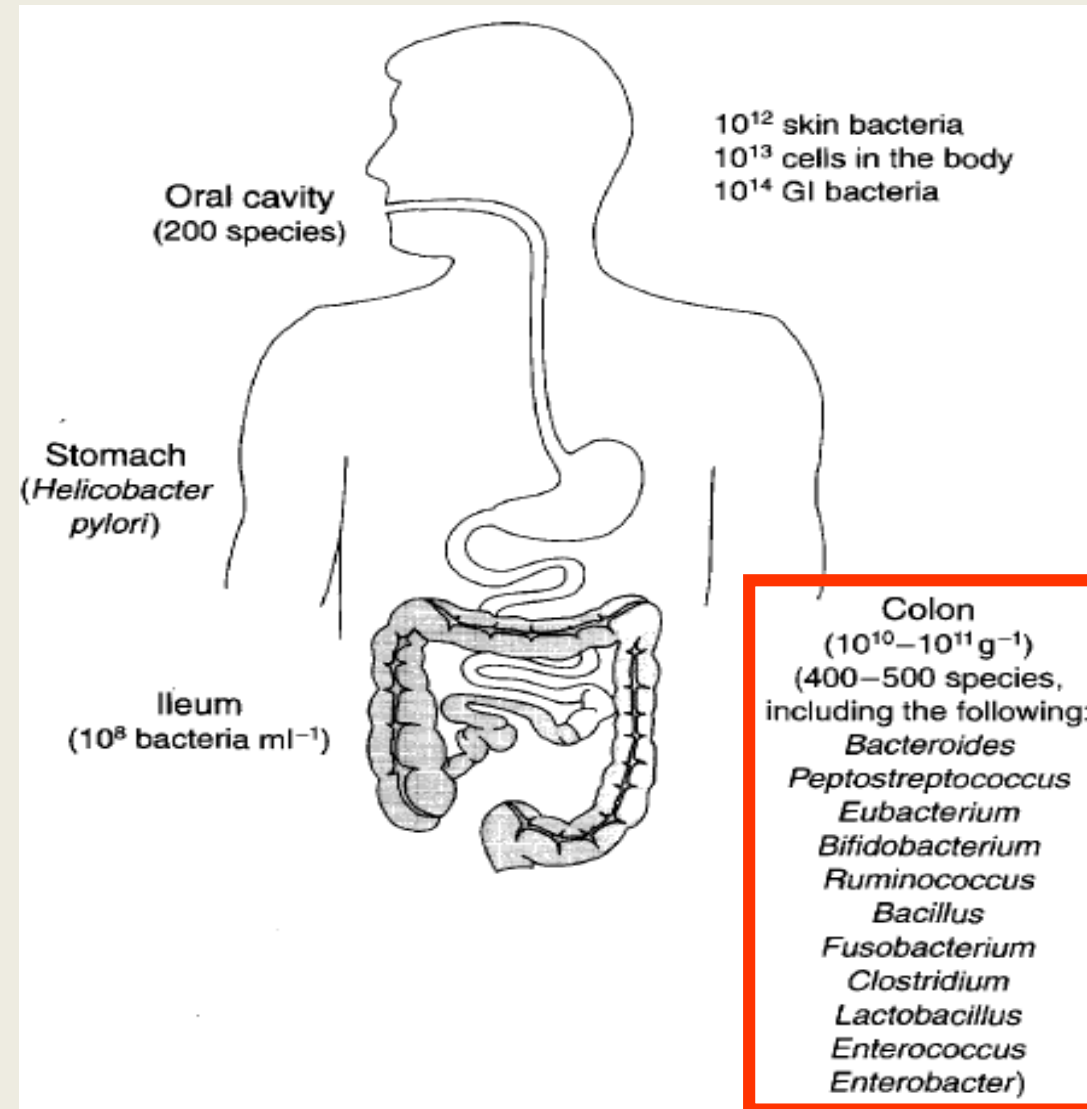


MICROBIOMA INTESTINALE: FALSITA' E VERITA' (NELL'INFEZIONE DA HIV)

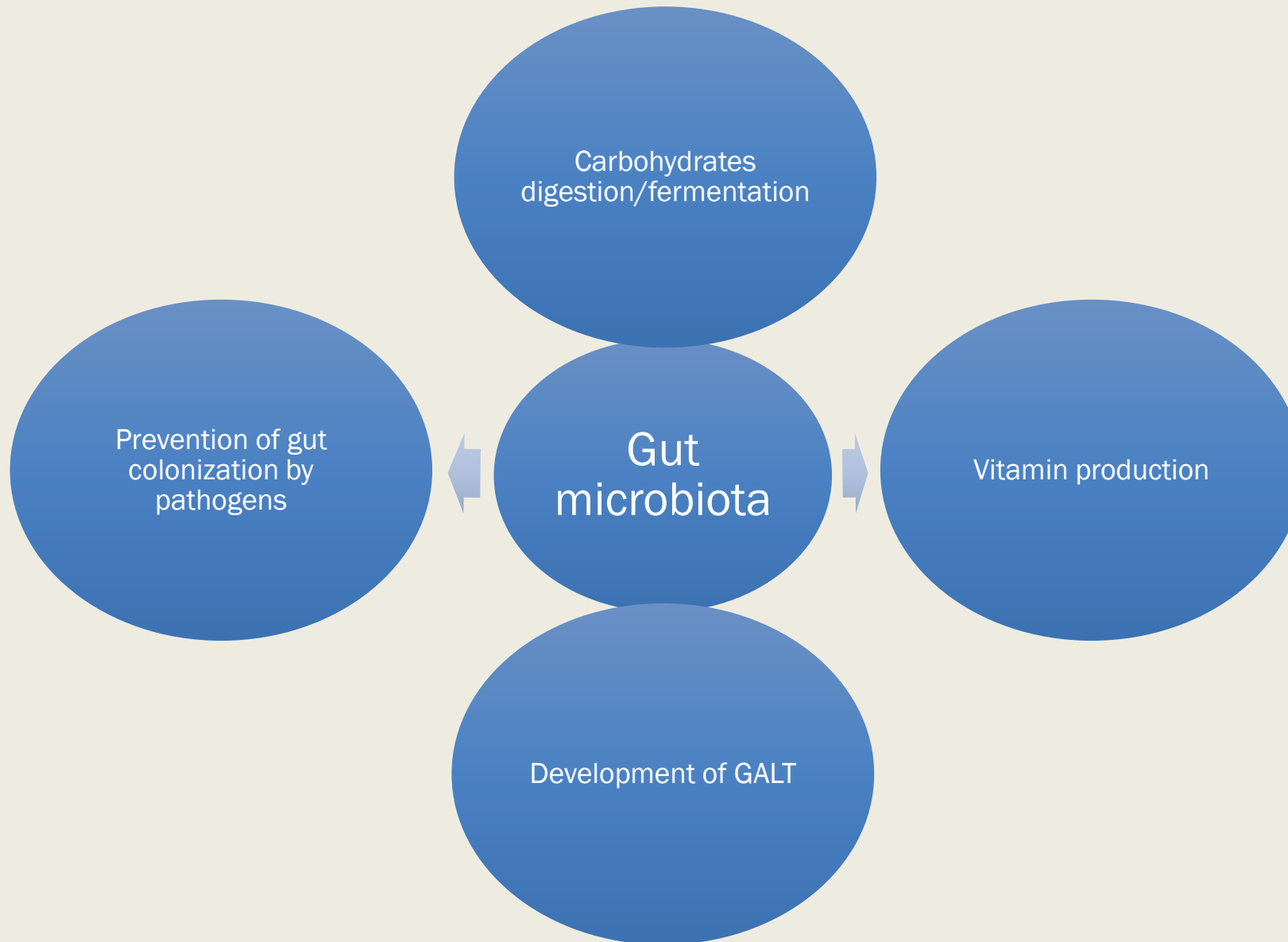
Prof.ssa Giulia Marchetti, MD, PhD

*Dip di Scienze della Salute, Clinica delle Malattie Infettive, Università
degli Studi di Milano – ASST Santi Paolo e Carlo, Milano*

Gut microbiota



Contributions of gut microbiota to the host



HIV, gut damage, dysbiosis, microbial translocation and systemic inflammation

The facts

The “yet to be demonstrated” facts

HIV, gut damage, dysbiosis, microbial translocation and systemic inflammation

The facts

The “yet to be demonstrated” facts

Fact #1

**HIV is a disease of
the gut**

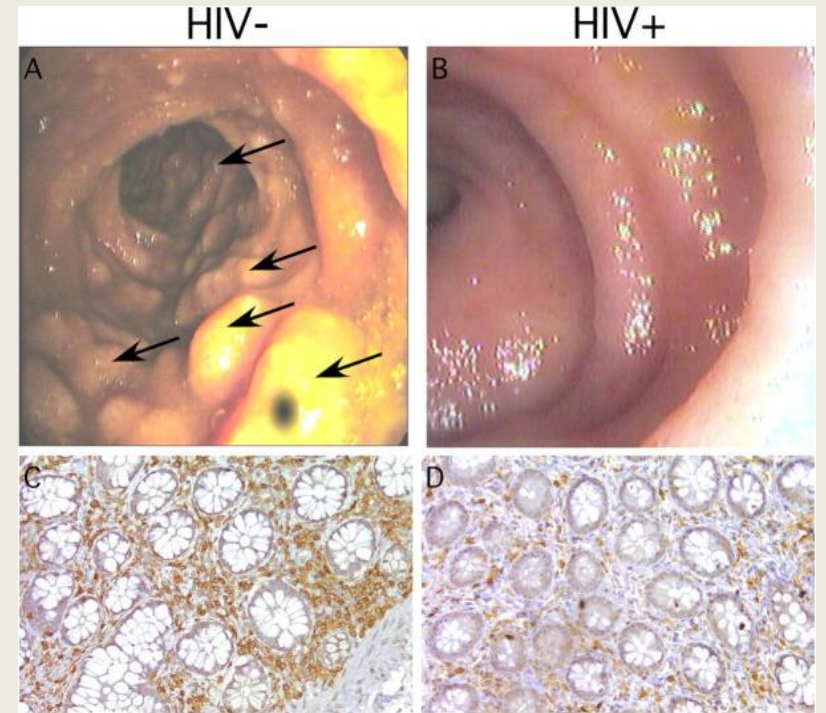
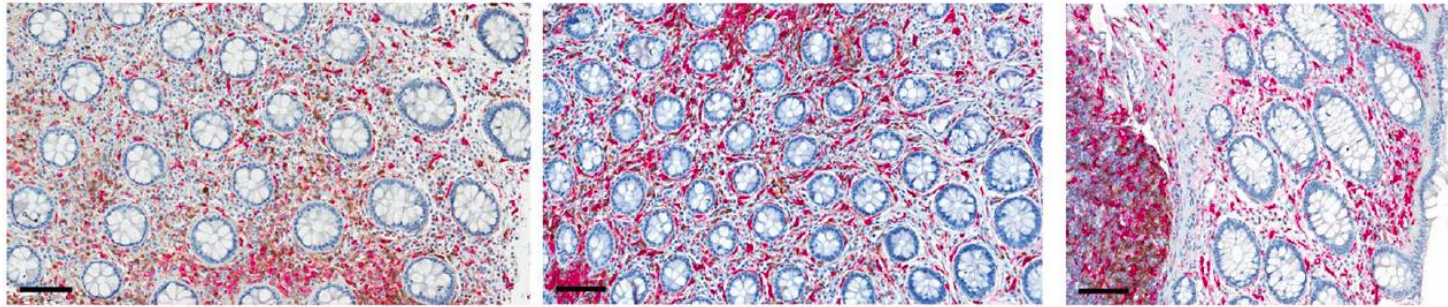
HIV & the gut: an old story

“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”

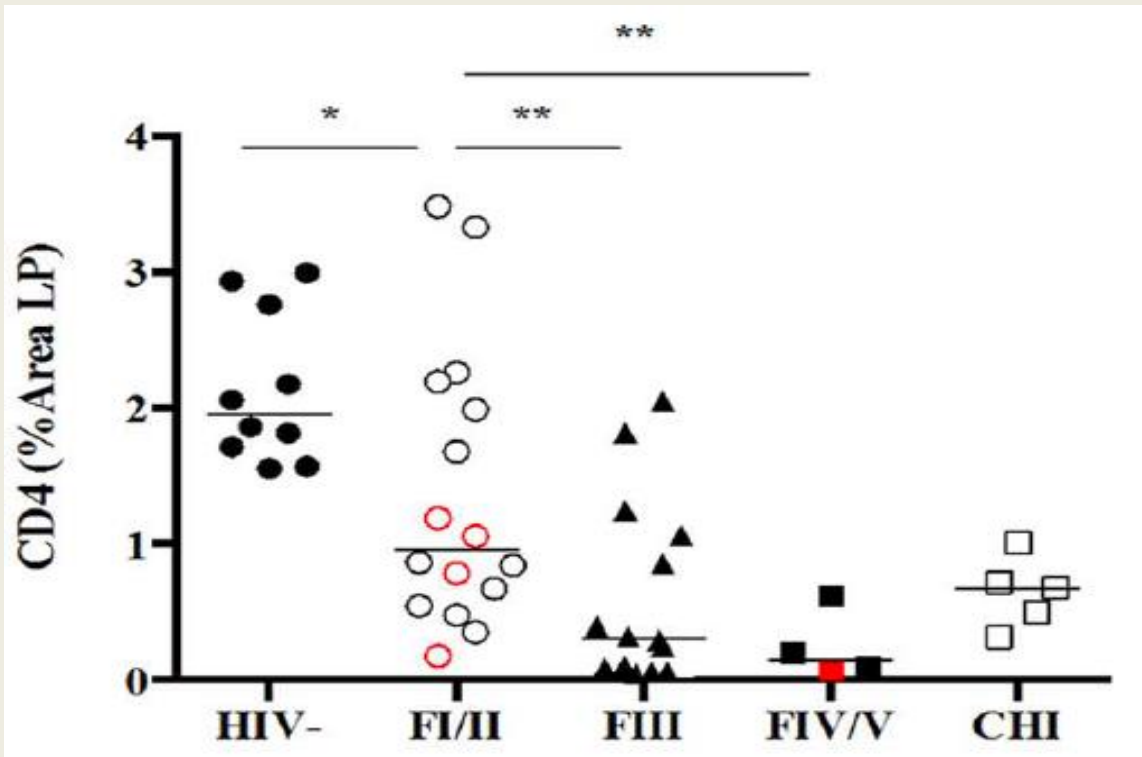
Kotler JID 1999;179 (suppl 3)

Earliest depletion of gut-associated CD4+

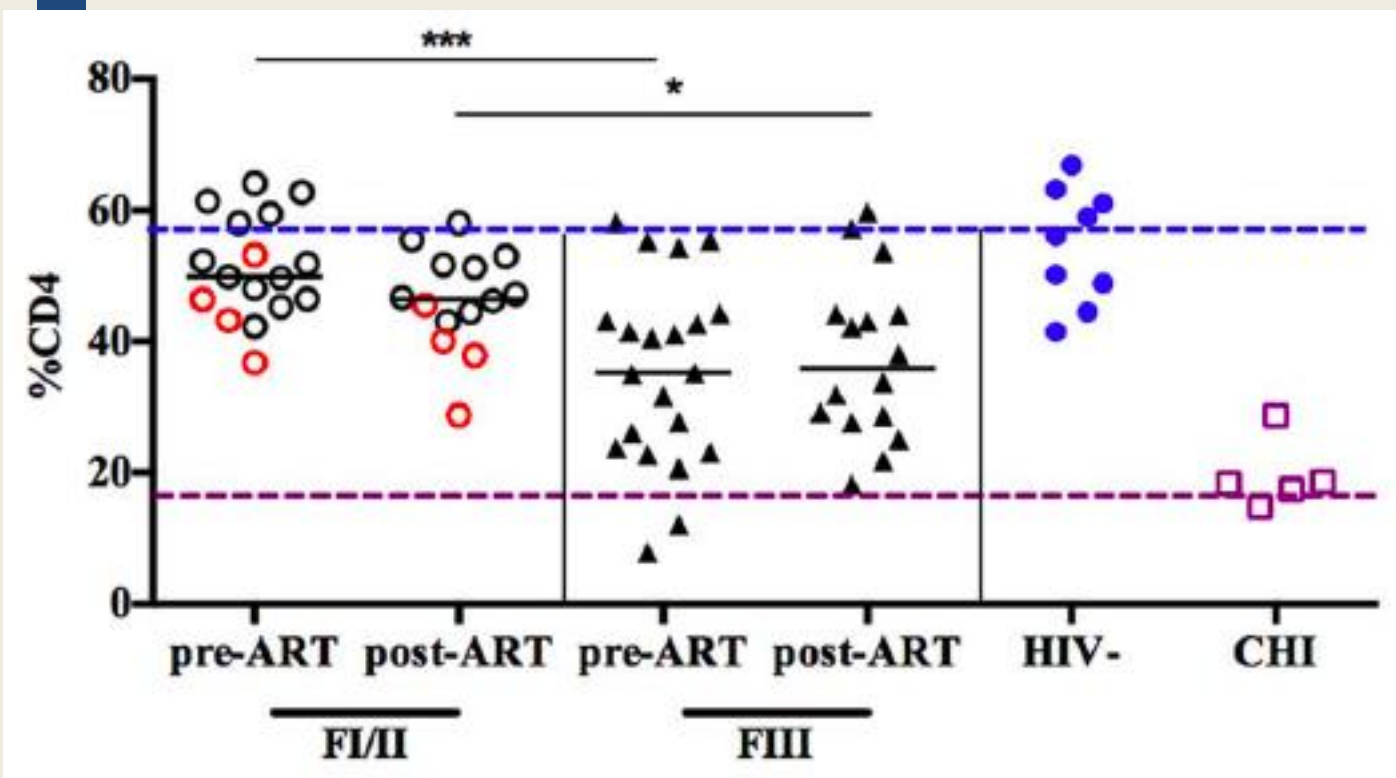
CD4+ T cells
(CD68/CD163)



