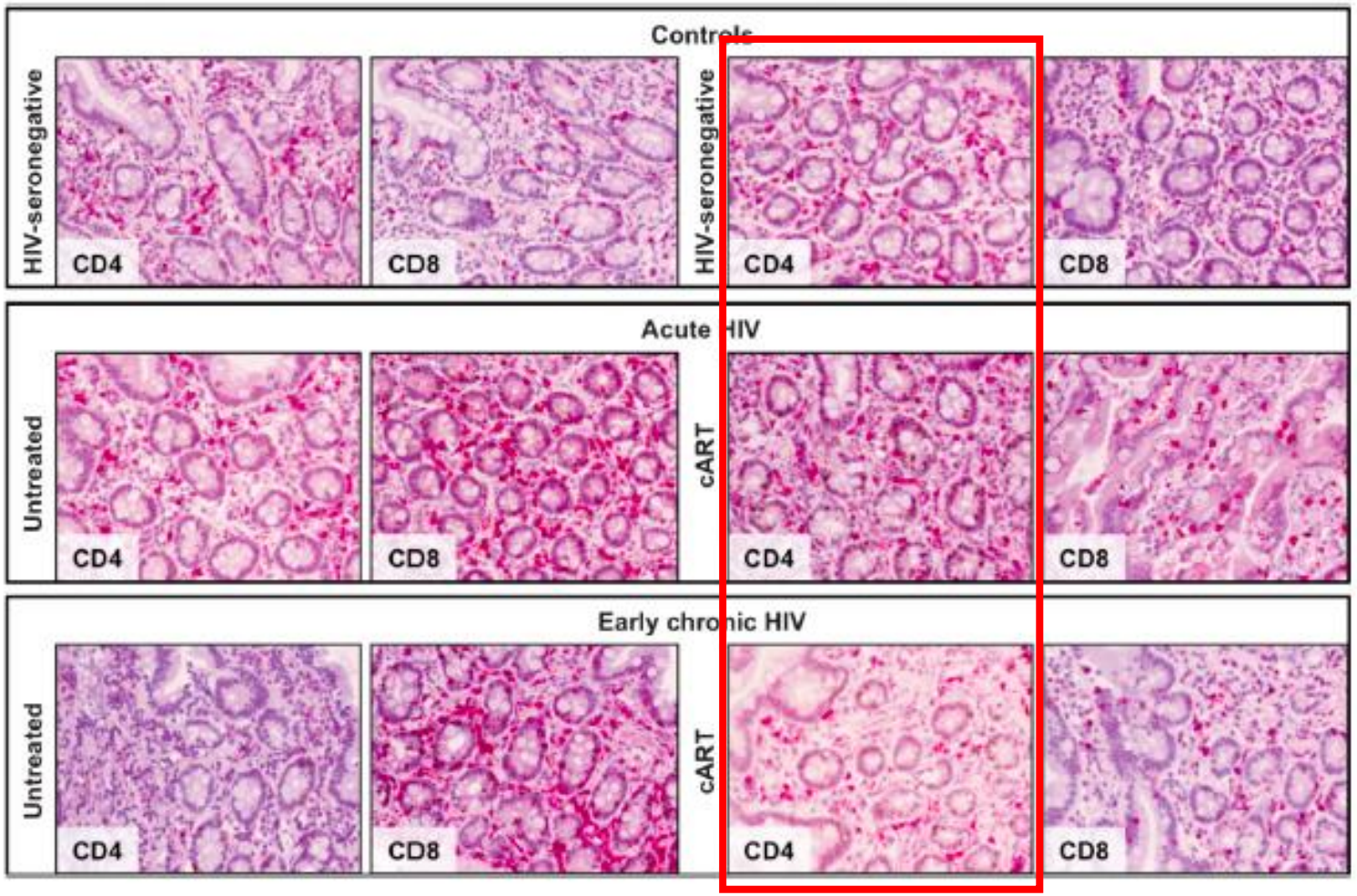
Brenchley et al., Nat Med 2006



Marchetti et al., AIDS 2008

(see also Jiang JID 2009)

Limited reconstitution of CD4+ T-cell in the gut during cART (acute vs chronic)

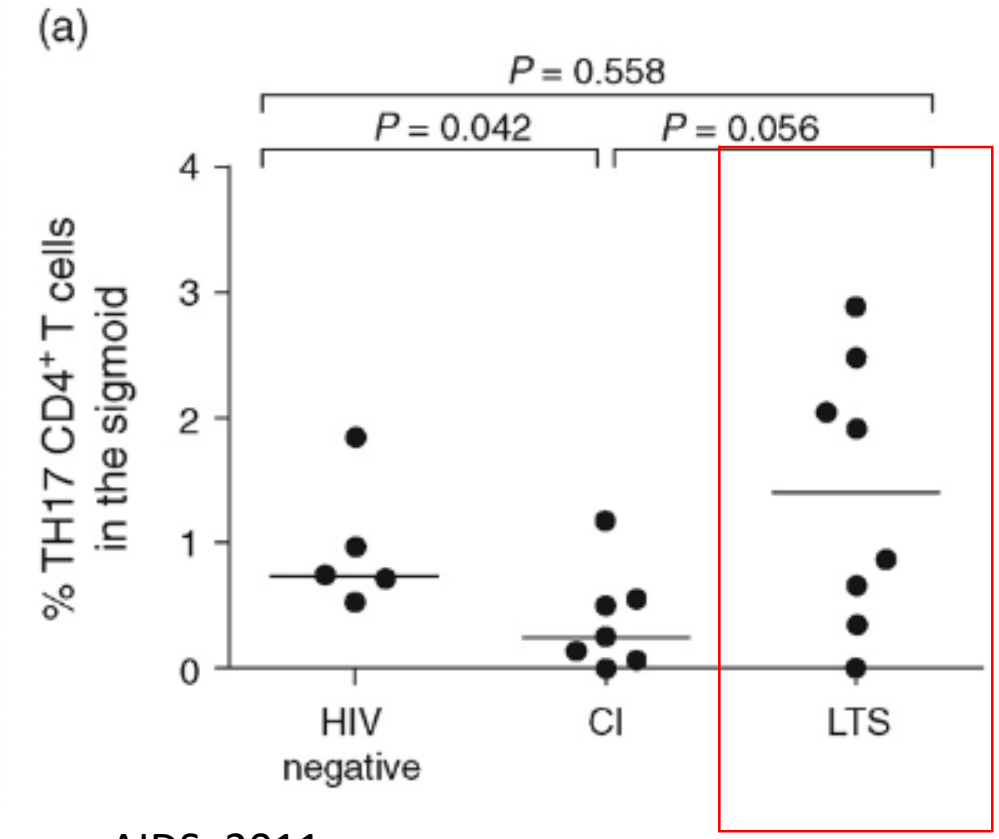


Timing of cART may influence CD4+ T reconstitution in the gut:

