Microbiome in HIV: chronic inflammation, immune activation and transmission

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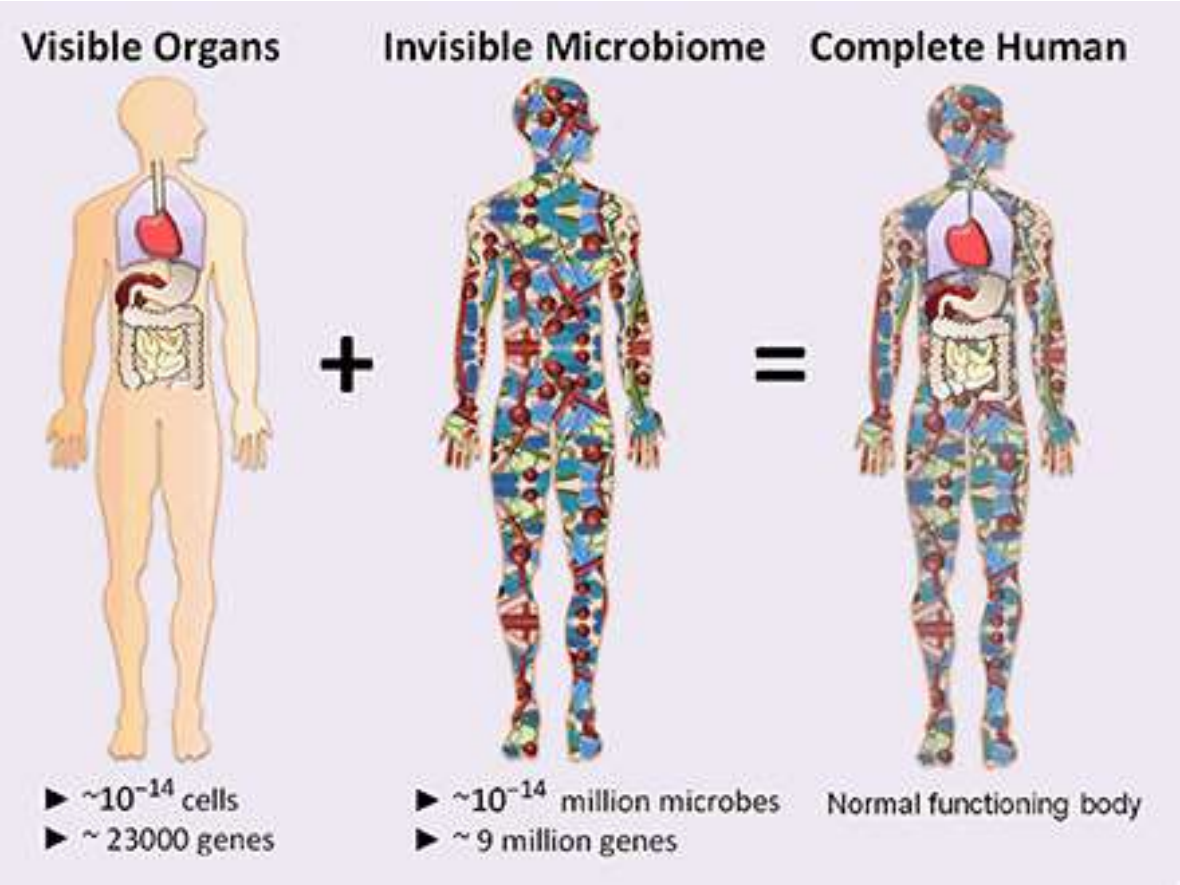
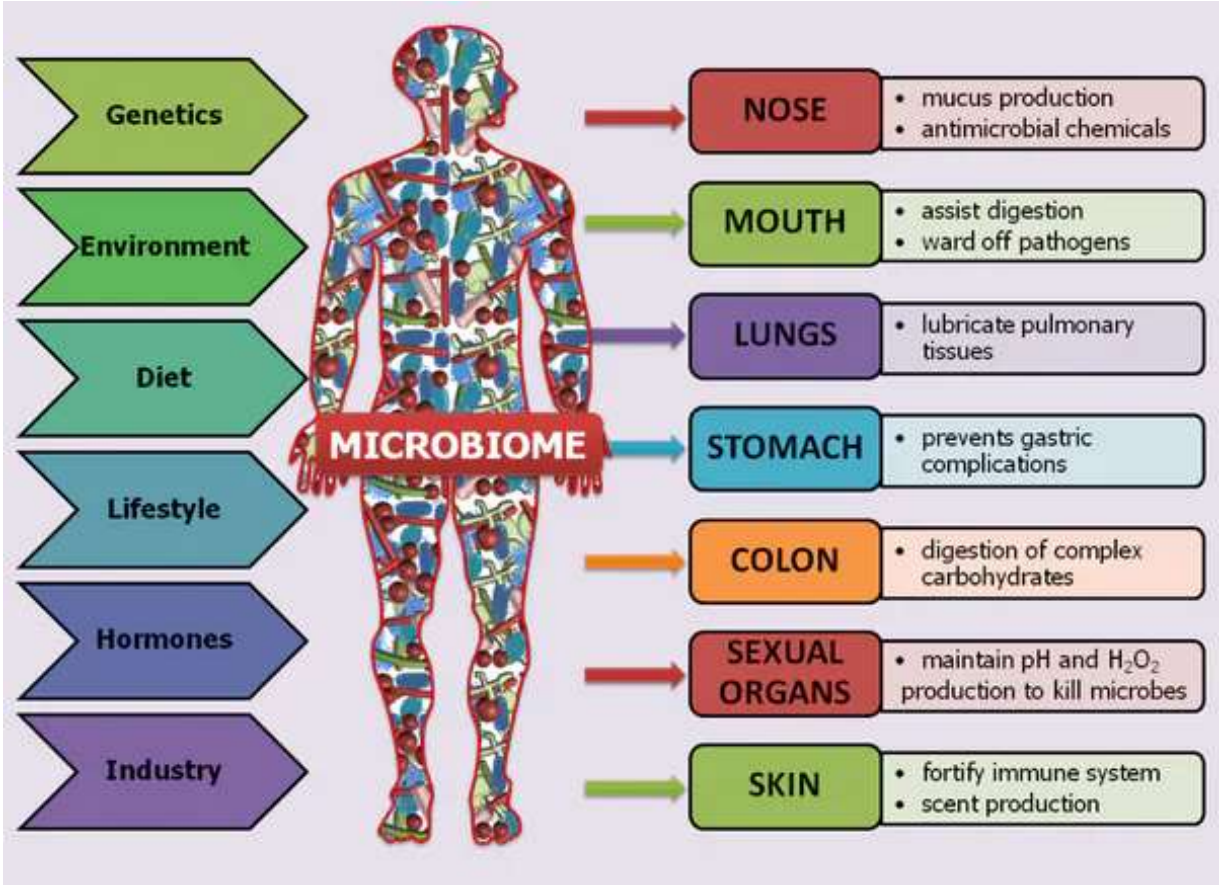
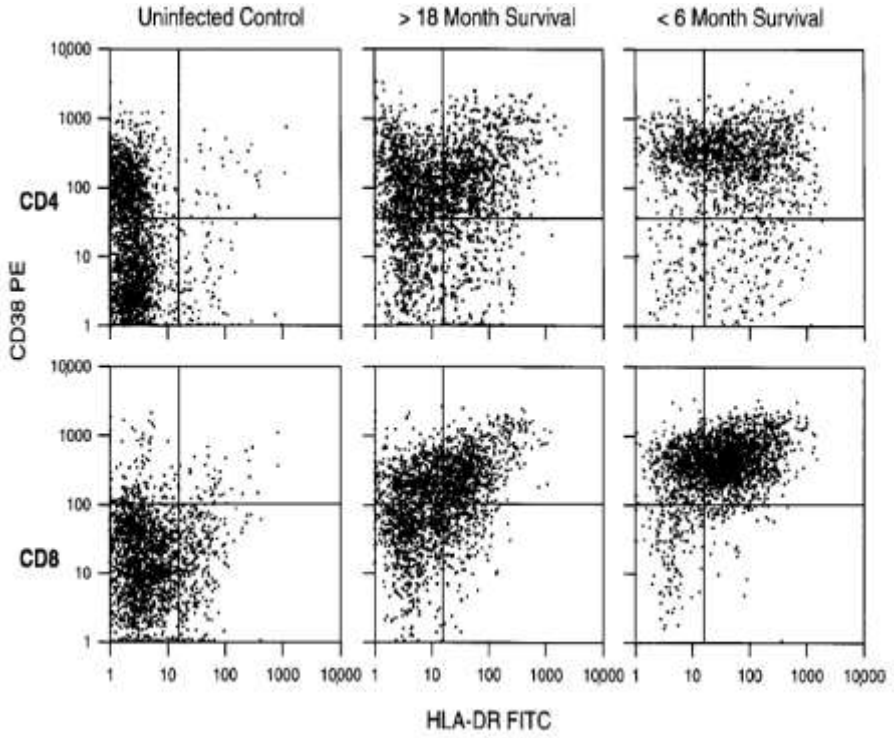
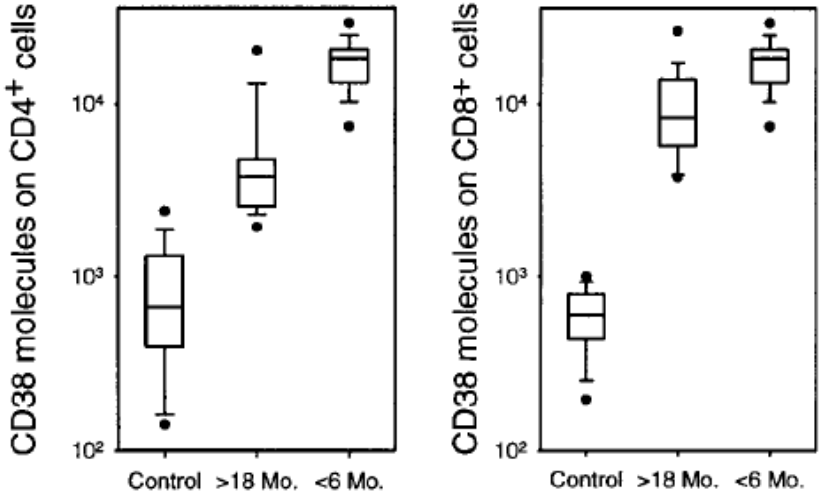




The human microbiome – Human Microbes 2018

HIV as a pro-inflammatory disease



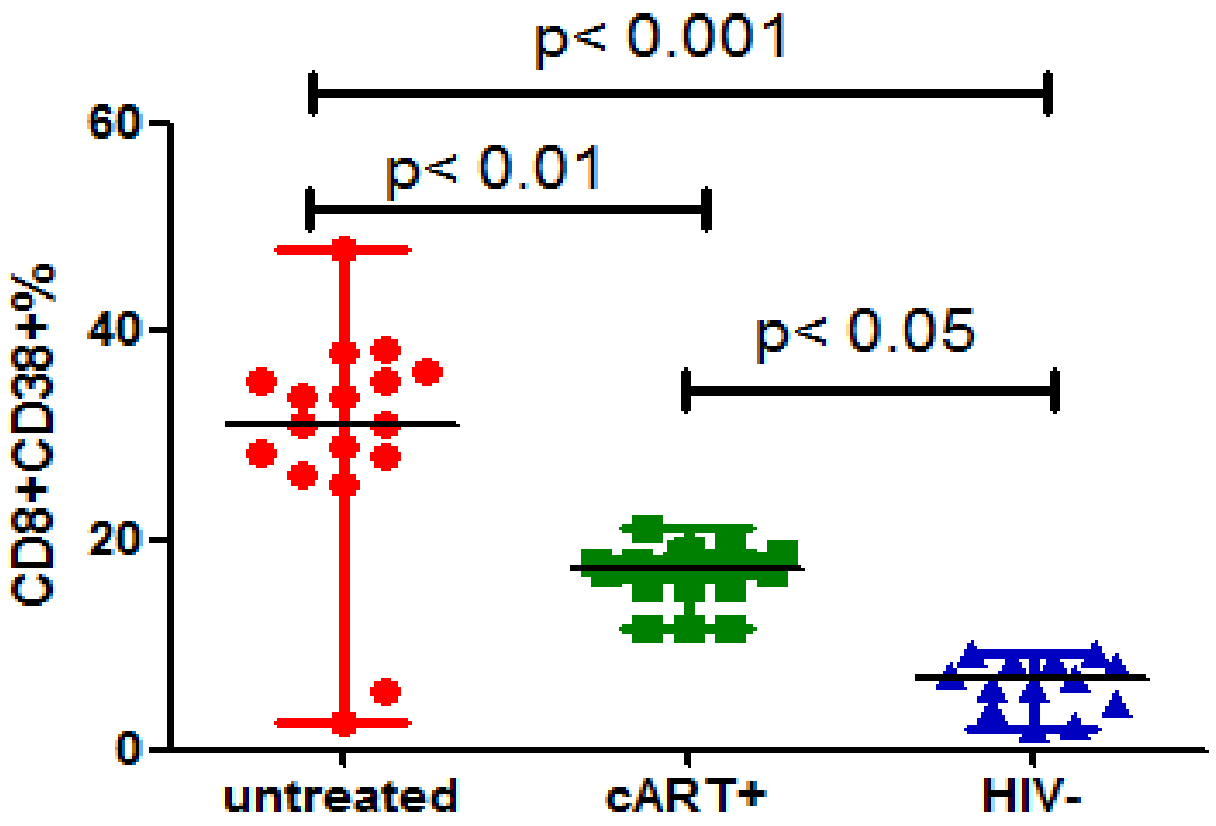


Giorgi, J et al. JID 1999

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

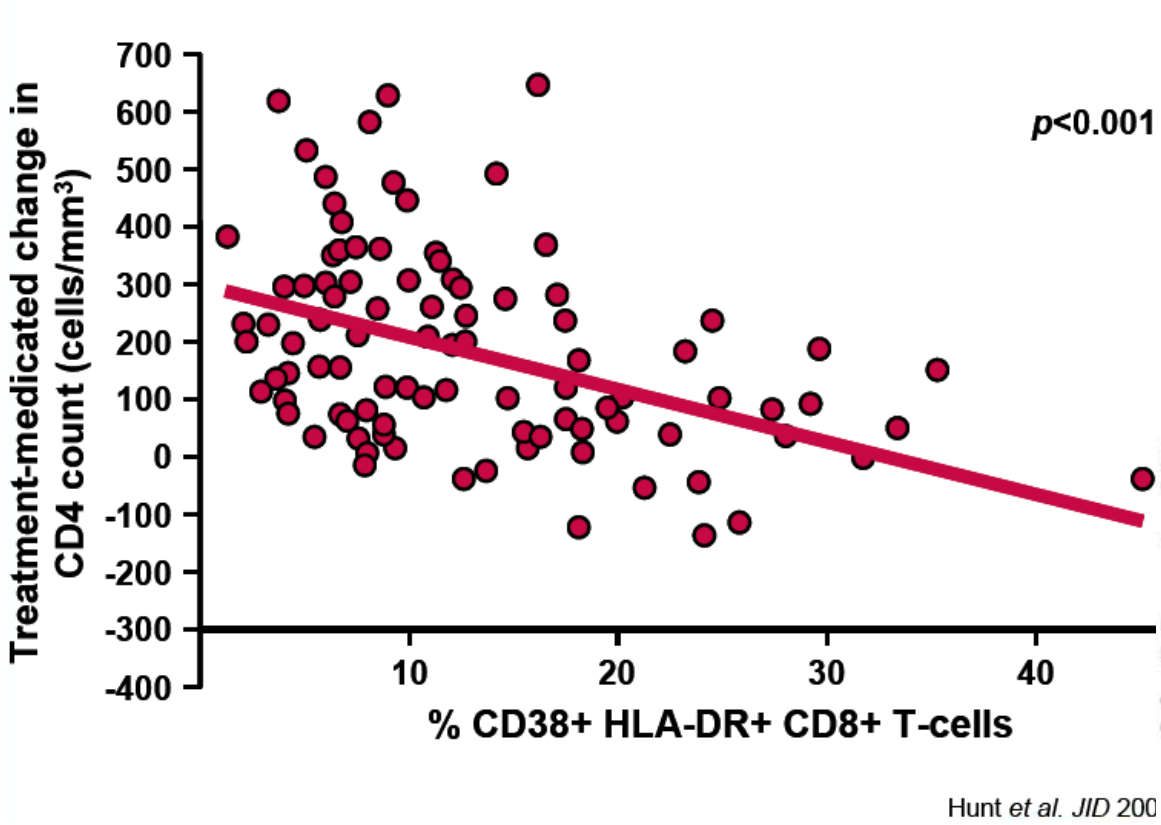
T-cell activation predicts CD4+ T-cell count over time

