

# The microbiota in health and disease: alteration and modulation of intestinal germs and bacteria

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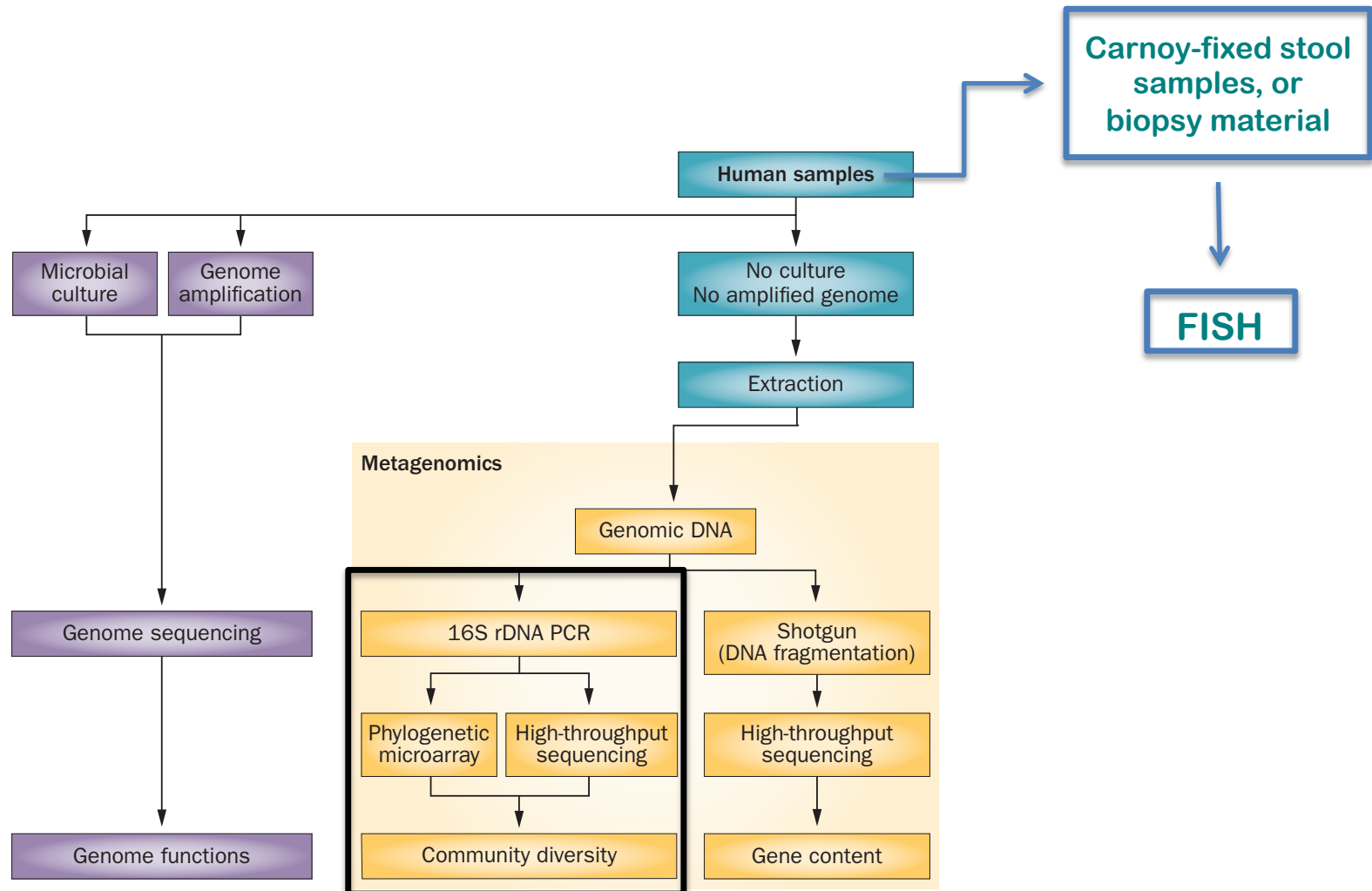


Christian Doppler  
Forschungsgesellschaft



We are not alone ...  
... but who is there?  
... and how many?

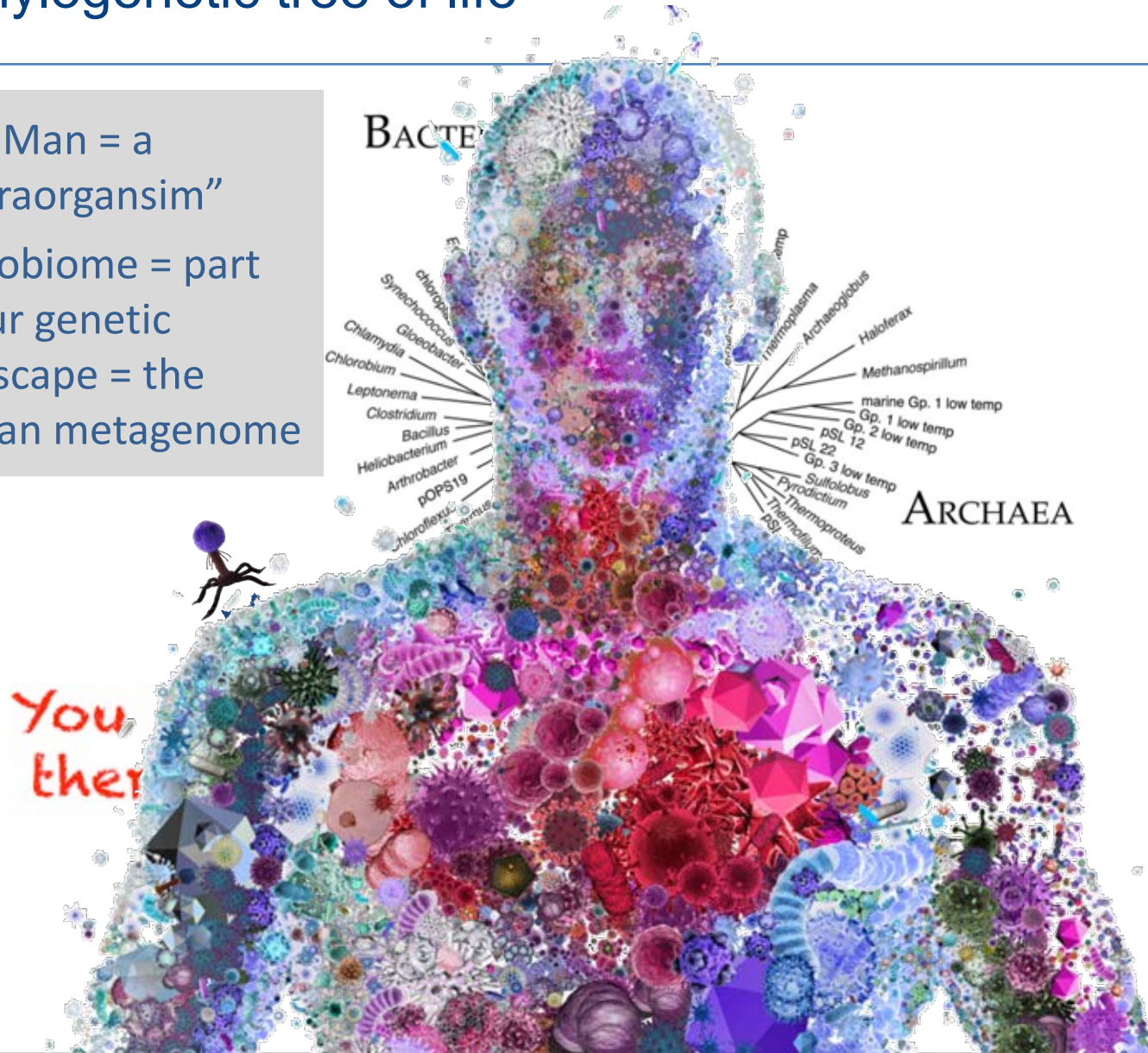
# Studying the human microbiome ...



# The phylogenetic tree of life



- (Wo)Man = a “supraorganism”
- Microbiome = part of our genetic landscape = the human metagenome





# Just bacteria?.... rather not ;-)

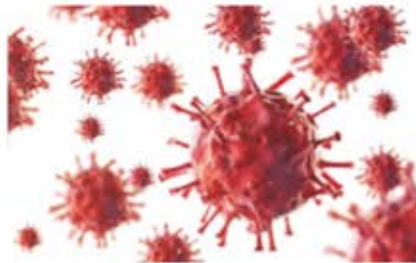


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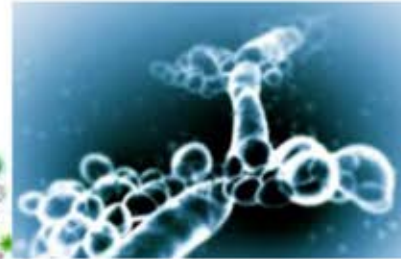
Bakterien



Viren



Pilze



Protozoen



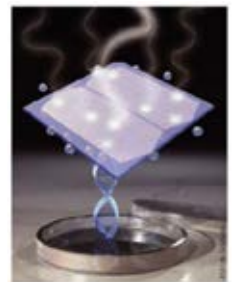
# Generation of reference gene catalogues: Human microbiomes differ in species & genes

- n=124 individuals (Europeans)
- 3.3 Mio bacterial genes (>99%)
- approx. 1150 bacteria
- 160 bacterial species/individual
- 57 species common to >90%
- 530,000 genes/individual



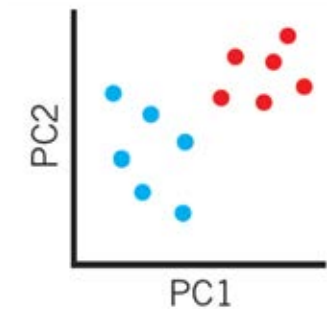
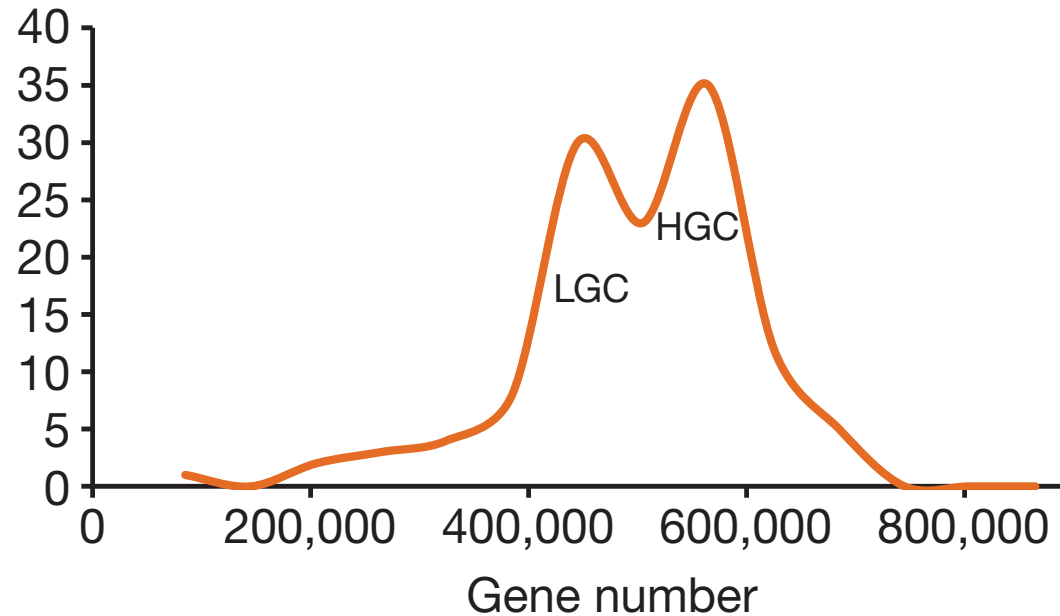
## ICG

- 10,000,000 bacterial genes in n=1267 individuals
- 500,000 genes per individuum
- approx. 50% of genes shared by 50% of individuals (**metagenomic core**)
- Comparable catalogues for Europeans, Americans, Japanese, Chinese



Qin J et al. Nature 2010; 464:59-65  
Li et al. Nat Biotechnol 2014; 32: 834-41

# Human microbiomes differ in gene counts – gut microbial structure as a biomarker?



- ✓ Gut microbial composition in **obese** and **non-obese** Danish sj (n=292)
- ✓ **Low gene count (LGC)**, 23% of population more adiposity, IR, dyslipidemia and a higher degree in inflammation
- ✓ **LGC** = Predictor for relaps in IBD, aggravation of chronic conditions, etc.

Cottillard A et al. Nature 2013; 500: 585-8  
Le Chatellier E et al. Nature 2013; 500: 541-6



# Low Species Richness and Low Gene Count (LGC) characterize dysbiosis and bad health

## Intestinal disorders

- Crohn's disease

Gevers *CH&M* 2014, Frank *PNAS* 2007, Becker *ILAR J* 2015, Qin *Nature* 2010

- Ulcer
- Colitis
- Irritable
- AA

Dysbiosis reflects an alteration of the mutualistic host-microbe relationship with recurring features

