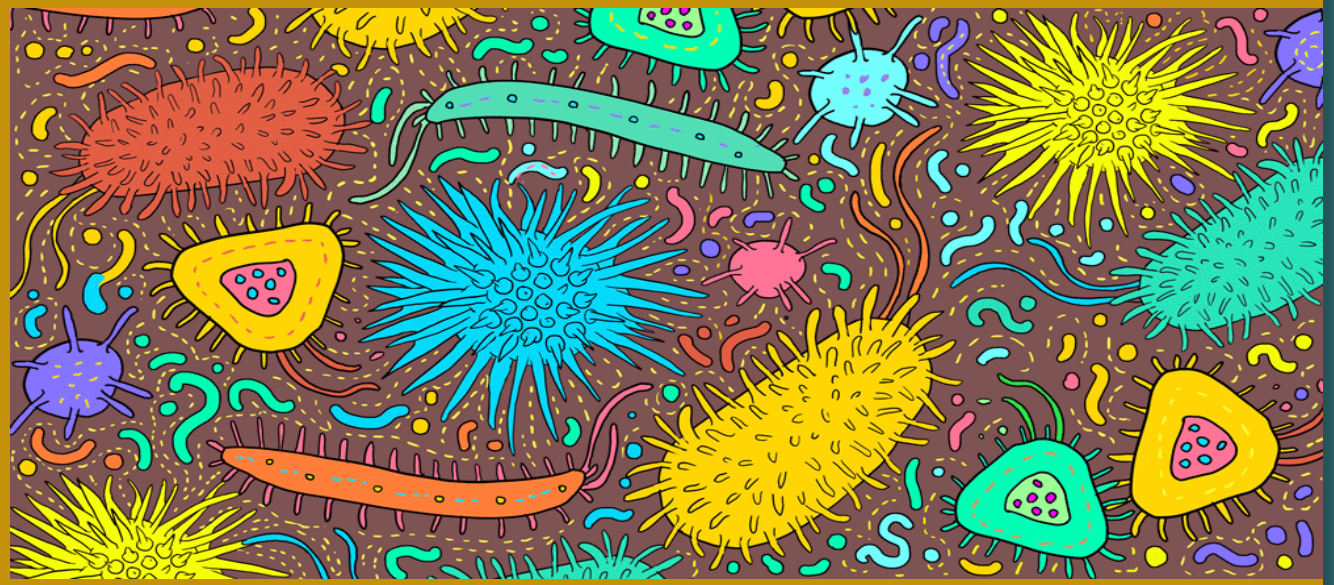


“The early life microbiome and disrupting exposures”

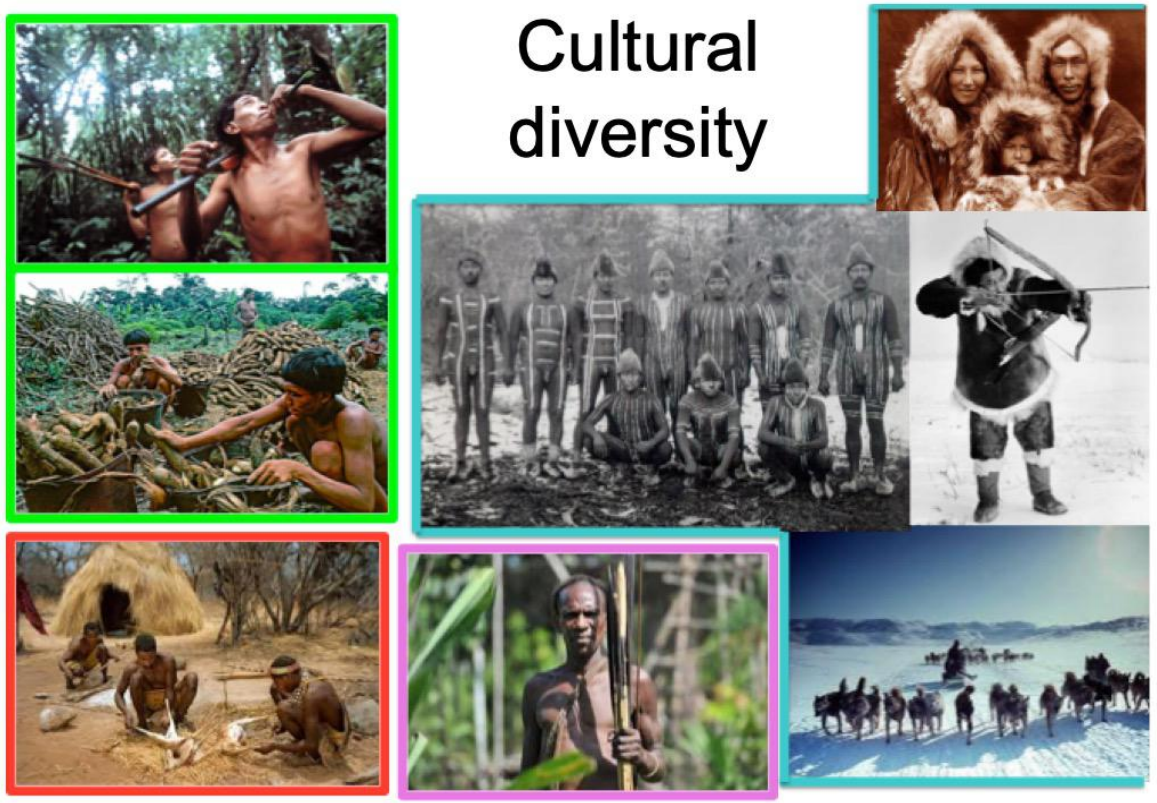
ECHO Discovery Series
Sept 11, 2024

María Gloria Domínguez-Bello

Henry Rutgers Professor of Microbiome and Health
CIFAR Fellow

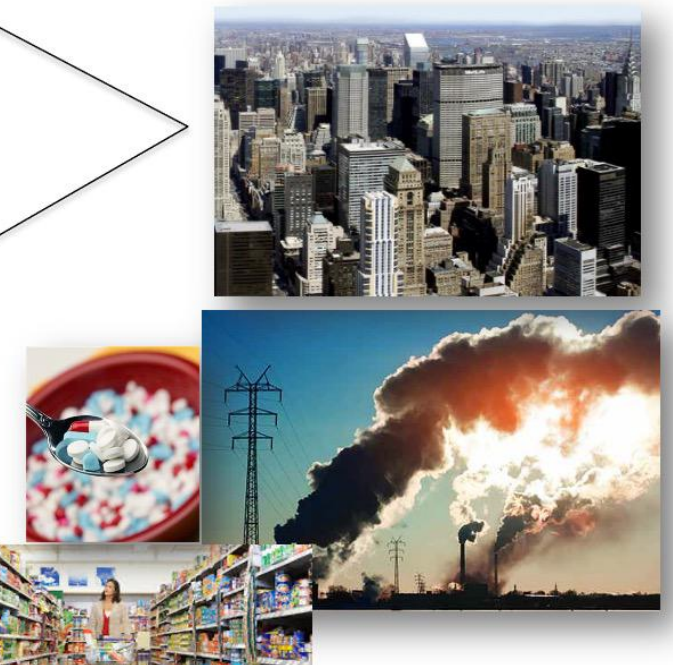
Department of Biochemistry and Microbiology,
Department of Anthropology
Institute for Food, Nutrition and Health
Rutgers University, USA