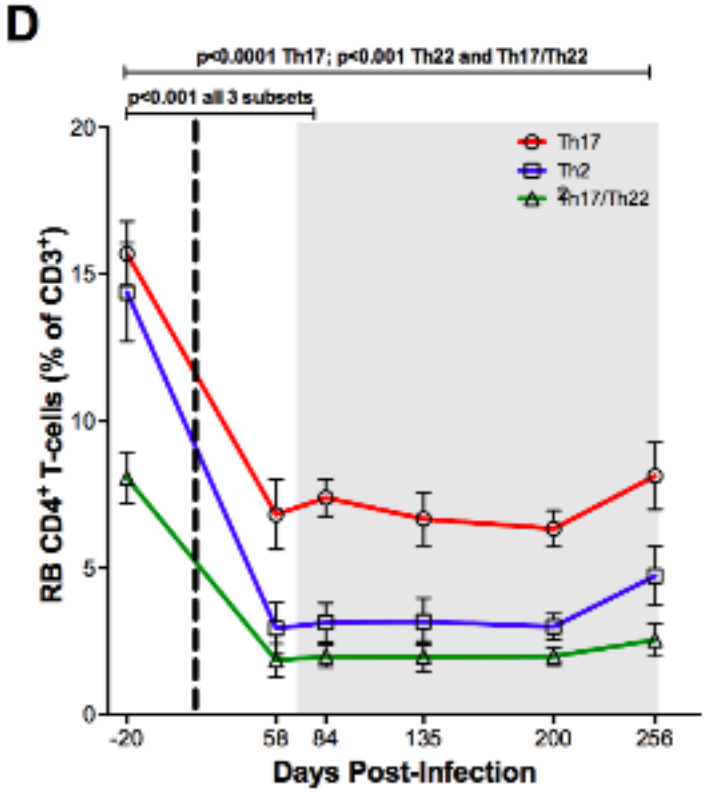
- acute: CD4 820/uL
- early chronic: CD4 > 350/uL

