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Role of the maternal microbiota in shaping gene expression and microbiota composition in the offspring intestine

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Are we human?

100 % Human ?

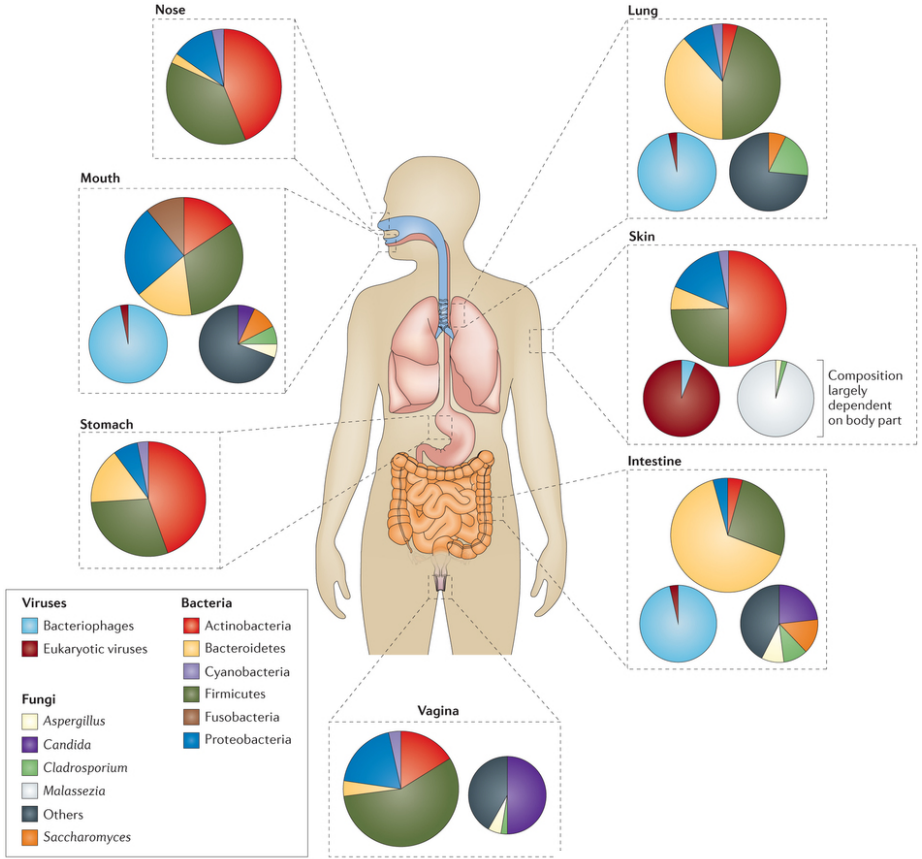
50% microbes

$10^{13} – 10^{14}$ microbes

$10^{12} – 10^{13}$ body cells

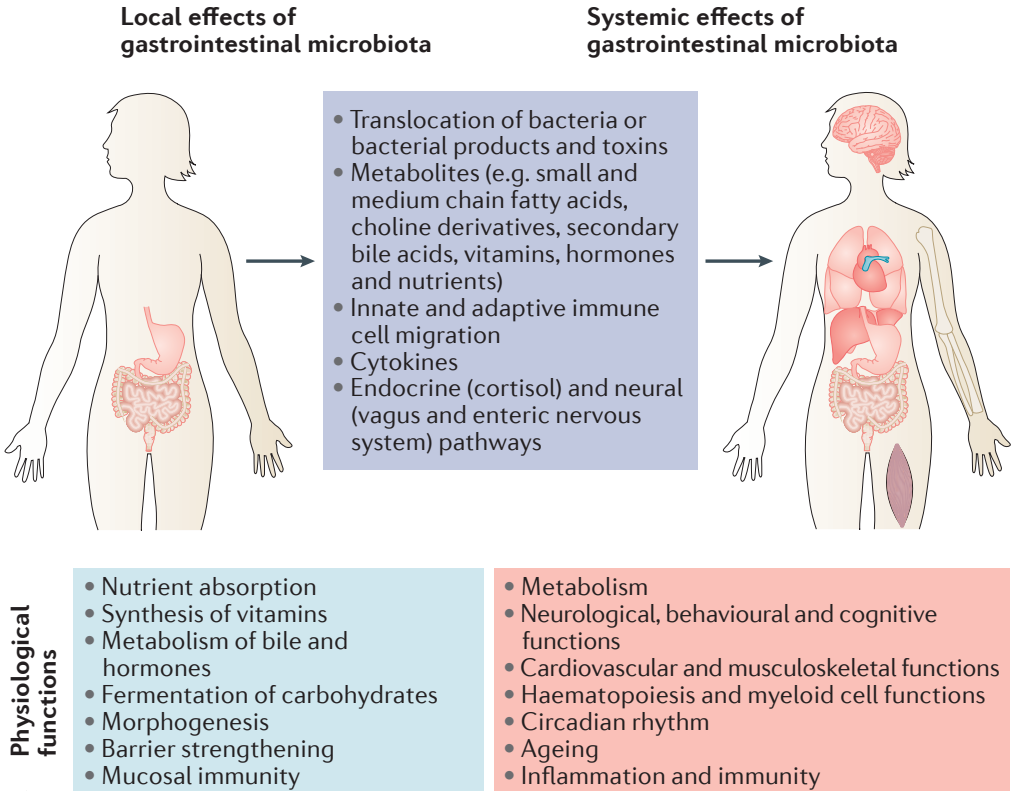
50% human cells

Sender et al., 2016