Converging life-styles

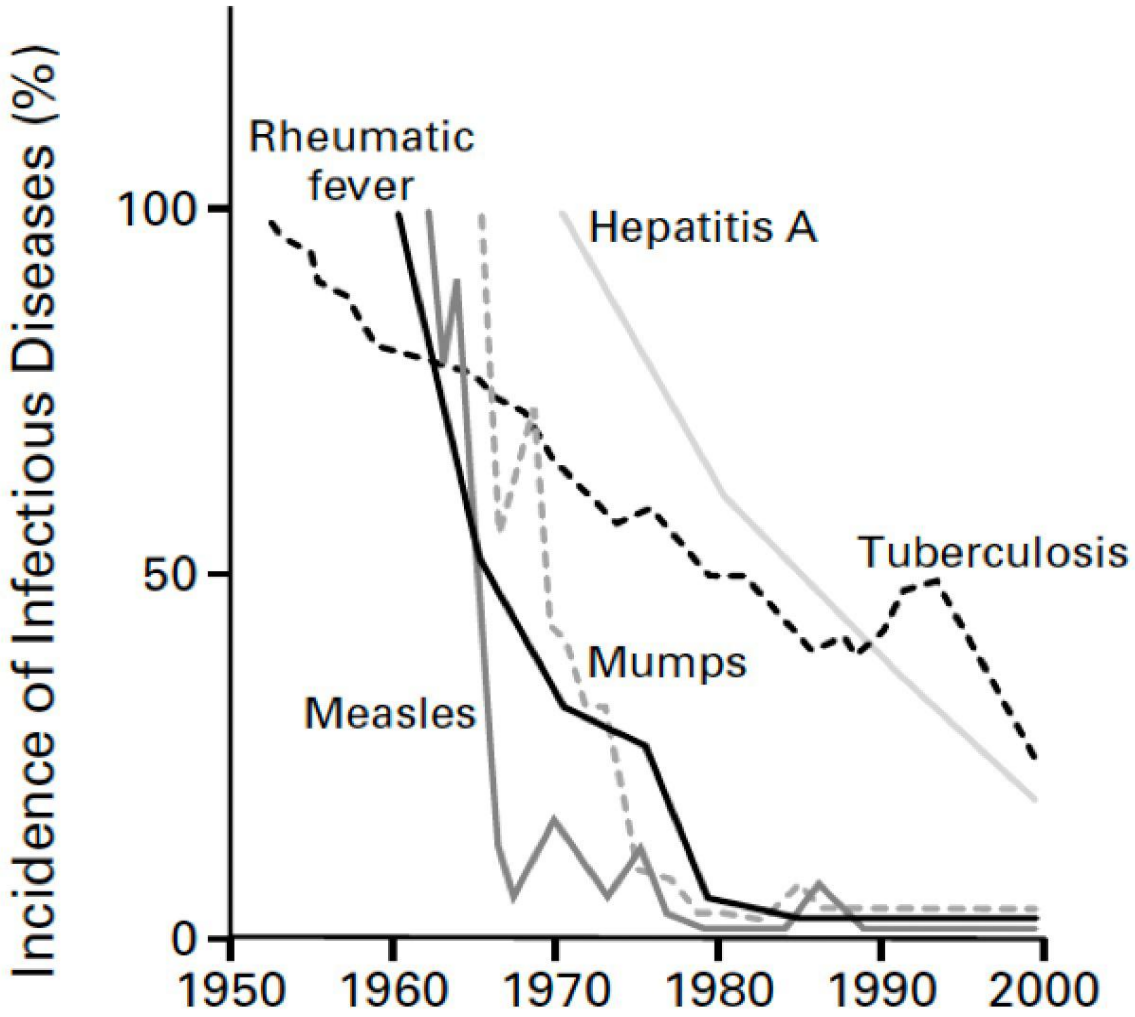


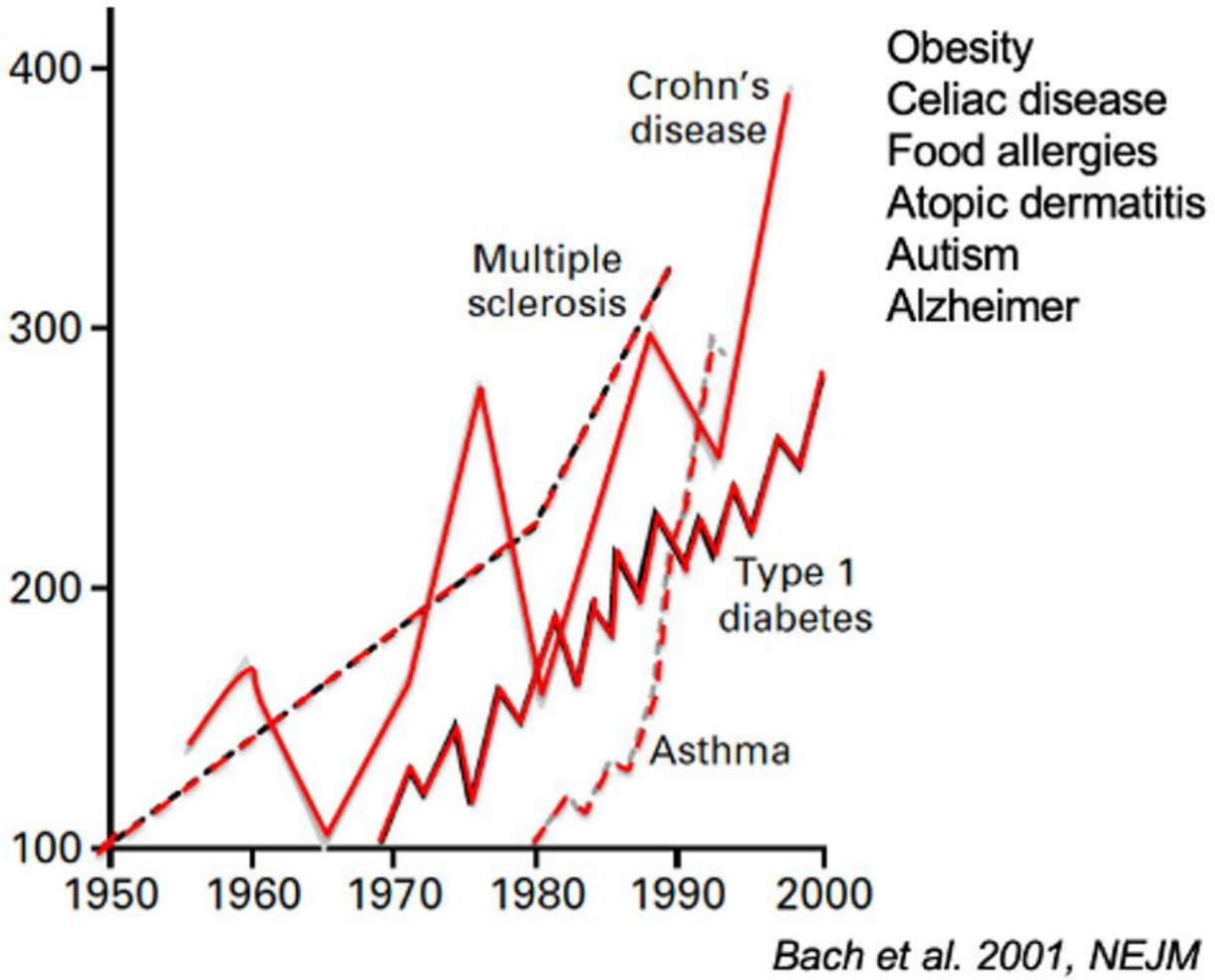
Social practices
Food production practices
Diet
Medicine

Antropocene



Disease trade of the 20th century





Bach 2001, NEJM

Hypotheses for immune/metabolic diseases

Disappearing microbiota hypothesis

HUMAN-ADAPTED MICROBES
→regulatory pathways in early life

Blaser 2006 EMBO Rep
Rook 2010 Clin Exp Immunol
von Hertzen et al. 2011 EMBO Rep.
Round et al. 2011 Science
Grainger, et al. 2010 J Exp Med
Atarashi et al. 2011 Science

Hygiene hypothesis

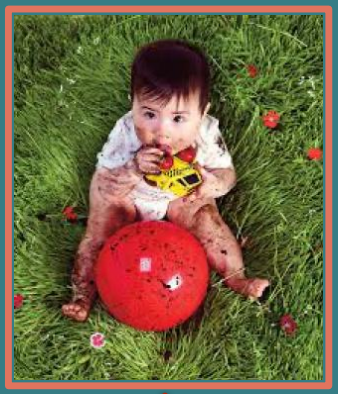
ENVIRONMENTAL MICROBES
< childhood asthma/allergies

Strachan 1989 Brit Med J
Rook 2009 Immunology
von Ehrenstein et al. 2000 Clin Exp Allergy
Riedler et al. 2001 Lancet
von Mutius, Vercelli, 2010 Nat Rev Immunol
Ownby et al. 2002 JAMA
Hanski I et al. 2012 PNAS

