

# Microbiome in HIV

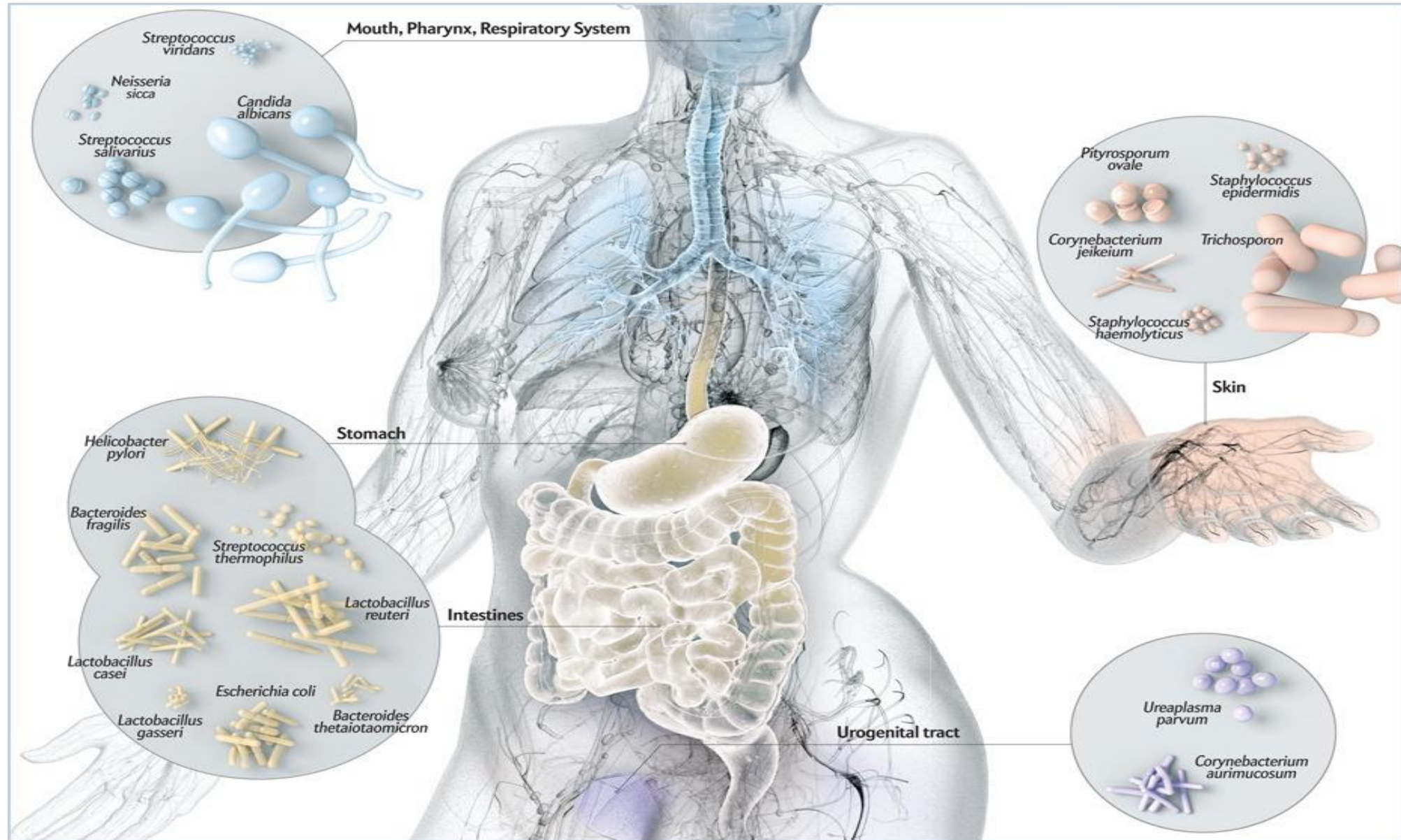
**Brent Palmer PhD**

**University of Colorado, USA**

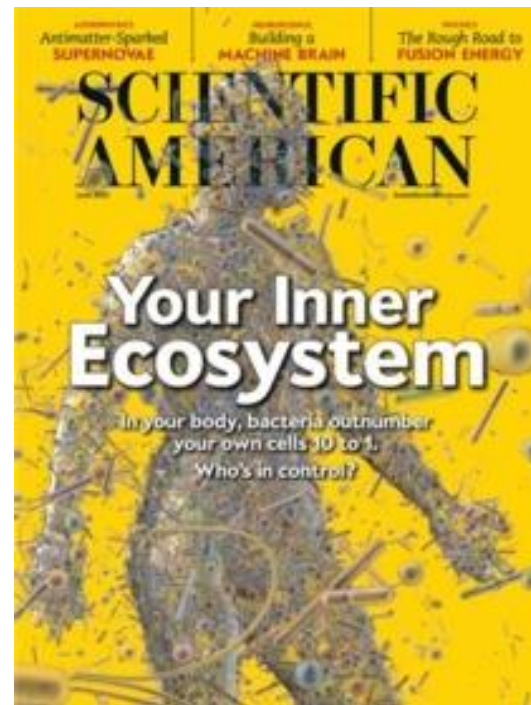
15<sup>th</sup> Residential Course on Clinical Pharmacology  
of Antiretrovirals

Torino, January 16, 2020

# The Human Microbiome







# The Human Gut Microbiome

- 100 trillion microbial cells
  - Weighs as much as the human brain
  - Genetic diversity is 1000X more rich
- Most gut microbes are harmless or beneficial
  - Extract dietary calories and vitamins
  - Protect against enteropathogens (colonization resistance)
  - “Train” immune system in early life
  - Prevent inflammation
- Dysbiosis (altered composition) co-occurs with gut Inflammation
  - Inflammatory Bowel Disease
  - Metabolic Disease
  - HIV-infection

# Microbiome can alter drug efficacy

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## Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota

Marie Vétizou<sup>1,2,3</sup>, Jonathan M. Pitt<sup>1,2,3</sup>, Romain Daillère<sup>1,2,3</sup>, Patricia Lepage<sup>4</sup>, Nadine Waldschmitt<sup>5</sup>, Caroline Flament<sup>1,2,6</sup>, ...

+ See all authors and affiliations

*Science* 27 Nov 2015:  
Vol. 350, Issue 6264, pp. 1079-1084  
DOI: 10.1126/science.aad1329

### REPORT

## Gut microbiome influences efficacy of PD-1 – based immunotherapy against epithelial tumors

Bertrand Routy<sup>1,2,3</sup>, Emmanuelle Le Chatelier<sup>4</sup>, Lisa Derosa<sup>1,2,3</sup>, Connie P. M. Duong<sup>1,2,5</sup>, Maryam Tidjani ...

+ See all authors and affiliations

*Science* 05 Jan 2018:  
Vol. 359, Issue 6371, pp. 91-97  
DOI: 10.1126/science.aan3706

## Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women

Nichole R. Klatt<sup>1,\*,†</sup>, Ryan Cheu<sup>1,‡</sup>, Kenzie Birse<sup>2,3,‡</sup>, Alexander S. Zevin<sup>1,‡</sup>, Michelle Perner<sup>2,3,‡</sup>, Laura Noël-Romas<sup>2,3,‡</sup>, Ann...

+ See all authors and affiliations

*Science* 02 Jun 2017:  
Vol. 356, Issue 6341, pp. 938-945  
DOI: 10.1126/science.aai9383

## Vaginal microbiome modulates topical antiretroviral drug pharmacokinetics

Ekaterina Taneva, ... , Marla J. Keller, Betsy C. Herold

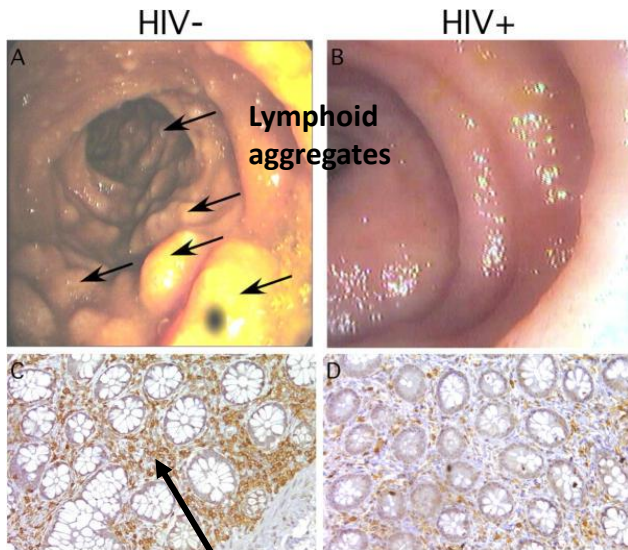
*JCI Insight*. 2018;**3**(13):e99545. <https://doi.org/10.1172/jci.insight.99545>.