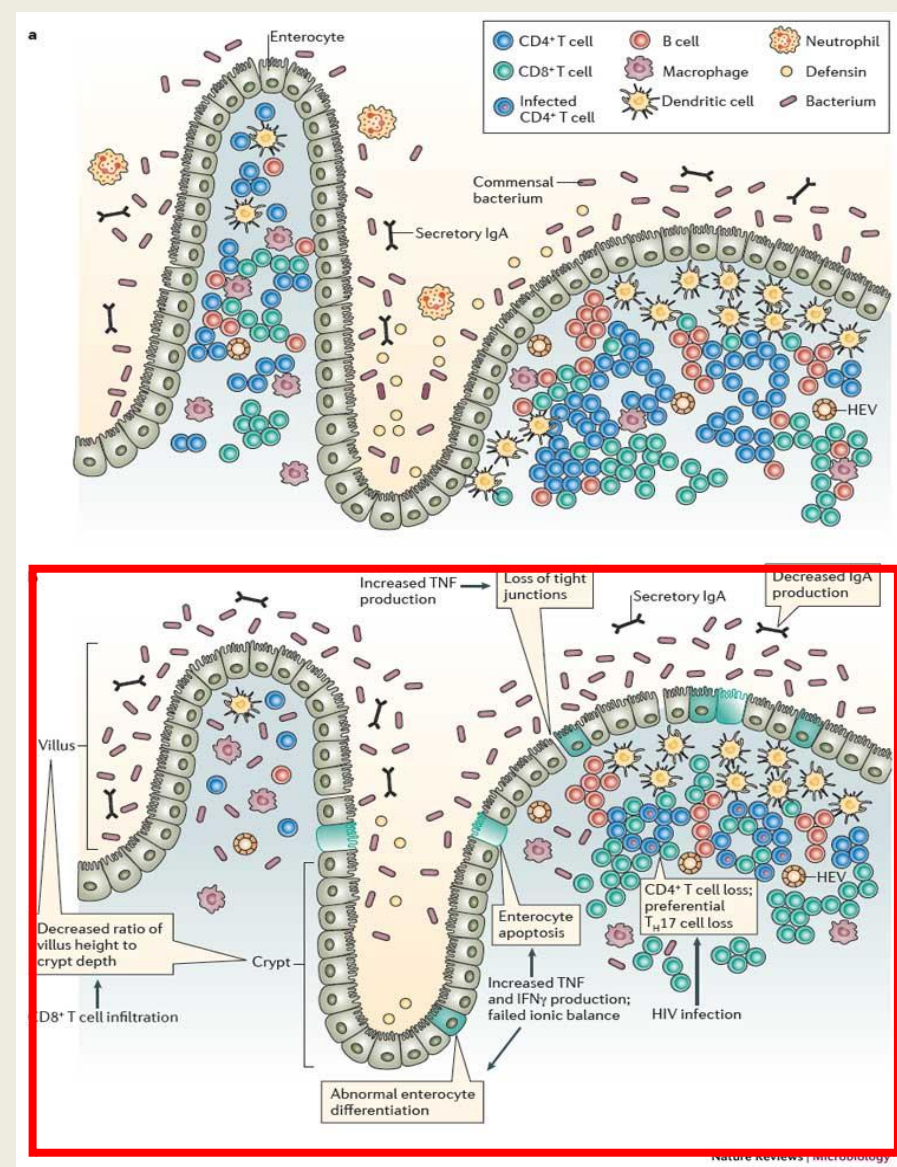
Brenchley et al. Nat Med 2006



Shuetz et al. Plos Path 2014

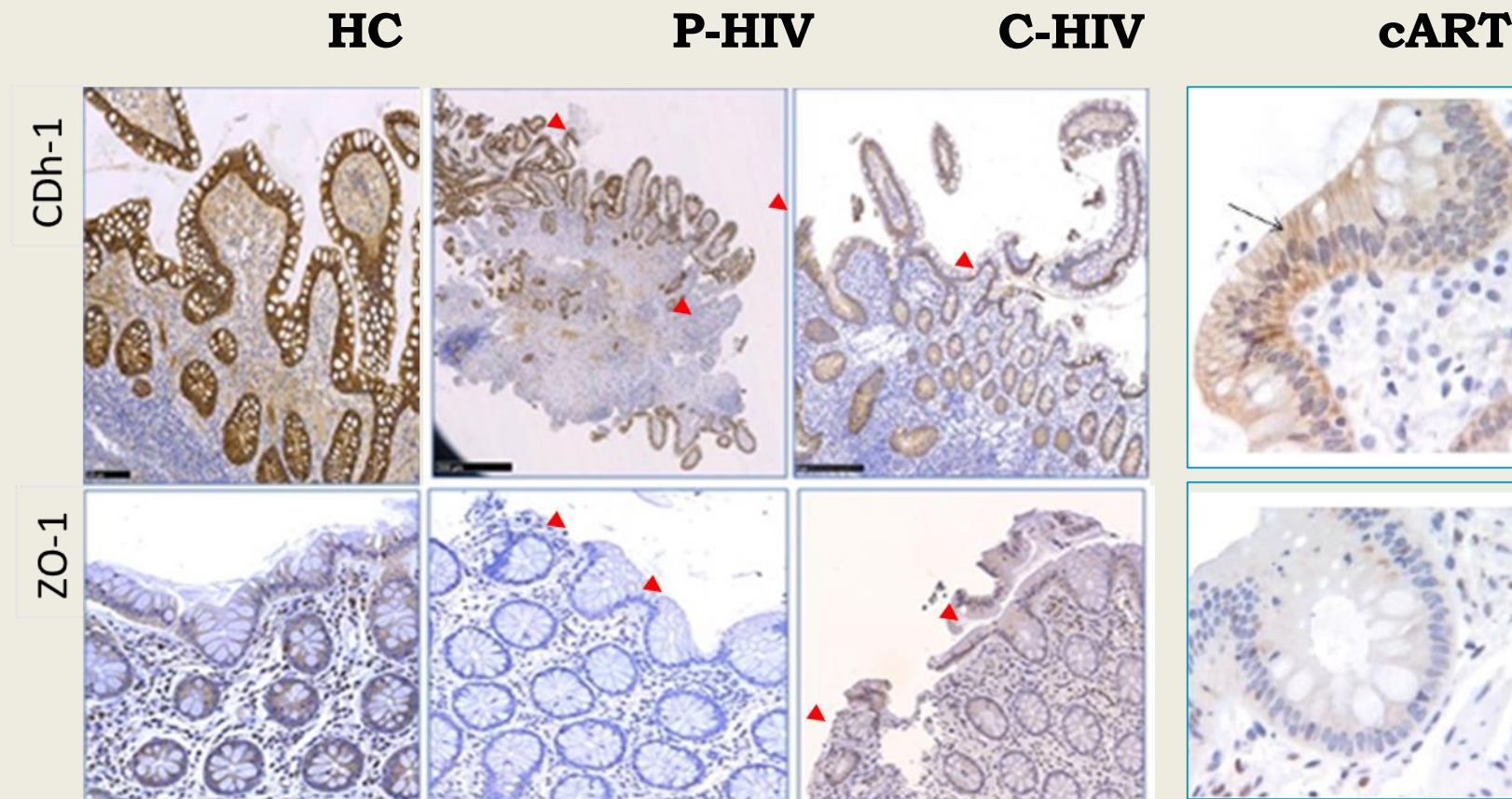


Shuetz et al. Plos Path 2014



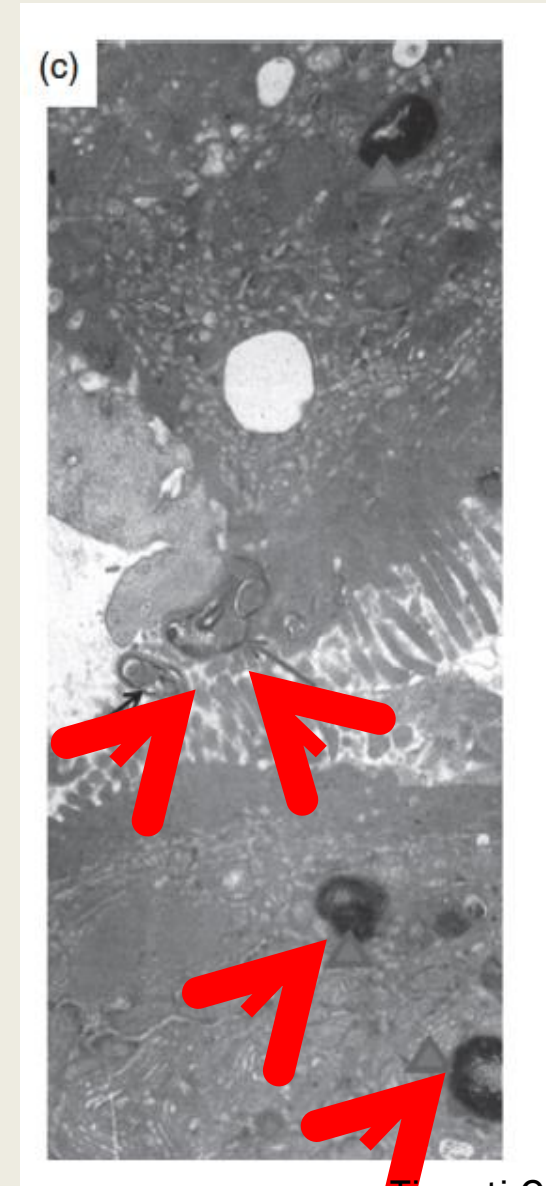
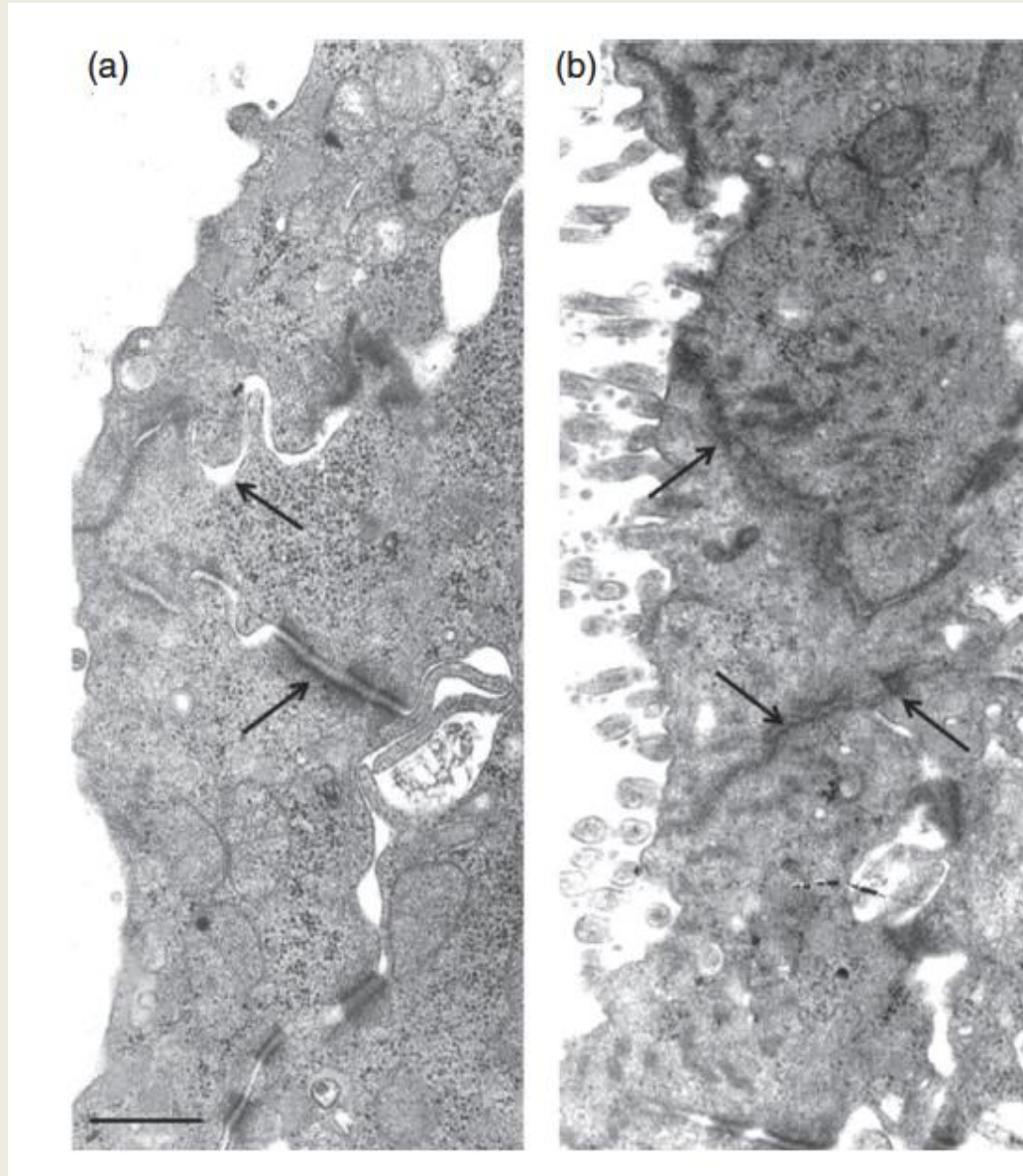
Sandler & Douek, Nat Reviews 2012

Early depletion of gut tight junctions that is not reverted by cART



Chronic HIV on virally-suppressive cART: nadir CD4 < 200/mm³

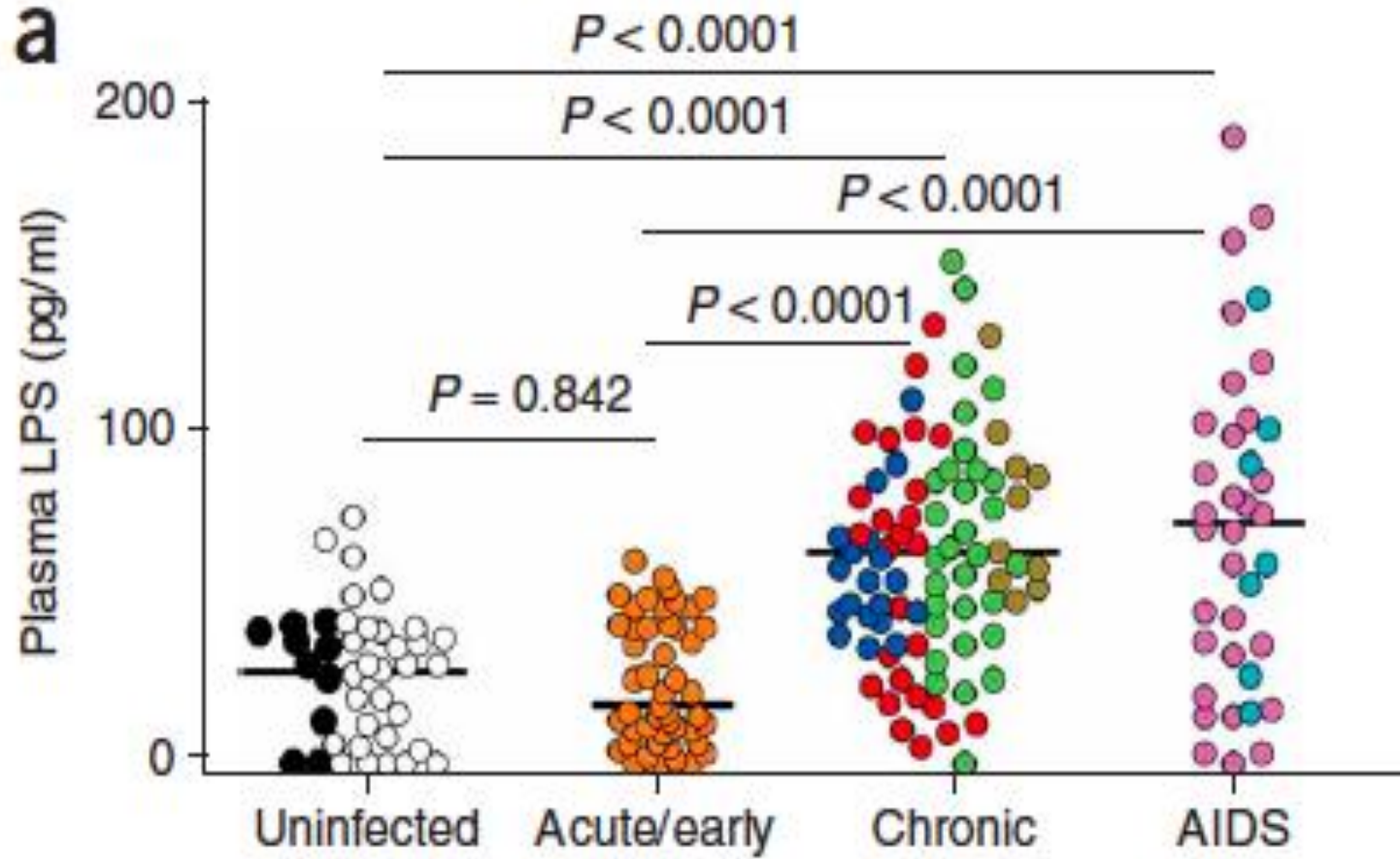
Healthy HIV uninfected



Fact #2

**HIV-associated gut
disruption leads to
microbial
translocation**

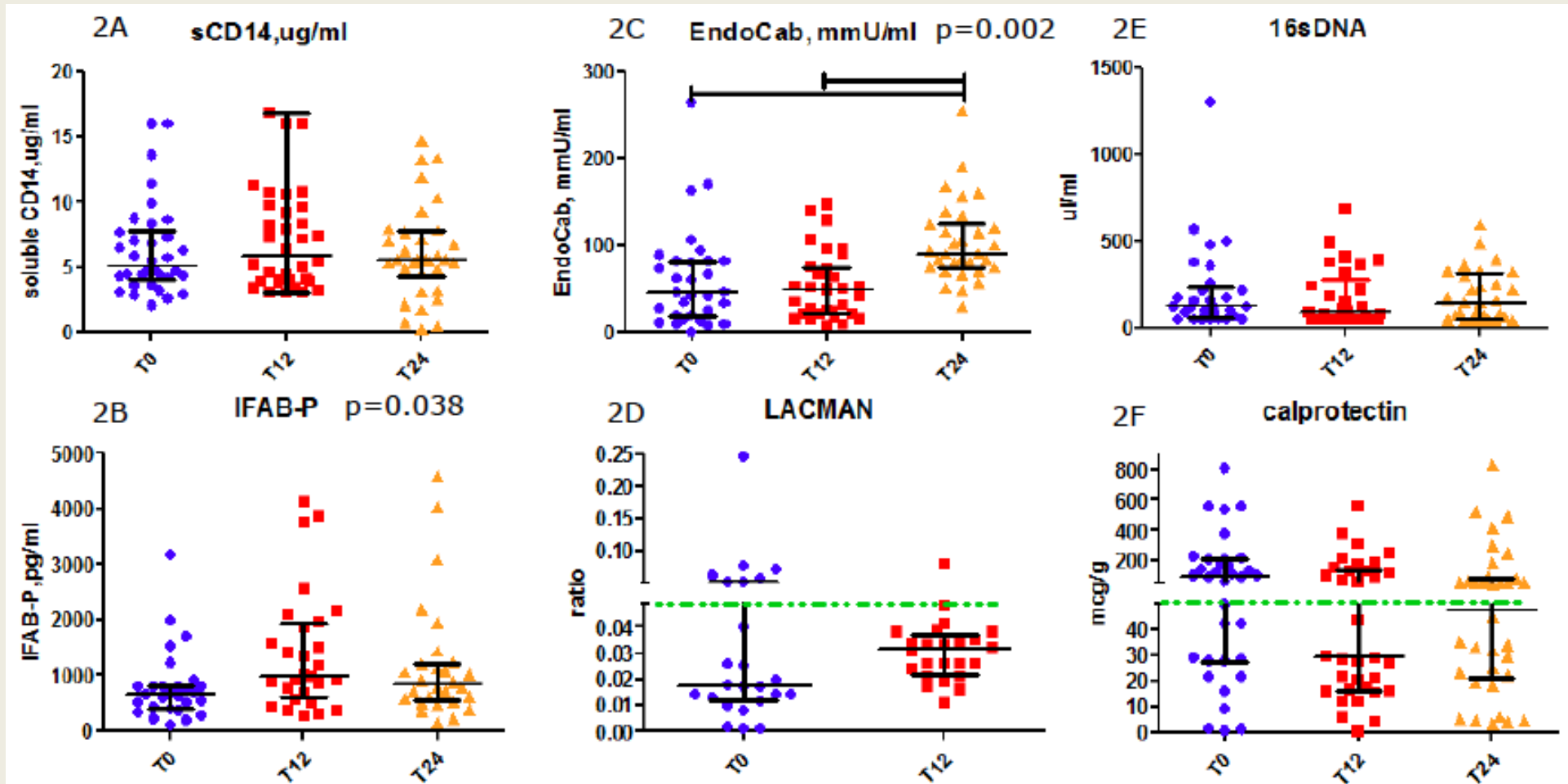
a



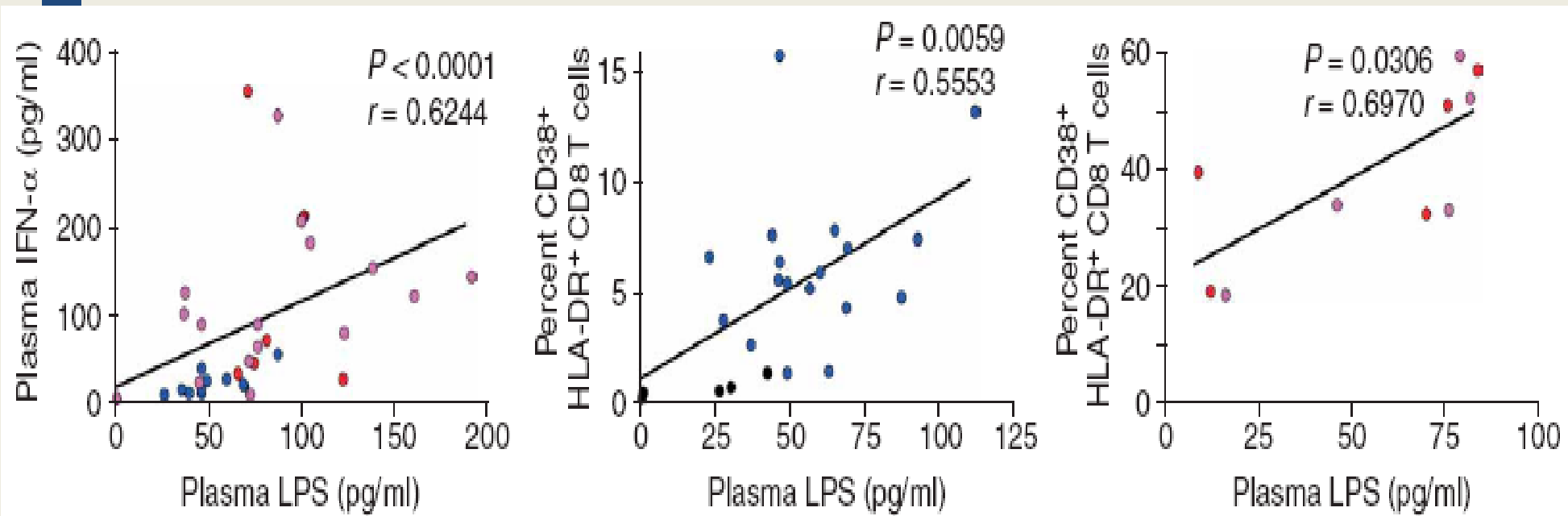
Brenchley et al. Nat Med 2006

No recovery of microbial translocation and gut damage on long-term cART started during chronic HIV

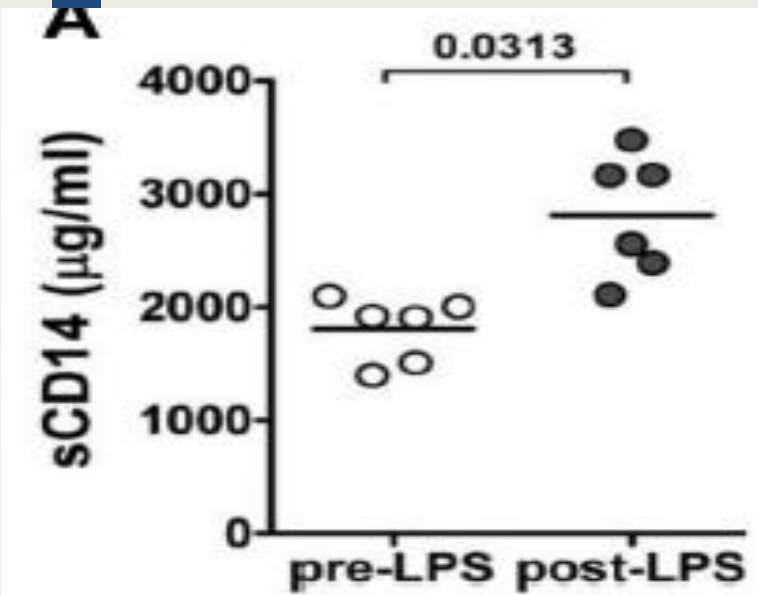
40 HIV+, nadir CD4 =300/ μ l



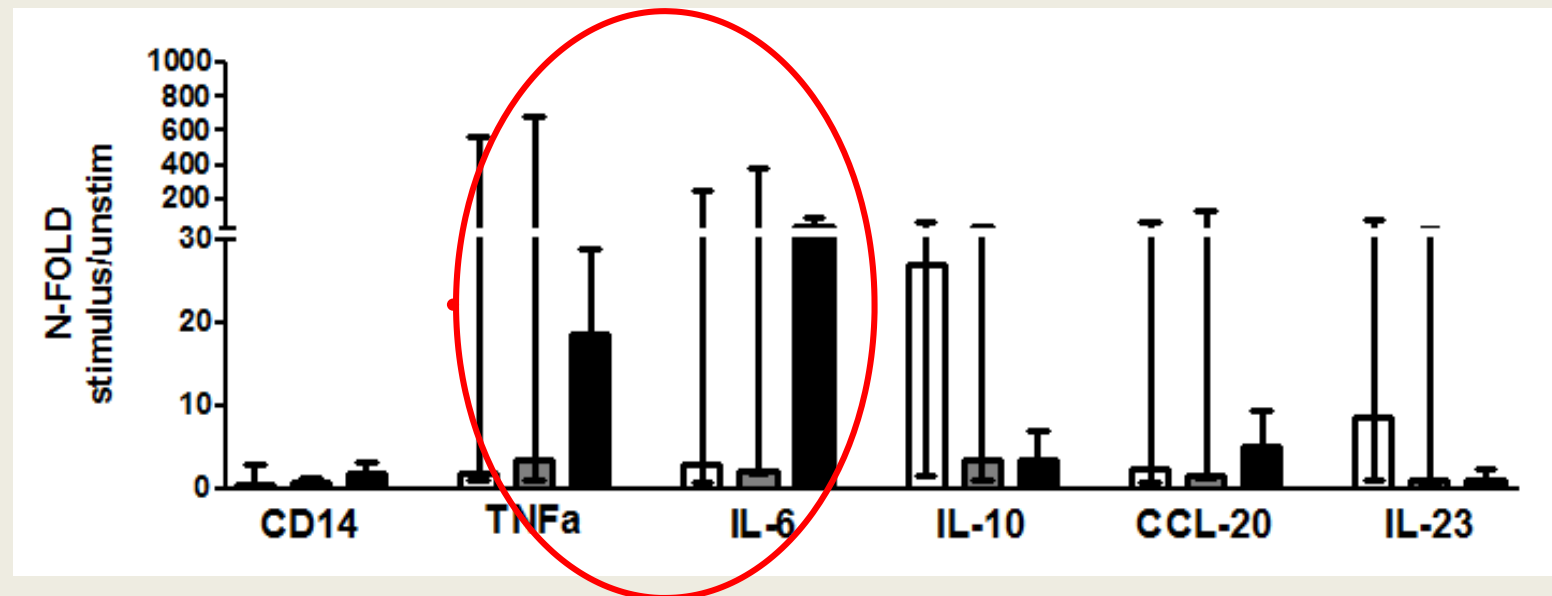
**Which consequences of
microbial translocation?**



Brenchley Nat Med 2006



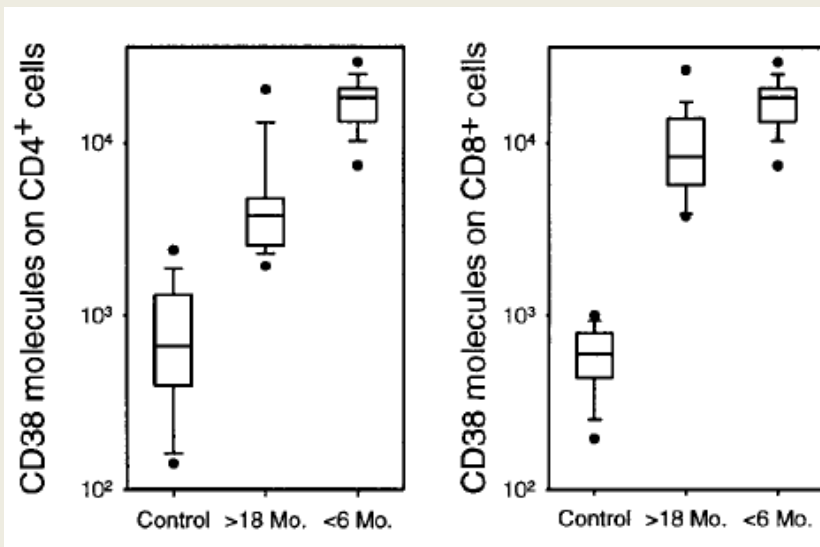
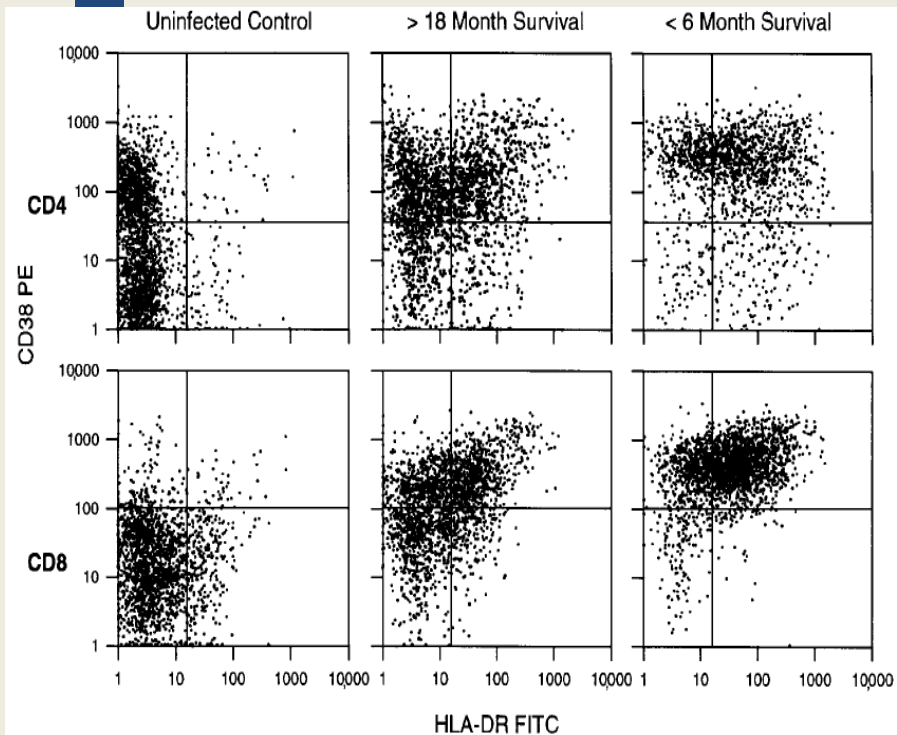
Pandrea et al Blood 2012