Cottrell *Nature* 2015, Turnbaugh *Nature* 2007

Low species richness and low *Faecalibacterium prausnitzii*

Low grade inflammation

Colorectal cancer

Feng *Nat Commun* 2015, Moschen *CH&M* 2016, Arthur *Science* 2012





# GOOD BUGS

# BAD BUGS



„symbionts“



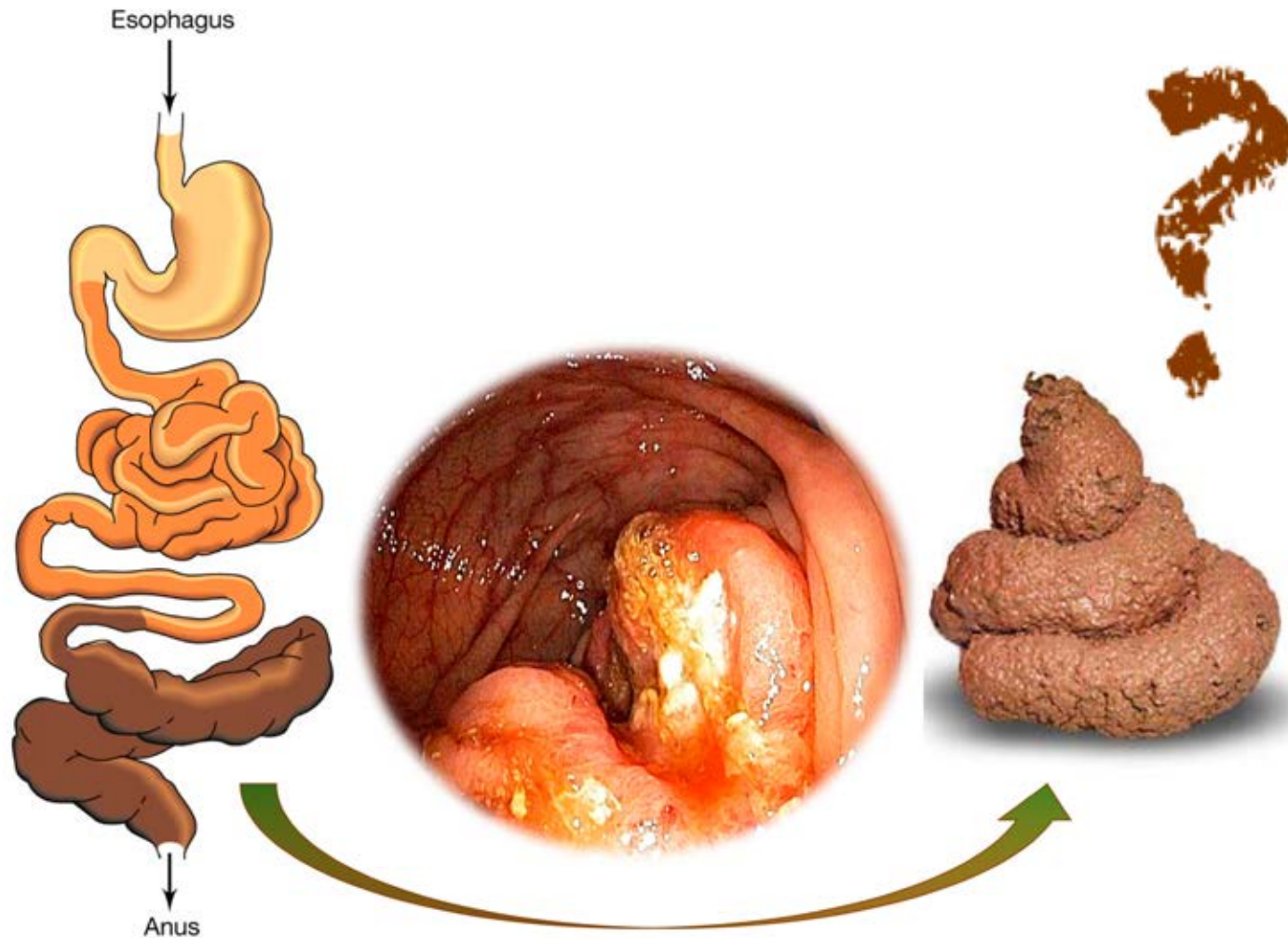
„pathobionts“



# How is CRC linked to our microbiome?



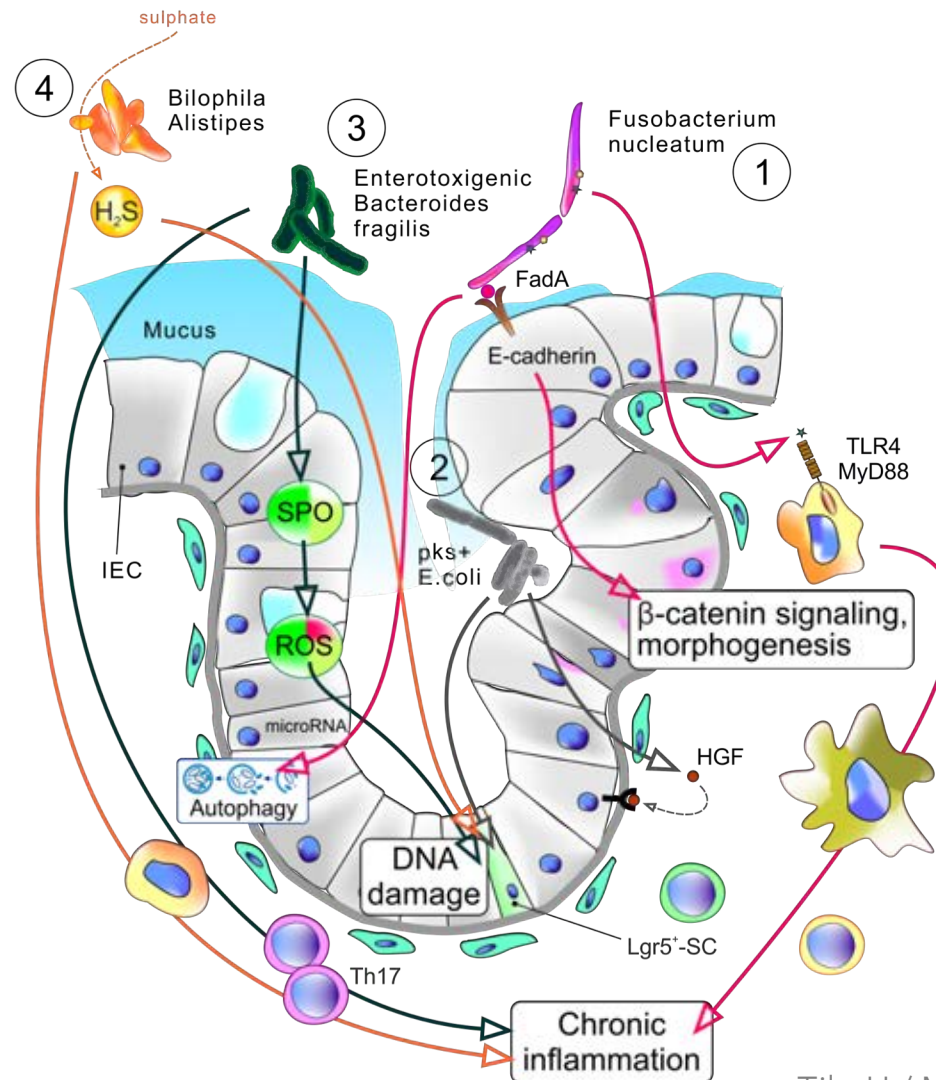
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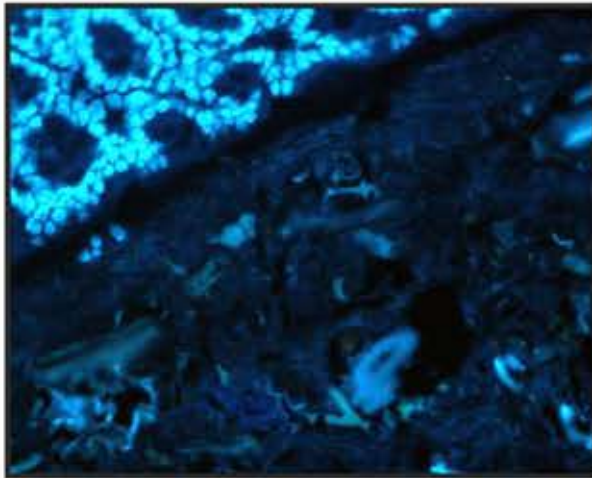
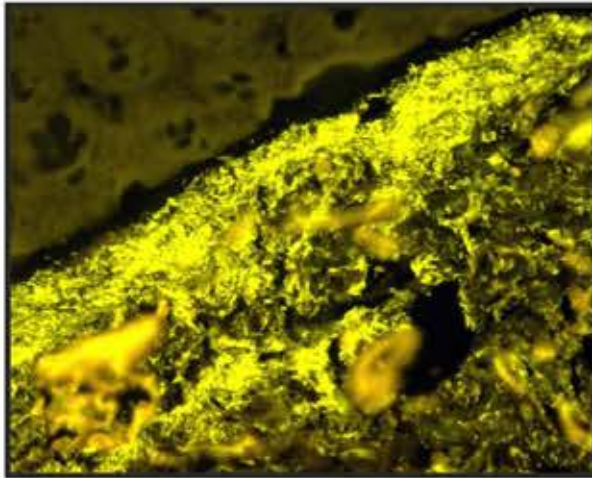
Feng et al. Nature Communication 2015

# Which are the bad bugs ?

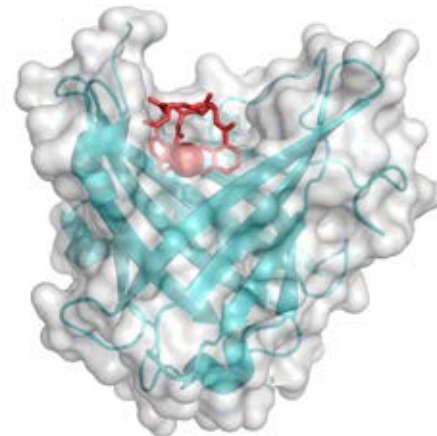
## Mechanism linking specific bacteria with CRC



Tilg H / Moschen AR. Cancer Cell 2018



Host factors shaping microbial communities are important for building and maintaining a homeostatic relationship and to defend host integrity from aggressive bacteria.

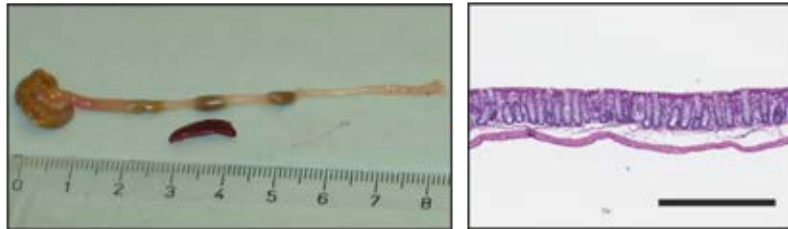


Moschen AR et al. unpublished

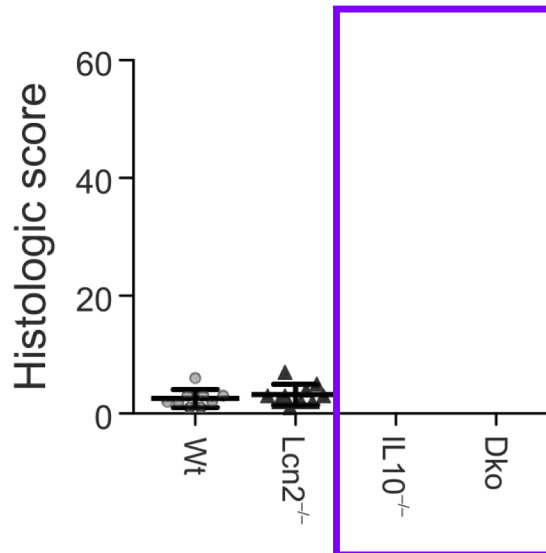
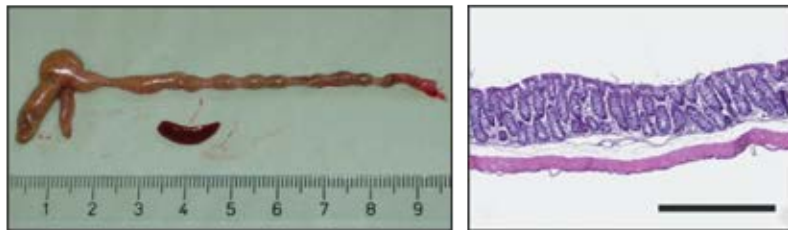


# In the steady-state $Lcn2^{-/-}$ mice are healthy, while $IL10^{-/-}/Lcn2^{-/-}$ show massive inflammation ...

Wildtype

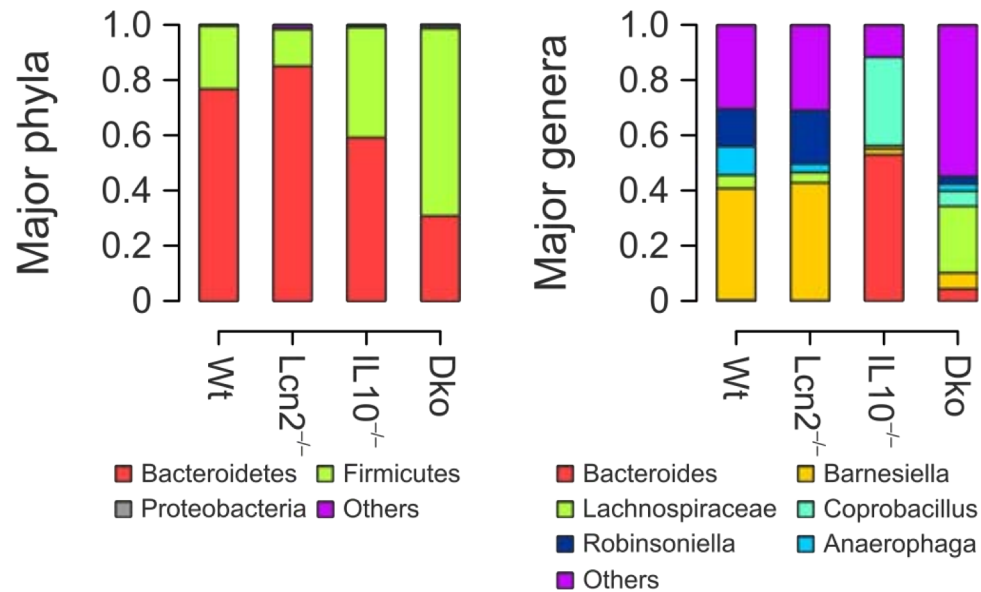


$Lcn2^{-/-}$



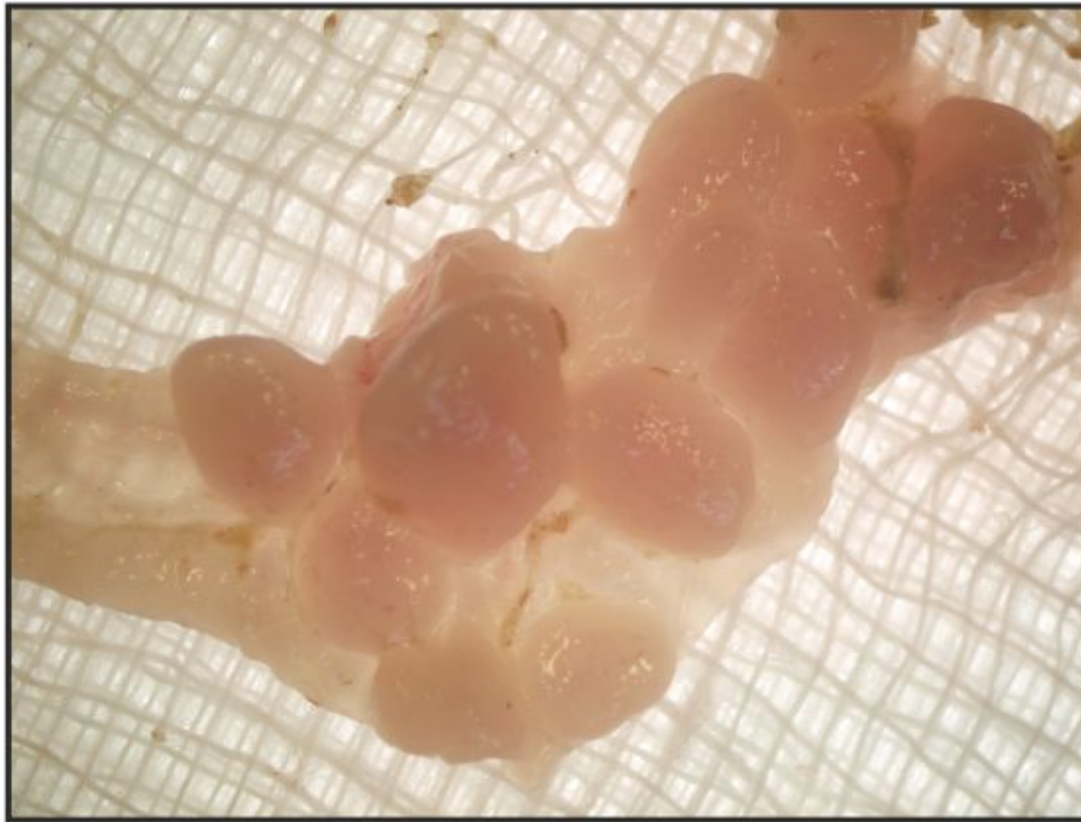
Moschen et al. Cell Host & Microbe 2016

# IL10<sup>-/-</sup>/Lcn2<sup>-/-</sup> animals exhibit an altered microbial ecology ...



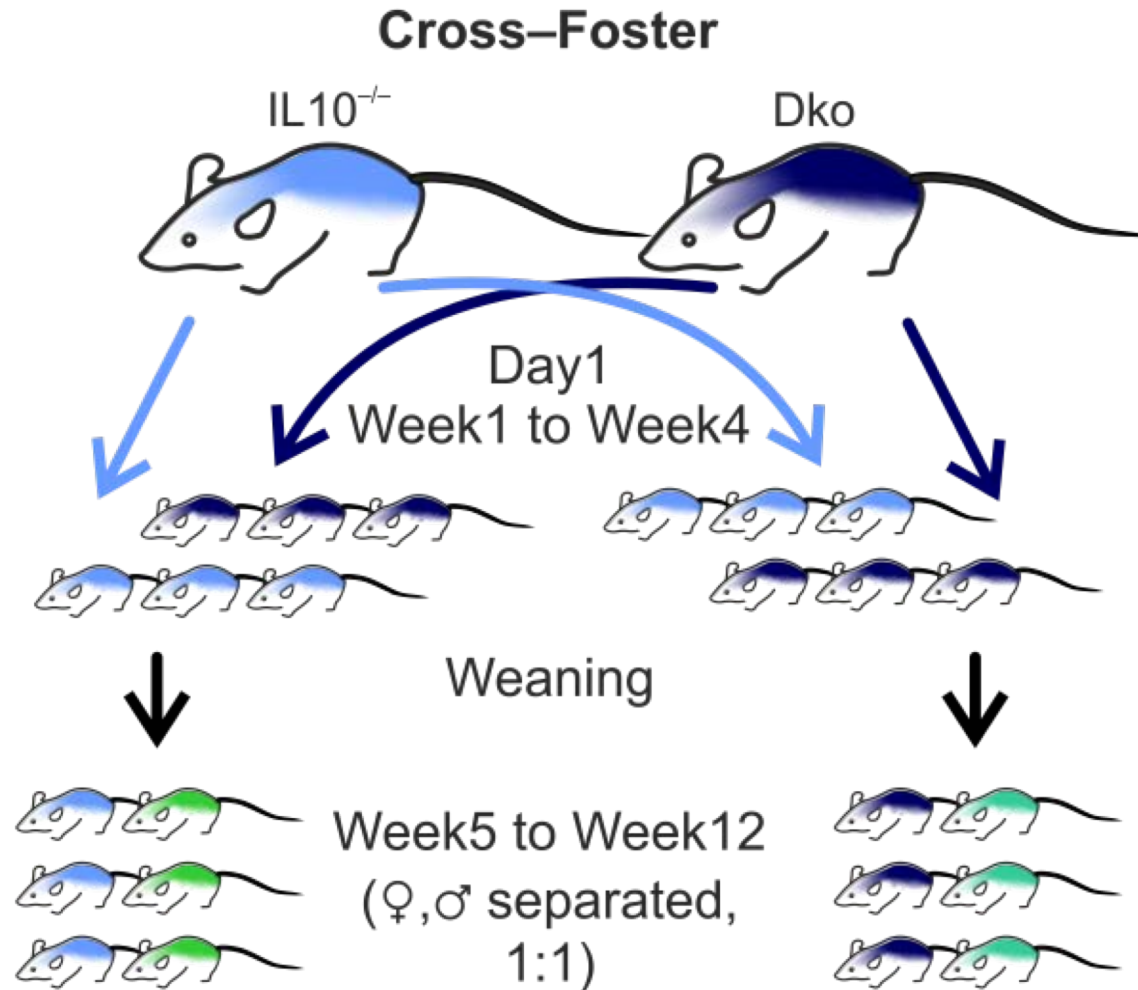
In the steady-state  $Lcn2^{-/-}$  mice are healthy, while  $IL10^{-/-}/Lcn2^{-/-}$  show massive inflammation ...

## **Lcn2/IL-10 dko**



Moschen et al. Cell Host & Microbe 2016

... and transmissible to cross-fostered animals.