# Gut microbiota and HIV-associated GI disease

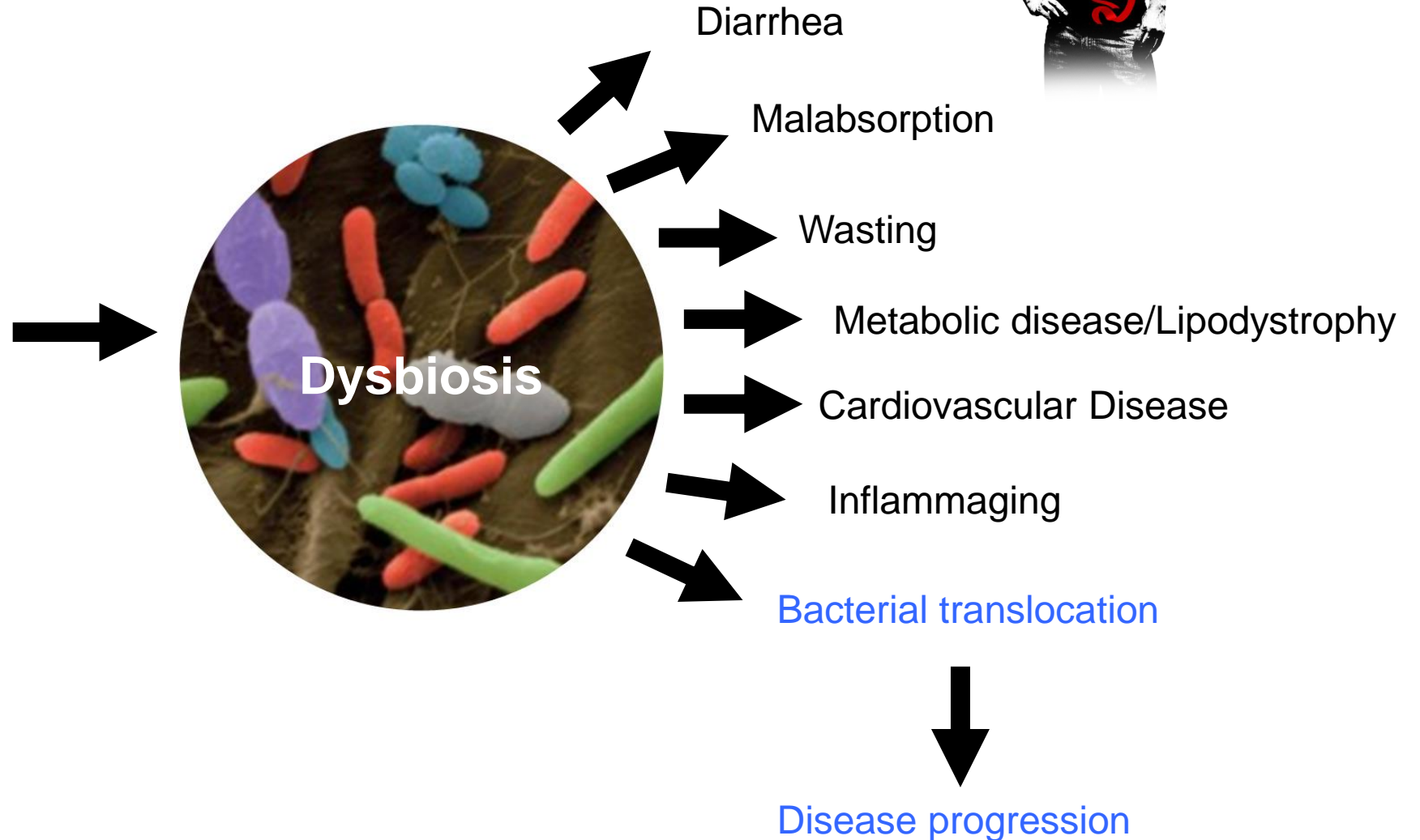


Profound depletion of CD4+ T cells in the GALT



Brenchley, JEM 2004

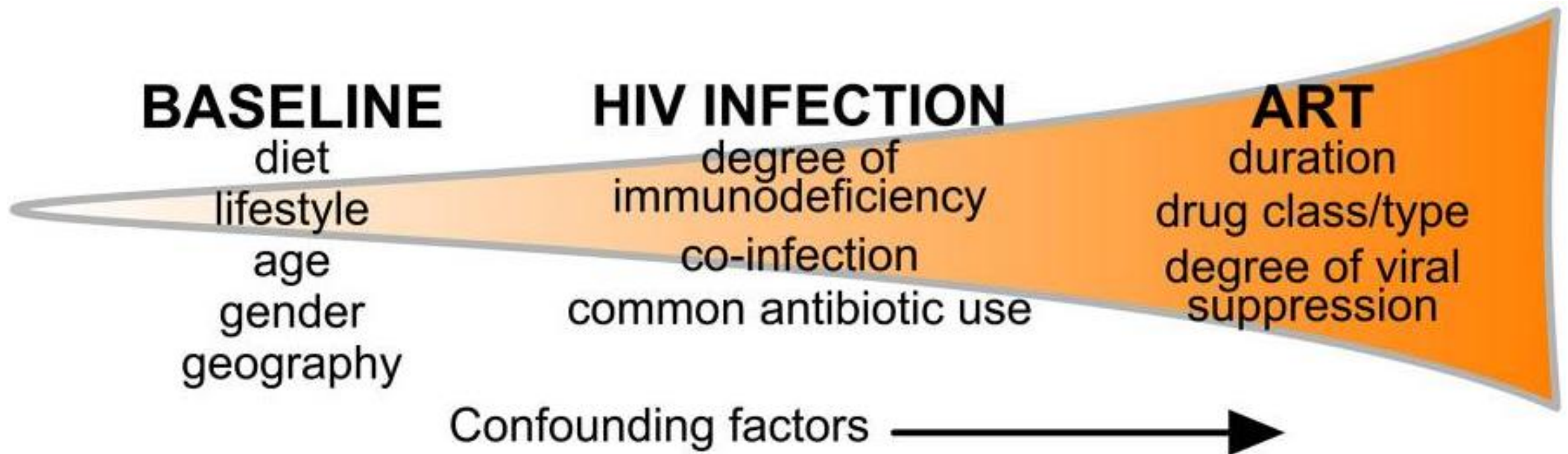
CD4+ T cells



# Questions

- Is the gut microbiome altered with HIV or ART?
- Is the HIV-associated gut microbiome pro-inflammatory?
  - What specific microbes are driving immune activation/inflammation?
  - By what mechanism?
- Does immune-modulation by these microbes influence HIV pathogenesis, transmission or viral persistence?

# Studies of the microbiome are confounded by many factors





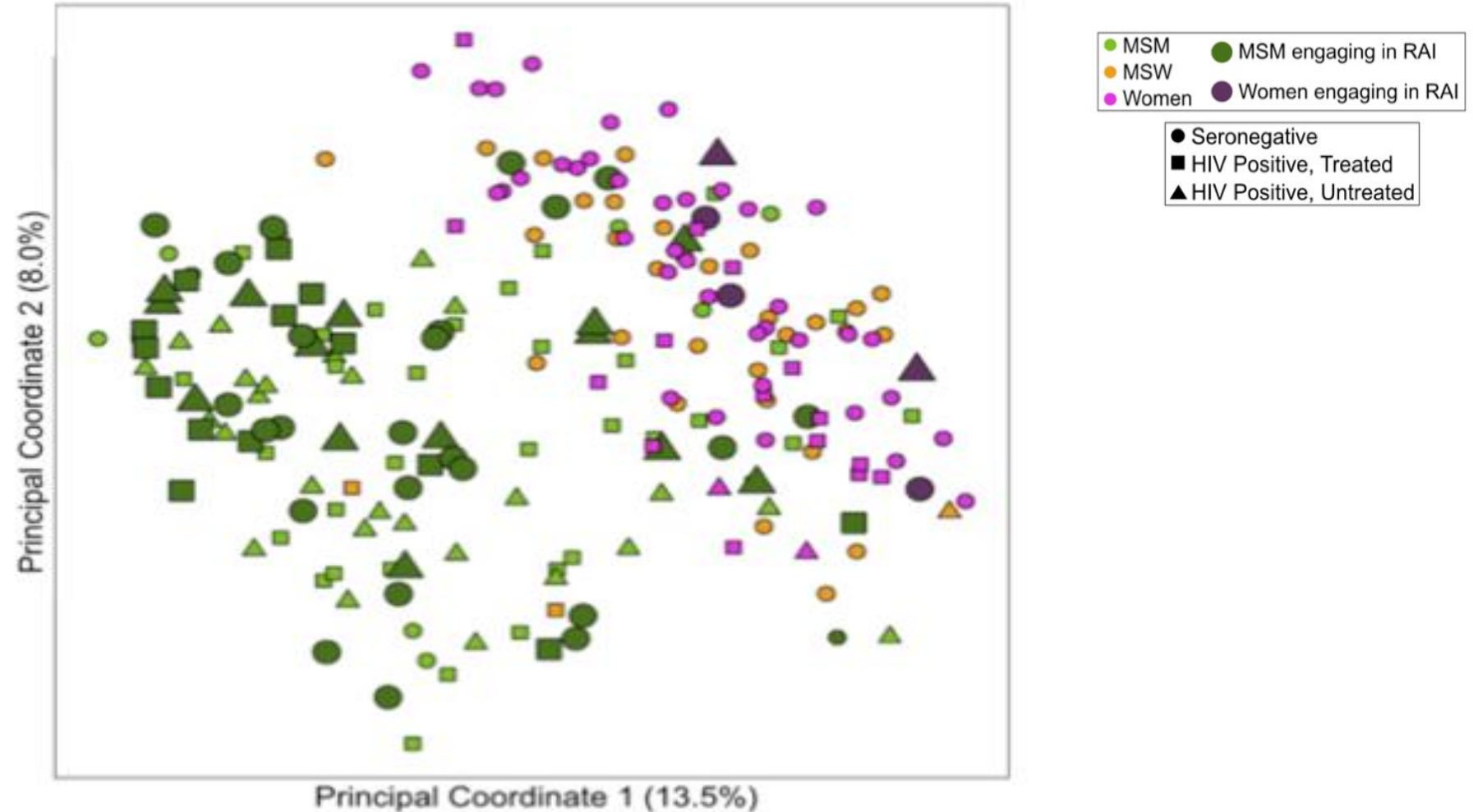
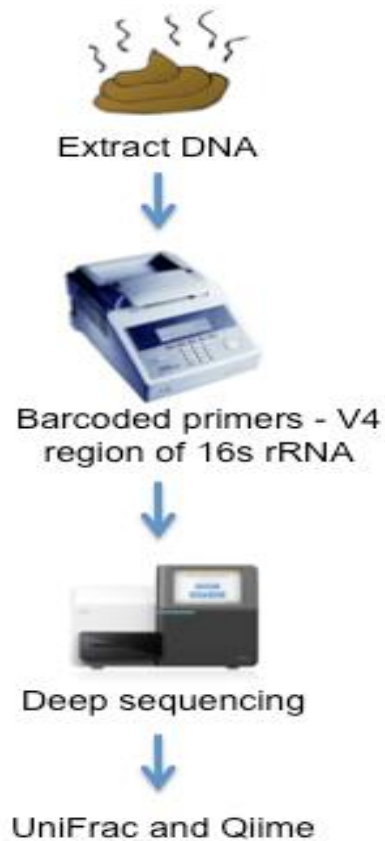
# What does 'dysbiosis' in the context of HIV infection look like?