Merlini E et al Frontiers Immunol 2016

Fact #3

**Microbial translocation
contributes to immune
activation/inflammation**

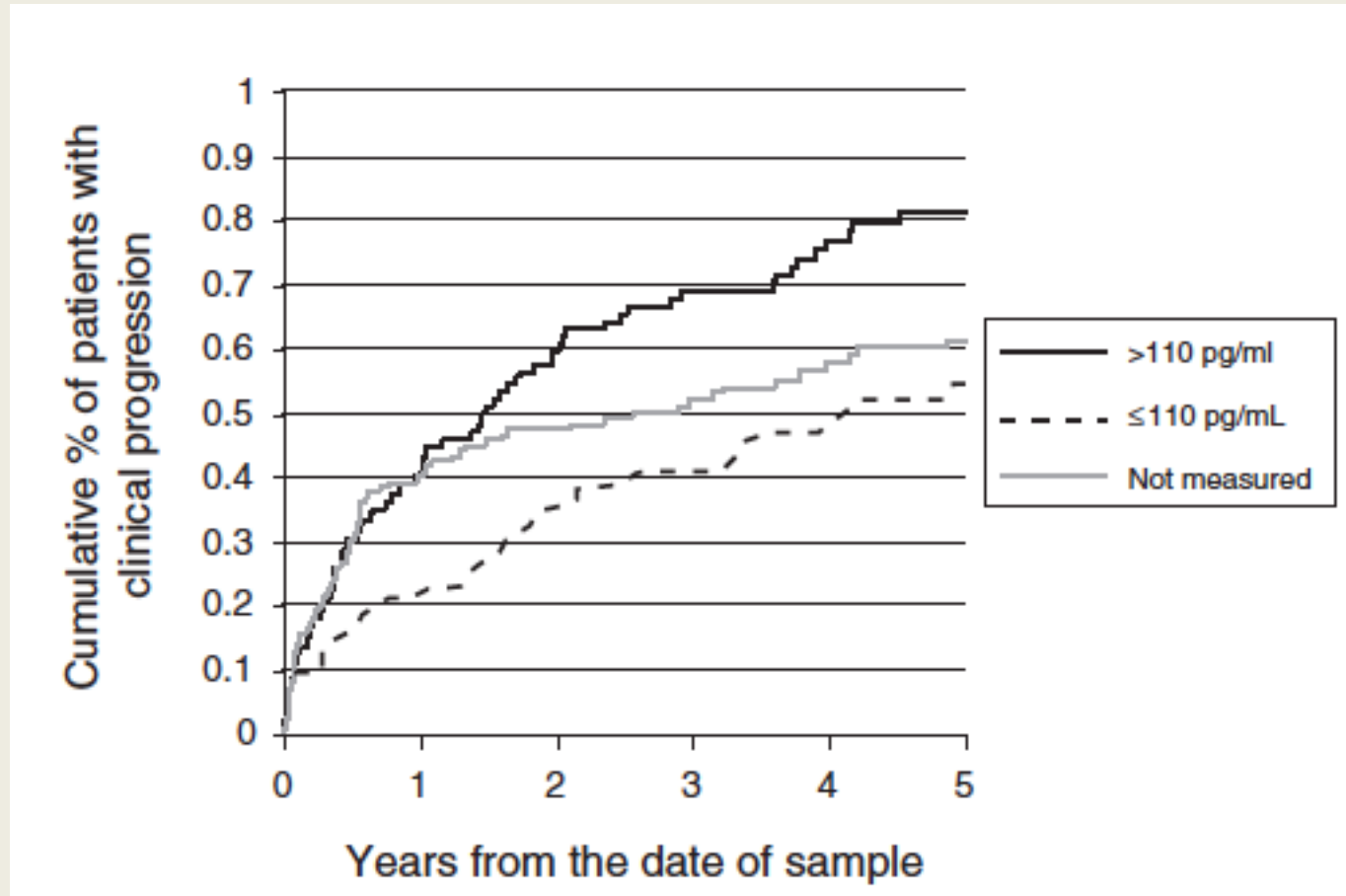


Giorgi, J et al. JID 1999

Parameter	Estimate	Standard error	P
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

Immune activation drives immune depression

Microbial translocation drives disease progression in untreated HIV



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

Fact #4

**Both immune
activation/inflammation
and microbial
translocation contributes
to disease progression**

Altered gut microbiota in SIV/HIV

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

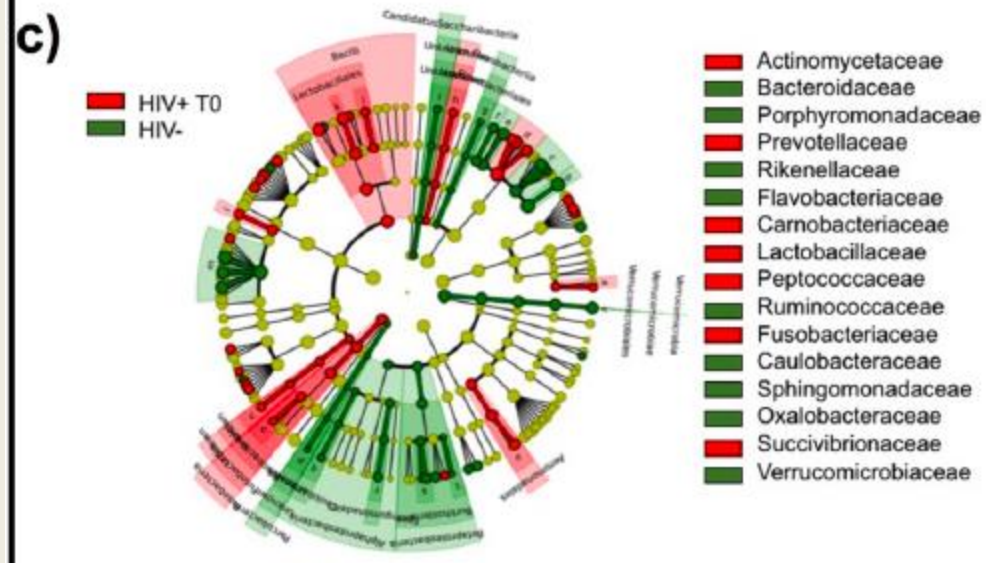
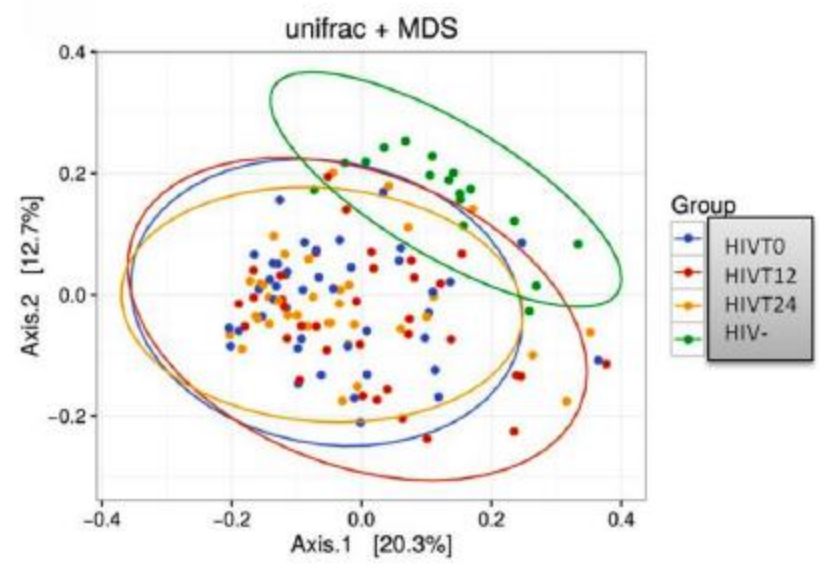
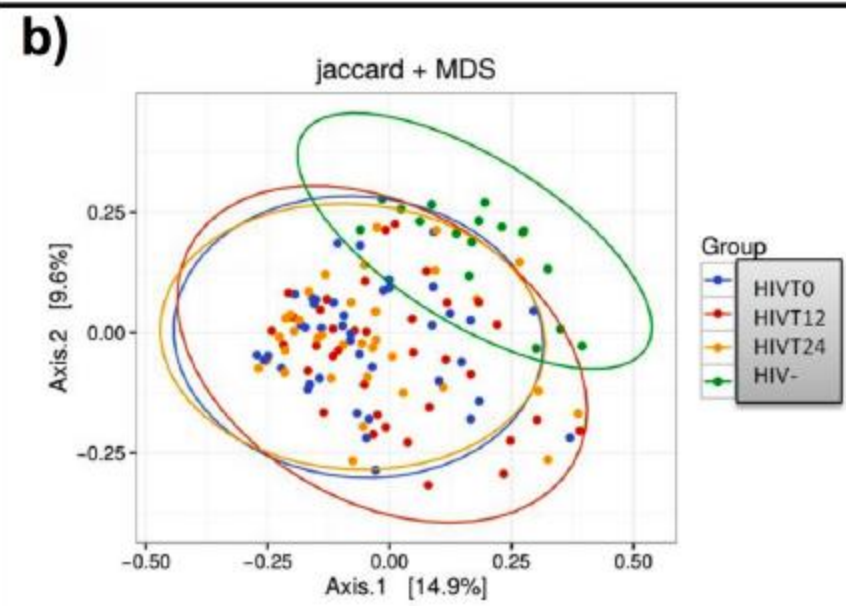
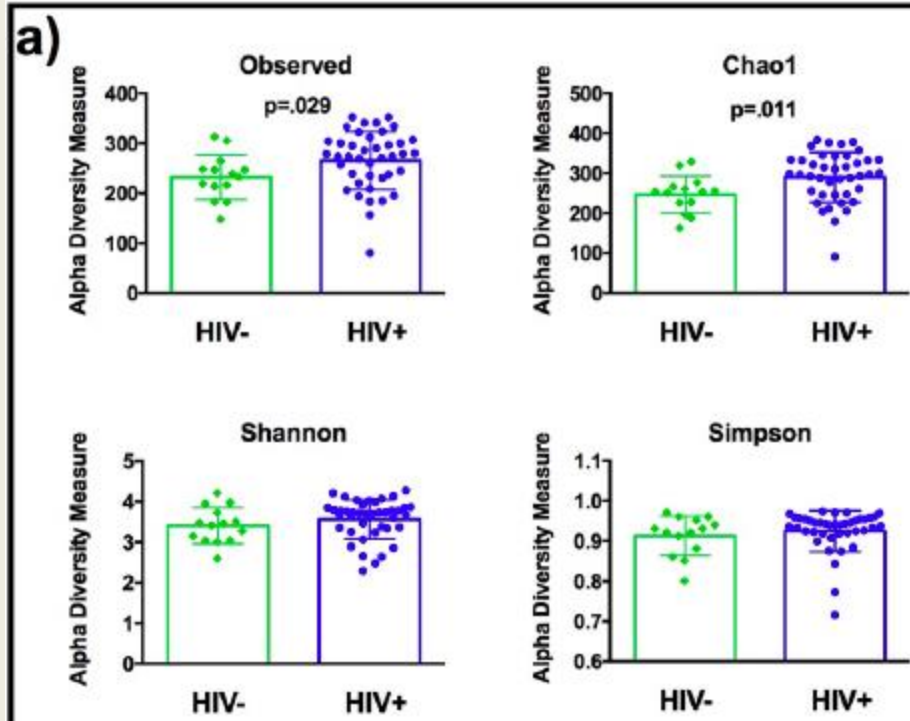
'+' signs signify relative amounts of bacterial species cultured

Brenchley Nat Med 2006; in humans: Gori et al. JCM 2008

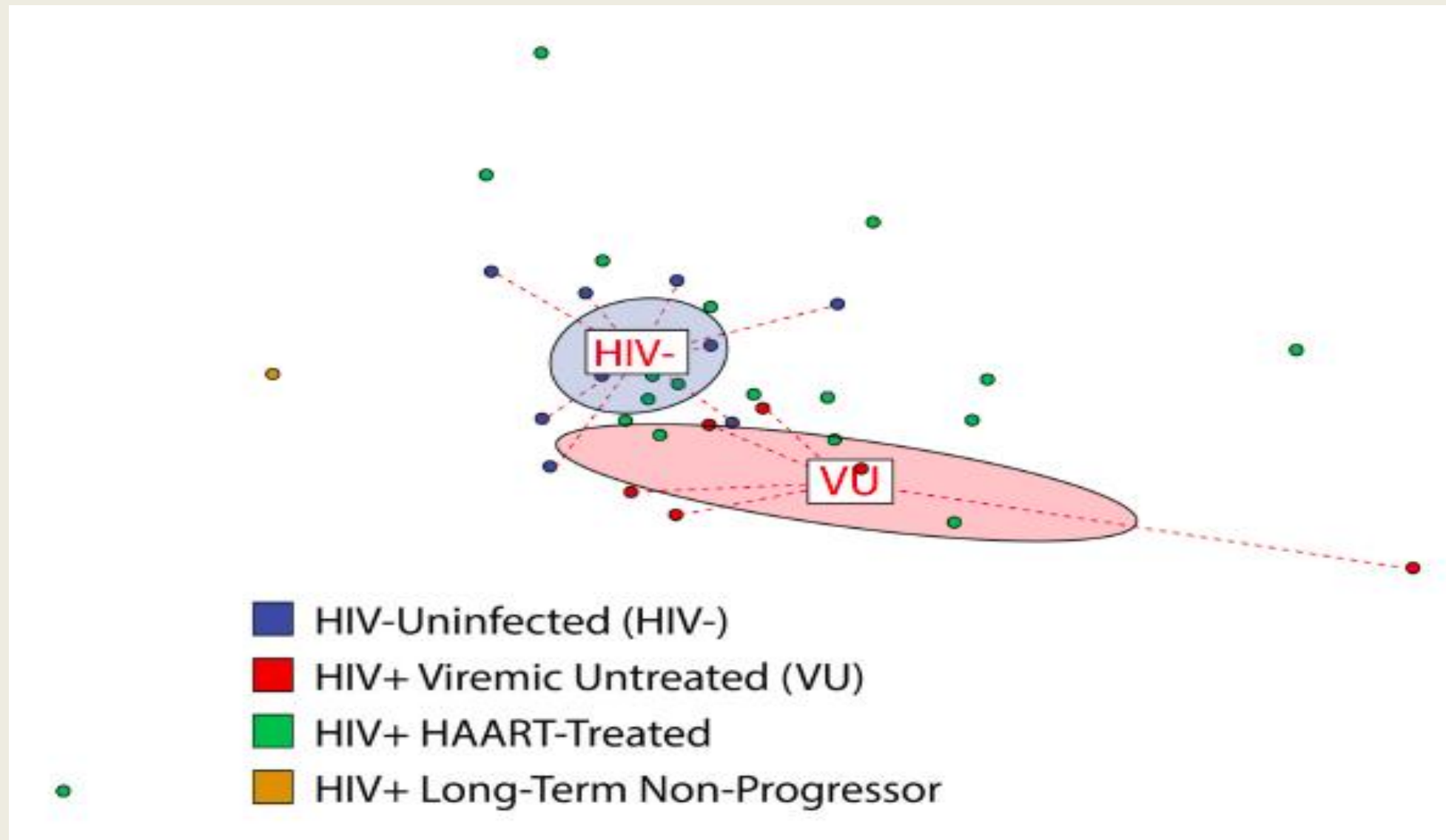
Fact #5

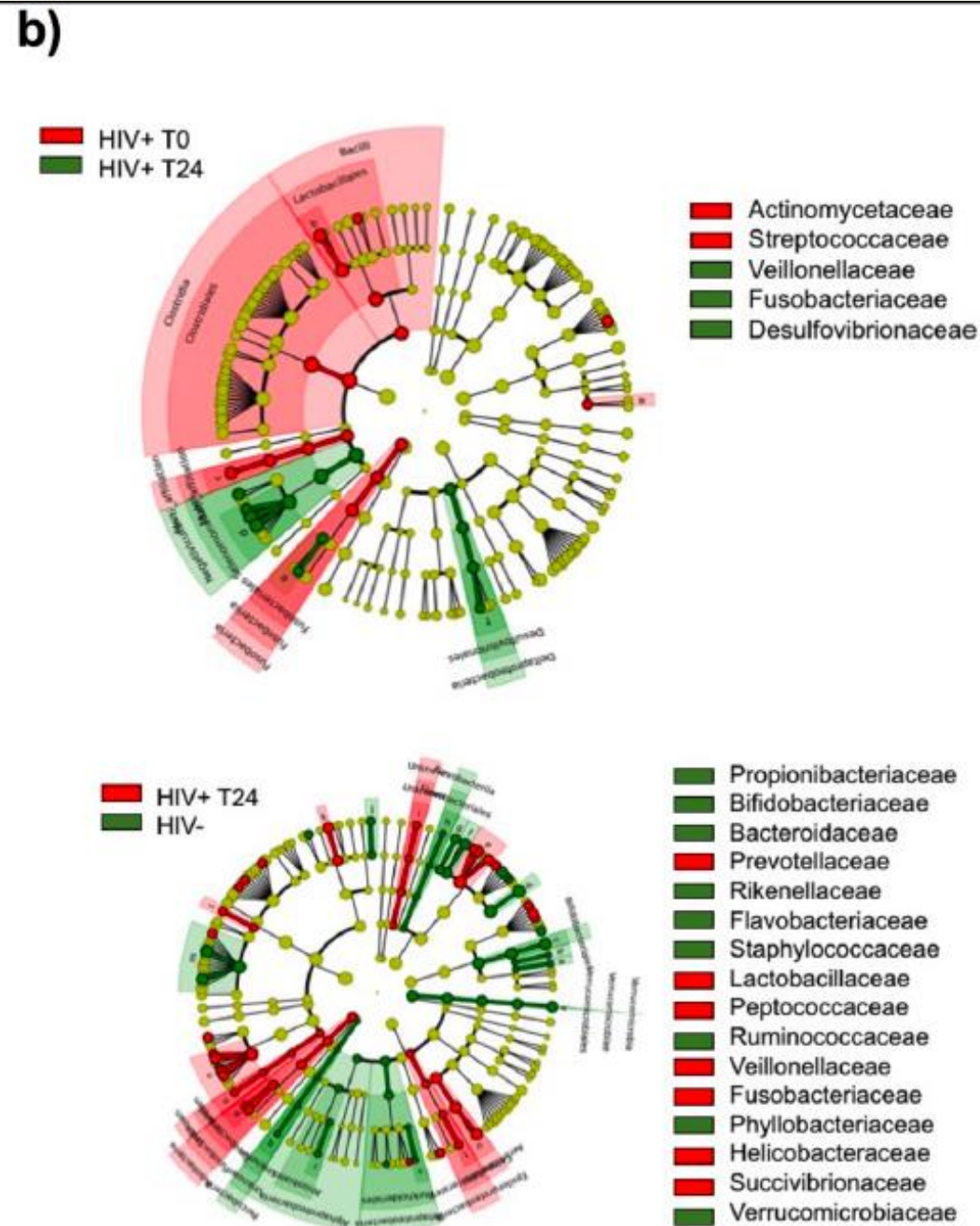
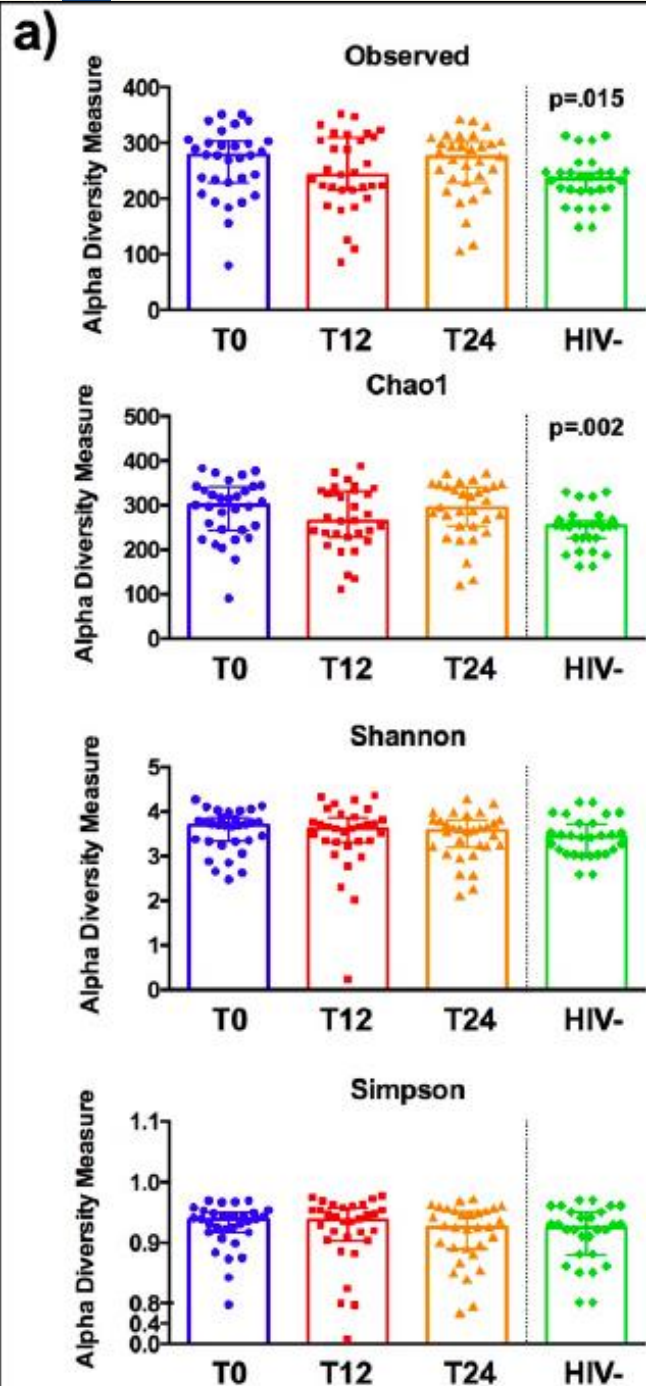
**Altered intestinal
microbioma in HIV**

