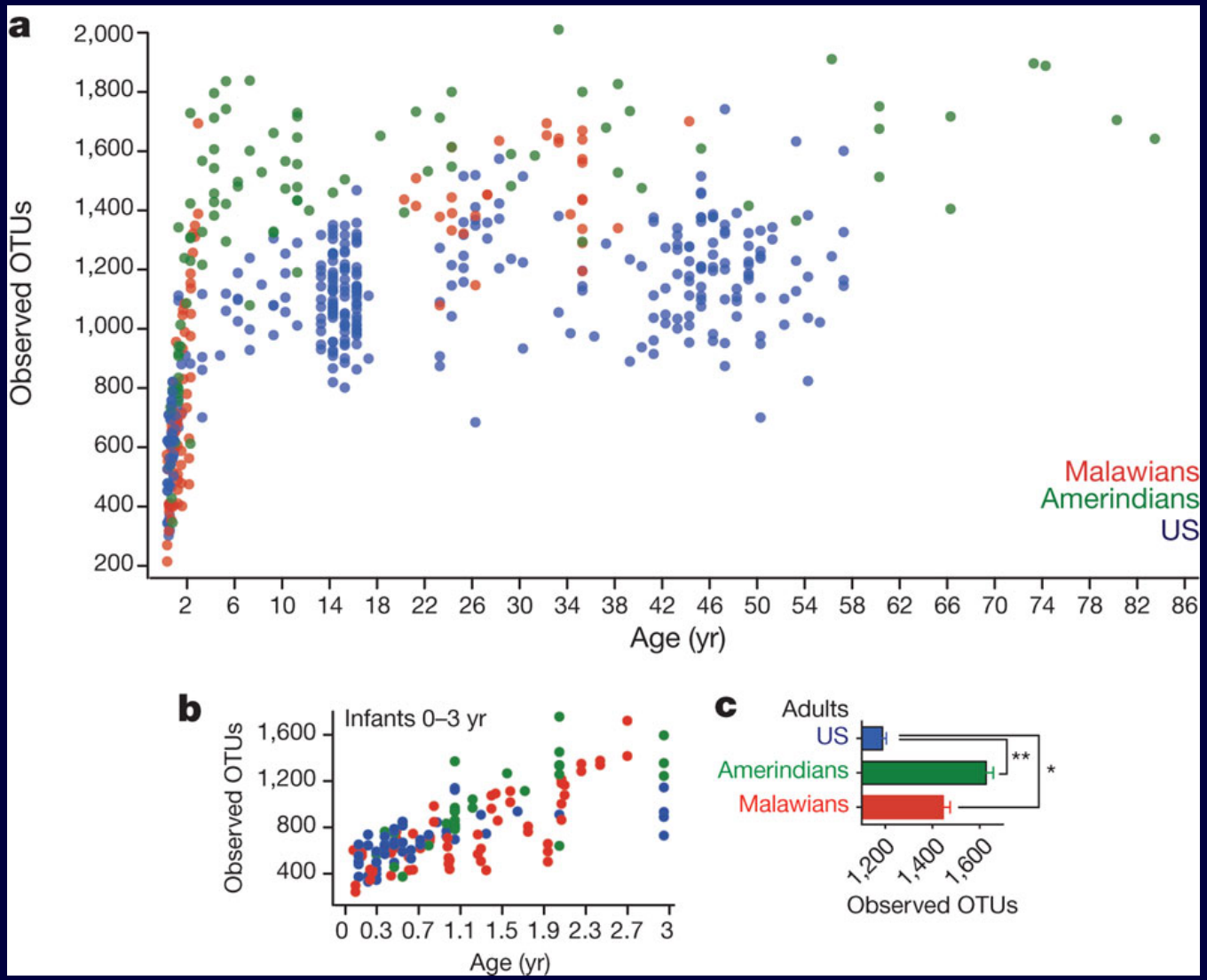
Microbiota, metabolism and immunity: the potential for early-life intervention.

Mick Bailey
Professor of Comparative Immunology,
Bristol University

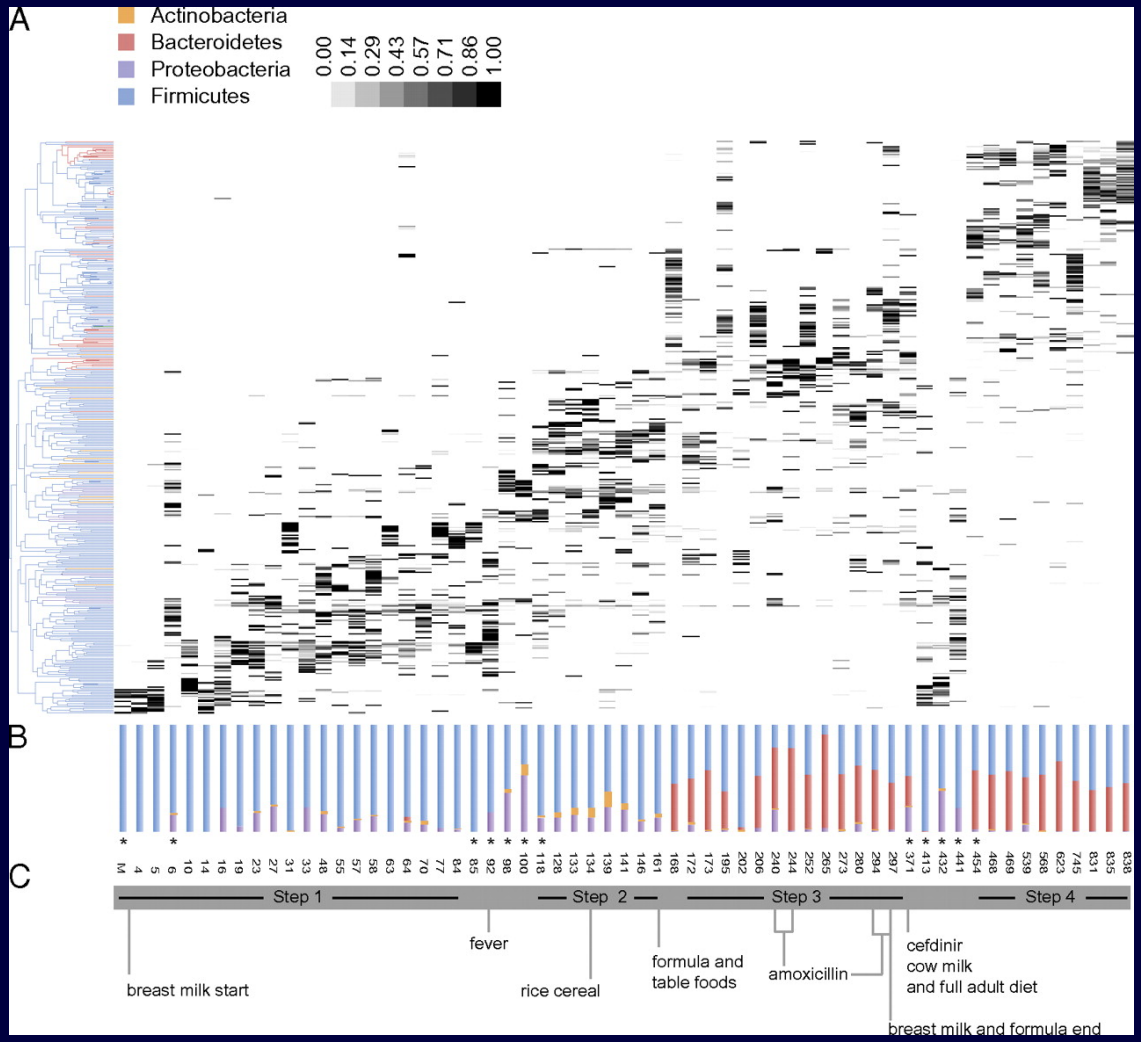
Bacterial diversity increases with age in human populations.



T Yatsunenko *et al. Nature* **000**, 1-7 (2012) doi:10.1038/nature11053

nature

Succession of microbial communities: OTU-based community structure and composition in the human gut microbiota changes with age, rather than just becomes more complex.



Koenig J E et al. PNAS 2011;108:4578-4585

PNAS

The ‘hygiene hypothesis’

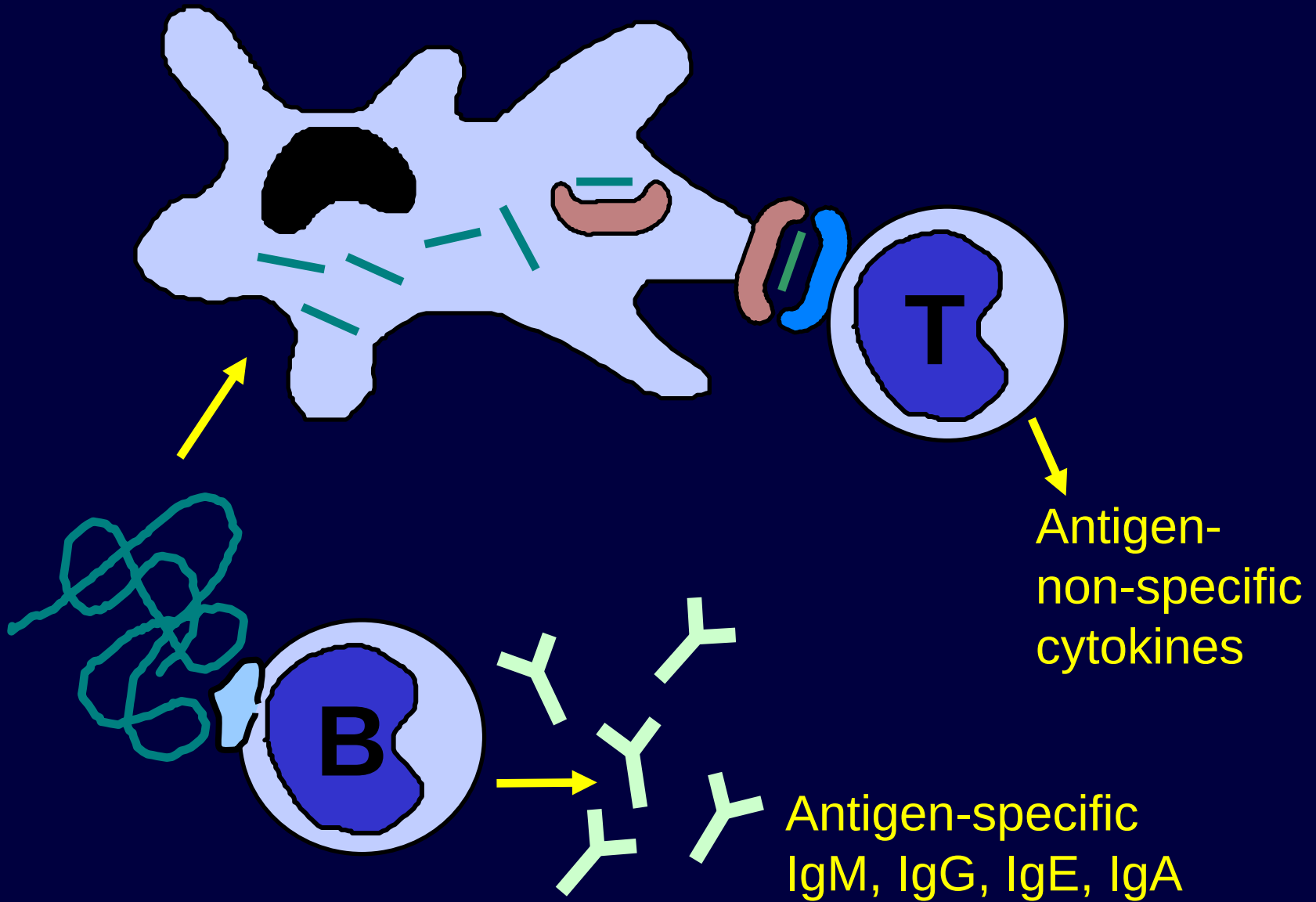
- Species co-evolve with their microbiota.
- The immune system ***requires*** interactions with commensal microbiota and with pathogens for it to express appropriate function (‘enteric health’).
- The absence of appropriate interactions predisposes to diseases of immune dysfunction (allergies, autoimmunity, unusual susceptibility to infectious diseases).
- Similarly, appropriate interactions with gut microbiota are required for development of normal metabolic systems.
- The ‘wrong’ interactions can predispose to metabolic disease in later life.