Natural microbial exposures

Human microbiome transmission

Environmental exposures



0-6 months

>6 months

Newborn

1 month

2 months

4 months

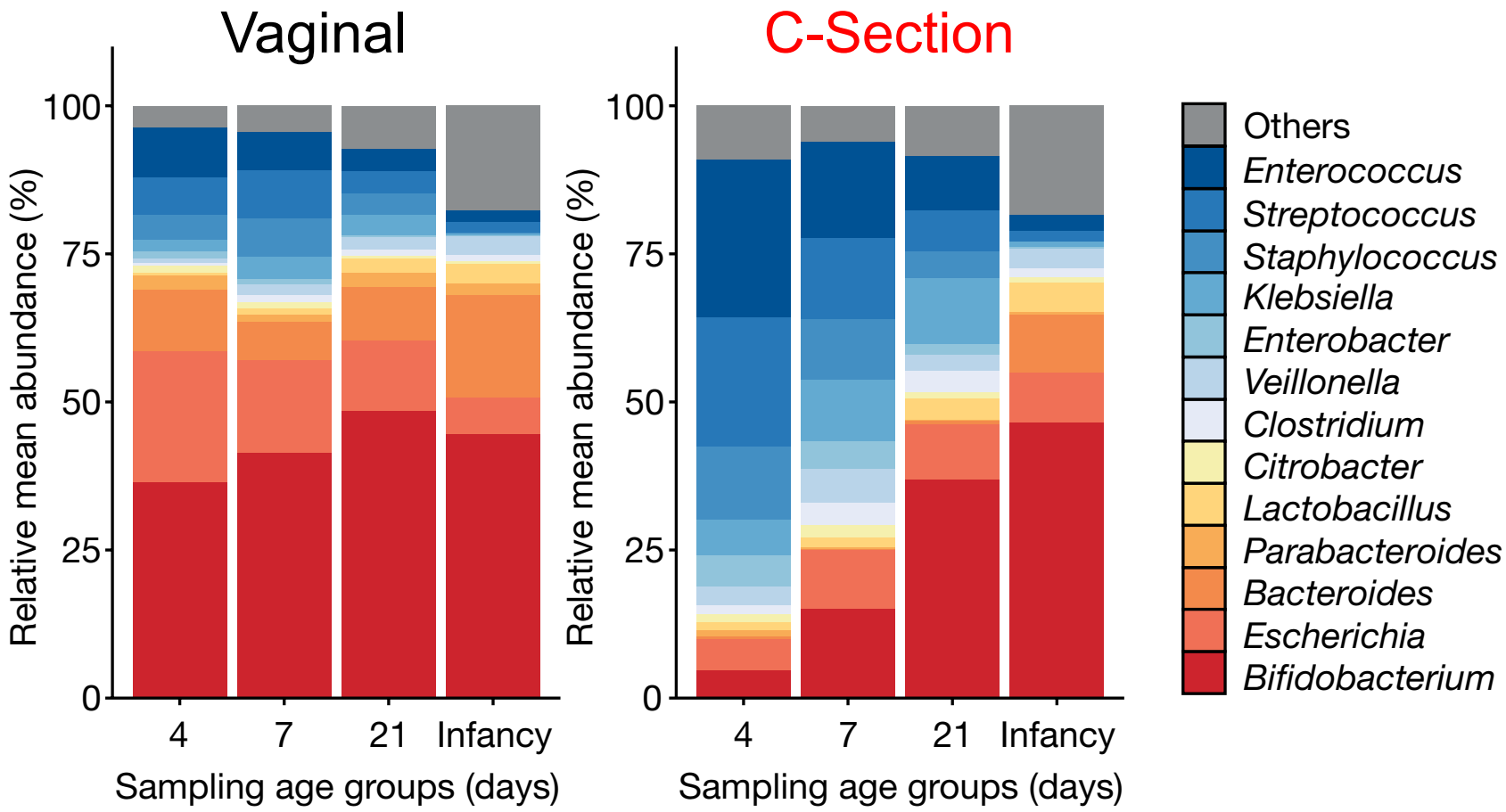
7 months

Microbiome stressors

Human microbiome transmission

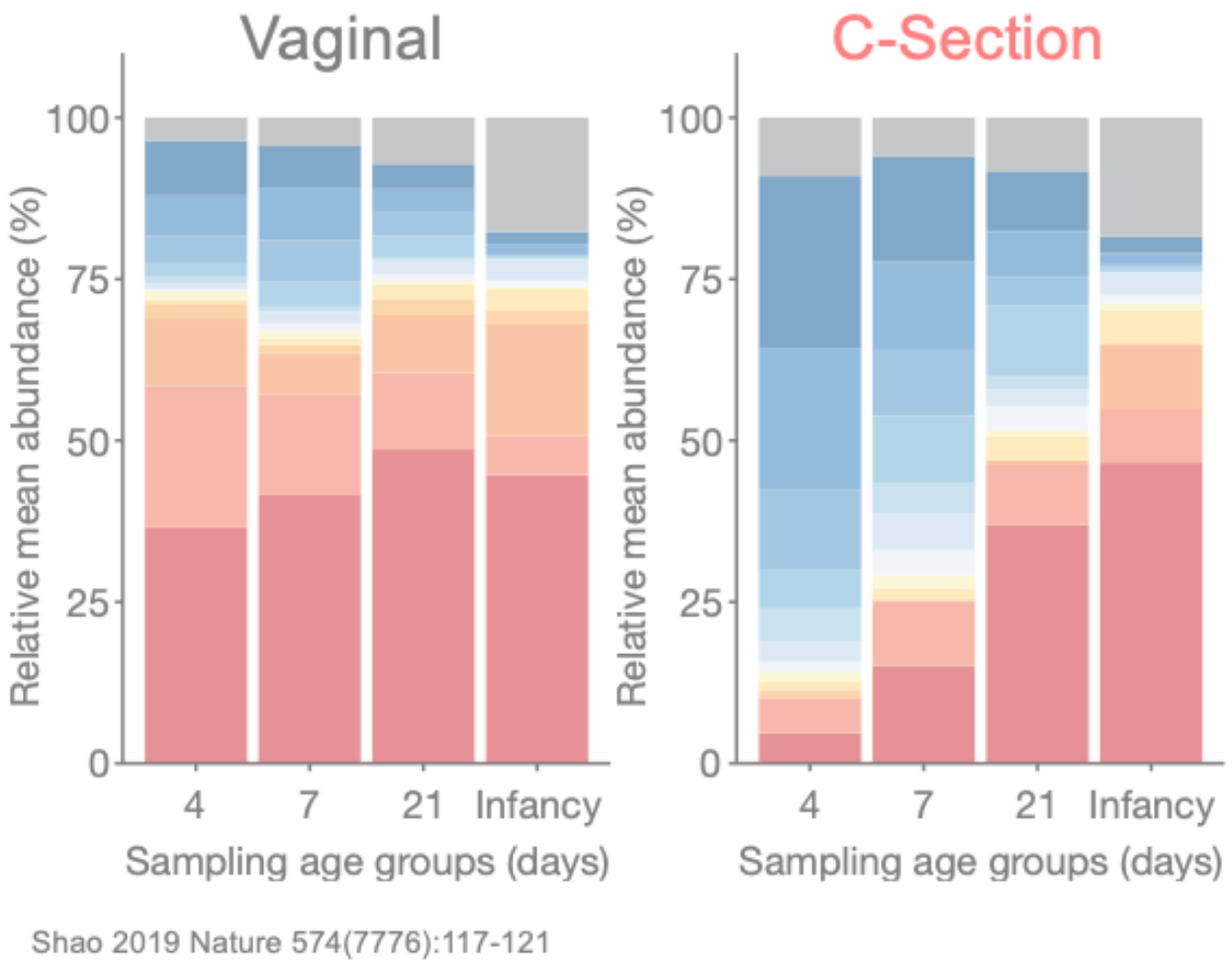
Environmental exposures

C-section alters the microbiome

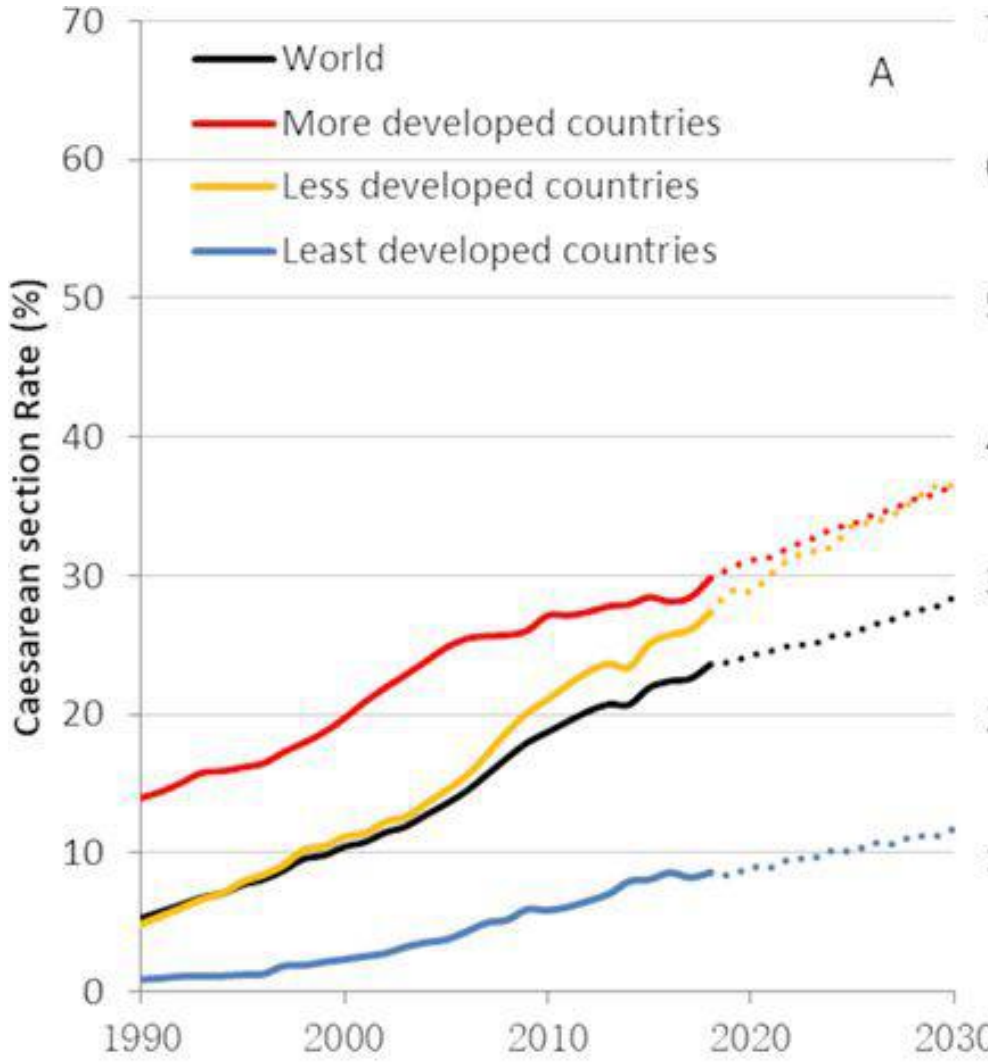


Shao 2019 Nature 574(7776):117-121

C-section alters the microbiome



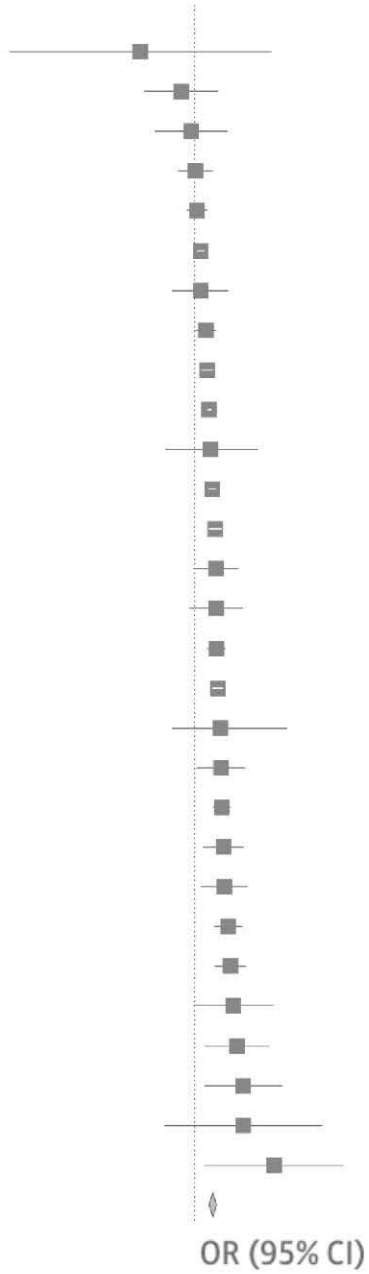
Shao 2019 Nature 574(7776):117-121



Betran AP, et al. BMJ Glob Health. 2021. PMID: 34130991.

CS and Autism Spectrum Diseases

Study	Outcome	Cesarean Delivery	Vaginal Delivery	OR (95% CI)
ASD				
Matsuishi et al,[31] 1999	ASD	1/18	25/205	0.42 (0.05-3.32)
Mrozek-Budzyn et al,[32] 2013	ASD	21/74	67/204	0.81 (0.45-1.45)
Hamadé et al,[83] 2013	ASD	24/74	62/184	0.94 (0.53-1.68)