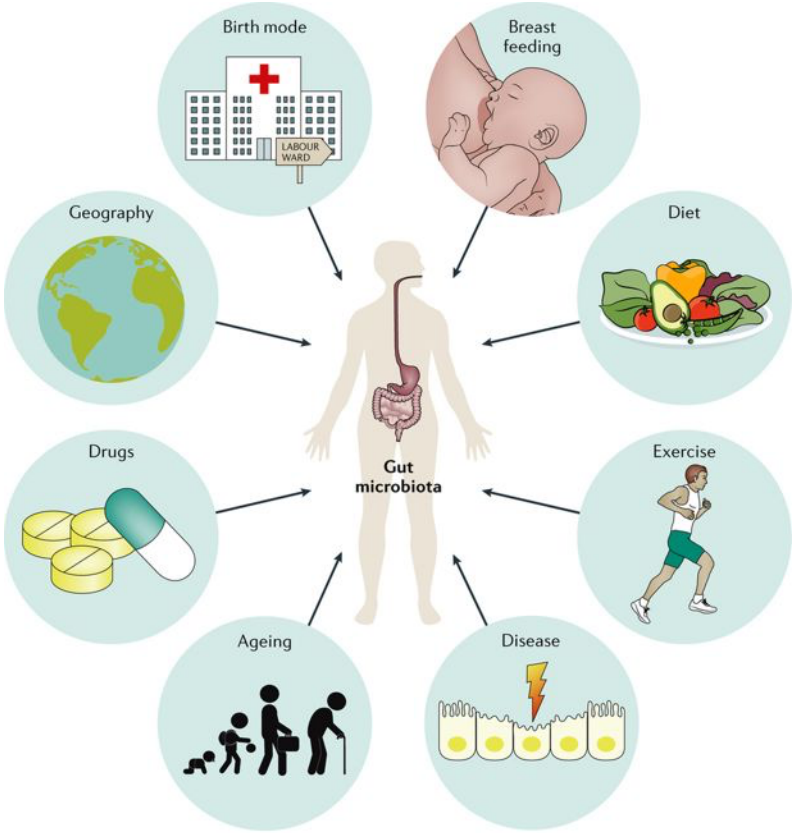
Marsland and Gollwitzer, 2014

The microbiome and host function



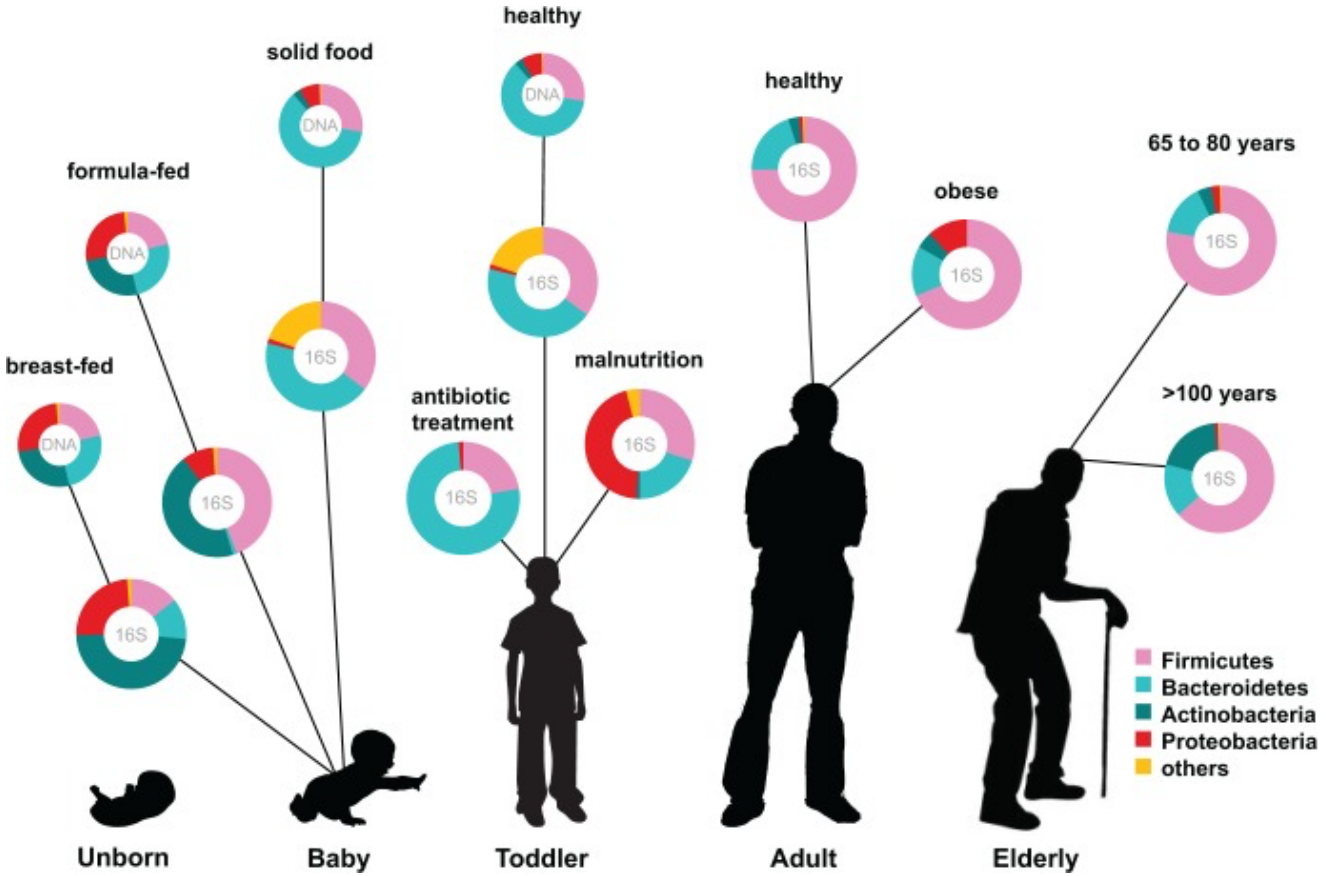
Roy et al., 2017

Commensal microbiota is shaped throughout life



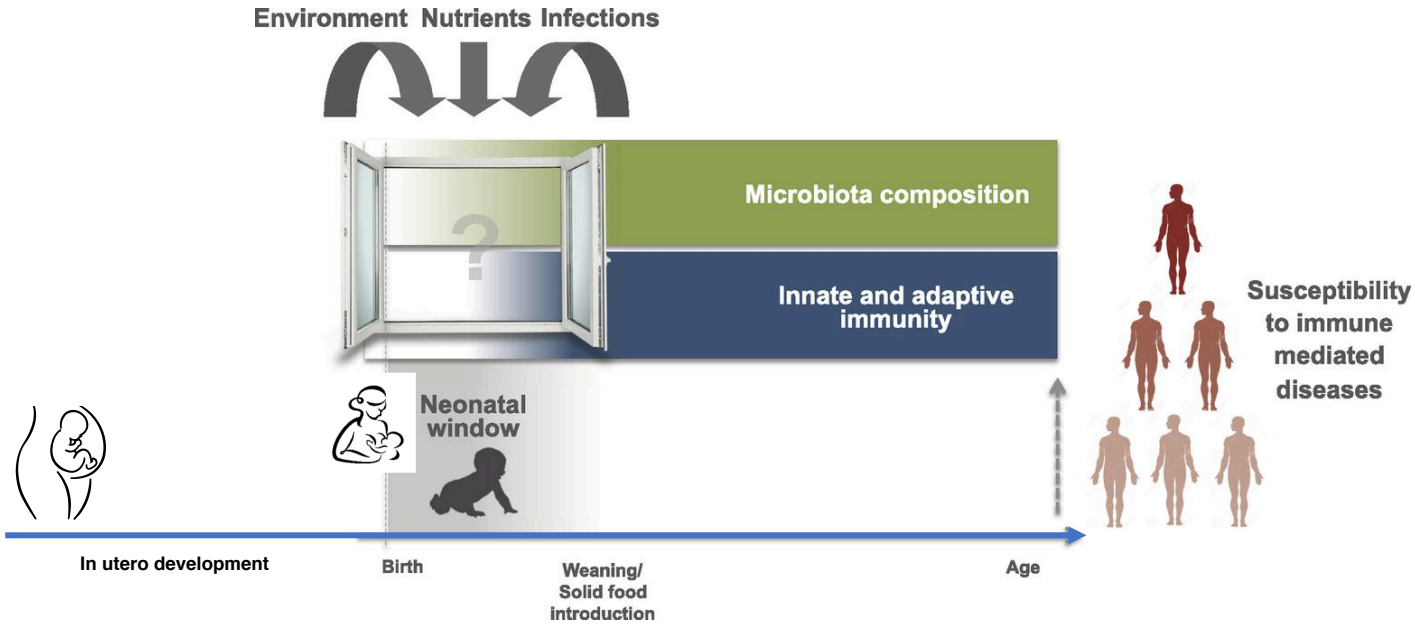
Quigley et al., 2017

Commensal microbiota is shaped throughout life



Ottman et al., 2012

The neonatal **window of opportunity**



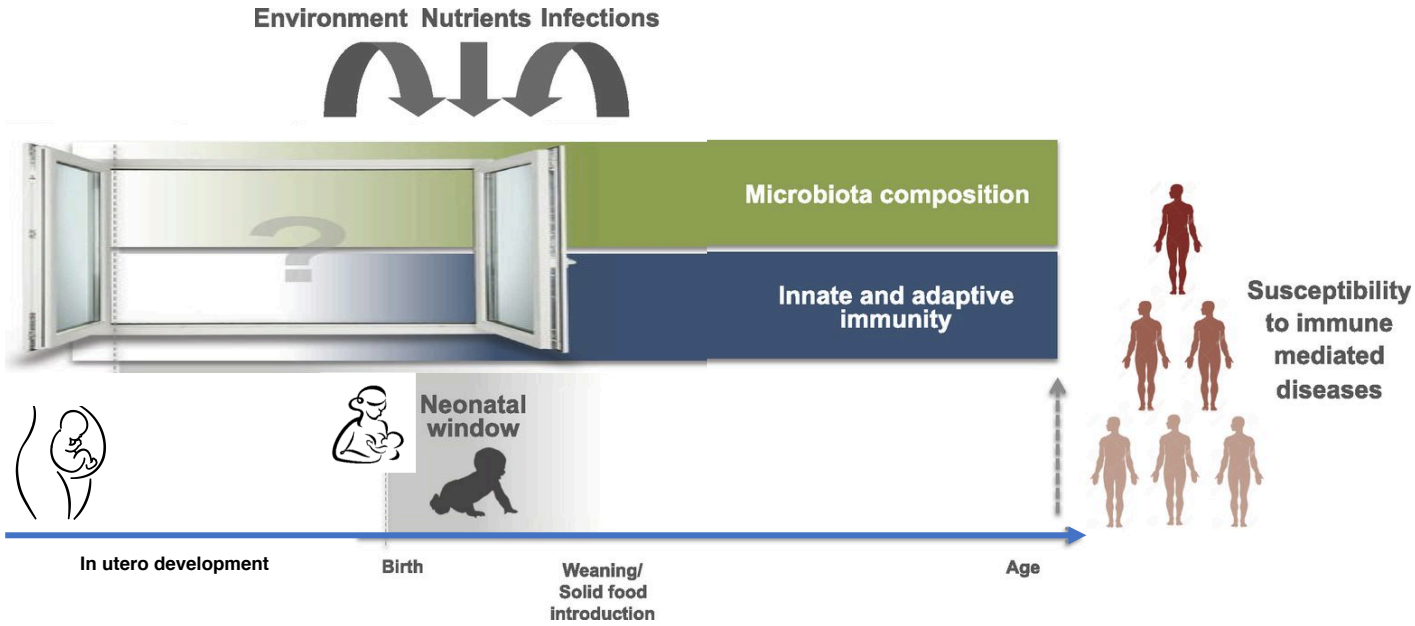
Early life microbial signals

IMMUNE SYSTEM ?