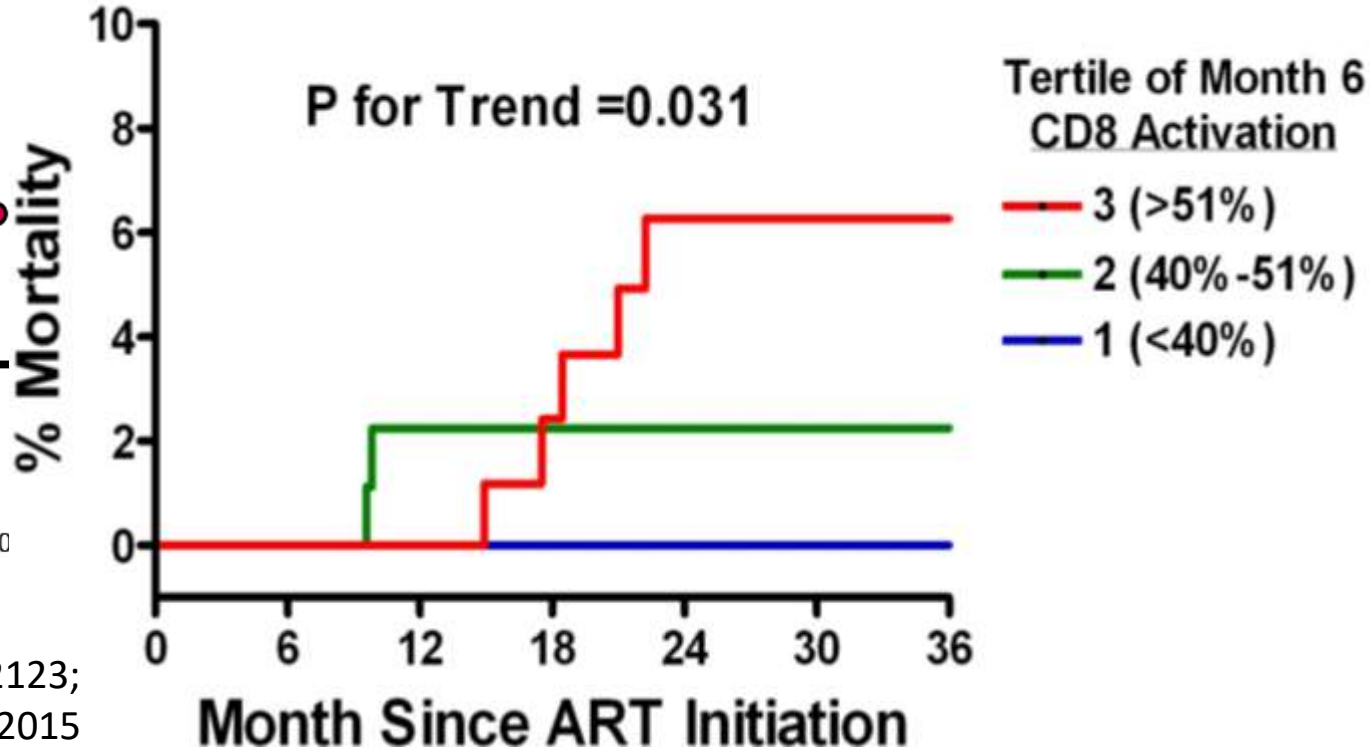
Deeks et al. Blood 2004



Cannizzo et al. JID 2015

Immune activation affects immune reconstitution and disease progression on cART

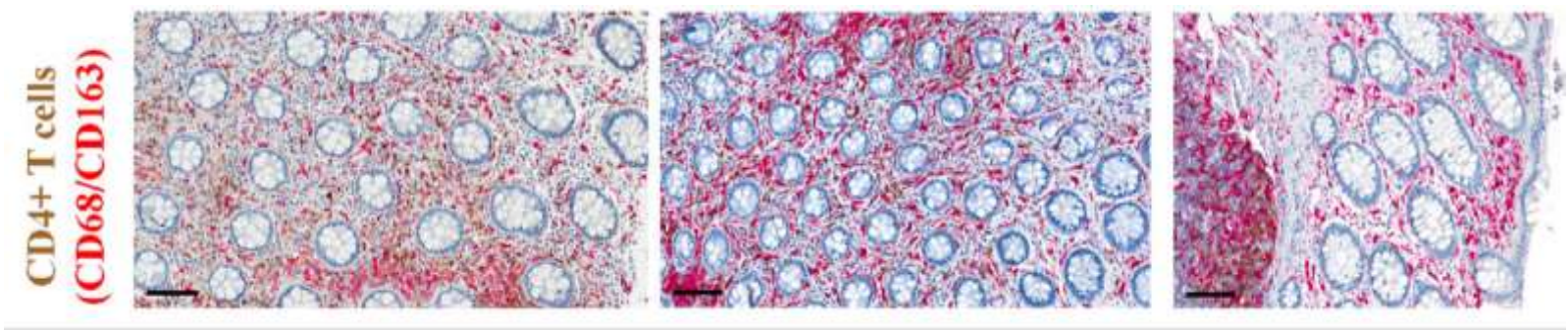


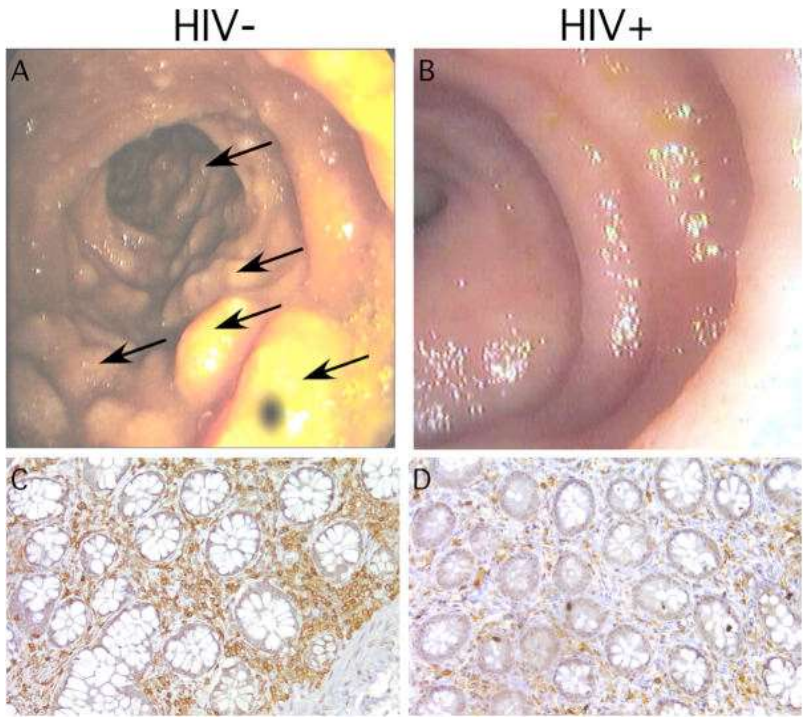


Hunt et al, AIDS 2011 25:2123;
also: Balagopal JAIDS 2015

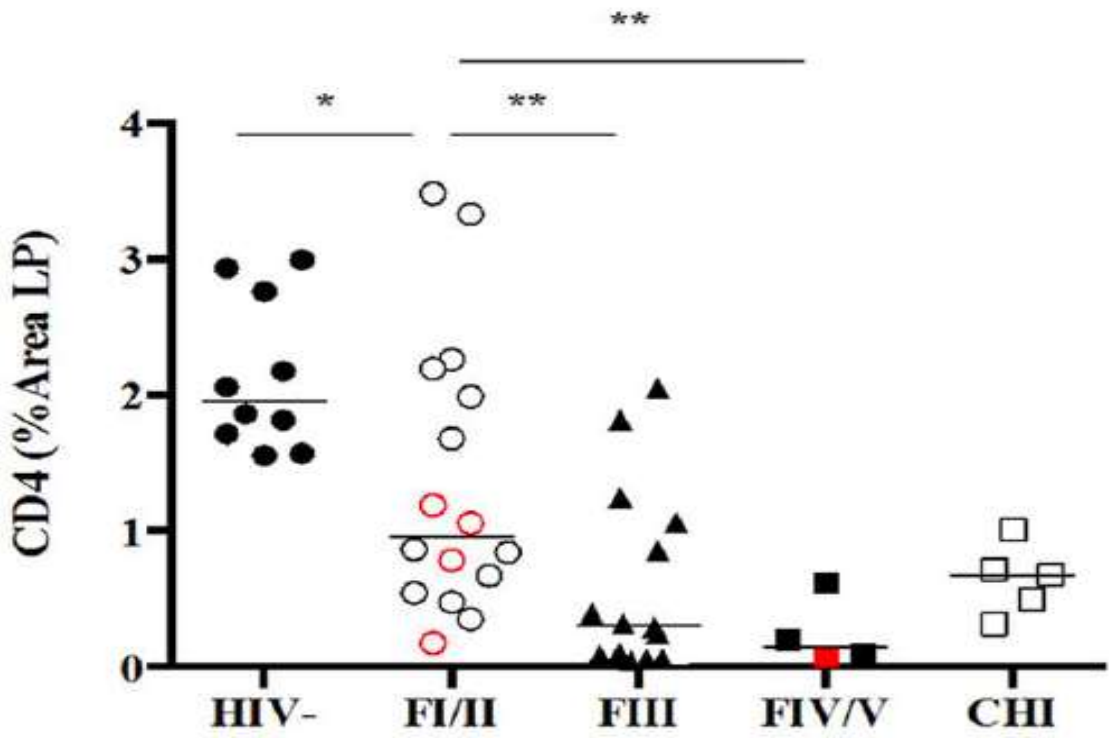
HIV as a disease of the gut

Earliest depletion of gut-associated CD4+

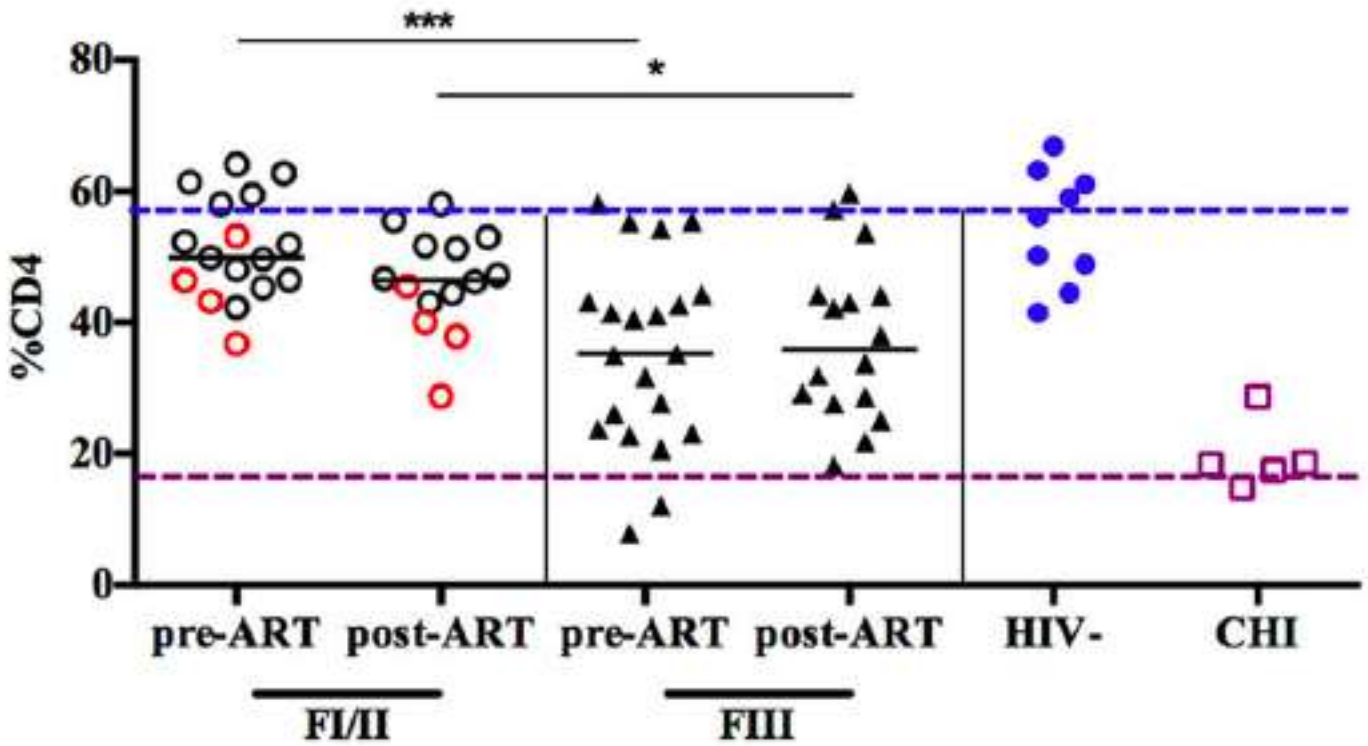




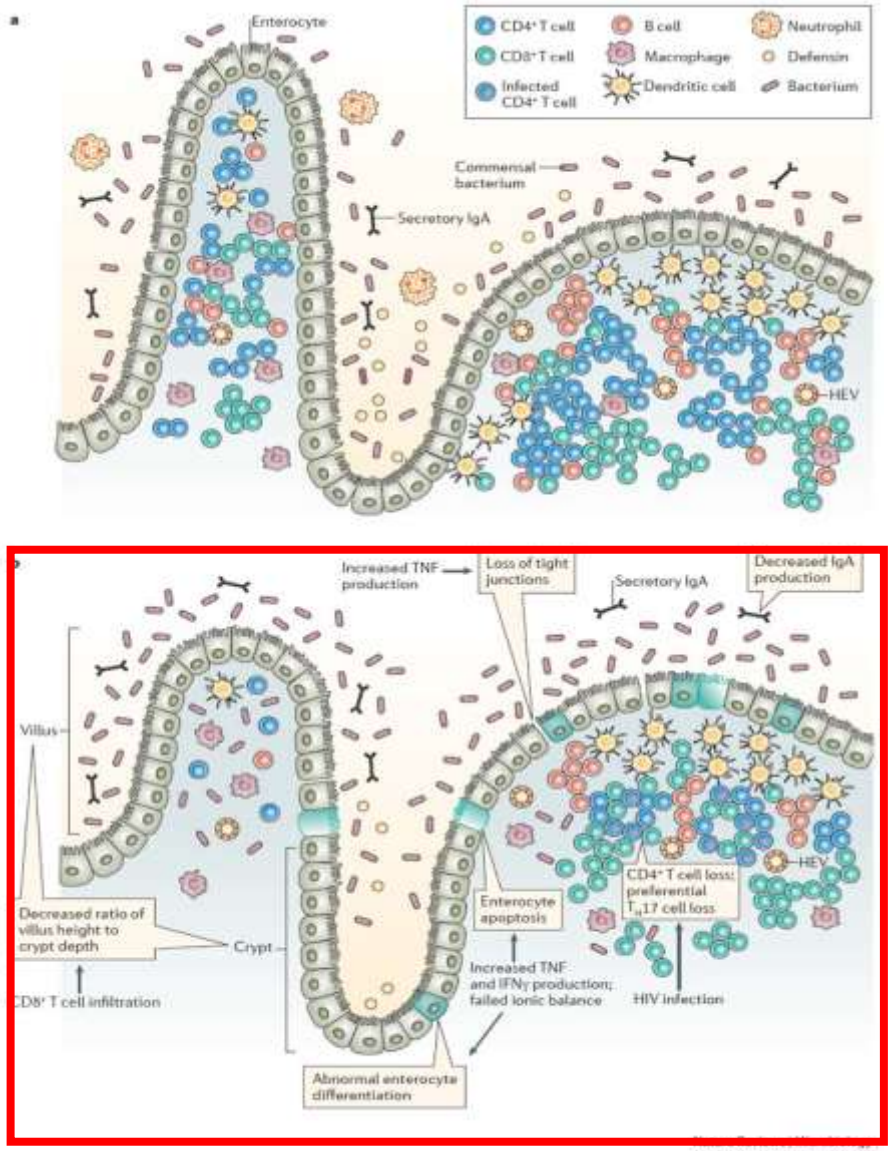
Brenchley et al. Nat Med 2006



Shuetz et al. Plos Path 2014



Shuetz et al. Plos Path 2014



Sandler & Douek, Nat Reviews 2012

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"

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)

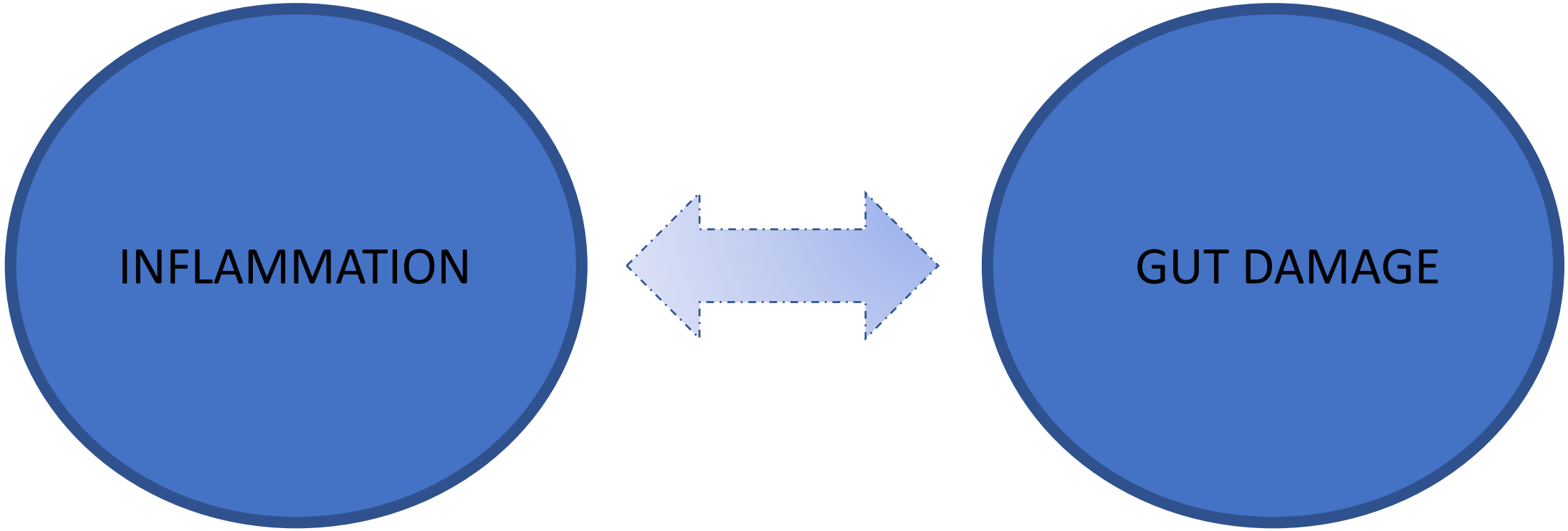
Giorgi et al. J Immunol 1993

Conclusion

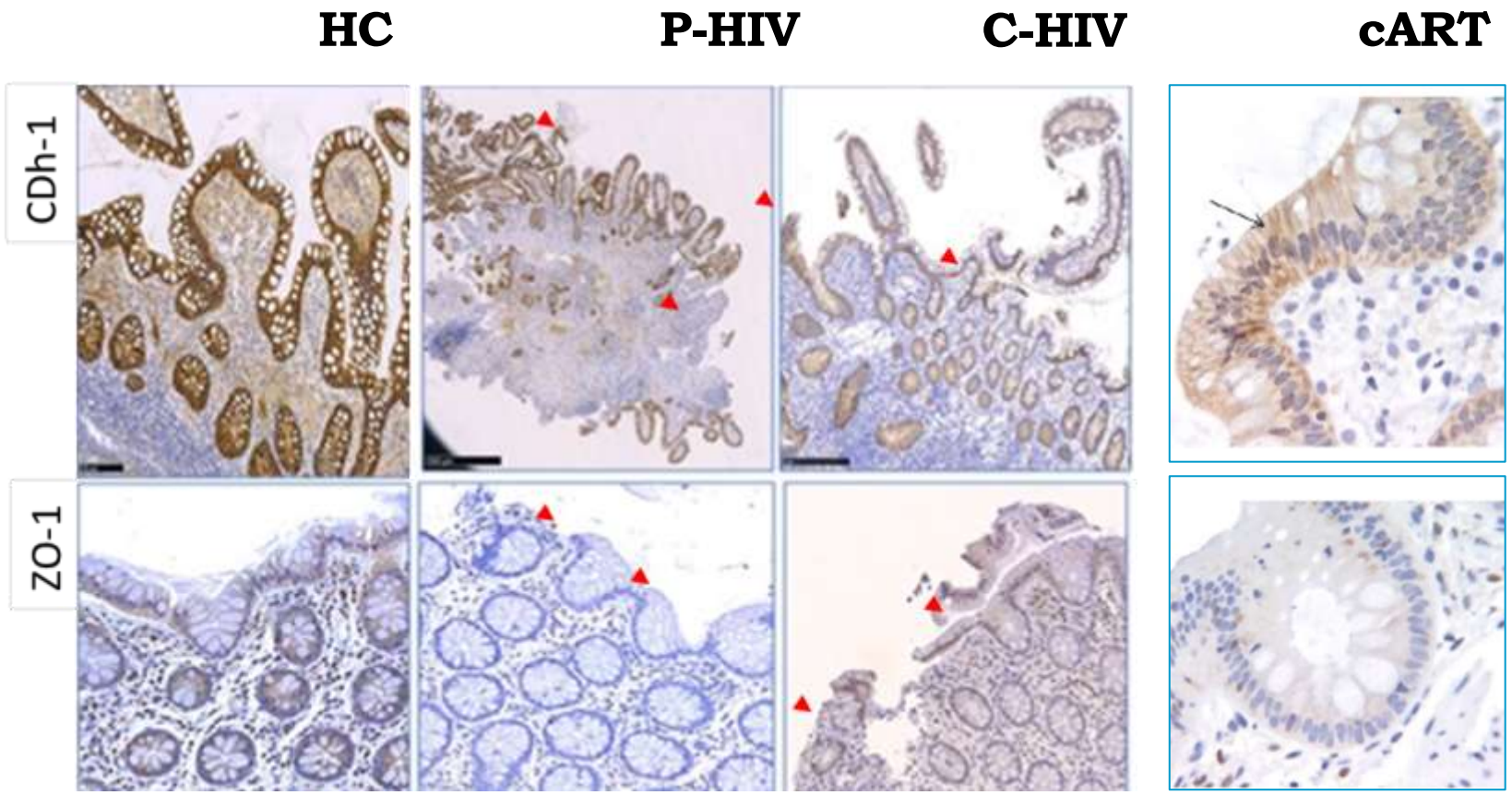
Further studies are required to define the precise mechanism for HIV-associated intestinal injury and its relationship to HIV replication. Evidence from this study and others [14] suggests

Kotler JID 1999;179 (suppl 3)

HIV: INFLAMMATION AND GUT DAMAGE



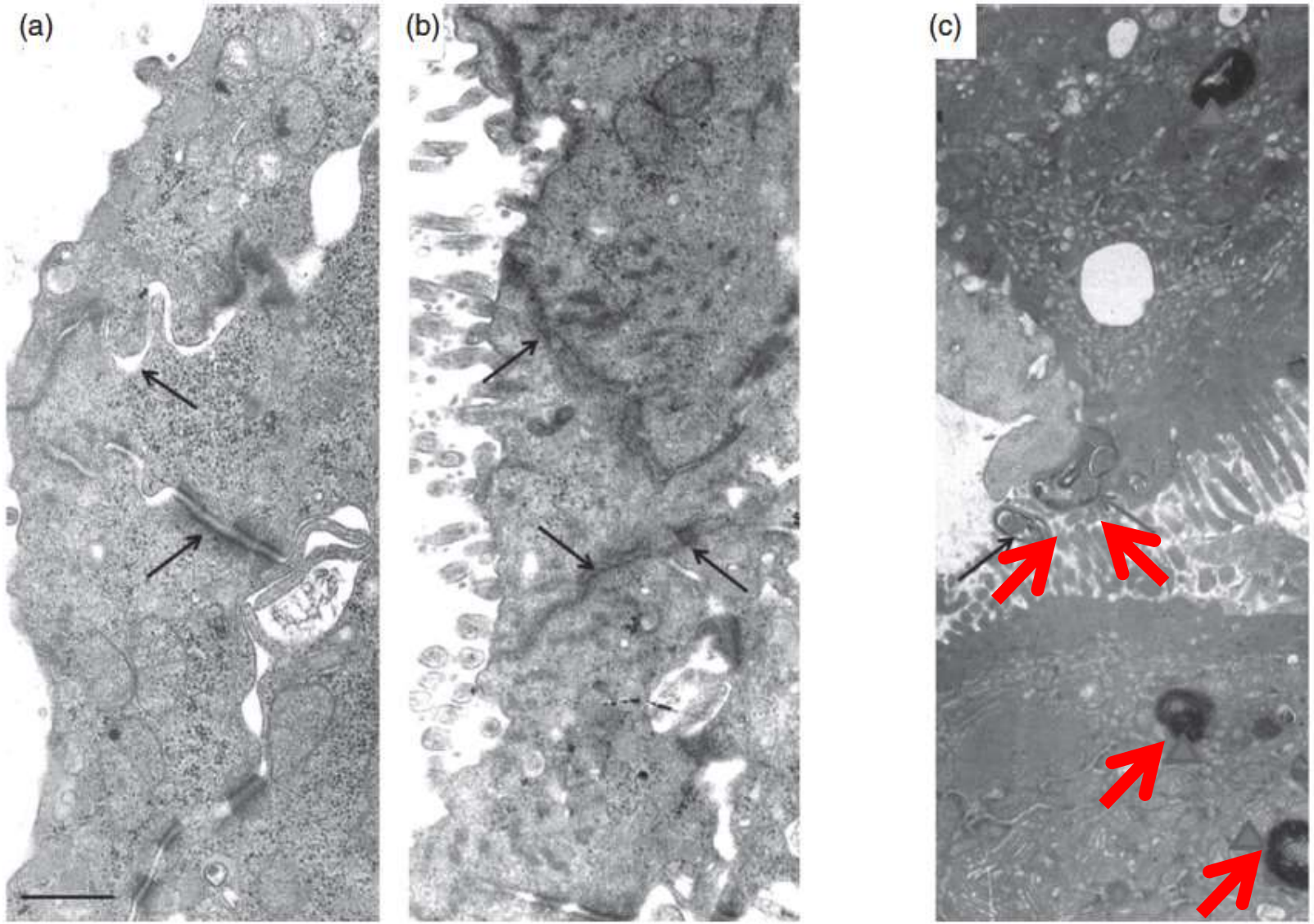
Early depletion of gut tight junctions that is not reverted by cART



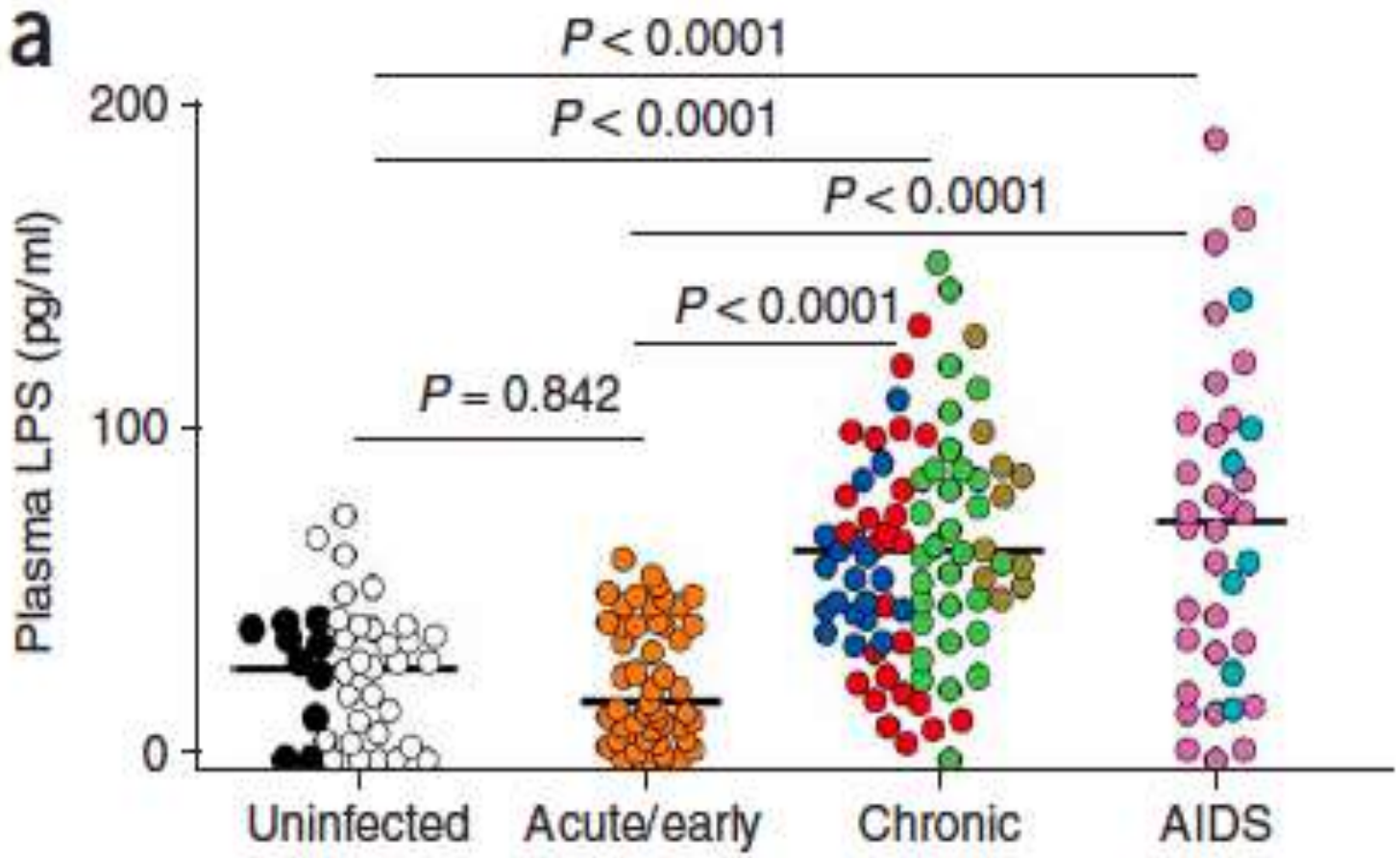
Cannizzo et al. CROI 2018

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

Healthy HIV uninfected



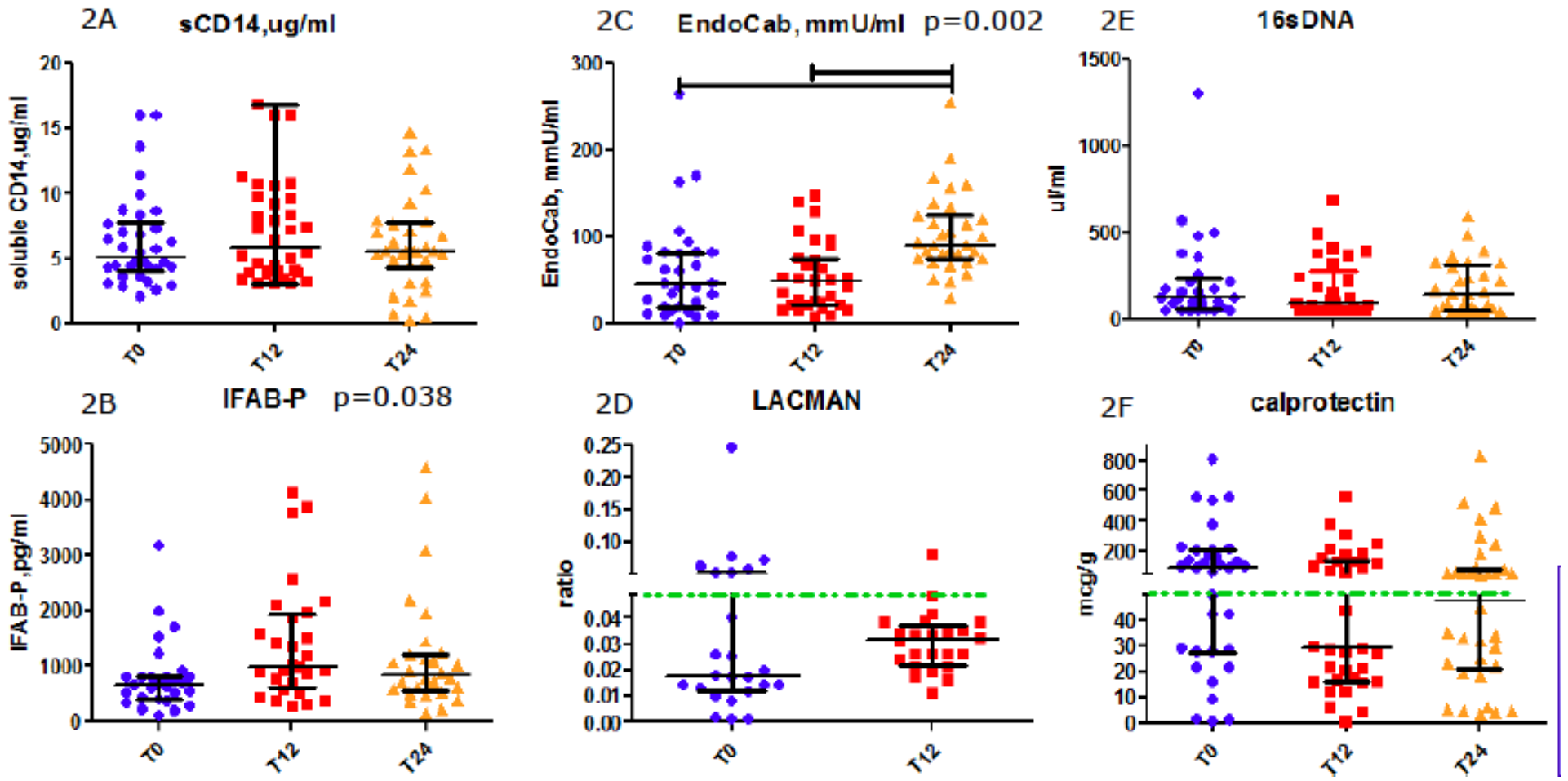
Tincati C et al. AIDS 2016



Brenchley et al. Nat Med 2006

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

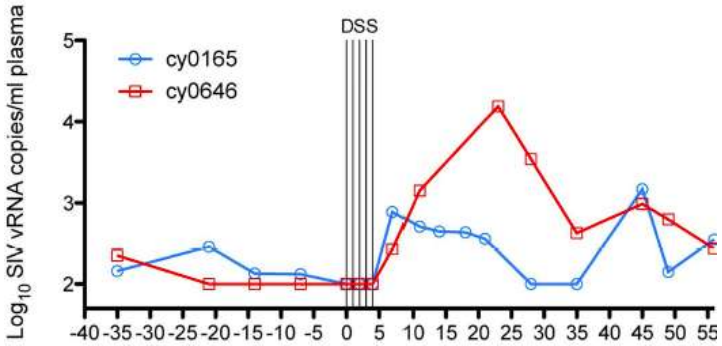
40 HIV+, nadir CD4 =300/µl

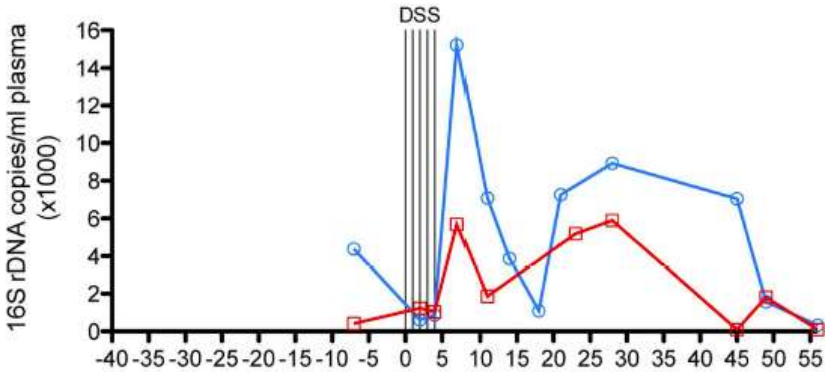


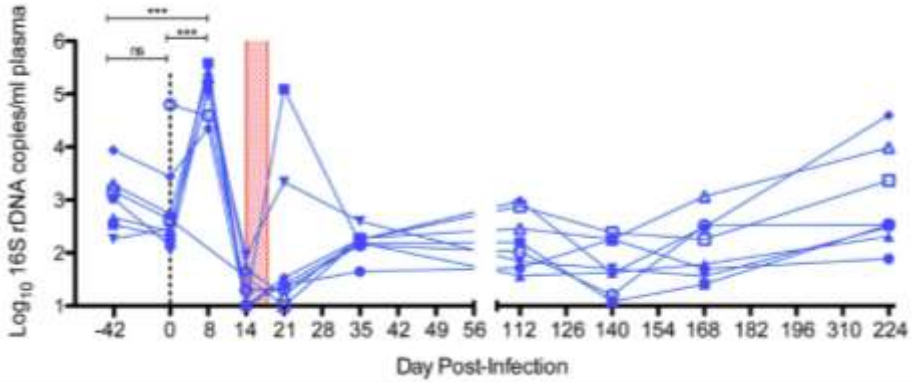
Tincati T et al. CROI 2017 #215, paper in preparation