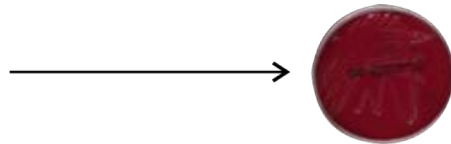






# So what next ... ?

*Alistipes* spp.



whole genome  
sequencing



metatranscriptomics

metabolomics

**identification of  
metabolites,  
metabolic  
pathways,  
genes of  
interest**

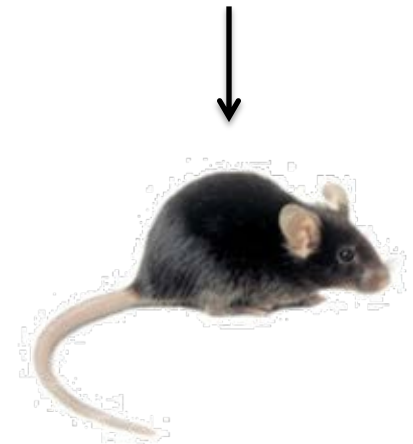
„in vitro“ environments

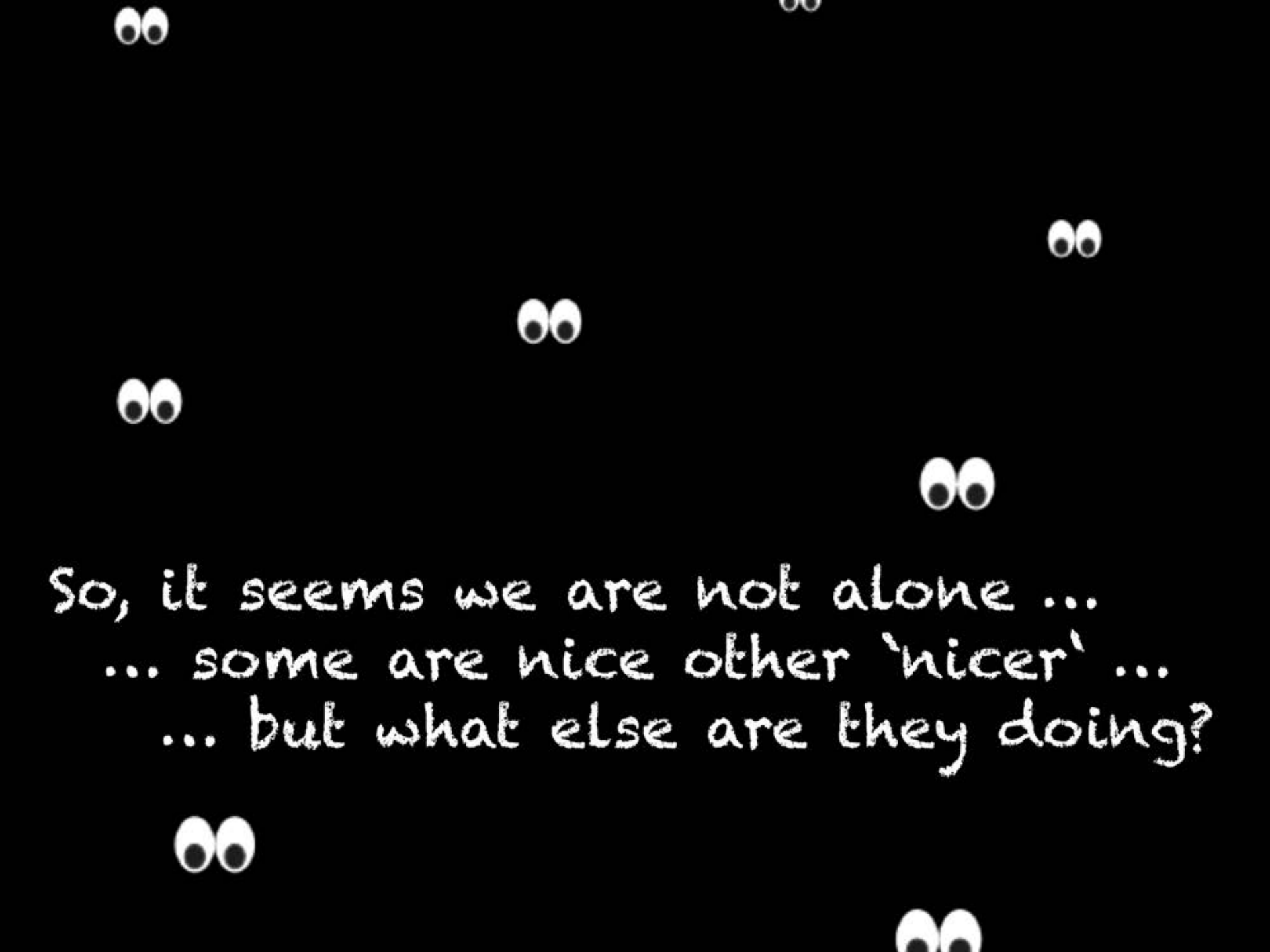
- O<sub>2</sub> saturation
- pH
- bile acids
- animal fat/protein
- MAC

**human/mouse  
organoid cultures**



**specific mutants**

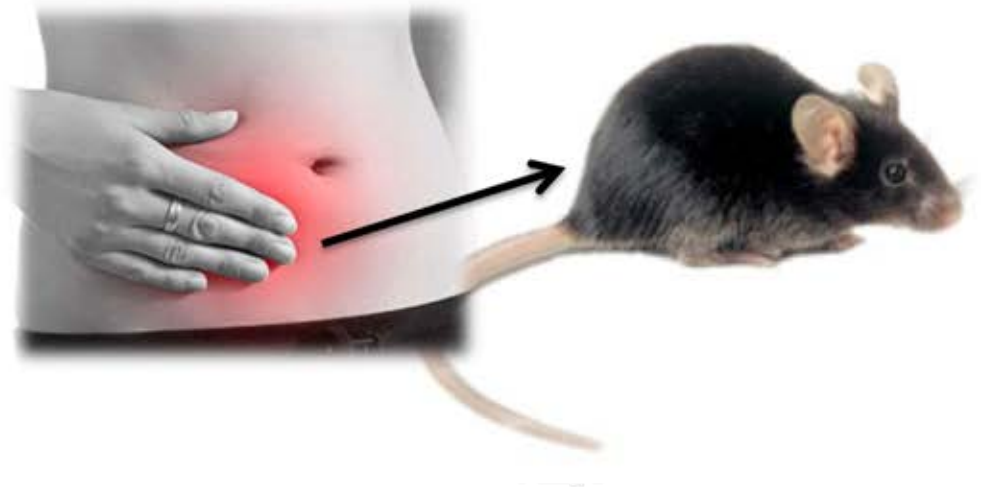




So, it seems we are not alone ...  
... some are nice other 'nicer' ...  
... but what else are they doing?

# Transmitting gut function and behavior from IBS patients to mice ... microbial composition not relevant!

- FMT from IBS-D patients plus/minus anxious behavior and healthy controls to GF mice
- Study intestinal biology and behavior.



De Palma et al. Sci Transl Med 2017

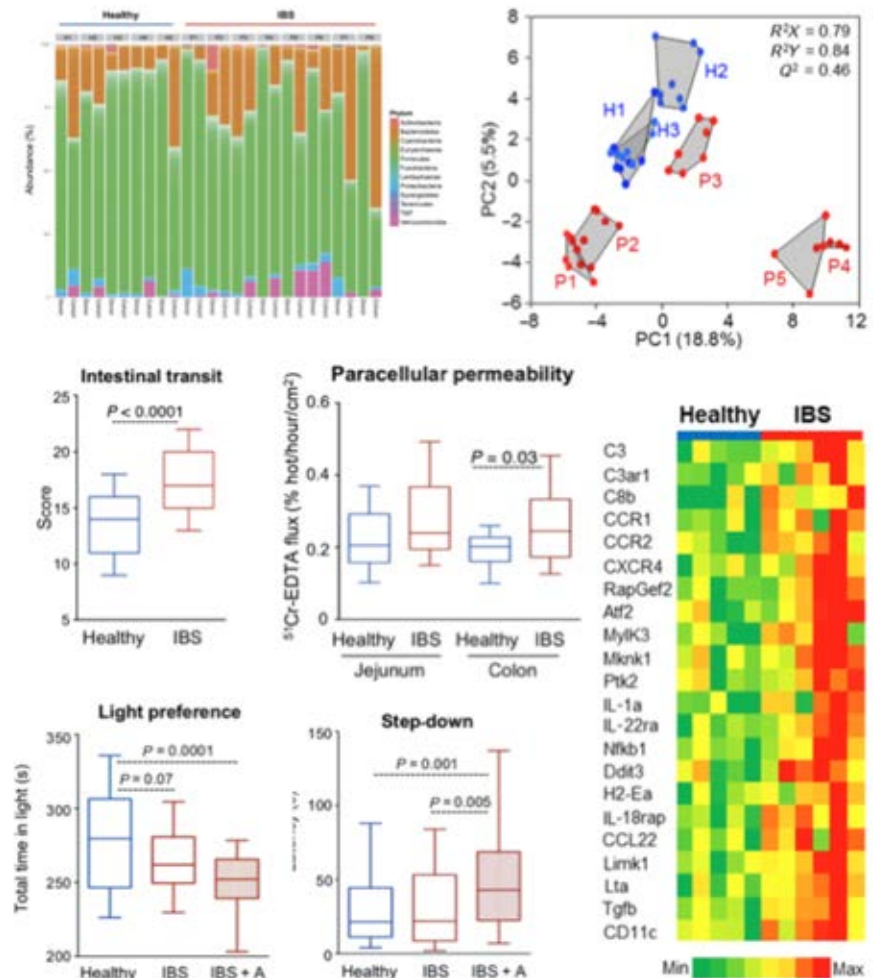


# Transmitting gut function and behavior from IBS patients to mice ... microbial composition not relevant!



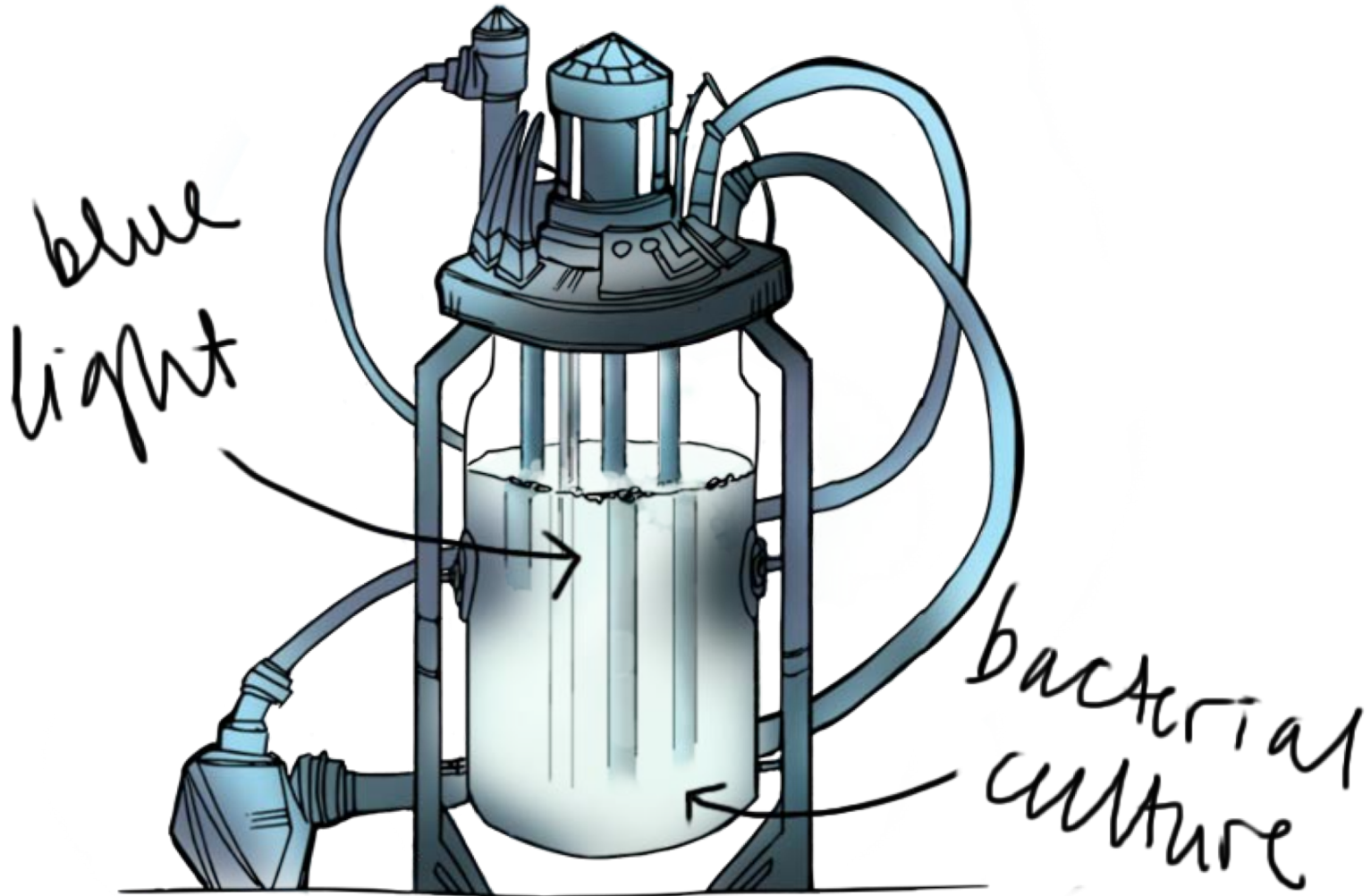
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- No difference in taxonomic composition between IBS-D and control recipient mice.
- Differences in the profile of serum metabolites.
- IBS-D microbiota induces:
  - ✓ Shorter GI transit time
  - ✓ Impaired gut barrier function („leaky gut“)
  - ✓ Higher grade of immune activation
  - ✓ More anxiety

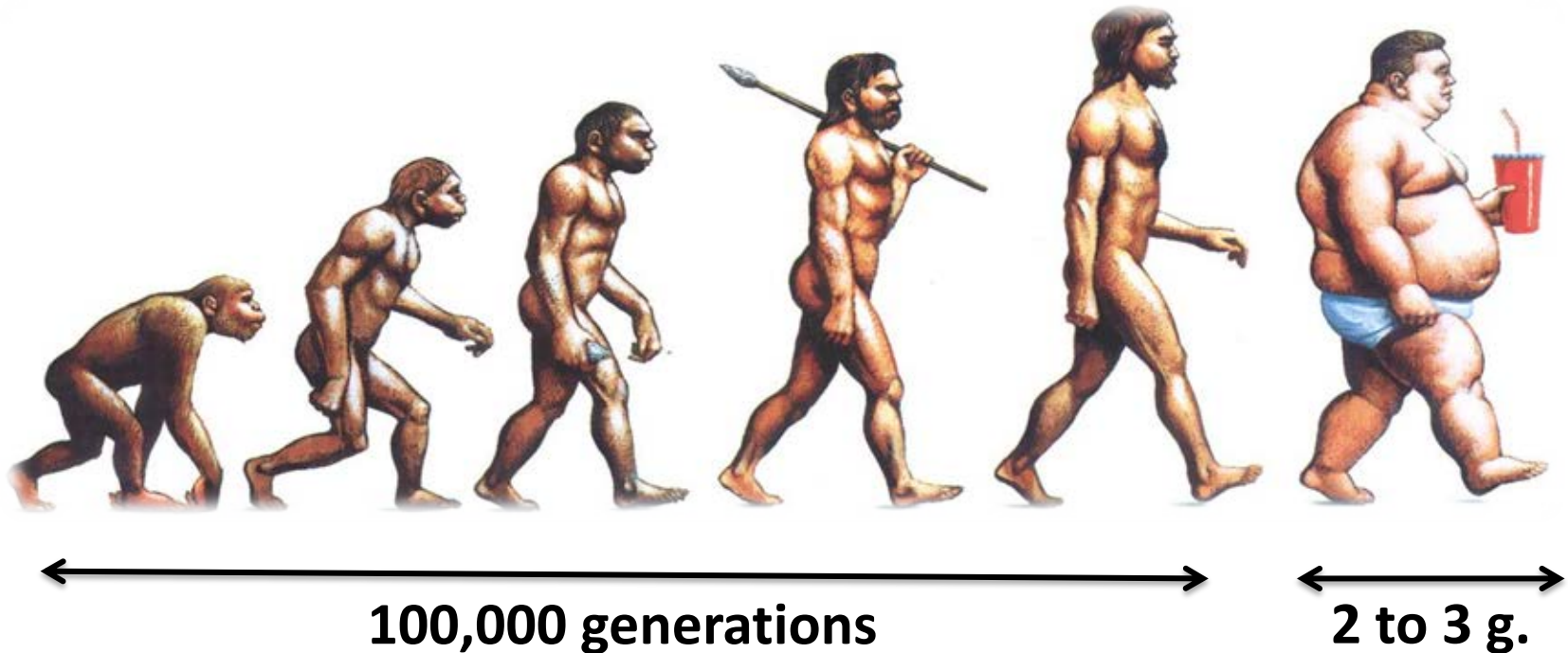


De Palma et al. Sci Transl Med 2017

# Our GI tract is, so to say, a huge bioreactor ...



***A co-evolution from the very beginning  
accounts for a high grade of mutualism***



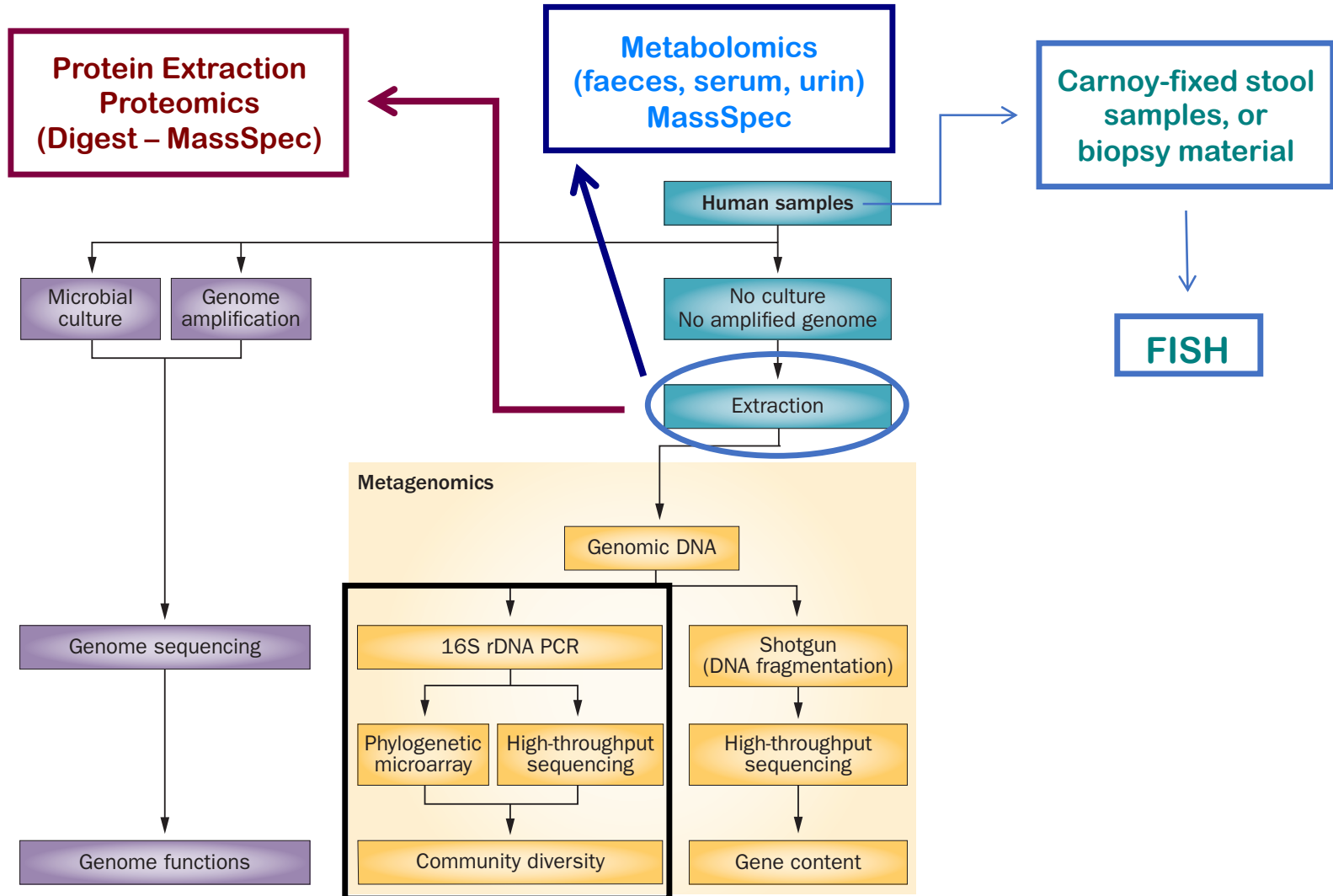
# PROBLEM: „mechanism“ gap in current microbiome studies