Maramara et al,[33] 2014	ASD	73/31872	190/84023	1.01 (0.77-1.33)
Burstyn et al,[34] 2010	ASD	304/49456	834/170572	1.04 (0.88-1.22)
Axelsson et al,[20] 2019	ASD	1564/119433	6703/560440	1.10 (1.04-1.16)
Maimburg et al,[35] 2006	ASD	76/633	371/4099	1.10 (0.70-1.70)
Dodds et al,[36] 2011	ASD	218/26754	706/102948	1.19 (1.02-1.39)
Chien et al,[37] 2015	ASD	684/174376	1166/362297	1.22 (1.11-1.34)
Curran et al,[24] 2015	ASD	4330/340108	23960/2357206	1.26 (1.22-1.30)
Guisso et al,[38] 2018	ASD	64/125	72/189	1.28 (0.63-2.70)
Yip et al,[39] 2017 (Finland)	ASD	1331/156863	5753/891684	1.32 (1.24-1.40)
Schieve et al,[40] 2014	ASD	NA	NA	1.38 (1.25-1.52)
Haglund et al,[41] 2011	ASD	36/7434	214/61530	1.39 (0.98-1.99)
Chen et al,[42] 2017	ASD	36/6285	52/12698	1.40 (0.91-2.15)
Yip et al,[39] 2017 (Norway)	ASD	213/127922	1090/924475	1.41 (1.22-1.64)