The ‘hygiene hypothesis’

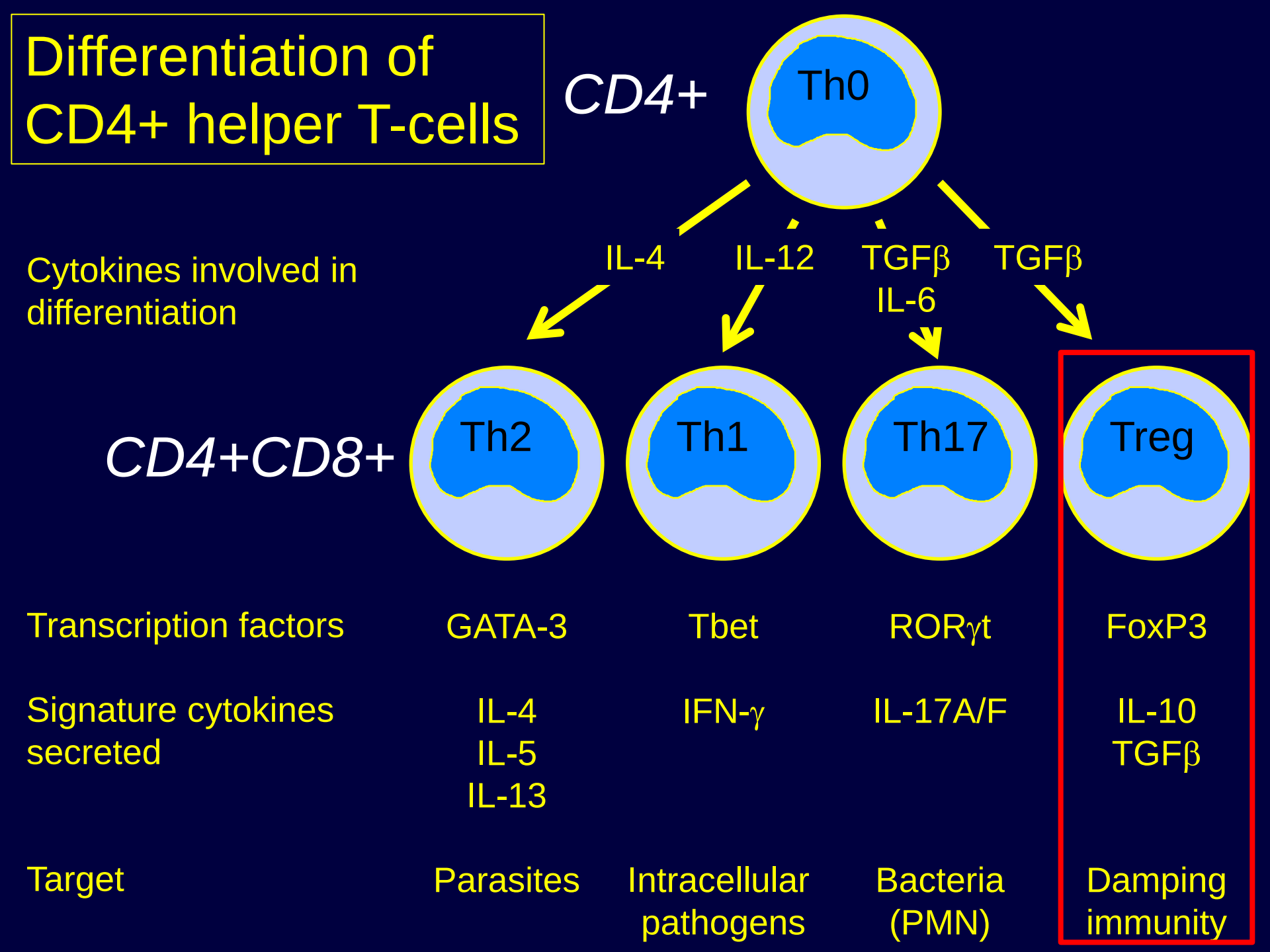
- **Early-life colonisation by microbiota can have long-lasting effects on immunity and metabolism, which may be categorised as ‘beneficial’ or ‘detrimental’ under particular circumstances.**
- **This is as relevant to our domesticated species as to humans.**