Patterson/Turnbaugh. Cell Metabol 2014



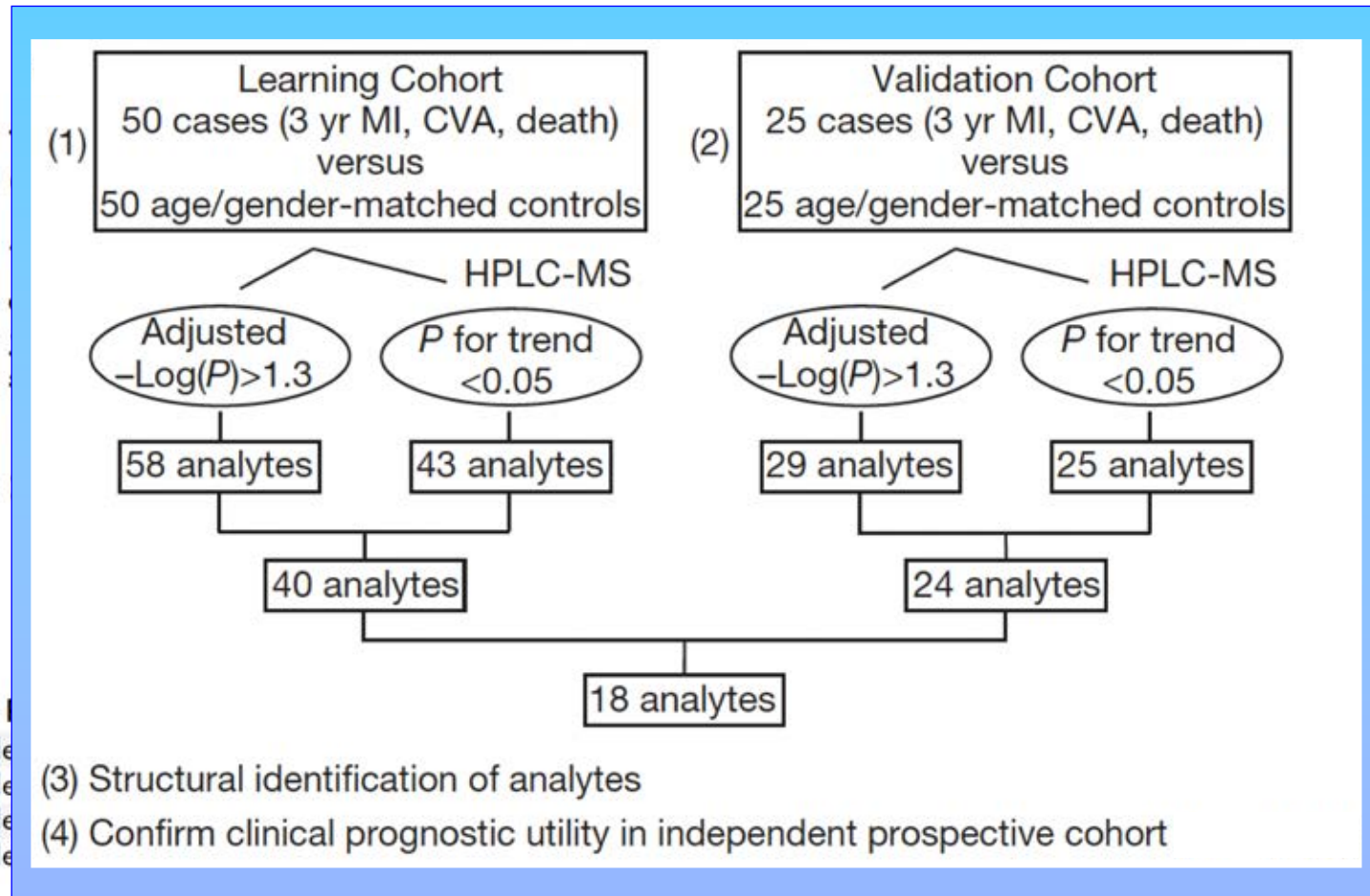
# Studying the human microbiome ... the multi-‘omic’ approach



# Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk



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No. at  
Quartile  
Quartile  
Quartile  
Quartile

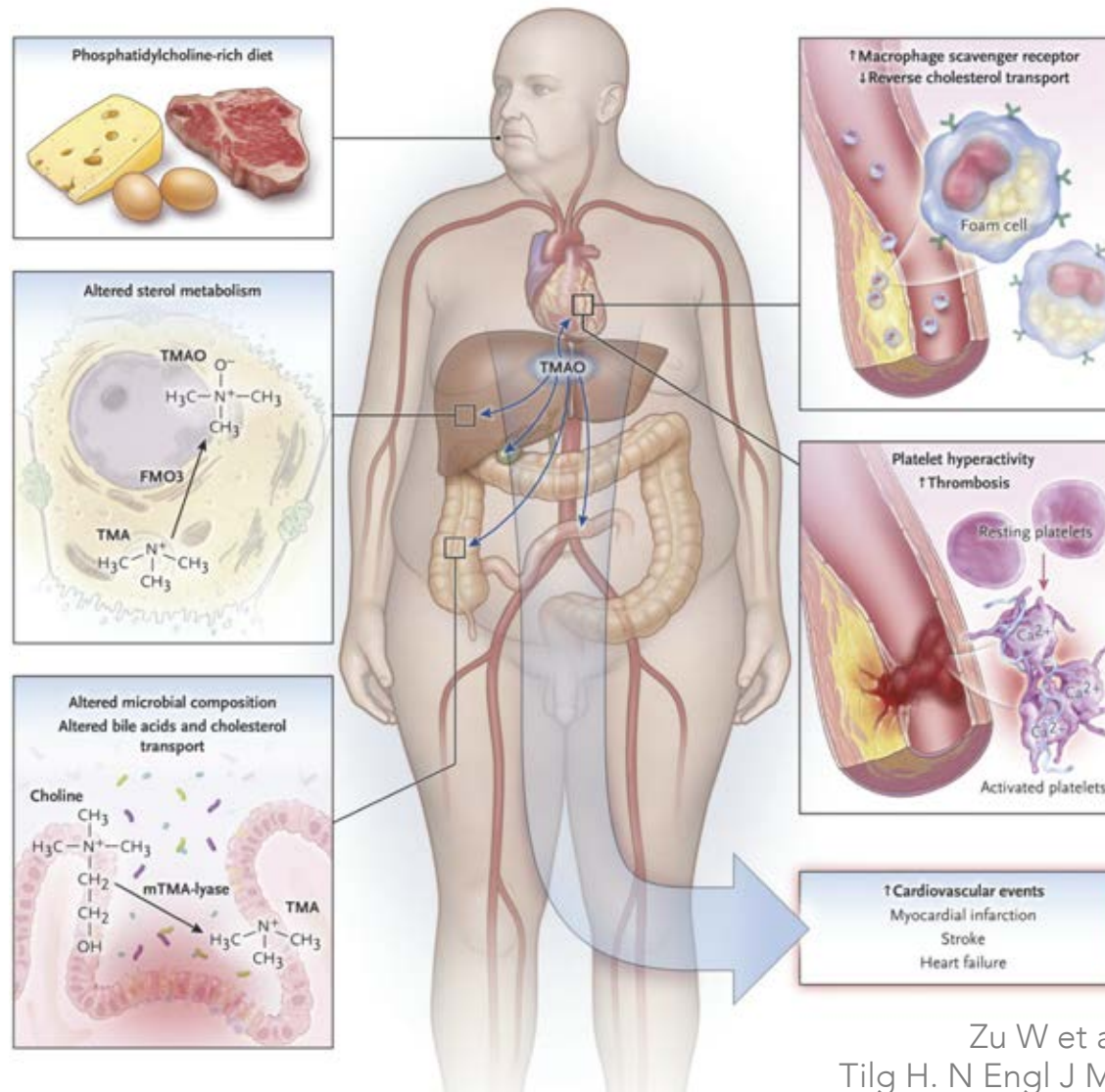
MAO  
death

Wang Z et al. Nature 2011; 452:57-63  
Tang WHW et al. N Engl J Med 2013; 368:1575-84

# Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk



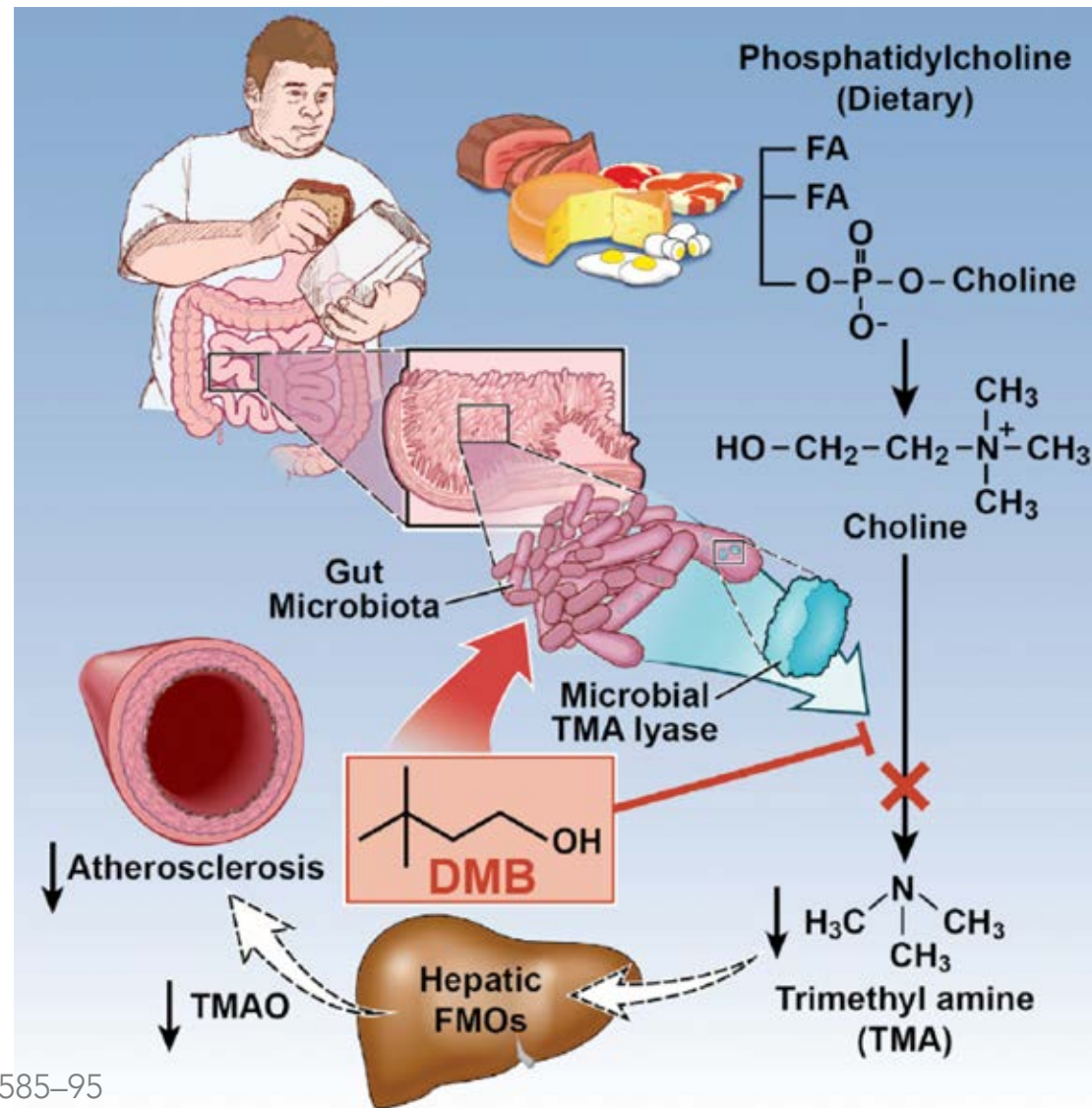
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Zu W et al. Cell 2016;165:111-124  
Tilg H. N Engl J Med 2016; 374:2494-2496

# Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk

**Non-lethal Inhibition of Gut Microbial  
Trimethylamine Production for the Treatment of  
Atherosclerosis**

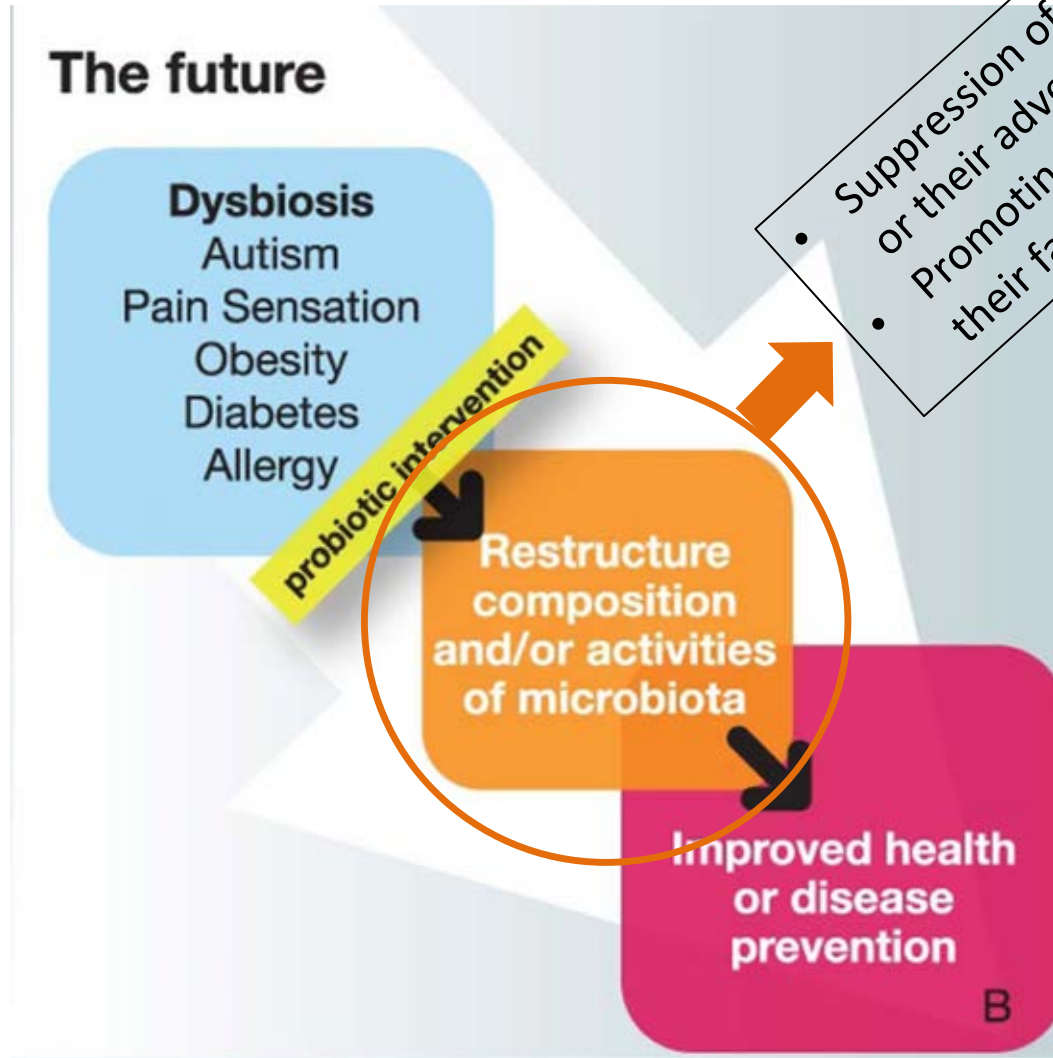


Wang et al. Cell 2015;163:1585–95

How to translate this knowledge  
into clinical practice ... ?



# Intervening in the microbiome ...



## Diet and supplementations

- Composition of diet (low fiber, low FODMAP, GFD)
  - Calories, minerals, vitamins, trace elements
  - Fasting periods ?