Allers, Mucosal Immunology, 2016

Impaired reconstitution of IL-17 producing cells in the gut

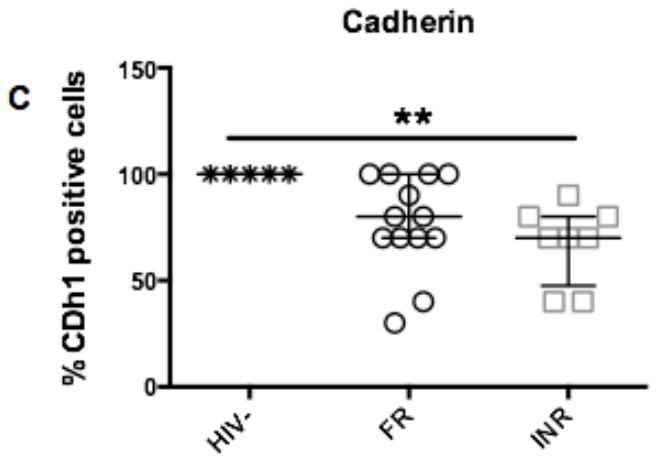


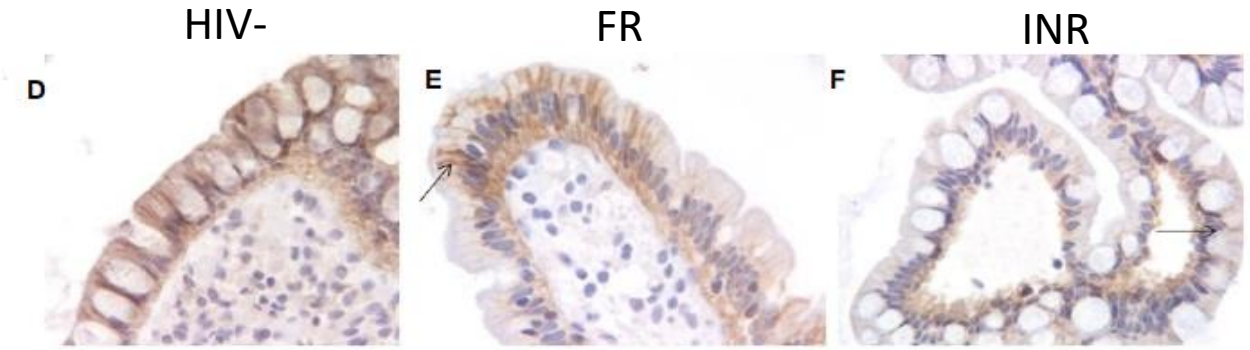
Chege, AIDS, 2011

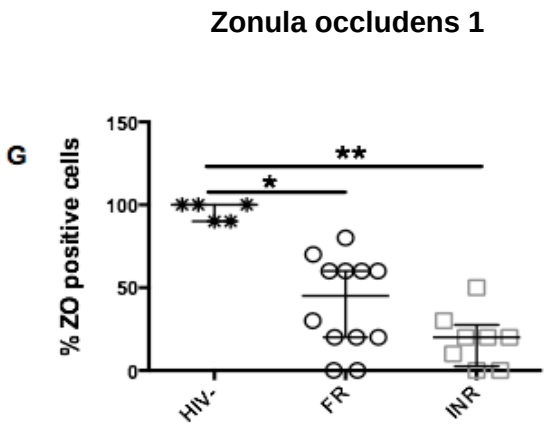


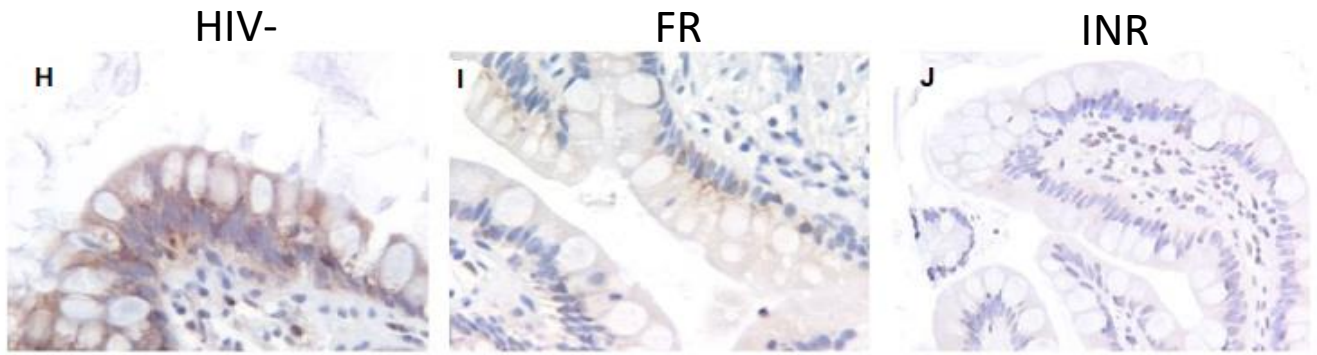
Ryan, Plos Path 2016

INR display impairment of ileum JC





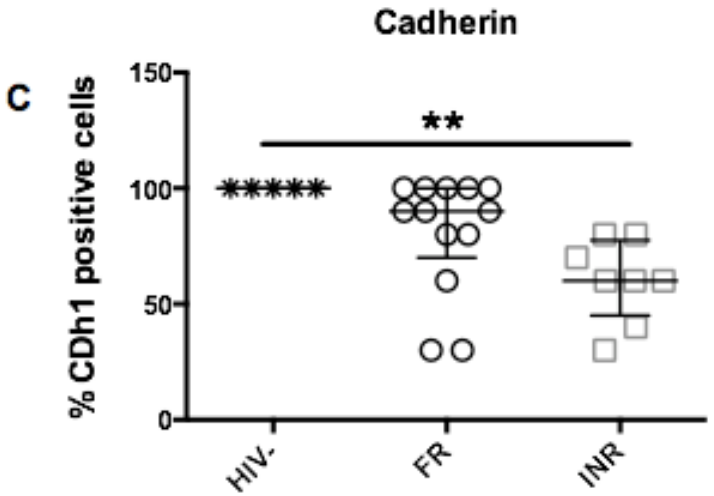


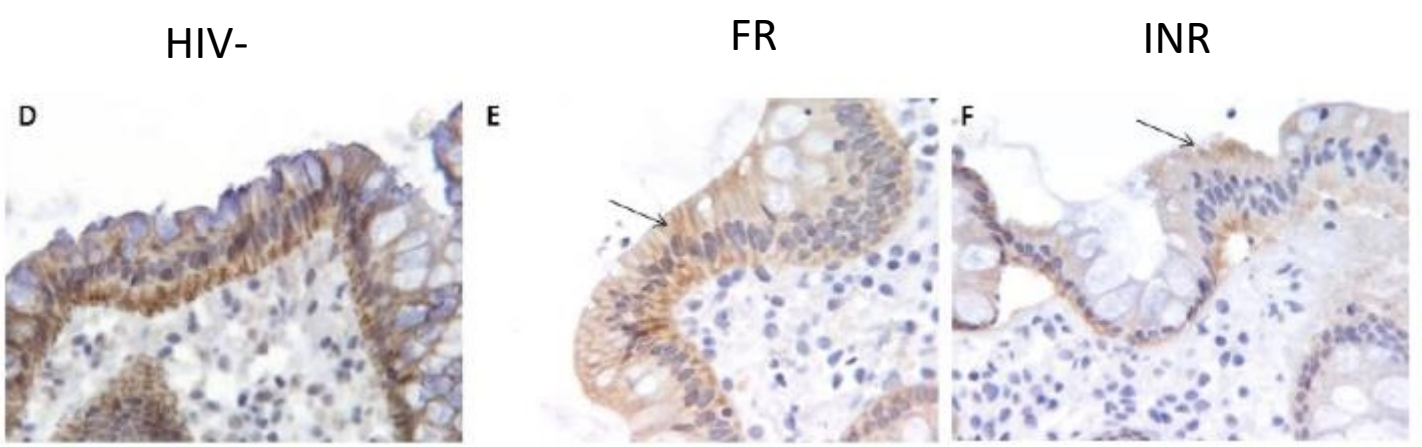


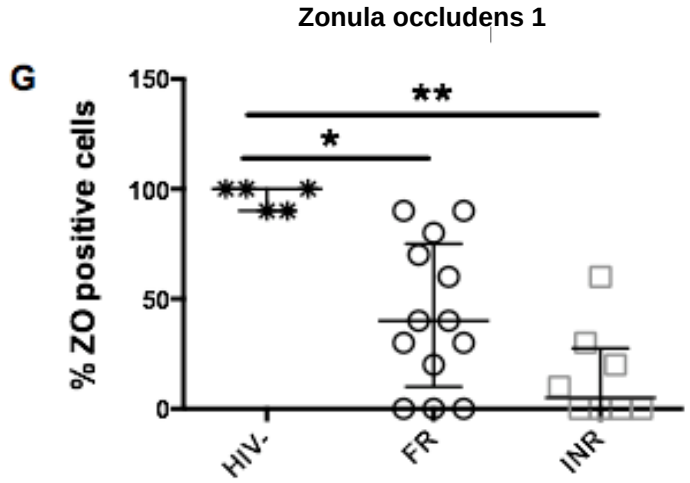
Tincati et al.; AIDS 2016

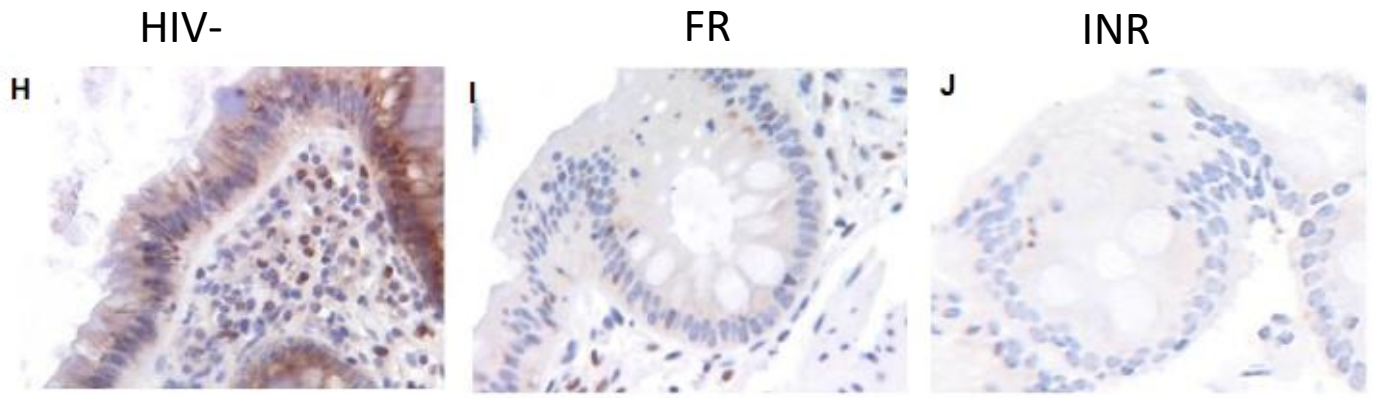
See also: Chung et al. Plos Path 2014; Somsouk et al. AIDS 2015