	<u>HIV Negative</u>			<u>HIV Positive</u>					
	<u>MSM</u>	<u>MSW</u>	<u>Women</u>	<u>MSM</u>	<u>ART Treated</u> <u>MSW</u>	<u>Women</u>	<u>MSM</u>	<u>ART Naïve</u> <u>MSW</u>	<u>Women</u>
Number	35	29	41	50	2	15	40	1	4
IVDU	0	0	0	1 (2.0%)	0	0	2 (5.0%)	0	1 (25.0%)
BMI (kg/m2)	26.1 +/- 4.2	25.7 +/- 3.1	24.7 +/- 3.4	26.3 +/- 4.0	27.1 +/- 0.6	30.0 +/- 6.5	24.4 +/- 4.3	26.8 +/- 0.0	27.5 +/- 7.2
CD4 (cell/uL)	NA	NA	NA	699 +/- 311 (177-1297)	962 +/- 344 (619-1306)	816 +/- 336 (187-1417)	560 +/- 206 (201-1095)	551	600 +/- 357 (103-1071)
Viral Load (Copies/mL)	0	0	0	12 +/- 14 (0-44)	10 +/- 10 (0-20)	35 +/- 80 (0-297)	126714 +/- 189725 (159-856000)	3880	33250 +/- 40880 (7000-104000)

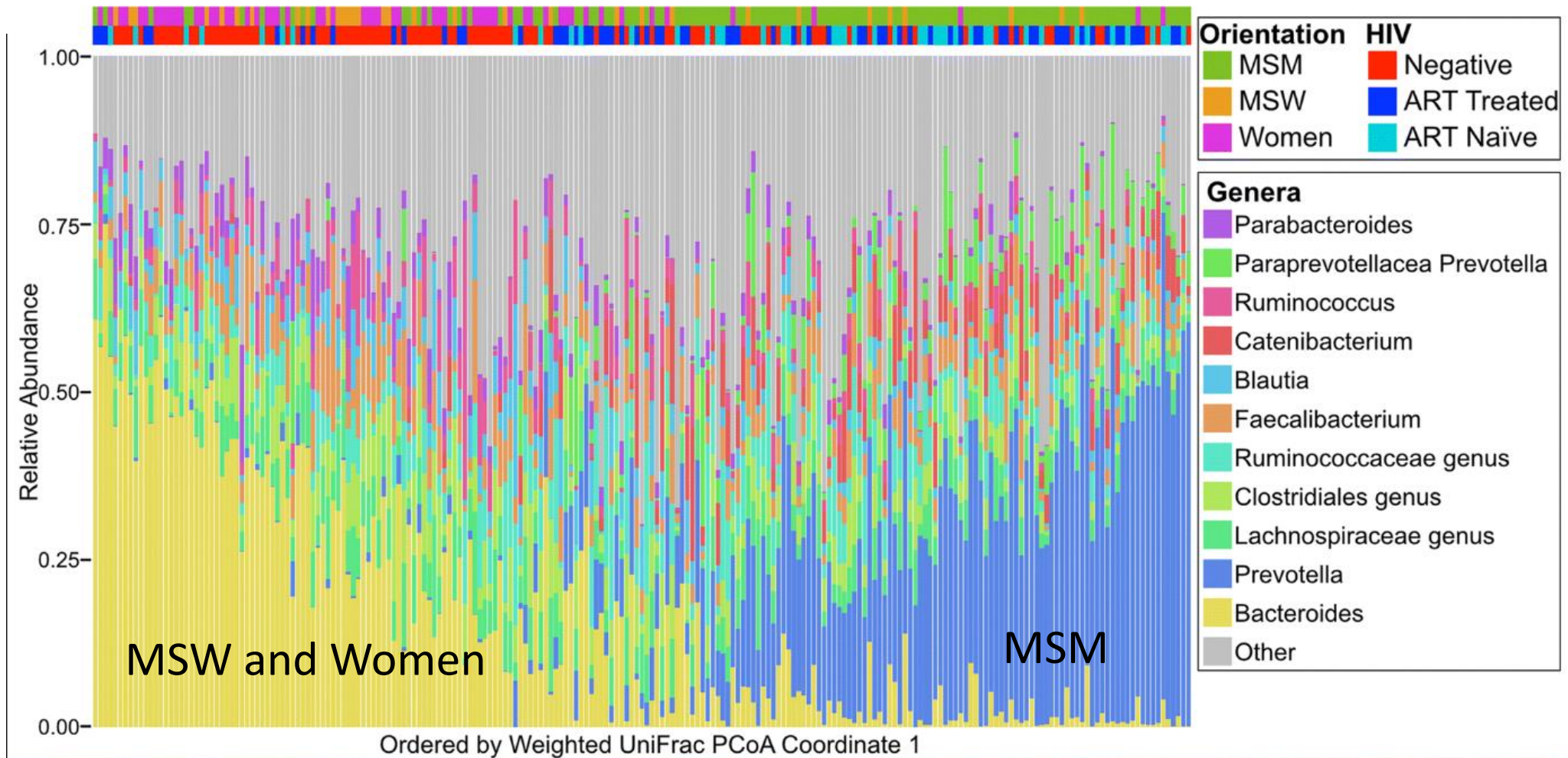
- Analysis of the fecal microbiome of 217 individuals
- HIV+ Cohort dominated by MSM
- Control population of high risk MSM
- All recruited from the same geographic area
- ART naïve and ART treated

# Separation by sexual behavior and not HIV status

## Weighted UniFrac PCoA



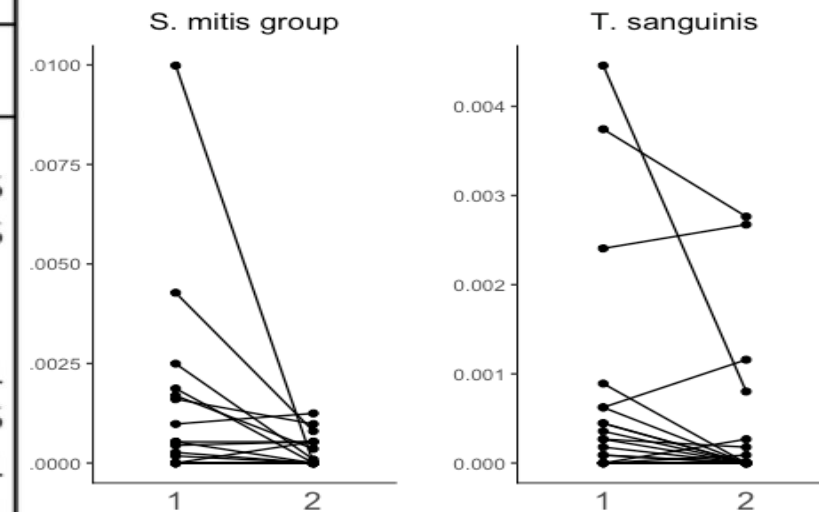
# Prevotella/Bacteroides “enterotypes” drive sexual behavior clustering: MSM are Prevotella-rich