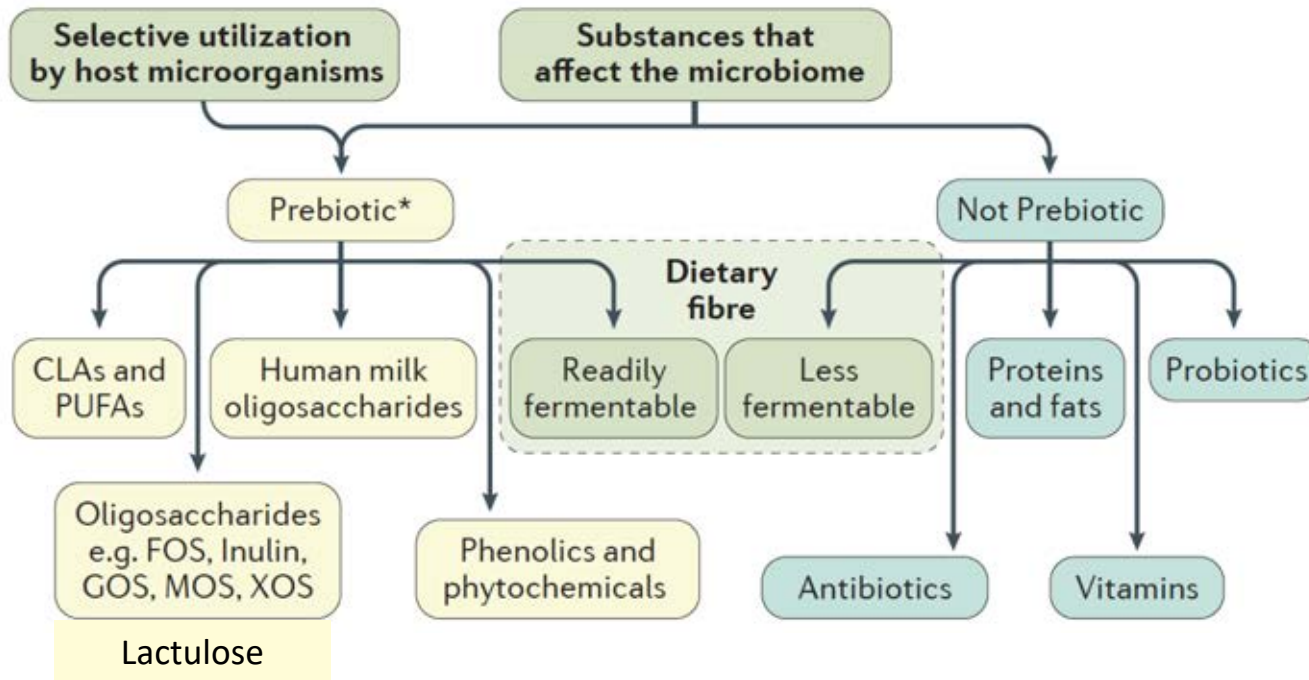
## Removal of predisposing factors

- Treatment of hyperglycemia, endocrine/motility dysfunction
  - Stopp PPIs, antibiotics, immunosuppressives, NSAIDs etc.

## Interventions

- Biotherapy: Prebiotics, Probiotics, (Synbiotics, Postbiotics)
  - Fecal microbiome transplantation
    - Antibiotics
  - Precision editing ?

# What are prebiotics ?

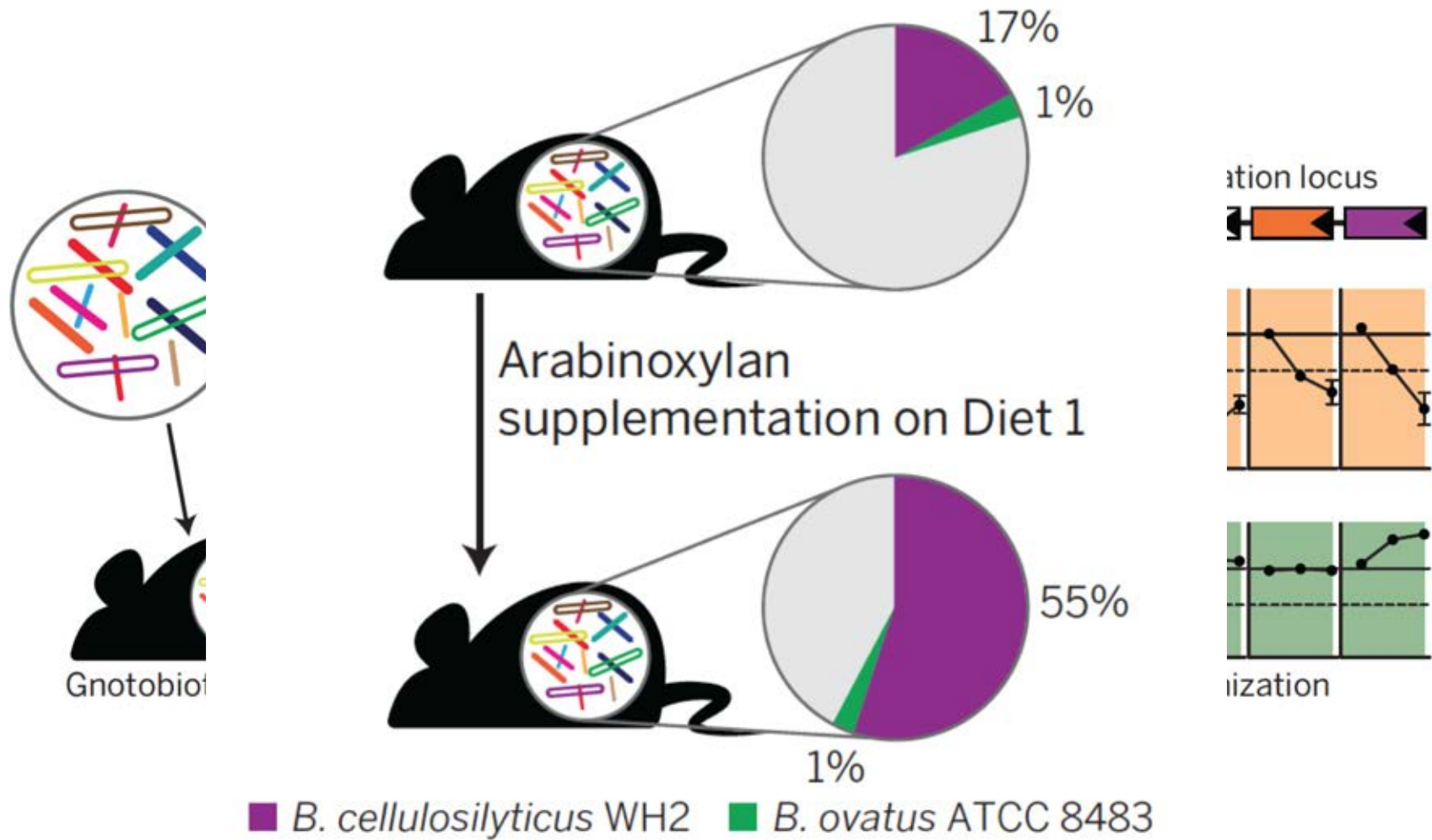


International  
Scientific  
Association  
for Probiotics  
and  
Prebiotics  
(ISAPP)  
Consensus

- „Prebiotics are fermentable, non-digestible oligosaccharides that increase the activity and number of some bacteria in the intestine, thus, promote the host health“
- ... ‘a substrate that is selectively utilized by host microorganisms conferring a health benefit’ ...

Olano & Corzo. J Sci Food Agriculture 2009, 89(12), 1987-1990  
Gibson GR et al. Nat Rev Gastroenterol Hepatol 2017

# Next generation prebiotics ?

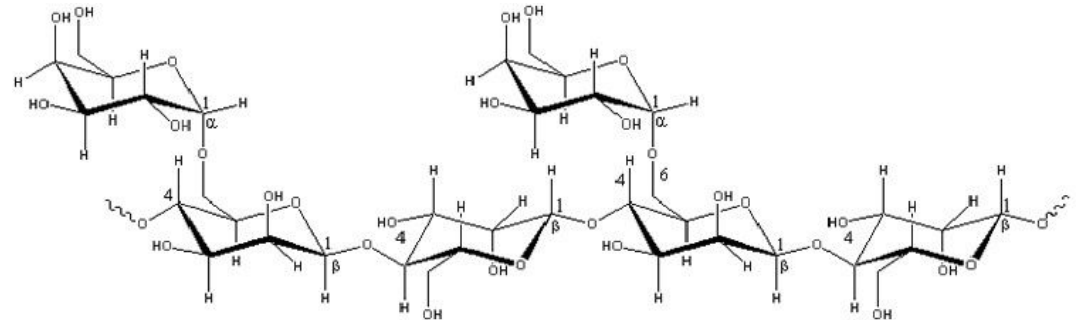


Wu M, ... & Gordon JI. Science 2015; 350:55

# The PAGODA study

Linking PHGG-induced alterations in gut microbial composition with genus- and species-specific fitness determinants on the levels of bacterial gene expression and metabolism

PAGODA STUDIE



Metatranscriptomics

Metabolomics  
(MR Spektroskopie,  
Gaschromatographie)

Metagenomics (16S)

Mikrobiom



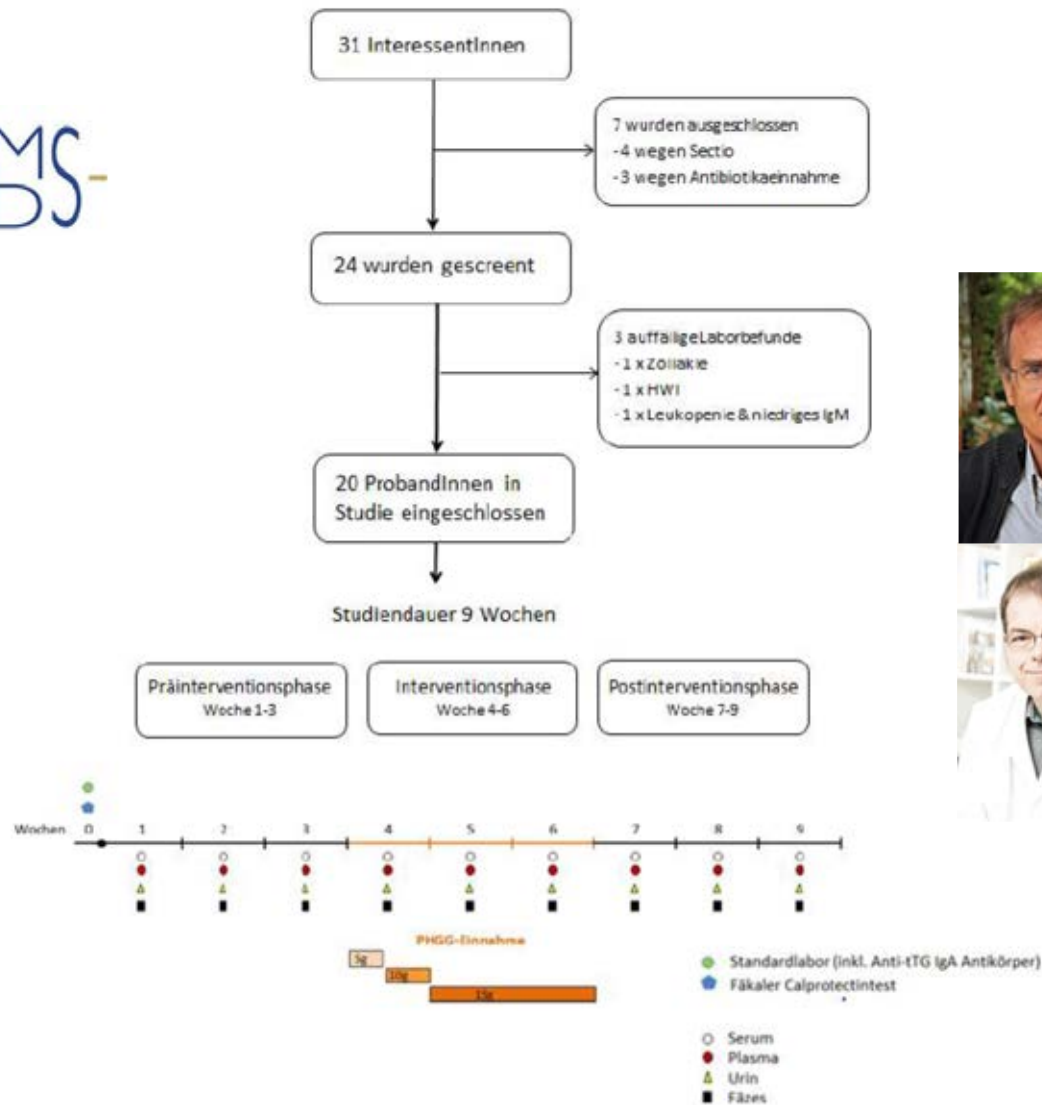


# The PAGODA study



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Judith Tragust




# The PAGODA study – clinical outcomes



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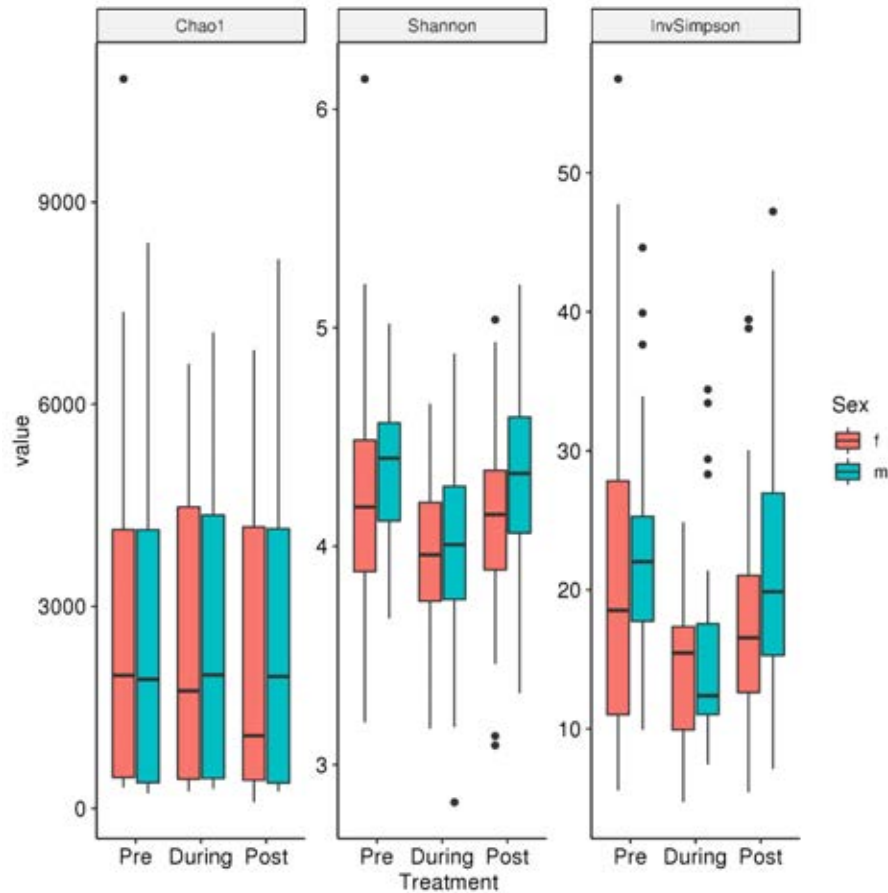
Parameter	Study Period			P Values		
	"Pre"	"During Intervention"	"Post"	pre - during i.	during i. - post	pre - post
Stool Frequency						
total (n = 19)	1.13 ±0.46	1.27 ± 0.47	1.22 ± 0.41	0.002	0.172	0.066
male (n = 8)	1.11 ± 0.37	1.38 ± 0.55	1.26 ± 0.45	< 0.001	0.077	0.050
female (n = 11)	1.15 ± 0.53	1.18 ± 0.41	1.18 ± 0.39	0.980	0.576	0.593
Stool Consistency						
total (n = 19)	3.48 ± 1.04	3.72 ± 0.84	3.48 ± 0.82	0.020	0.030	0.840
male (n = 8)	3.27 ± 0.97	3.91 ± 0.89	3.47 ± 0.87	< 0.001	0.020	0.290
female (n = 11)	3.64 ± 1.08	3.59 ± 0.79	3.48 ± 0.81	0.770	0.510	0.340

## Bristol Stool Chart

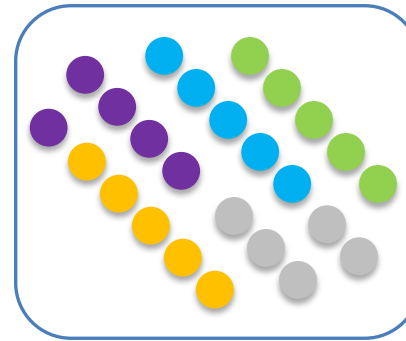
Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clean-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. Entirely Liquid