INR display impairment of colon JC





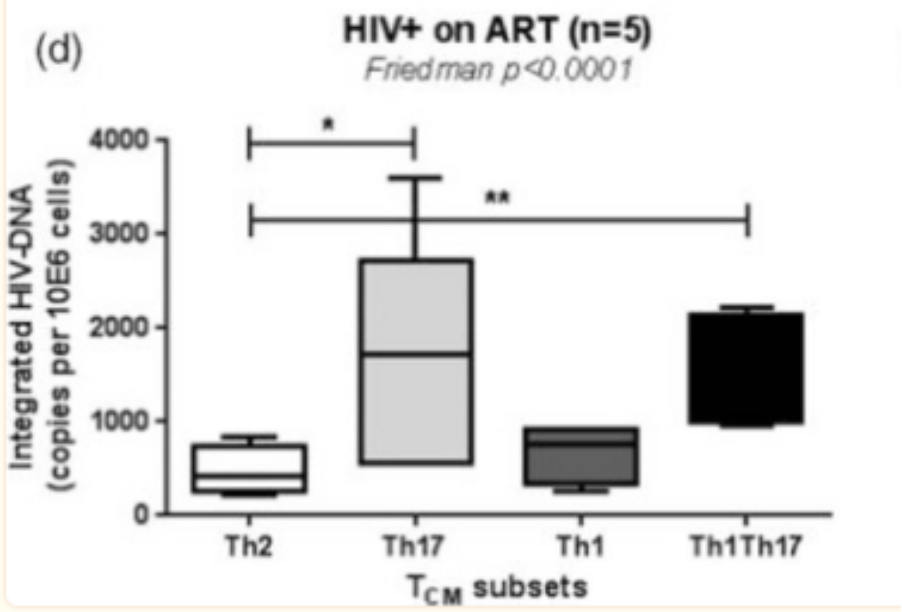




Tincati et al.; AIDS 2016

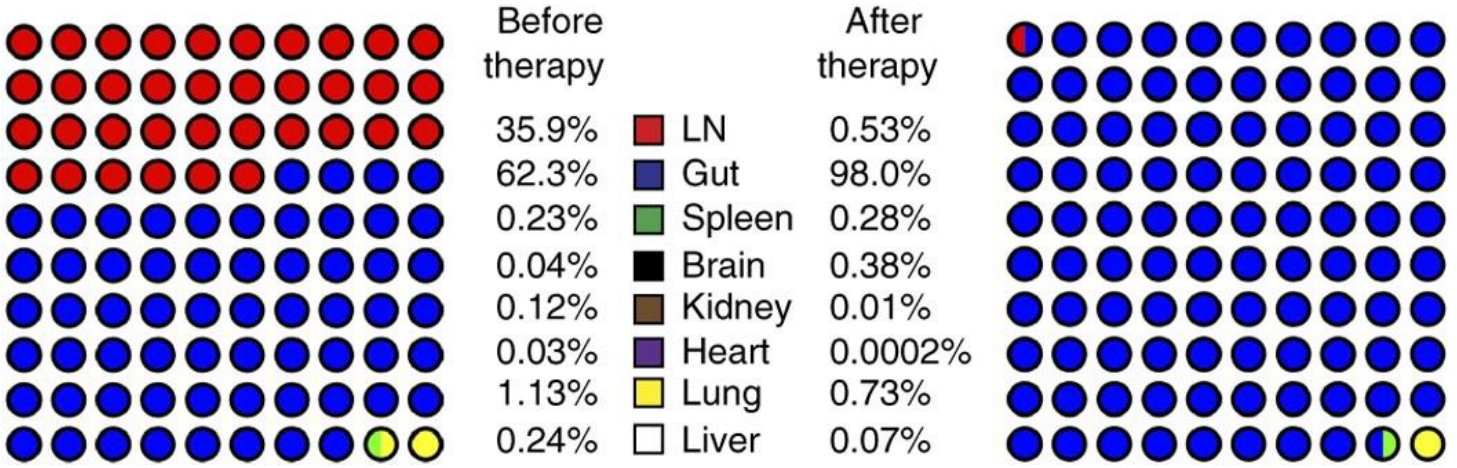
See also: Chung et al. Plos Path 2014; Somsouk et al. AIDS 2015

HIV persistence in Th17 cells



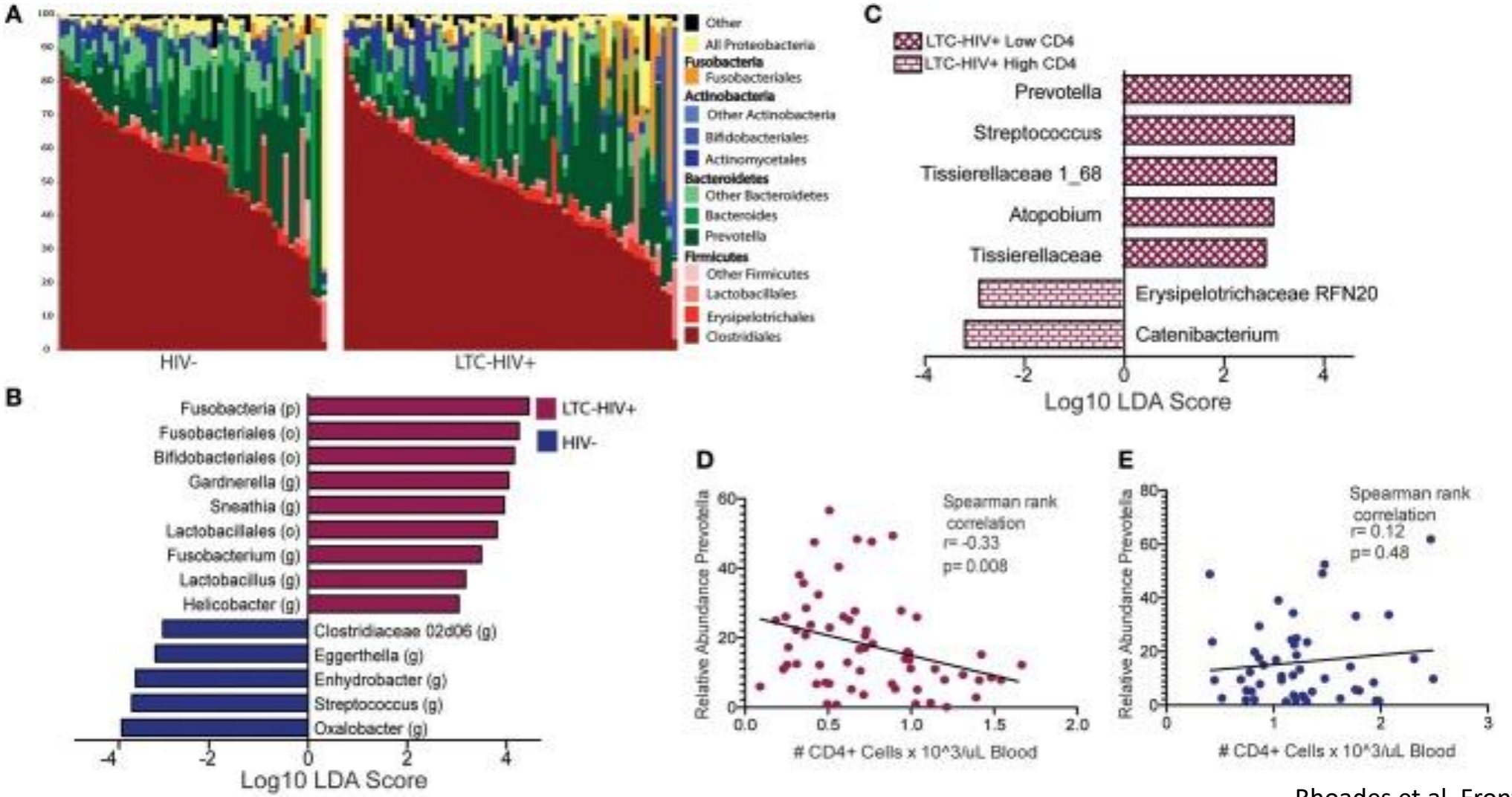
Gosselin, AIDS, 2017

Proportion of infected cells (vRNA+) cells in each organ system before and during suppressive ART