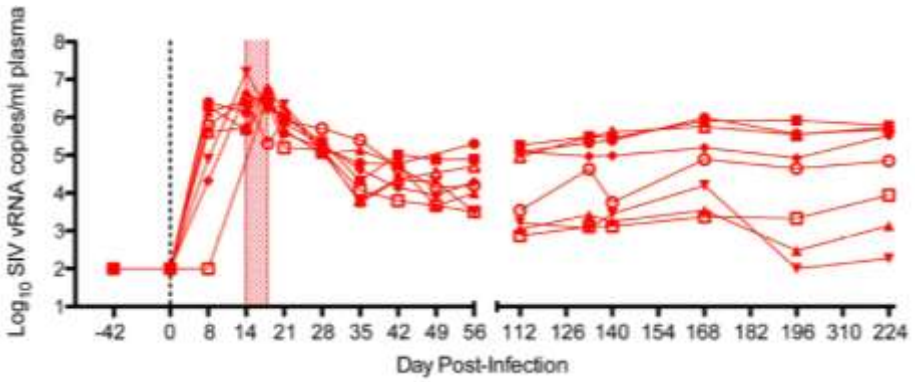
Hyperacute microbial translocation precedes viremia

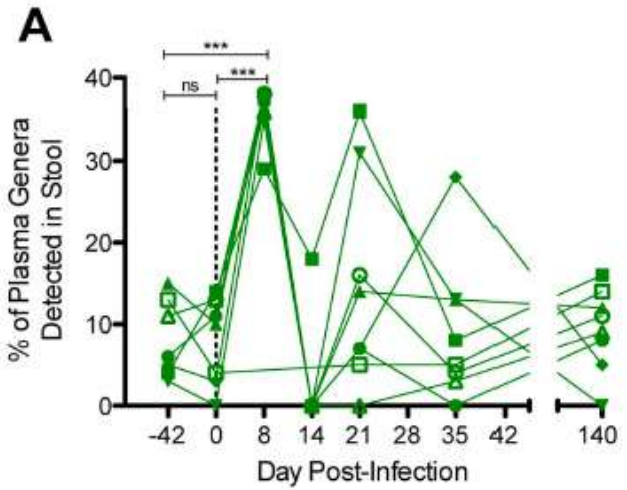
8 macaques infected with SIVmac239





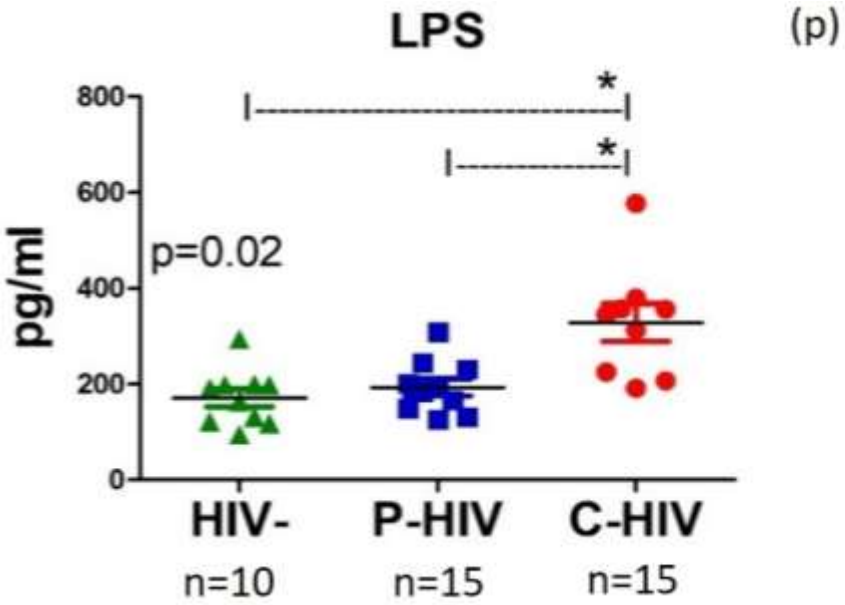






Ericsen et al., Plos Path 2016

Cannizzo et al. CROI 2018



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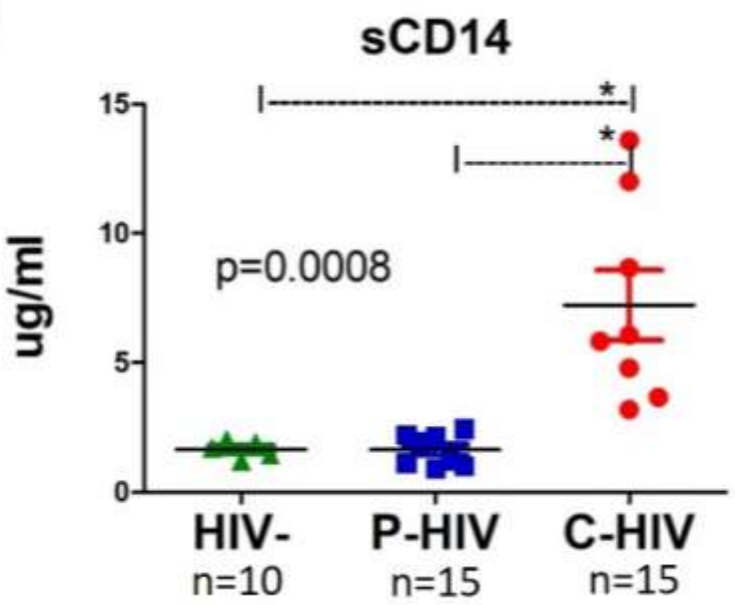

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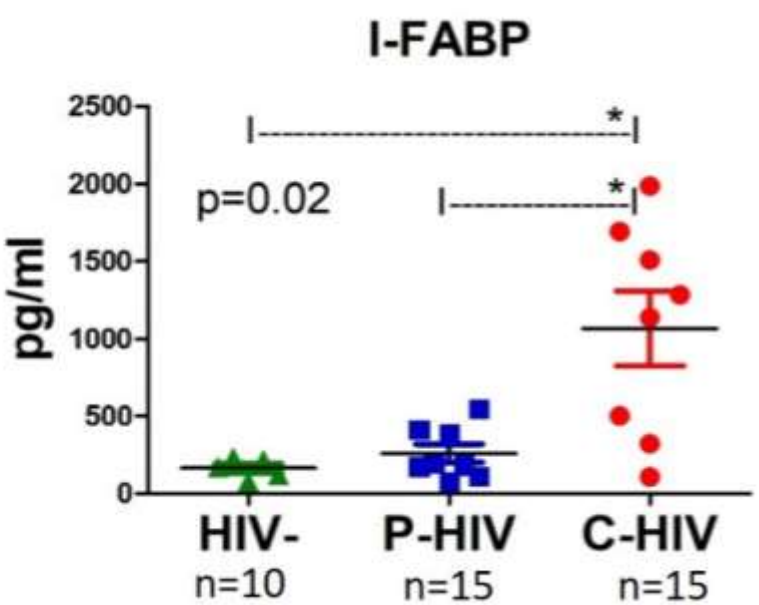
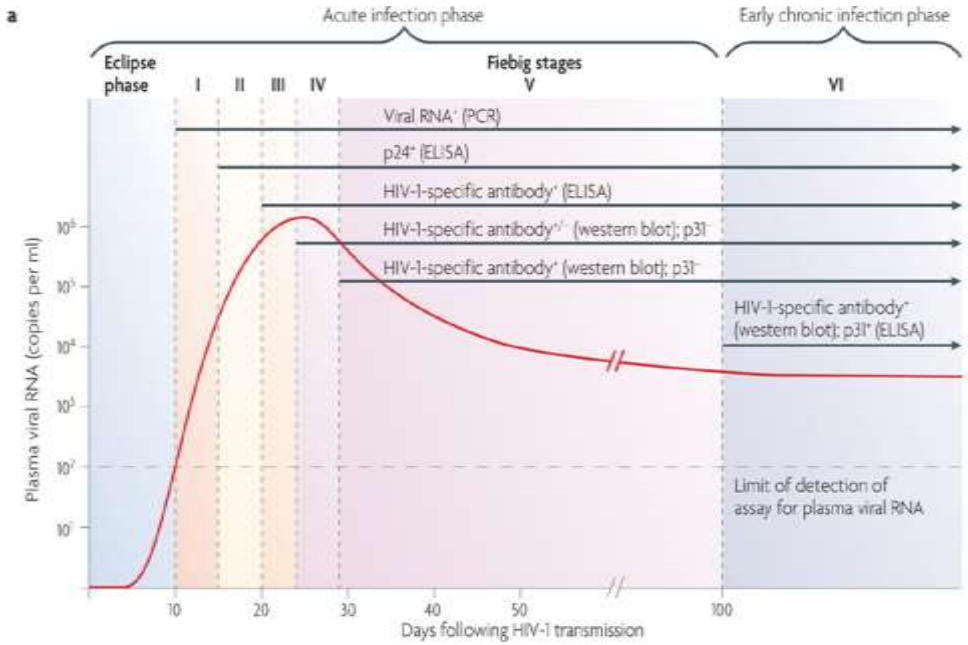
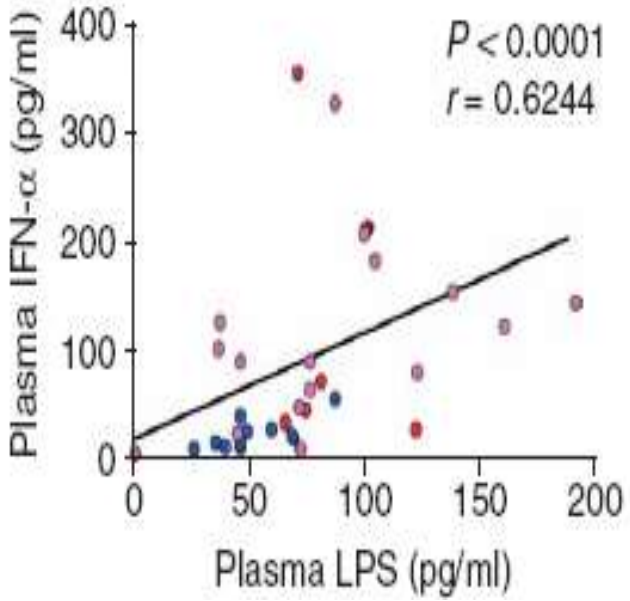
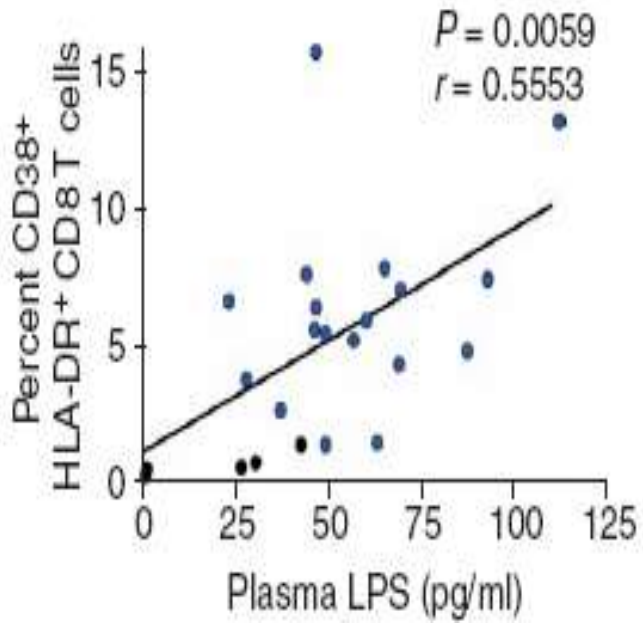


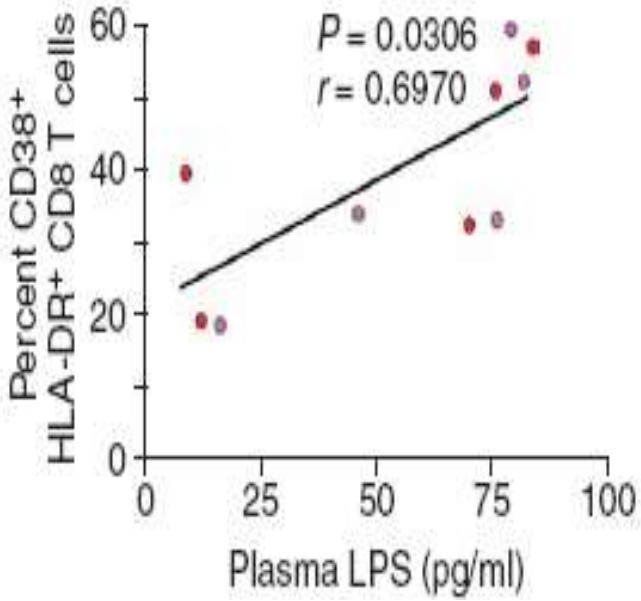
Fiebig Stage, n	
I-II	2
III	5
IV-V	8 (3 IV; 5 V)

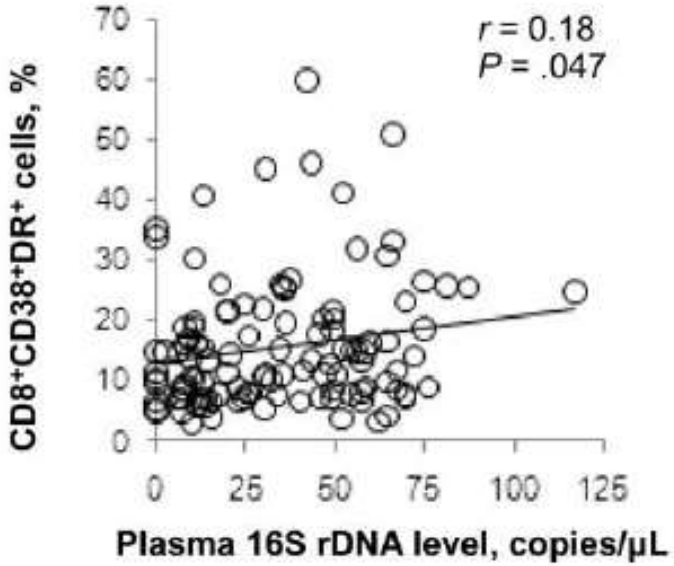


Microbial translocation as continuous challenge to immune activation



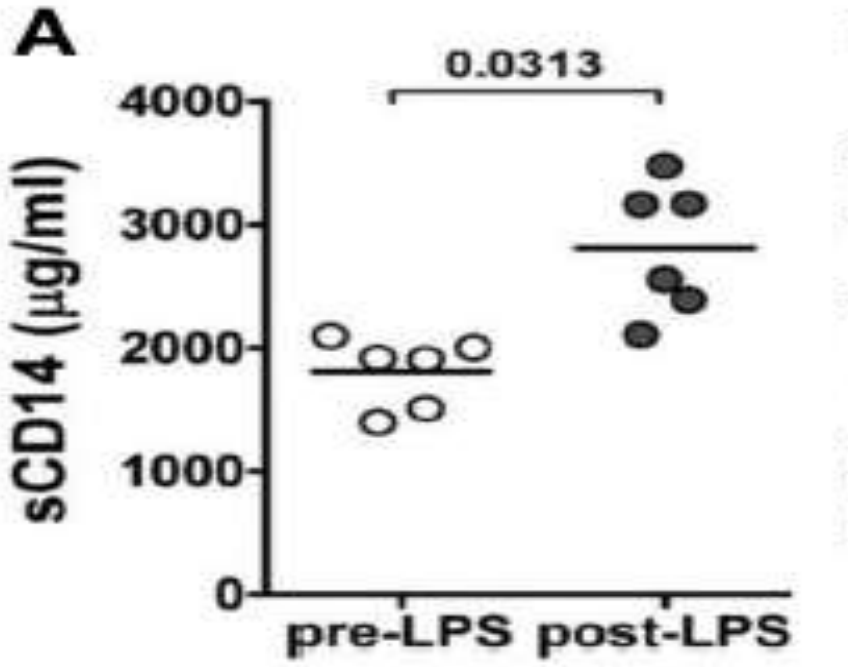






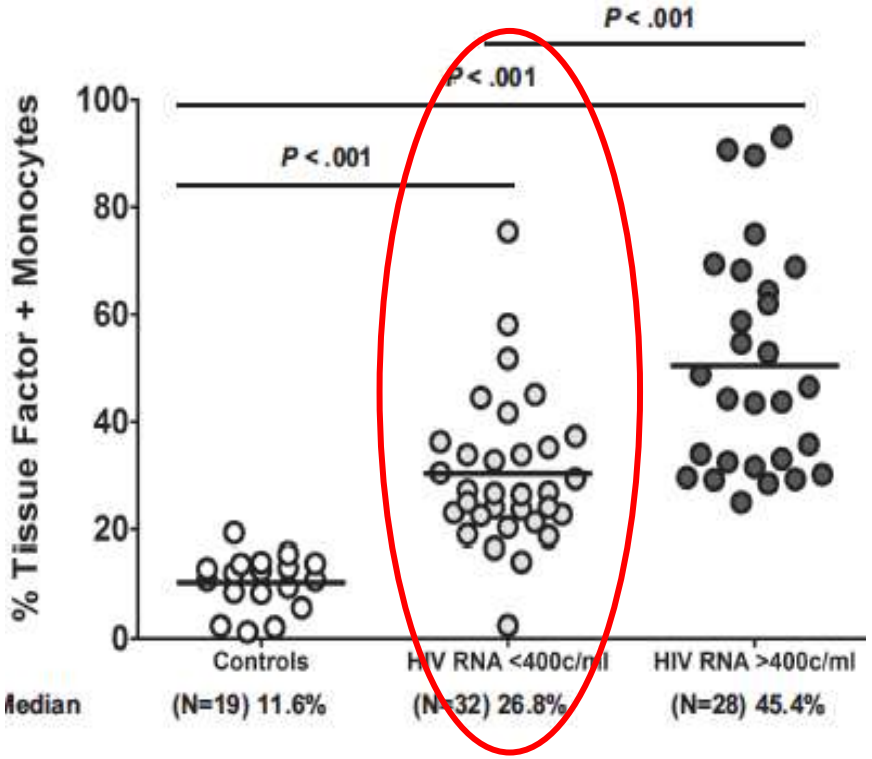
Brenchley Nat Med 2006; Jiang et al. JID 2009

In vivo LPS administration enhances immune activation



Pandrea et al Blood 2012

Ex vivo LPS stimulation enhances monocyte activation

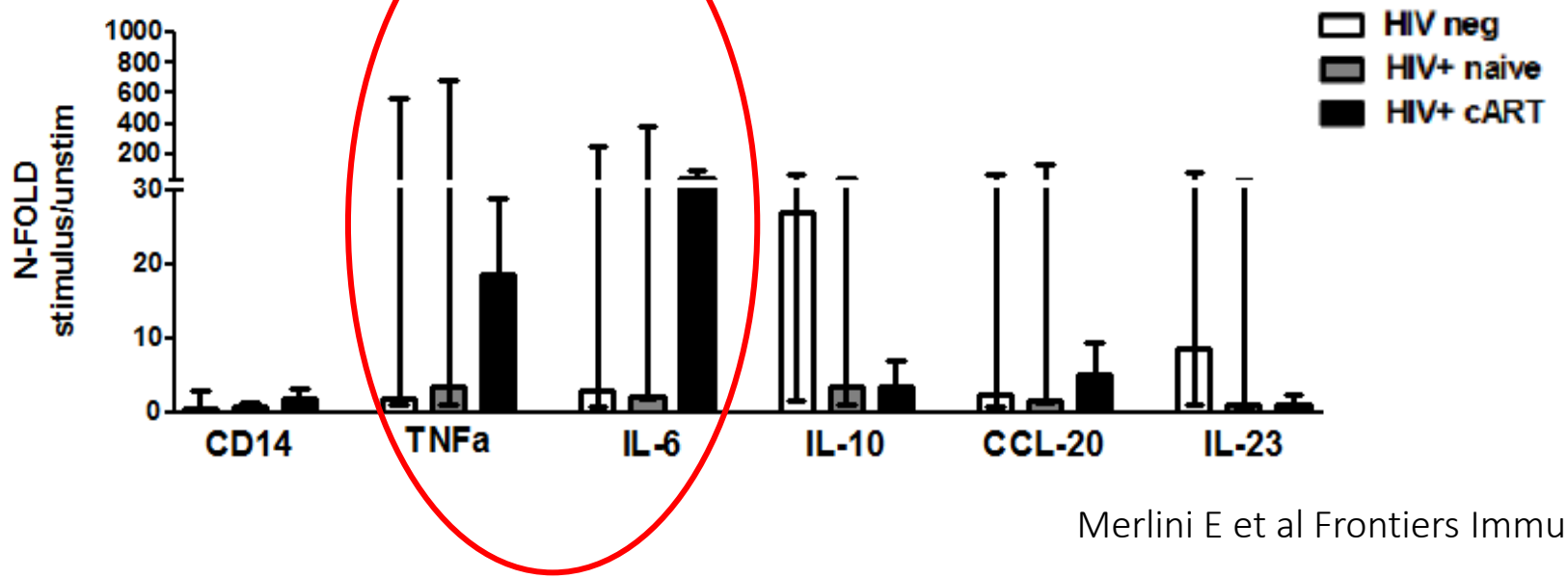


Funderburg N et al Blood 2010

Ex vivo LPS stimulation drives monocyte activation

35 HIV+ cART-treated

	HIV negative (n=16)	HIV+ naive (n=28)	HIV+ cART (n=35)	p
LPS, pg/ml (IQR)	75 (75-81)	187 (97-427)	75 (75-147)	.012
sCD14, ug/ml (IQR)	1.96 (1.39-2.10)	4.56 (2.96-9.67)	4.77 (3-12.14)	.0002