# Some opportunistic pathogens increase with HIV infection and decrease with ART

MSM					
Taxonomy	Mean Relative Abundance			FDR	
	HIV Negative	Untreated	Treated	P-value	P-value
<b>No difference in HIV and significant reduction in ART treatment ( <math>\searrow</math> )</b>					
<i>Fenollaria massiliensis</i>	1.76E-03	2.00E-03	3.01E-04	<0.001	<0.05
<i>Peptoniphilus</i> sp.	1.44E-03	2.41E-03	5.28E-04	<0.001	<0.05
<b>Significant increase in HIV and significant reduction in ART treatment ( <math>\nearrow</math> )</b>					
<i>Streptococcus</i> sp.	5.09E-05	1.84E-03	2.32E-05	<0.001	<0.01
<i>Fusobacterium equinum</i>	1.17E-03	7.77E-03	1.84E-04	<0.001	<0.05
<i>Turicibacter sanguinis</i>	5.55E-04	6.02E-04	3.17E-04	<0.01	<0.1
<i>Finegoldia magna</i>	5.60E-03	8.26E-03	5.35E-03	<0.01	<0.1

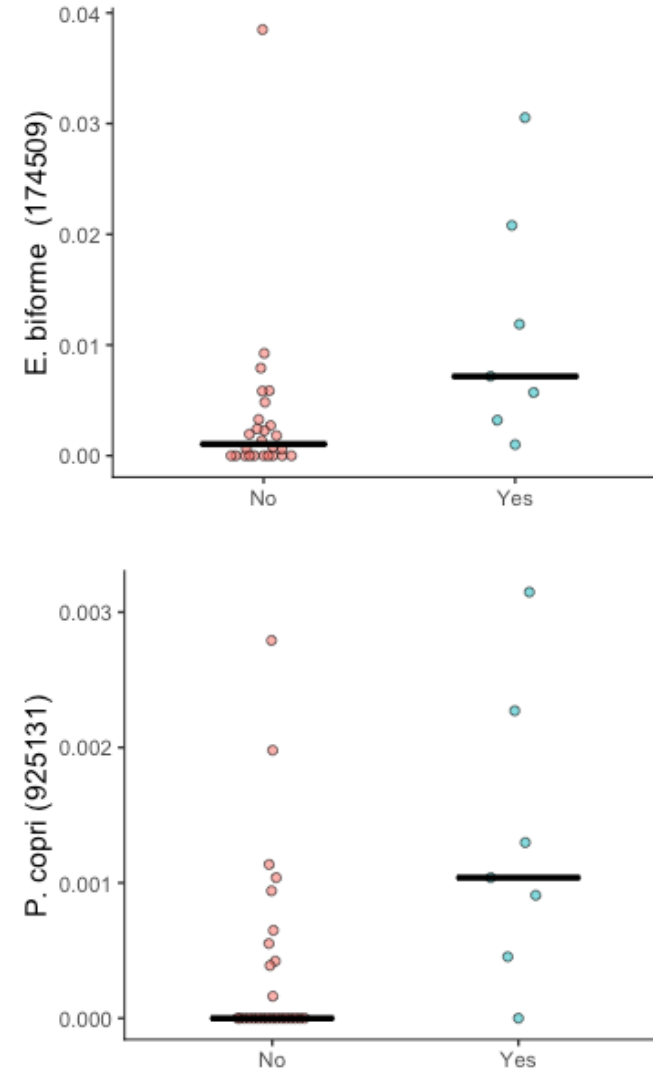
Before and after ART





# PrEP is associate with alterations in relative abundance of certain bacteria taxa in the gut

Daily HIV pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate-emtricitabine reduced *Streptococcus* and increased *Erysipelotrichaceae* in rectal microbiota. **Dube et al. Scientific Report 2018**



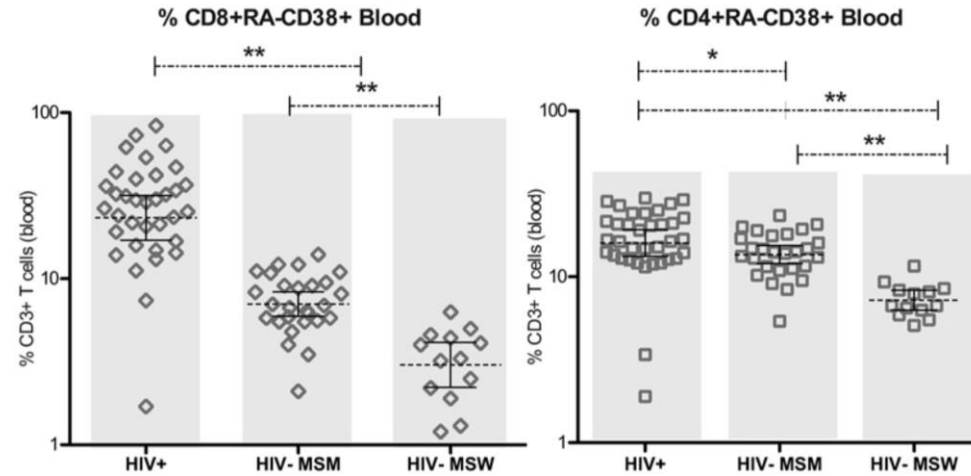
Enrichment of gut bacteria already associated with MSM.

Is it due to ART itself or change in behavior?

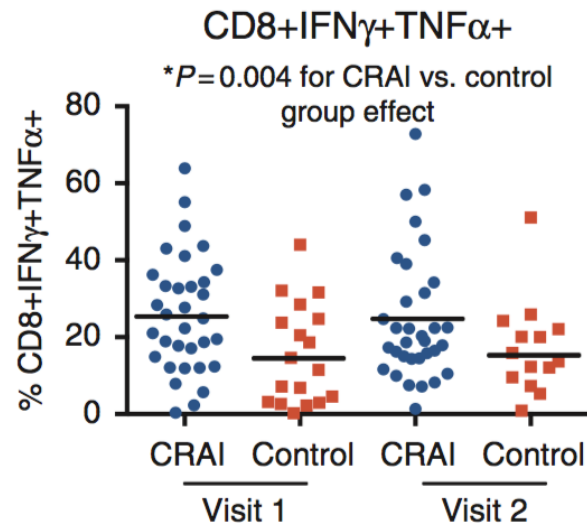
# Summary Part 1

- High-risk MSM, regardless of HIV status, have profoundly altered gut microbiome composition compared to low risk controls.
- HIV infection is associated with relatively subtle microbiome differences.
  - AIDS is associated with more profound gut microbiome dysbiosis.
  - Some studies have suggested stronger HIV associated dysbiosis in intestinal biopsy (versus feces).
- ART is associated with unique microbiome differences
  - Some correction of HIV driven changes.
  - Some unique changes driven by the drugs themselves.
- What are the functional implications???