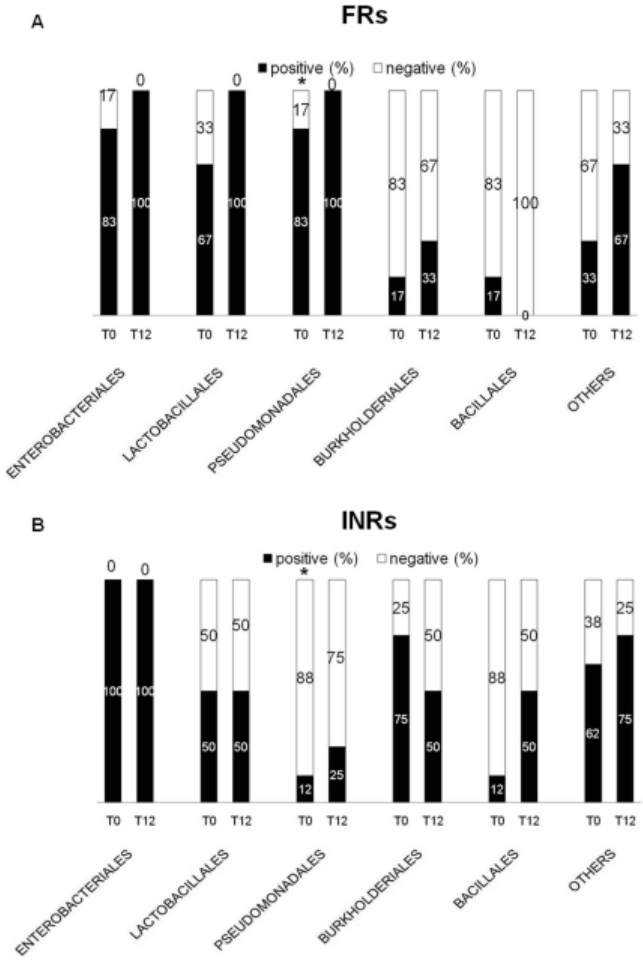
Estes et al. Nat Med, 2017

LTC-HIV+ are enriched in gut Gardnerella, Snethia, Fusobacterium, Lactobacillus, and Helicobacter; Prevotella is more abundant in individuals with a low CD4+ and negatively correlates with CD4+ T cell count