Merlini E et al Frontiers Immunol 2016

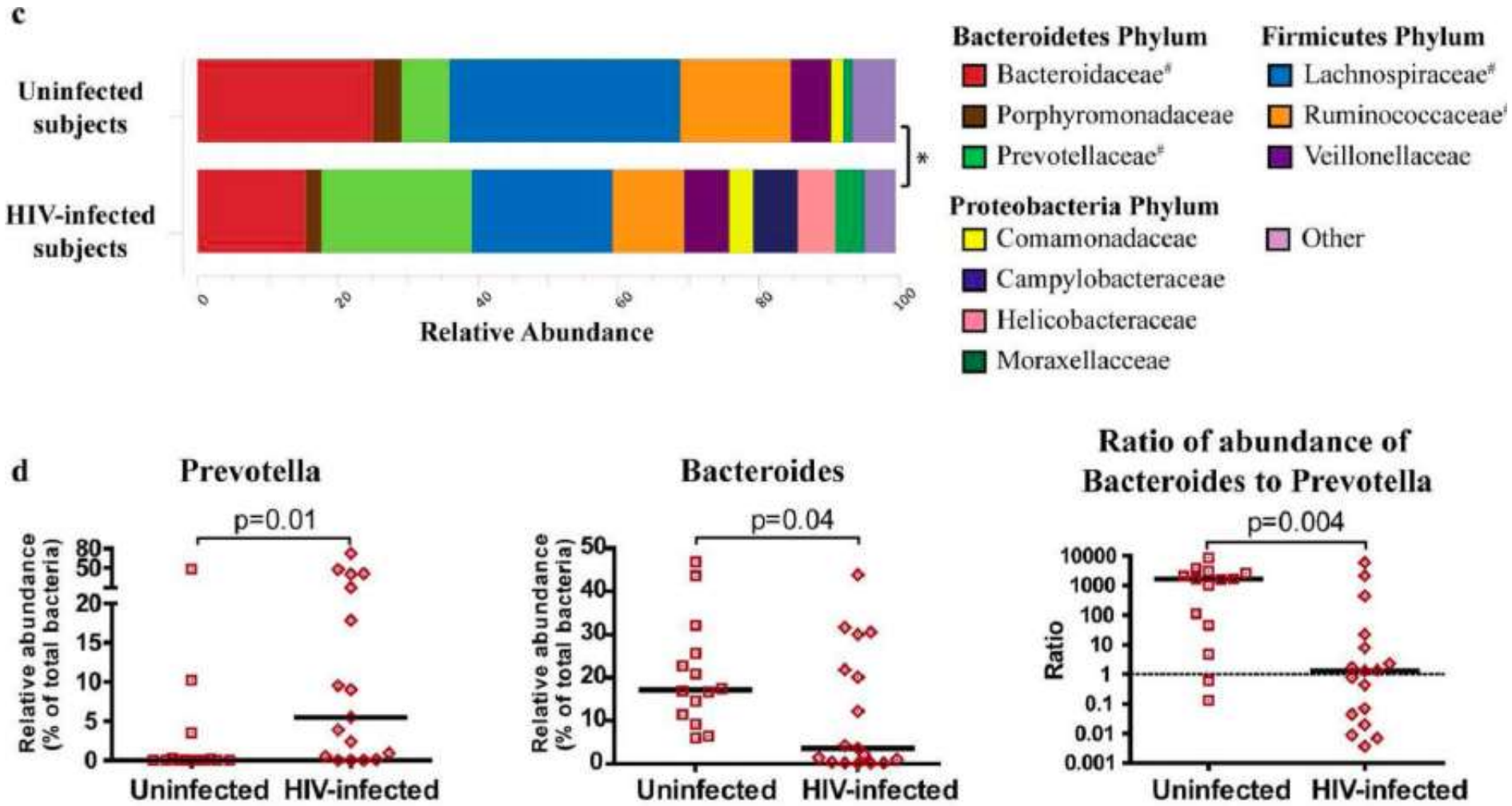
Altered gut microbiota in SIV/HIV

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

Colon biopsies from 17 untreated HIV; 14 uninfected controls

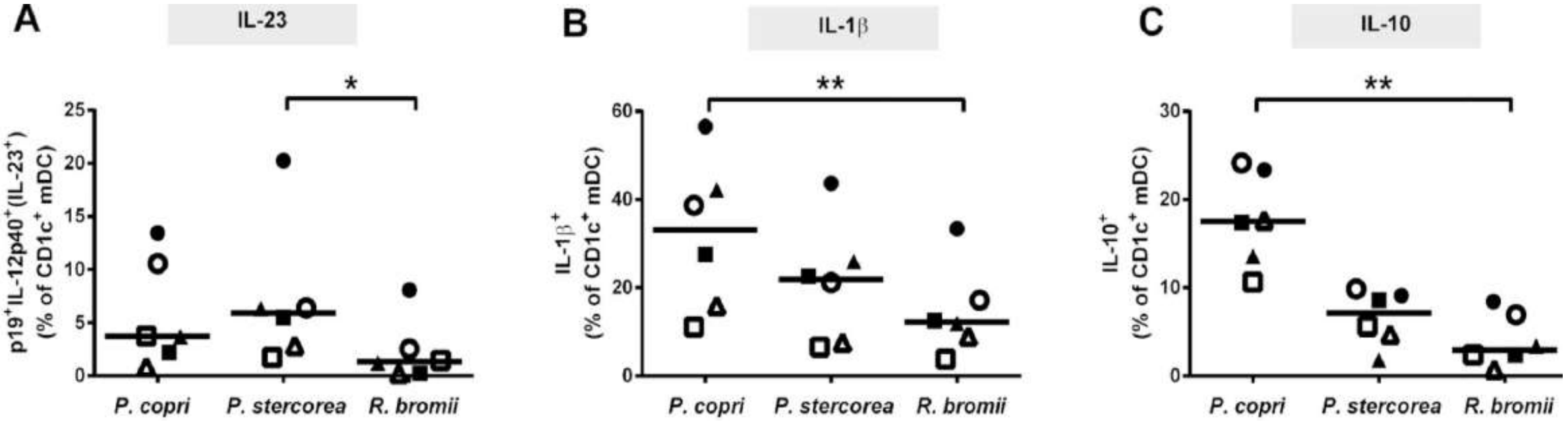


Dillon et al., Mucosal Immunol, 2014

Prevotella-rich, Bacteroides-poor gut microbioma

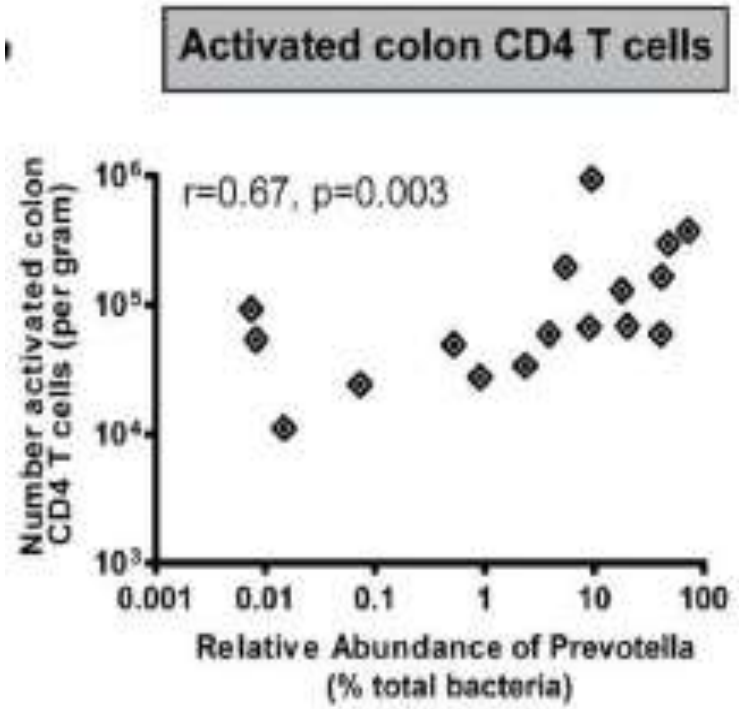
Gut dysbiosis as driver of immune activation

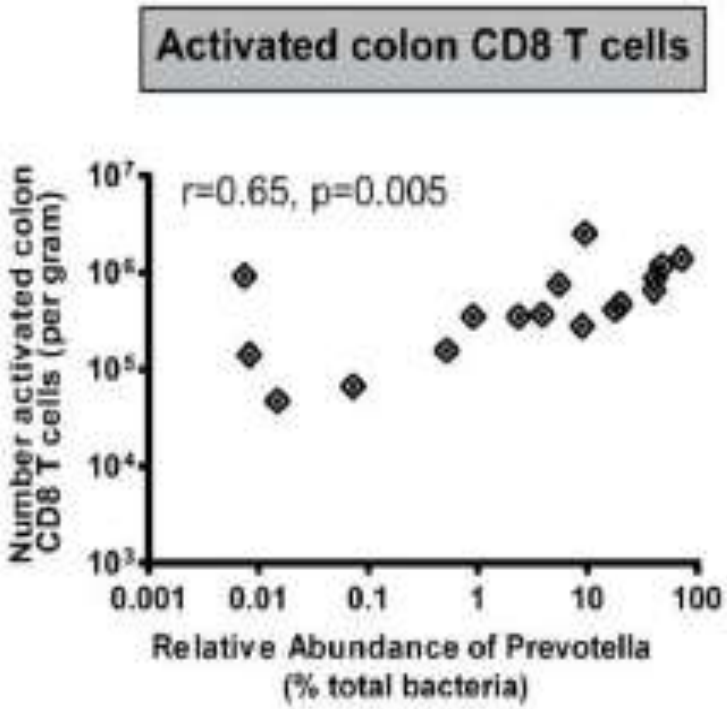
Colonic LPMC exposed to several HAMB (*HIV Altered Mucosal Barrier*)

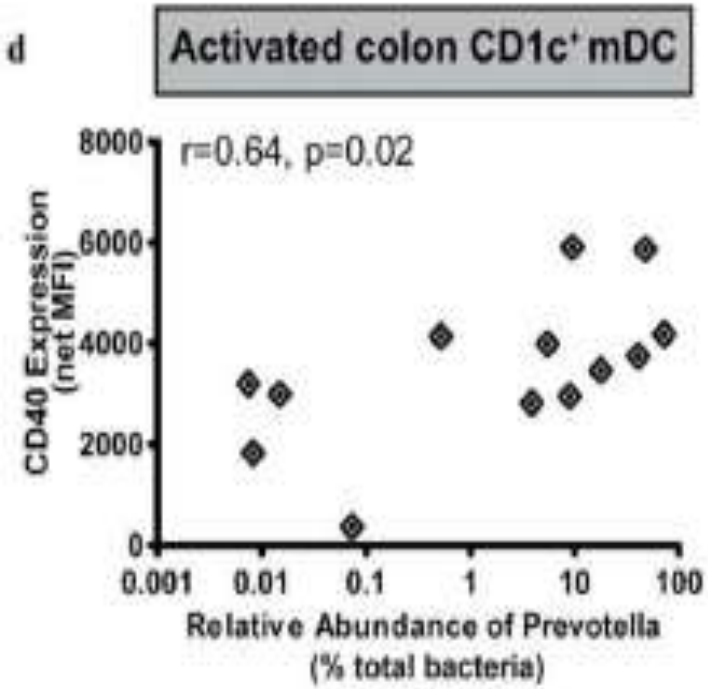


Prevotella spp. drive ex vivo increased production of pro-inflammatory cytokines by LP mDCs

Dillon et al., Mucosal Immunol, 2016





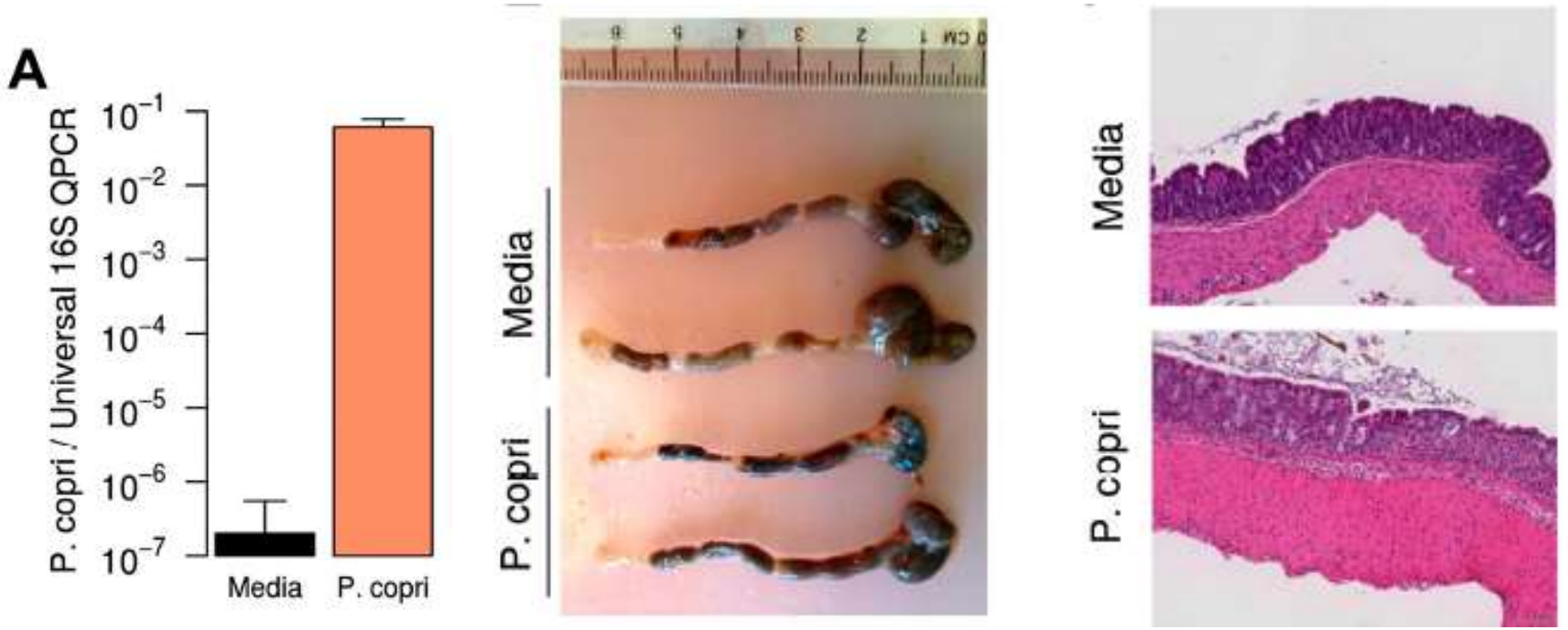


Dillon et al., Mucosal Immunol, 2014; also Vasquez-Castellanos Mucosal Immunol 2015

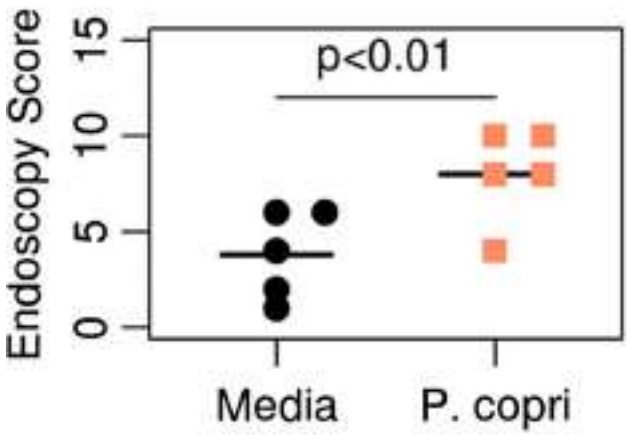
Taxon	P77 inflammation		P178 inflammation	
	Yes	No	Yes	No
Bacteroidetes	74	42	54	61
'*Clostridia*'	10	53	40	33
Enterobacteriaceae	16	5	0	0
Other bacteria	0	0	6	6

Patients with ulcerative colitis

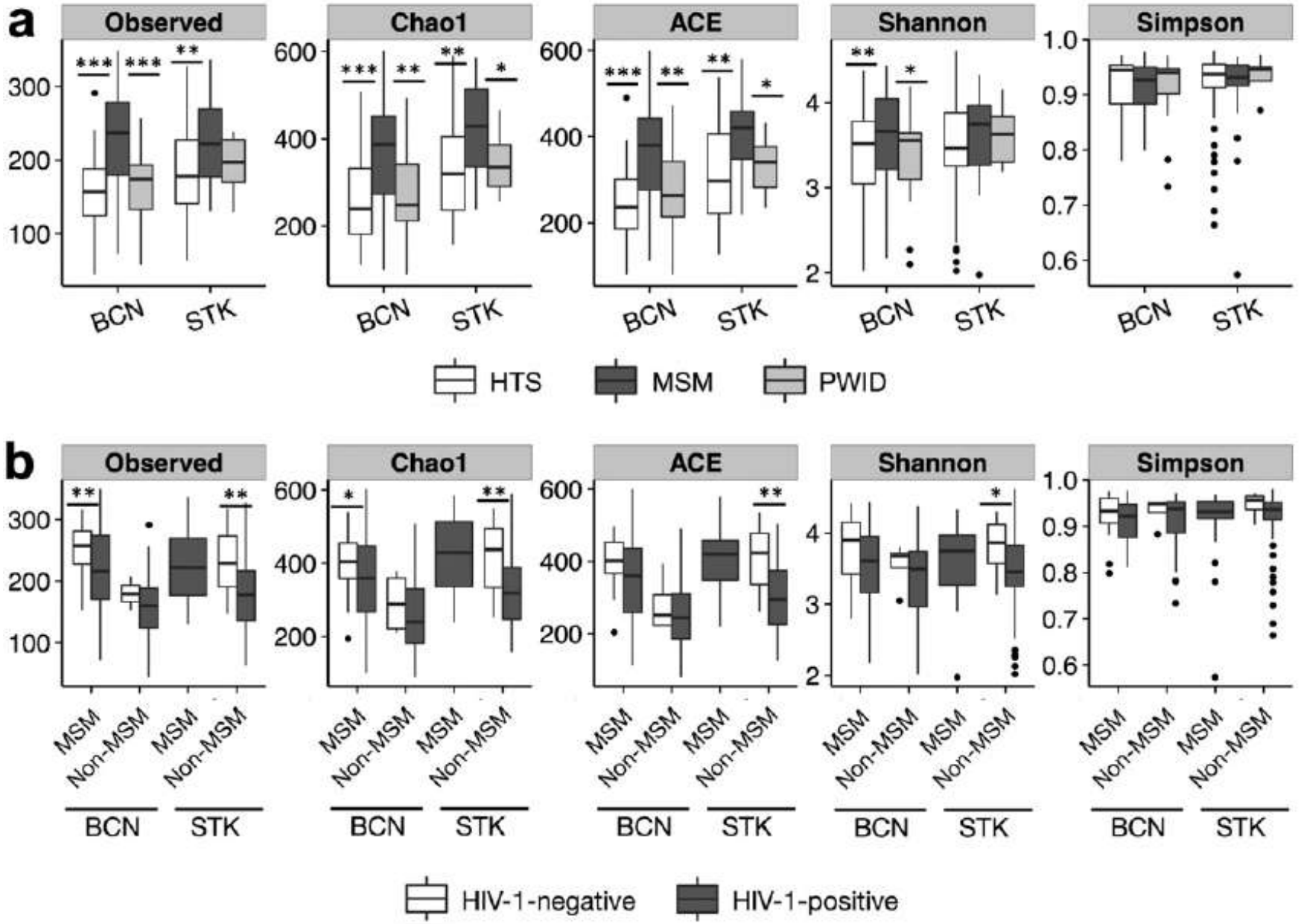
Lucke et al. J Med Microbiol 2006



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



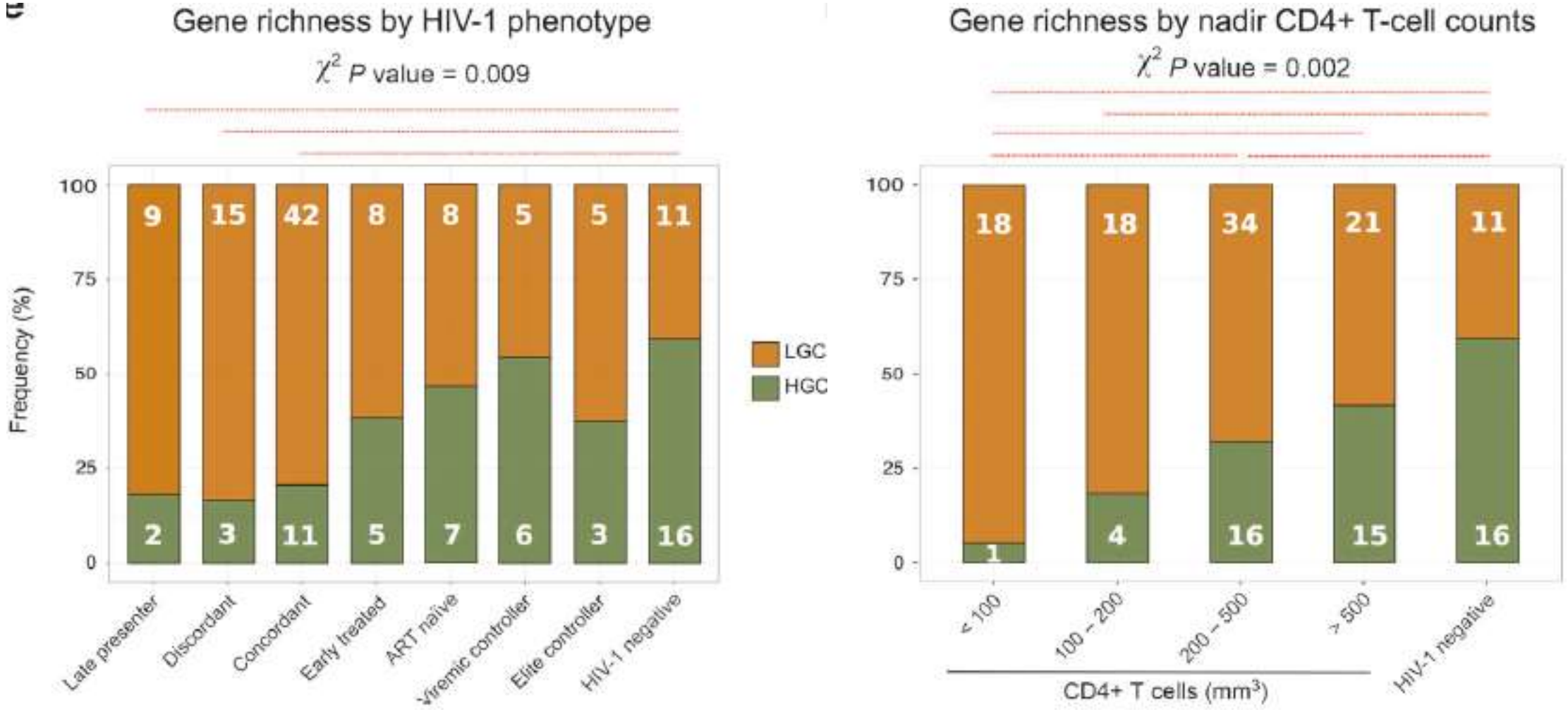
Scher et al. Elife 2013



Changes in microbiota associated to sexual preference (and HIV)

Noguera Julian Ebiomedicine 2016

Fecal whole metagenome shotgun sequencing



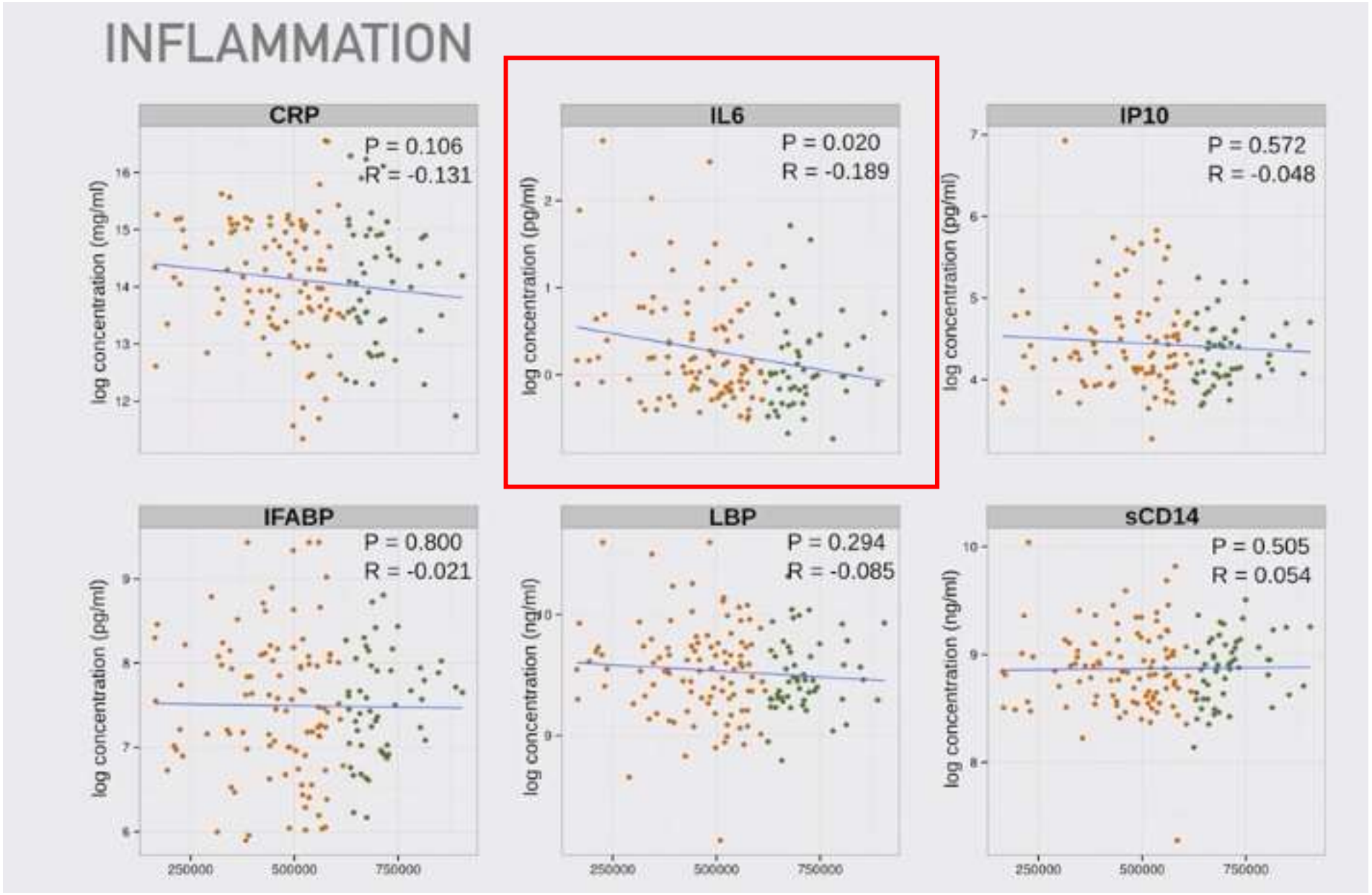
Guillen et al. Mucosal Immunol 2019

Table 2. Factors associated with low-microbial gene counts[a]