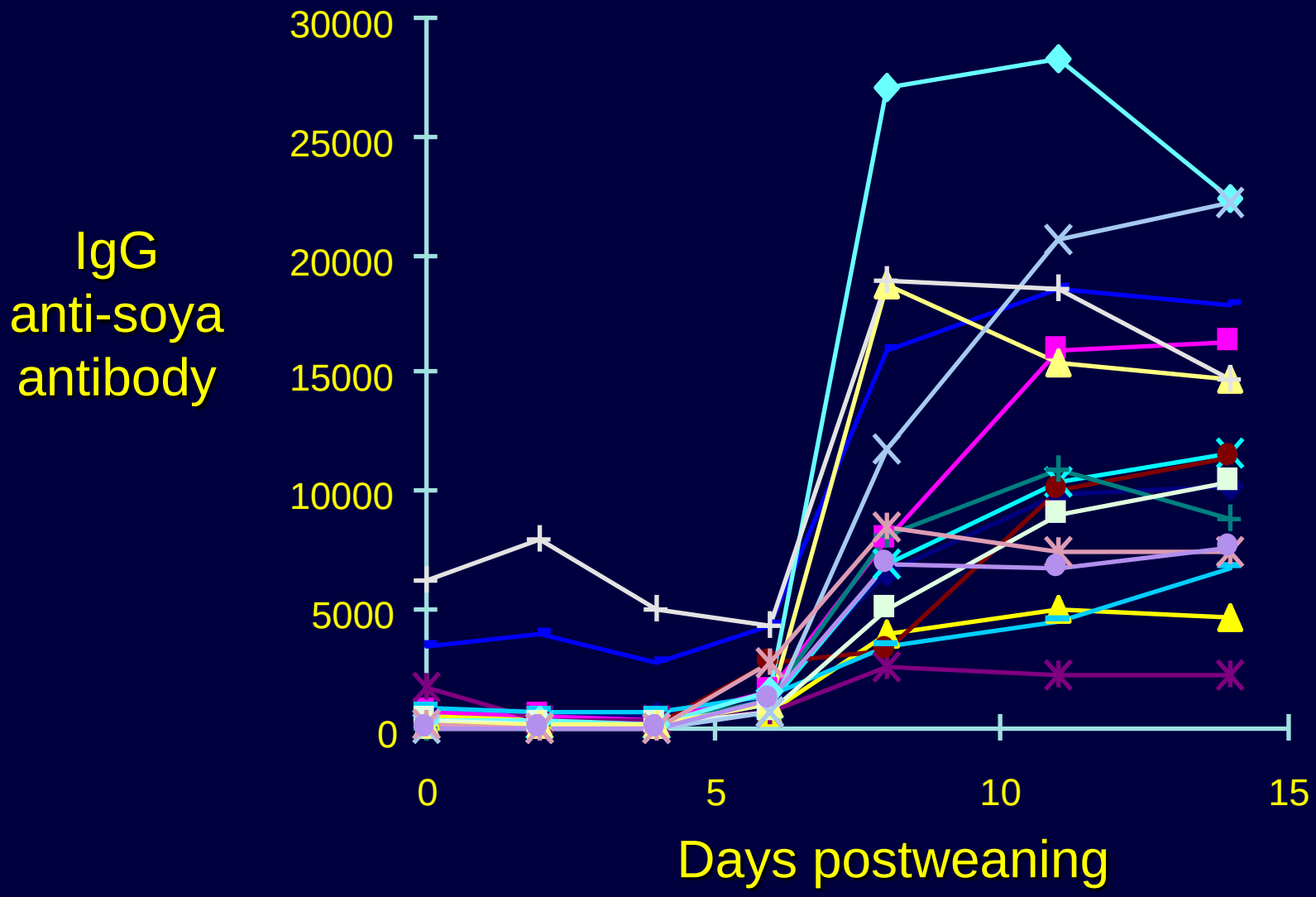

CD4+ helper T cells require MHCII-restricted presentation of peptides



Differentiation of CD4+ helper T-cells

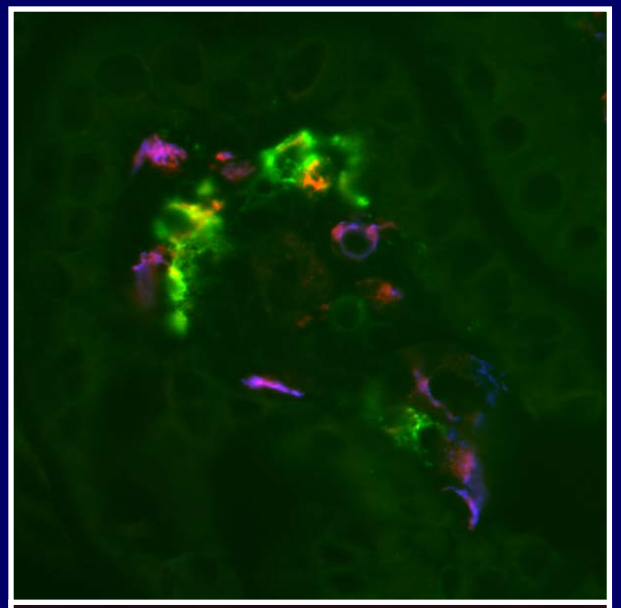


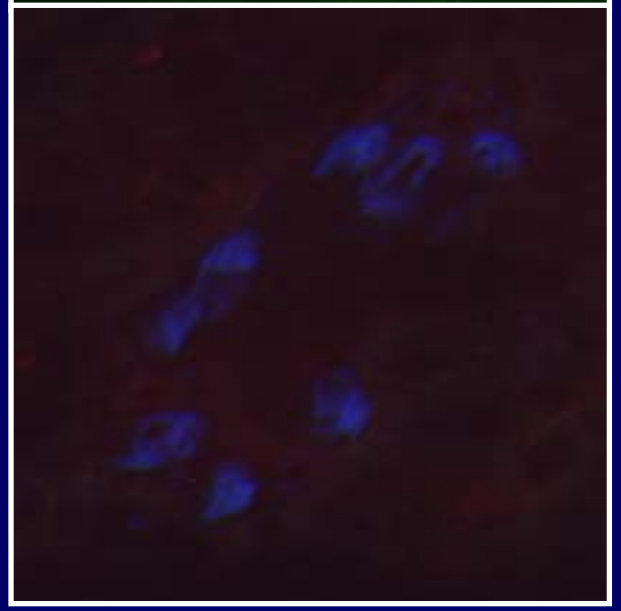
Novel food proteins trigger inappropriate immune responses in early-weaned piglets



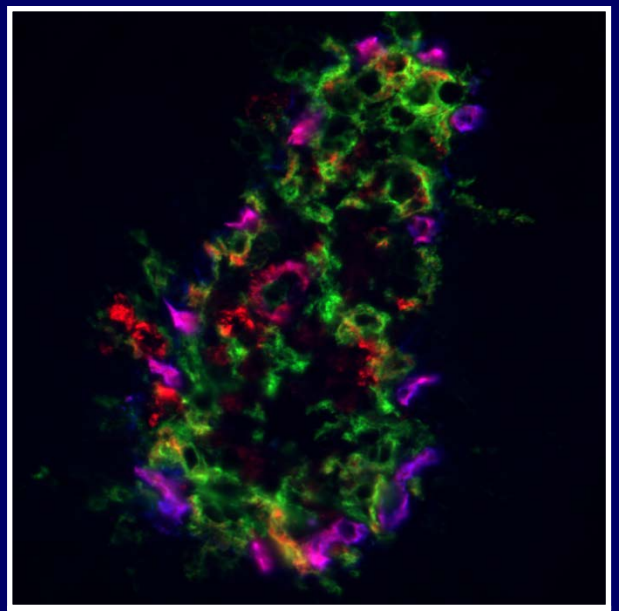
The ability to mount appropriate responses is critical for ‘enteric health’ – IgG, IgE or IgA; Th_1, Th_2, Th_{17} or T_{reg}

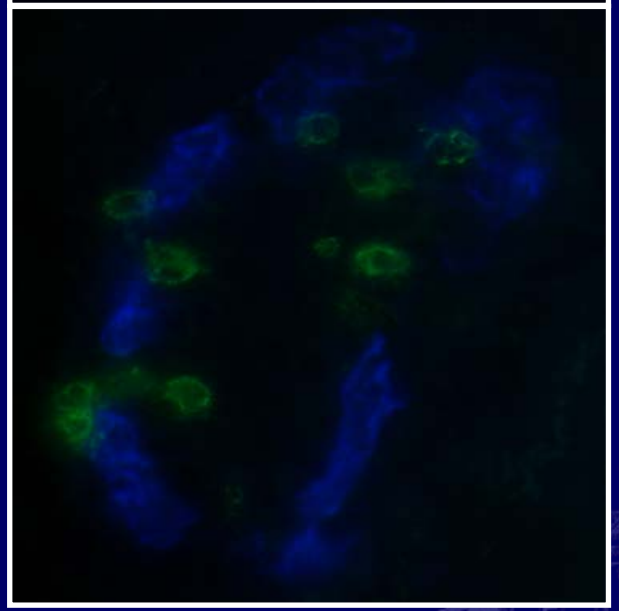
The structure of the mucosal immune system determines the efficiency of responses to food, pathogen and commensal microbiota.





Day 0





Day 28

MHC II

CD16

MIL11

CD8

CD4

MIL11

Stages in the development of the mucosal immune system of the pig

1. Rudimentary Peyers patches, essentially no mucosal T cells. Limited B-cell repertoire. Few dendritic cells but MHCII on endothelial cells. The newborn pig.

2. Non-specific expansion of B-cells and Peyers patches. Appearance of early, activated T-cells, influx of MHCII+ cells. 1 days to 2 weeks.

3. Appearance of CD4+ T cells. 2 weeks to 4 weeks.

4. Antigen-specific B-cell responses. Appearance of CD8+ T cells. 4 weeks to 6 weeks.

Most of this expansion of the adaptive immune system is driven by microbiota.

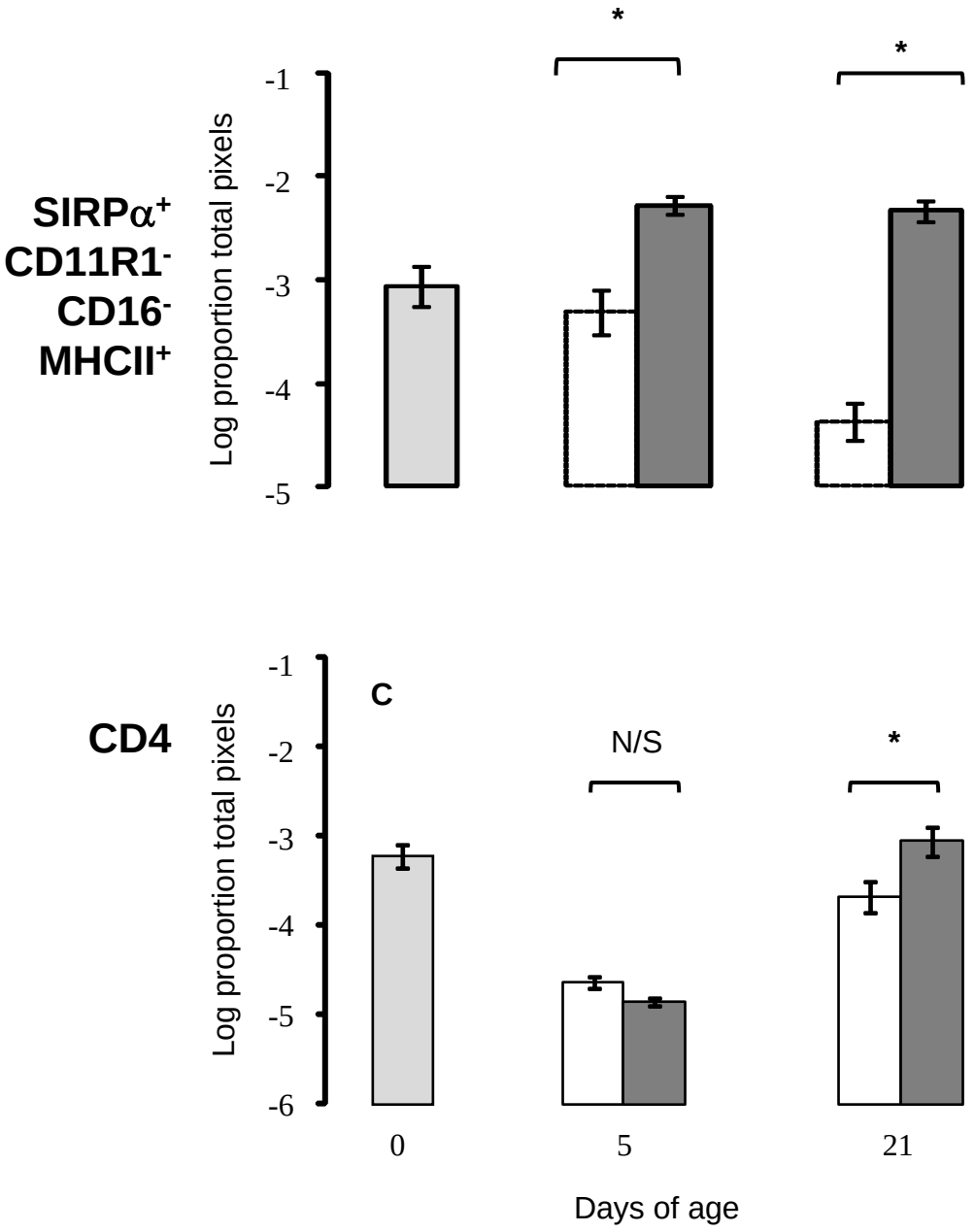
Colonisation of newborn, gnotobiotic piglets with defined microbiota results in expansion of the mucosal immune system which replicates, approximately, that in conventional pigs.