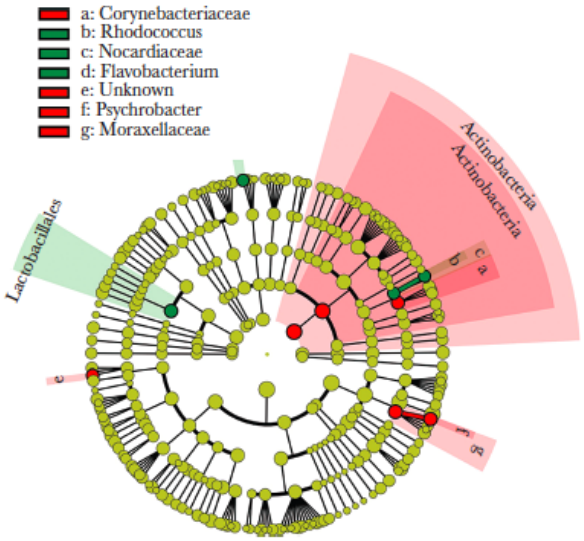


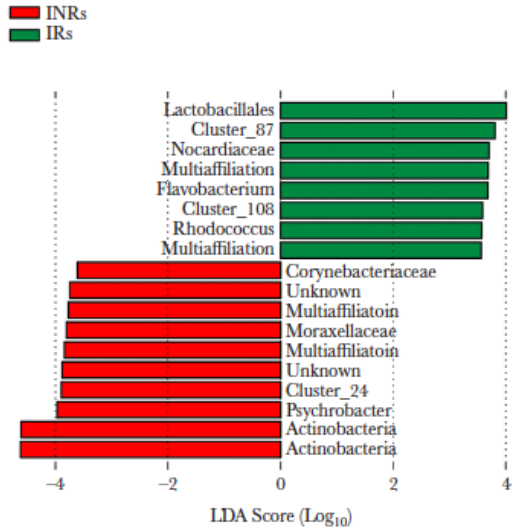
Rhoades et al. Front Immunol 2019

Translocating bacteria in the blood impact CD4+ recovery

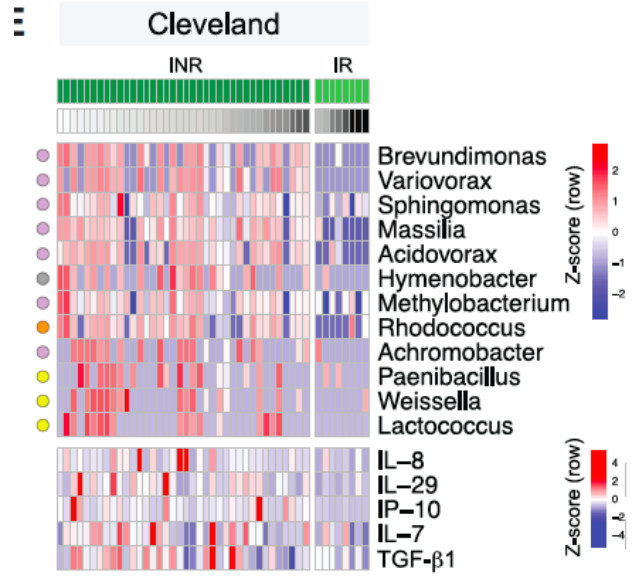


Merlini, PlosOne 2011



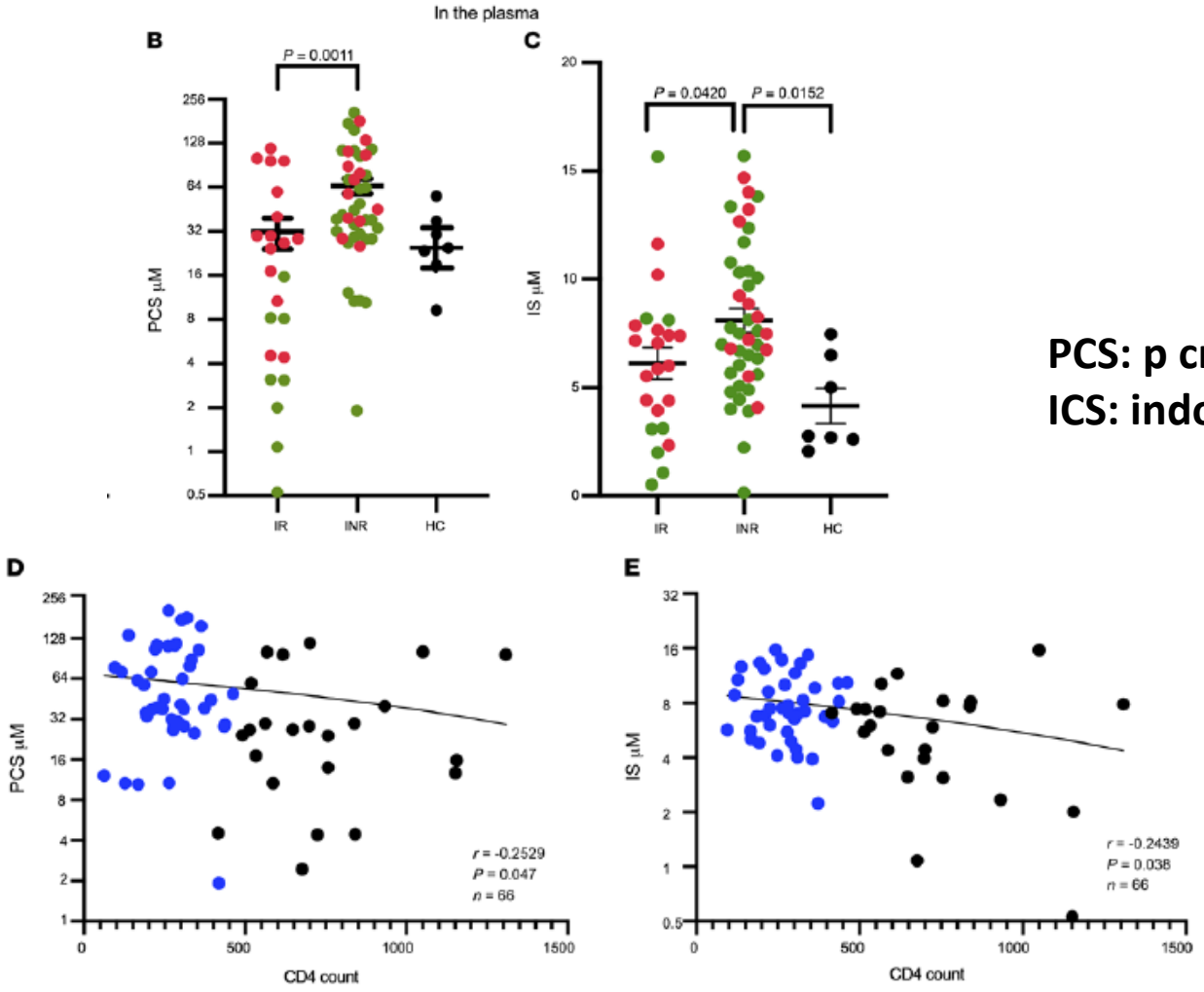


Serrano-Villar, JID, 2021

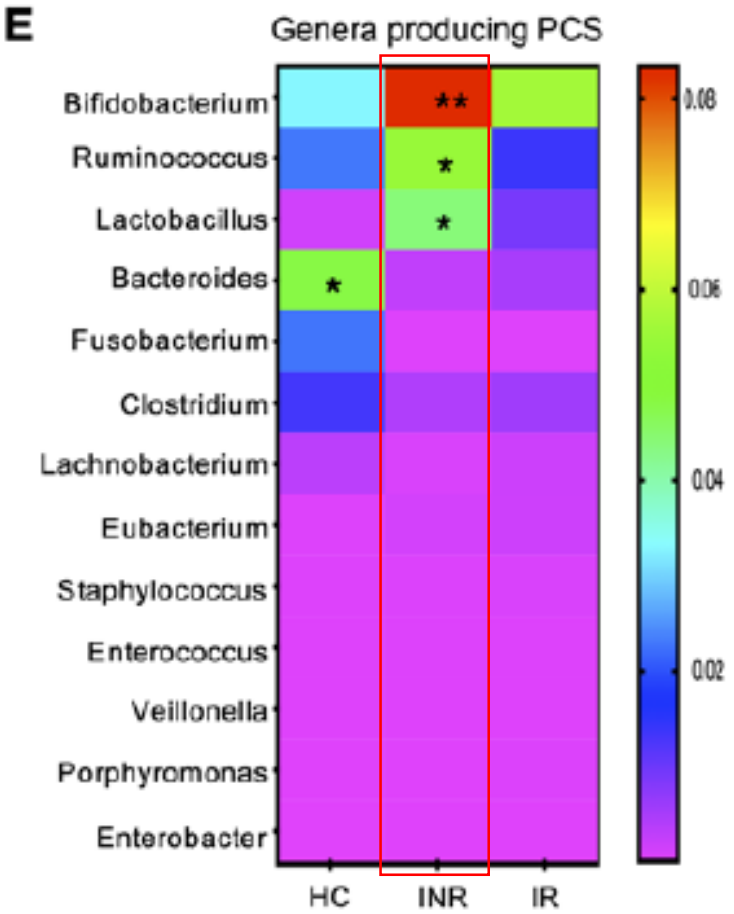