		Univariate			Multivariate		
		OR	95% CI	*p* Value	OR	95% CI	*p* Value
Age	Per each additional year	1.03	[1–1.07]	0.074	–	–	–
Gender	Female	1					
	Male	0.32	[0.10–0.82]	0.028	–	–	–
	Transgender woman	–	–	–			
Ethnic Group	Caucasian	1			1		
	Hispanic-Latin	0.27	[0.11–0.64]	0.003	0.26	[0.10–0.67]	0.006
	Asiatic and others[b]	–	–	–	–	–	–
HIV-1 risk group	Non-MSM	1			1		
	MSM	0.17	[0.06–0.39]	<0.001	0.20	[0.07–0.51]	0.002
HIV-1 status	Negative	1					
	Positive	3.68	[1.57–8.89]	0.003	–	–	–
Nadir CD4+ T-cell count, cells/mm^3	HIV-1 negative	1			1		
	>500	2.04	[0.75–5.74]	0.169	2.13	[0.73–6.45]	0.173
	200–500	3.09	[1.19–8.37]	0.006	2.92	[1.03–8.62]	0.047
	100–200	6.55	[1.86–27.71]	0.023	5.55	[1.40–26.15]	0.020
	<100	26.18	[4.40–506.6]	0.003	14.00	[2.02–288.71]	0.023

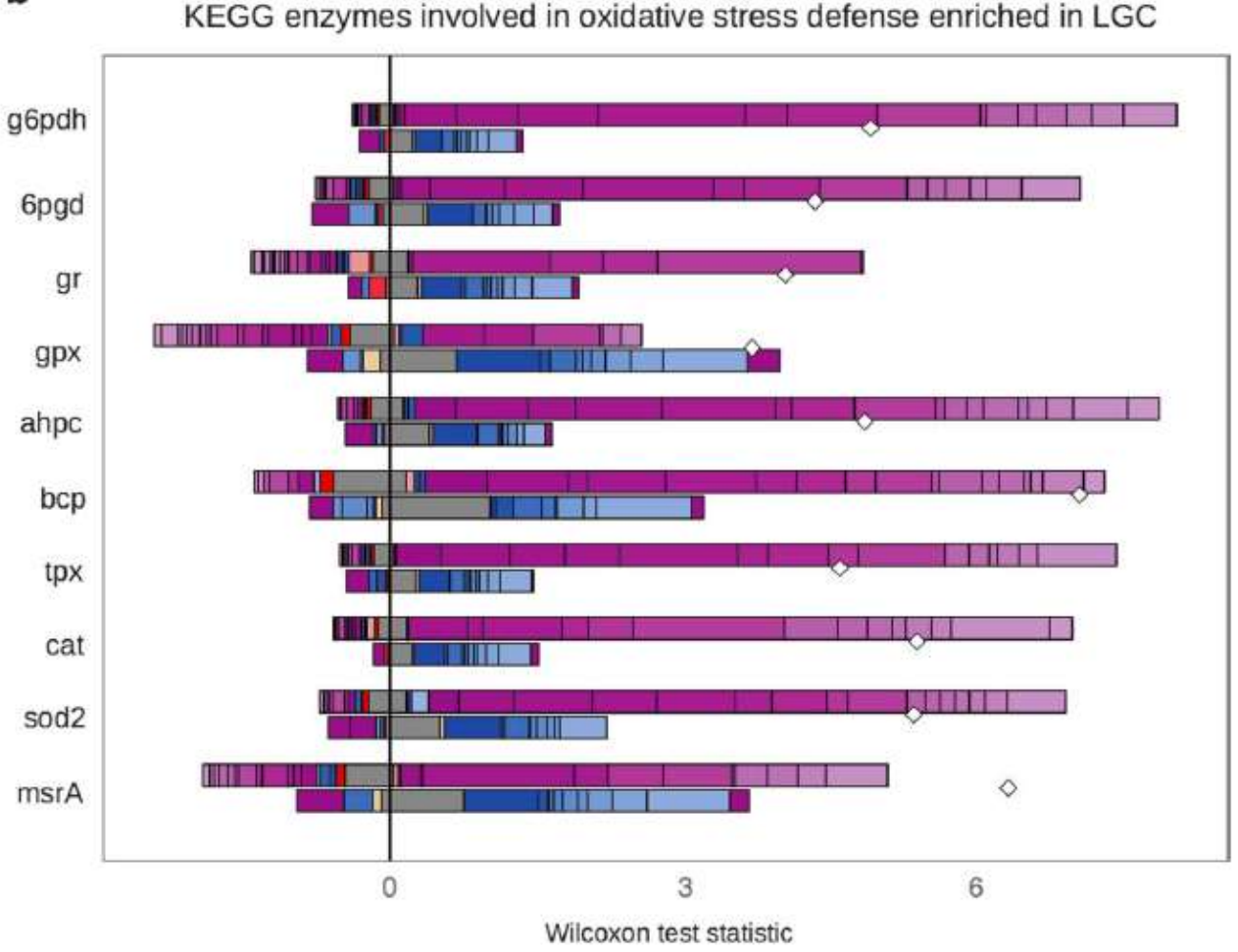
[a]Full dataset analysis, $n = 156$ subjects
[b]Analysis does not apply because all subjects are included in the same response group
MSM men-who-have-sex-with-men

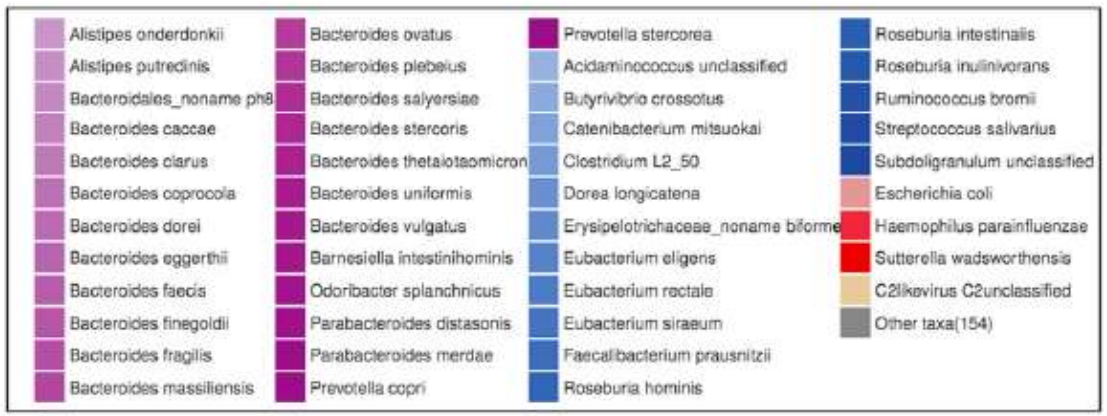
Guillen et al. Mucosal Immunol 2019



Lower genetic richness is associated with inflammation

Guillen et al. Mucosal Immunol 2019



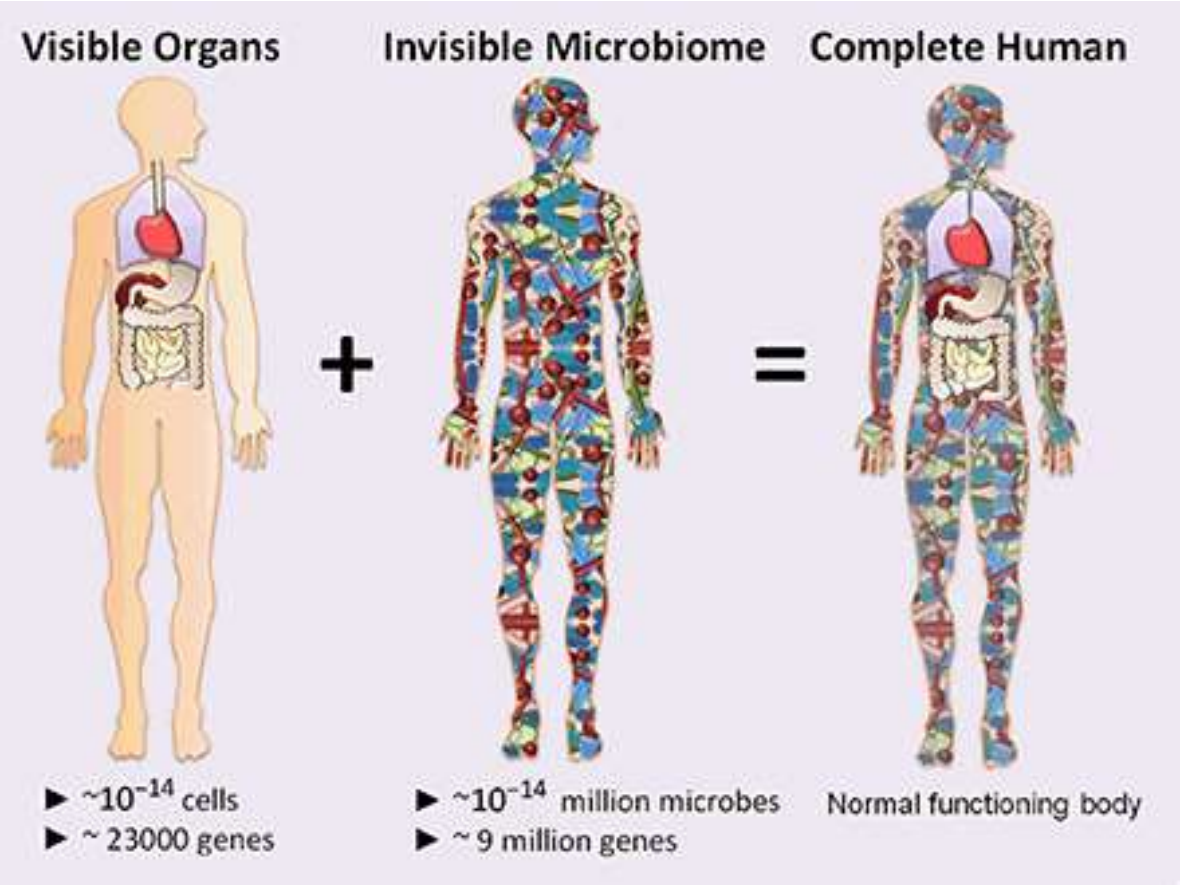


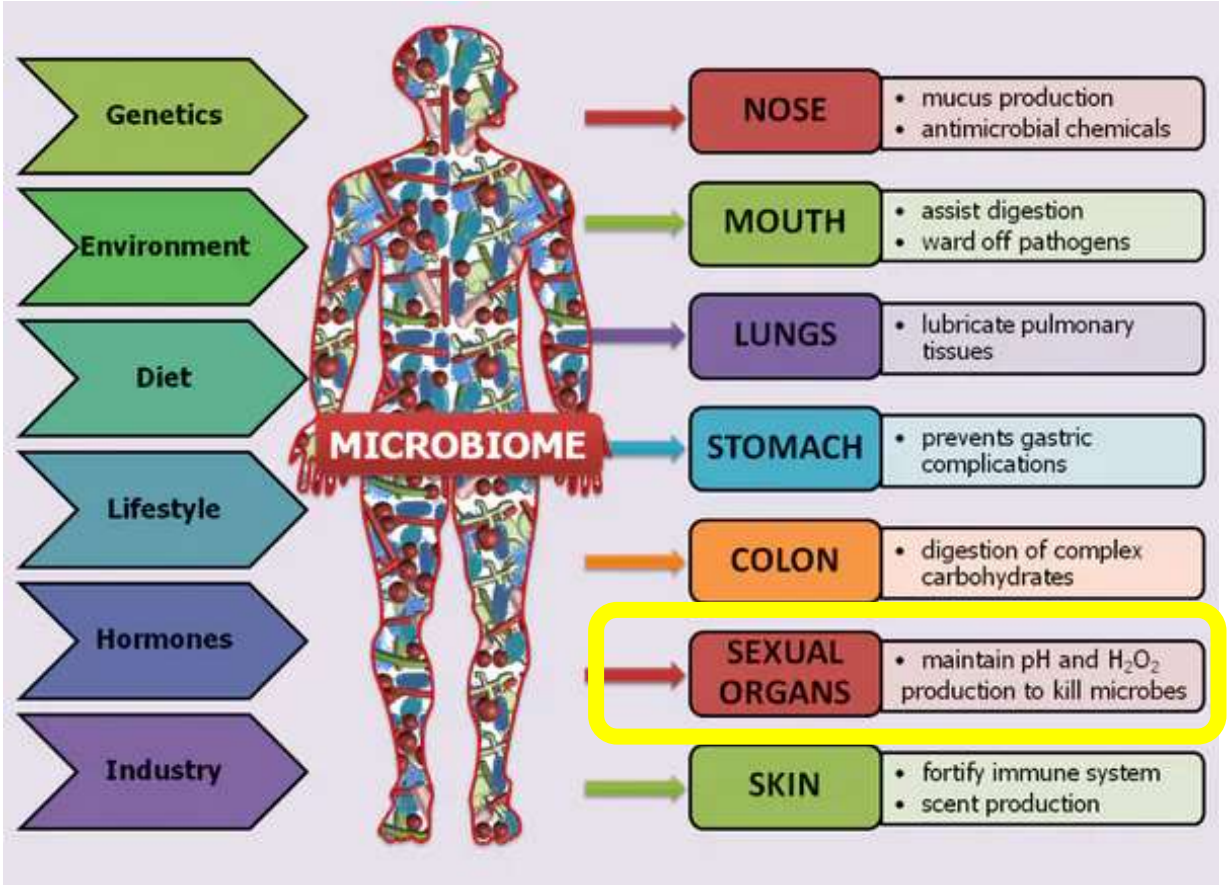
Low gene content were enriched in genes involved in reactive oxygen and nitrogen (ROS/RNS) metabolism encoded by Bacteroides and Proteobacteria

Adaptation of the gut microbic ecosystem to continuous (HIV-driven) oxidative stress

Guillen et al. Mucosal Immunol 2019

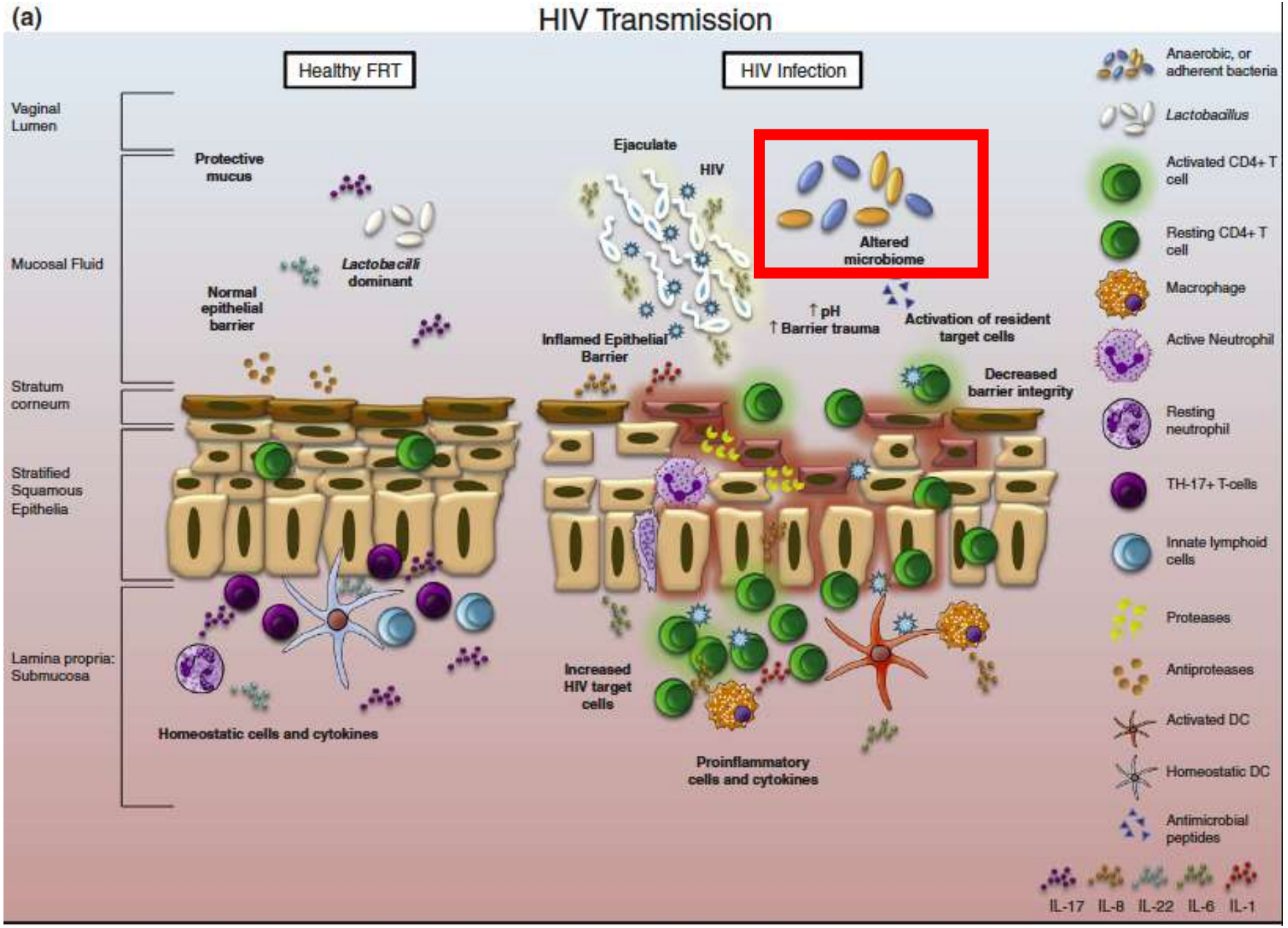
Dysbiosis and HIV aquisition?





The human microbiome – Human Microbes 2018

Clinical question: does microbiome affect HIV acquisition? – issue for PreP

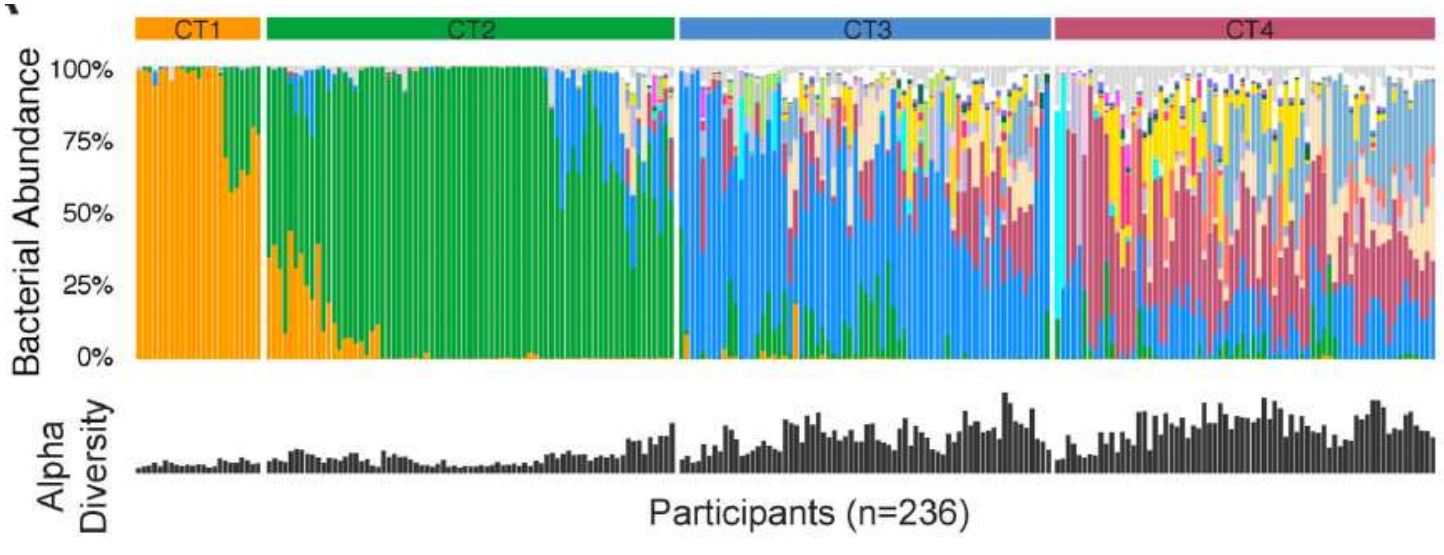


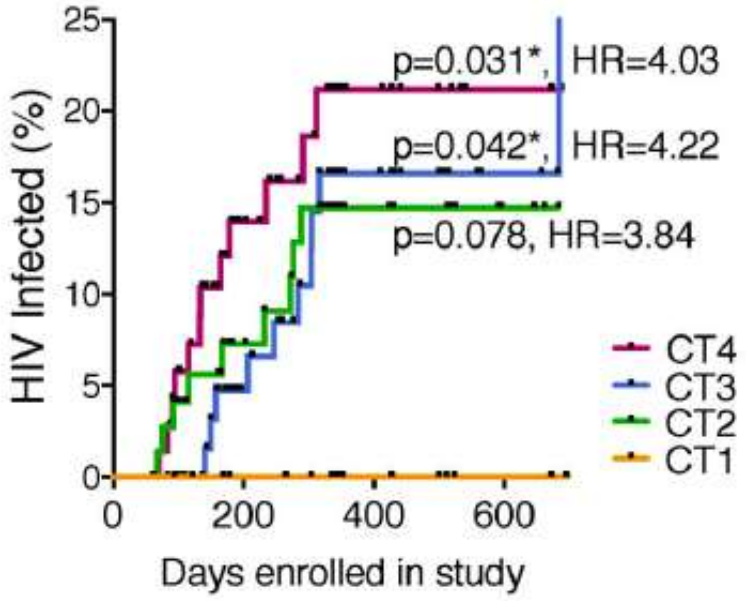
Putative mechanisms behind HIV transmission in the female reproductive tract (FRT)

Burgener A et al. Curr Opin Immunol 2015

Vaginal microbiome: possible cervicotypes (CT) influence HIV acquisition

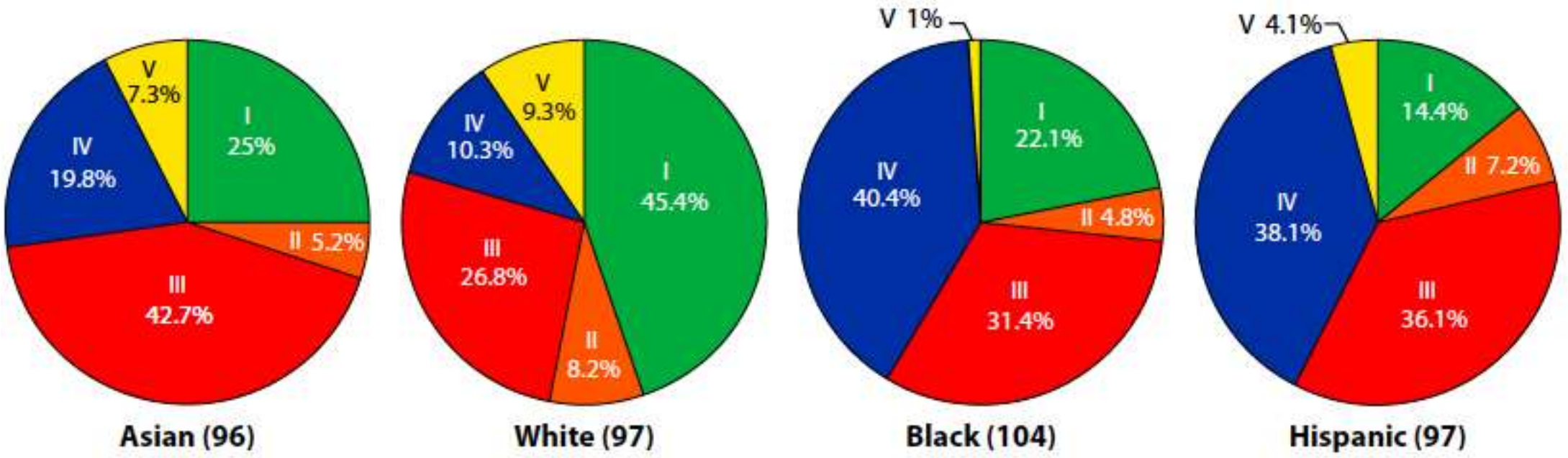
FRESH cohort (South Africa), 236 HIV uninfected women (31 became infected)