Inman et al, 2012 PLoS One; Laycock et al, 2012 Vet Immunol Immunopathol

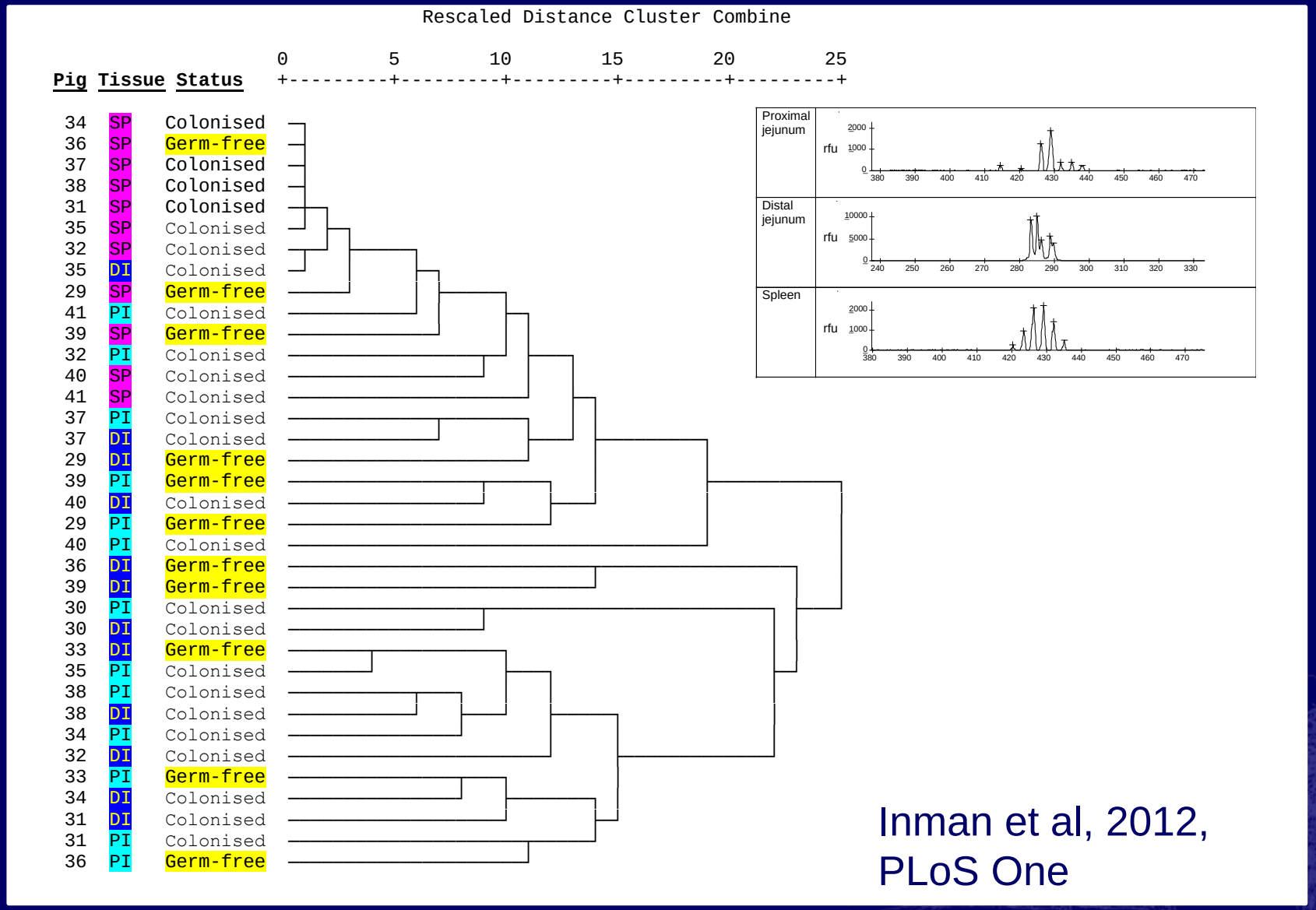
Defined colonisation of gnotobiotic piglets expands mucosal SIRPα^+ DC first, then CD4$^+$ T-cells (Inman, 2012, PLoS One)

Colonised

Germ-free



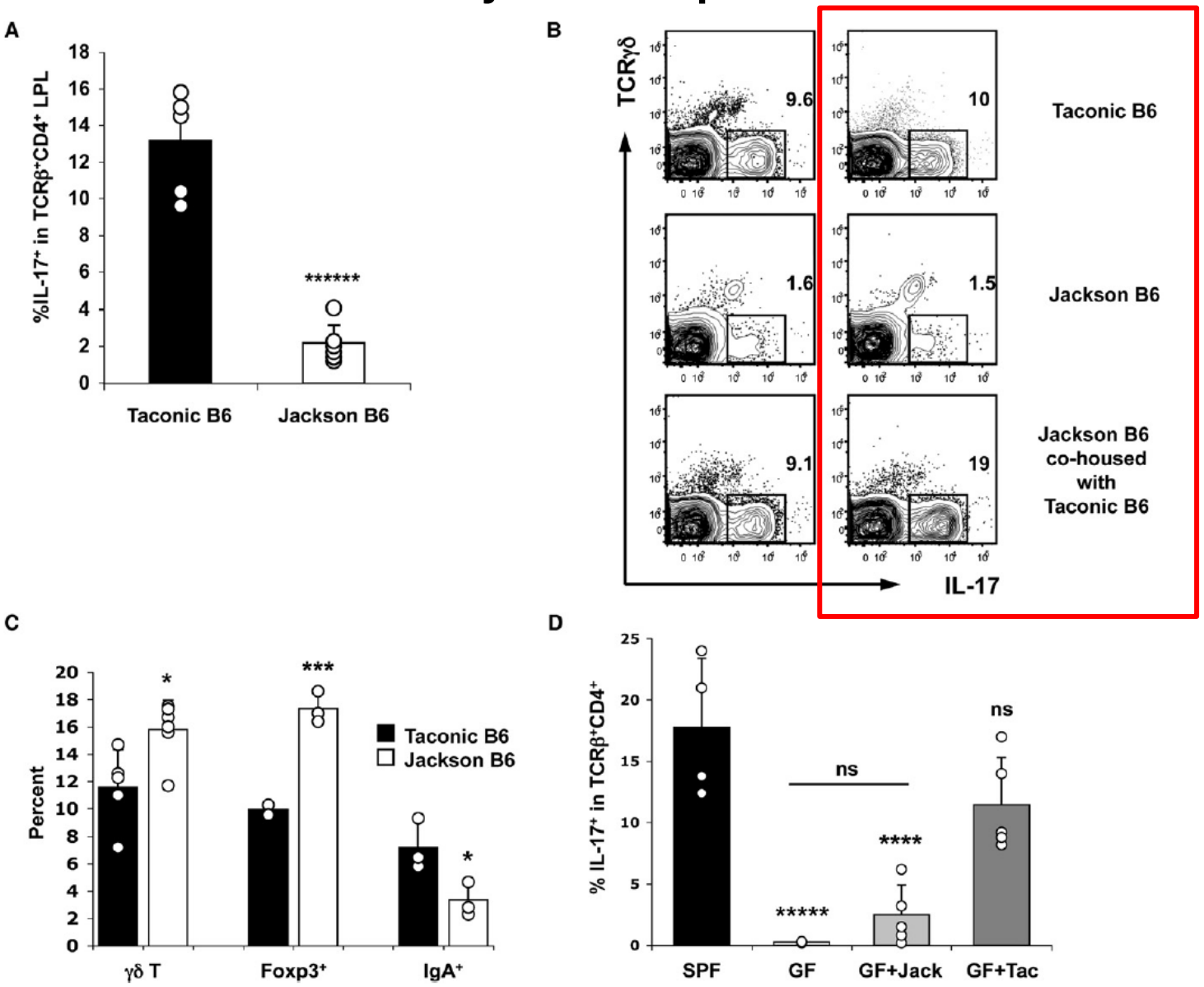
T-cell receptor repertoire is not skewed in fully MHC-inbred, colonised, gnotobiotic piglets



Inman et al, 2012, PLoS One

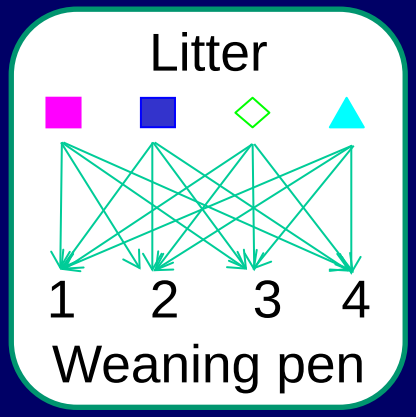
Are studies in gnotobiotic mice and piglets relevant to anything that looks vaguely like the real thing?

Is it important? Genetically identical mice from different suppliers have different immune systems dependant on their microbiota



Ivanov et al, 2008 Cell Host and Microbe 4:337

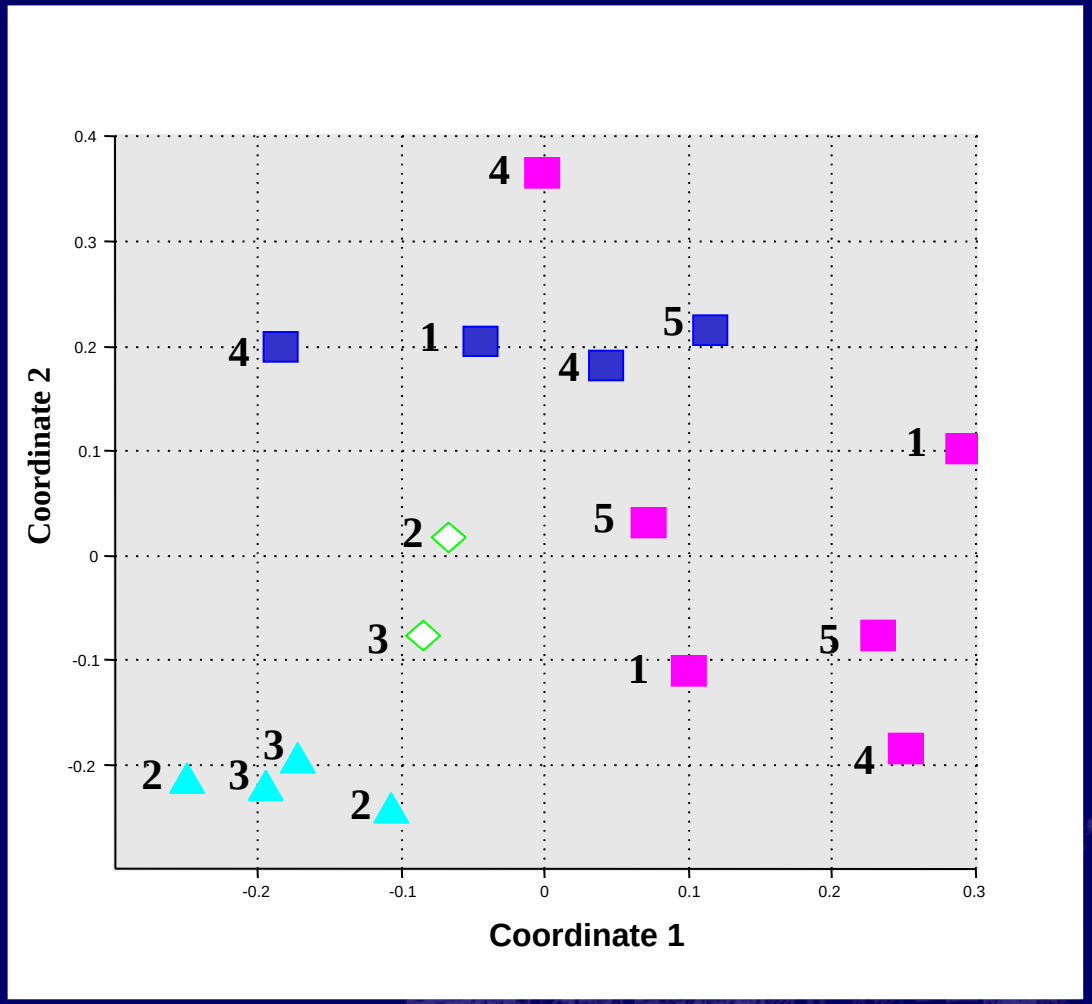
Is it important? Microbiota is ‘imprinted’ before weaning in neonatal piglets