**Altered gut
microbioma in
untreated HIV....**



**....that seems not
recovered by long
term cART**





41 HIV+ patients before
and at 1 and 2-3 year of
cART

Persistent
dysbiosis
despite 2-year
successful
cART

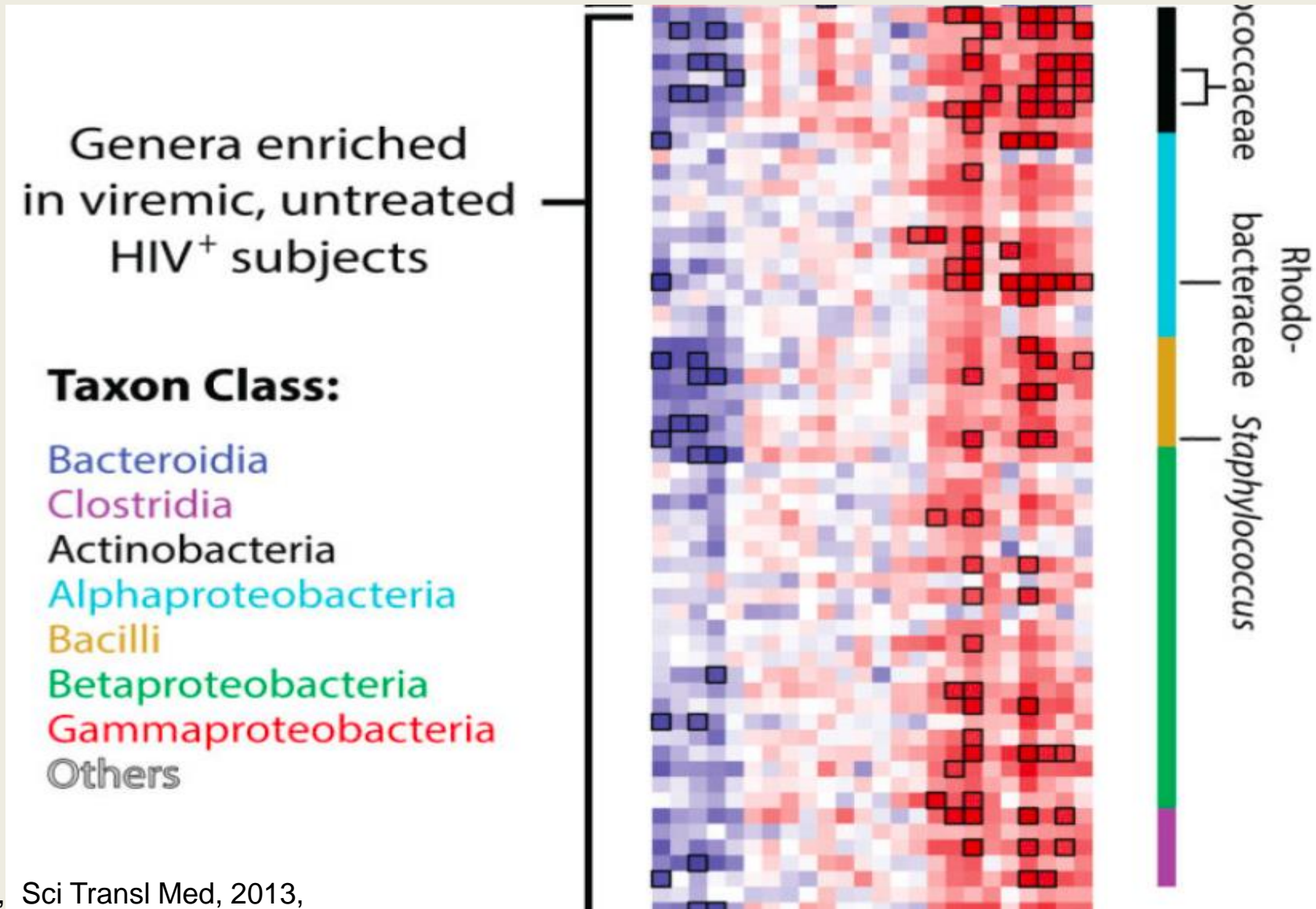
Ancona et al. CROI 2017 #215

**Which consequences of
gut dysbiosis?**

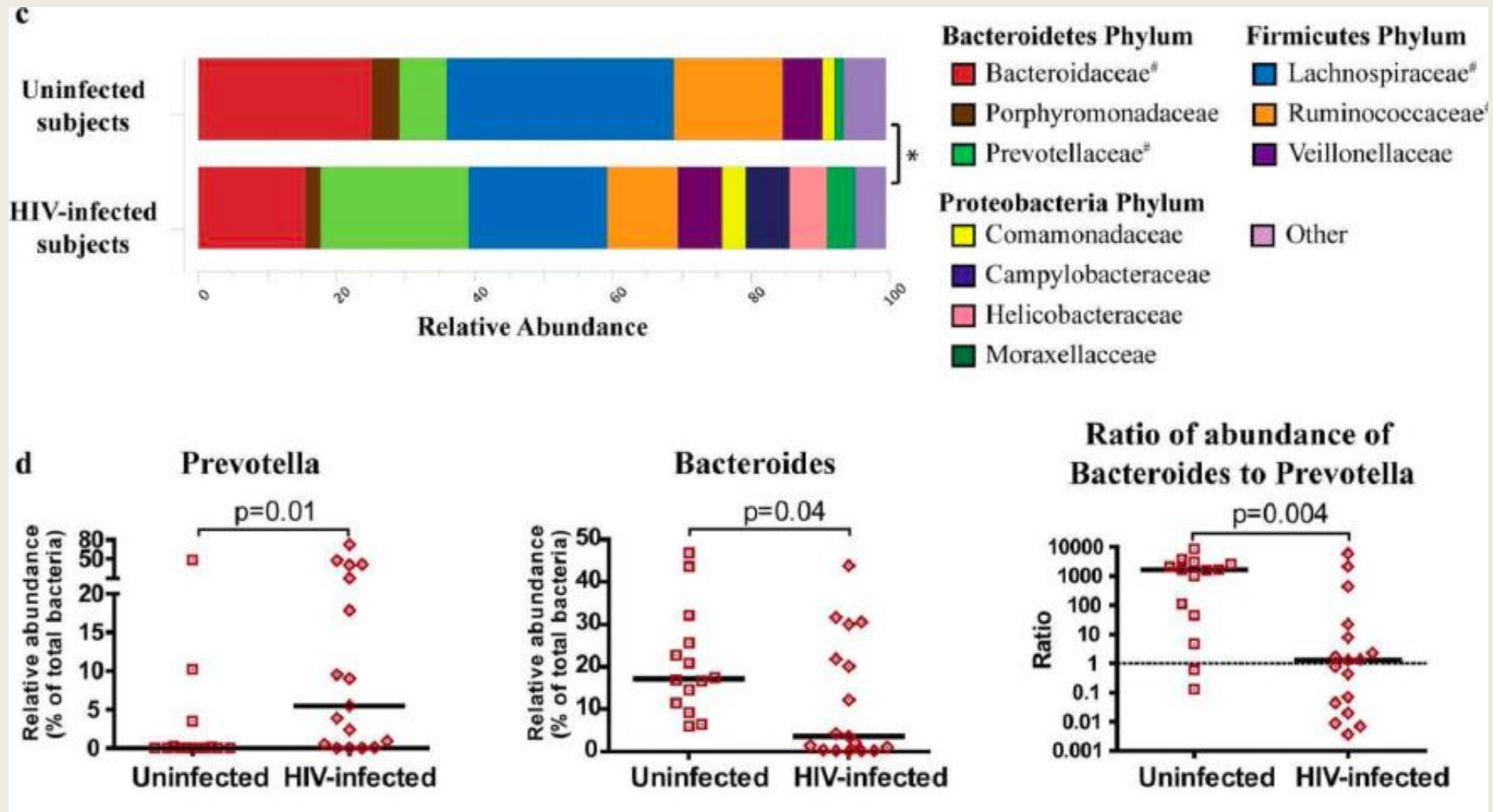


Gut-barrier- enhancing
cytokine secretion (IL-17, IL-
22)

T-cell activation in blood
and GALT, serum
inflammatory markers



Colon biopsies from 17 untreated HIV; 14
uninfected controls



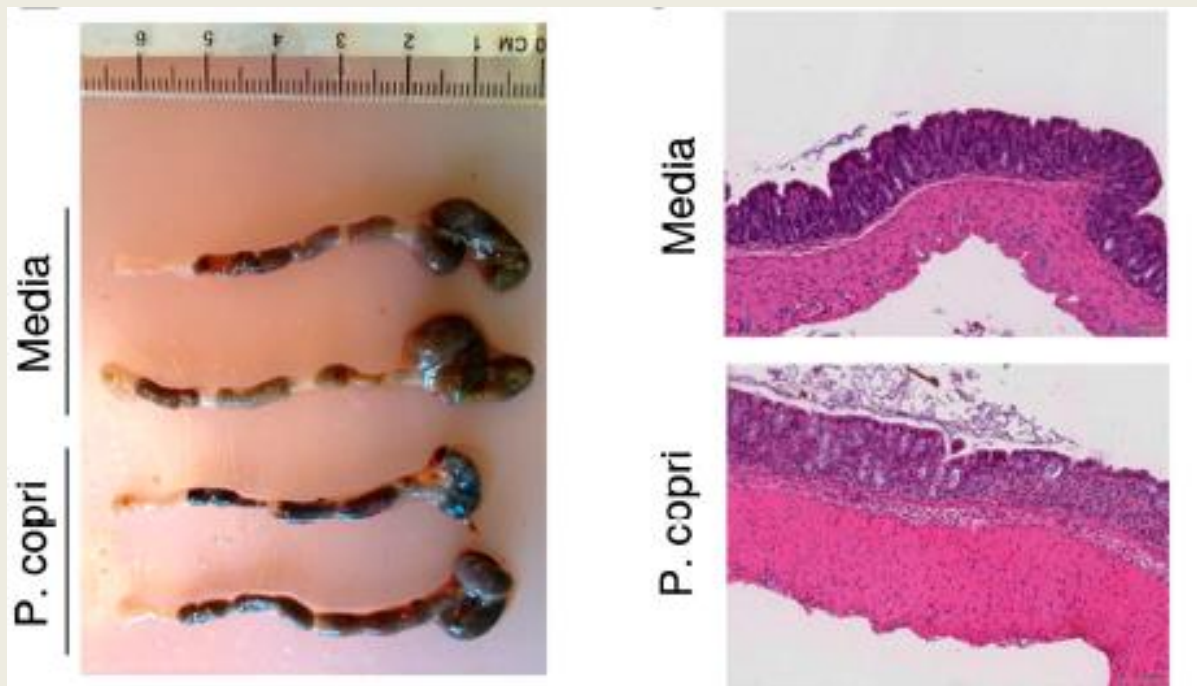
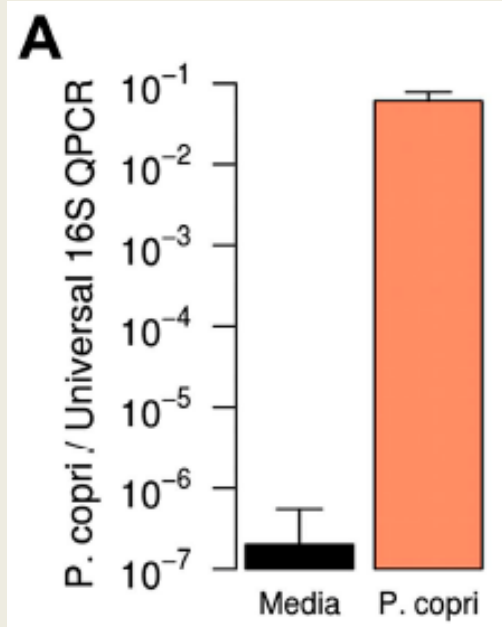
Dillon et al., Mucosal Immunol, 2014

Prevotella-rich, Bacteroides-poor gut microbioma

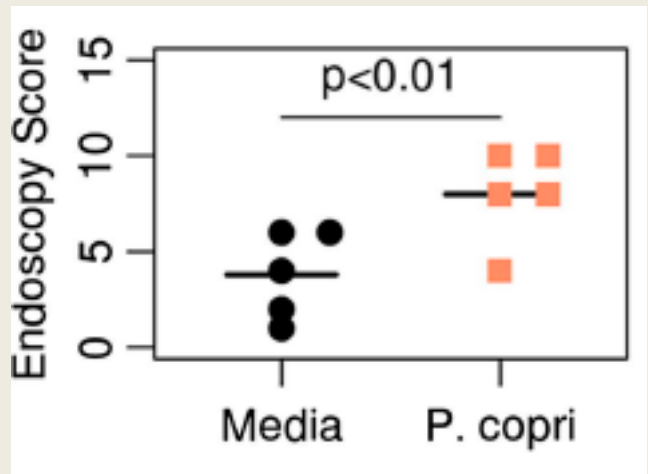
Taxon	P77 inflammation		P178 inflammation	
	Yes	No	Yes	No
<i>Bacteroidetes</i>	74	42	54	61
' <i>Clostridia</i> '	10	53	40	33
<i>Enterobacteriaceae</i>	16	5	0	0
Other bacteria	0	0	6	6

Patients with
ulcerative colitis

Lucke et al. J Med Microbiol 2006

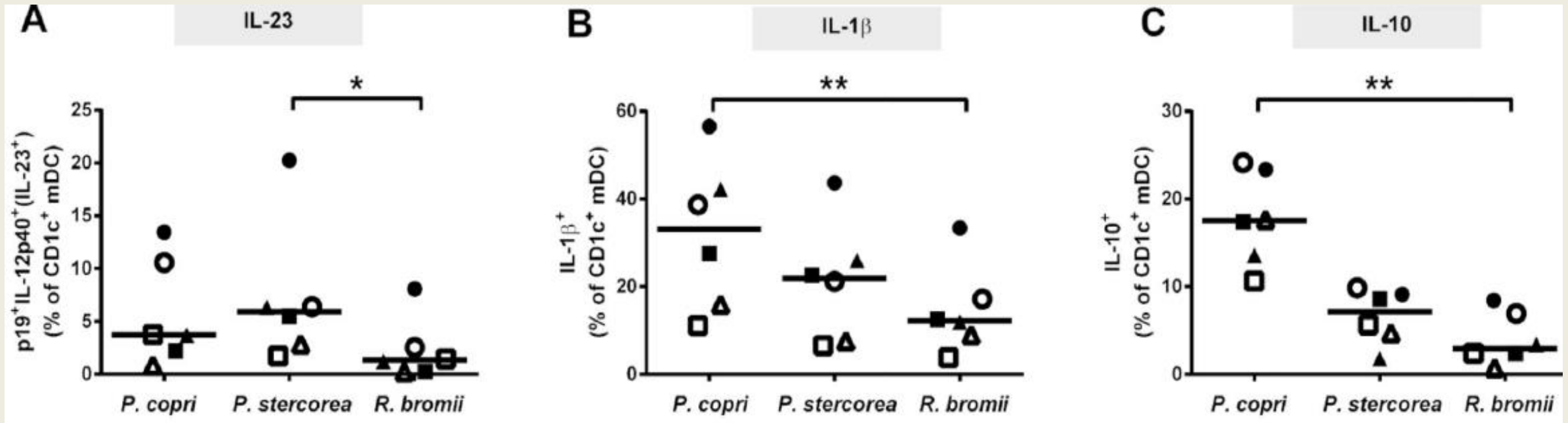


Antibiotic-treated mice
colonized with *P. copri*;
DSS-induced colitis

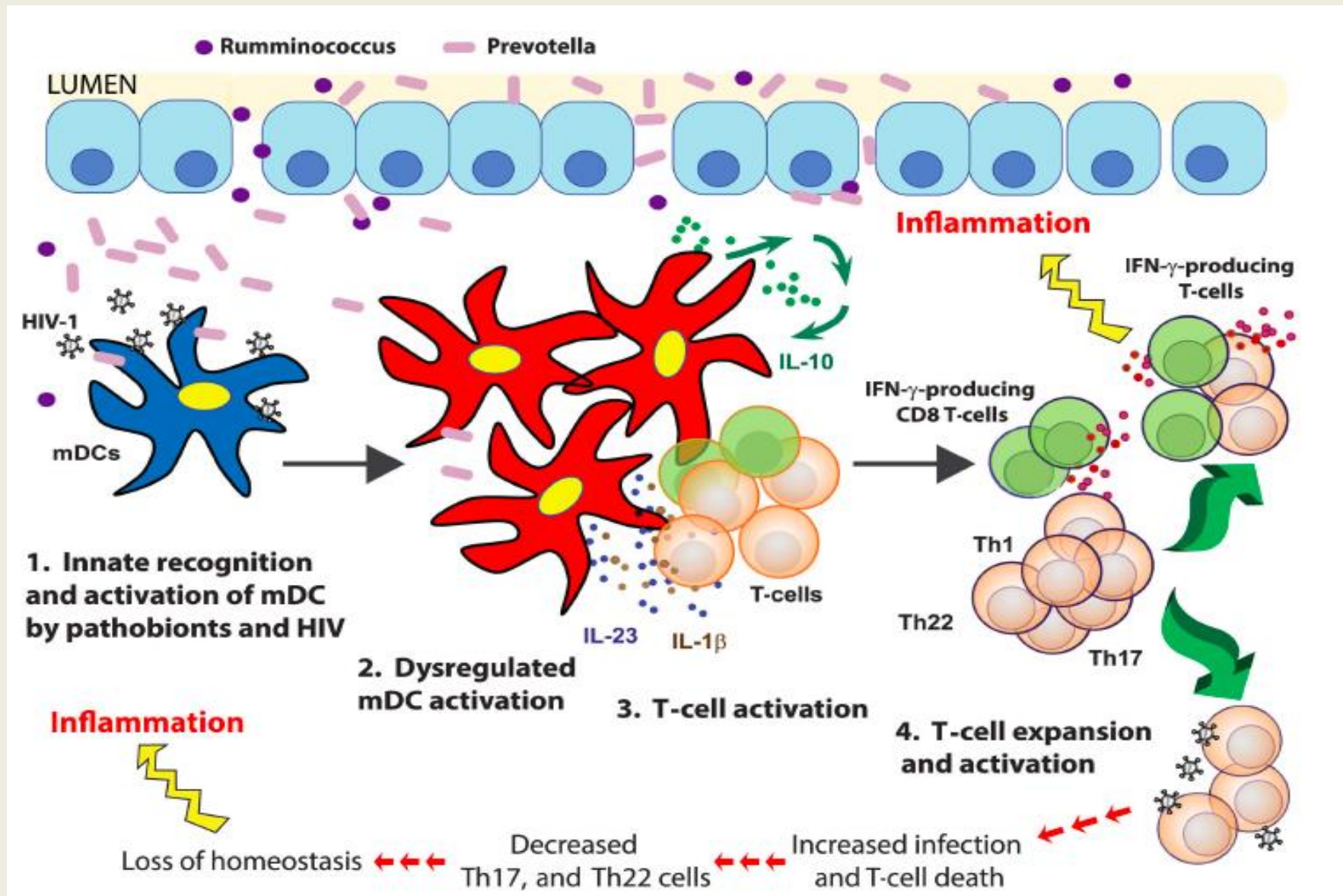


Scher et al. Elife 2013

Colonic LPMC exposed to several HAMB
(*HIV Altered Mucosal Barrier*)



Prevotella spp. drive ex vivo increased production of pro-inflammatory cytokines by LP mDCs

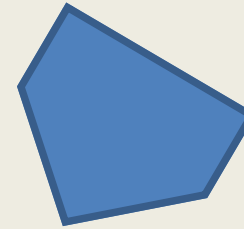
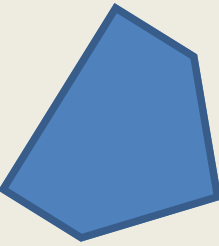


Fact #6

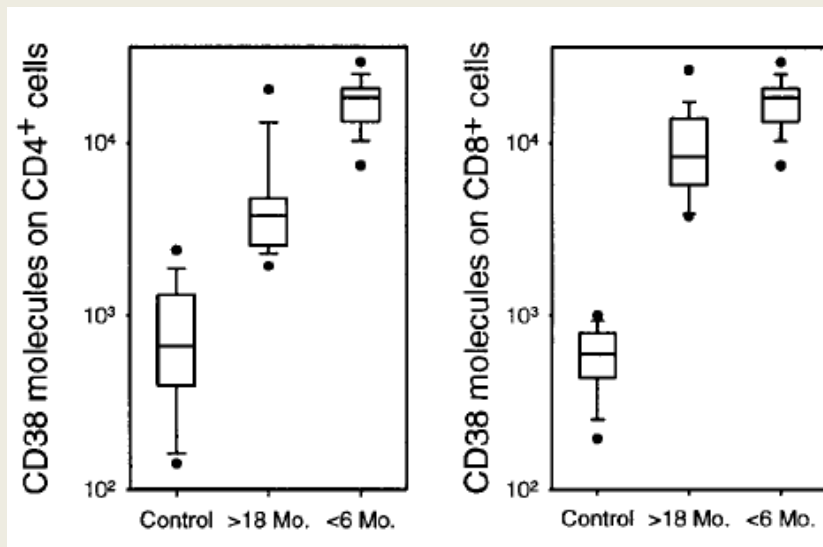
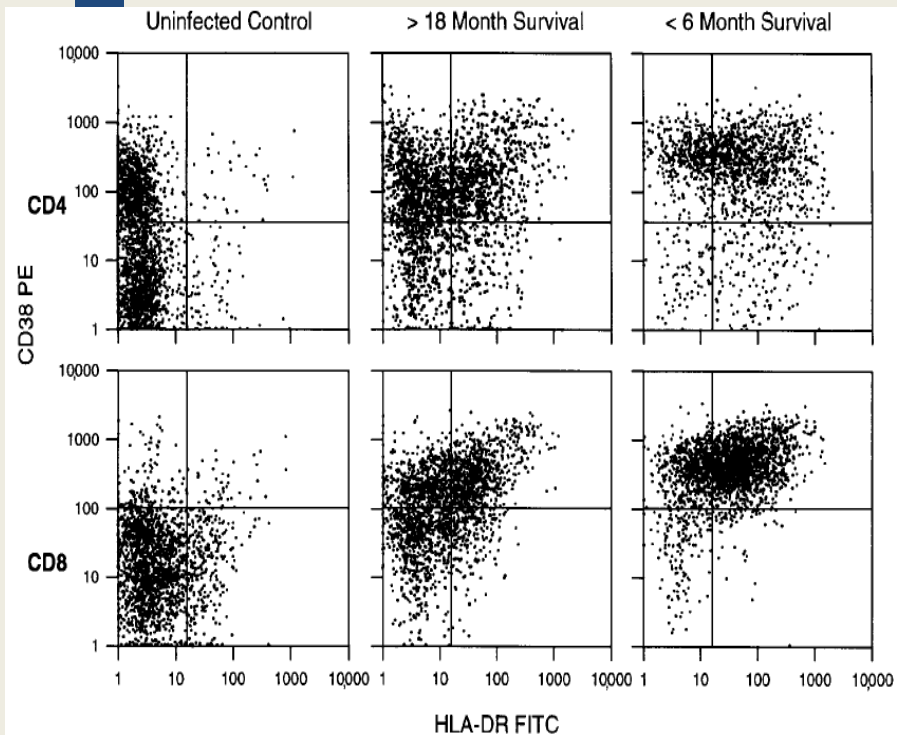
**Altered intestinal
microbioma as
driver of
inflammation**

Dysbiosis

Microbial
translocation



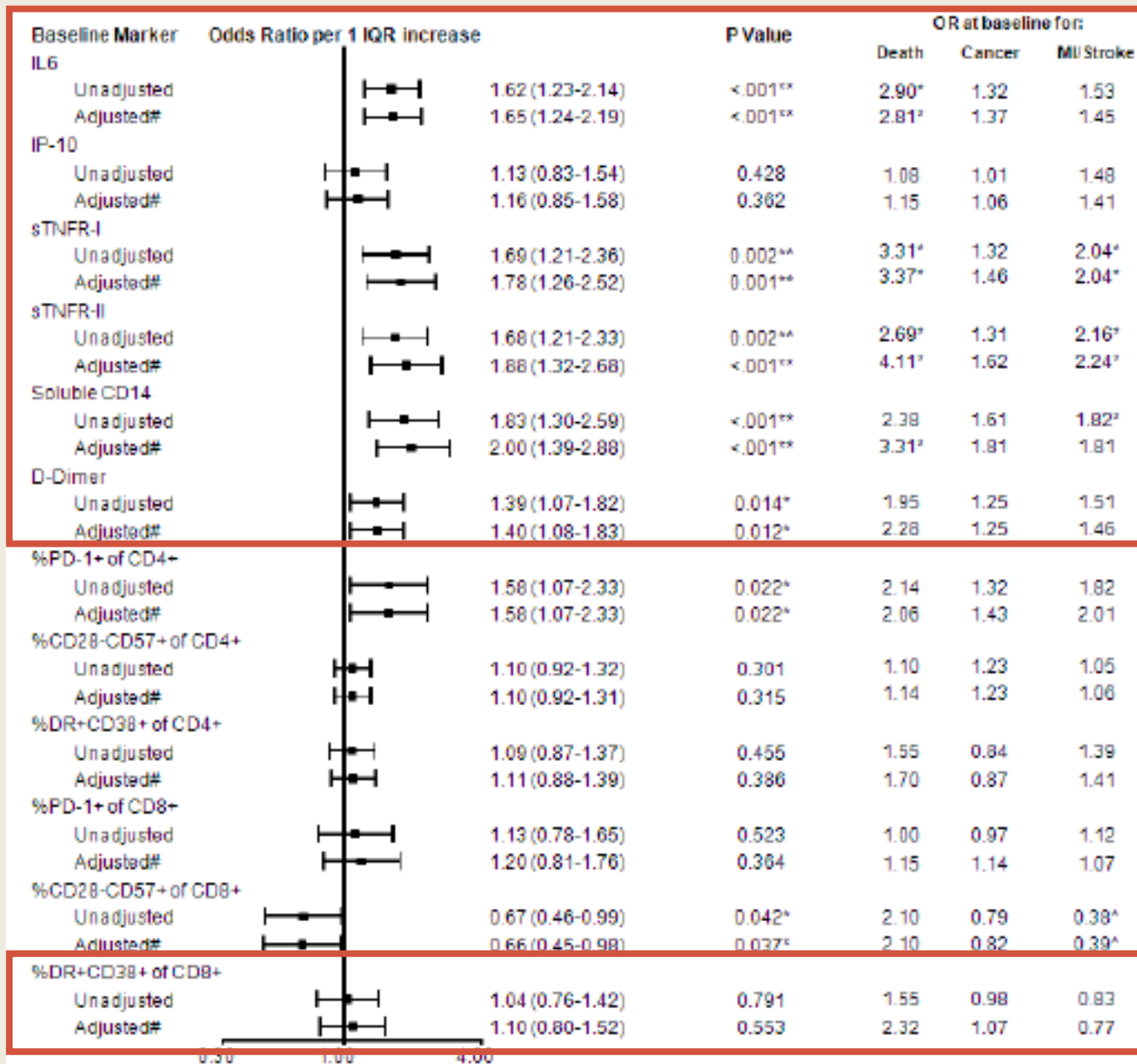
Systemic
inflammation/immune
activation



Giorgi, J et al. JID 1999

Parameter	Estimate	Standard error	P
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
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CD8 ⁺ T-cell activation, log ₁₀	−0.033	0.015	.027
CD4 ⁺ T-cell activation, log ₁₀	−0.013	0.019	.474