Polo-Kantola et al,[43] 2014	ASD	860/3349	3328/17117	1.43 (1.31-1.56)
Duan et al,[44] 2014	ASD	152/287	134/285	1.50 (0.70-4.30)
Durkin et al,[45] 2015	ASD	33/4624	127/26843	1.51 (1.03-2.22)
Yip et al,[39] 2017 (Western Australia)	ASD	289/65953	802/279228	1.53 (1.34-1.75)
Eriksson et al,[46] 2012	ASD	52/23286	134/94242	1.57 (1.14-2.17)
Hultman et al,[47] 2002	ASD	87/352	321/2096	1.60 (1.10-2.30)
Kissin et al,[48] 2015	ASD	328/27152	109/15231	1.70 (1.36-2.11)
Glasson et al,[49] 2004	ASD	134/380	331/1398	1.76 (1.38-2.24)
Zhang et al,[50] 2010	ASD	43/77	43/104	1.83 (0.98-3.43)
El-Baz et al,[51] 2011	ASD	39/89	61/213	1.94 (1.16-3.25)
Ji et al,[52] 2018	ASD	22/214	22/434	2.15 (1.16-3.97)
Winkler-Schwartz et al,[53] 2014	ASD	7/35	5/48	2.15 (0.62-7.45)
Al-Jammas et al,[54] 2012	ASD	14/19	36/81	3.50 (1.15-10.63)
Subtotal $I^2 = 69.5\%$, $P < .001$				1.33 (1.25-1.41)