# Immune differences in HIV- high-risk MSM

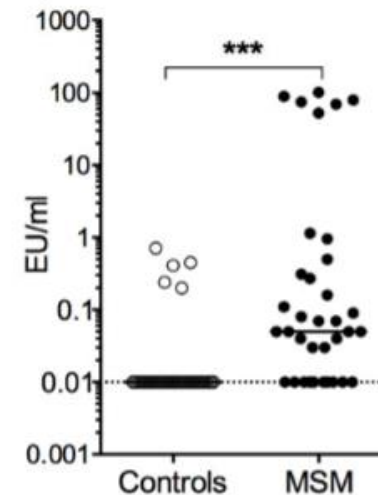


Gianella, J. Virology 2012

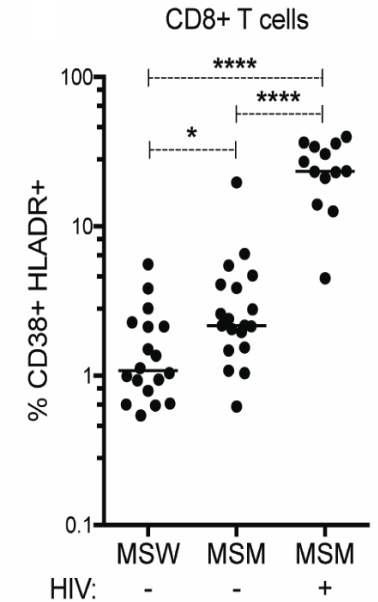


Kelley, Mucosal Immunology 2016

## Blood LPS levels



Palmer, AIDS 2014



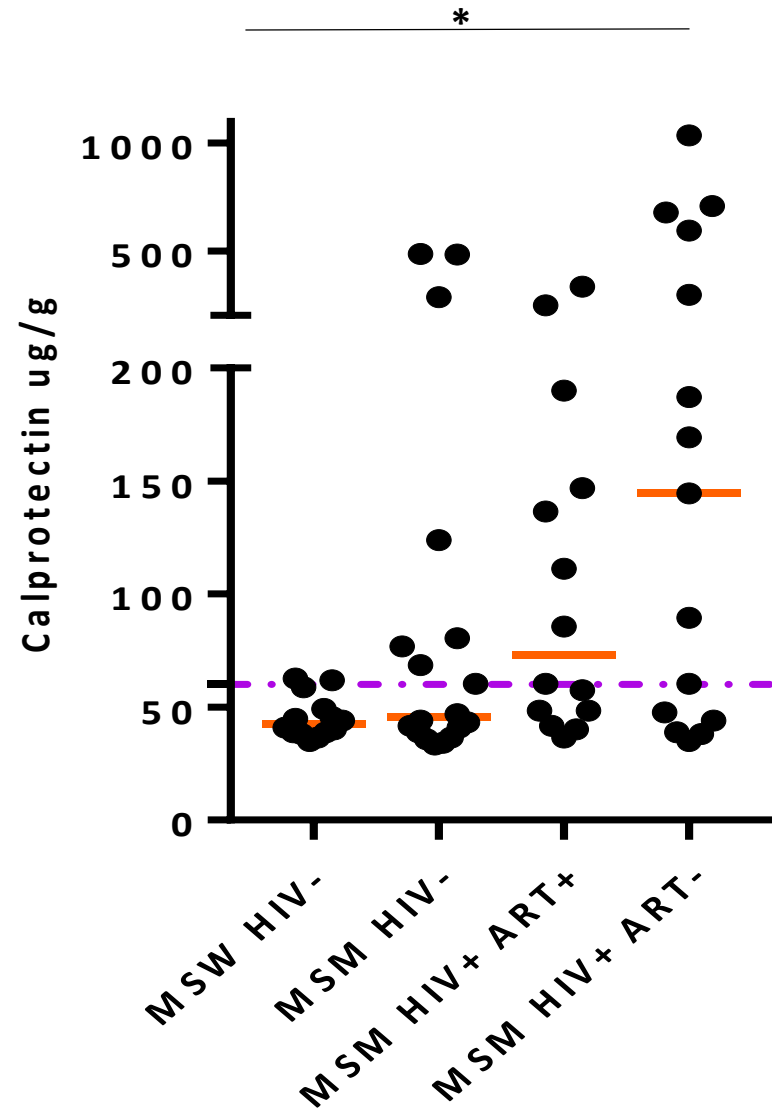
Li SX, PLOSPathogens 2019

# Does gut microbiome composition impact immune activation?

- We hypothesize that MSM, HIV and ART related alterations in gut microbiota composition induce gastrointestinal immune activation and inflammation.
- Approach:
  - *Ex vivo*: Analysis of stool cytokine/chemokines/biomarkers of inflammation.
  - *In vitro*: Bacterial communities isolated from stool to stimulate human immune cells
  - *In vivo*: Gnotobiotic mice and fecal transplants



# GI Inflammation is Elevated in HIV+/- MSM

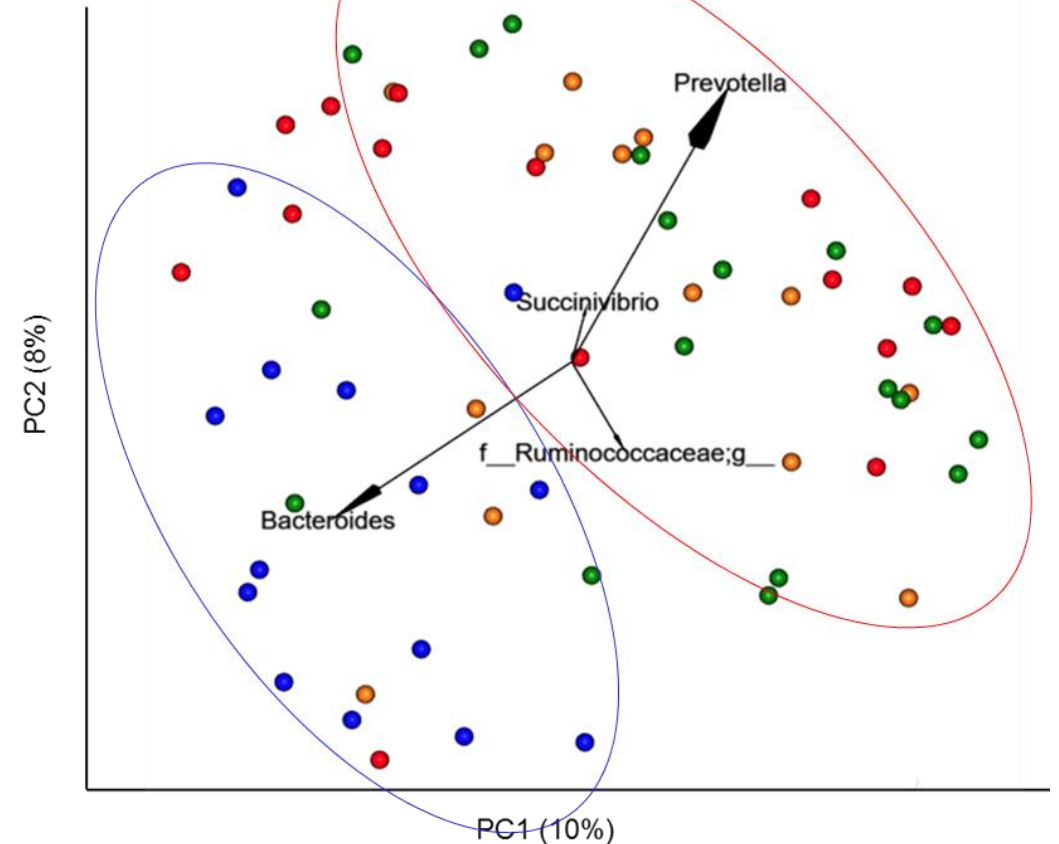
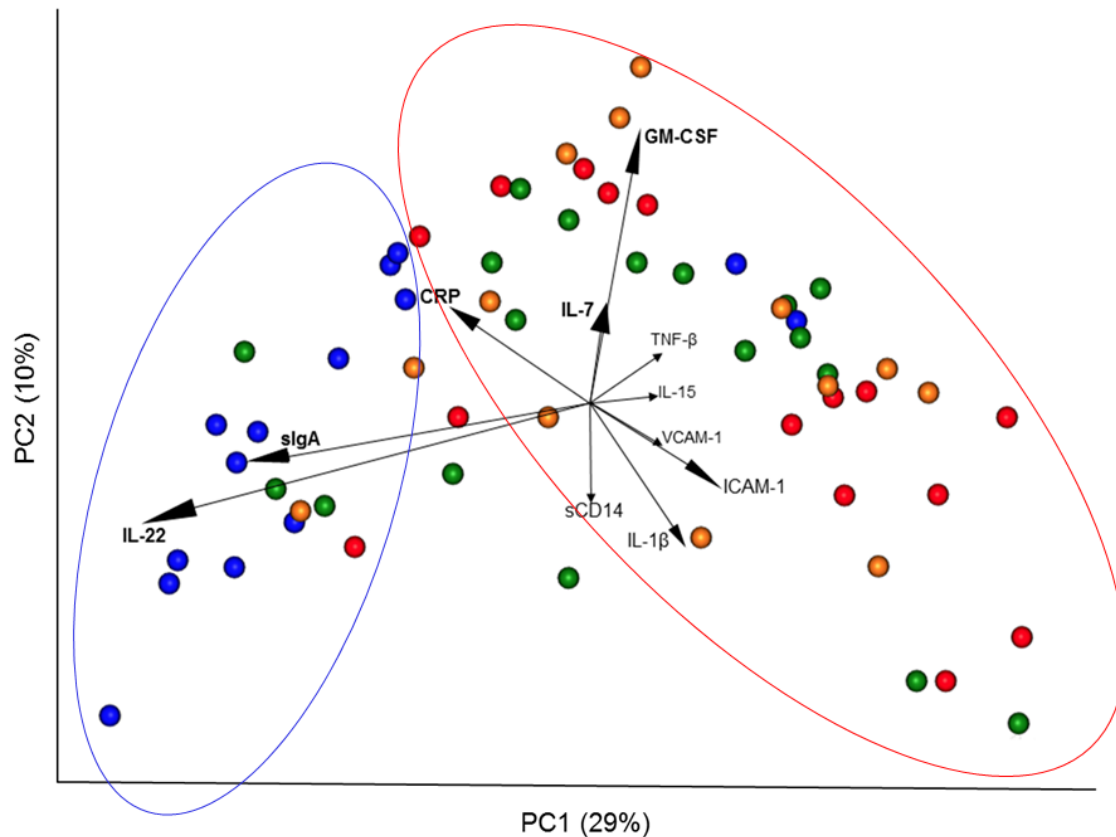


- Calprotectin or S100A8/A9 excreted by activated neutrophils and other granulocytes.
- Measure of disease severity (Crohn's or IBD)
- $\geq 60$  ug/g clinical level indicative of inflammation.