Immune
activation
drives immune
depression



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

Immune activation drives residual disease progression on cART

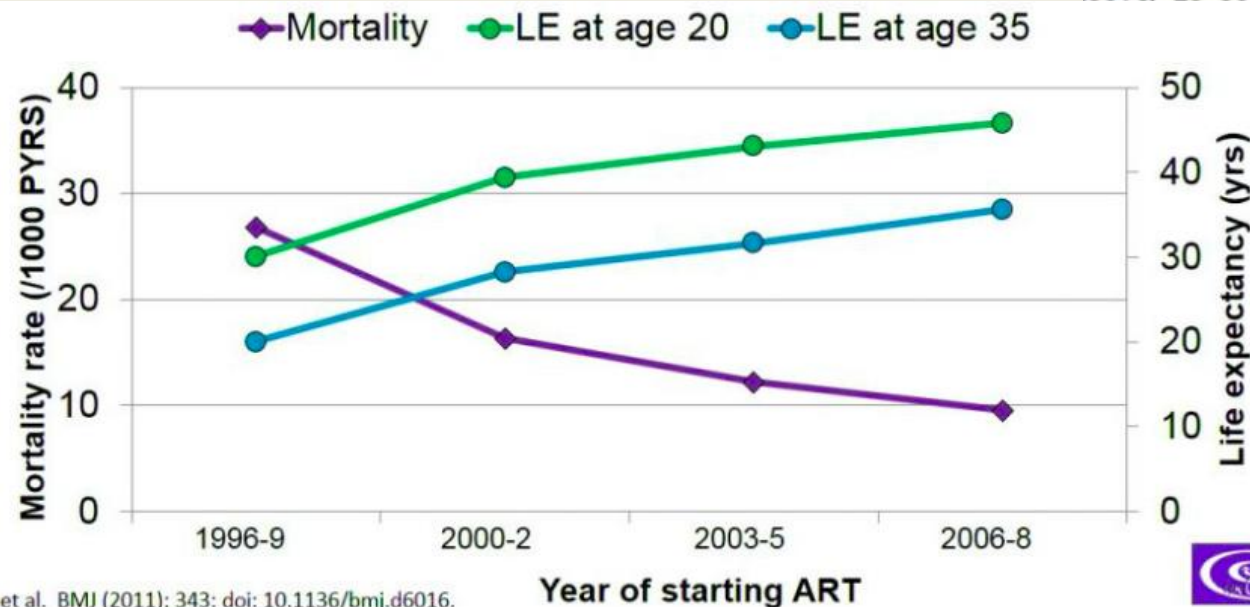
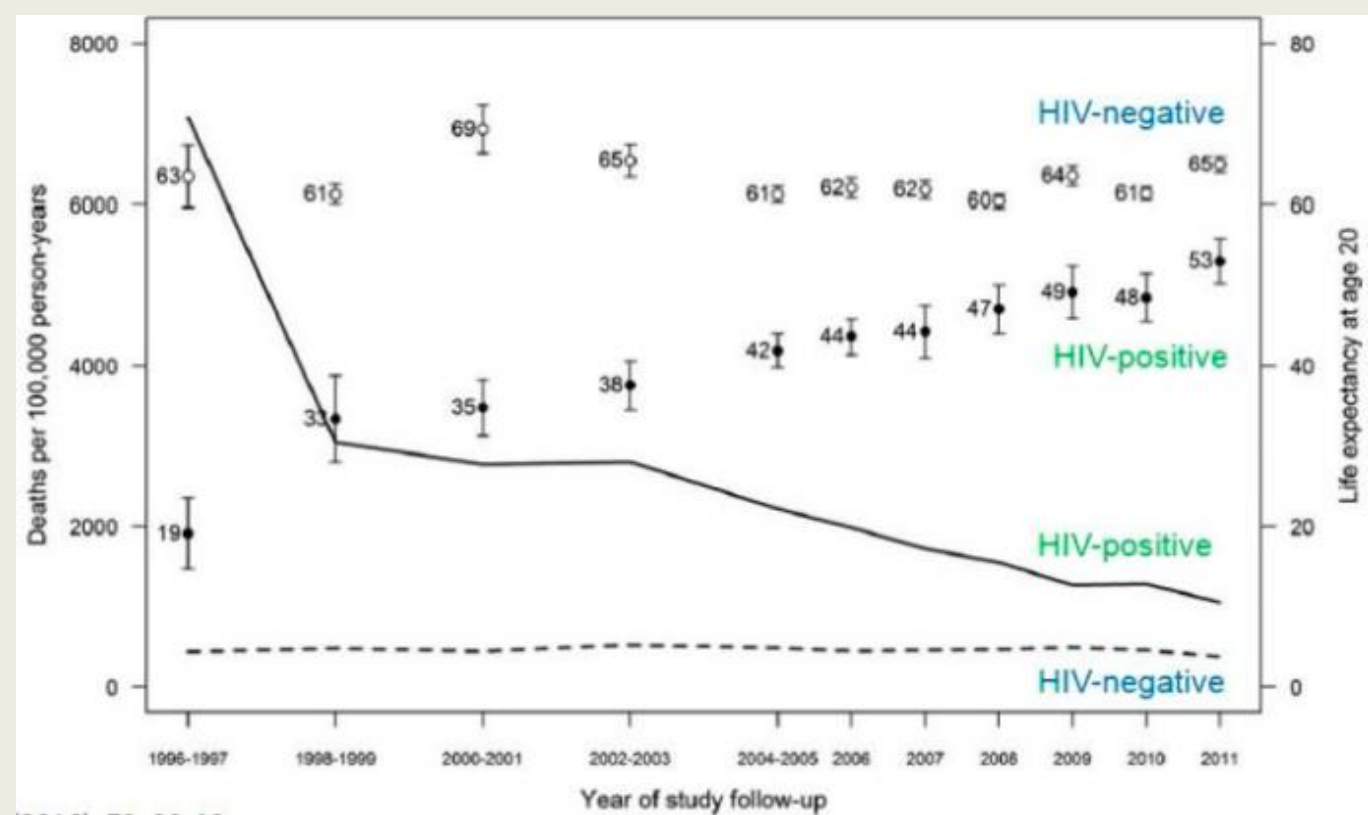
Dysbiosis as driver of inflammation

- Research hypothesis:

- *Dysbiosis in HIV progression ? The issue of residual disease on cART*

Kaiser-Permanente, California

Reduced mortality
and increased life
expentancy (LE)

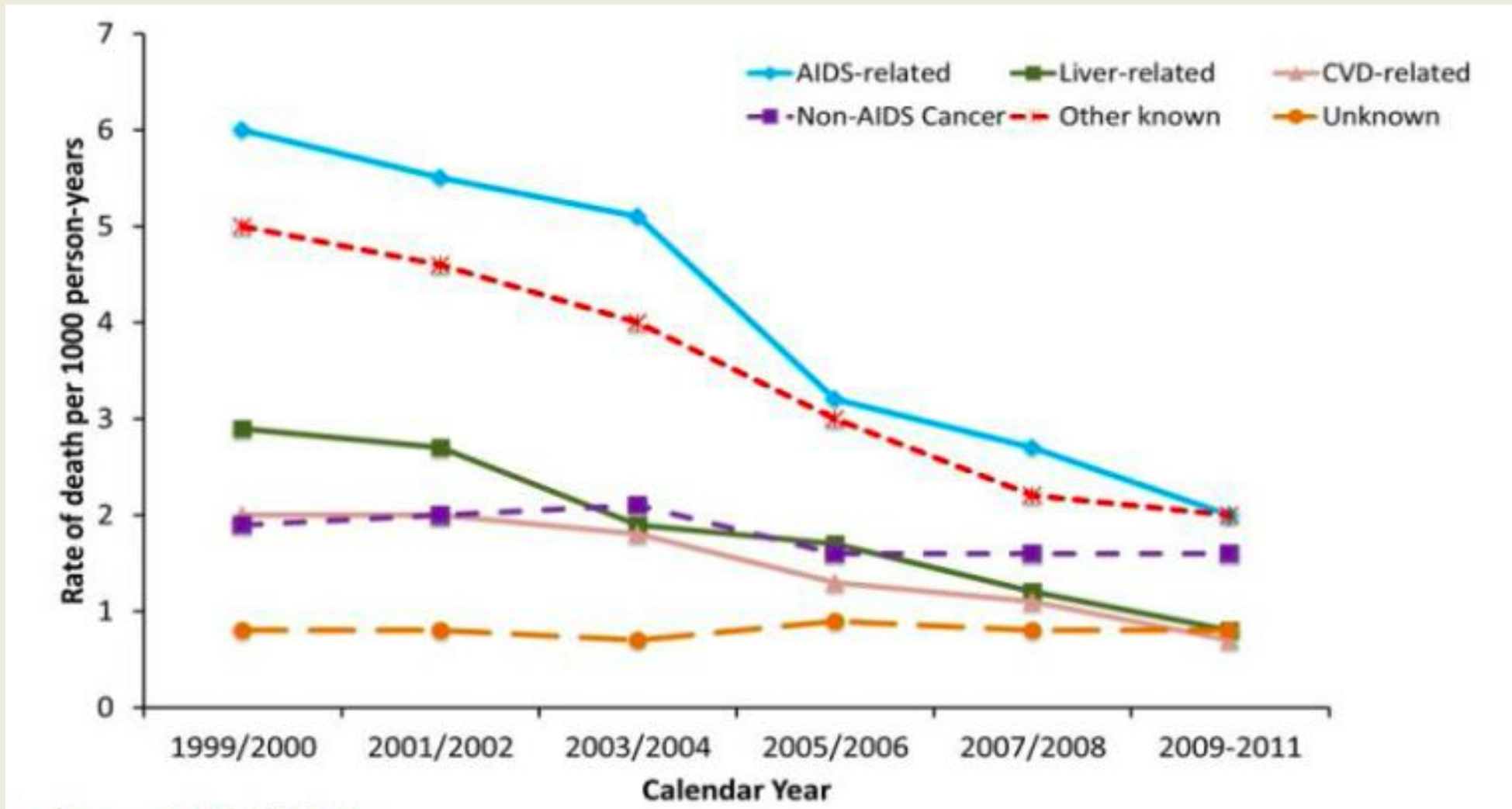


May M et al. BMJ (2011); 343: doi: 10.1136/bmj.d6016.

UK CHIC

Marcus JL JAIDS 2016

What do people die of in modern ART era?



HIV, gut damage, dysbiosis, microbial translocation and systemic inflammation

The facts

**The “yet to be demonstrated”
facts**

**Any evidence for
microbioma as driver
of residual disease?**

Taxa_Feces	L_FS (n=29)	IH_FS (n=12)	p	Taxa_Feces	N_nIMT (n=15)	I_IMT/P (n=5)	p
Order				Order			
Actinomycetales	0,03 (0,01-0,07)	0,01 (0-0,03)	0,023	Actinomycetales	0,03 (0,02-0,05)	0 (0-0,001)	0,001
Family				Family			
Actinomycetaceae	0,023 (0,01-0,04)	0,01 (0-0,02)	0,042	Actinomycetaceae	0,03 (0,02-0,04)	0 (0-0,001)	0,001
Carnobacteriaceae	0,03 (0,01-0,05)	0,01 (0,003-0,01)	0,037	Carnobacteriaceae	0,03 (0,01-0,06)	0,003 (0-0,01)	0,028
Enterococcaceae	0,01 (0,004-0,25)	0 (0-0,04)	0,032	Lachnospiraceae	19,38 (7,99-30,24)	11,91 (6,45-15,89)	0,067
Genus				Genus			
Clostridium XIVb	0,01 (0-0,05)	0 (0-0,01)	0,041	Alloprevotella	0,21 (0,01-0,72)	0,85 (0,26-1,70)	0,081
Enterococcus	0,01 (0,001-0,17)	0 (0-0,03)	0,027	Blautia	4,73 (1,25-5,49)	1,07 (0,82-1,66)	0,045
Granulicatella	0,03 (0,01-0,05)	0,01 (0,003-0,01)	0,037	Flavonifractors	0,13 (0,07-0,19)	0,02 (0,004-0,07)	0,014
Ruminococcus	0,55 (0,17-3,59)	0,11 (0,01-0,88)	0,041	Granulicatella	0,03 (0,01-0,06)	0,002 (0-0,01)	0,028
				Ruminococcus	0,71 (0,18-3,90)	0,07 (0,005-0,77)	0,097
				Ruminococcus2	1,63 (1,14-2,54)	0,65 (0,21-1,15)	0,029
Species				Species			
Megasphaera sp	0 (0-0,005)	0,02 (0-0,08)	0,045	Blautia obeum	1,64 (0,72-2,55)	0,49 (0,28-0,59)	0,014
				Ruminococcus Faecis	1,22 (0,78-2,06)	0,27 (0,19-0,89)	0,055

41 naive patients, 29 Framingham <10, 12 Framingham ≥10

20/41: 15 normal intima media thickmness (IMT<1mm), 5 abnormal IMT/plaque

high_risk
low_risk

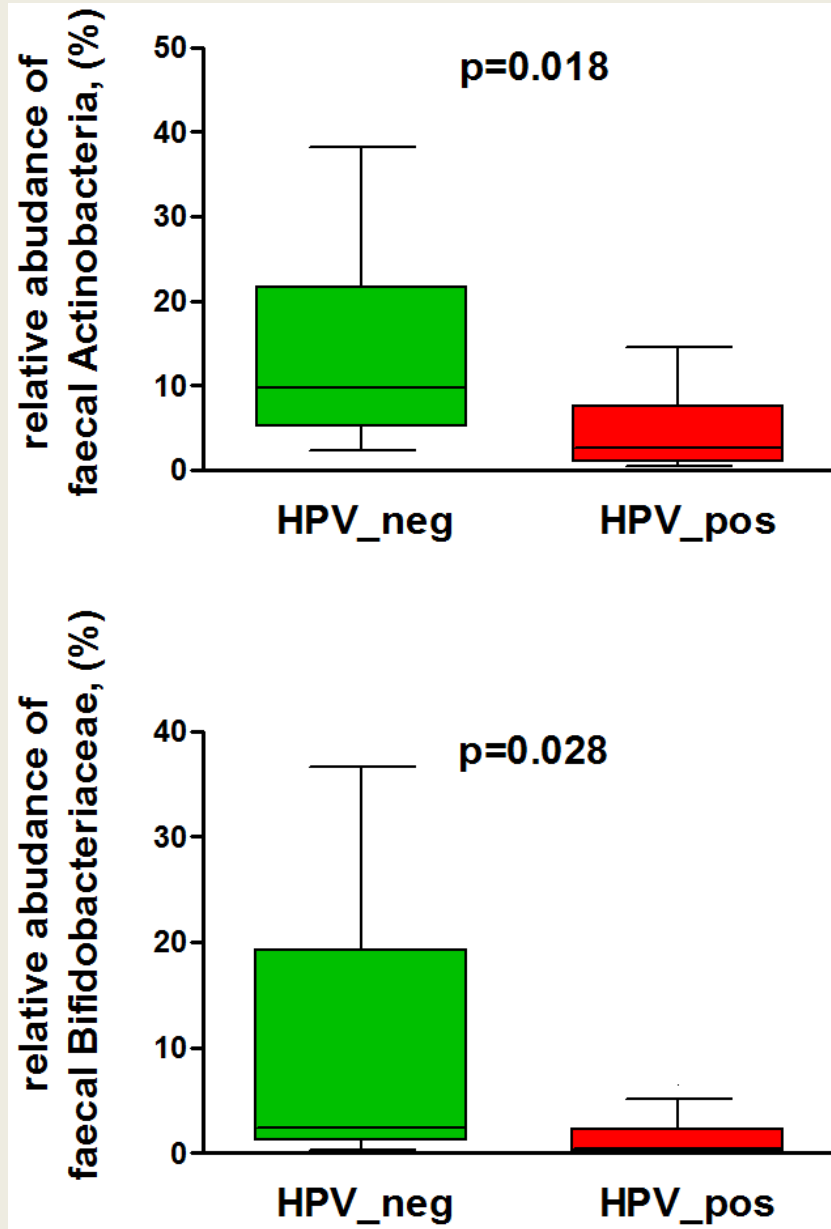
a: Actinomycetaceae
b: Actinomycetales
c: Granulicatella
d: Carnobacteriaceae
e: Multi_affiliation
f: Ruminococcus
g: Fusobacteriaceae
h: Fusobacteriales

highIMT/plaque
normal_IMT

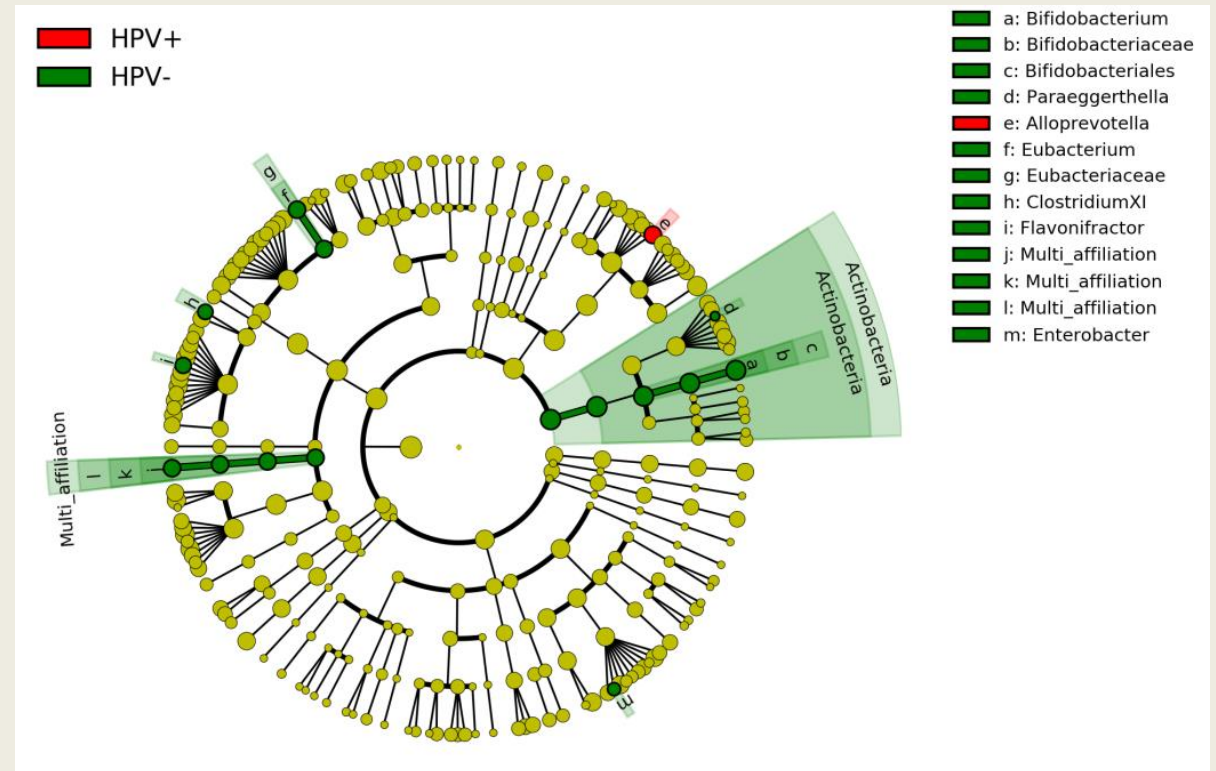
a: Actinomyces
b: Actinomycetaceae
c: Actinomycetales
d: Granulicatella
e: Carnobacteriaceae
f: Lactococcus
g: Multi_affiliation
h: Clostridiaceae1
i: Blautia
j: Multi_affiliation
k: Ruminococcus2
l: Flavonifractor
m: Multi_affiliation
n: Multi_affiliation
o: Multi_affiliation

36 HIV+
males
cART+:

30/36
HPV+;
18/30
abnormal
cytology



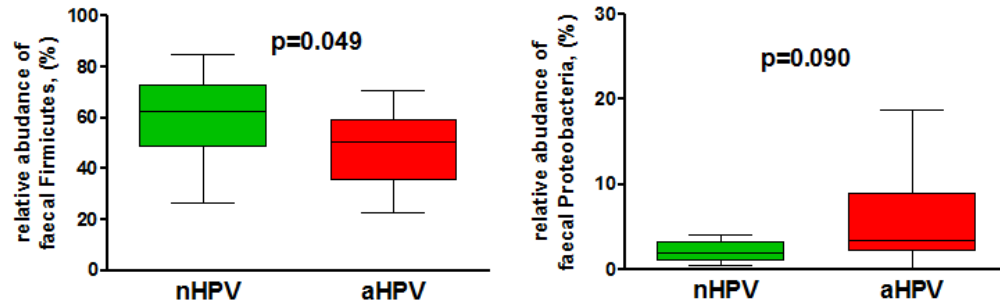
Different fecal microbiota in HIV+ HPV+ versus HPV- patients



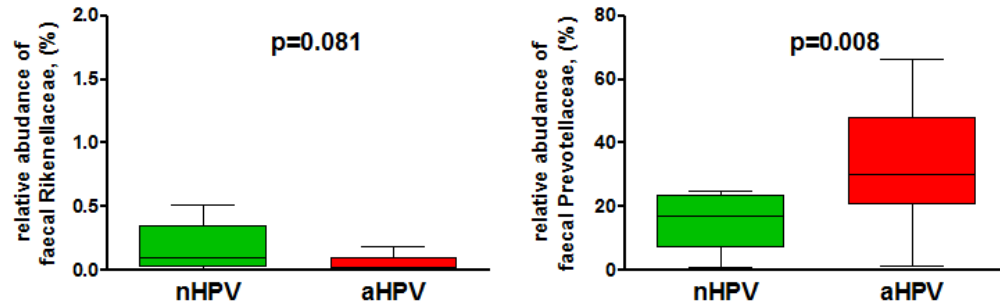
Merlini et al. ICAR 2017

No differences in alpha diversity indexes (Observed, Chao1, Shannon, Simpson)