Zhang, et al, 2019
JAMA Netw Open
2(8):e1910236

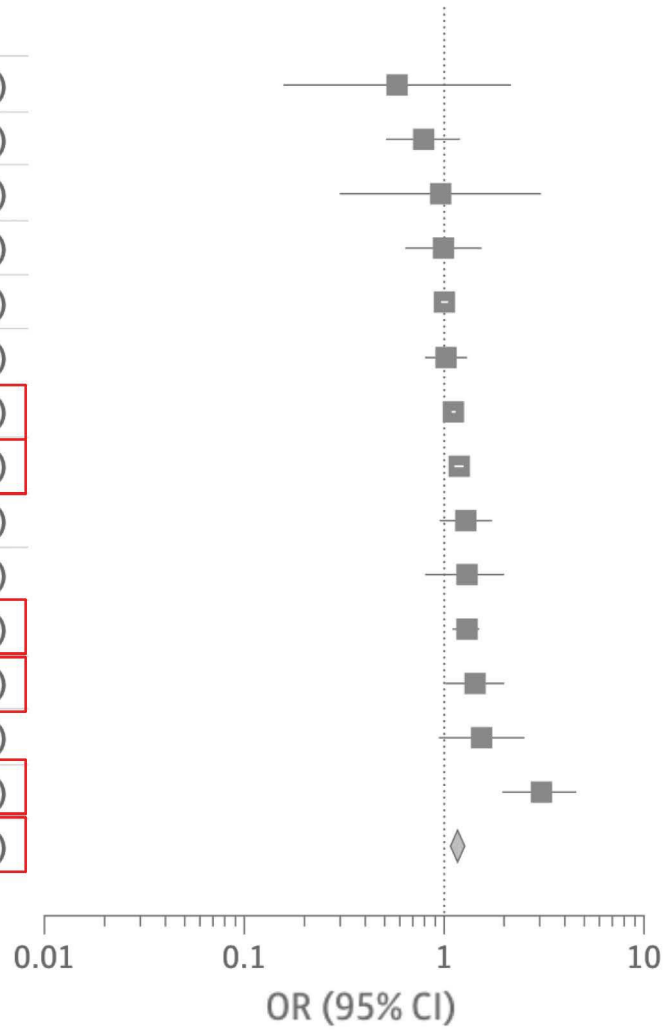
CS and Neurodevelopmental Diseases

ADHD

Çak et al,[55] 2013	ADHD	18/80	4/12	0.58 (0.16-2.15)
Murray et al,[56] 2016 (Pelotas)	ADHD	NA	NA	0.79 (0.51-1.20)
Yeo et al,[57] 2015	ADHD	24/34	34/46	0.96 (0.30-3.04)
Gustafsson and Källén et al,[58] 2011	ADHD	22/2996	215/29016	0.99 (0.64-1.54)
Axelsson et al,[19] 2019	ADHD	3167/117863	14804/553727	1.01 (0.97-1.05)
Silva et al,[59] 2014	ADHD	2819/8863	10363/34829	1.02 (0.80-1.30)
Curran et al,[24] 2016	ADHD	7230/238687	40548/1483861	1.11 (1.08-1.14)
Sucksdorff et al,[60] 2018	ADHD	1892/8034	8428/40963	1.19 (1.12-1.26)
Chen et al,[42] 2017	ADHD	71/6320	112/12758	1.28 (0.95-1.73)
Ketzer et al,[61] 2012	ADHD	NA	NA	1.30 (0.80-2.00)
Halmøy et al,[62] 2012	ADHD	186/69051	2137/1103345	1.30 (1.10-1.50)
Ji et al,[52] 2018	ADHD	75/267	113/525	1.42 (1.02-2.00)
Murray et al,[56] 2016 (ALSPAC)	ADHD	NA	NA	1.54 (0.94-2.52)
Amiri et al,[63] 2012	ADHD	103/162	61/168	3.06 (1.96-4.80)
Subtotal $I^2 = 79.2\%$, $P < .001$				1.17 (1.07-1.26)