Cahenzli et al., 2013
Olszak et al., 2012
Kearney et al., 2015
Torow et al., 2017

Modified from Torow et al., 2017

The neonatal **window of opportunity**

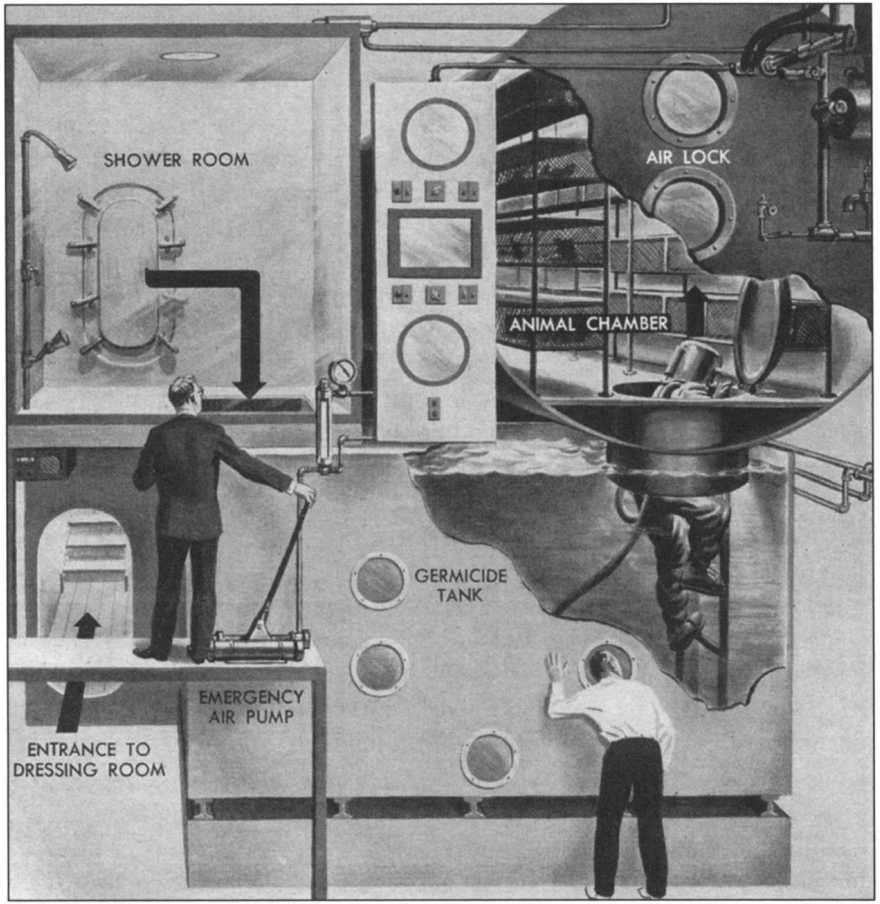


Signals originating from maternal microbiota

OFFSPRING IMMUNE SYSTEM ?

Modified from Torow et al., 2017

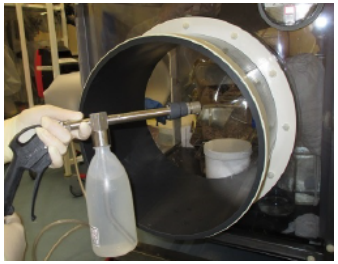
The first germ-free isolators



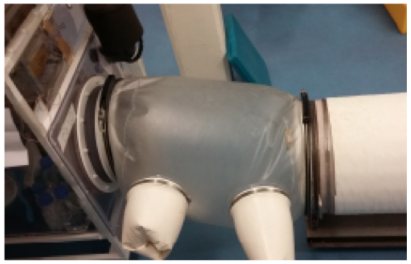
Kirk, 2012

Work with germ-free isolators in the **Clean Mouse Facility Bern**

Autoclaved food and water drums



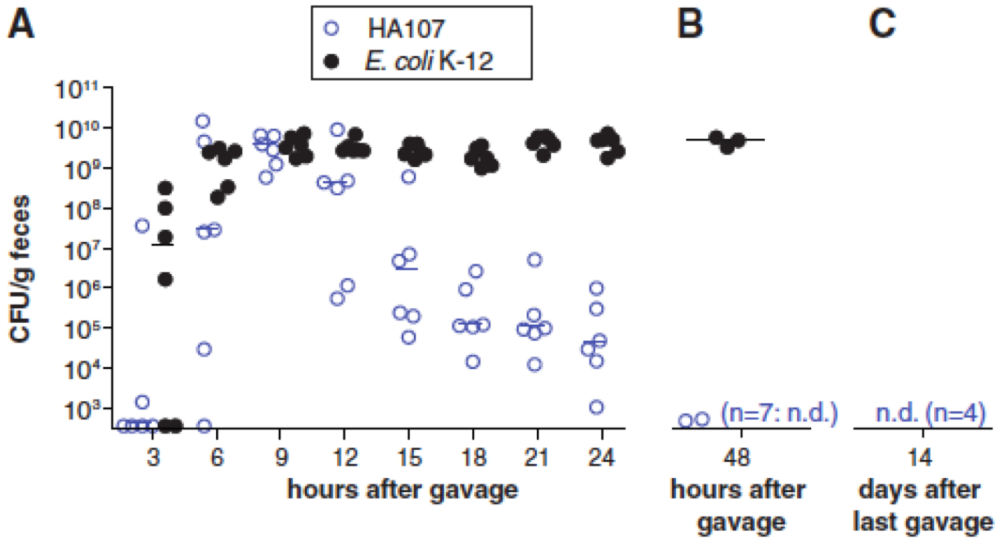
Peracetic acid sterilization



Isolator-drum connection

Reversible colonization of germ-free mice with *E. coli* HA107

- *Escherichia Coli* K-12 JM83
- auxotrophic deficiency in meso-diaminopimelic acid (m-DAP) and D-Ala synthesis
- reversibly colonizes germ-free mice for 12 - 48 hours