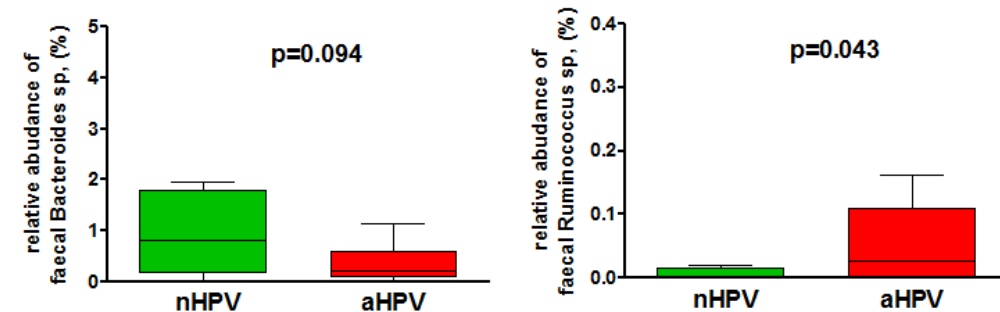
TAXA: Phylum



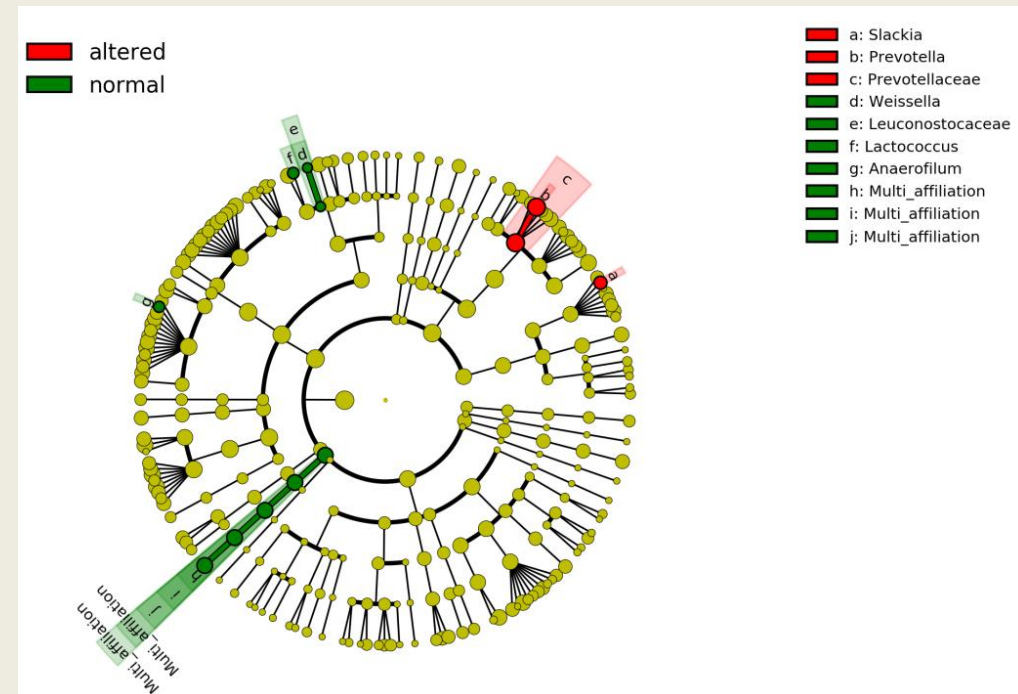
TAXA: Family



TAXA: Specie



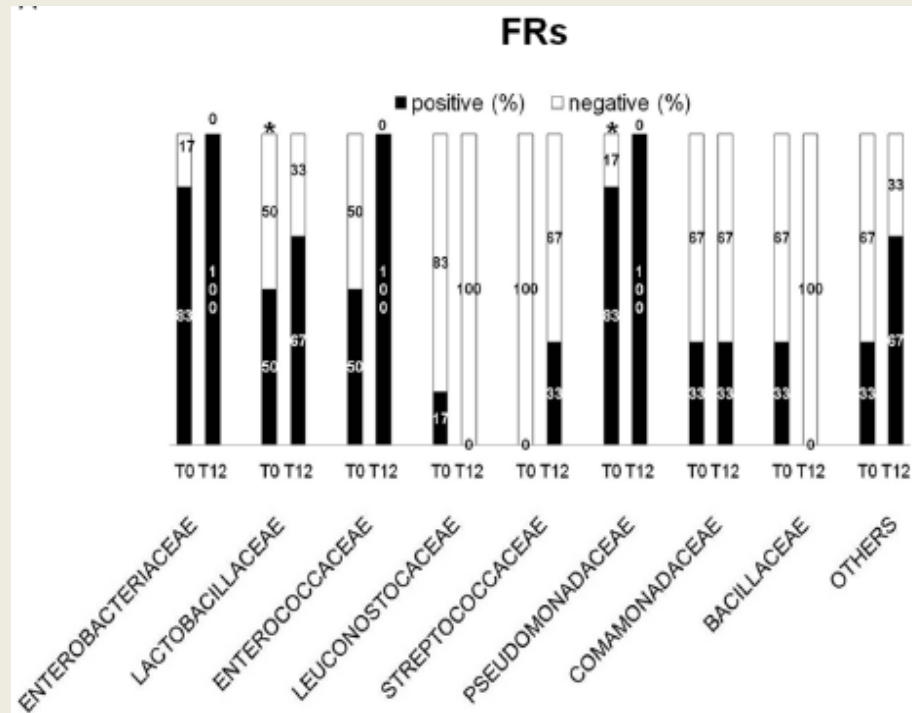
HPV+ with altered cytology show less bacteria with immune regulatory function and higher abundance of pro-inflammatory bacteria



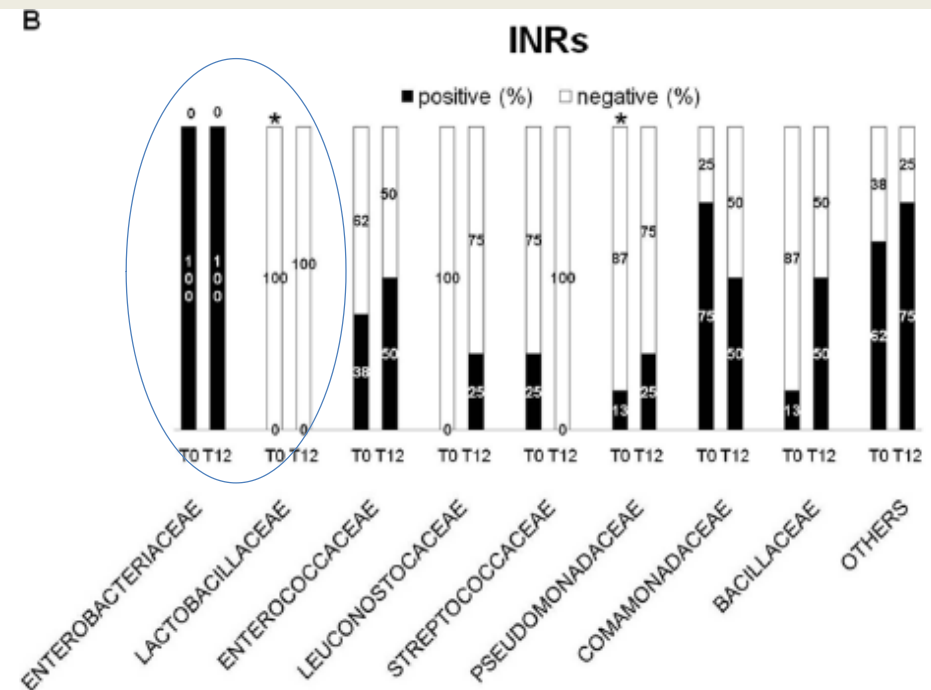


Translocating microbiota might also affect immune reconstitution

Good response to therapy



Scarce response to therapy

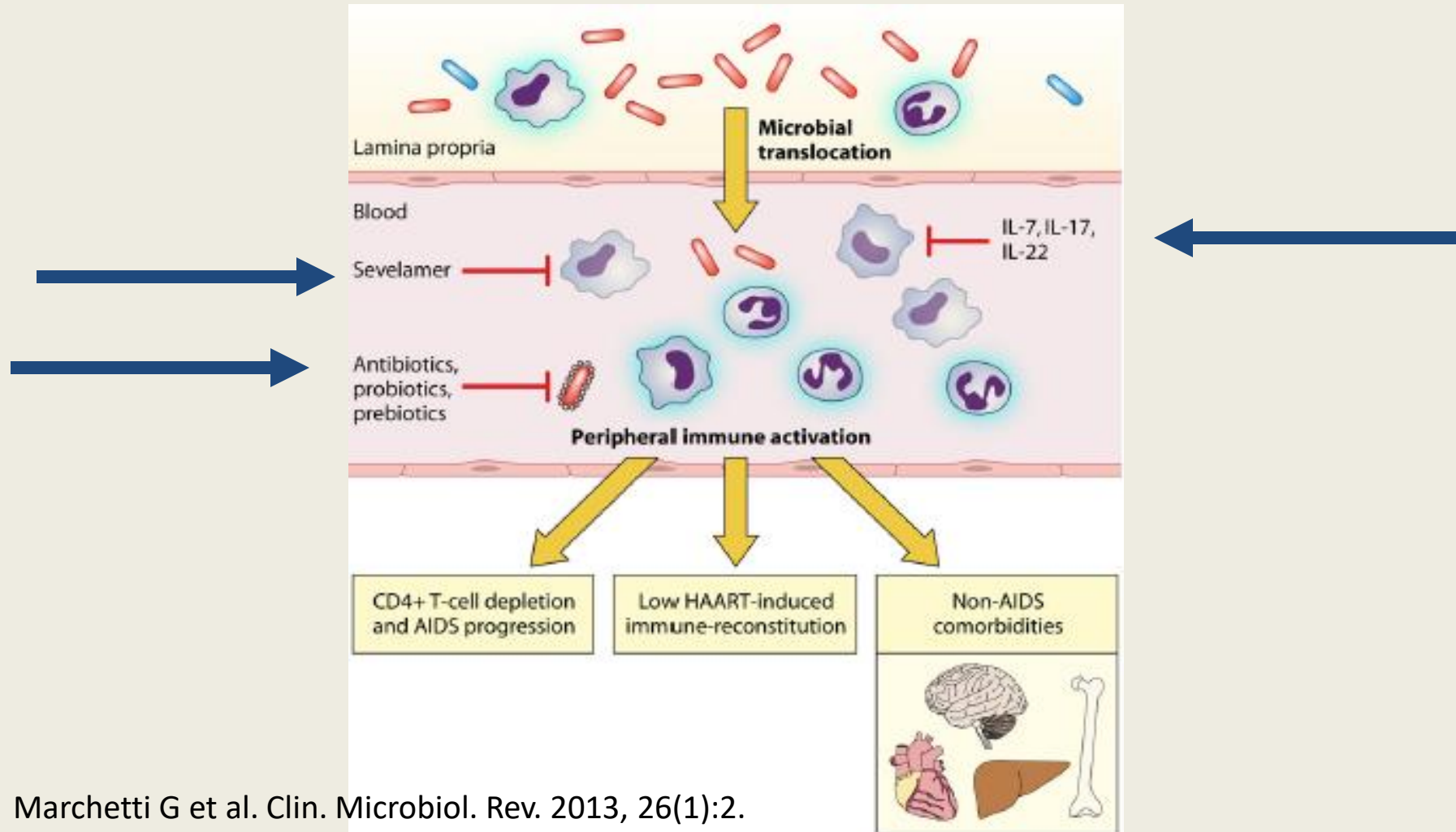


“Yet to be demonstrated” fact #1

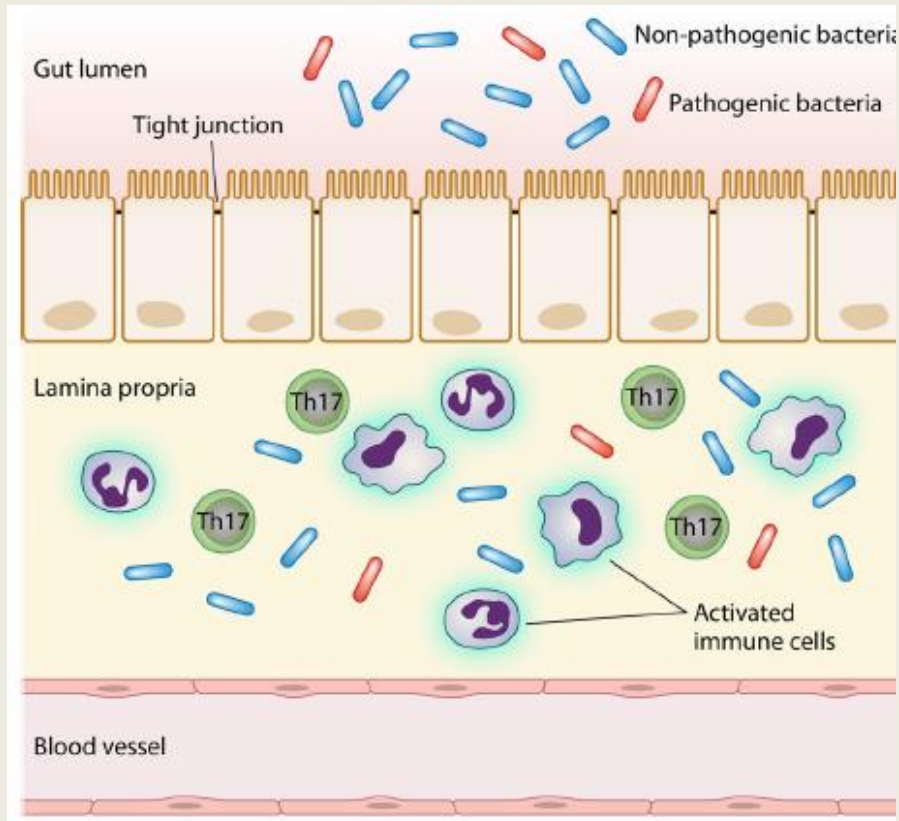
**Any evidence for
microbioma as driver
of residual disease?**

**Any chance to treat
the gut?**

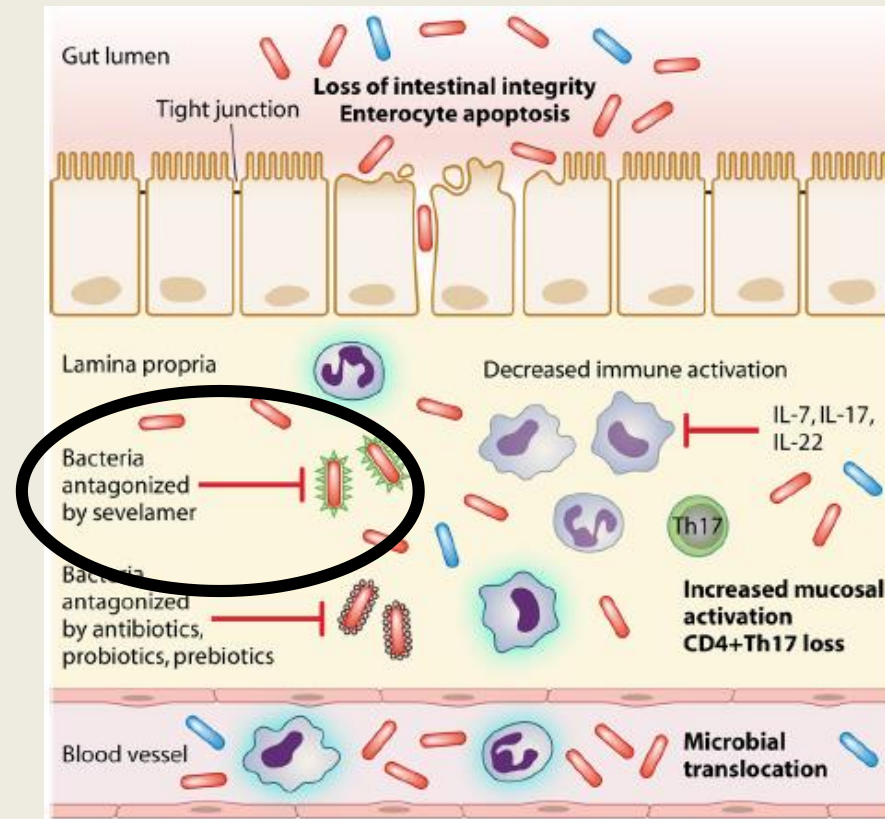
Approaches to the treatment of gut impairment



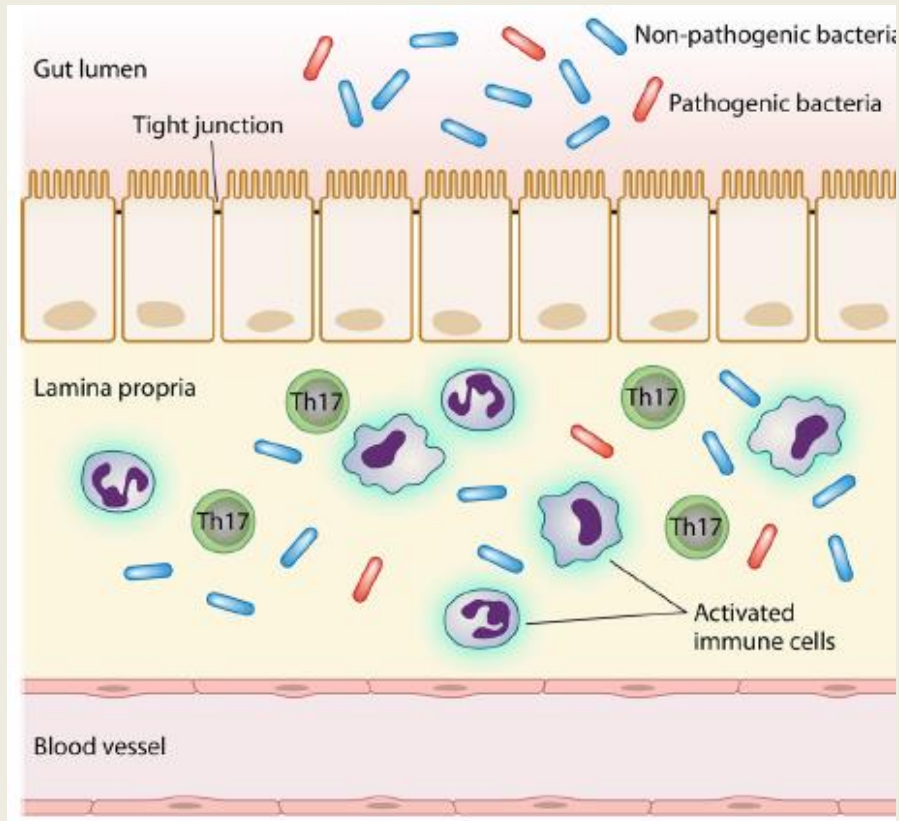
HIV-



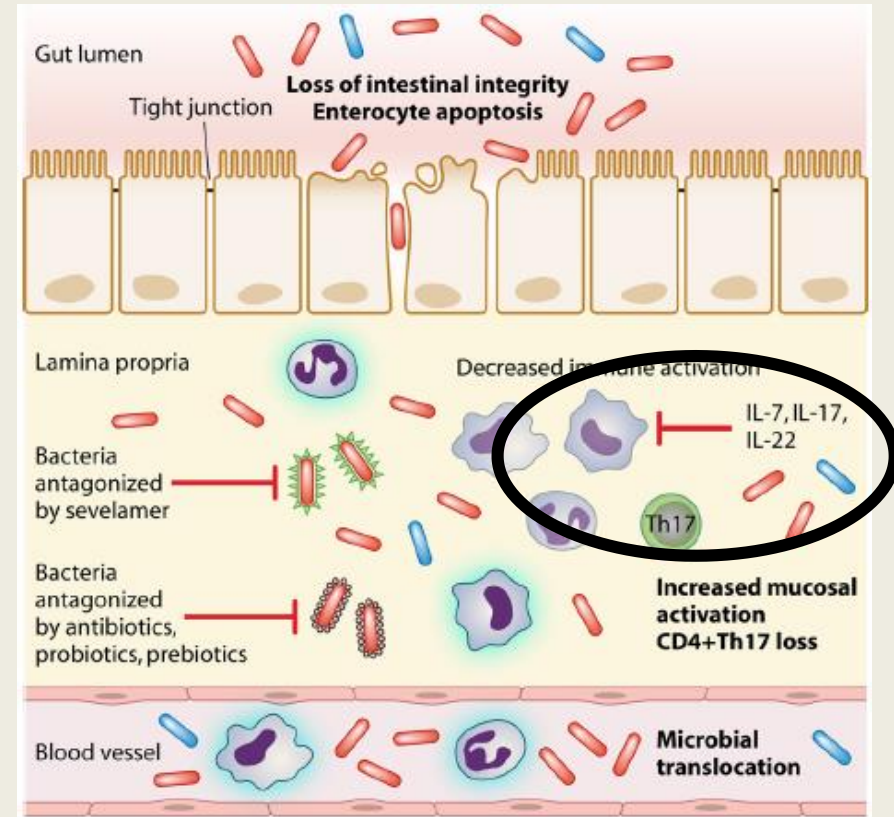
HIV+



HIV-

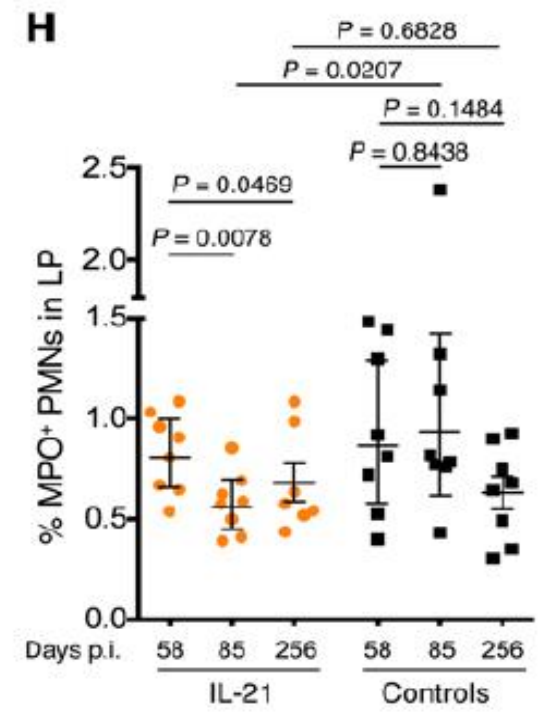
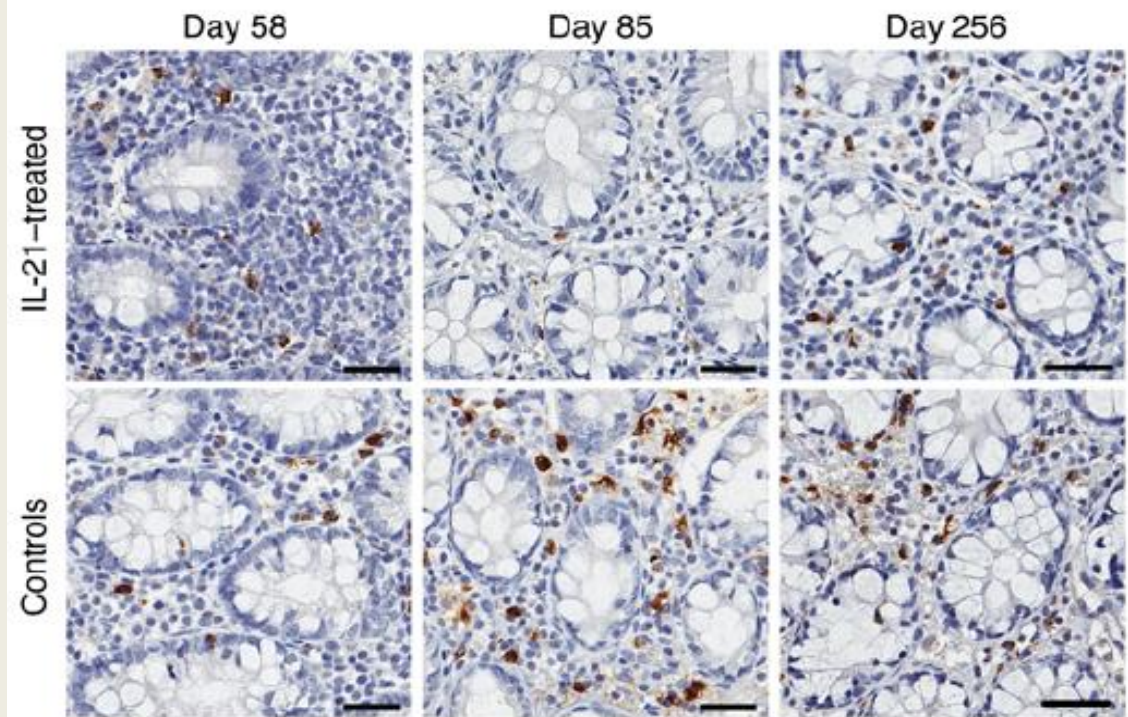
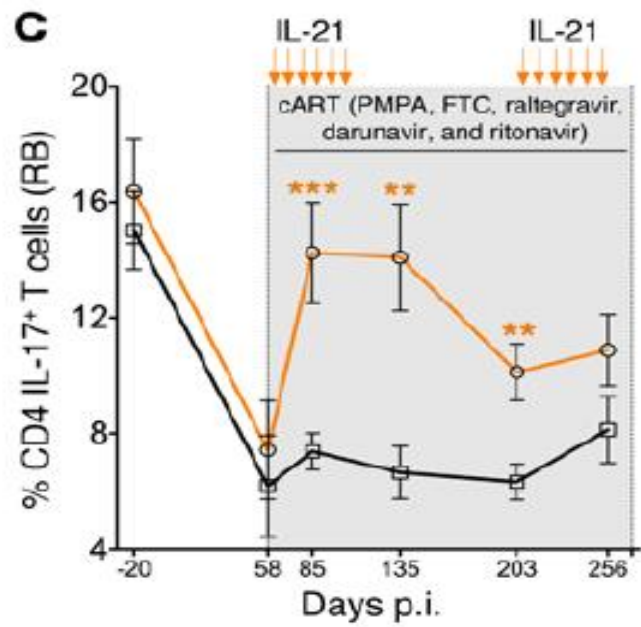