ADHD

Attention Deficit Hyperactivity Disorder



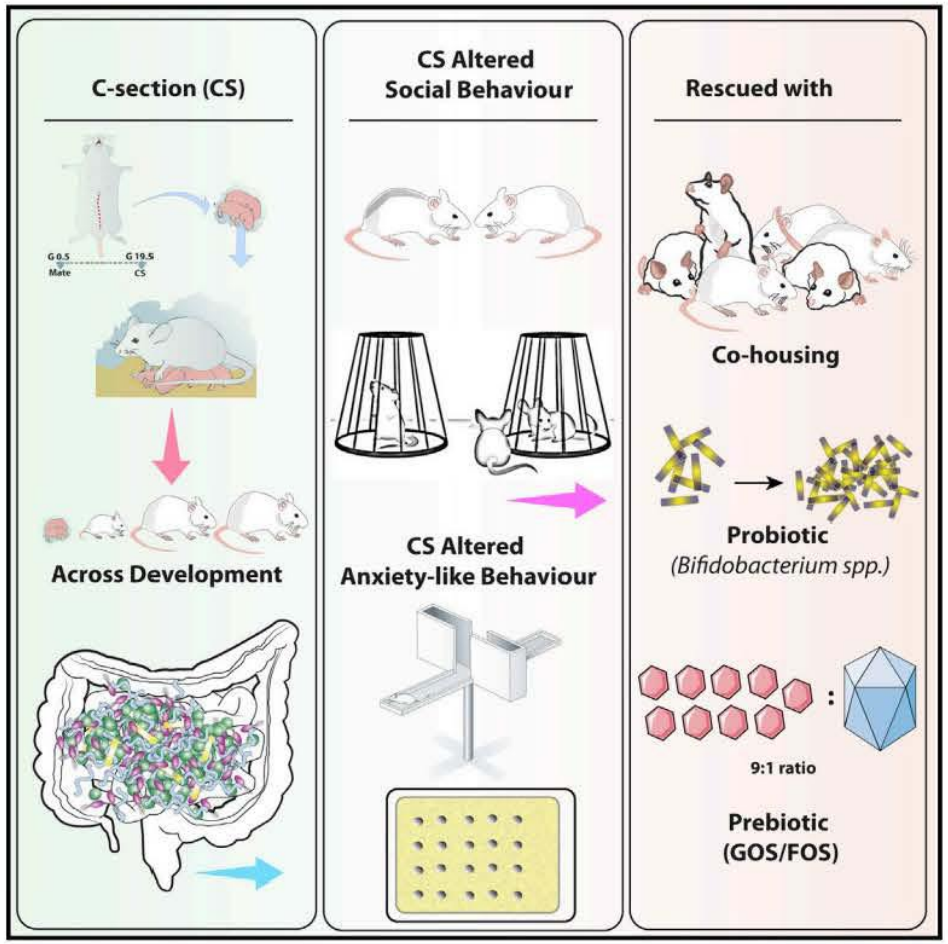
Zhang 2019 JAMA Netw Open 2(8):e1910236

Article

Current Biology

Enduring Behavioral Effects Induced by Birth by Caesarean Section in the Mouse

Graphical Abstract



Authors

Livia H. Morais, Anna V. Golubeva, Gerard M. Moloney, ..., Catherine Stanton, Timothy G. Dinan, John F. Cryan

Correspondence

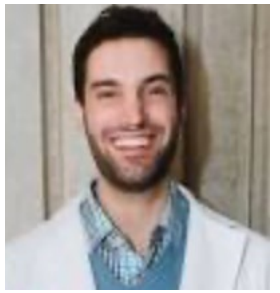
j.cryan@ucc.ie

In Brief

Recent evidence points to an important role for the microbiome in regulating brain function and behavior. Here, Morais *et al.* show that birth by C-section results in a different pattern of microbiota colonization with long-term behavioral consequences in the mouse. Targeting the gut microbiota reverses social behavioral effects of C-section.

Morais et al., 2020, Current Biology

Experimental Design: brain and the gut microbiome



Jeremy Lessing

E0
Mate

E19-21
Birth mode

P7

P21

WT (16p$^{+/+}$)

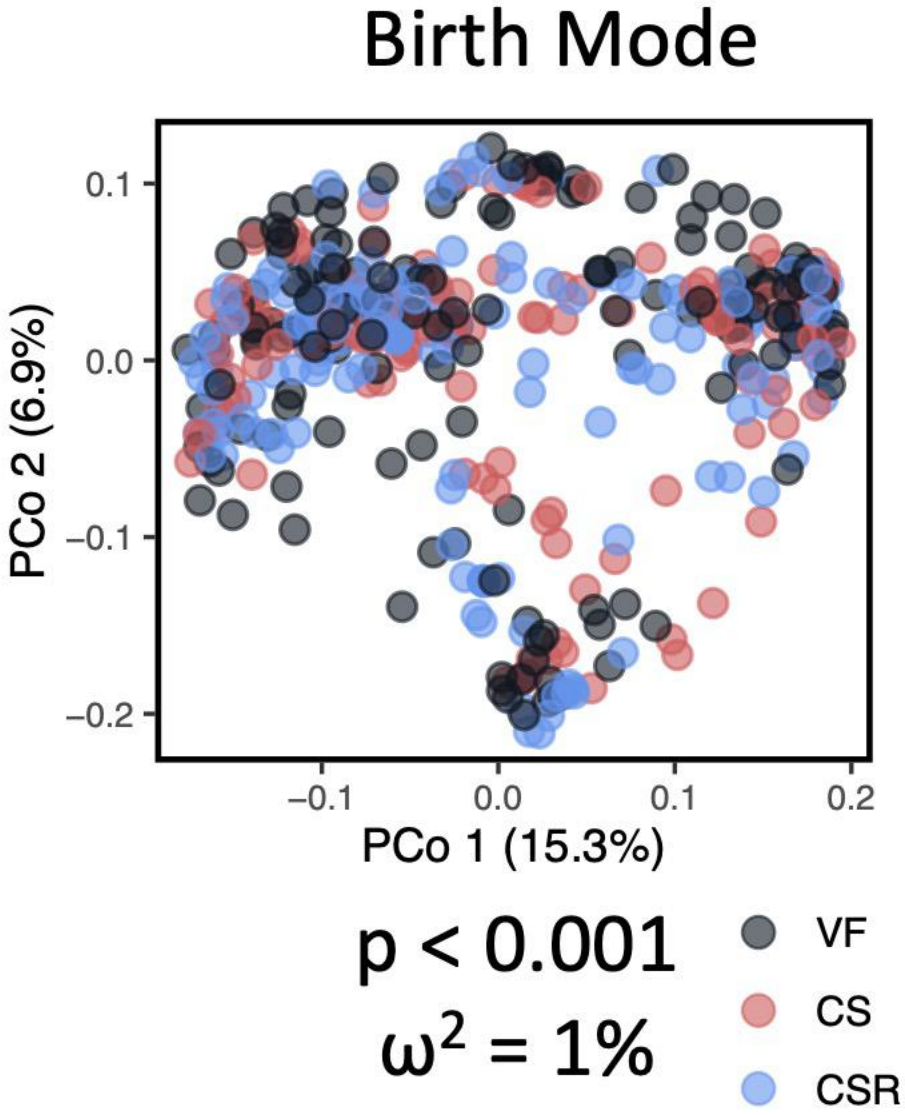
Het (16p $^{+/-}$)