Non-metric, multidimensional scaling analysis

Tighter clustering for siblings than pen mates

4 different litters (weaning + 21 days)



Is it important? Manipulation of the microbiota and the mucosal immune system by rearing macro-environment (GUTWEAN)

Mulder et al (2009). Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biology (7).

Inman et al (2010). Rearing environment affects development of the immune system in neonates. Clinical and Experimental Immunology 160: 431-439

Mulder et al (2011) Restricting microbial exposure in early life negates the immune benefits associated with gut colonization in environments of high microbial diversity. PLoS One.

Schmidt et al, (2011) Establishment of normal gut microbiota is compromised under excessive hygiene conditions. PLoS One.

Is it important? Variation between commercial farms in development of the immune system may also be attributable to farm-specific microbiota

Appearance of memory/effector T-cells (CD4+CD8+) with age (Griersen, Banks, Haverson, Bailey)

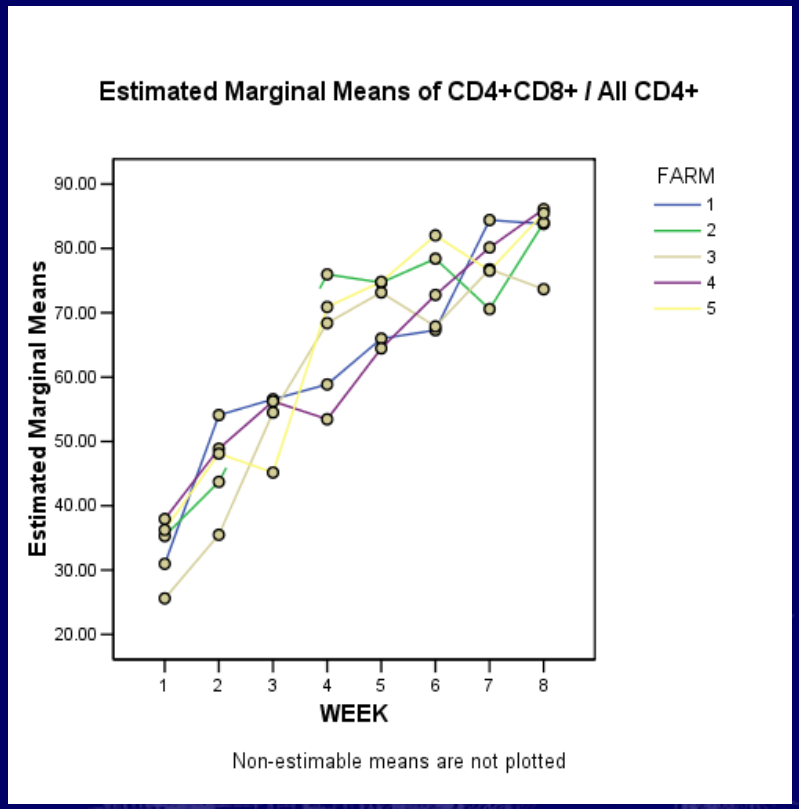
Tests of Between-Subjects Effects

Dependent Variable: CD4+CD8+ / All CD4+

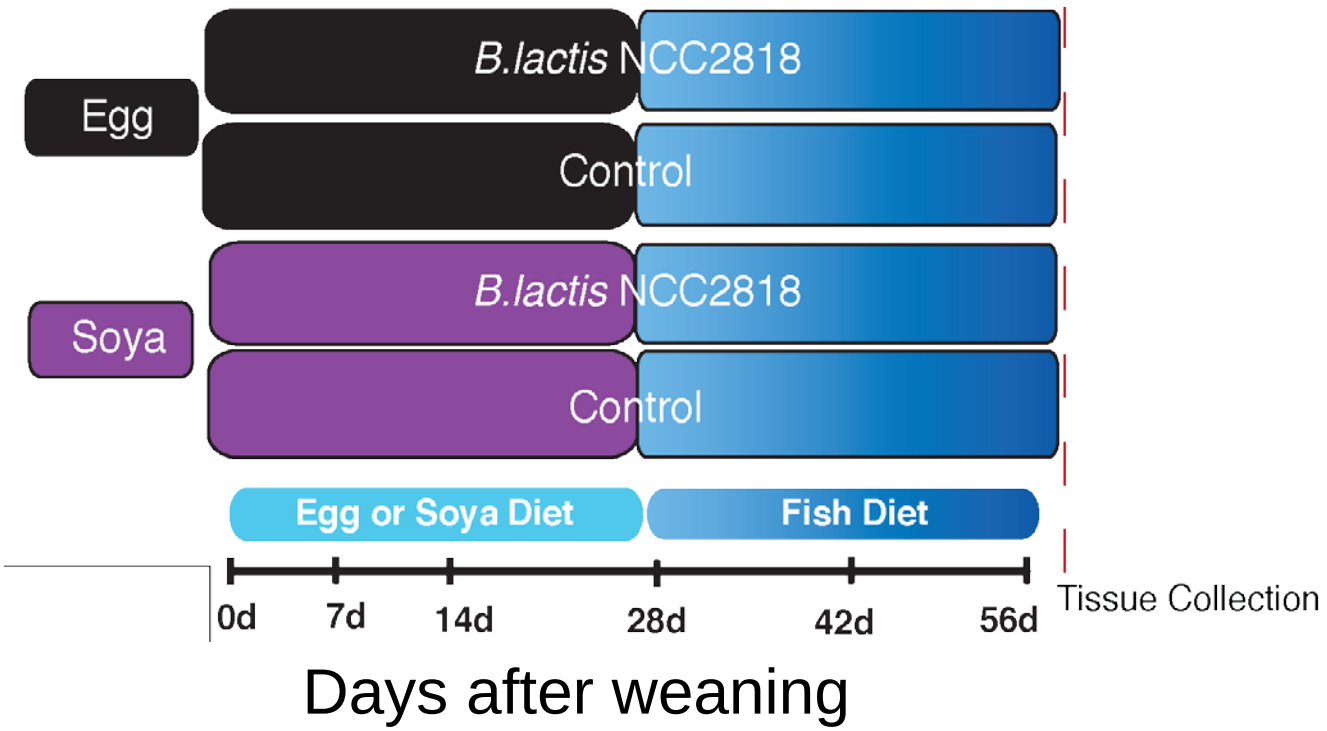
Source	Type IV Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	145383.169[a]	38	3825.873	26.416	.000
Intercept	1872181.253	1	1872181.253	12926.805	.000
WEEK	121856.891[b]	7	17408.127	120.197	.000
FARM	462.841[b]	4	115.710	.799	.526
WEEK * FARM	14587.177	27	540.266	3.730	.000
Error	68504.300	473	144.829		
Total	2297958.454	512			
Corrected Total	213887.469	511			

a. R Squared = .680 (Adjusted R Squared = .654)

b. The Type IV testable hypothesis is not unique.

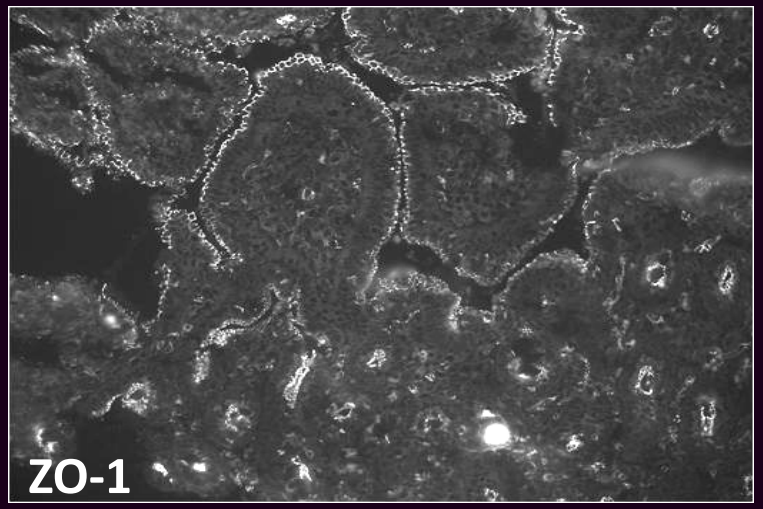


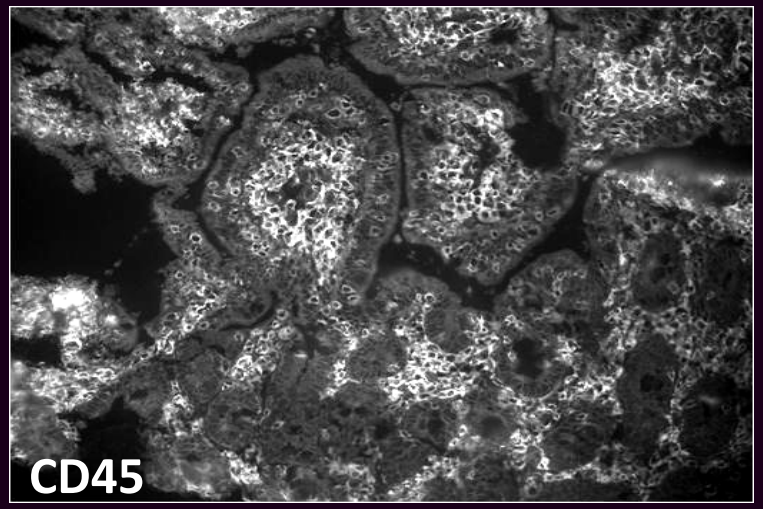
Can we manipulate this directly in conventional animals with complex, highly variable microbiota?