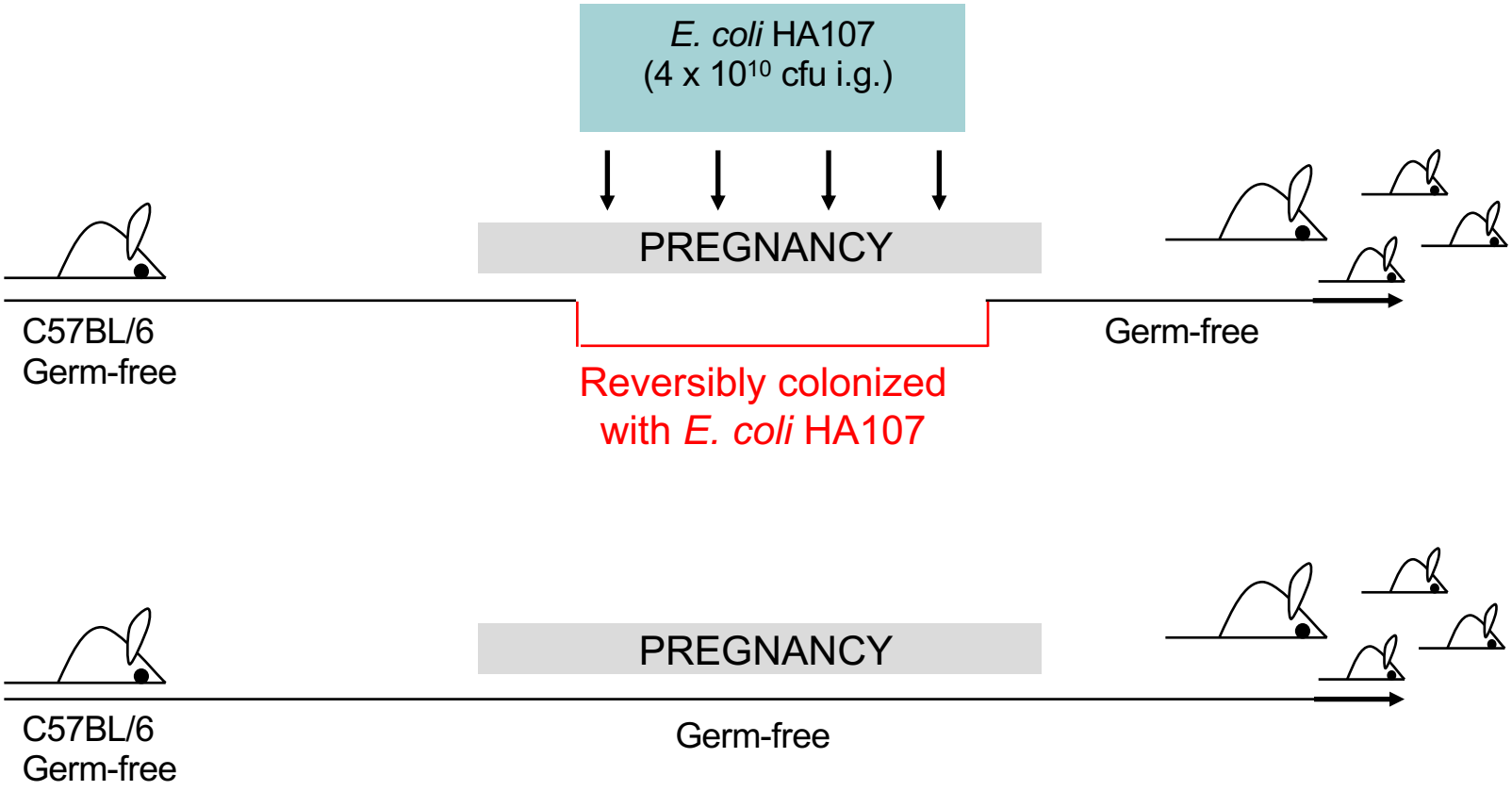
→ Defined dose

→ Defined time

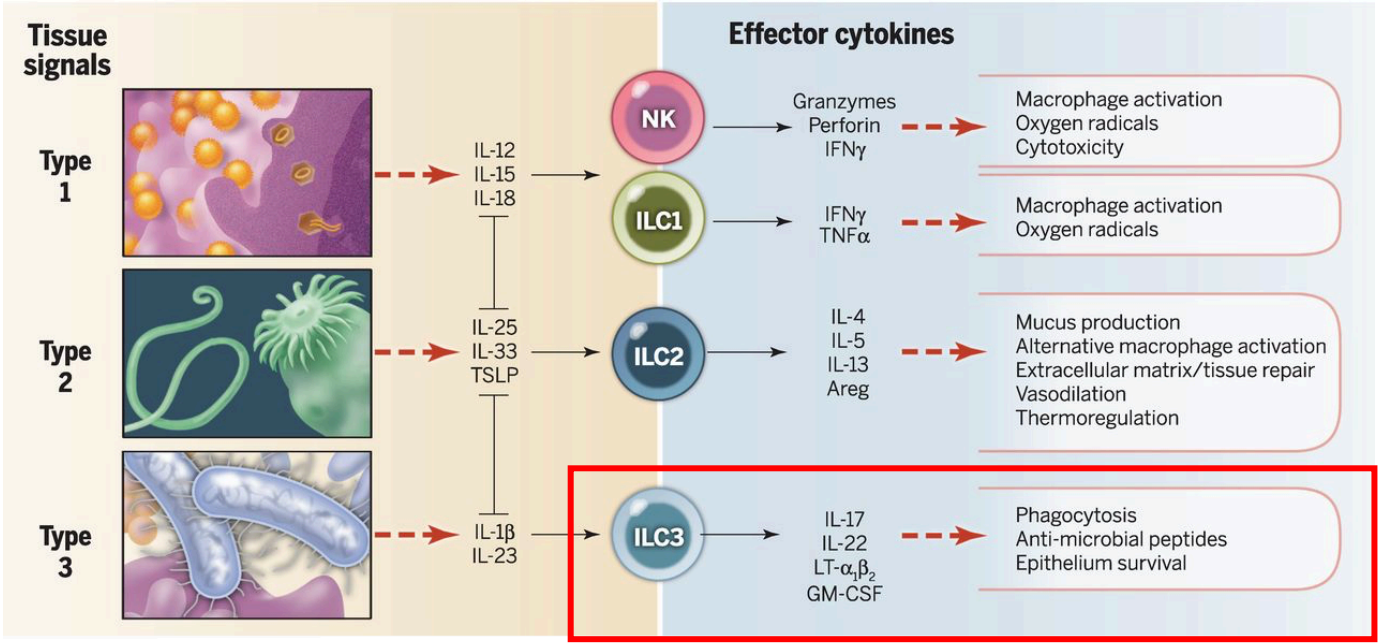
→ Defined stage of life

Hapfelmeier et al., Science, 2010

Model of gestational colonization with auxotrophic *E. coli* HA107

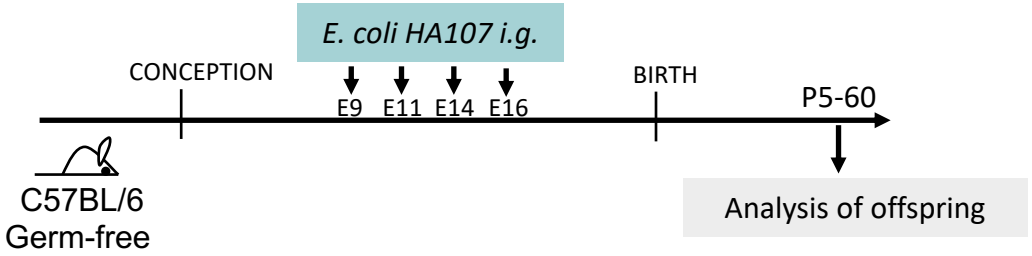


Innate lymphoid cells (ILC) are mainly present at mucosal sites and can be classified into 3 subgroups

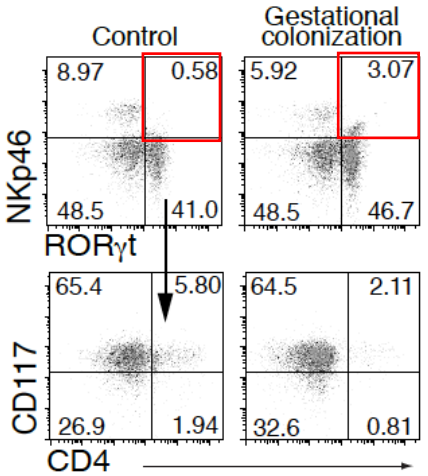


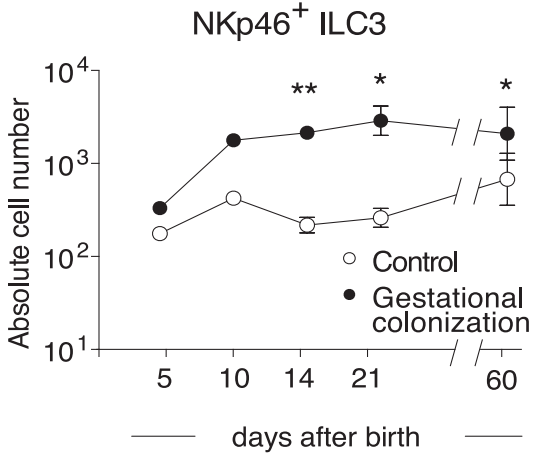
Eberl et al., Science, 2015

Maternal colonization during pregnancy increases the number of intestinal NKp46^{+} ILC3s in the offspring



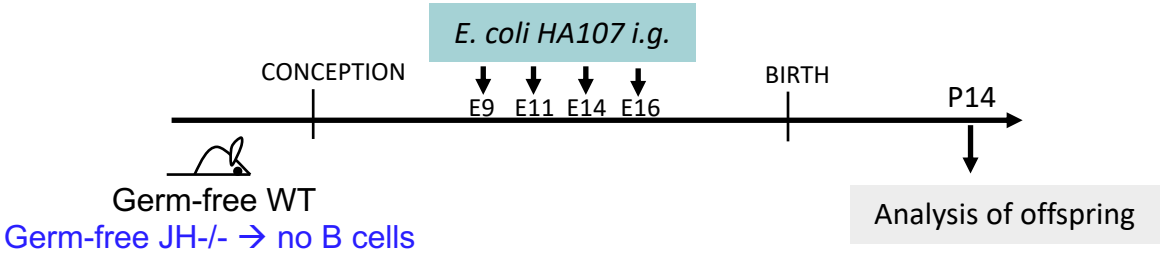
OFFSPRING small intestine lamina propria, Lin^{-} cells:



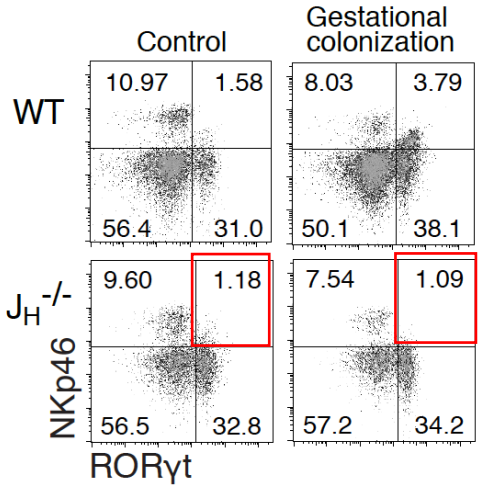


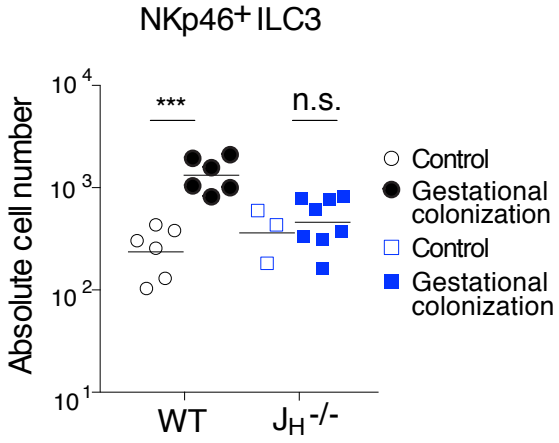
Gomez de Agüero, Ganal-Vonarburg et al., Science, 2016

Maternal colonization during pregnancy increases the number of intestinal NKp46⁺ ILC3s in the offspring



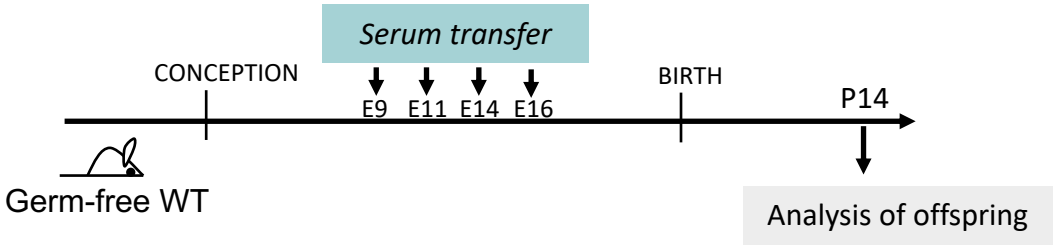
OFFSPRING small intestine lamina propria, Lin⁻ cells:

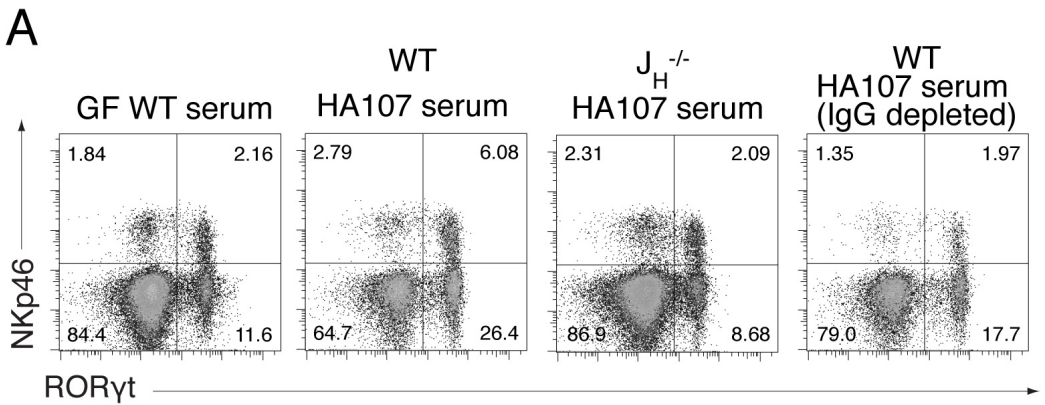


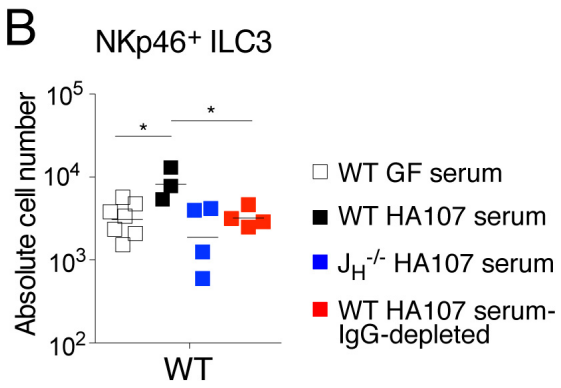


Gomez de Agüero, Ganal-Vonarburg et al., Science, 2016

Maternal colonization during pregnancy increases the number of intestinal NKp46⁺ ILC3s in the offspring