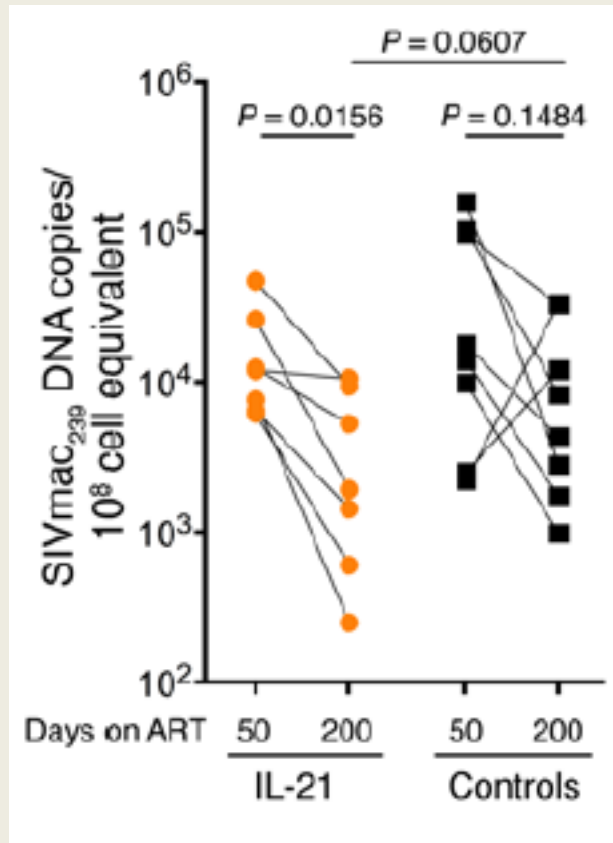
HIV+



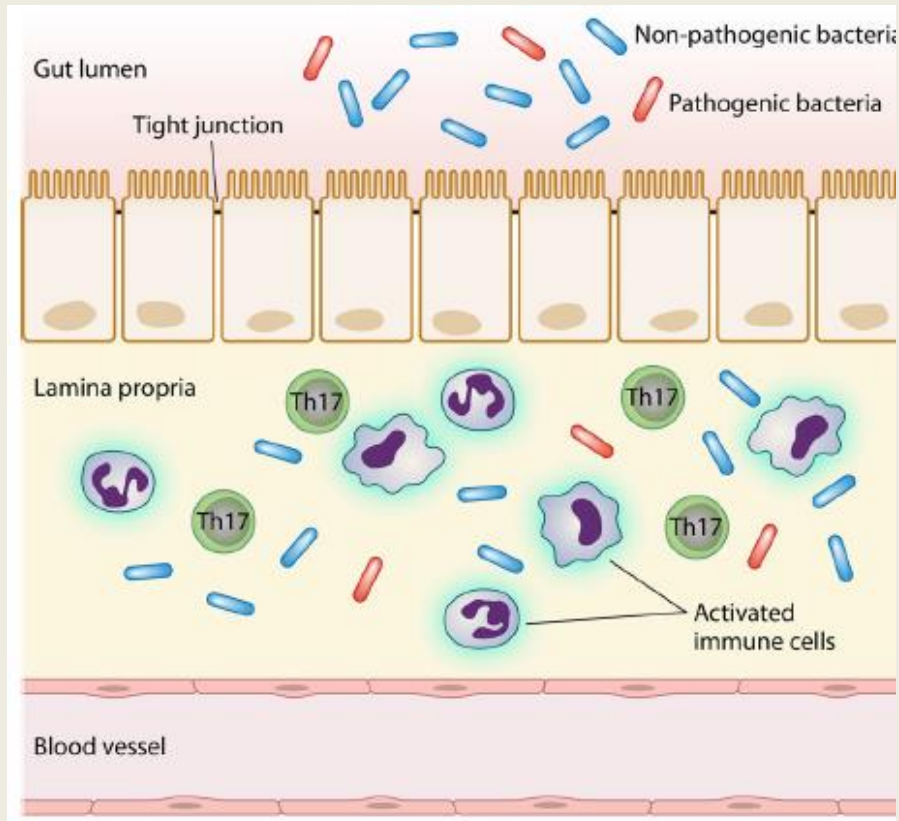


; Micci, JCI 2015

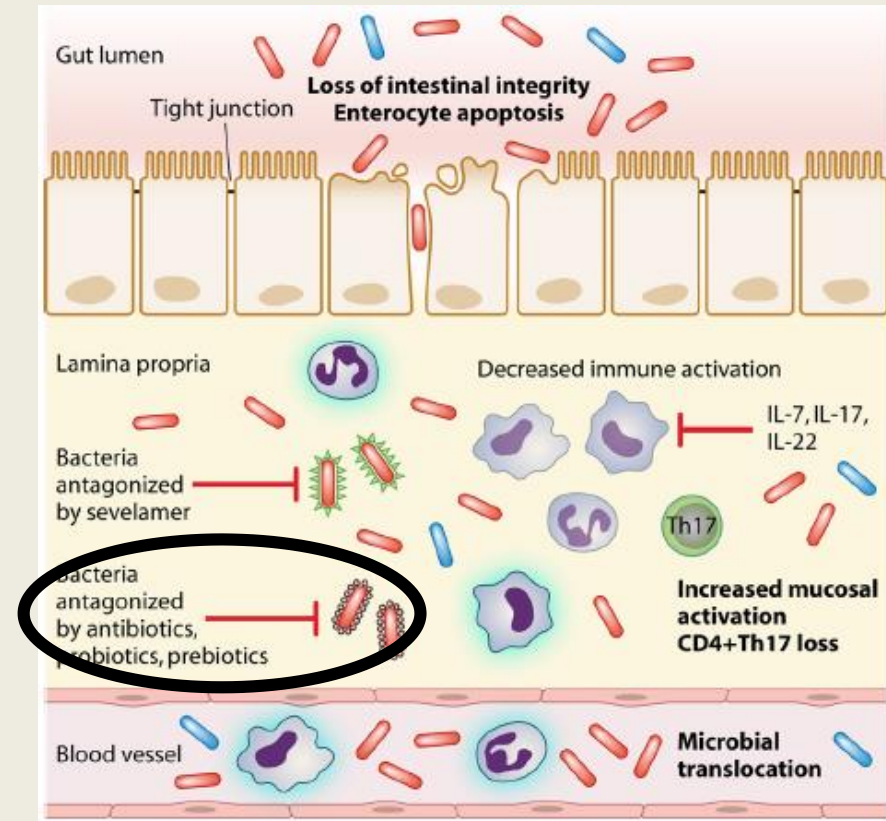




HIV-

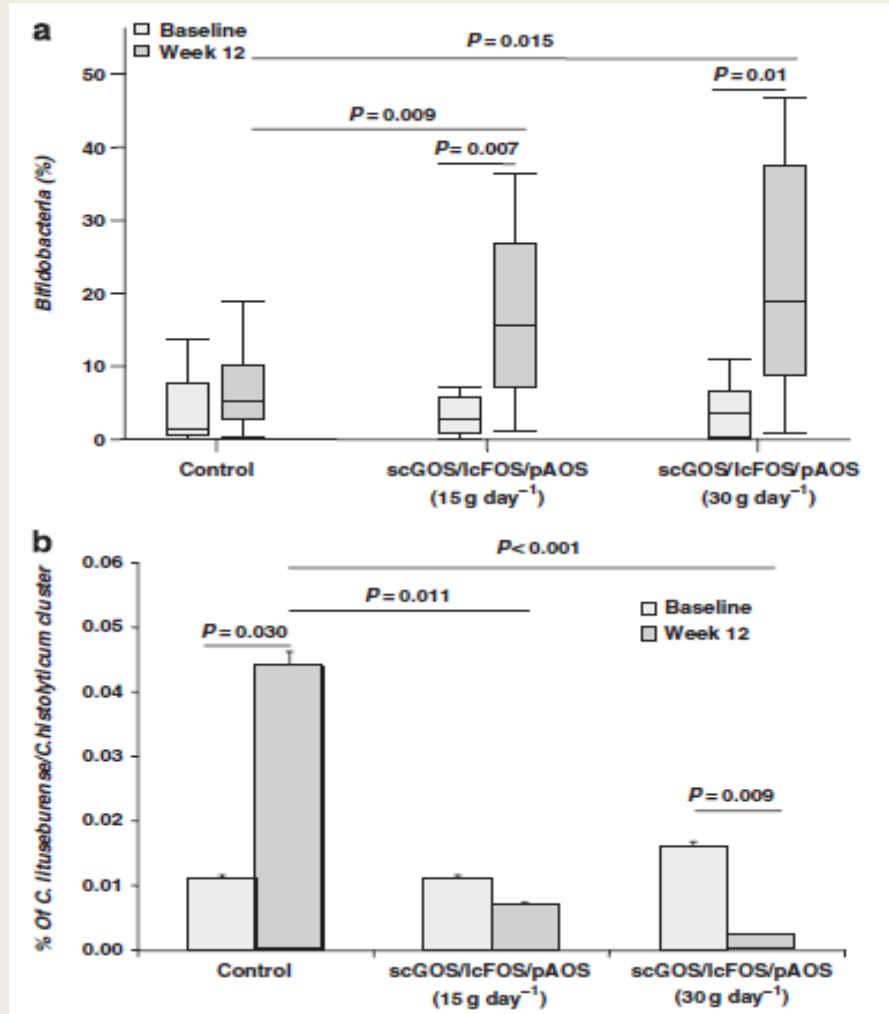


HIV+

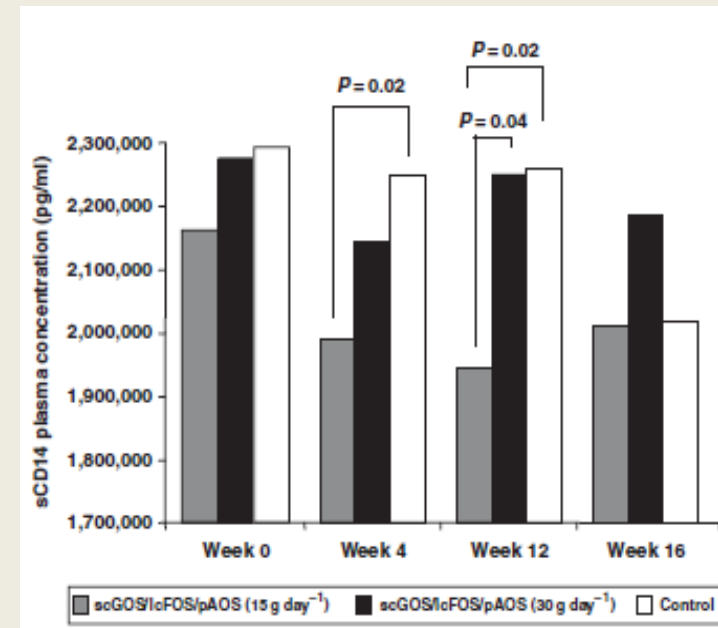


Giulia Marchetti, Camilla Tincati and Guido Silvestri
Clin. Microbiol. Rev. 2013, 26(1):2. DOI:

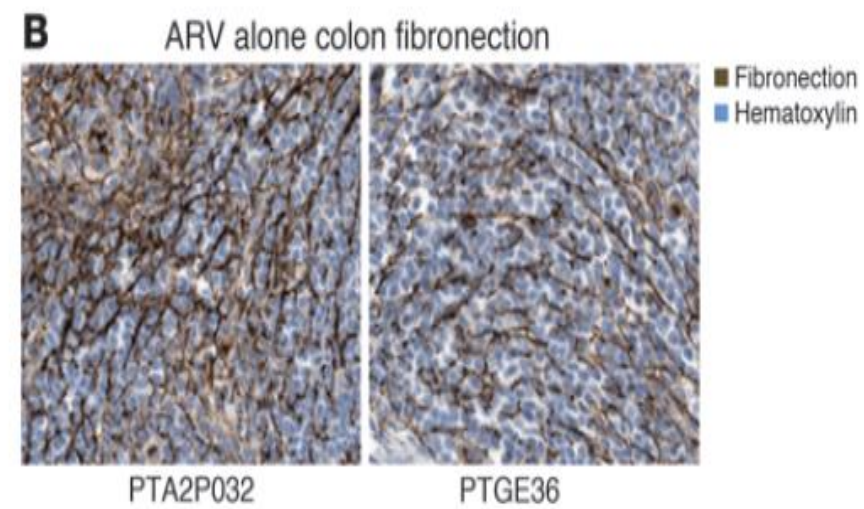
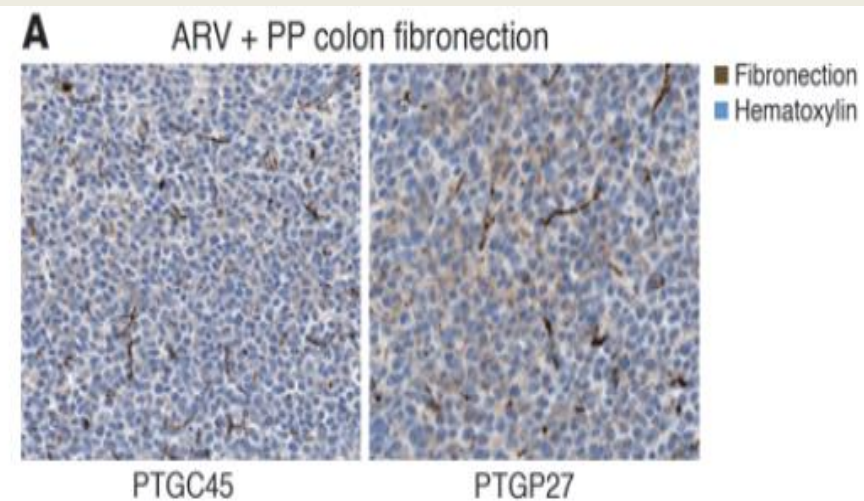
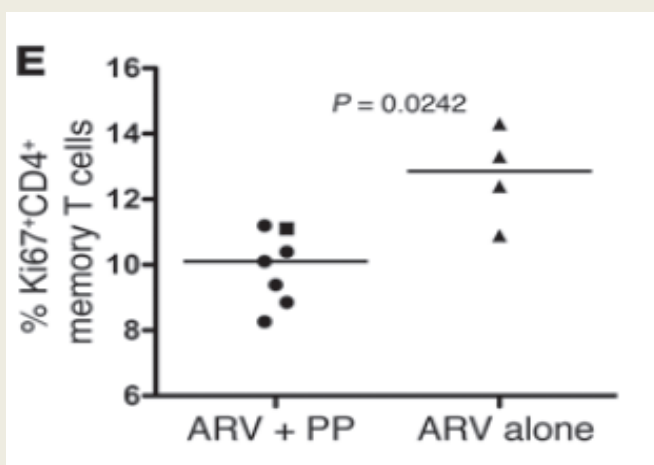
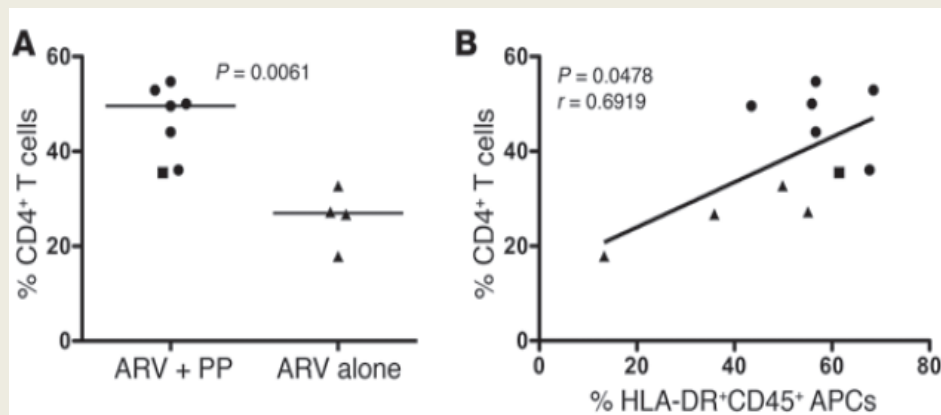
Prebiotics: amelioration of “quality” of gut microbioma and reduction of circulating sCD14



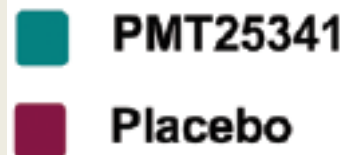
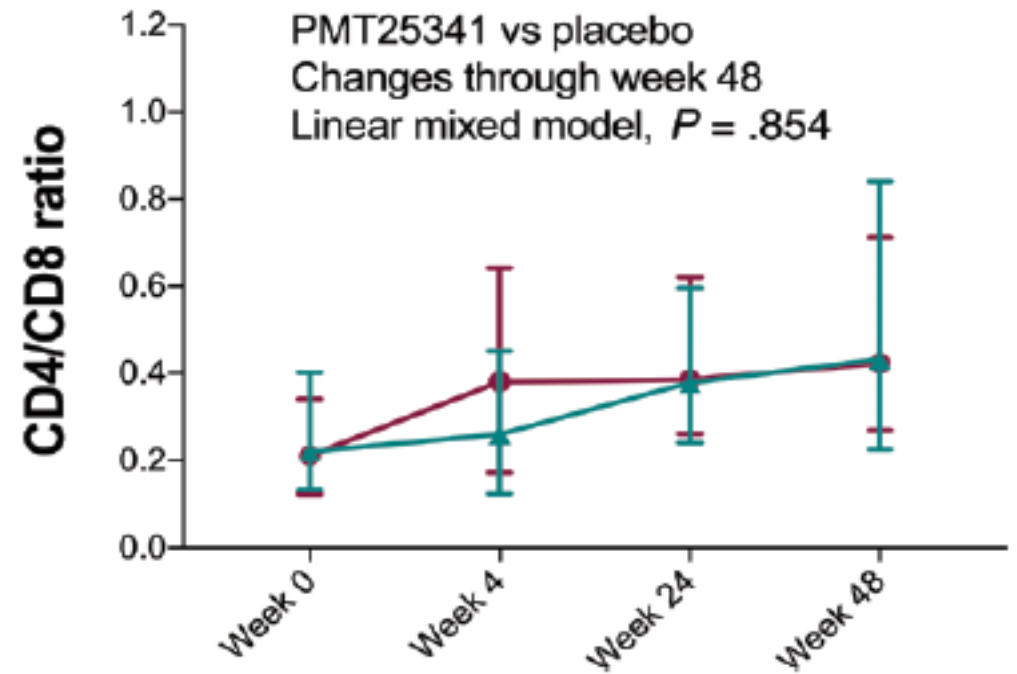
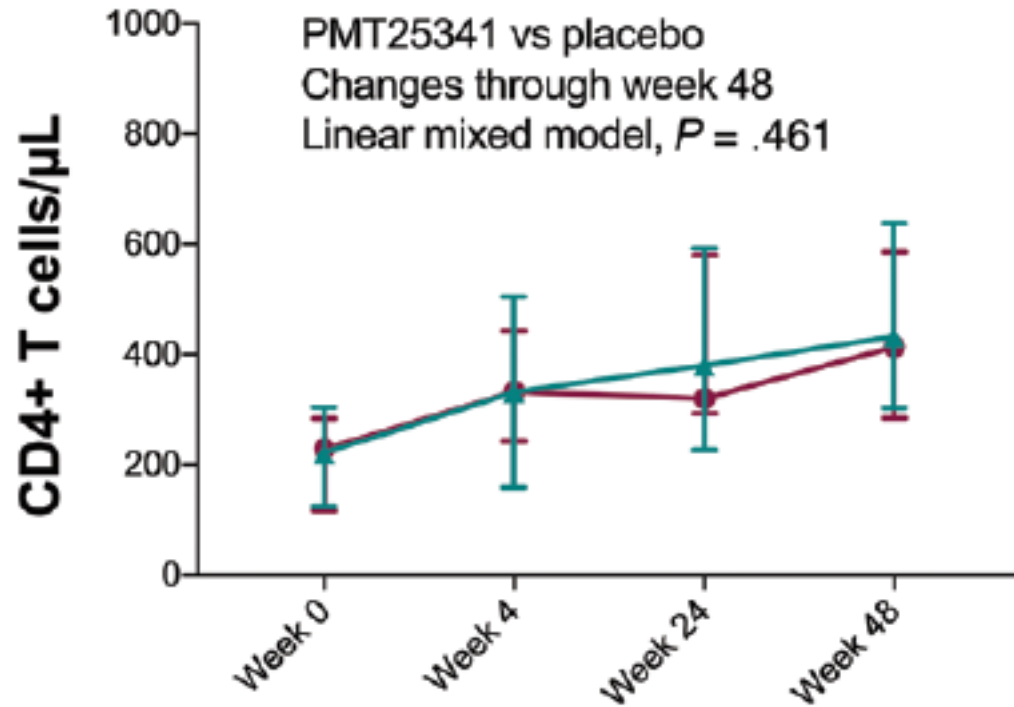
57 HAART-naïve HIV-1-infected patients: oligosaccharide mixture versus placebo for 12 weeks

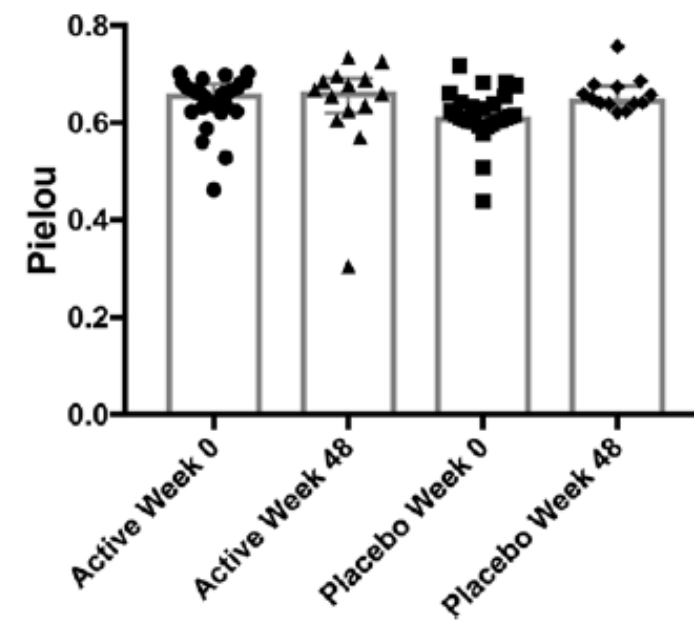
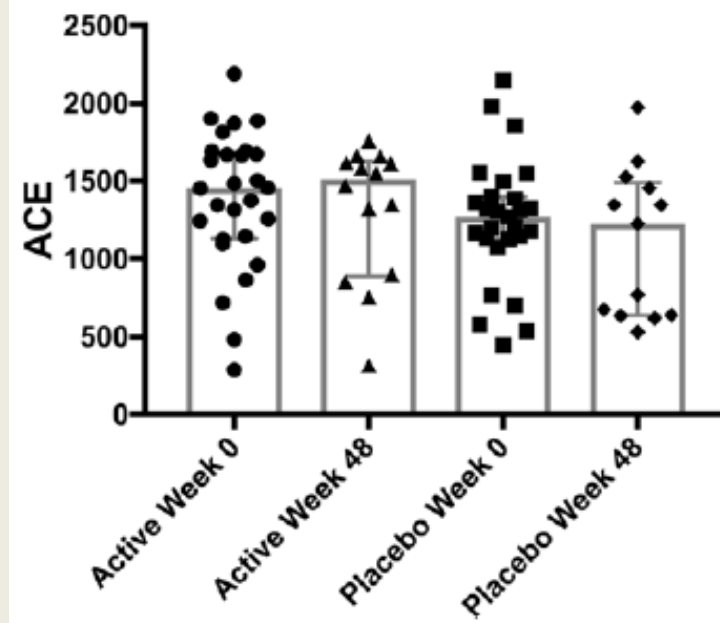
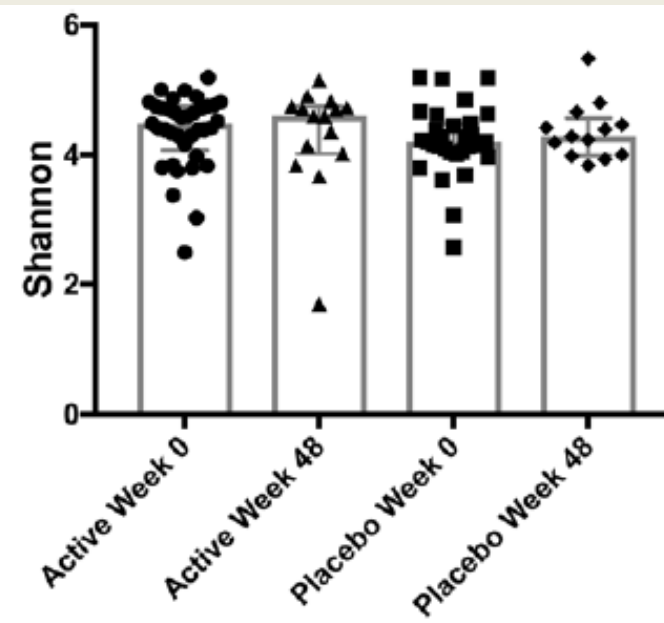
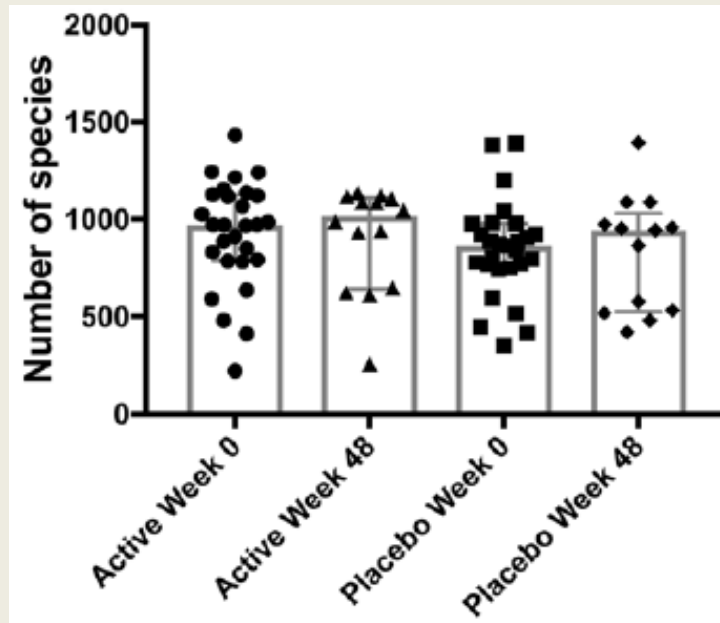