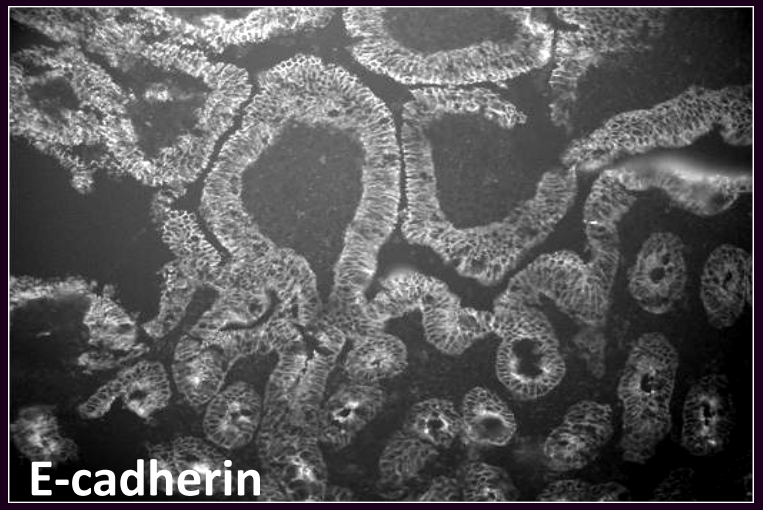


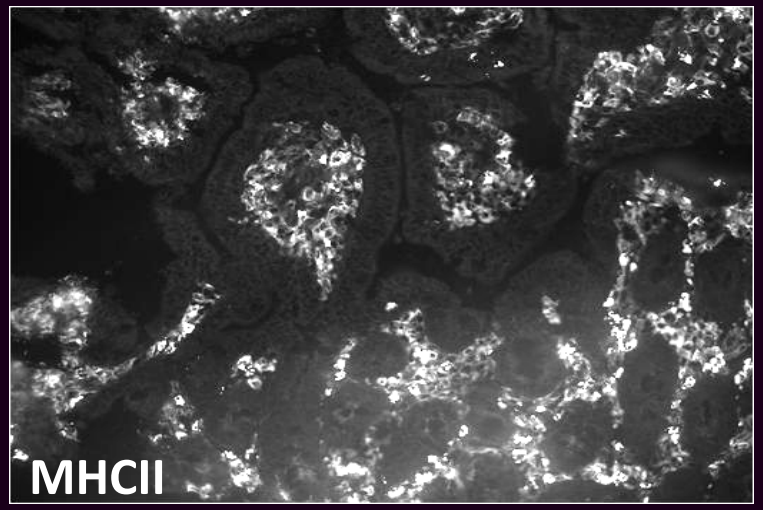
Merrifield, Lewis, et al, 2013, Gut (in press)