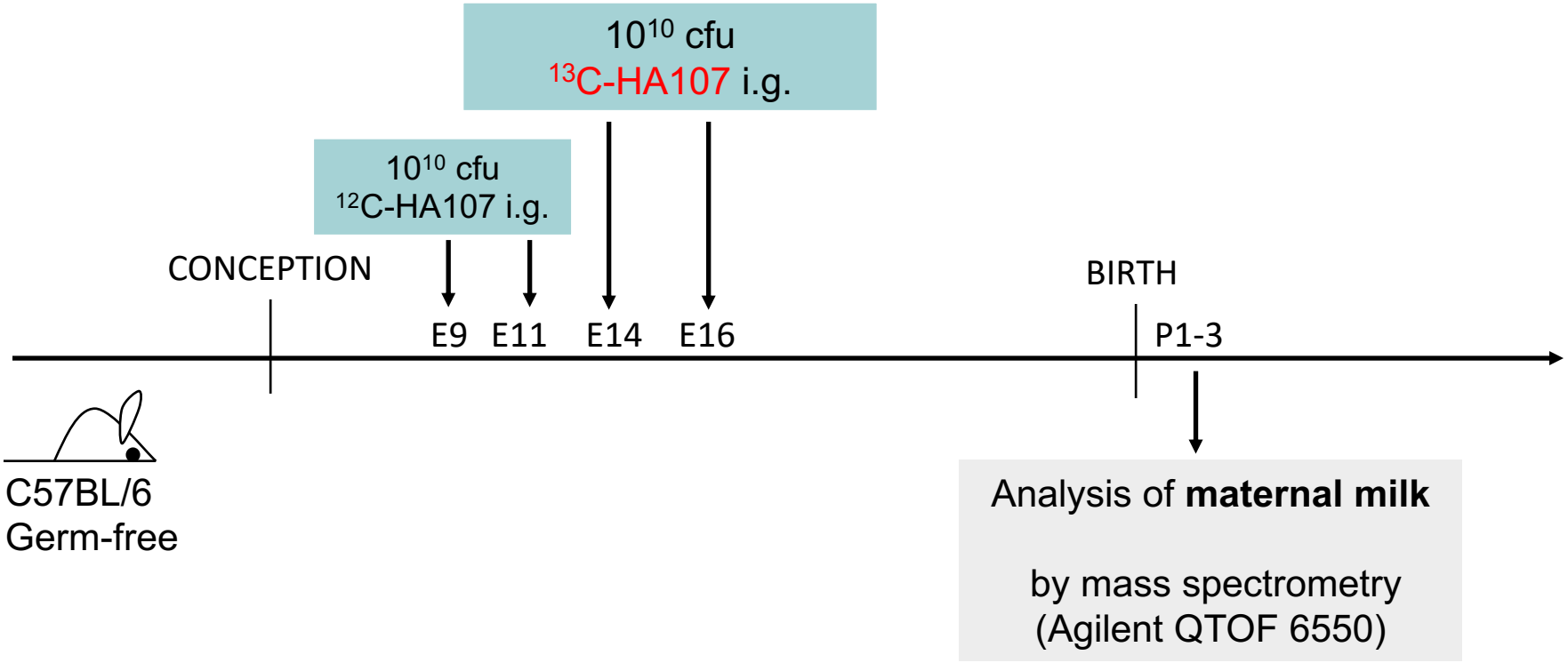




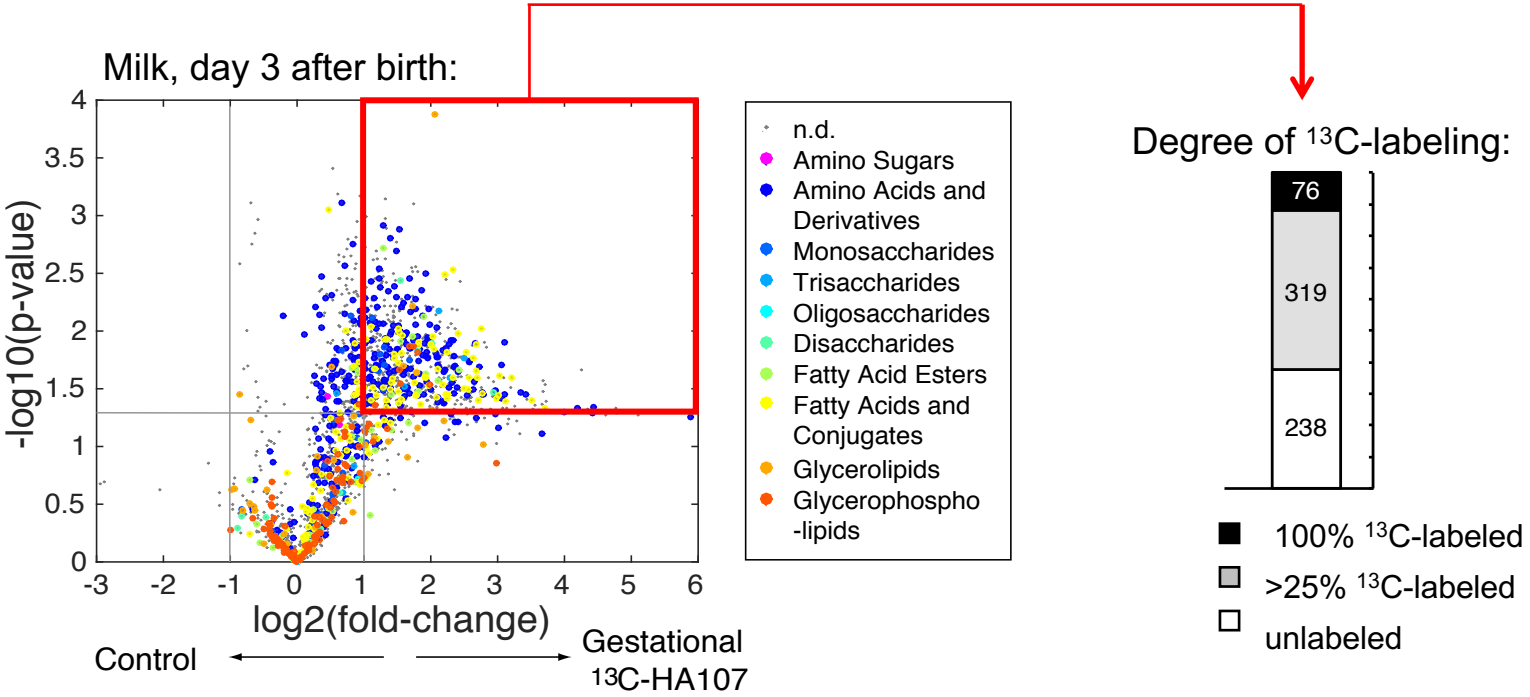


Gomez de Agüero, Ganal-Vonarburg et al., Science, 2016

Use of ^{13}C-labeled *E. coli* HA107 and mass spectrometry to identify microbiota-derived metabolites that are transferred to the offspring

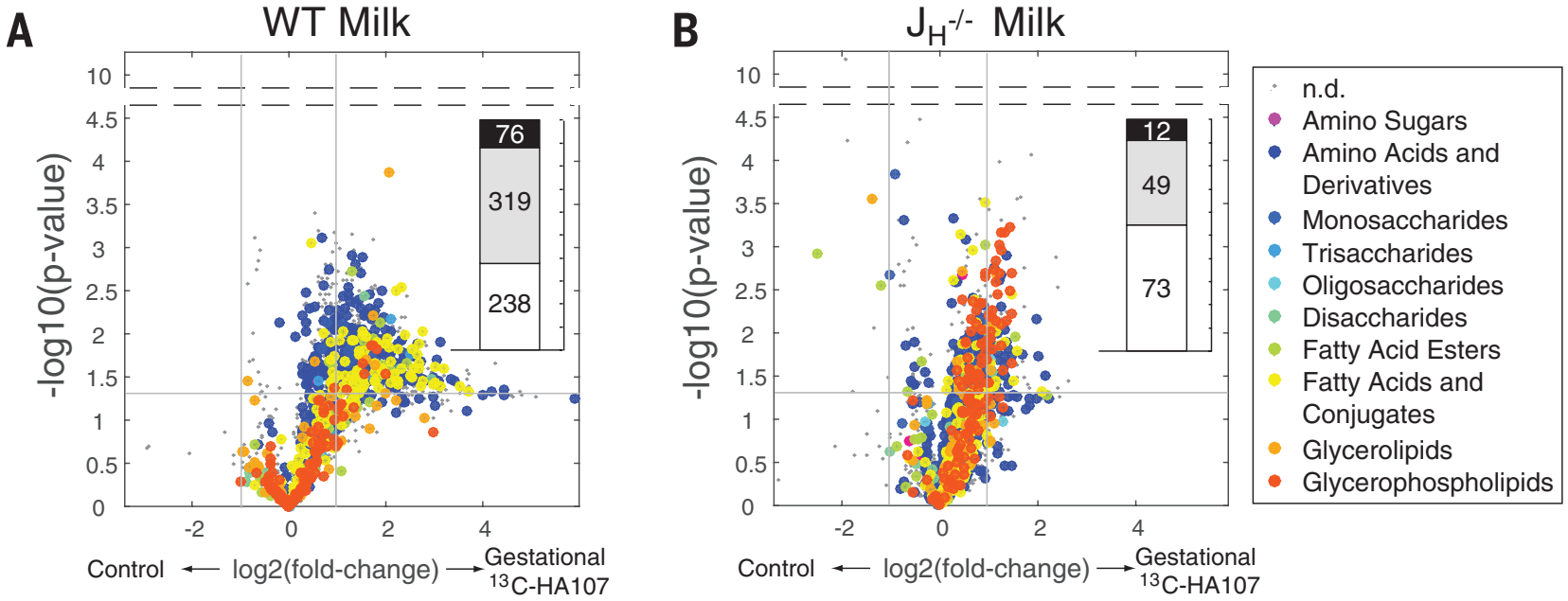


Milk of gestationally colonized dams is metabolically different and contains bacterial metabolites



Gomez de Agüero, Ganal-Vonarburg et al., Science, 2016

The presence of maternal antibodies significantly alters the composition of the milk



Gomez de Agüero, Ganal-Vonarburg et al., Science, 2016

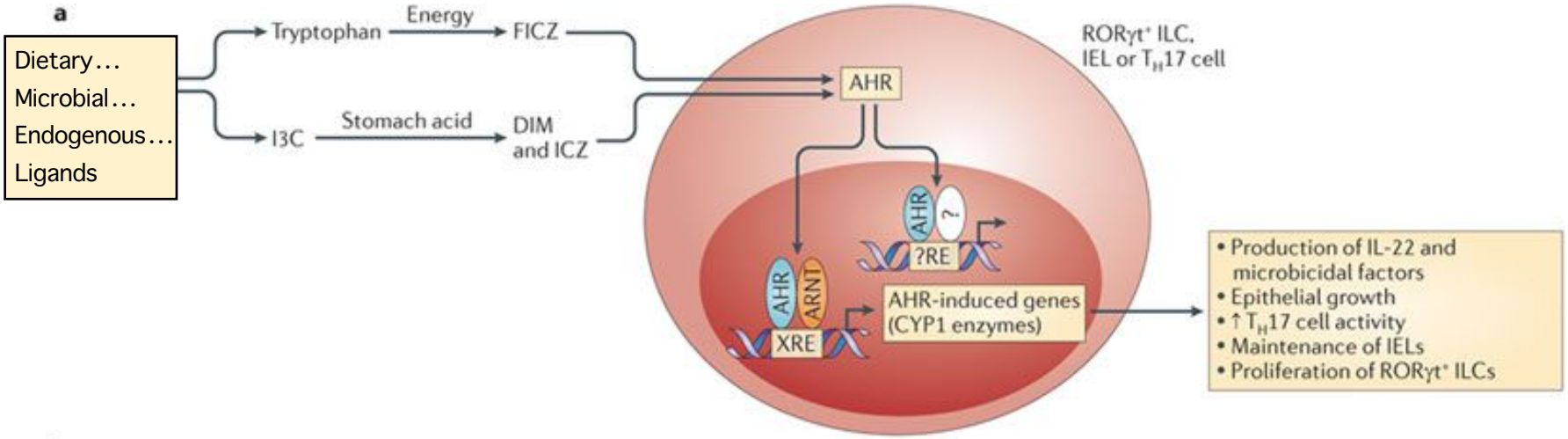
The milk of gestationally colonized mice is significantly enriched in bacteria-derived indoles/tryptophan metabolites

Tissue	Compound	Sum formula	WT		JH-/-	
			fold-change	p-value	fold-change	p-value
Milk	Kynurenine	[+10] C10H12N2O3	7.807	0.0325	2.529	> 0.05
	Indolelactic acid	[+11] C11H11NO3	6.790	0.0341	n.d.	
	5-Hydroxy-L-tryptophan	[+11] C11H12N2O3	5.248	0.0308	1.791	> 0.05
	Hydroxykynurenamine	[+9] C9H12N2O2	4.973	0.0278	0.974	> 0.05
	Hydroxykynurenine	[+10] C10H12N2O4	4.539	0.0406	1.457	> 0.05
	5-Methoxytryptamine	[+11] C11H14N2O	4.181	0.0380	1.190	> 0.05
	5-Methoxytryptophol	[+11] C11H13NO2	3.808	0.0109	1.235	> 0.05
	Indole-5,6-quinone	[+8] C8H5NO2	2.024	0.0019	1.307	0.0057
Neonatal liver	Hydroxykynurenine	[+10] C10H12N2O4	2.394	< 0.0001	1.679	0.0015
Neonatal spleen	Hydroxykynurenine	[+10] C10H12N2O4	2.754	< 0.0001	1.656	0.0015