Nganou, Cell, 2021

Bacterial toxins may mediate poor CD4+ T-cell gain

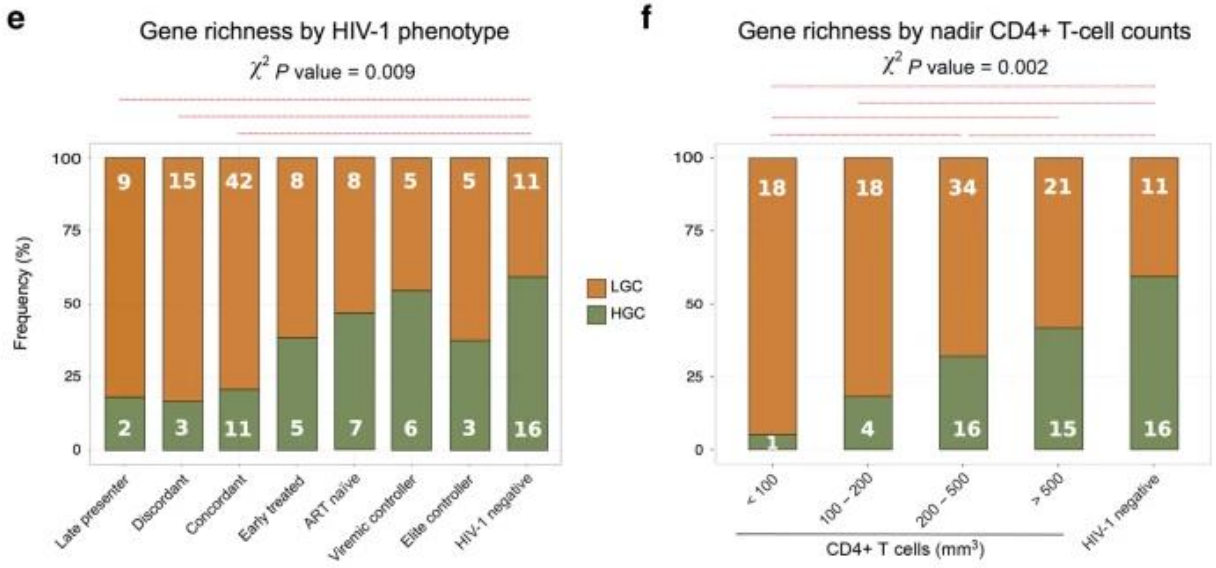


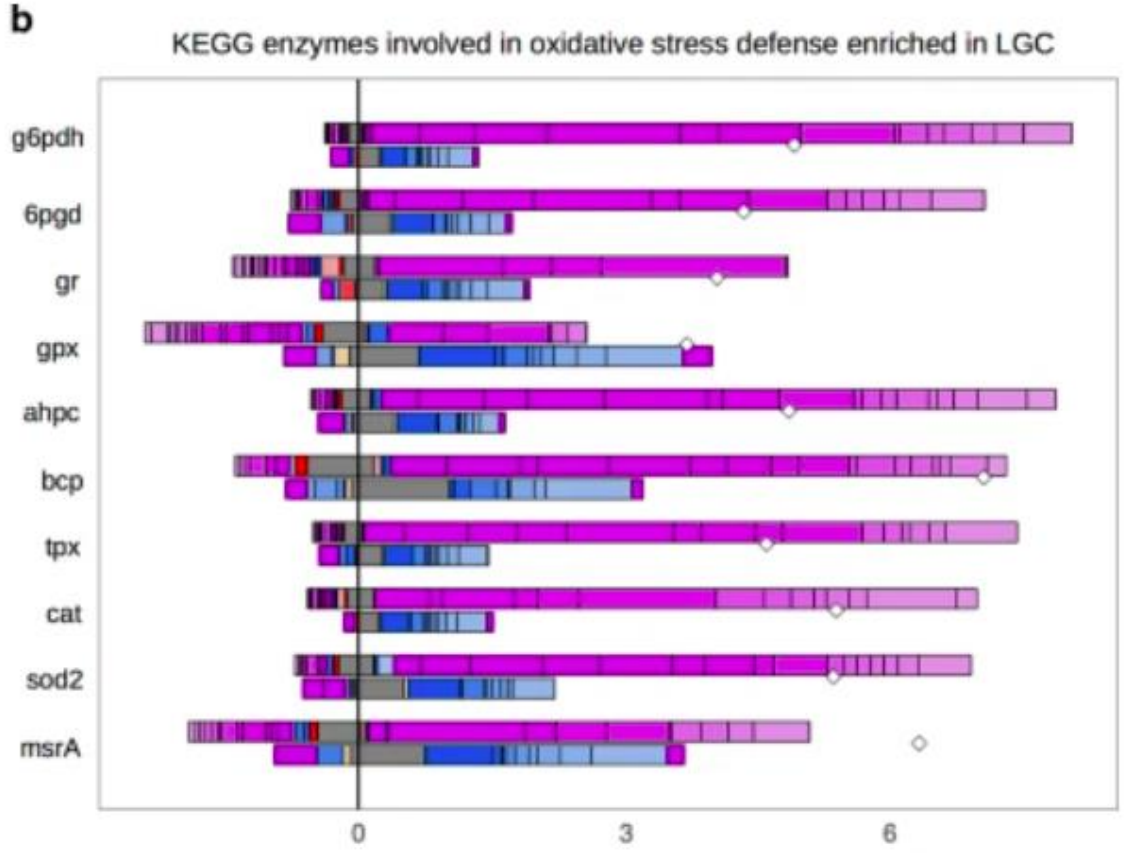
PCS: p cresol-sulfate
ICS: indoxyl sulfate



Ferrari, JCI, 2021 (see also Nystrom, OFID 2021)

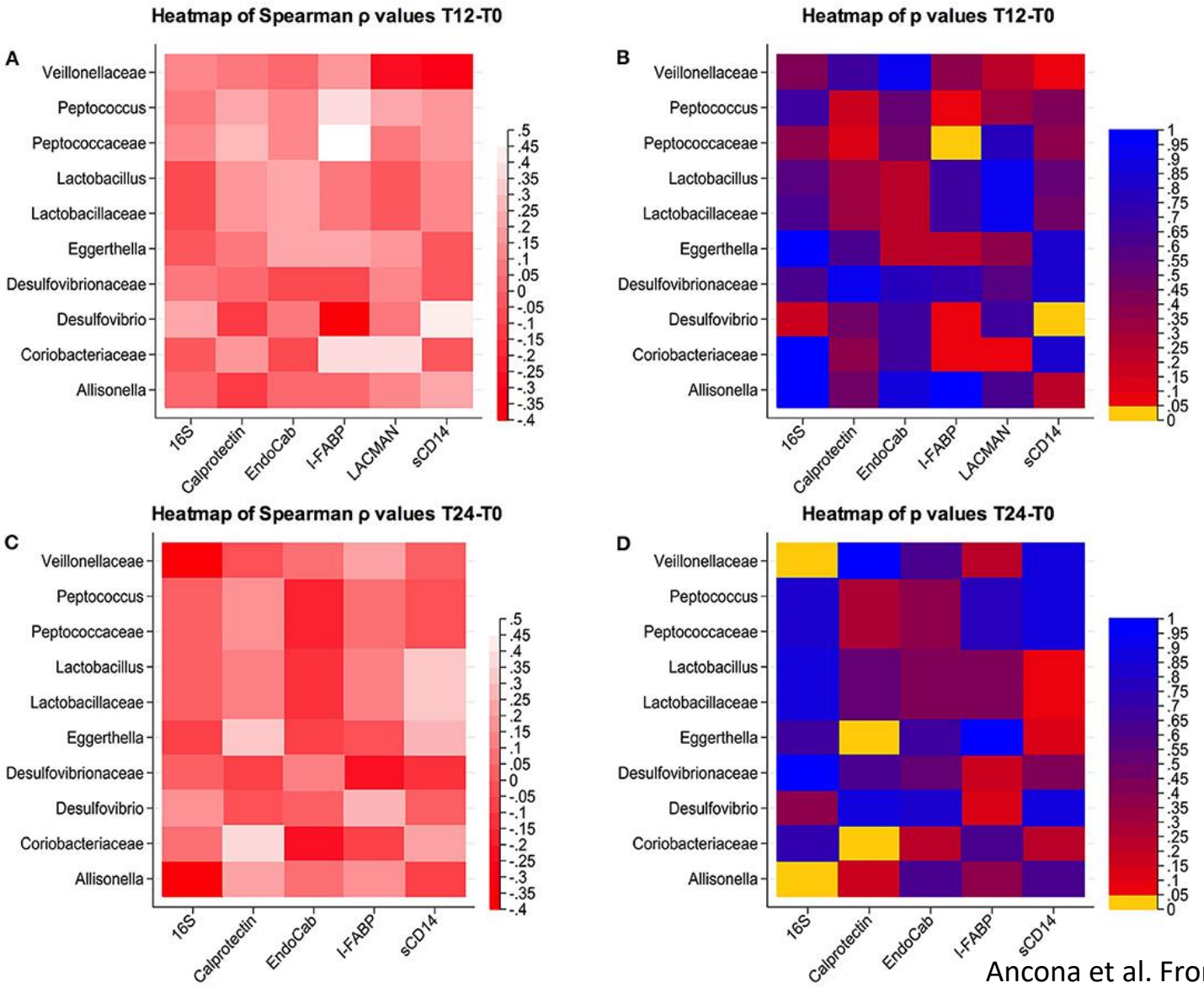
Low CD4+ nadir: low-microbial gene richness linked to ROS gene enrichment