Intestinal Barrier Function

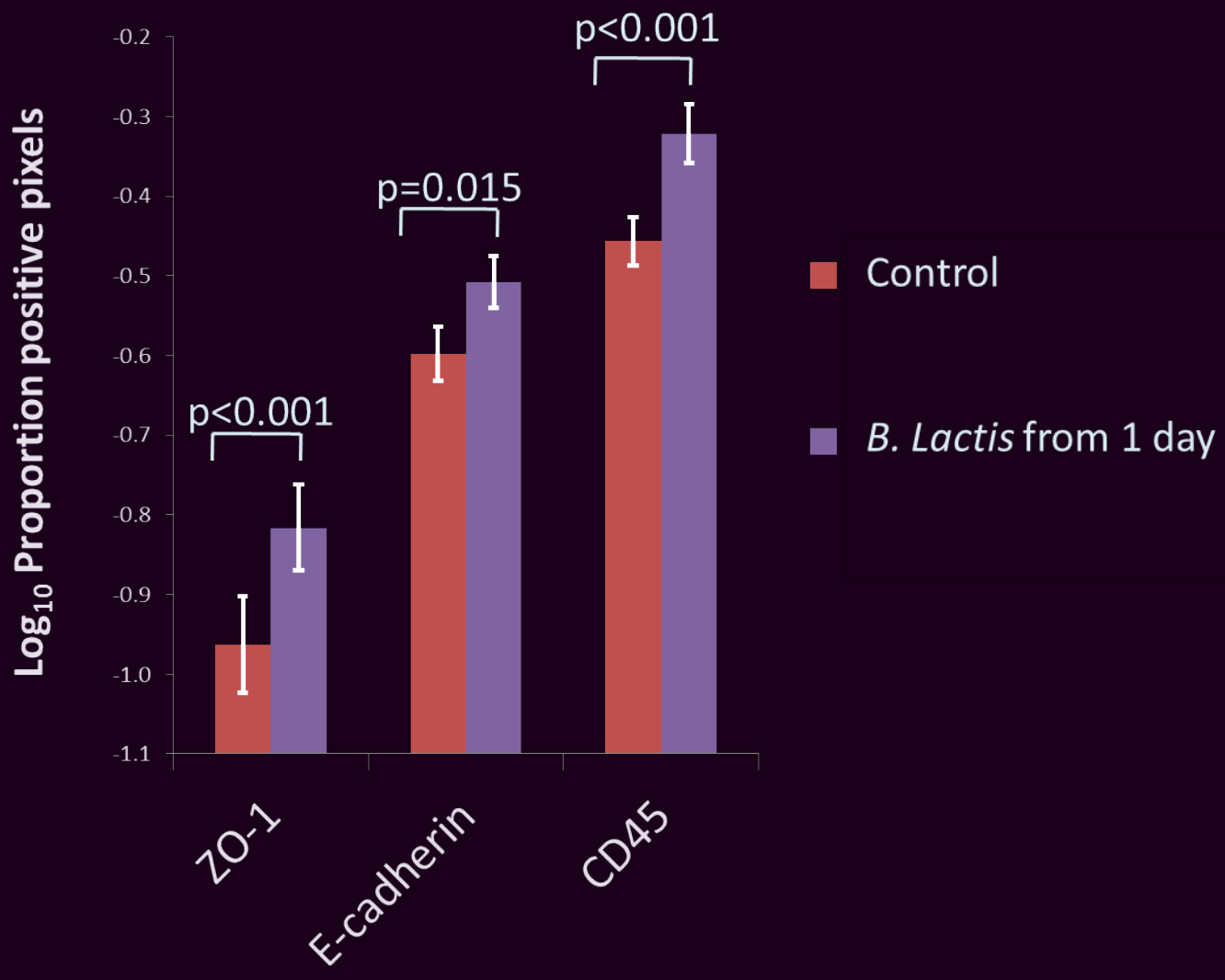




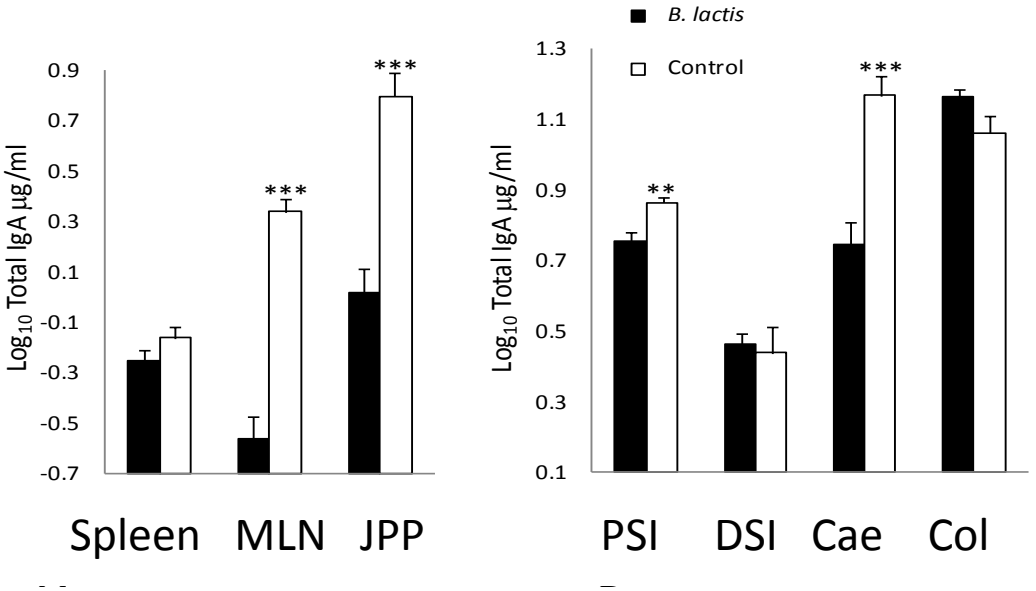




Increased epithelial barrier function after 'probiotic' administration



Decreased local IgA and IgM synthesis after 'probiotic' administration



MLN – mesenteric lymph node

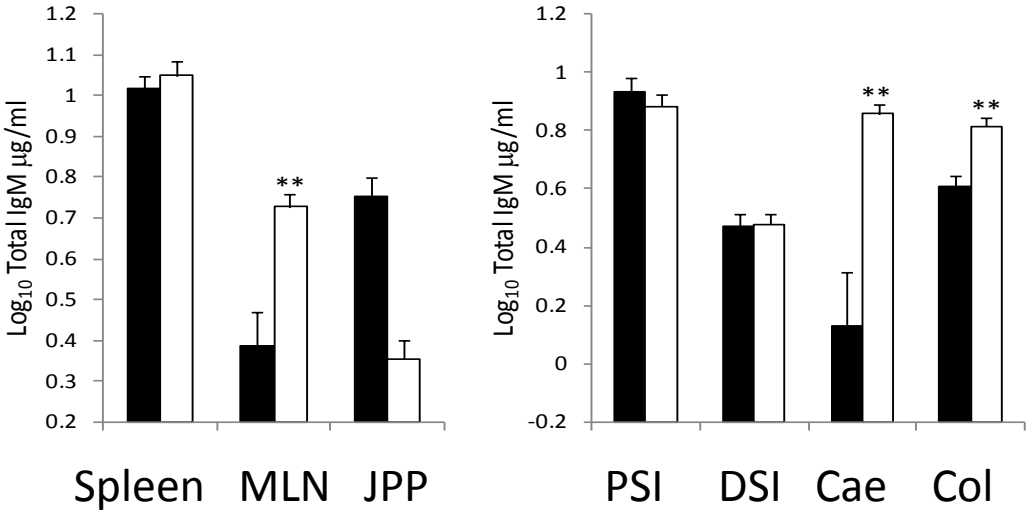
JPP – jejunal Peyers patch

PSI – upper jejunum

DSI – lower jejunum

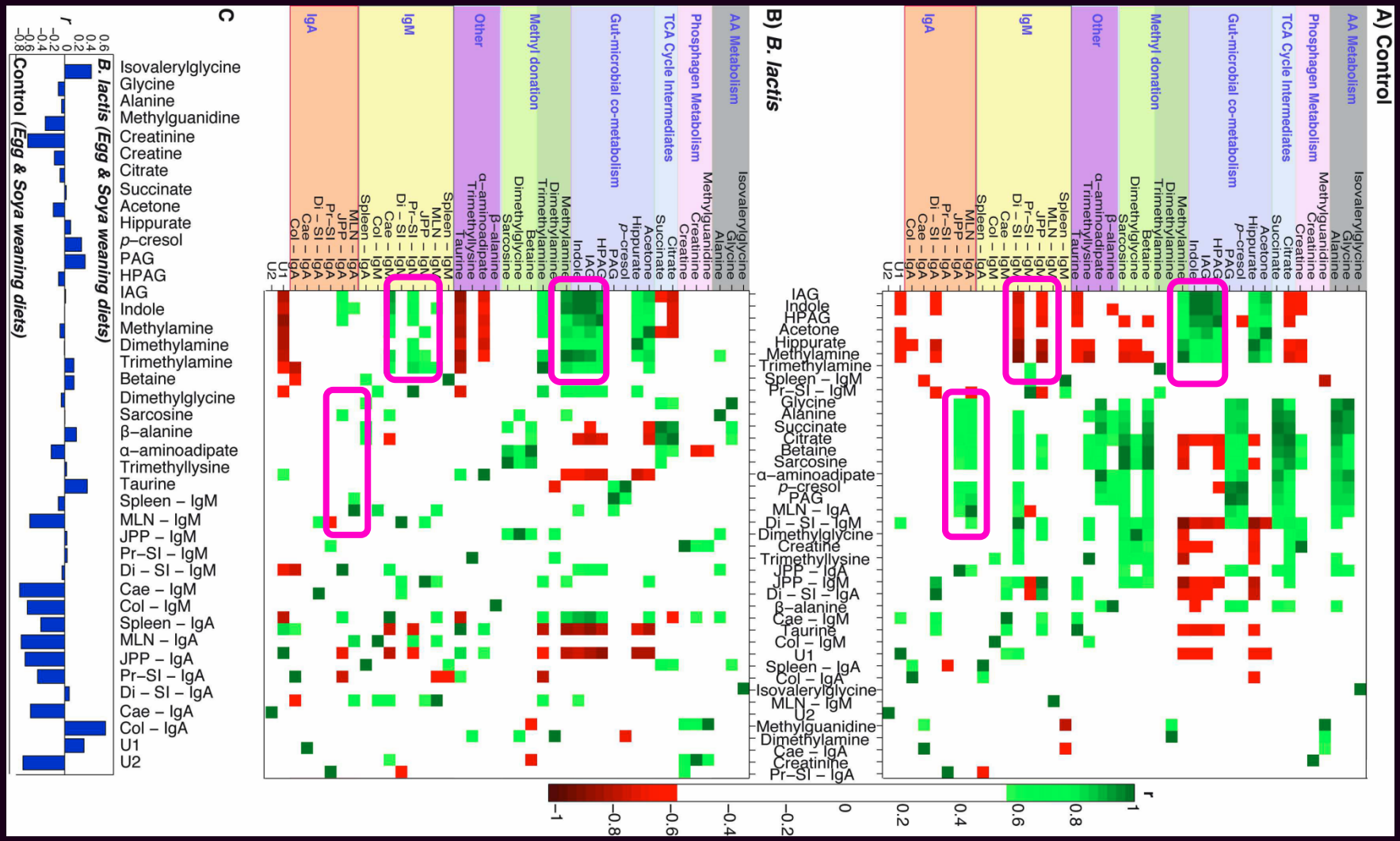
Cae – caecum

Col - colon



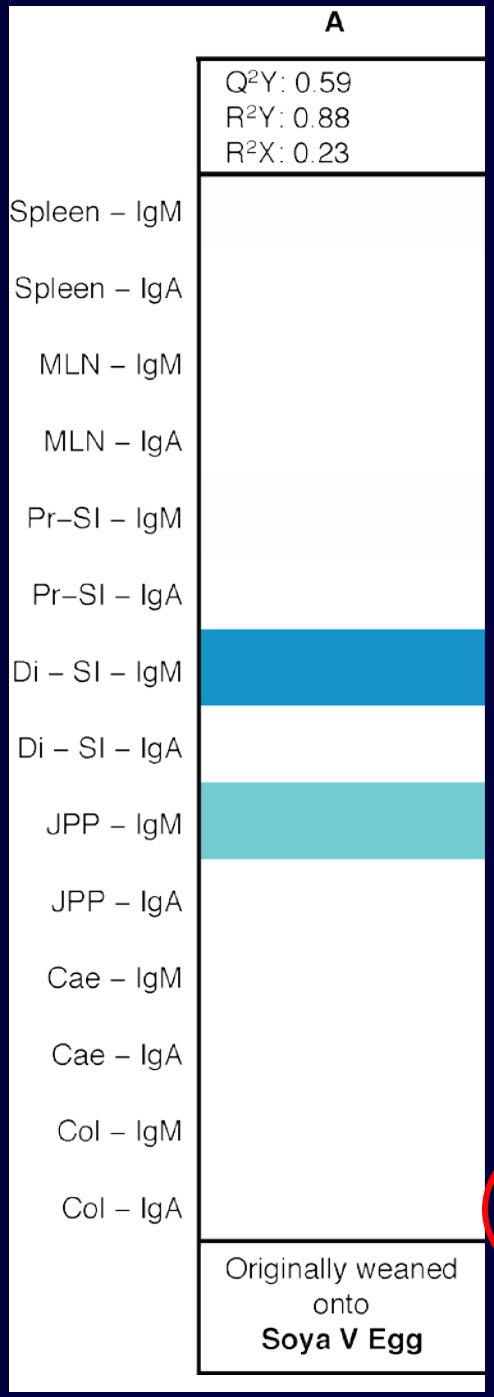
(Lewis et al, in press, BJN)

Probiotic supplementation changes the structure of immuno-metabolic correlations.



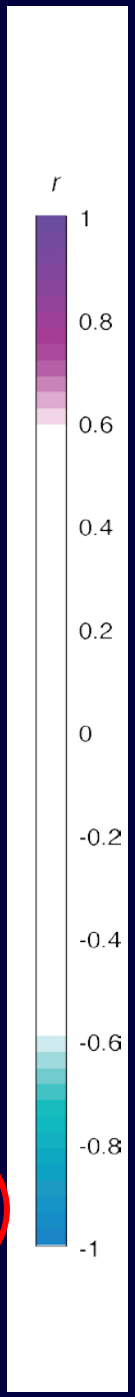
B. lactis

Control



Soya

Egg



+ B. lactis

- B. lactis

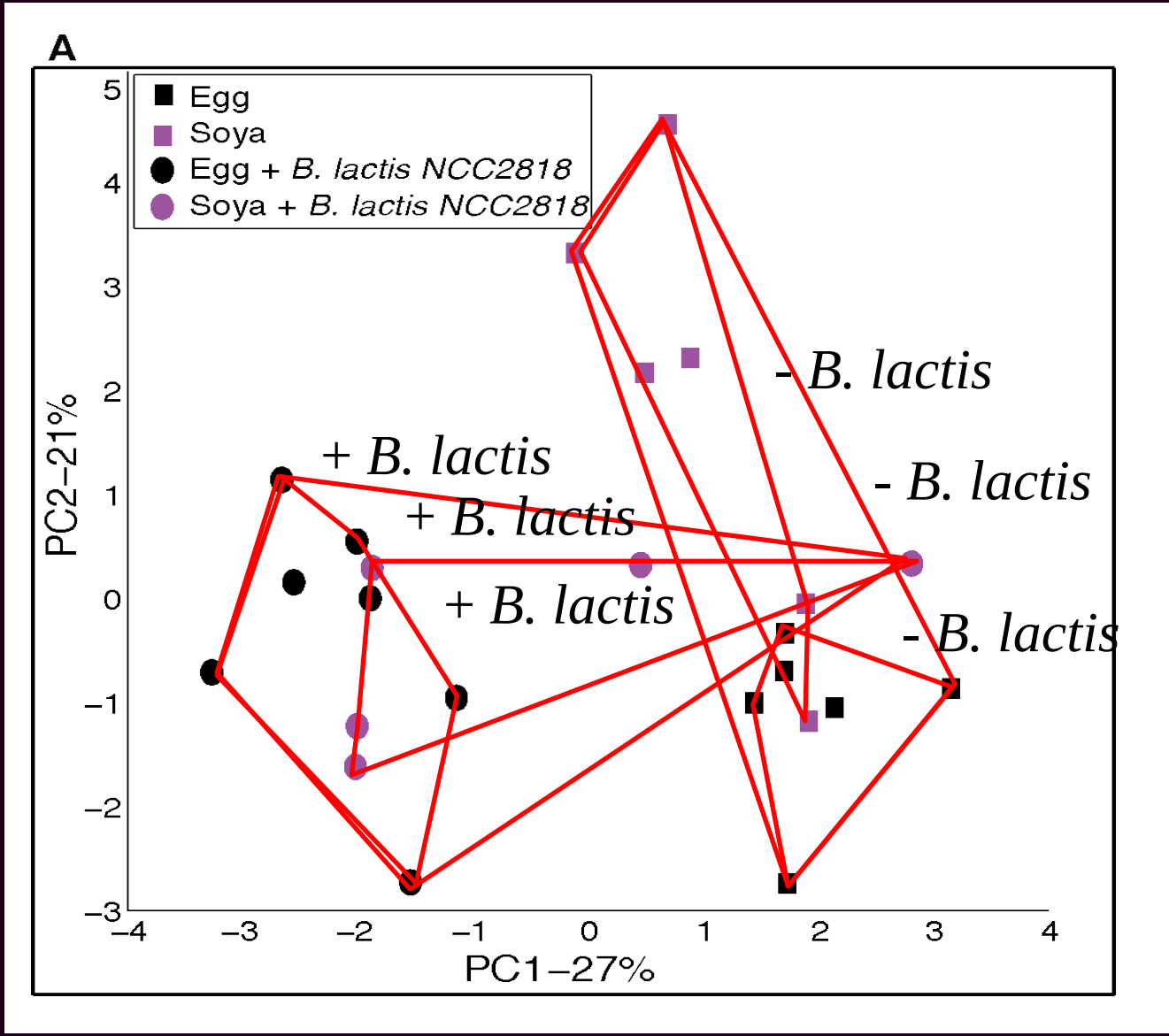
Mucosal synthesis of immunoglobulins is linked both to probiotic and to weaning diet.