# The PAGODA study – community structure „alpha diversity“

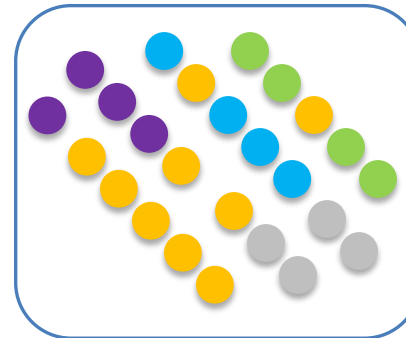
## V1-V3



before



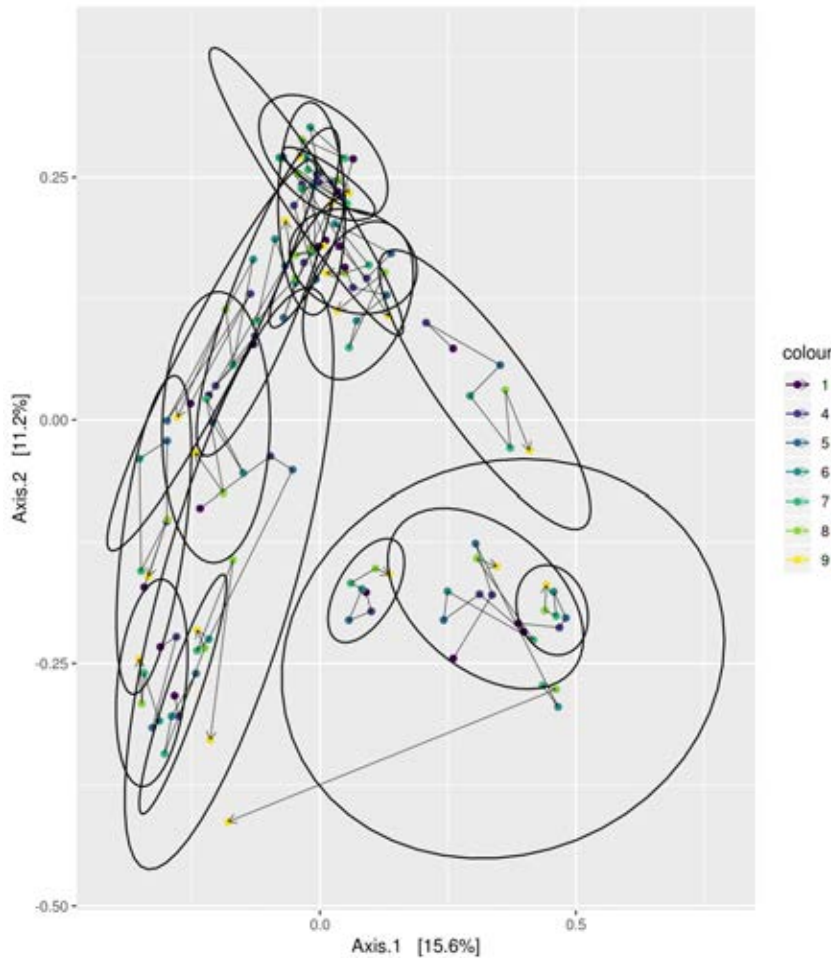
during



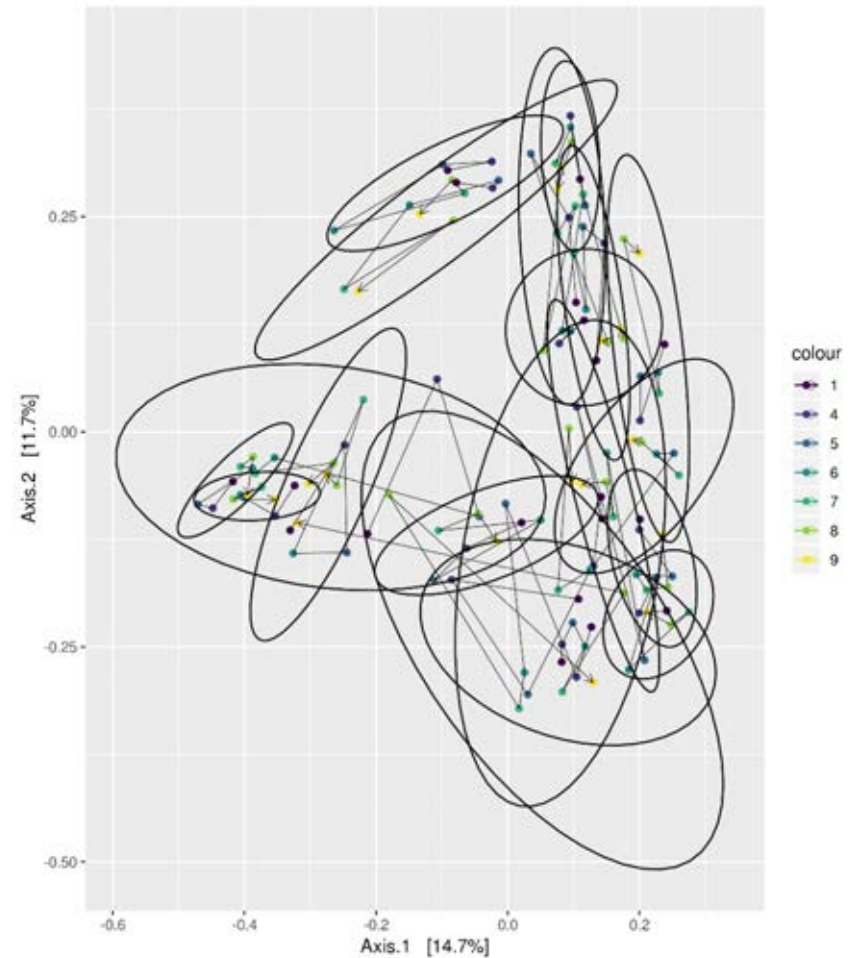
Dr Simon  
Reider

# The PAGODA study – community structure „NMDS and PCoA plots of Bray-Curtis distance“

V1-V3



V3-V4



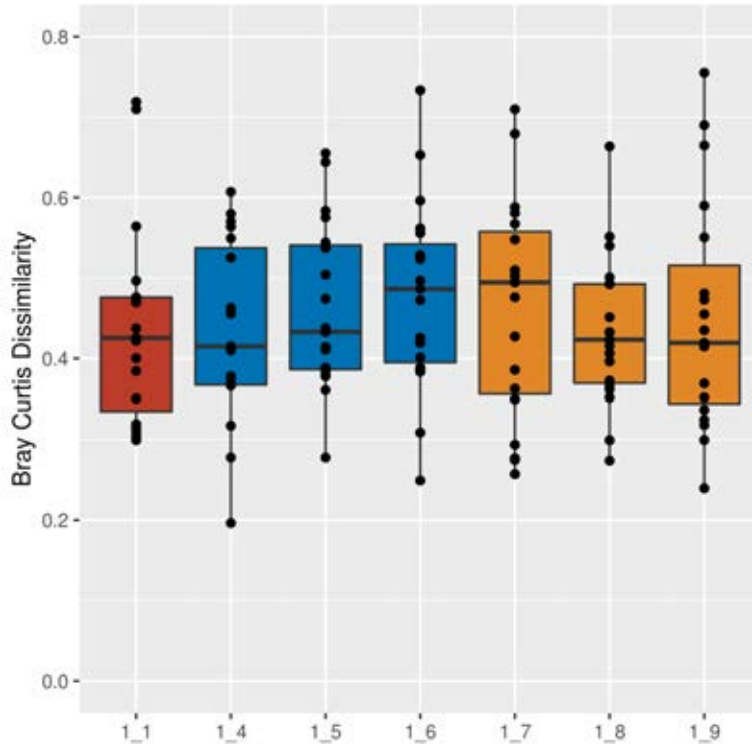
Note: subject had a predictor value (r-squared) of 0.69 and 0.67 ( $p < 0.01$ ) while timepoint (i.e. week) had an r-squared of 0.026 and 0.021 ( $p < 0.01$ )



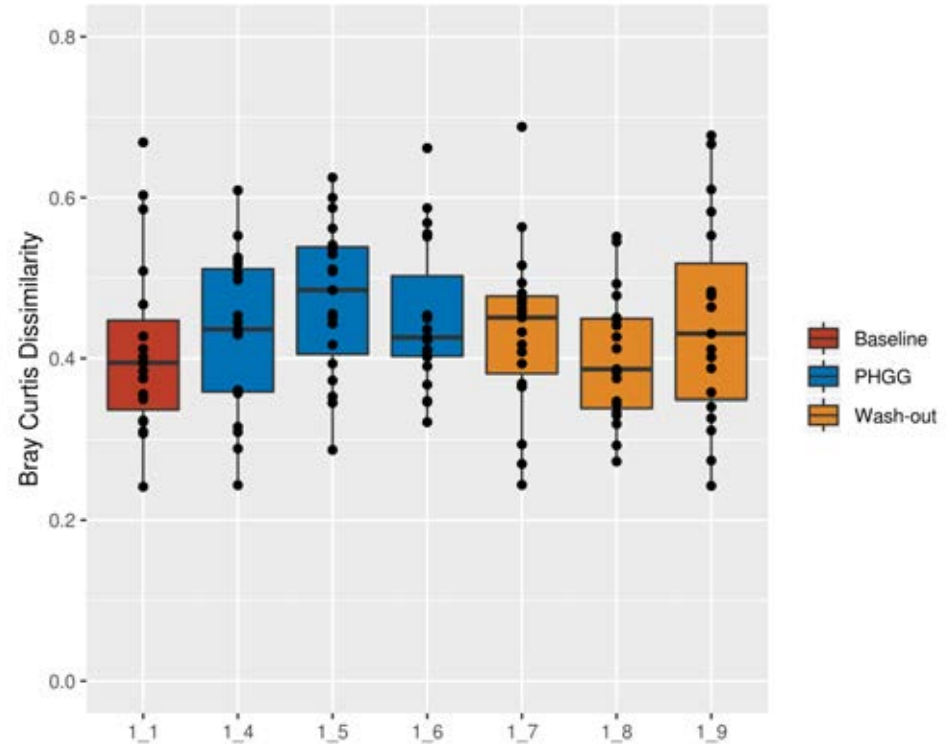
# The PAGODA study – community structure

## „Changes of Bray-Curtis distances over time“

V1-V3



V3-V4

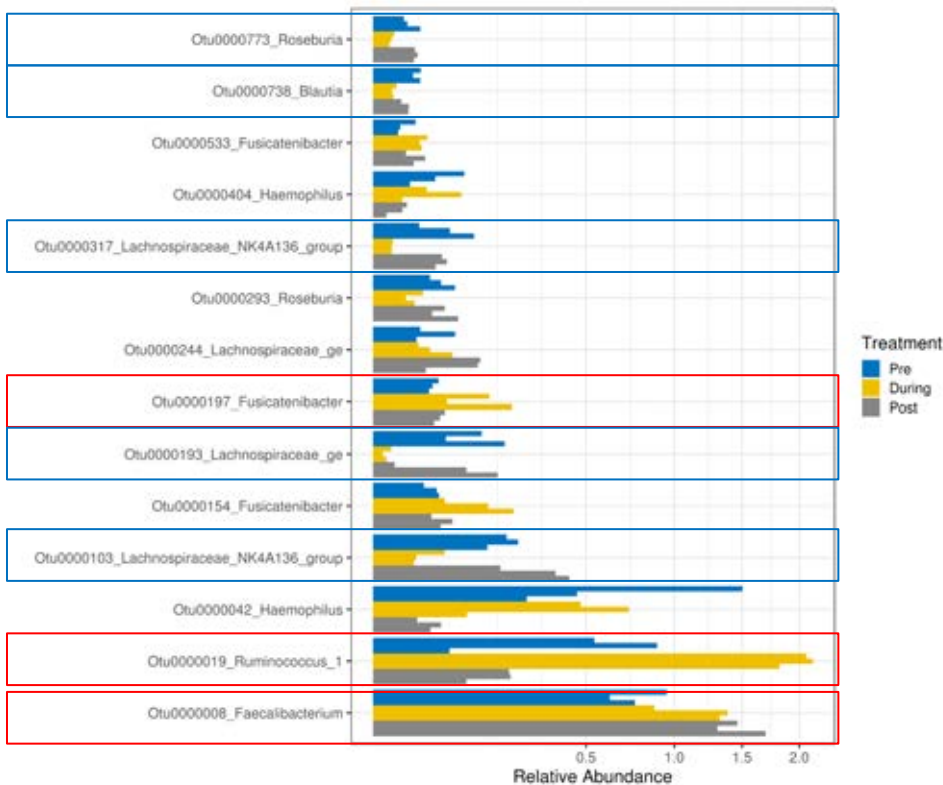


Note: PHGG induces changes in microbial community structure from the baseline configuration, that revert back to the original state after the intervention.

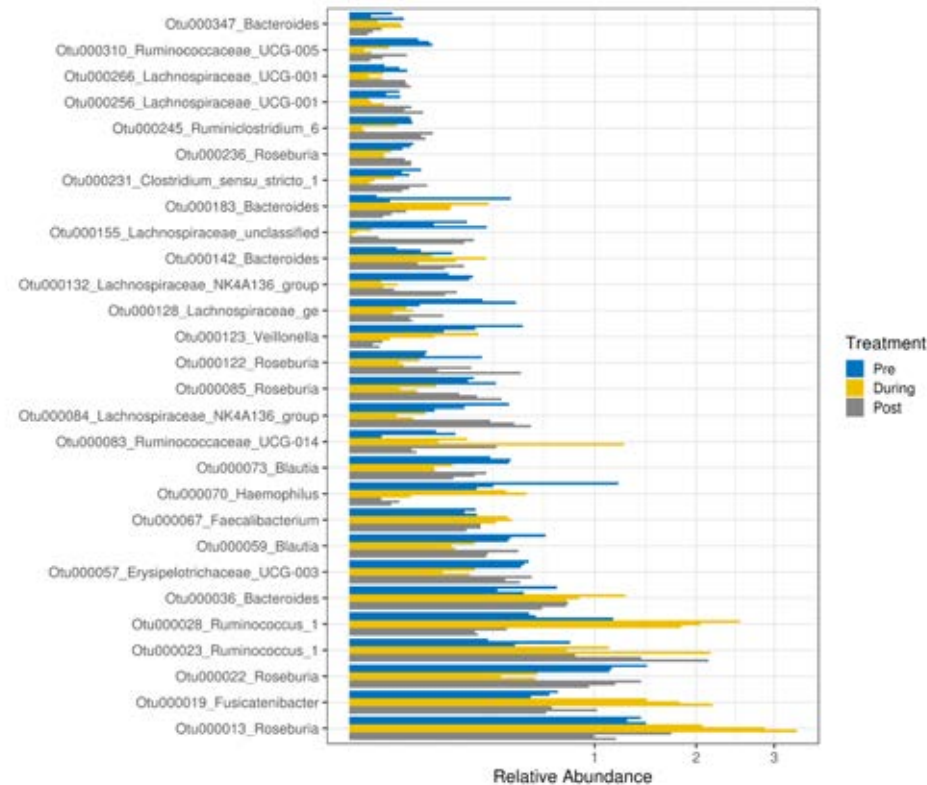
# The PAGODA study – (DeSeQ2)

## „16S Data: Differential abundance at the OTU level“

V1-V3



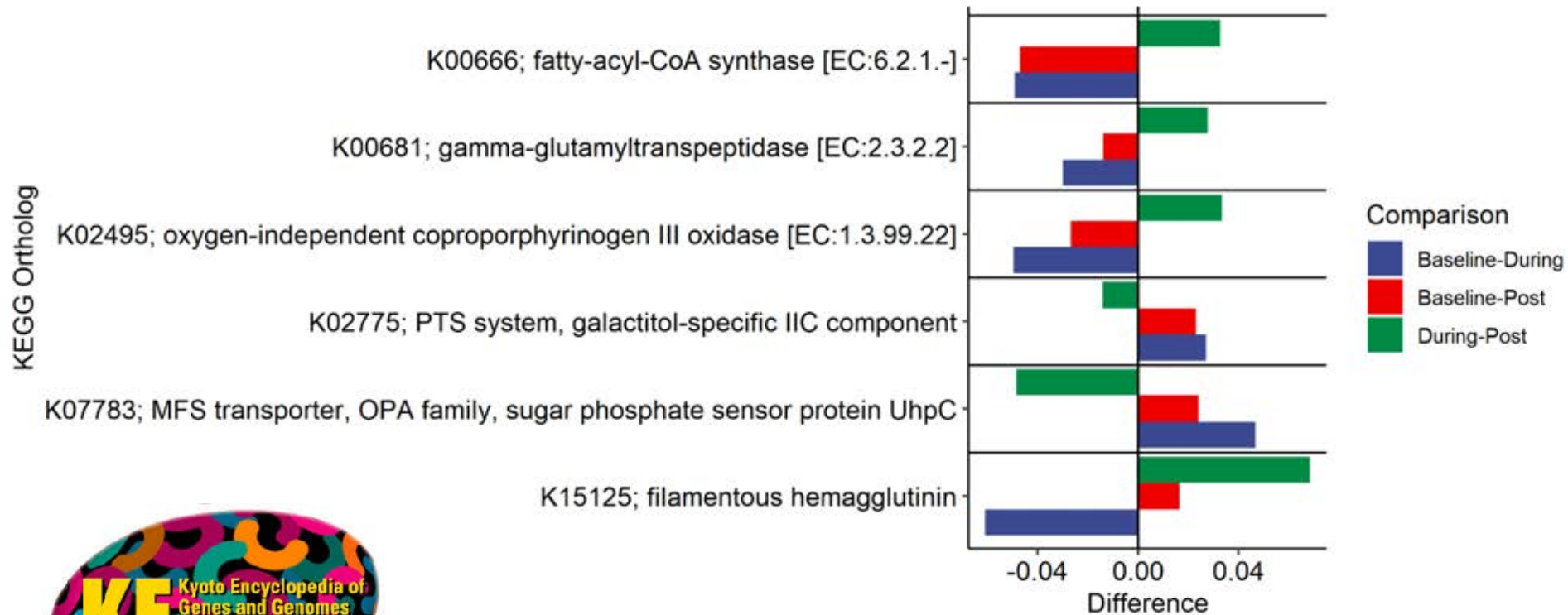
V3-V4



Note: Small but specific changes in taxa abundance by PHGG: “bloom” of Ruminococcus, Fusicatenibacter and Faecalibacterium, suppression of certain Lachnospiraceae, Roseburia, and Blautia.