7 SIV-infected PTM received
probiotics/prebiotics (PP)-ARV
for 60 days

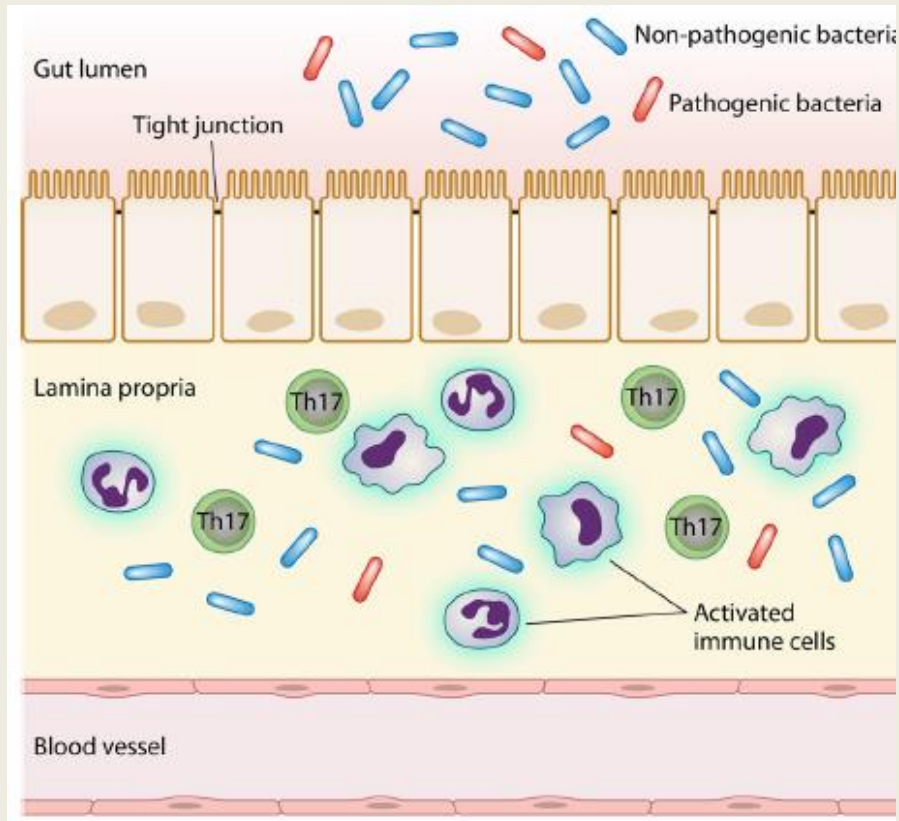


78 HIV+ naive, CD4 <350, starting ART with either placebo or PMT25341 (mixture of synbiotics, omega-3/6 fatty acids, amino acids) for 48 wks

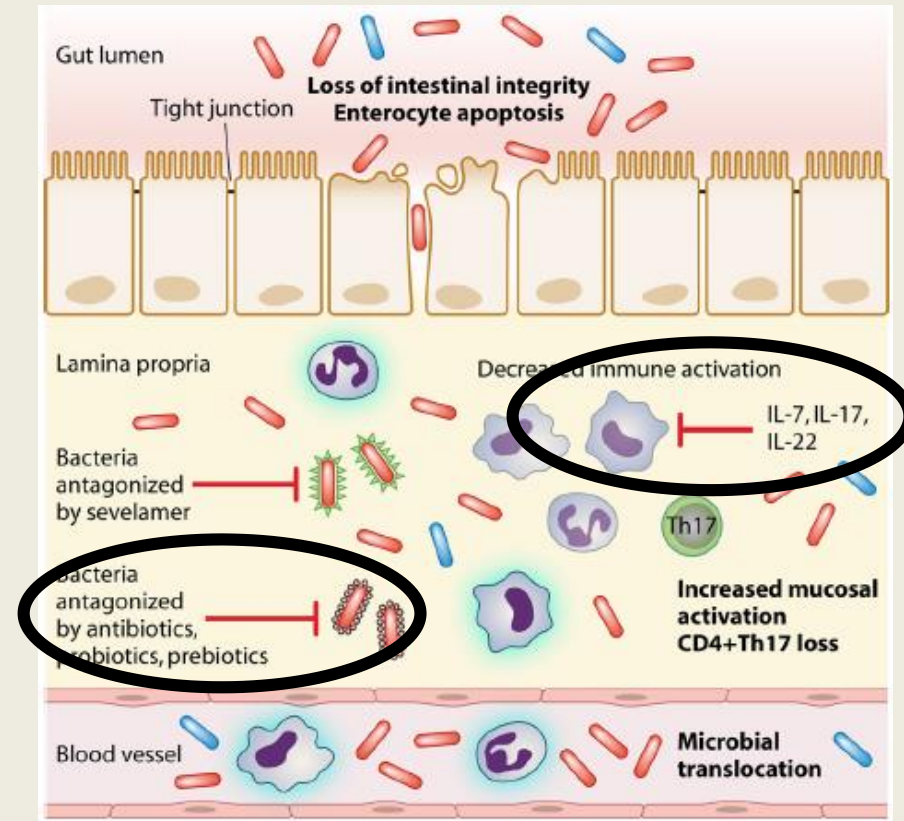


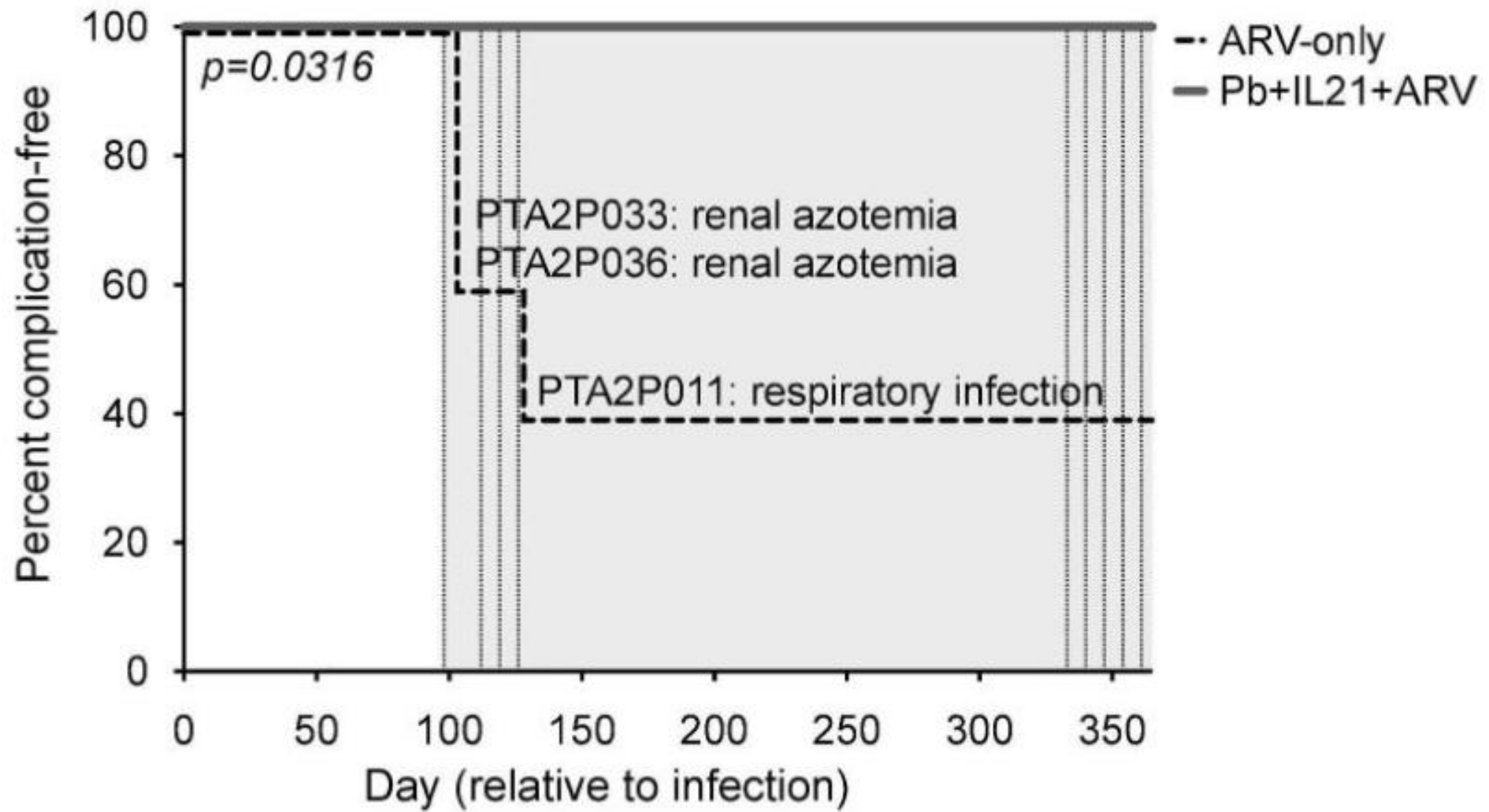


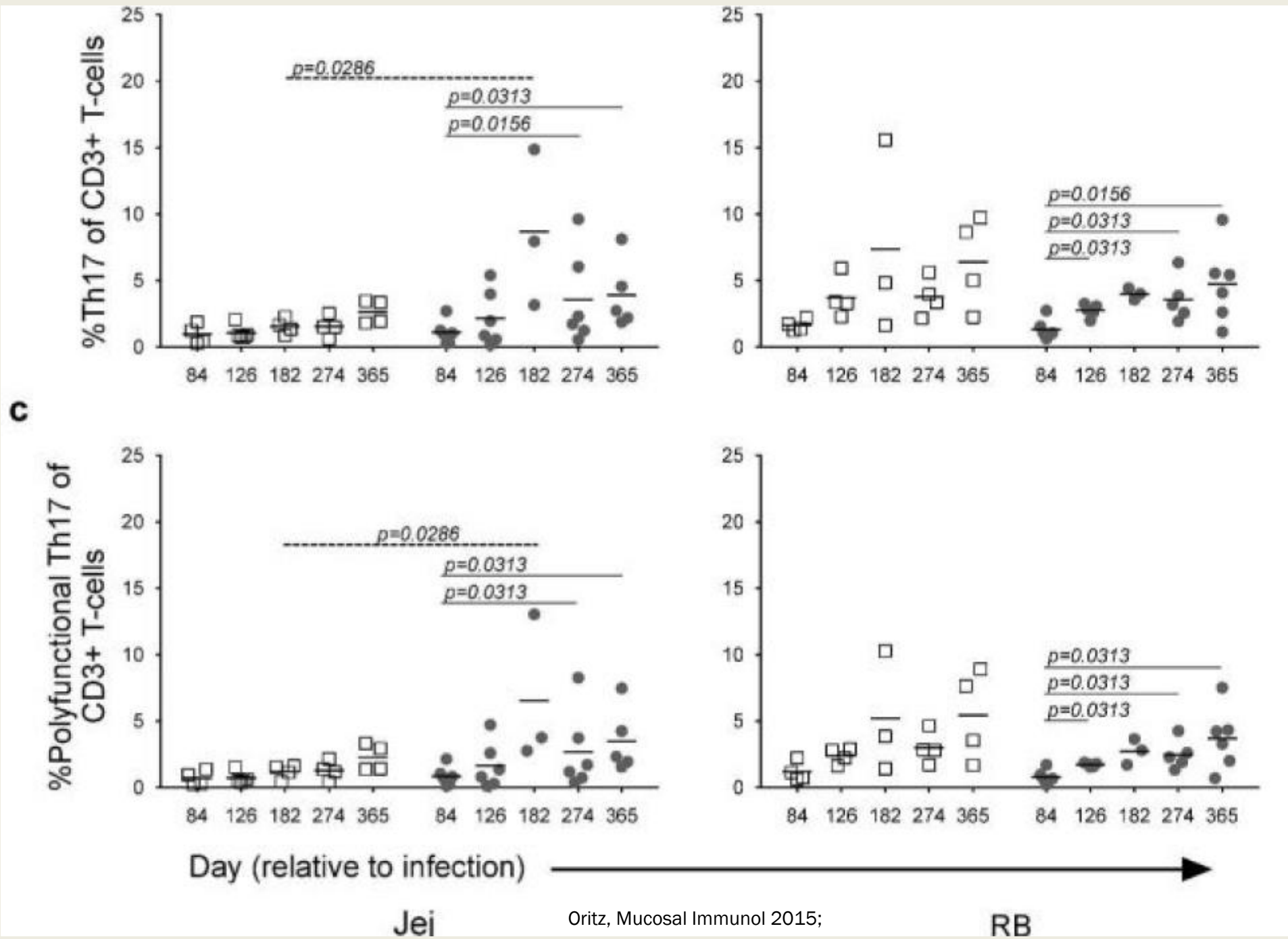
HIV-



HIV+







“Yet to be demonstrated” fact #2

**Any chance to treat
the gut?**

Targeting the microbiome for HIV Cure?

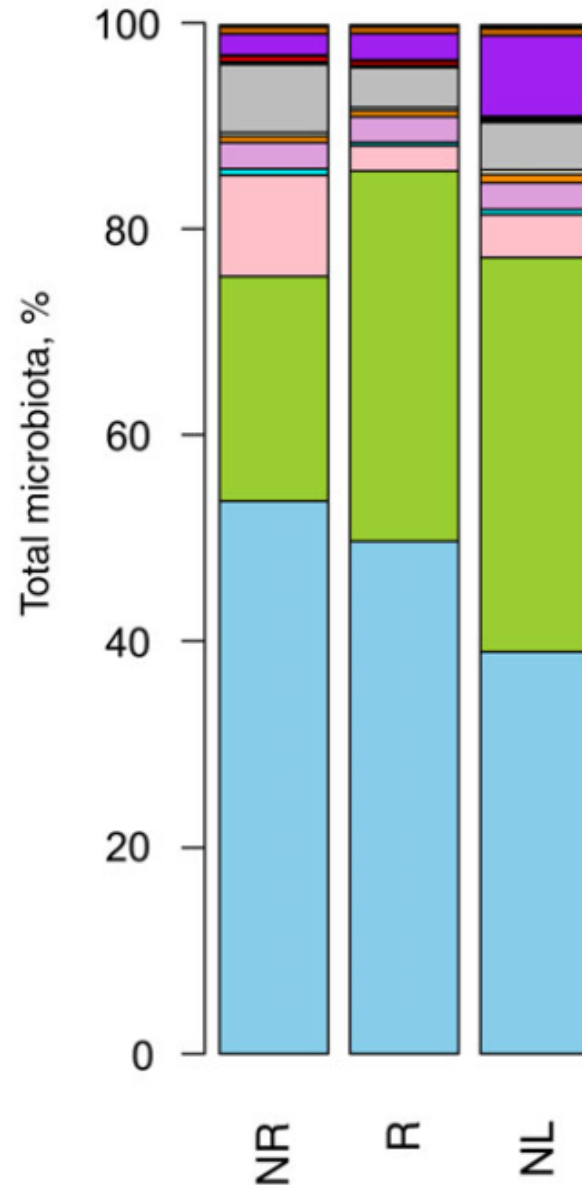
“More physiologic” microbiome in exposed uninfected infants and ECs

	Year	Location	Sample size	Population of interest	Comparison group	Enriched	Depleted	Summary
HEU infants								
Bender <i>et al.</i> [44 [■]]	2016	Haiti	25 HEU; 25 HUU	HEU infants	HUU infants	Pseudomonadaceae Oxalobacterae Rhodobacteraceae Thermaceae Bacillaceae Corynebacteriaceae Chloroacidobacteria Alicyclobacillaceae Sphingomonadaceae Enterobacteriaceae	Prevotellaceae Alcaligenaceae Desulfovibrionaceae Tissierellaceae Bacteroidiaceae Paraprevotellaceae Porphyromonadaceae Campylobacteraceae Erysipelotrichaceae Gemellaceae Bacilli (class)	Maternal HIV infection is associated with changes in the microbiome of HEU infants. Human breast milk oligosaccharides differ in mothers depending on their HIV status, and is associated with bacterial species found in the infant's gut microbiome.
Elite controllers								
Vesterbacka <i>et al.</i> [43 [■]]	2017	Sweden	16 EC, 32 HIV-infected (HIV+) ART-naïve, 16 HIV-uninfected (HIV-)	EC adults	HIV+ ART naïve, HIV-	EC compared to ART naïve and HIV-: <i>Succinivibrio</i> <i>Sutterella</i> EC compared to ART naïve: <i>Rhizobium</i> <i>Delftia</i> <i>Anaerofilum</i> <i>Oscillospira</i>	EC compared to ART naïve: <i>Blautia</i> <i>Anaerostipes</i>	ECs have a richer gut microbiota than untreated HIV adults, and their microbiome is more similar to HIV-uninfected adults than untreated HIV adults.
Nowak <i>et al.</i> [45]	2015	Sweden	28 HIV+, 3 EC, 9 HIV-	EC adults	HIV+, HIV-	EC compared to HIV+ Bacteroidetes[45]	EC compared to HIV+ Actinobacteria	The microbiome of ECs is significantly different from viremic

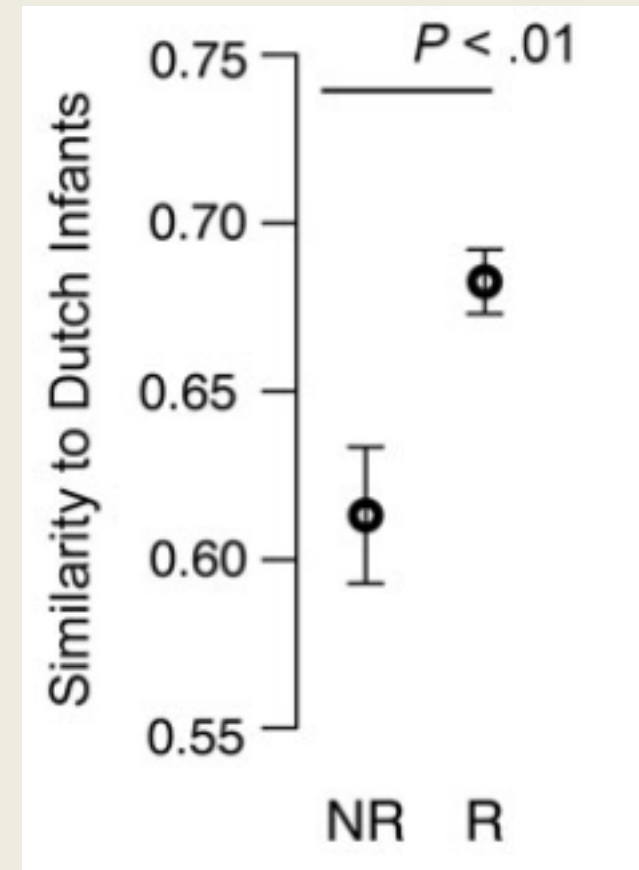
Impact of gut microbiome on response to Rotavirus vaccination

A

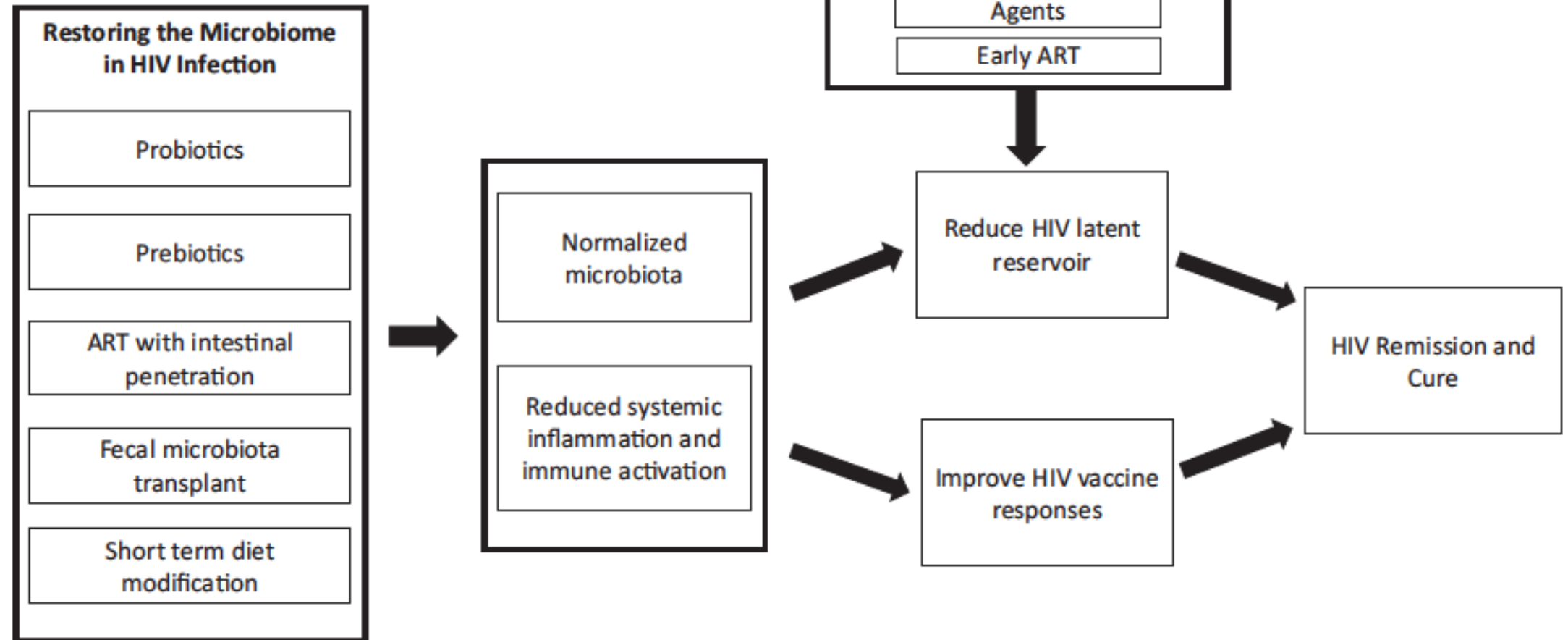
- Verrucomicrobia
- Uncultured Mollicutes
- Uncultured Clostridiales
- Proteobacteria
- Fusobacteria
- *Clostridium* cluster XVIII
- *Clostridium* cluster XVI
- *Clostridium* cluster XV
- *Clostridium* cluster XIVa
- *Clostridium* cluster XI
- *Clostridium* cluster IX
- *Clostridium* cluster IV
- *Clostridium* cluster I
- Bacteroidetes
- Bacilli
- Actinobacteria



78, 6-week old Ghanaian receiving Rotavirus vaccine: 39/78 R
154 Dutch age-matched, all R



Targeting the microbiome for HIV cure?



“Yet to be demonstrated” fact #3

**Targeting the
microbiome for HIV
Cure?**

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Ministero della Salute



UNIVERSITÀ DEGLI STUDI DI MILANO



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ITALIAN COHORT NAIVE ANTIRETROVIRALS



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