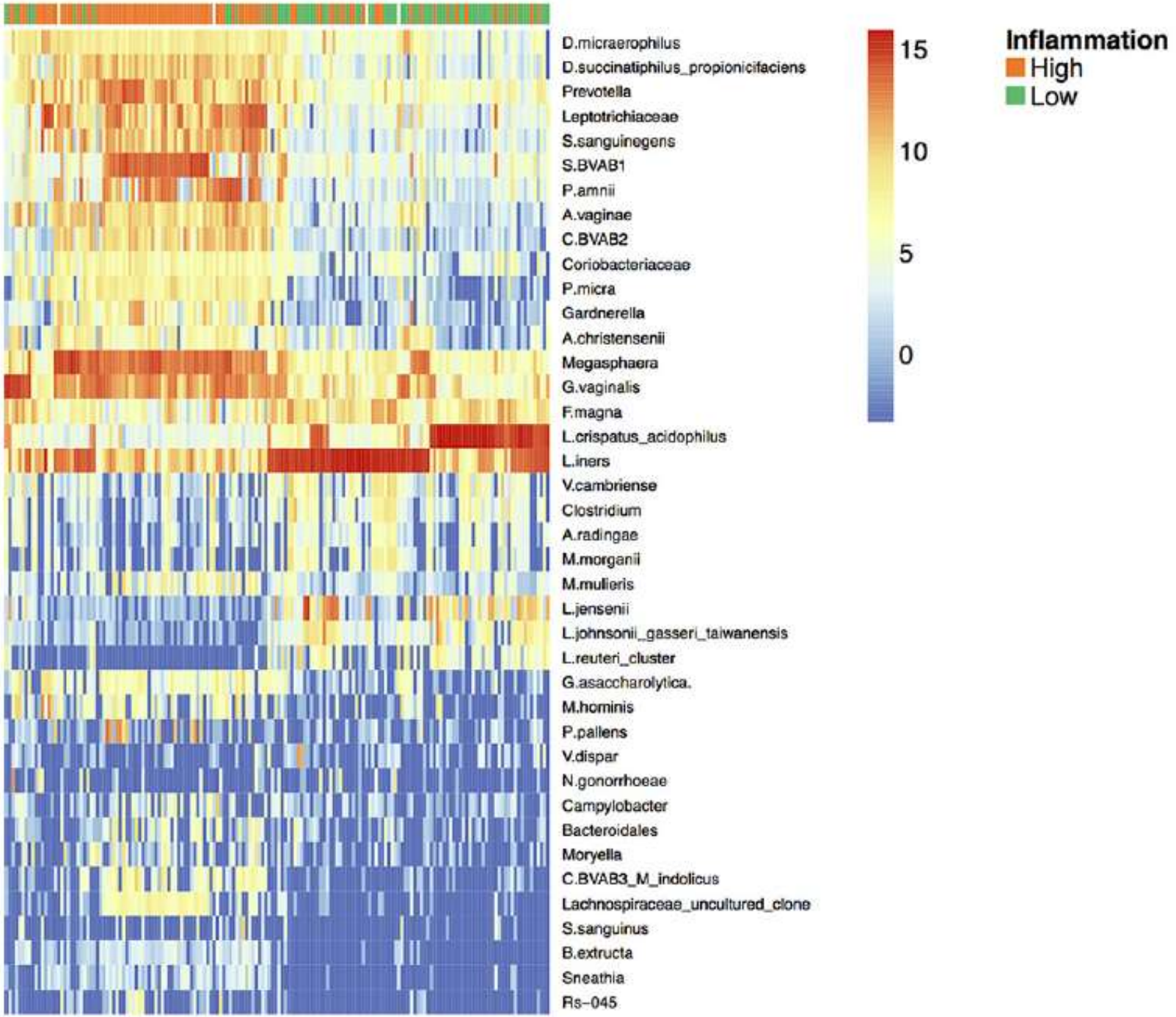
Gosman et al. Immunity 2017

Diverse vaginal microbiome across ethnicities



Ravel et al. PNAS 2011

Which mechanism(s) by which vaginal bacterial dysbiosis increases HIV transmission?



WISH cohort: 168 HIV-uninfected women in South Africa

Vaginal dysbiosis is associated to genital inflammation

Lennard et al. Infec and Immunity 2018

Vaginal dysbiosis is a cause of genital inflammation

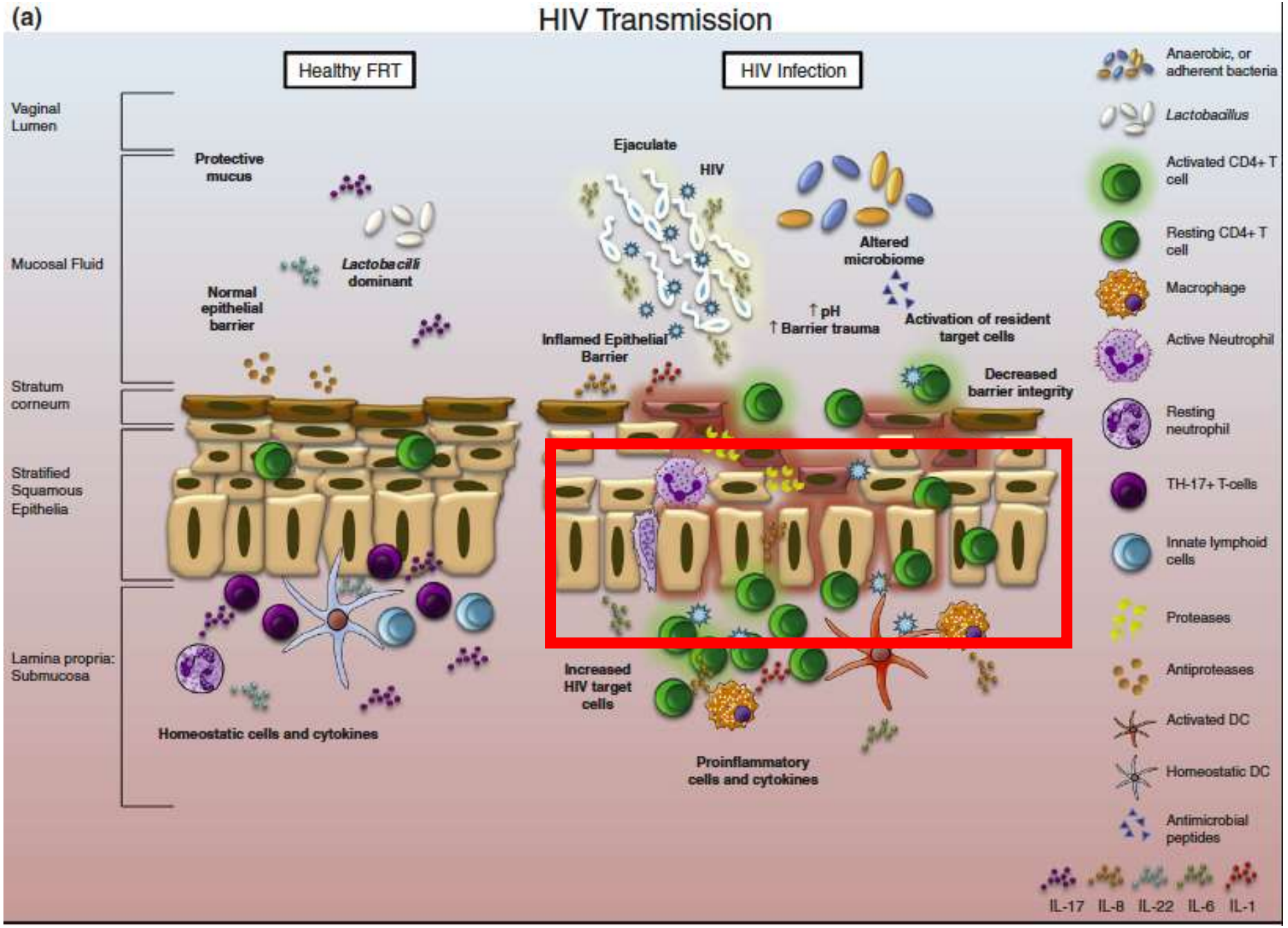
TABLE 2 Multivariate logistic regression analysis of predictors of genital inflammation

Predictor[b]	Coefficient	*P* value	Adjusted odds ratio[a]	95% CI
Location (JHB)	0.7	0.4		
Hormonal contraception (yes)	2.6	9.00e−04	14	3–72
STI (any)	0.4	0.4		
Microbiota subtype (C1 vs C2/C3)	3.1	3.00e−07	23	8–84
Ethnicity (Xhosa vs other)	0.3	0.7		
BMI	−0.02	0.7		

[a]Adjusted for all other variables in the table.
[b]STI, sexually transmitted infection; JHB, Johannesburg.

C2/C3= *Lactobacillus*-rich cervicotypes

Lennard et al. Infec and Immunity 2018



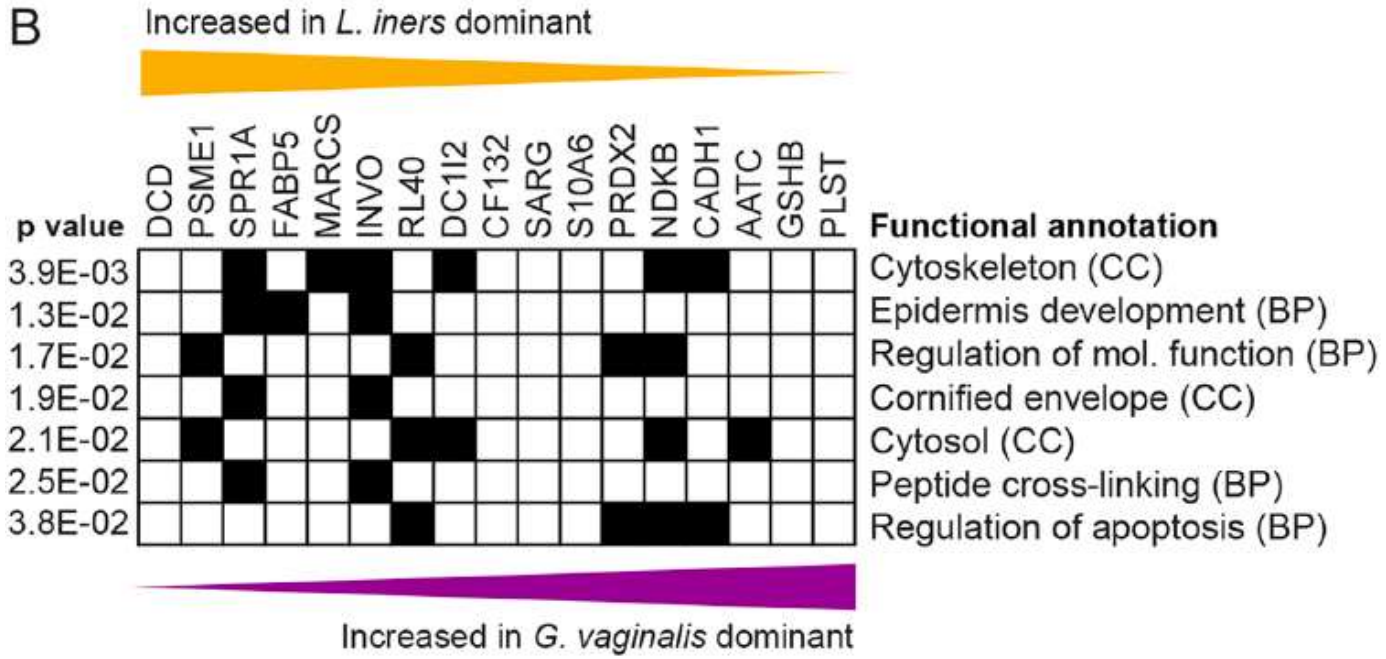
Vaginal dysbiosis and epithelial barrier dirsuption?

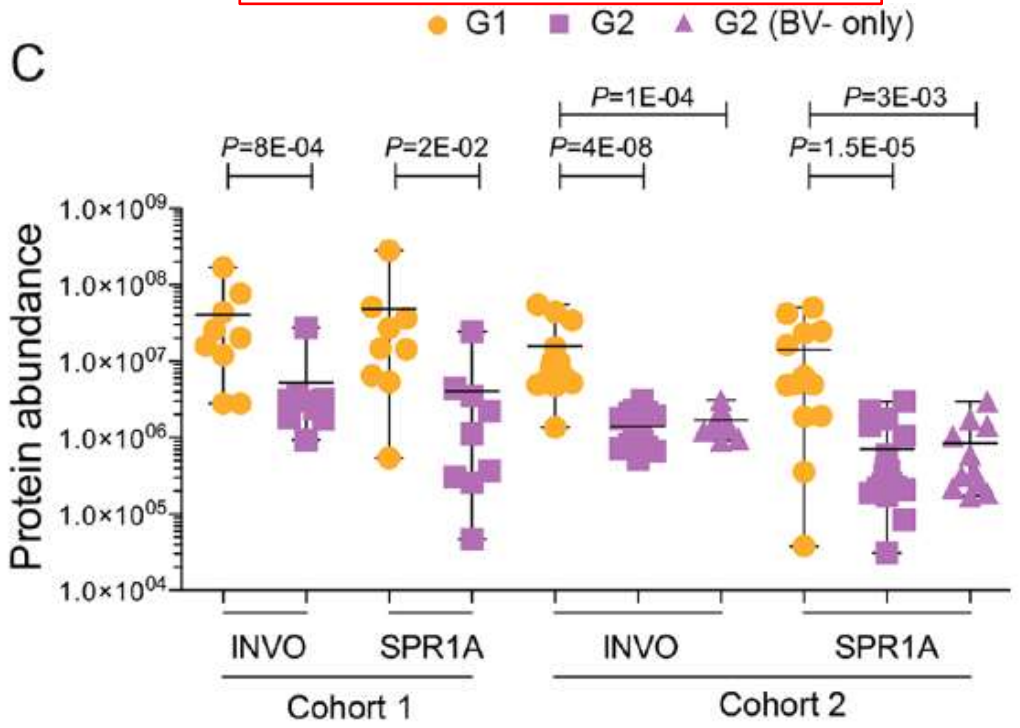
Burgener A et al. Curr Opin Immunol 2015

Vaginal epithelial barrier proteins are lower in *Gardnerella*- vs *Lactobacillus*-enriched cervicotypes

Cohort1: female partners in Partner PreP (Kenya); Cohort 2: uninfected women (US)

G1: *Lactobacillus*-enriched;
G2: *Gardnerella*-enriched

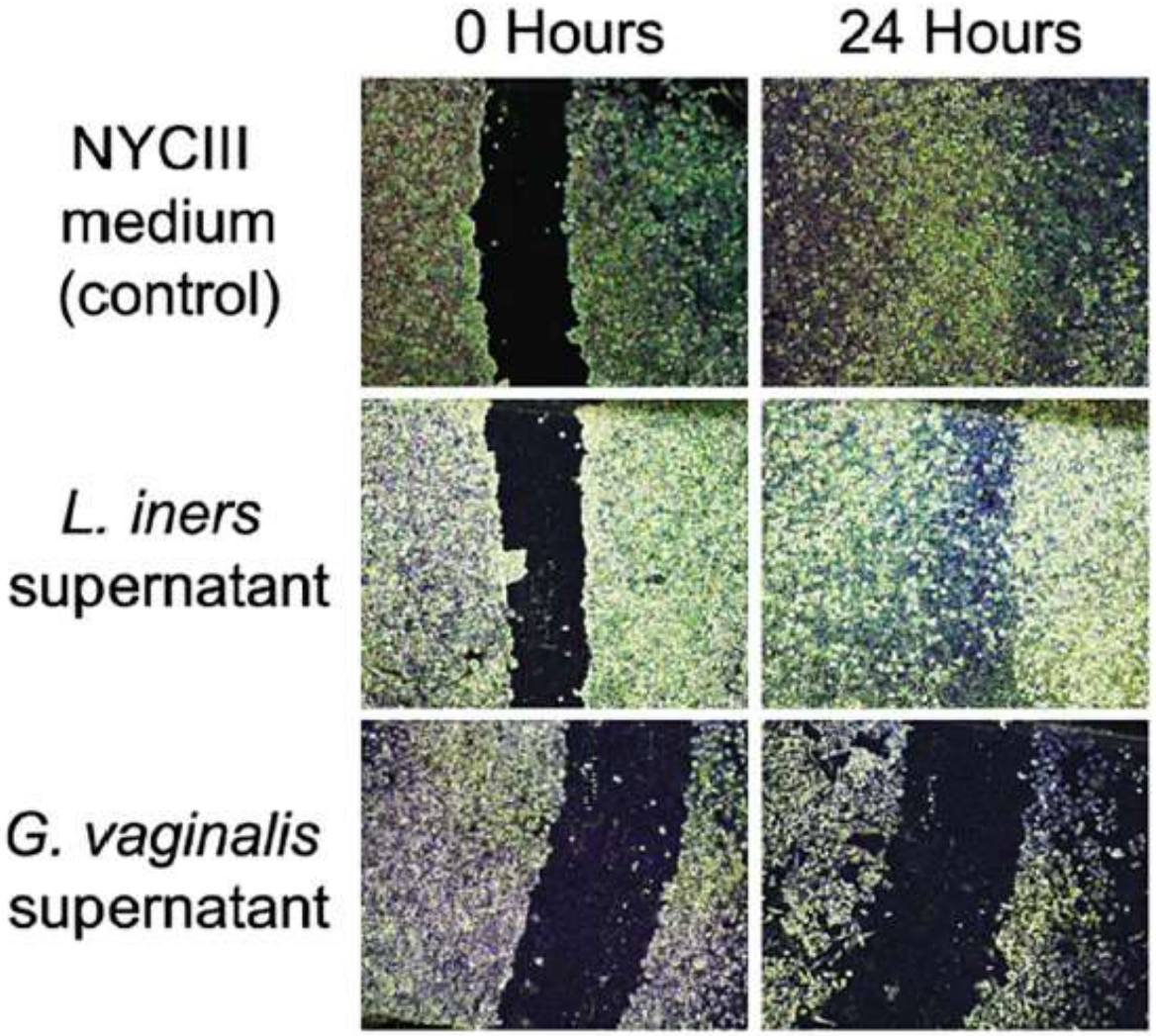


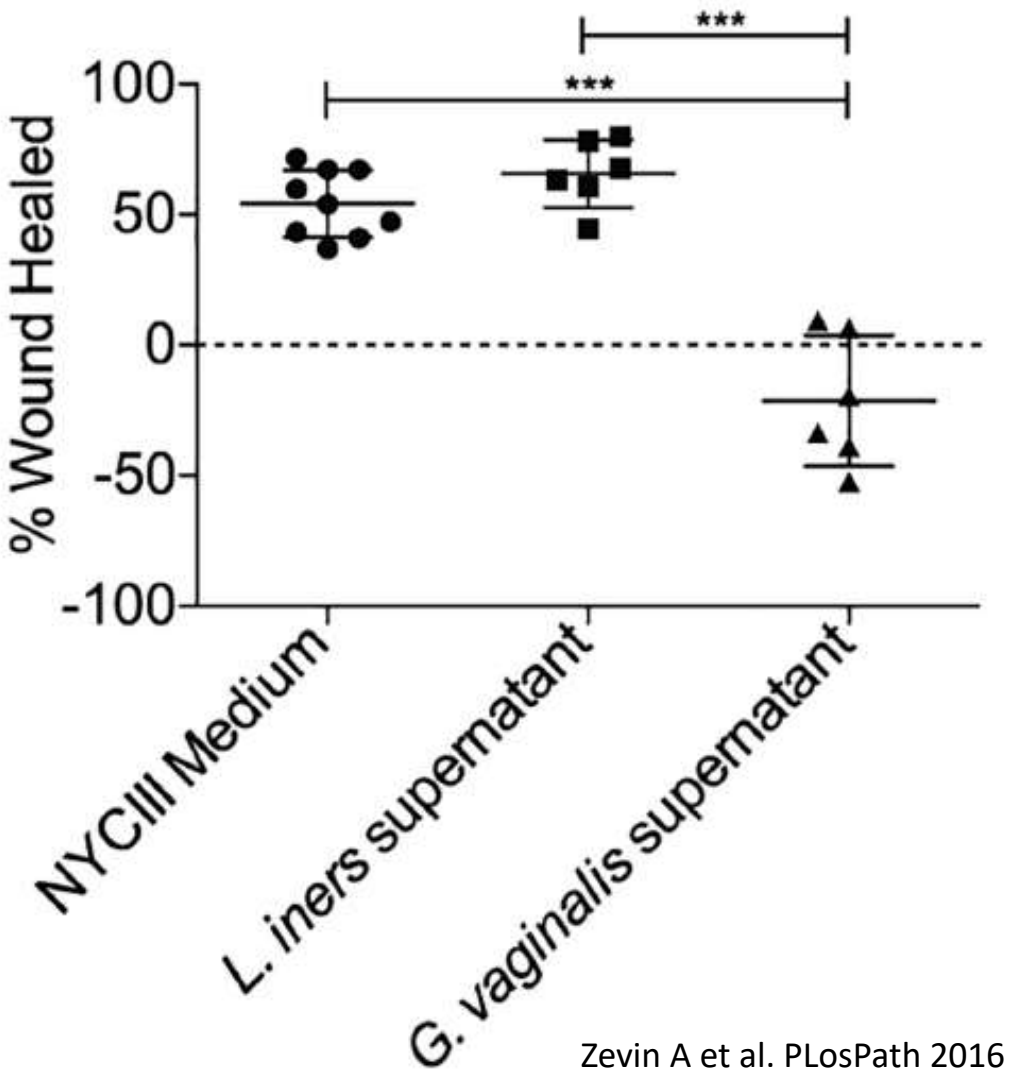


Cornified envelope proteins: involucrin (INVO); Small Prolin-Rich Protein 1A (SPR1A)

Zevin A et al. PLosPath 2016

Bacterial species drive FRT epithelial barrier repair





Zevin A et al. PLosPath 2016

Vaginal dysbiosis reduces mucosal epithelial integrity

HIV-driven dysbiosis:

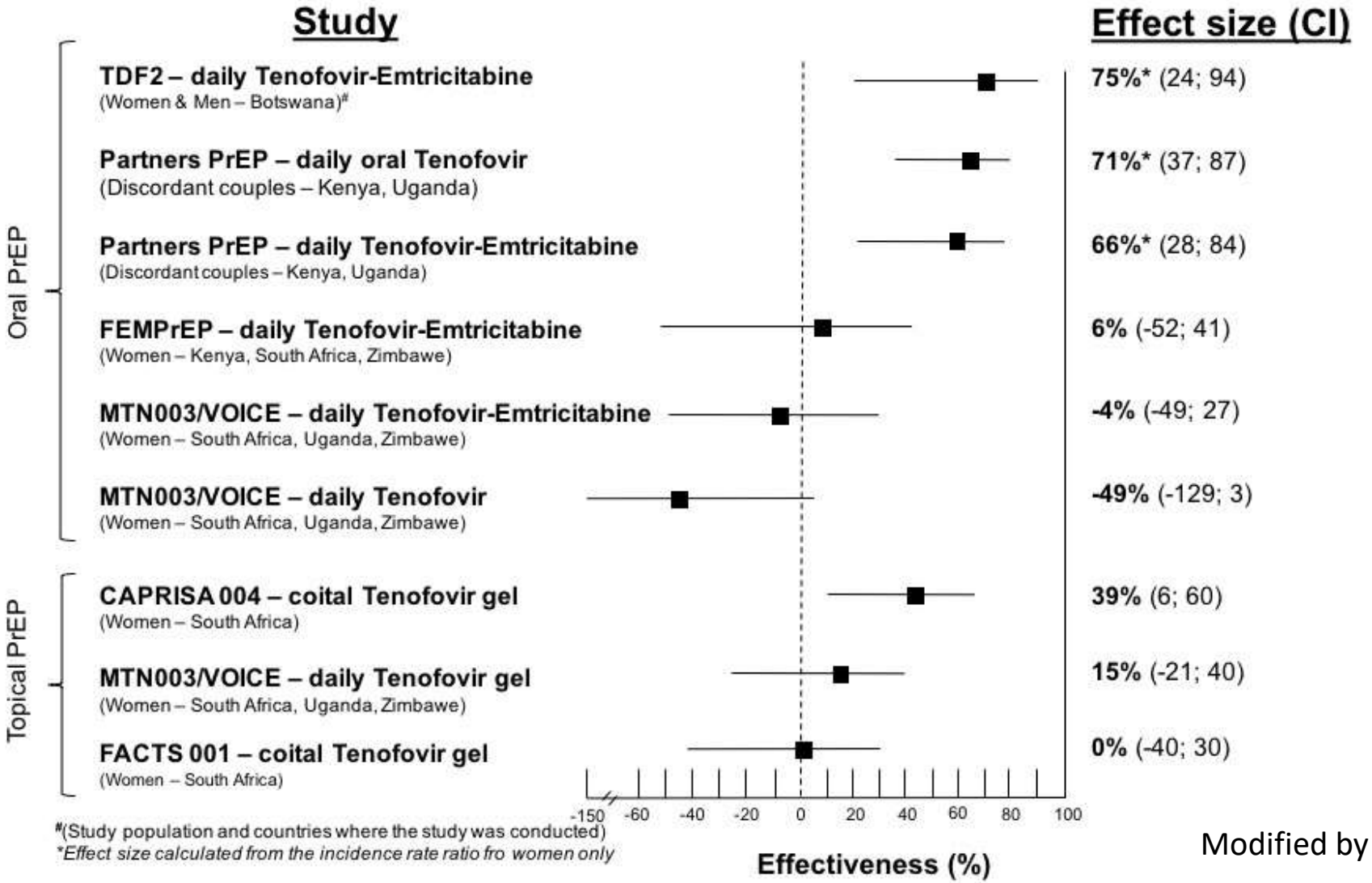
induces mucosal inflammation

reduces mucosal epithelial integrity

Facilitates HIV mucosal infection/transmission

The clinical question: does dysbiosis affect PrEP efficay?