Cohorts	Subjects with inflammation (>60ug/g)
HIV- MSW (14)	14.3
HIV- MSM (18)	44.4
HIV+ ART+ MSM (14)	57.1
HIV+ ART- MSM (15)	66.7

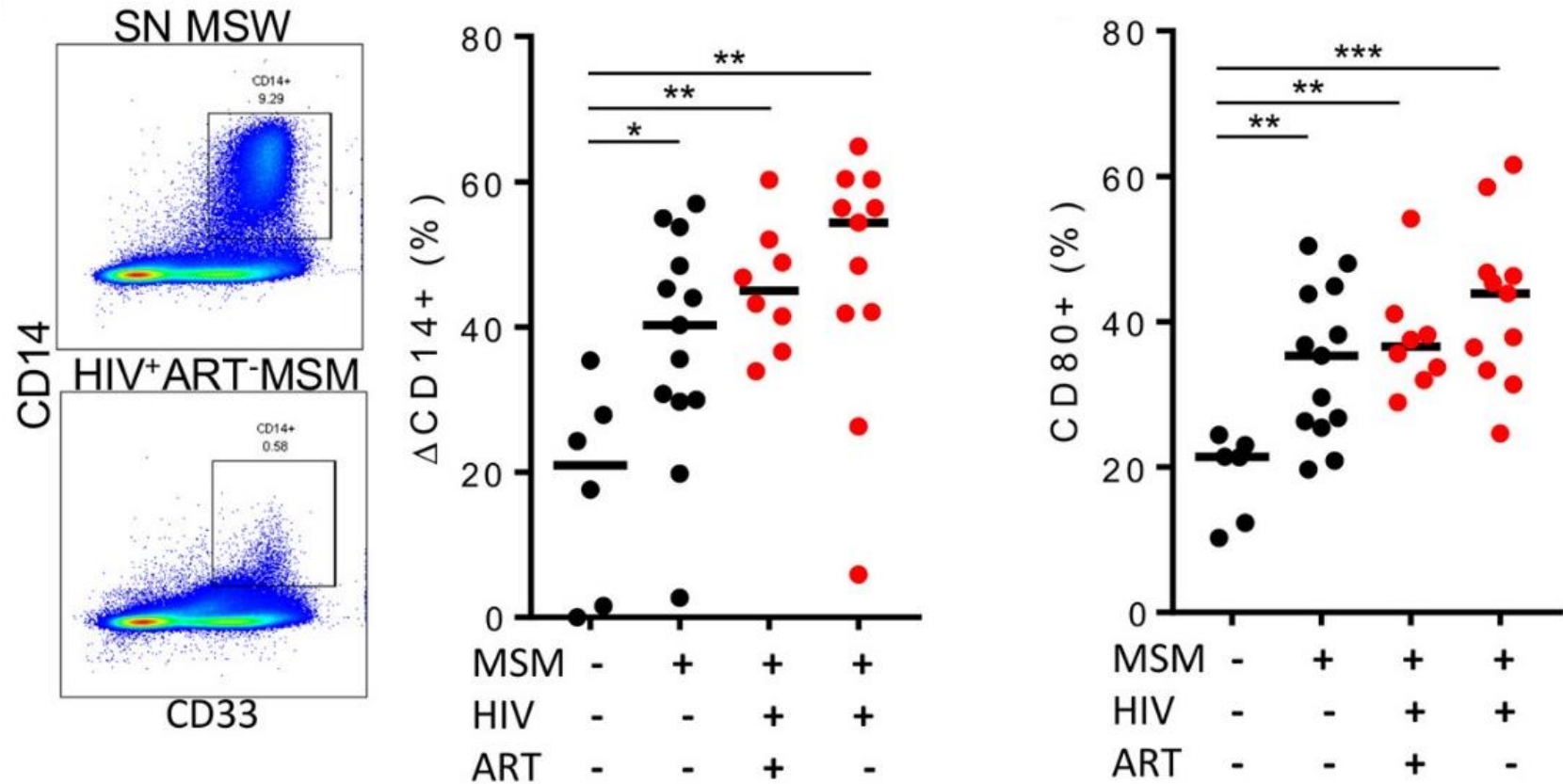
# Fecal Biomarkers Segregate by MSM Regardless of HIV Status

- HIV- MSW
- HIV- MSM
- HIV+ ART+ MSM
- HIV+ ART- MSM

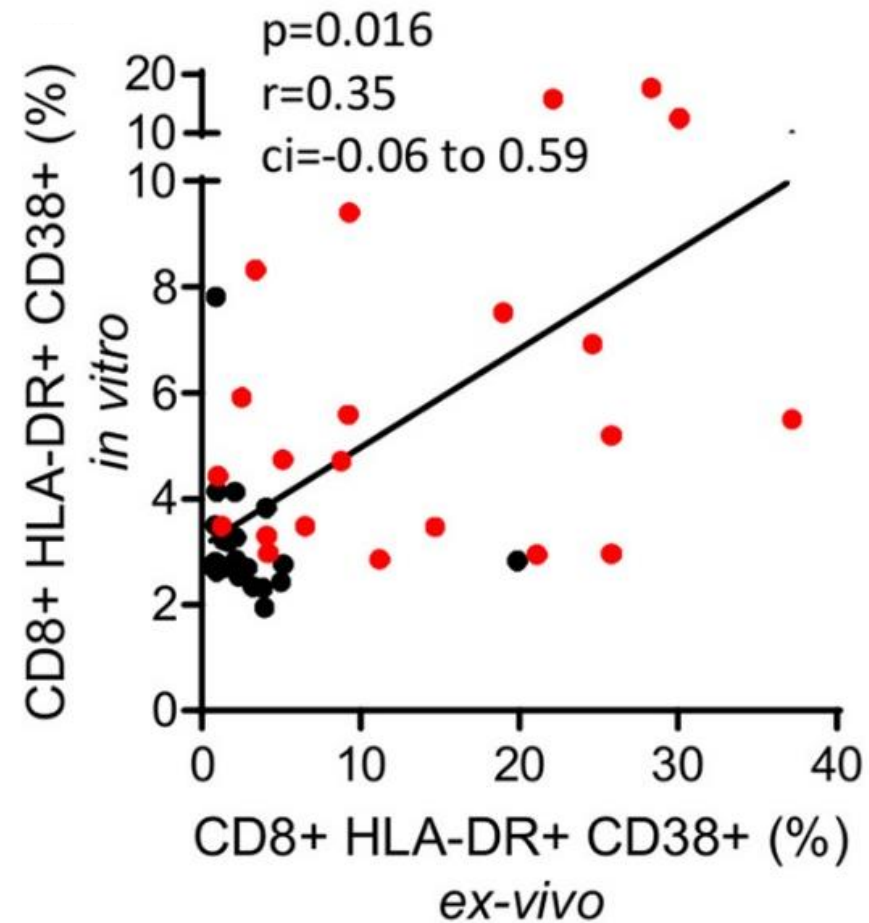
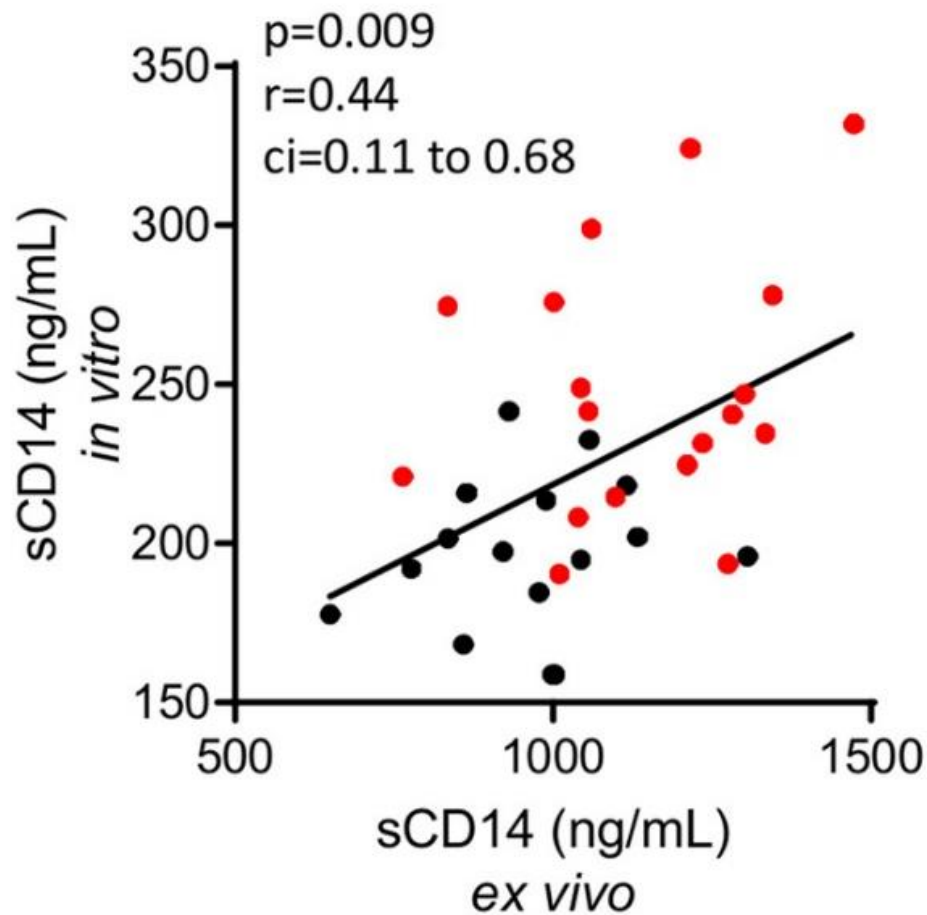