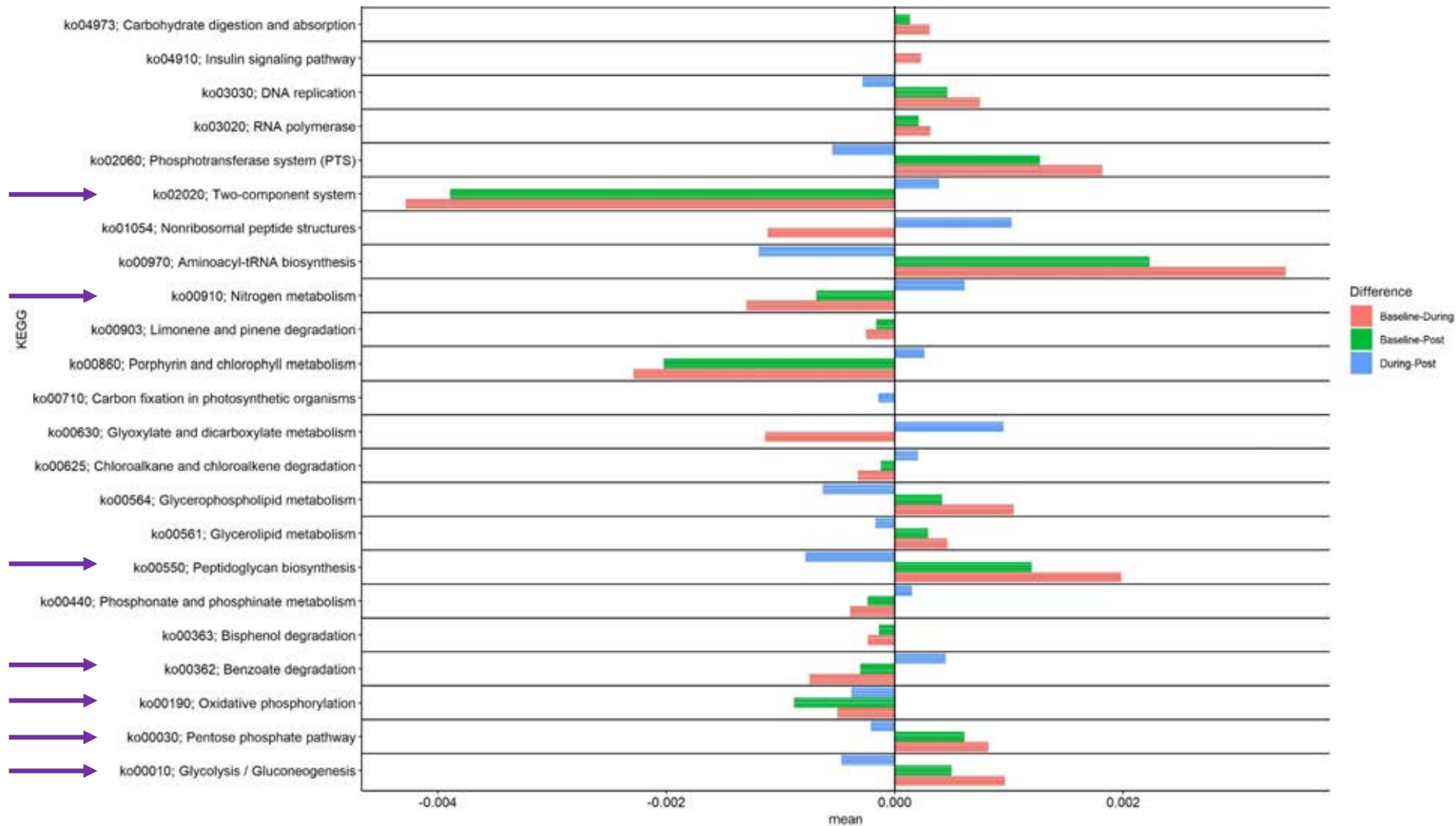
# The PAGODA study – (Tax4Fun)

## 16S Data: Mapping to KEGG orthologues (enzymes)



# The PAGODA study – (Tax4Fun)

## 16S Data: Mapping to KEGG functional profiles





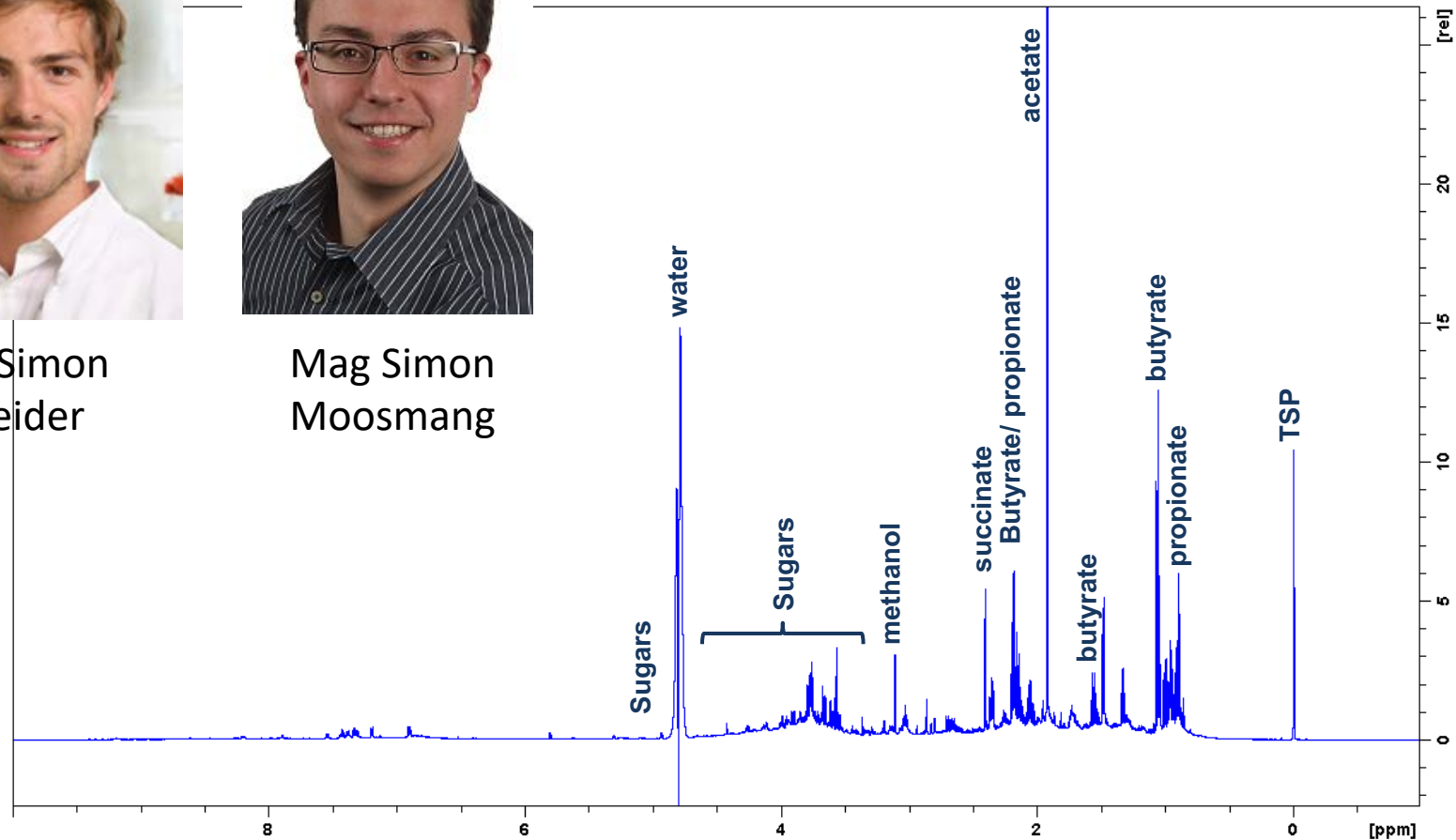
# PAGODA Studie – NMR profiles



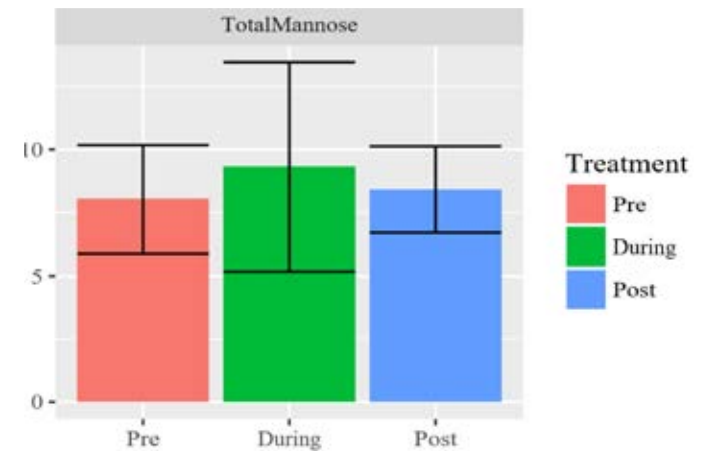
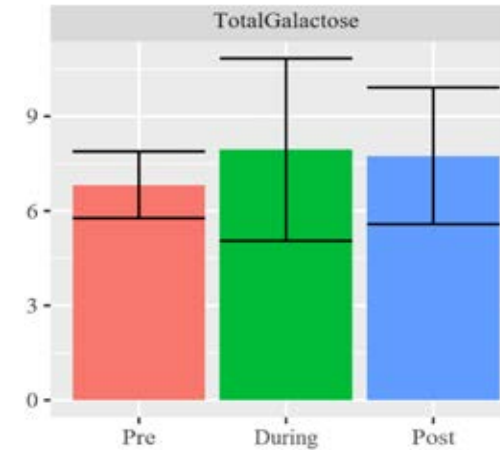
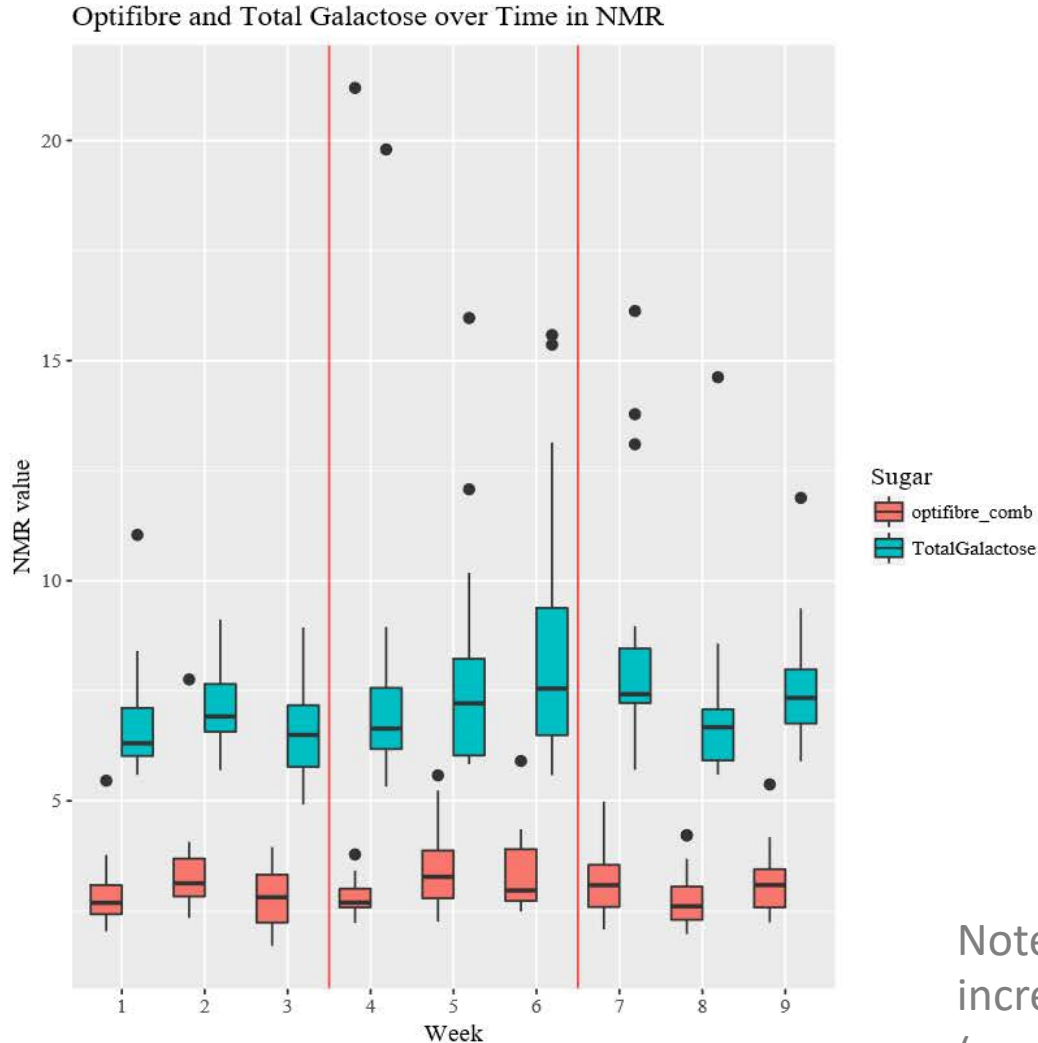
Dr Simon  
Reider



Mag Simon  
Moosmang

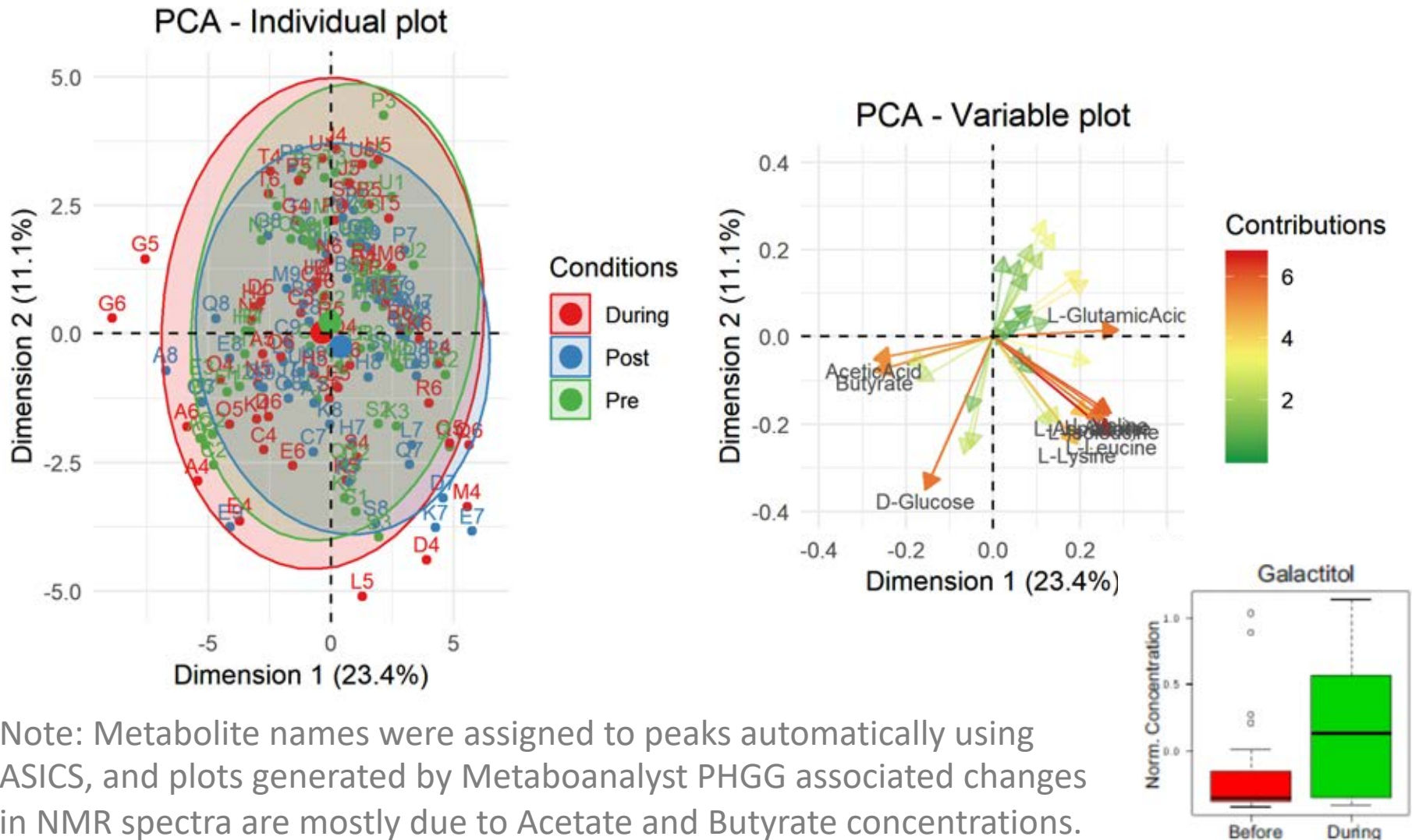


# The PAGODA study – Metabolome: PHGG – associated changes



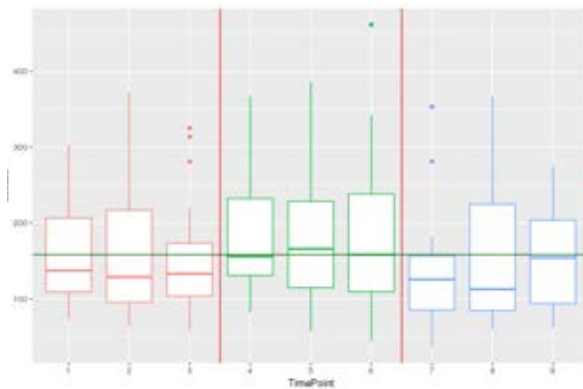
Note: Known metabolites of PHGG increase during the intervention (supports the credibility).