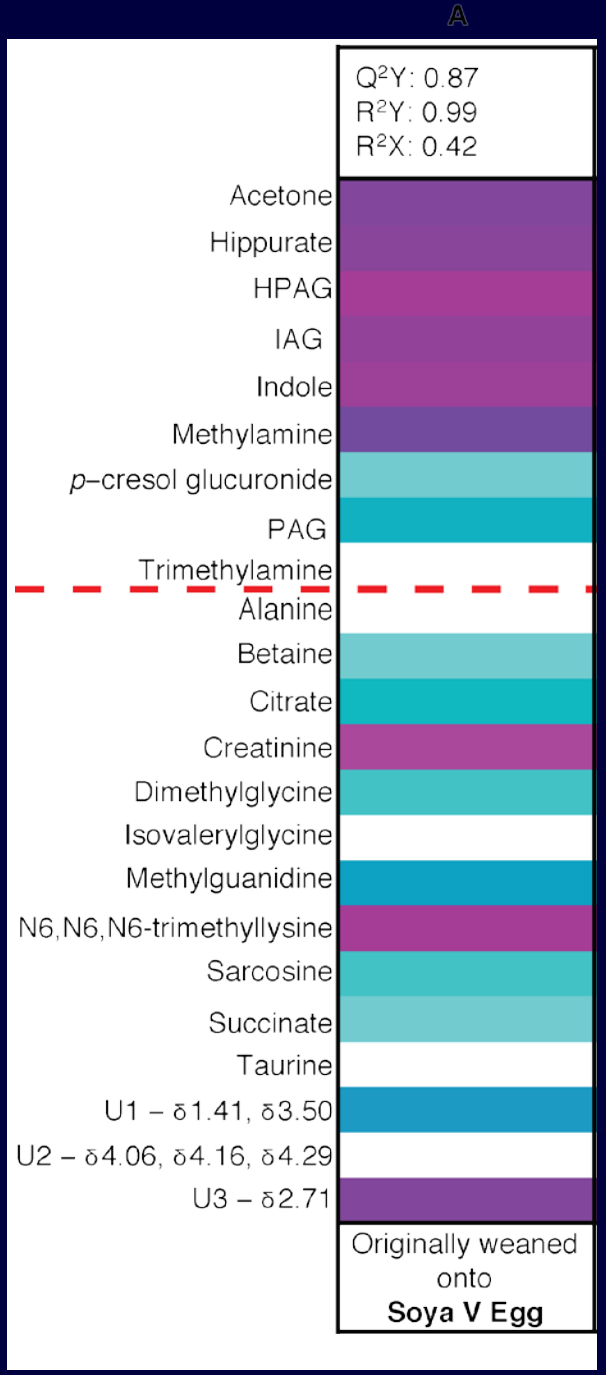
The effect of probiotic is different between diets.

Merrifield et al, Gut, in press.

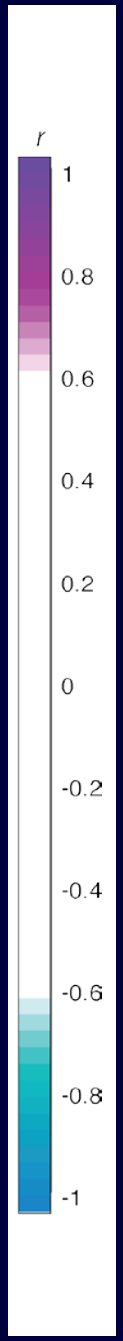
B. lactis, diet and Ig synthesis

Merrifield et al, Gut, in press





Soya



Egg

+ *B. lactis*

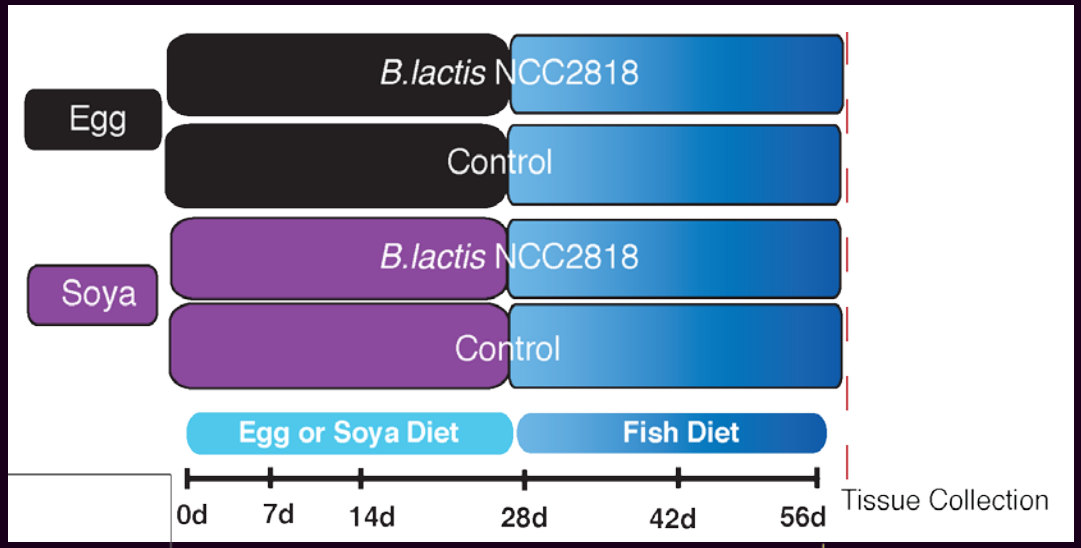
- *B. lactis*

The effect of probiotic on urinary metabolites also differs between diets.

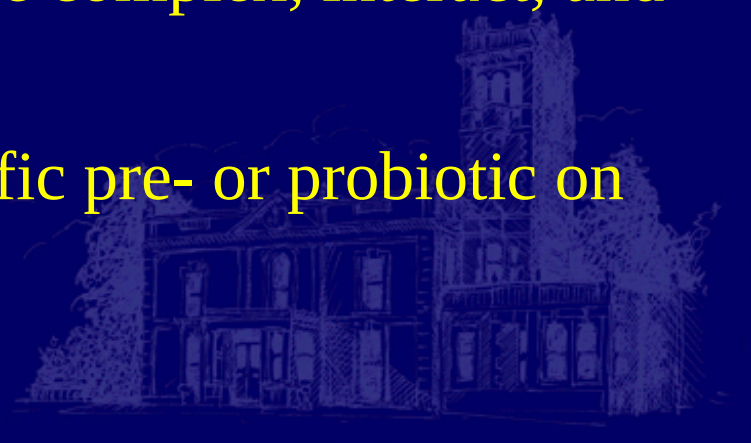
Merrifield et al, Gut, in press.

The effects of this probiotic on metabolic and immune function differ between weaning diets:

- The effect is far more obvious on piglets weaned onto egg than those weaned onto soya.
- This is despite the fact that egg and soya were fed for only 4 weeks after weaning.
- At time of sampling (12 weeks old), all piglets had been on the same diet, based on fishmeal, for 4 weeks.



1. Microbiota drives establishment of the architecture of the mucosal immune system.
2. (Different patterns of colonisation, as a consequence of different rearing environments, drive development of different mucosal immune systems.)
3. This can be manipulated by diet and/or probiotic (and/or prebiotic) in early life
4. Birth and weaning seem to be times of particular susceptibility to changes.
5. However, the patterns of change are complex, interact, and are affected by multiple factors.
6. At present, the effects of any specific pre- or probiotic on specific farms are hard to predict.



University of BRISTOL

bbsrc biotechnology and biological sciences research council

Bristol University

Mick Bailey
Paul Bland
Tom Humphrey
Steve Cose
Ross Harley
Charlotte Inman
Paniotis Tourlomousis
Jenny Bailey
Phil Jones
Christine Whiting
Philippa Lait
Martin Kenny
Sakon Singha
Anusha Edwards
Louisa Rees
John Tarleton
Chris Stokes
Karin Haverson
Frieda Jurgenson
Bevis Miller
Zoe Christoforidou
Marie Lewis
Chelsea Hicks
Cecilia Harris
Steve Wilson
Georgina Laycock
Ben Bradley
Andy Weale
Martin Birchall
Emma Barker

Wageningen University

Hauke Smidt
Odette Perez
Sergei Konstantinov

I.A.H. Compton

Jamie Leigh
Jeremy Morgan
Mark Stevens
Pam Norton
Adrian Smith
Pauline Vandiemen

Imperial College, London

Jeremy Nicholson
Claire Merrifield
Elaine Holmes
Olivier Cloarec

Roslin Institute

Liz Glass
Mary Clapperton
Steve Bishop
Alan Archibald

Aberdeen University

Denise Kelly
Bettina Schmidt
Imke Mulder

Nestec

Adrian Zuercher
Annick Mercenier
Swantje Duncker

J.S.R.

Annabelle Hoste

defra Department for Environment Food and Rural Affairs

BPEX