Mantel test  $p = 0.029$

# Fecal bacterial communities from HIV+/- MSM induce monocyte activation

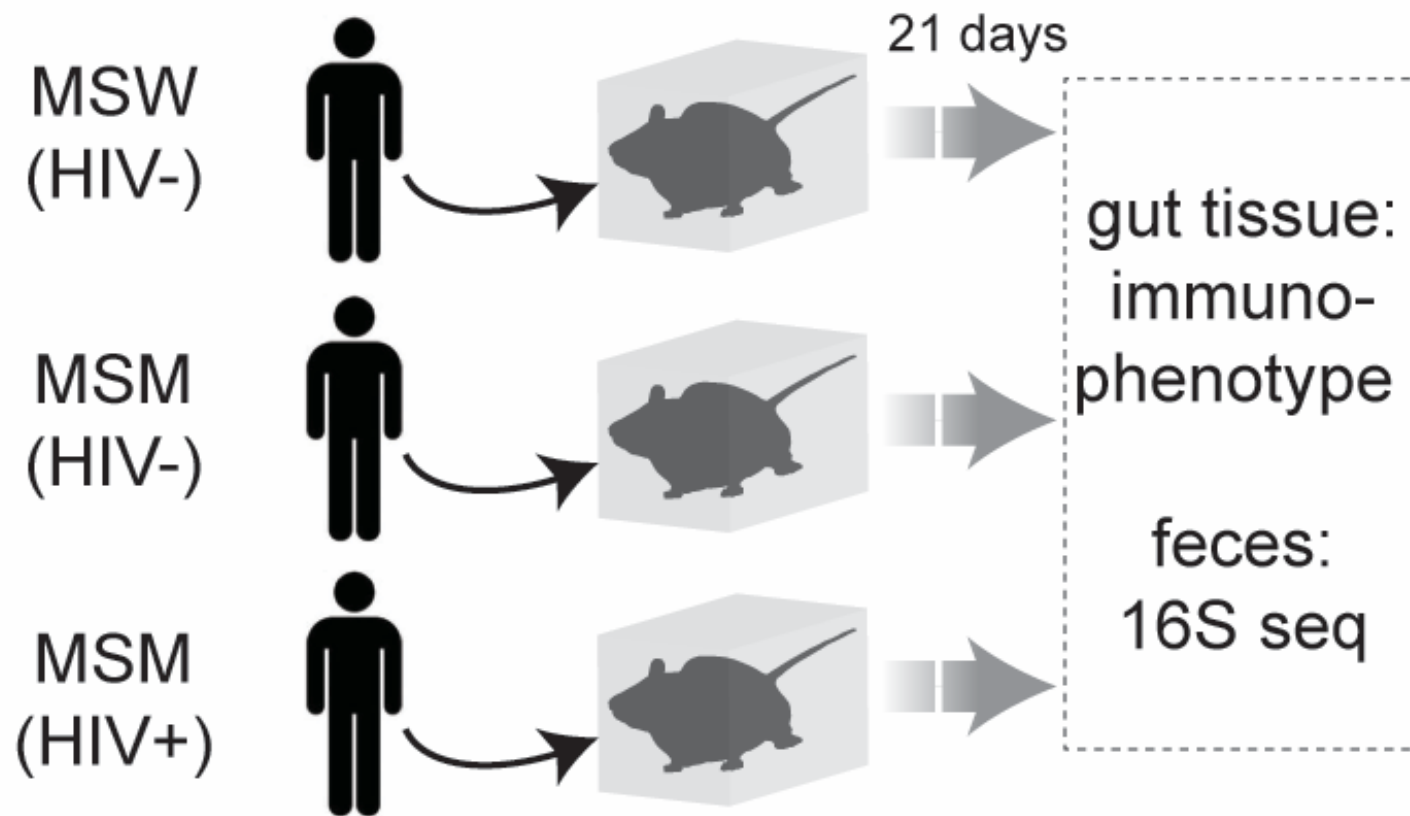


## *In vitro* and *in vivo* markers of inflammation are correlated

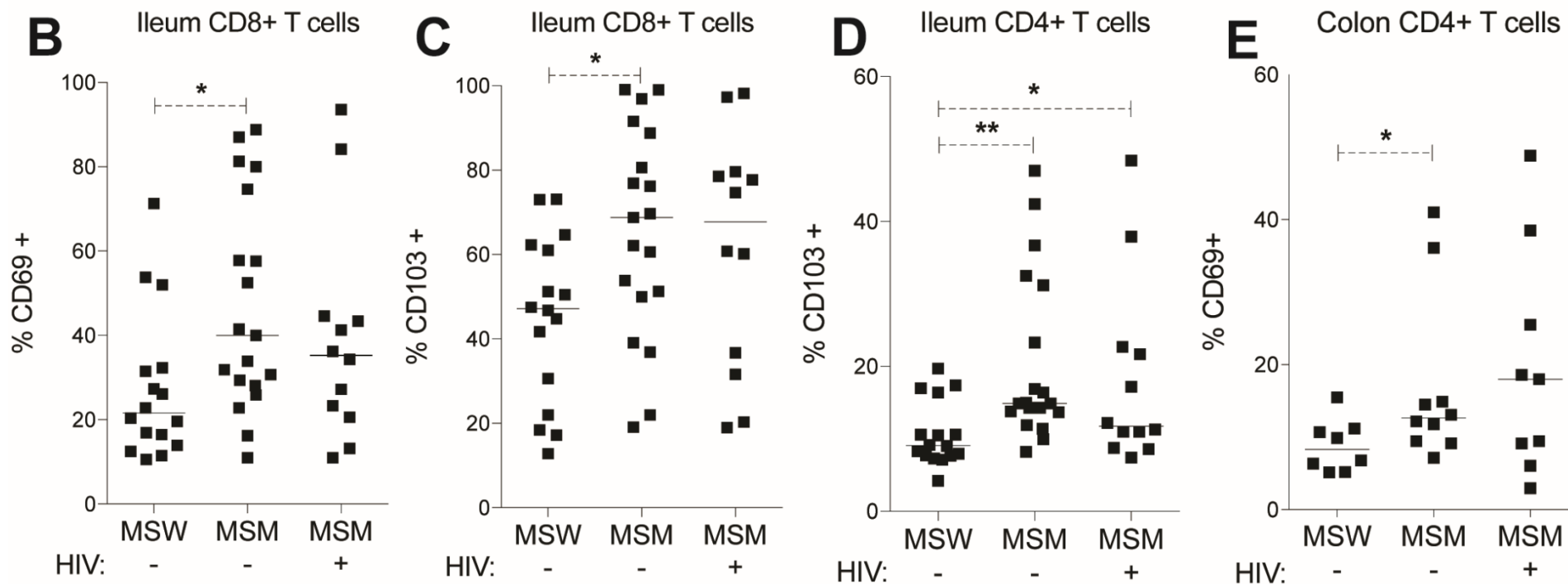




# Fecal microbiota transplant to germ-free mice



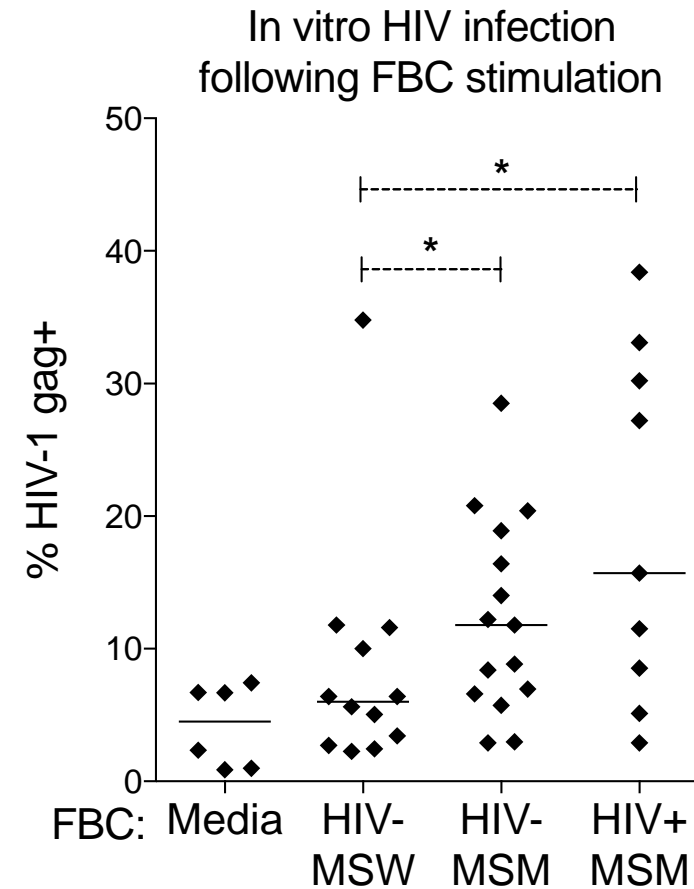
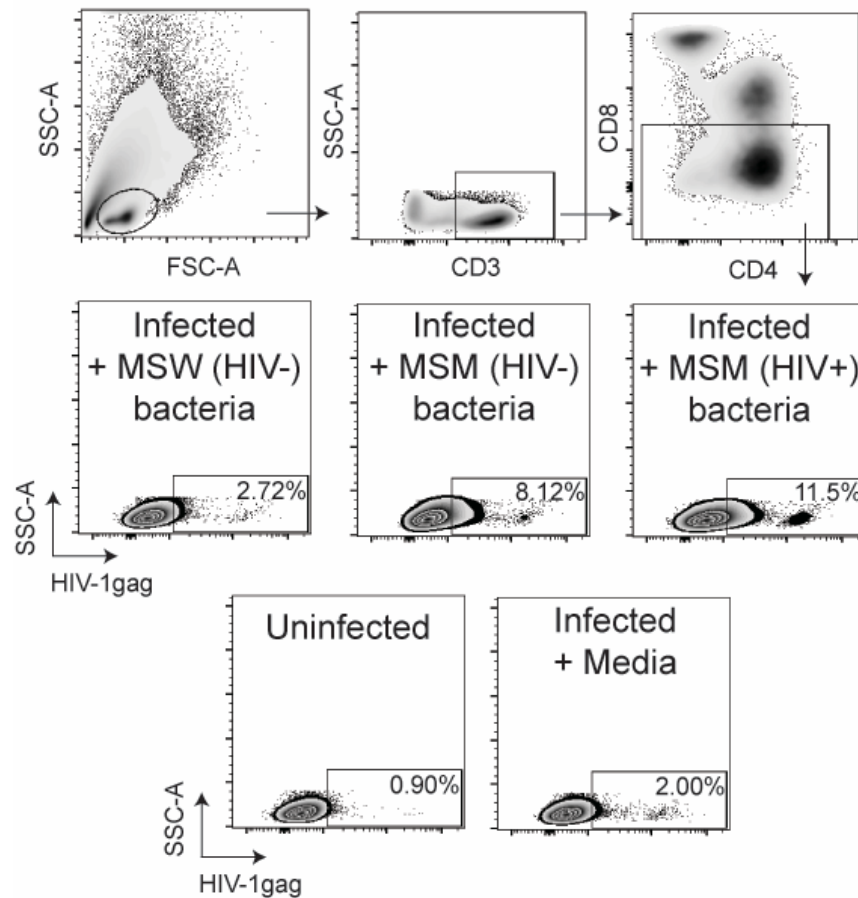
# Immune activation is higher in recipients of HIV- and HIV+ MSM



# Vaginal microbiome and immune activation impacts HIV transmission in women

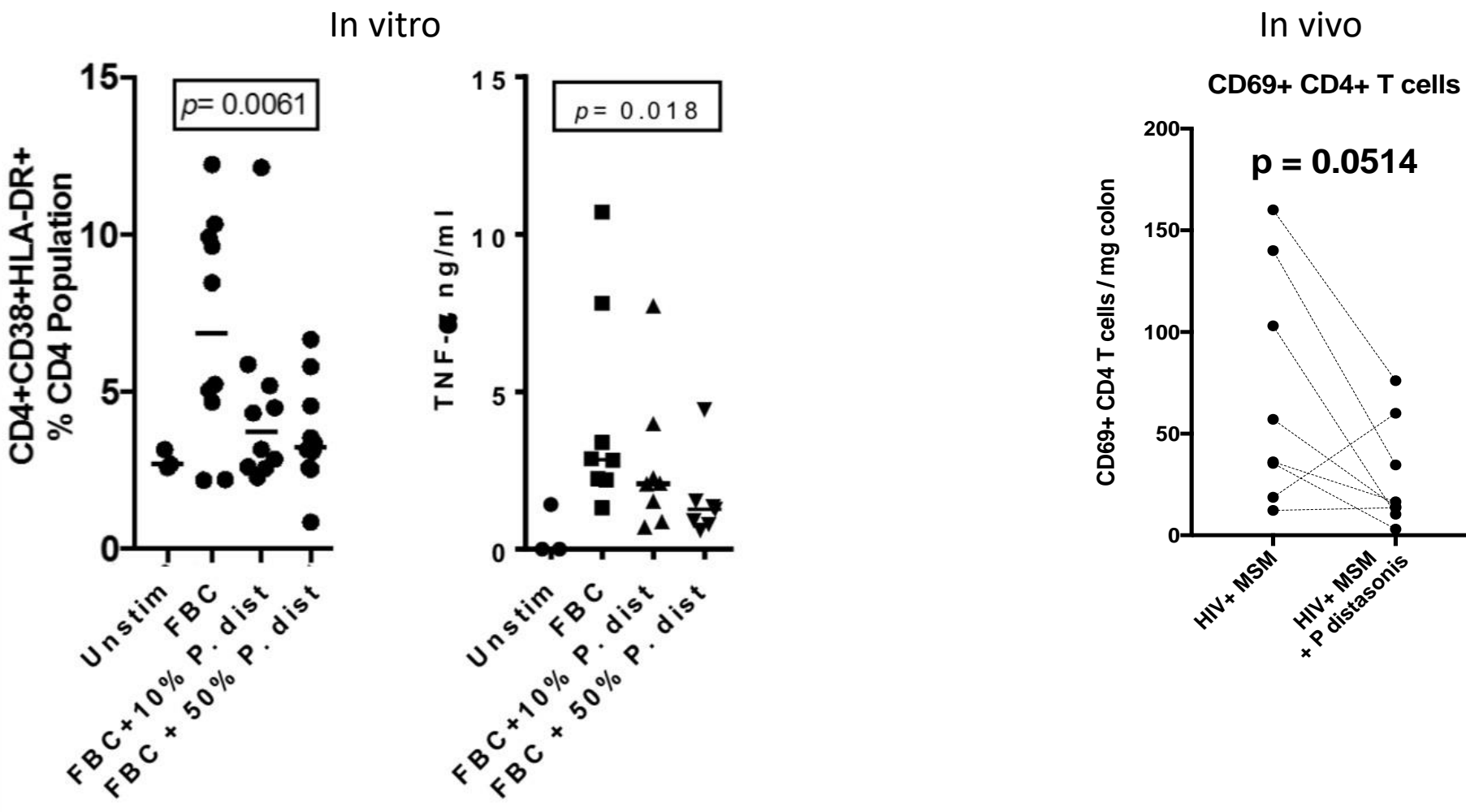
- Bacterial vaginosis increases HIV risk in pregnant and postnatal women in Malawi
  - Taha et al 1998 AIDS
- Systemic inflammatory cytokines and NK cell activation associated with HIV acquisition in South African women
  - Naranbhai et al 2012 JID
- Vaginal inflammatory cytokines associated with HIV risk in South African women
  - Masson et al 2015 CID
- High diversity vaginal microbiomes in asymptomatic South African women associated with vaginal inflammation
  - Anahtar et al 2015 Immunity

# HIV- and HIV+ MSM microbiota stimulate *in vitro* HIV infection





# *P. distasonis* reduces MSM microbiota-induced CD4+ T cell activation



## Summary (Part 2)

- Fecal water cytokines and markers of T cell activation are elevated in HIV-/+ MSM and associated with microbiome composition.