Arylhydrocarbon receptor ligands

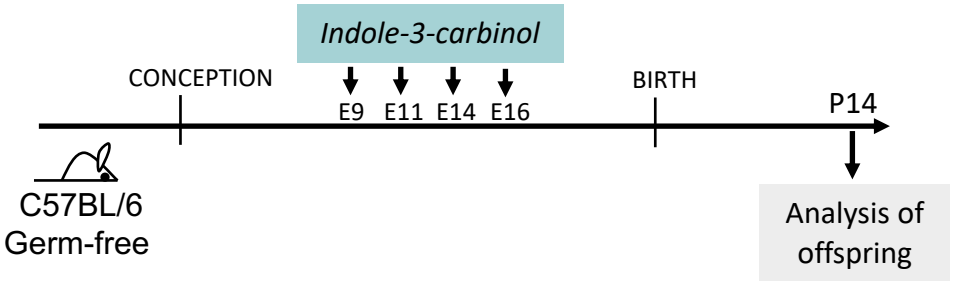
Gomez de Agüero, Ganal-Vonarburg et al., Science, 2016

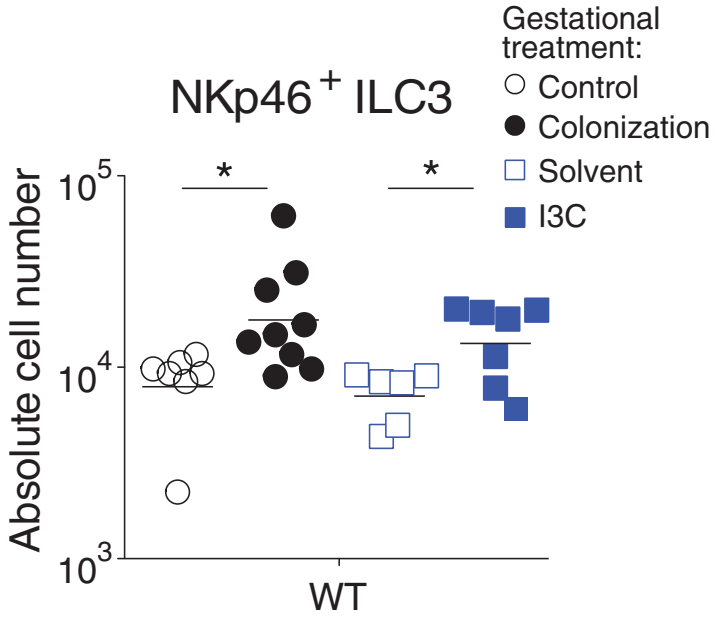
The Arylhydrocarbon Receptor (AhR) and its ligands



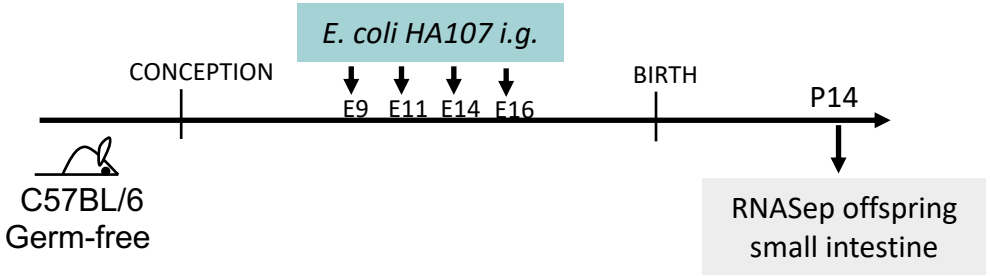
Modified from Veldhoen et al., 2012

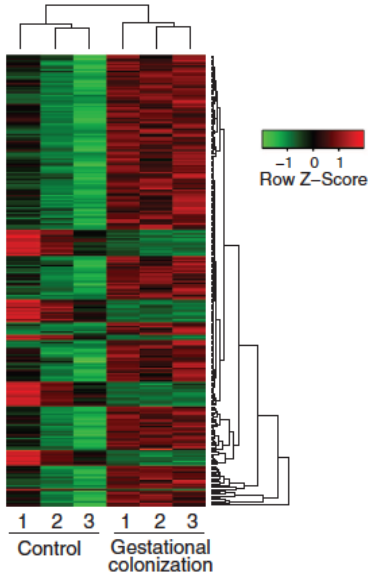
Gestational application of a purified AhR ligand (I3C) recapitulates changes in the offspring immune system observed after gestational colonization



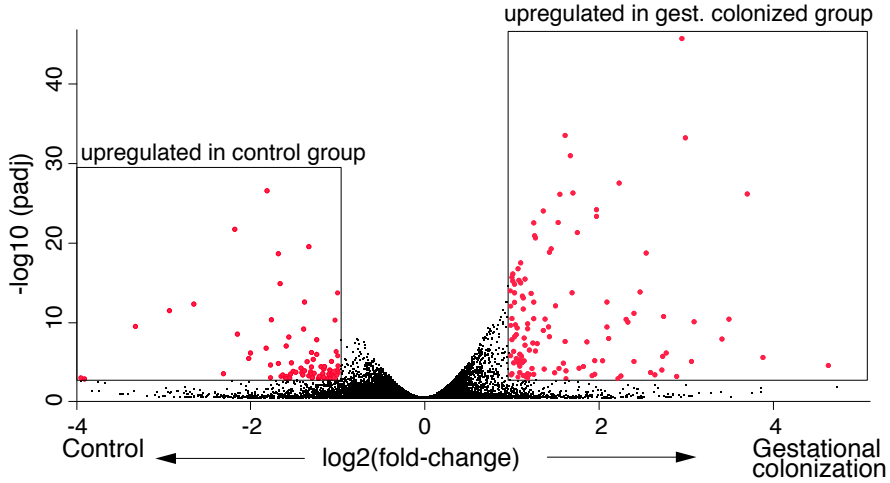


Gestational colonization widely alters intestinal gene expression profile in the offspring

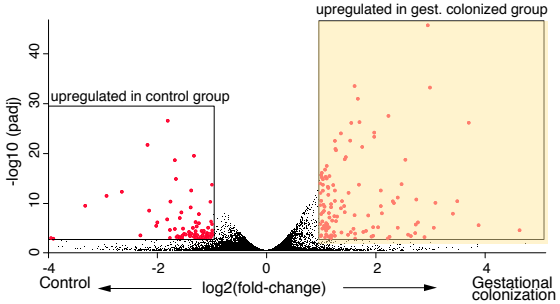




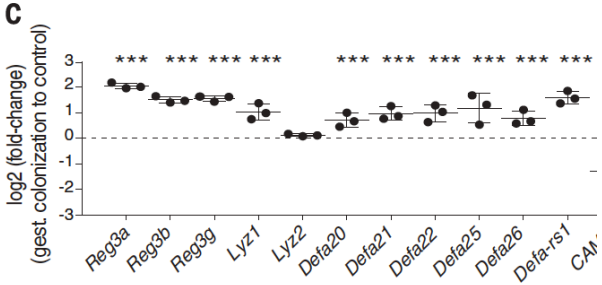
> 2000 genes significantly altered



Gestational colonization widely alters intestinal gene expression profile in the offspring