Lower PrEP effectiveness in women versus men



Modified by Klatt CROI 2018

Lower PrEP effectiveness in women versus men

Adherence?

Mucosal drug penetrance/pharmacokynetics? [Seifert et al. AIDS Hum Retrov 2016]

Other biological mechanism(s)? Vaginal microbioma? Vaginal inflammation?

The Caprisa-004 Tenofovir gel PreP trial

Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women

Quarraisha Abdool Karim,[1,2*†] Salim S. Abdool Karim,[1,2,3*] Janet A. Frohlich,[1] Anneke C. Grobler,[1] Cheryl Baxter,[1] Leila E. Mansoor,[1] Ayesha B. M. Kharsany,[1] Sengeziwe Sibeko,[1] Koleka P. Mlisana,[1] Zaheen Omar,[1] Tanuja N. Gengiah,[1] Silvia Maarschalk,[1] Natasha Arulappan,[1] Mukelisiwe Mlotshwa,[1] Lynn Morris,[4] Douglas Taylor,[5] on behalf of the CAPRISA 004 Trial Group‡

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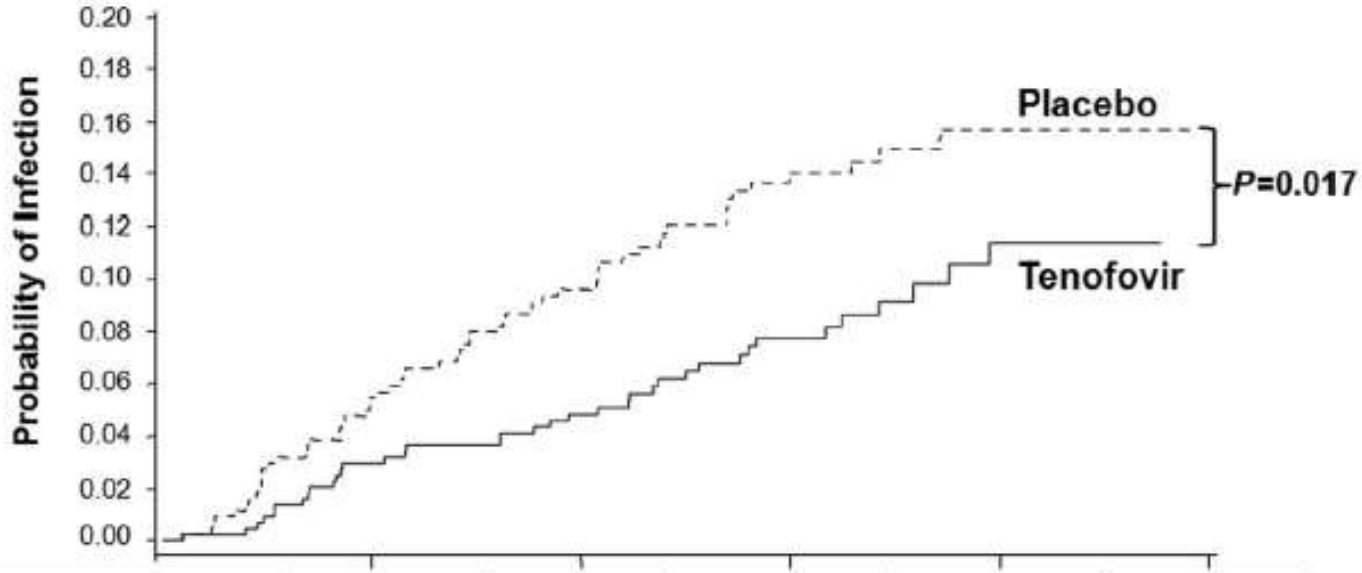
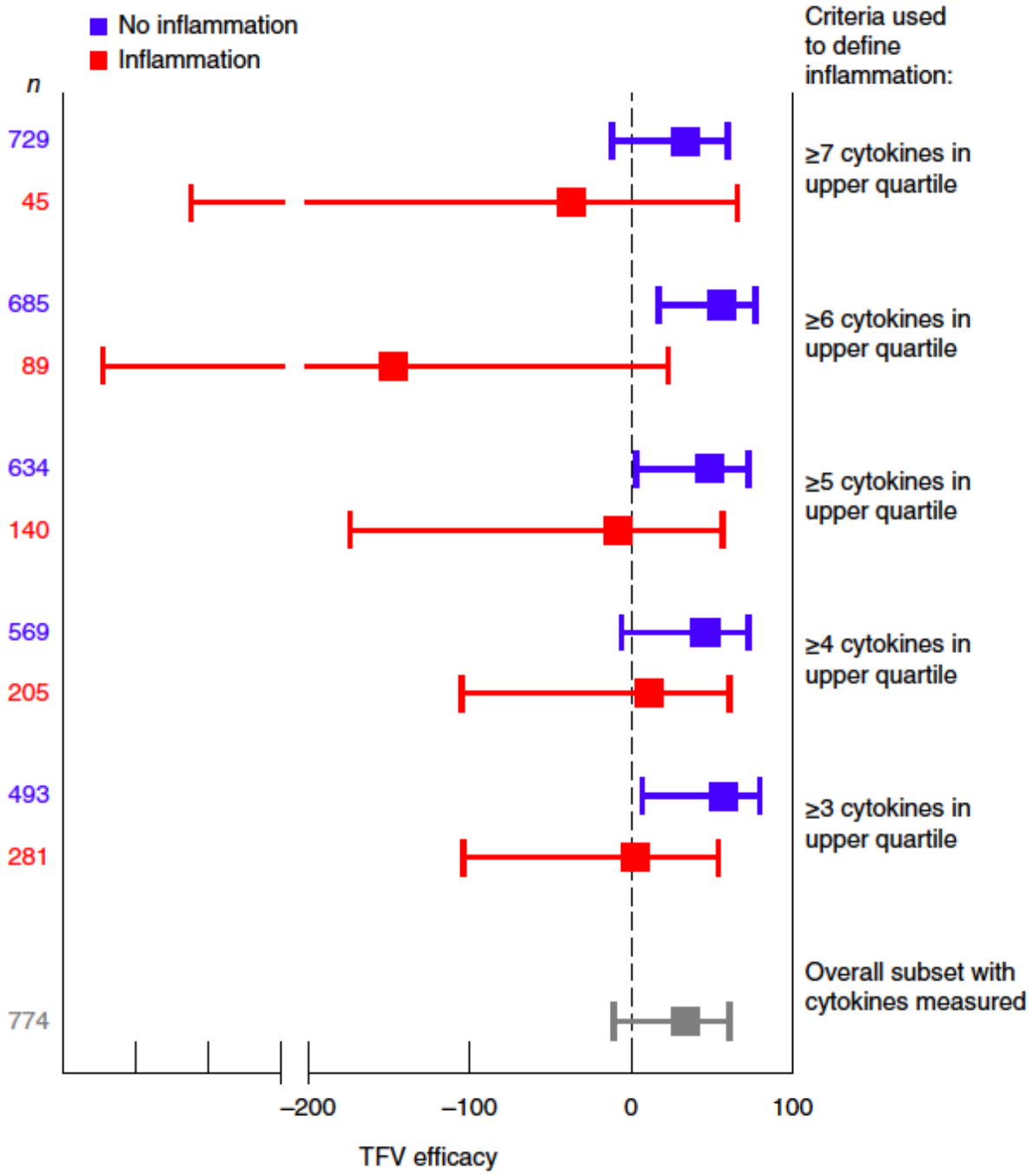


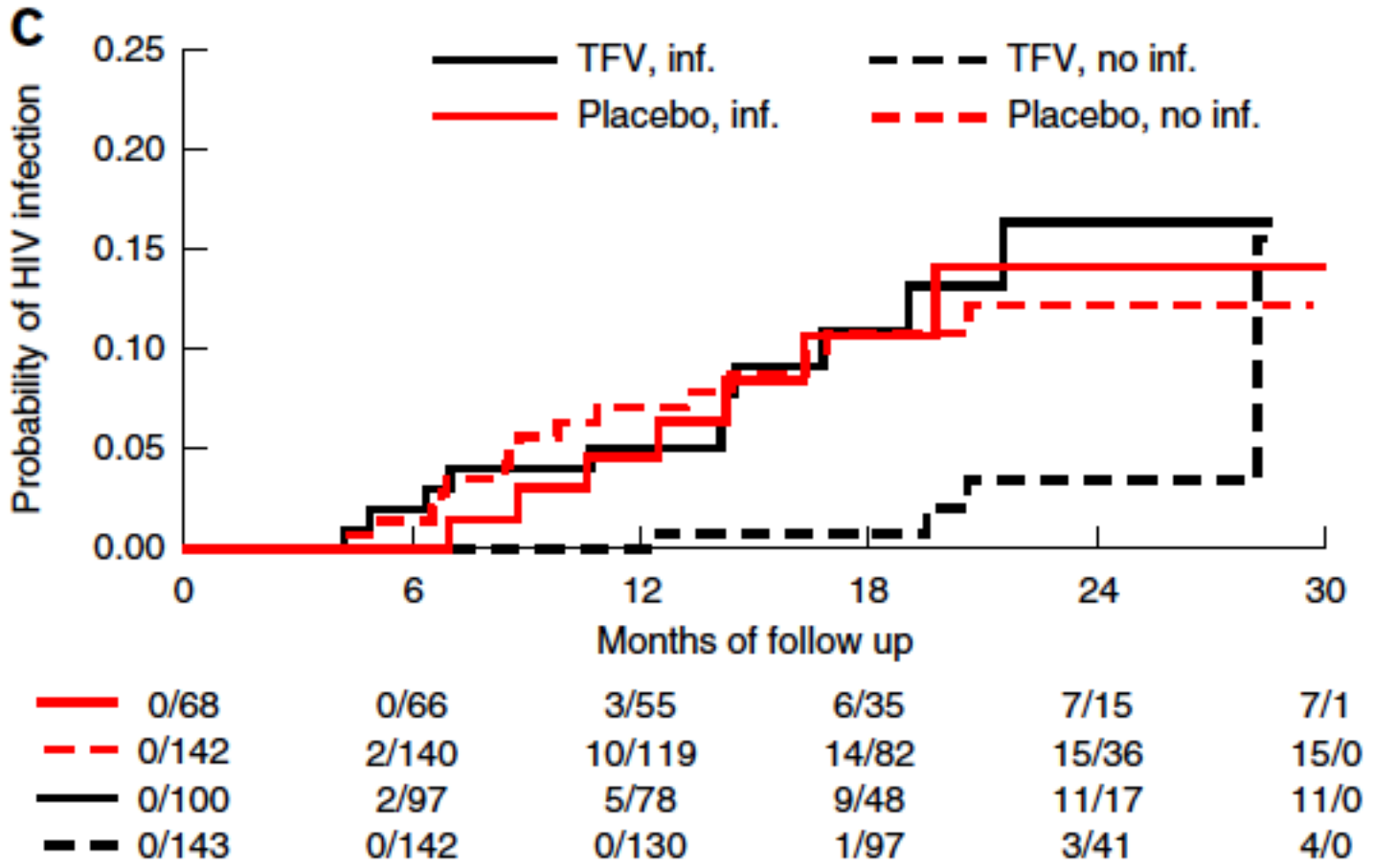
Months of follow-up	6	12	18	24	30
Cumulative HIV endpoints	37	65	88	97	98
Cumulative women-years	432	833	1143	1305	1341
HIV incidence rates (Tenofovir vs Placebo)	6.0 vs 11.2	5.2 vs 10.5	5.3 vs 10.2	5.6 vs 10.2	5.6 vs 9.1
Effectiveness (*P*-value)	**47% (0.064)**	**50% (0.007)**	**47% (0.004)**	**40% (0.013)**	**39% (0.017)**



Post-hoc prospective analysis of CAPRISA-004 women (n= 774)

FGT inflammation predicts TFV gel efficacy in PrEP

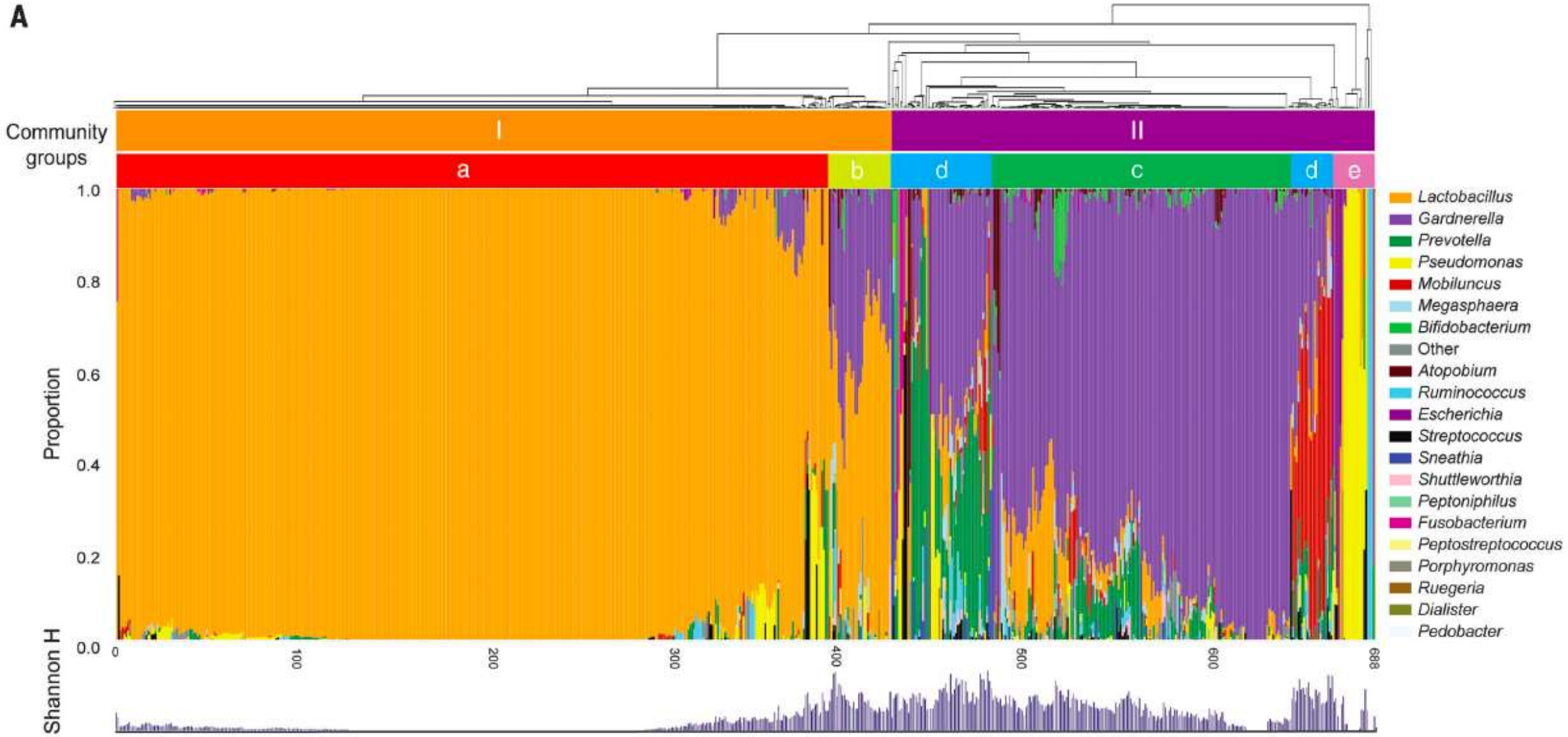
McKinnon et al. Nature Med 2018



Maximal efficacy in TFV-receiving women with no FGT inflammation

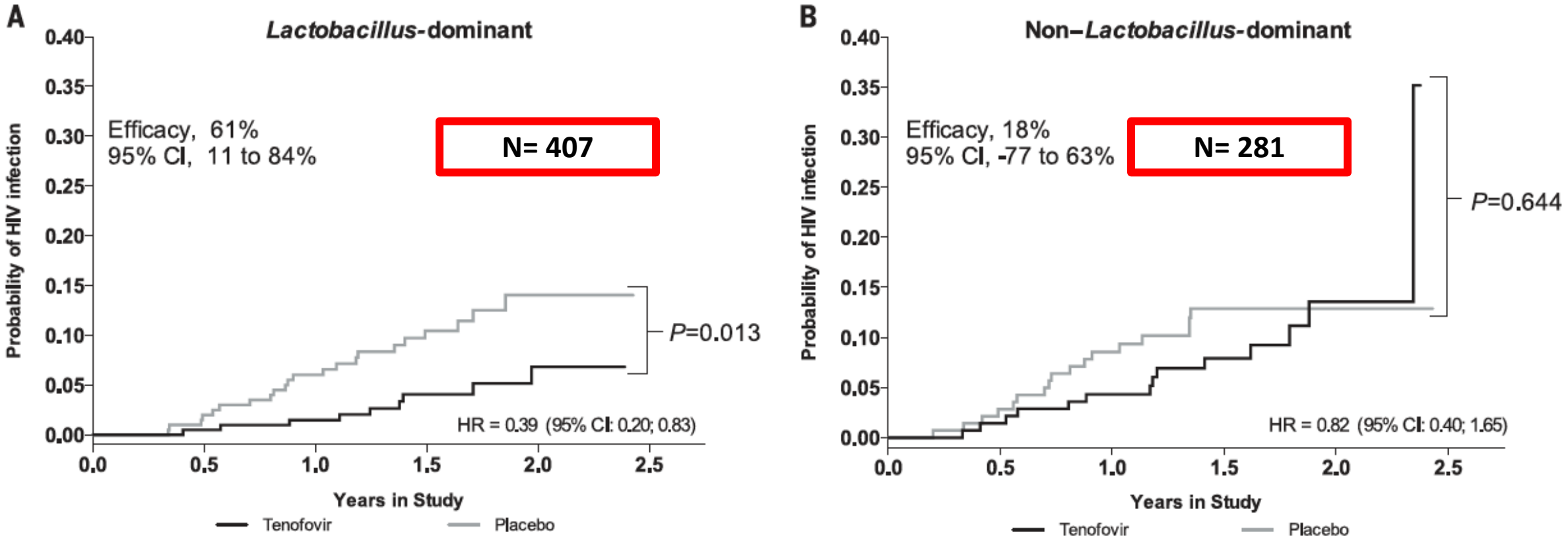
McKinnon et al. Nature Med 2018

688 women from CAPRISA-004



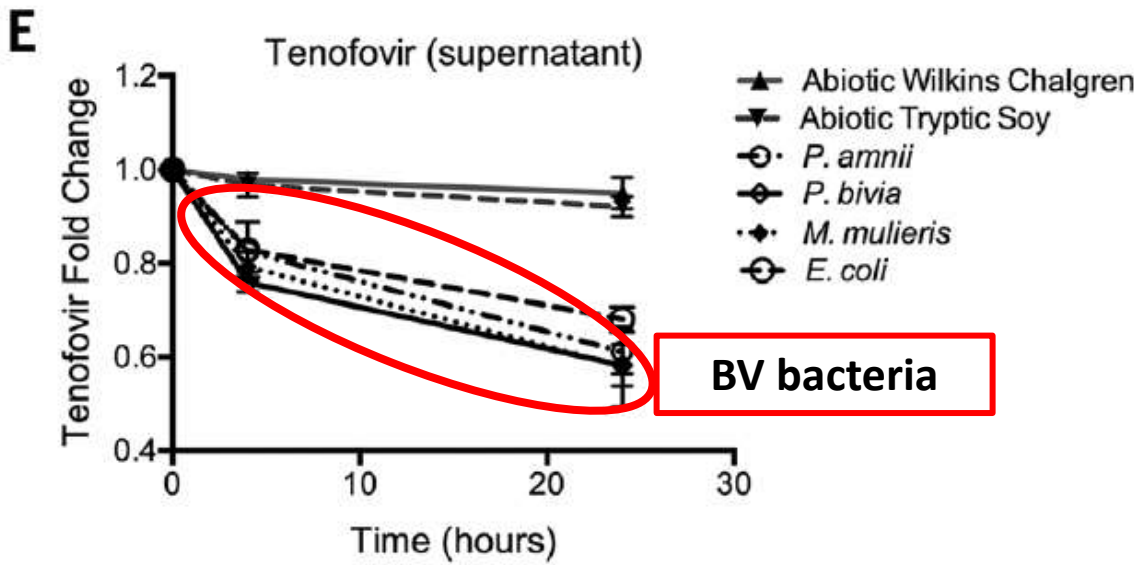
Klatt et al. Science 2017

Vaginal microbioma dramatically influences PreP efficacy

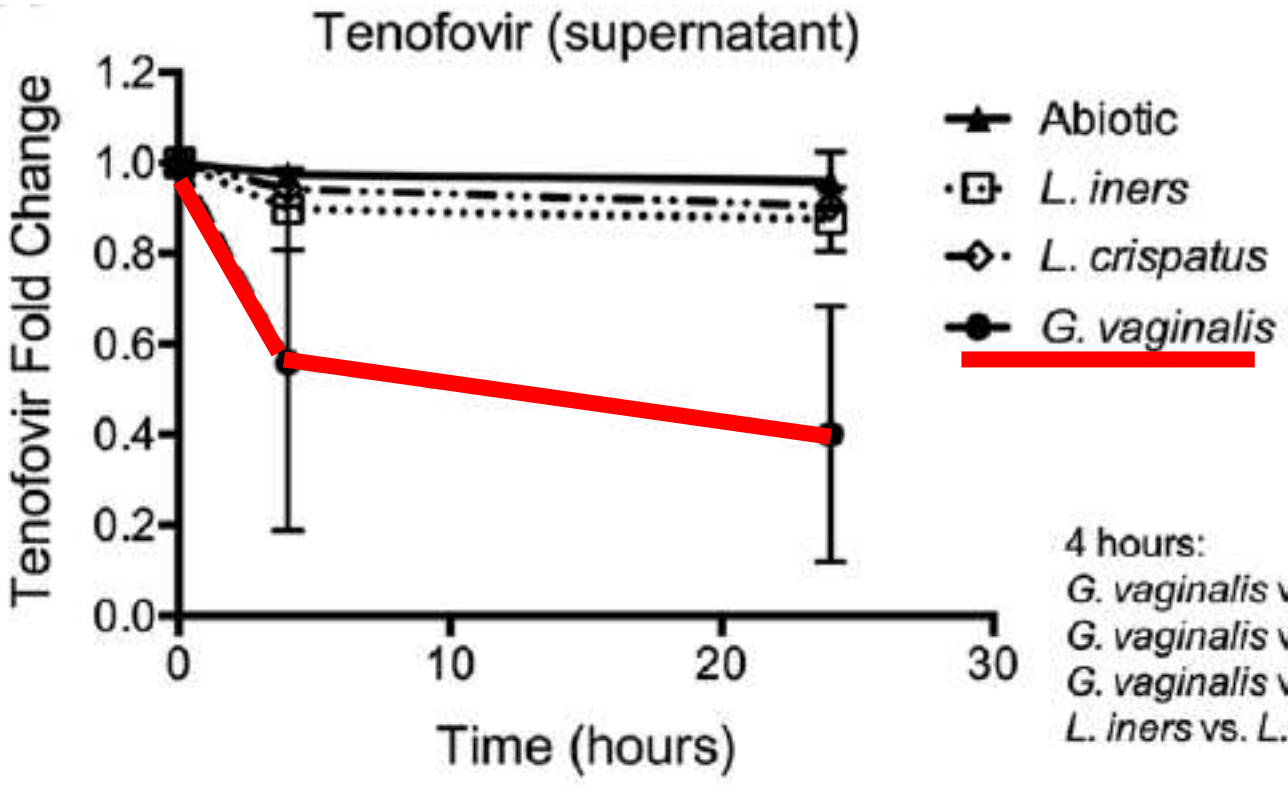


Klatt et al. Science 2017

Bacterial vaginosis-associated bacteria rapidly metabolize tenofovir, reducing extracellular drug availability



24 hours:
P. amnii vs. Abiotic (WC): *P*=0.0007
P. bivia vs. Abiotic (WC): *P*=0.0007
M. mulieris vs. Abiotic (WC): *P*=0.0007
E. coli vs. Abiotic (TS): *P*=0.1000