# The PAGODA study – Metabolome: PHGG – associated changes

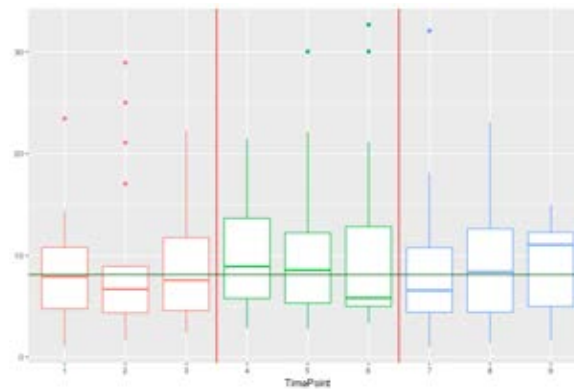


# The PAGODA study – Metabolome: PHGG – associated changes in SCFA

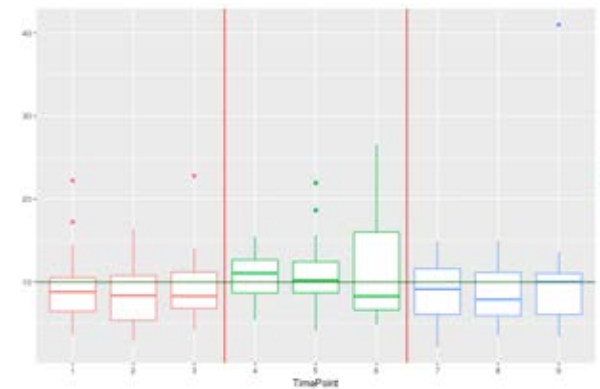
**Acetate**



**Butyrate**



**Propionate**



# The PAGODA study – Metabolome: PHGG – associated changes in SCFA

		FOLD CHANGE Acetate								
		Time Point 1	Time Point 2	Time Point 3	Time Point 4	Time Point 5	Time Point 6	Time Point 7	Time Point 8	Time Point 9
Male	A	0.02	0.38	-0.40	0.76	0.32	0.43	-0.16	0.82	0.11
	E	-0.08	-0.26	0.34	0.00	-0.39	-0.30	-0.45	-0.20	0.18
	K	-0.29	0.25	0.04	0.90	0.43	-0.28	0.05	0.30	0.48
	L	0.43	-0.15	-0.27	-0.29	-0.07	-0.10	-0.53	0.04	0.60
	M	0.22	0.01	-0.23	1.03	0.07	-0.42	-0.51	0.10	0.61
	N	-0.17	0.03	0.14	0.84	0.83	-0.12	-0.03	-0.08	-0.29
	O	-0.11	0.56	-0.44	0.89	0.25	0.59	0.81	0.44	-0.50
	U	-0.05	-0.17	0.22	0.67	-0.26	0.90	0.62	-0.23	0.99
Female	B	0.22	-0.12	-0.10	1.16	0.23	0.02	0.04	1.45	-0.33
	C	-0.09	0.48	-0.39	0.05	-0.10	-0.20	-0.28	-0.63	-0.19
	D	-0.35	0.39	-0.04	-0.58	0.17	0.38	-0.63	-0.64	-0.64
	G	0.02	-0.27	0.25	0.31	1.19	1.62	0.60	0.23	0.03
	H	-0.04	-0.26	0.30	-0.17	0.03	-0.18	-0.50	-0.63	-0.19
	J	0.05	0.01	-0.07	0.23	0.44	-0.12	0.29	-0.26	0.22
	P	0.09	-0.07	-0.01	0.22	1.69	1.14	-0.25	2.27	1.27
	Q	-0.12	0.00	0.12	-0.47	-0.55	-0.56	-0.53	0.47	-0.60
	R	0.70	-0.59	-0.11	-0.37	-0.28	-0.18	-0.06	-0.60	-0.65
	S	0.05	-0.13	0.08	0.15	-0.26	0.45	-0.58	-0.42	-0.27
	T	0.01	0.10	-0.11	0.15	-0.17	1.52	-0.05	-0.20	0.20
Male	Mean	0.00	0.08	-0.08	0.60	0.15	0.09	-0.02	0.15	0.27
	Median	-0.06	0.02	-0.10	0.80	0.16	-0.11	-0.09	0.07	0.33
Female	Mean	0.05	-0.04	-0.01	0.06	0.22	0.35	-0.18	0.09	-0.10
	Median	0.02	-0.07	-0.04	0.15	0.03	0.02	-0.25	-0.26	-0.19
All Participants	Mean	0.03	0.01	-0.04	0.29	0.19	0.24	-0.11	0.12	0.05
	Median	0.01	0.00	-0.04	0.22	0.07	-0.10	-0.16	-0.08	0.03

Note: There seems to be a transient, sex-dependent effect of PHGG on acetate concentrations in the feces.

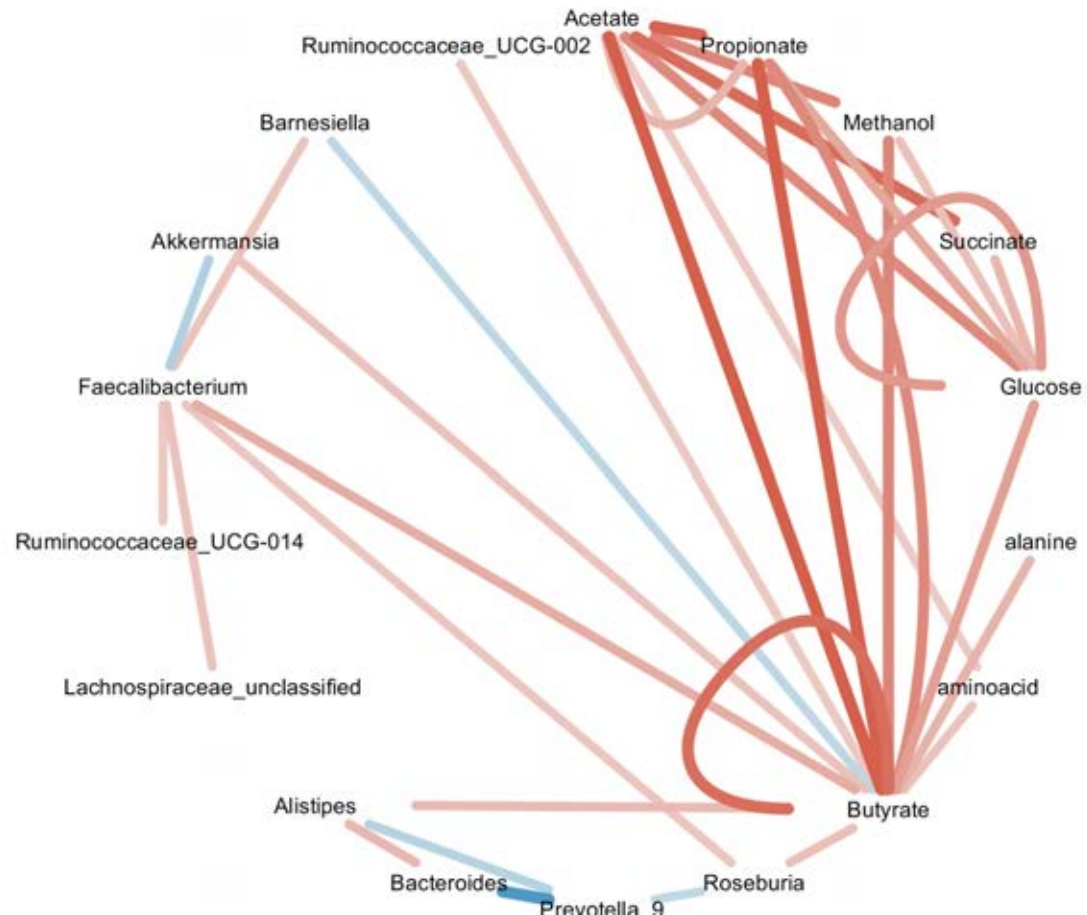


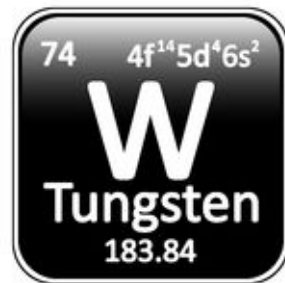
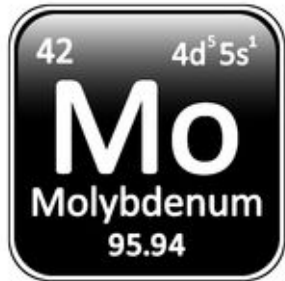
# The PAGODA study – (sparcc)

## Combining 16S metagenomics with NMR metabolomics

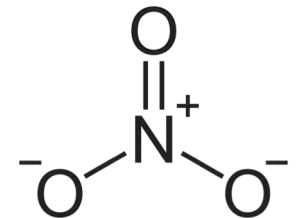
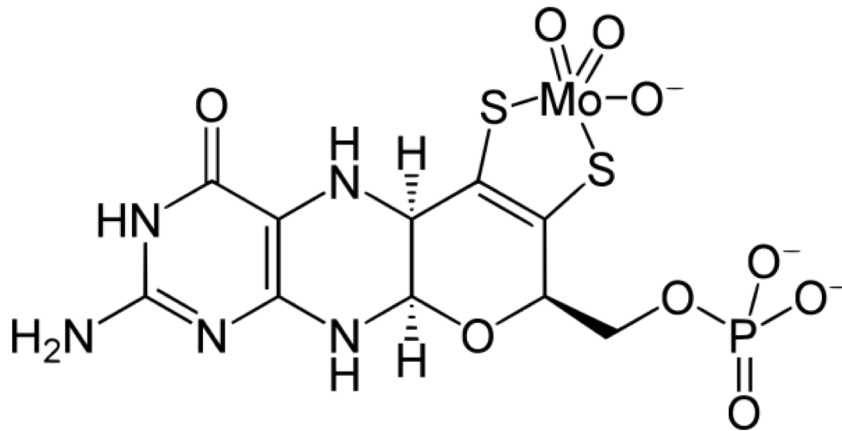
### Analysis of co-occurrence/co-exclusion

Note: Butyrate seems to be the key metabolite in PHGG associated metabolomic changes. It also shows strong links to certain bacterial taxa abundances, confirming results from DESEQ2 analysis.



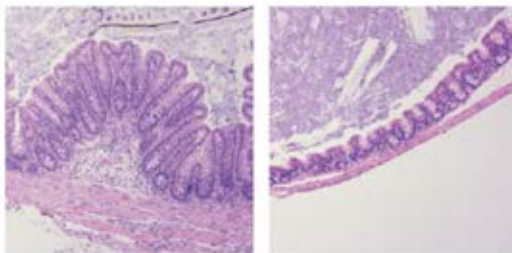
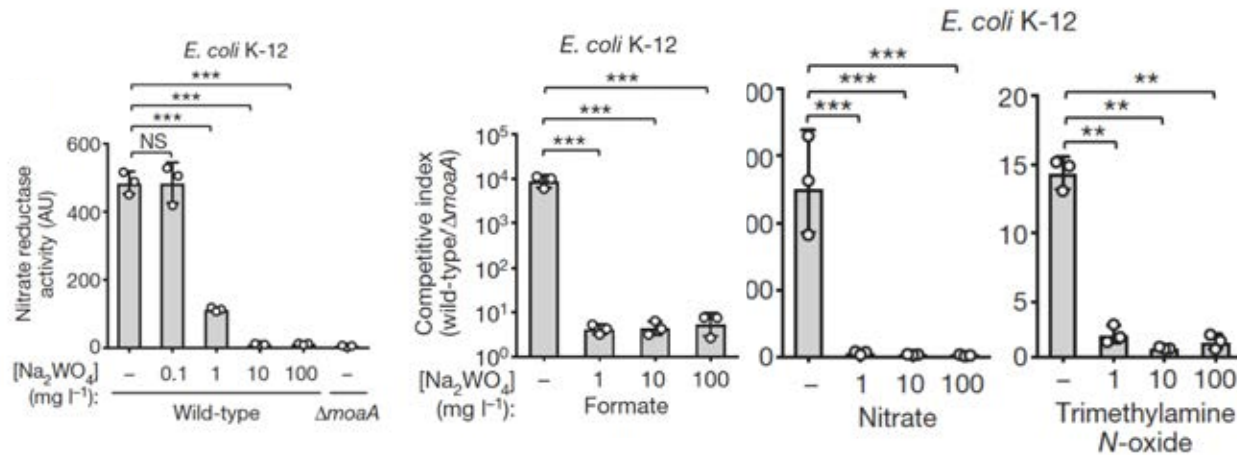


- Intestinale inflammation = metagenomics = penmanship of increase in molybdenum cofactor dependent metabolism
- Molybdenum cofactor dependent metabolic pathways contribute to fitness advantage of Enterobacteriaceae
- Molybdenum cofactor consists of molybdopterin and molybdenum oxyde
- Tungsten can edge out molybdenum from this chemical bond leading to its inactivation



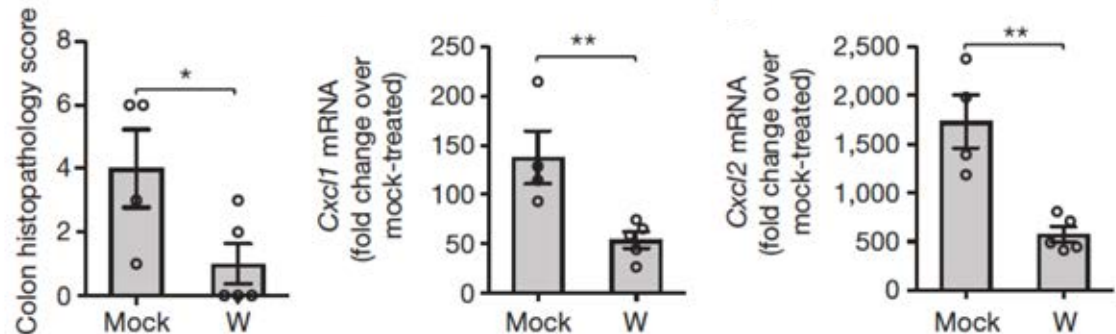
Hughes ER et al. Cell Host Microbe 2017;21:208–219  
Zhu W/Winter MG et al. Nature 2018;553:208–211

# Precision editing of the microbiome



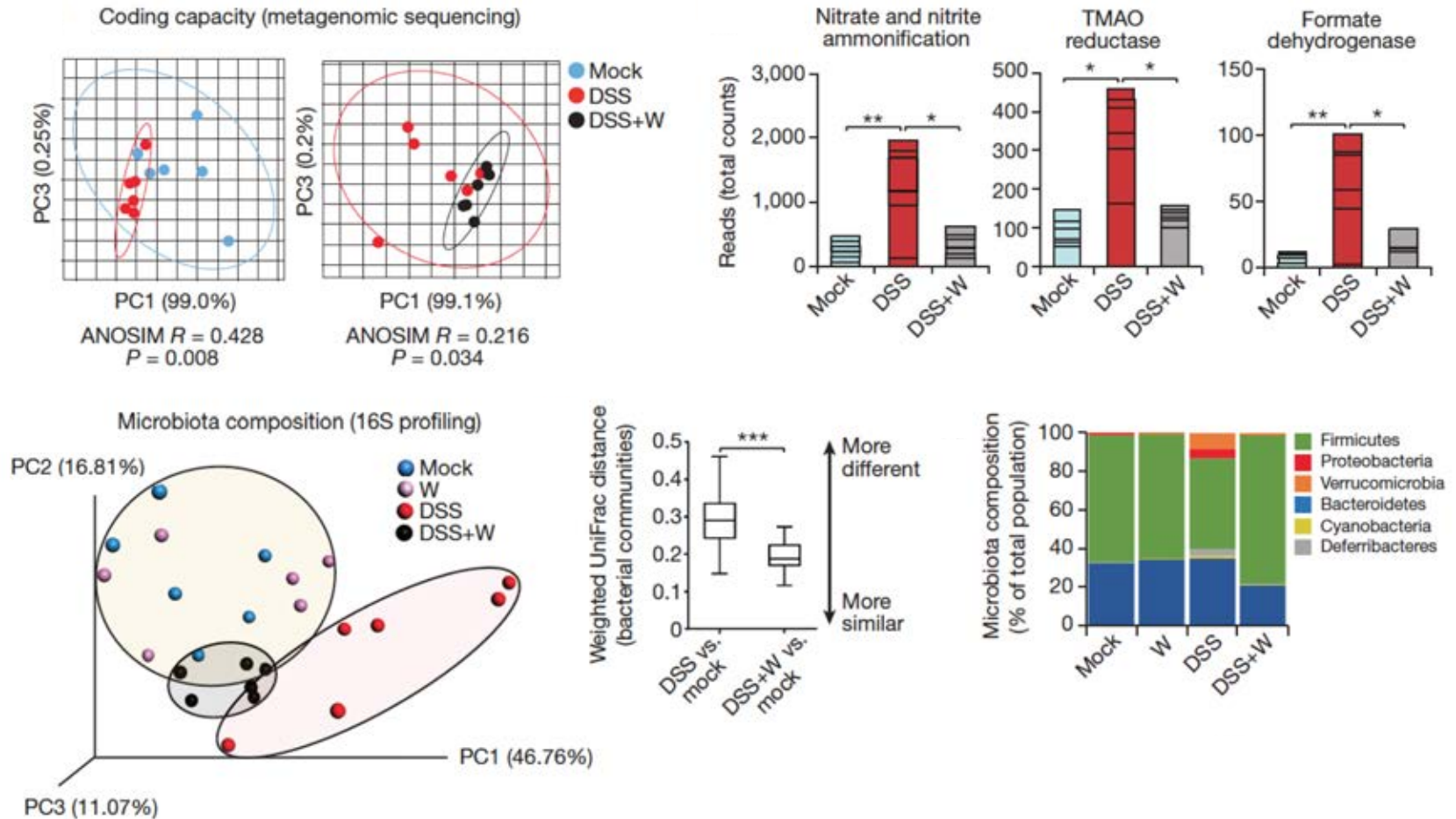
Mock

W



Hughes ER et al. Cell Host Microbe 2017;21:208–219  
Zhu W/Winter MG et al. Nature 2018;553:208–211

# Precision editing of the microbiome



Hughes ER et al. Cell Host Microbe 2017;21:208–219  
Zhu W/Winter MG et al. Nature 2018;553:208–211

- The microbiome represents a „powerful tool“ to intervene with human health in both in the positive and in the negative sense.
- Point and width of effect of microbiota-modulating strategies (alone or in combination with other therapeutics) seem an attractive strategies
- On the basis of the complexity of host-microbe, microbe-host, and microbe-microbe interactions, we still have to learn about long-term safety and efficacy, and the possibility of personalized approaches regarding microbiota-targeted therapies.





THANKS FOR LISTENING :-)