- Fecal bacteria from HIV+/- MSM induce immune activation *in vitro* and *in vivo*.
- MSM-associated microbiota enhance *in vitro* HIV infection of CD4+ T cells from the lamina propria - implications for transmission.
- *P. distasonis* reduces MSM-associated immune activation and is a potential therapeutic probiotic.

# Thanks!

# Study Subjects!



## University of Colorado

### • Palmer Lab

- Preston Neff
- Sam Li
- Jennifer Schneider
- Sharon Sen
- Owen Kruger
- Sara Coleman
- Sabrina Arif
- Kathy Xiong
- Eiko Yamada
- Henning Schade

### Lozupone Lab

Cathy Lozupone  
Jody Donnelly  
Nichole Nusbacher  
Abigail Armstrong  
Cuining Liu

### Tom Campbell

Christine Griesmer,  
Suzanne Fiorillo

### Kristi Kuhn

Erin Severs

### Blair Fenimore

Derek Linderman  
Janine Higgins (Nutrition Core)  
Tyson Marden

### University of Zimbabwe

Margaret Borok

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DIABETES AND DIGESTIVE  
AND KIDNEY DISEASES



Investigating  
HIV-Associated  
Lung Disease

# HIV infection is associated with loss of immune homeostasis and microbial dysbiosis