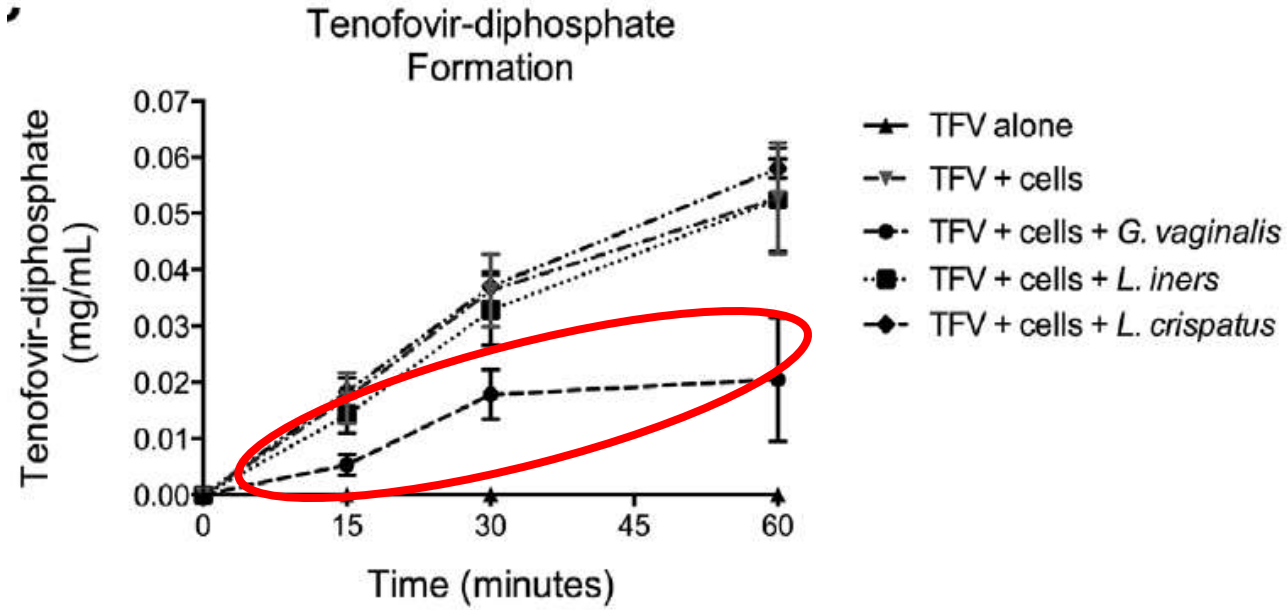


Klatt et al. Science 2017

4 hours:
G. vaginalis vs. Abiotic: *P*<0.0001
G. vaginalis vs. L. iners: *P*=0.0037
G. vaginalis vs. *L. crispatus*: *P*=0.0019
L. iners vs. *L. crispatus*: *P*=ns

24 hours:
G. vaginalis vs. Abiotic: *P*<0.0001
G. vaginalis vs. *L. iners*: *P*<0.0001
G. vaginalis vs. *L. crispatus*: *P*<0.0001
L. iners vs. *L. crispatus*: *P*=ns

Gardnerella decreases pharmacologically-active TDF by metabolizing tenofovir before cell uptake, in turn affecting gel adherence estimates based on vaginal drug levels [Kashuba et al. JAIDS 2015]



60 minutes:

G. vaginalis vs. TFV + cells: *P*=0.0002

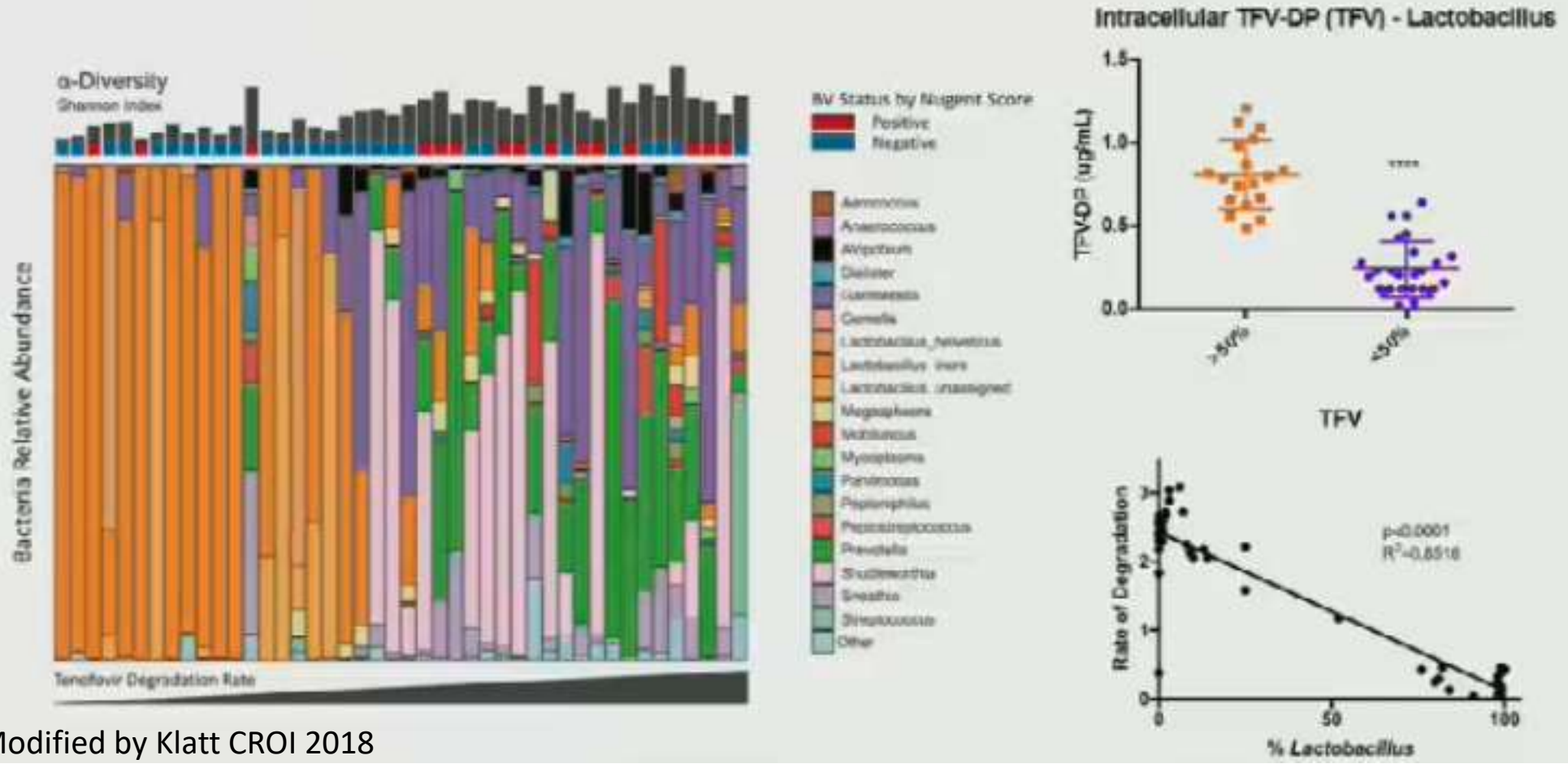
G. vaginalis vs. *L. iners*: *P*=0.0022

G. vaginalis vs. *L. crispatus*: *P*=0.0238

Lactobacillus spp. vs. TFV/cells: *P*=ns

Klatt et al. Science 2017

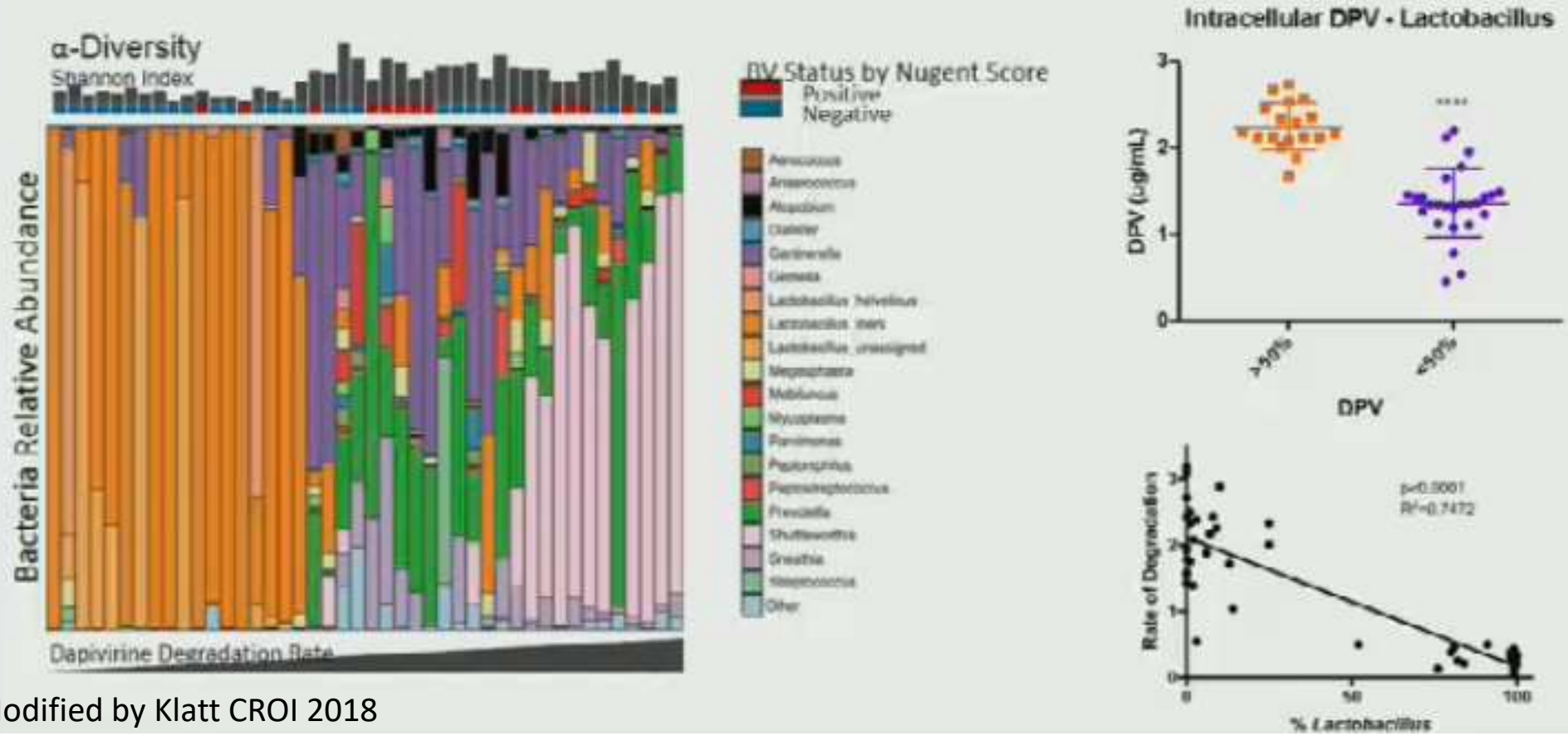
Dysbiotic bacteria metabolize **Tenofovir (TFV)**



Modified by Klatt CROI 2018

Potential reduction of TFV-based PreP efficacy in vivo!

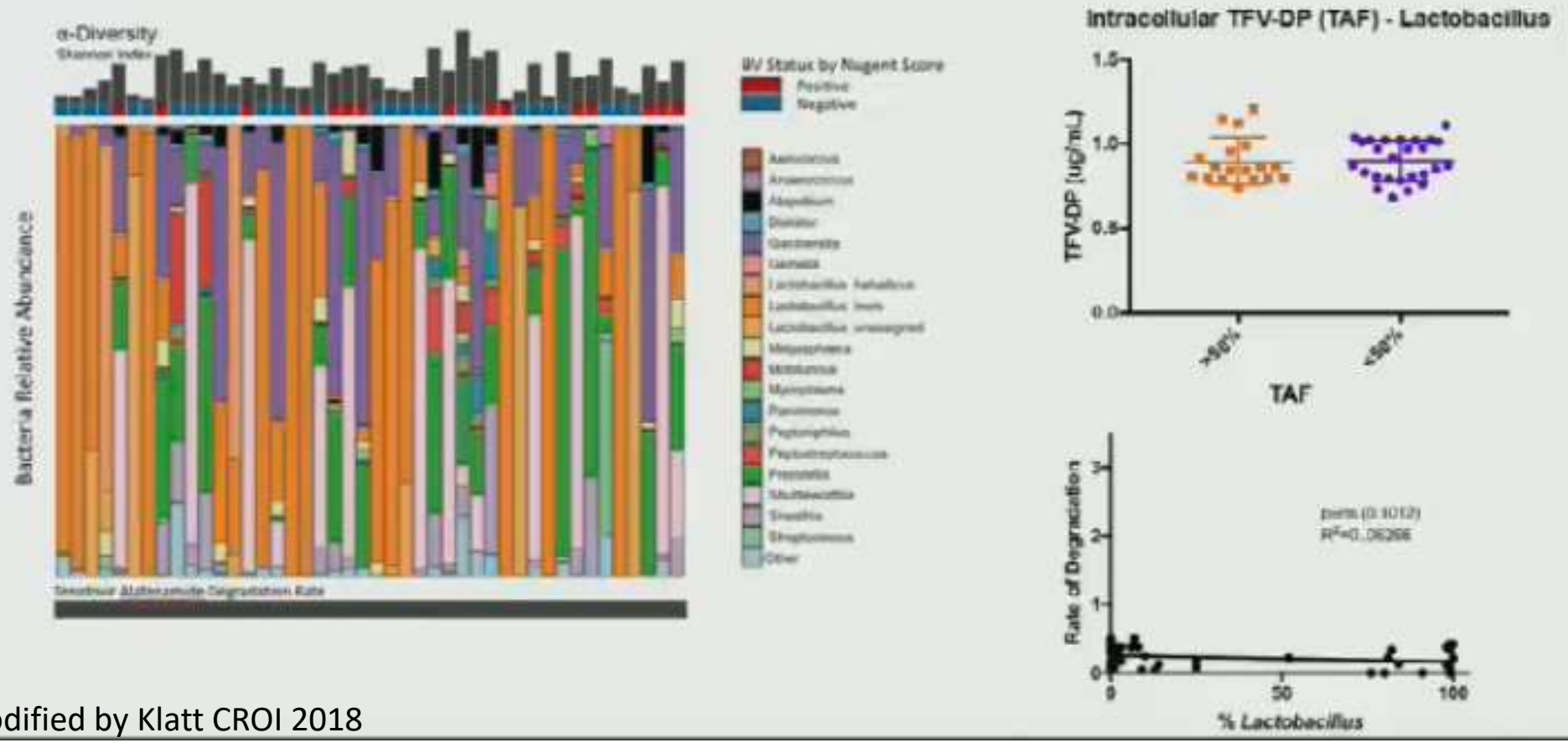
Dysbiotic bacteria metabolize **Dapivirine (DPV)**



Modified by Klatt CROI 2018

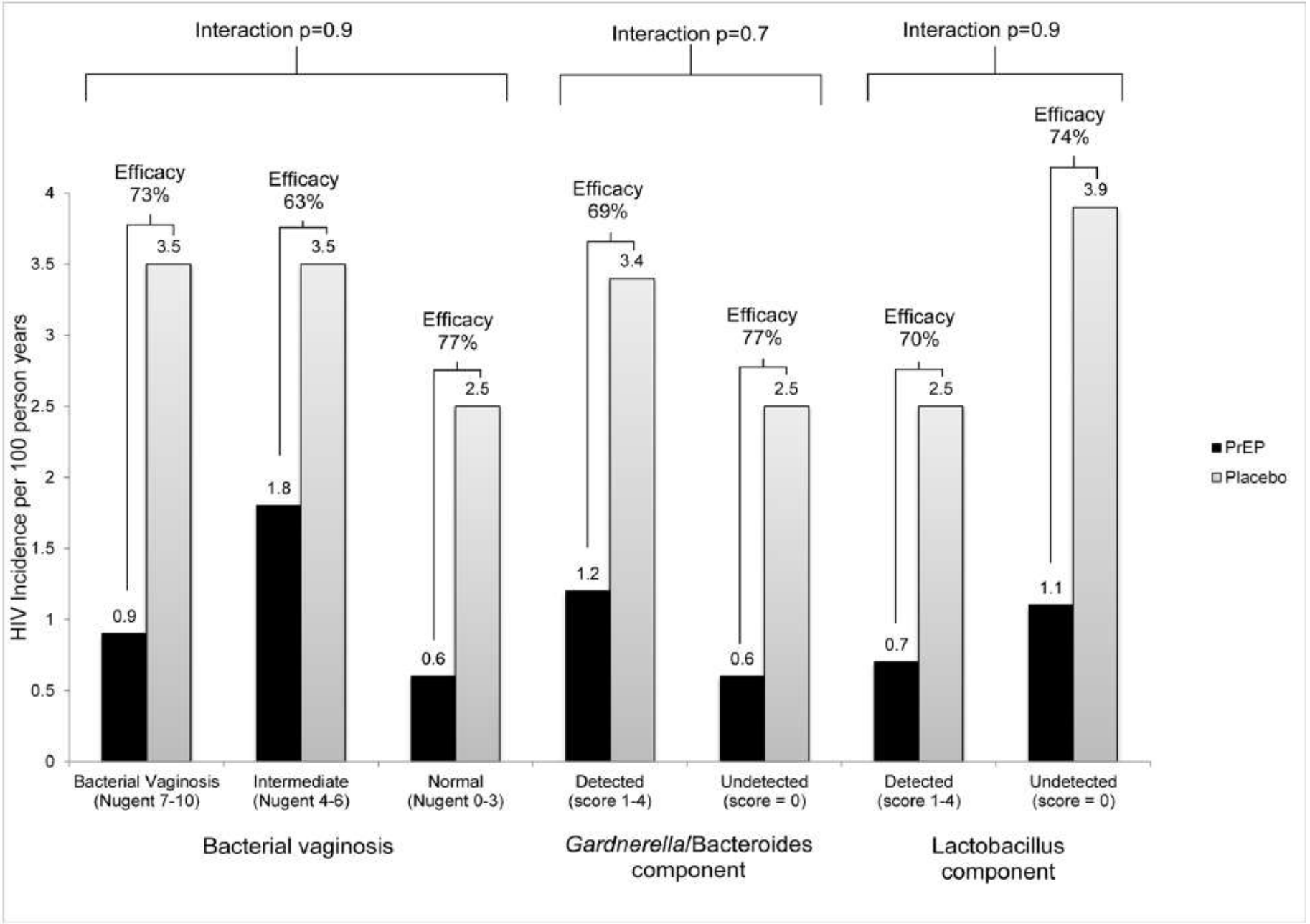
Potential reduction of DPV-based PreP efficacy in vivo!

Bacteria do not metabolize **Tenofovir Alafenamide (TAF)**



Modified by Klatt CROI 2018

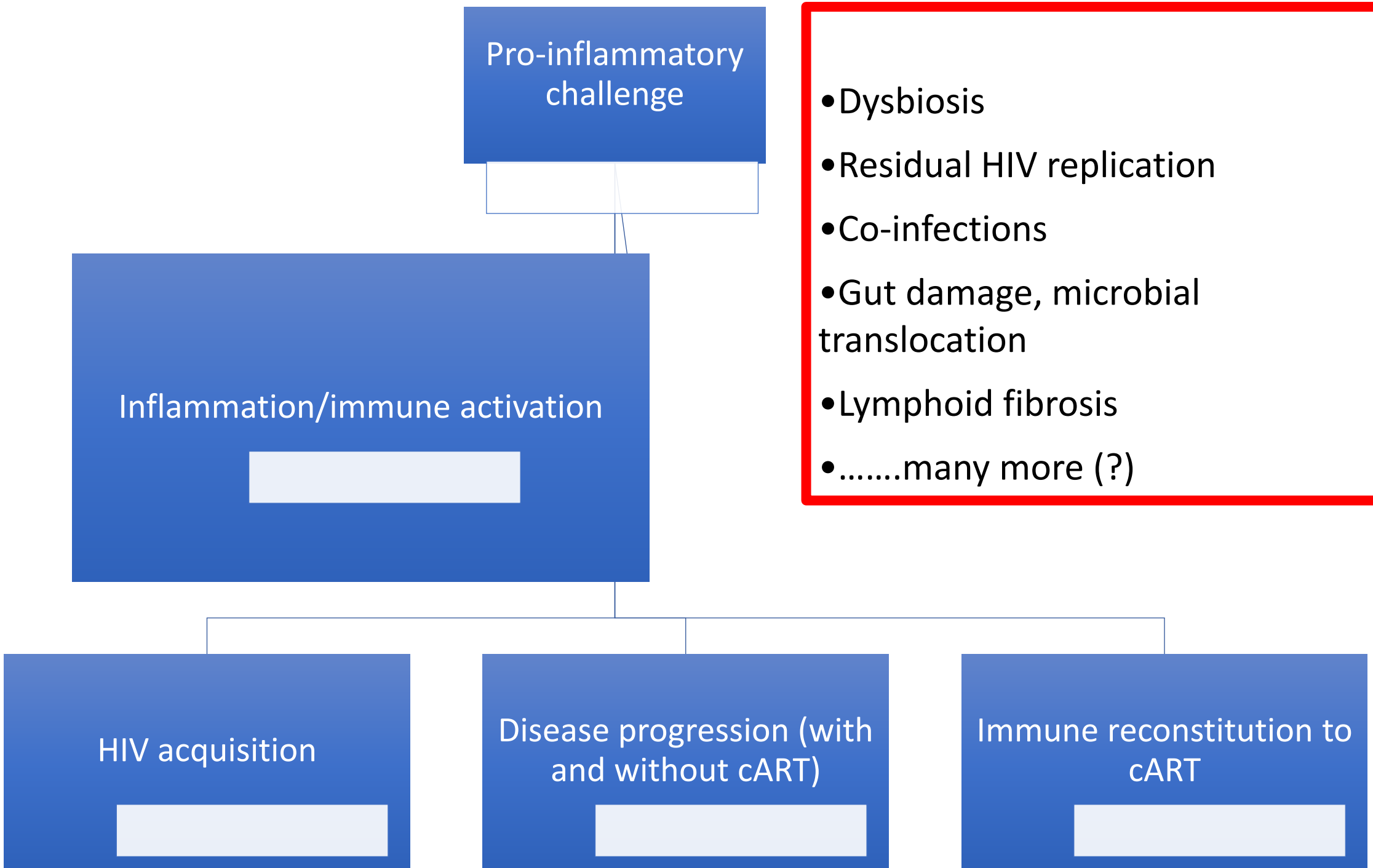
Higher efficacy for TAF-based PreP ?



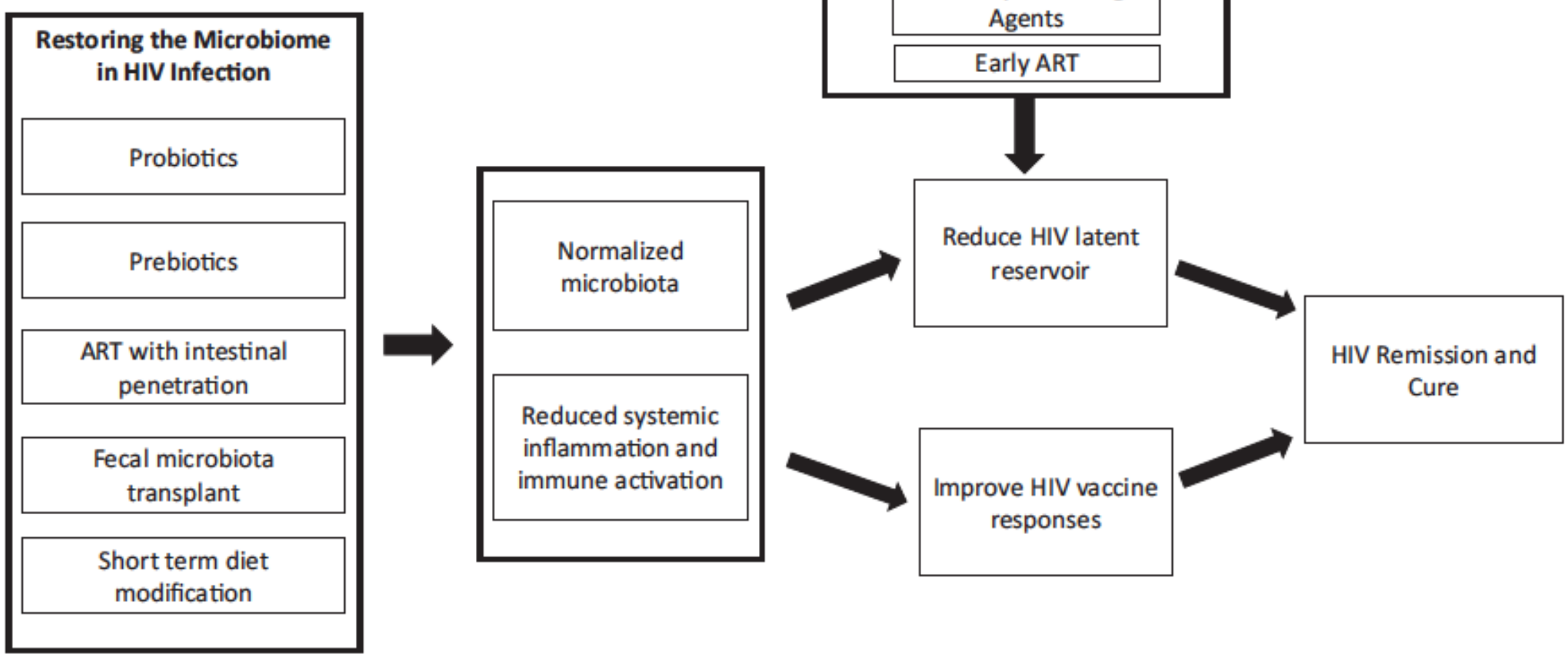
1470 women in Partners PrEP study

No influence of vaginal dysbiosis of oral PrEP effectiveness

Heffron et al. Lancet HIV 2017


Pro-inflammatory challenge
Inflammation/immune activation
HIV acquisition
Disease progression (with and without cART)
Immune reconstitution to cART
•Dysbiosis
•Residual HIV replication
•Co-infections
•Gut damage, microbial translocation
•Lymphoid fibrosis
•…….many more (?)

Targeting the microbiome for HIV cure?



Wei Li et al. Curr Opin HIV AIDS 2018

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•Patients and their families

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