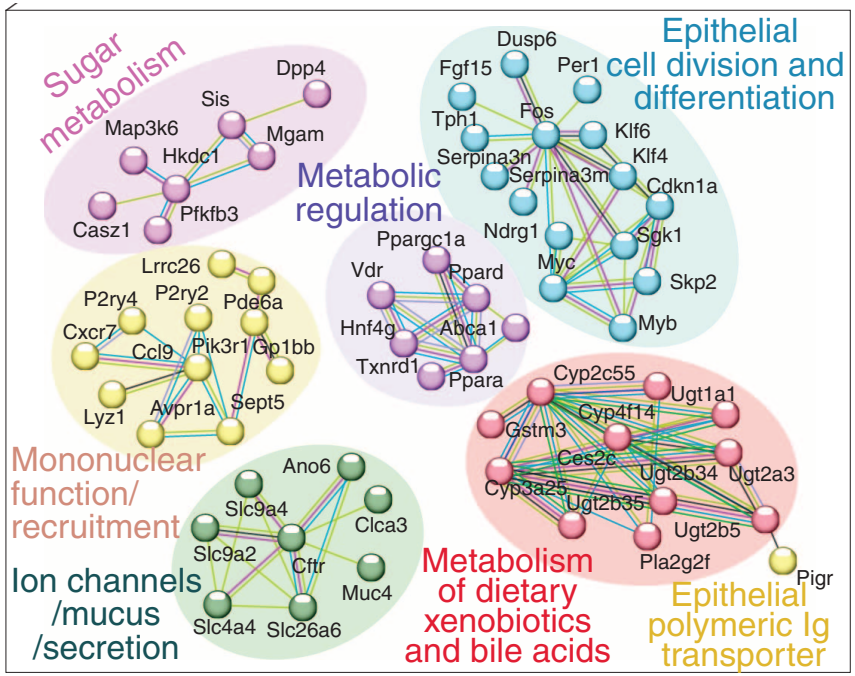


Expression of antimicrobial peptides

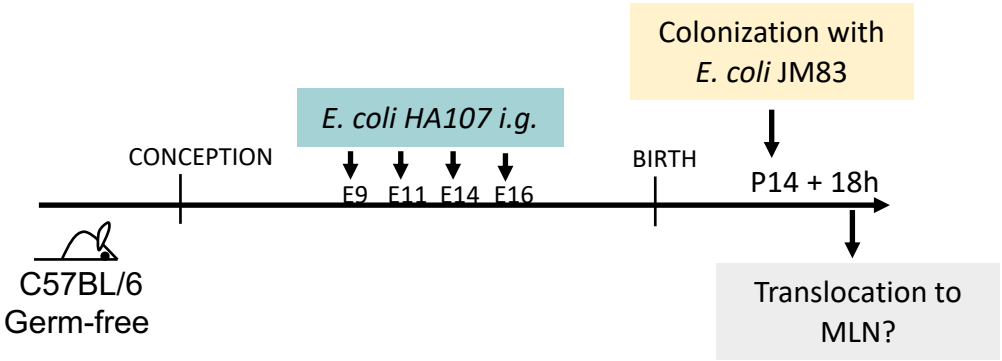


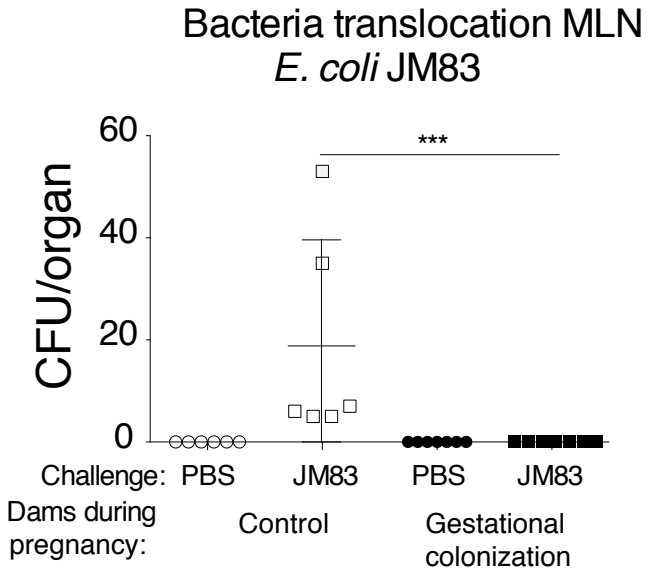
STRING analysis

fc ≤2, p-value<0.001



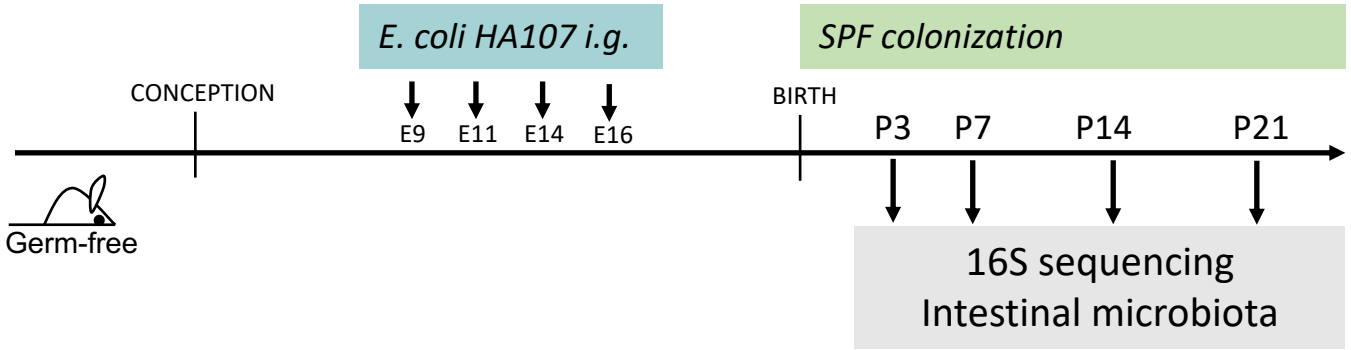
Gestational colonization prepares the offspring for colonization with microbes after birth





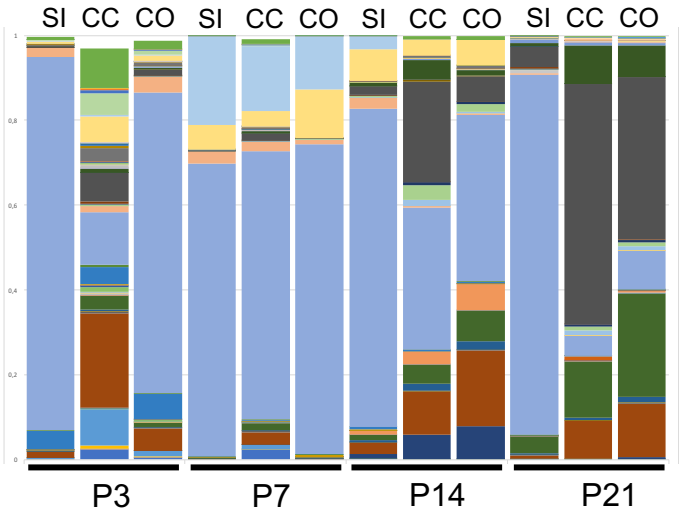
Gomez de Agüero, Ganal-Vonarburg et al., Science, 2016

Does the offspring born to gestationally colonized dams acquire a different commensal microbiota than the offspring born to germ-free control mice?



Offspring born to gestationally colonized dams exhibit a delayed maturation of intestinal commensal microbiota

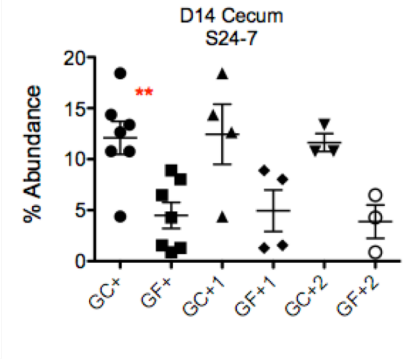
Succession of SPF microbiota after birth:

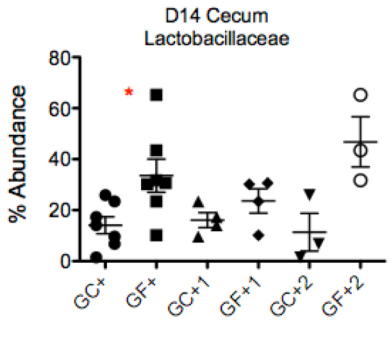


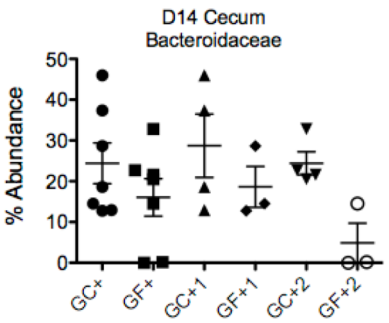
Lactobacillaceae
Verrucomicrobiaceae
Bacteroidaceae
Enterobacteriaceae
Lachnospiraceae
Streptococcaceae
Staphylococcaceae
S24-7
Pasteurellaceae
Paraprevotellaceae

Cecum P14:

Control
Gestational colonization







With Jakob Zimmermann

Summary

- We have established a reversible gestational colonization model using the auxotrophic *E. coli* HA107 strain

- Signals originating from the maternal intestinal microbiota contribute to the maturation of the offspring intestinal immune system

- Bacterial metabolites are transferred from the mother to the offspring with the help of maternal antibodies secreted into the breast milk

- The effects on the offspring immune system were long-lasting until adulthood and the offspring reacted differently to an incoming endogenous microbiota

- Epigenetics?
- Disease Models?

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