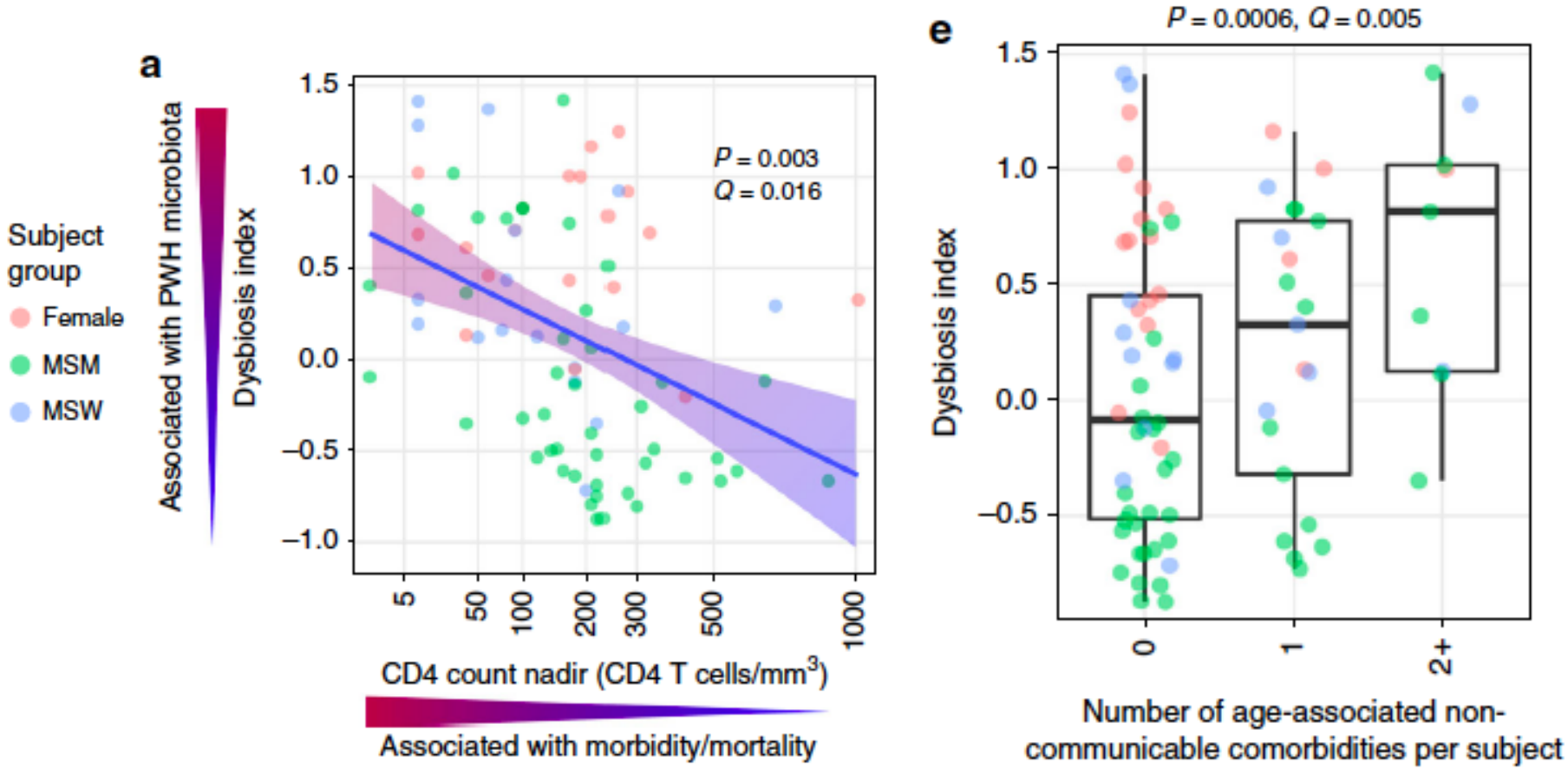
Guillen, Mucosal Immunology, 2019

Possible associations between bacterial composition and gut damage/microbial translocation

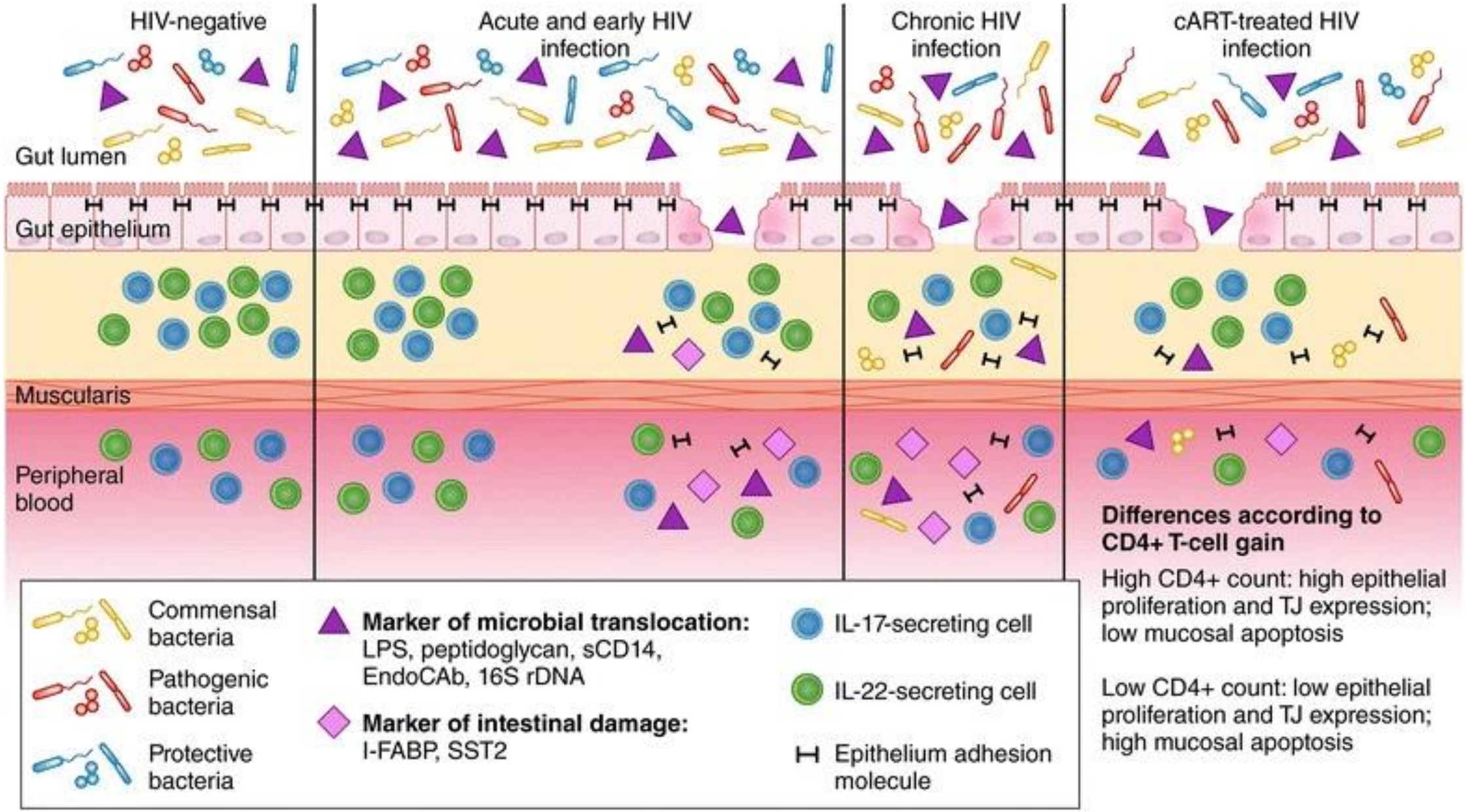


Ancona et al. Front Immunol 2021

HIV-associated gut microbiota features correlate with markers of HIV disease progression and comorbidity prevalence



Vujkovic-Cvijin et al. Nat Comm. 2020



Tincati, AIDS Res and Ther, 2016

Novel approaches to treat HIV (all failed)

- Immune activation: steroids, anti-TNF, statins
- Microbial translocation: sevelamer, rifaximin
- Immuno adjuvants: IL-2, IL-7, IL-22
- Dysbiosis: fecal transplantation
- Reservoirs: latency reverting agents

And many more….

Conclusions

- During HIV structural, viro-immunological and microbiologica changes occur in the gut→ site of pathogenesis

- cART partially reverts such abnormalities→implications in patient management and viral eradication:

- Which cART/adjuvant strategies? Impact on inflammation? Drug penetration in tissues?
- When should we treat? The earlier the better?

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TRANSCAN-2
ERA-NET: Aligning national/regional translational cancer research programmes and activities
TRANSCAN-2

Fondazione Regionale per la Ricerca Biomedica

Regione Lombardi

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