VD

CS

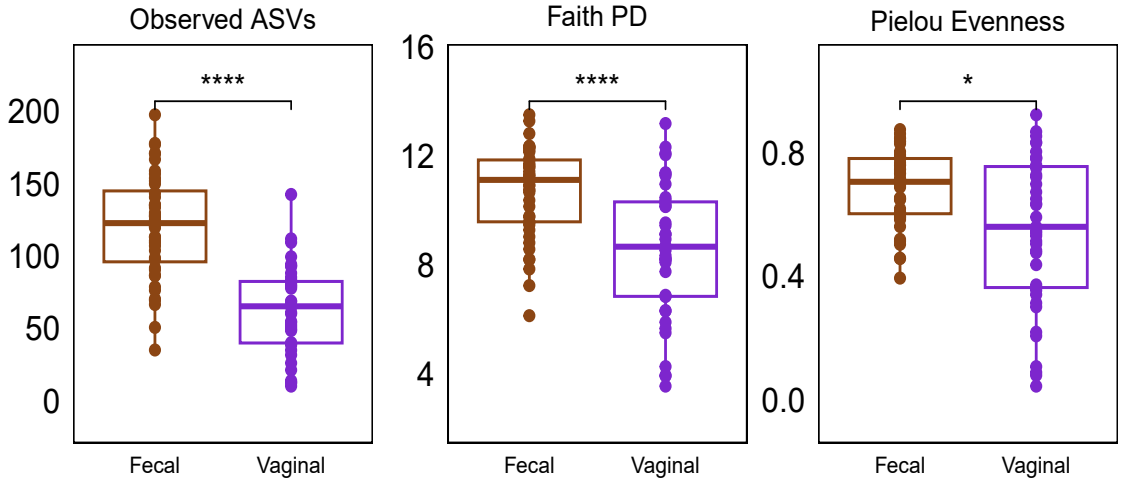
restored

Beta Diversity

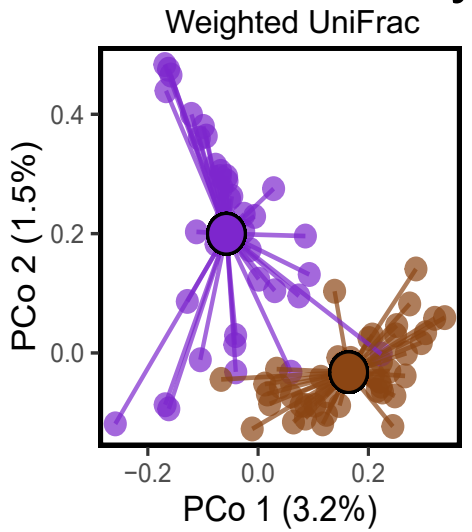


Vaginal community is distinct…

Alpha Diversity



Beta Diversity



fecal

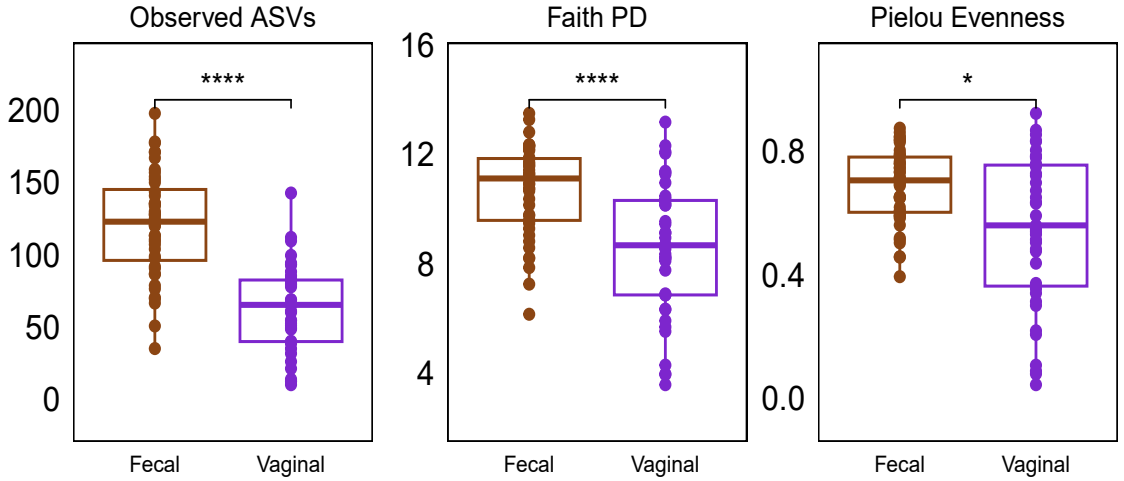
vaginal

* $p < 0.05$

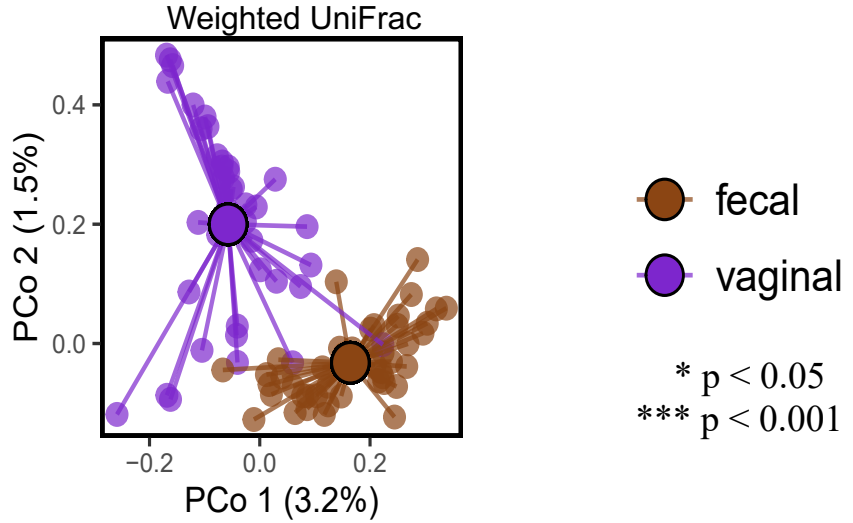
*** $p < 0.001$

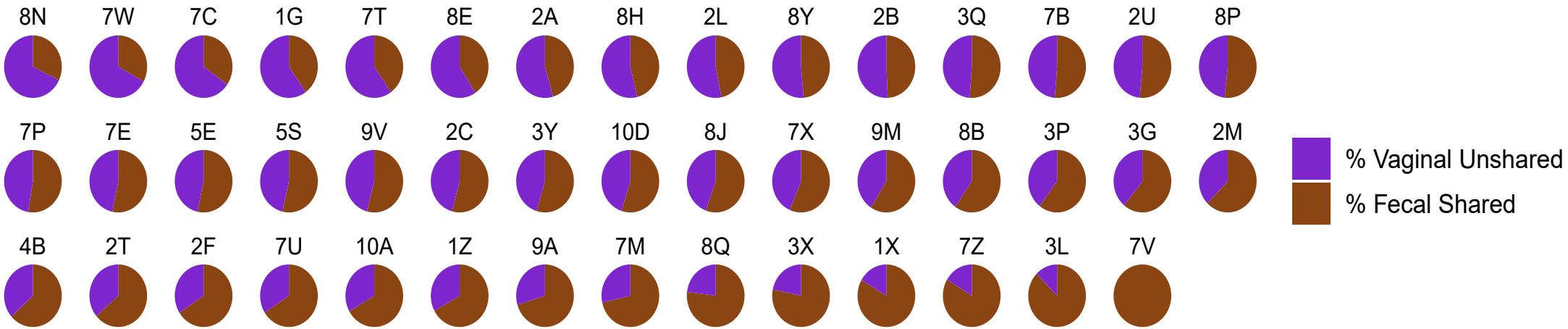
Vaginal community is distinct… but overlaps with Feces

Alpha Diversity

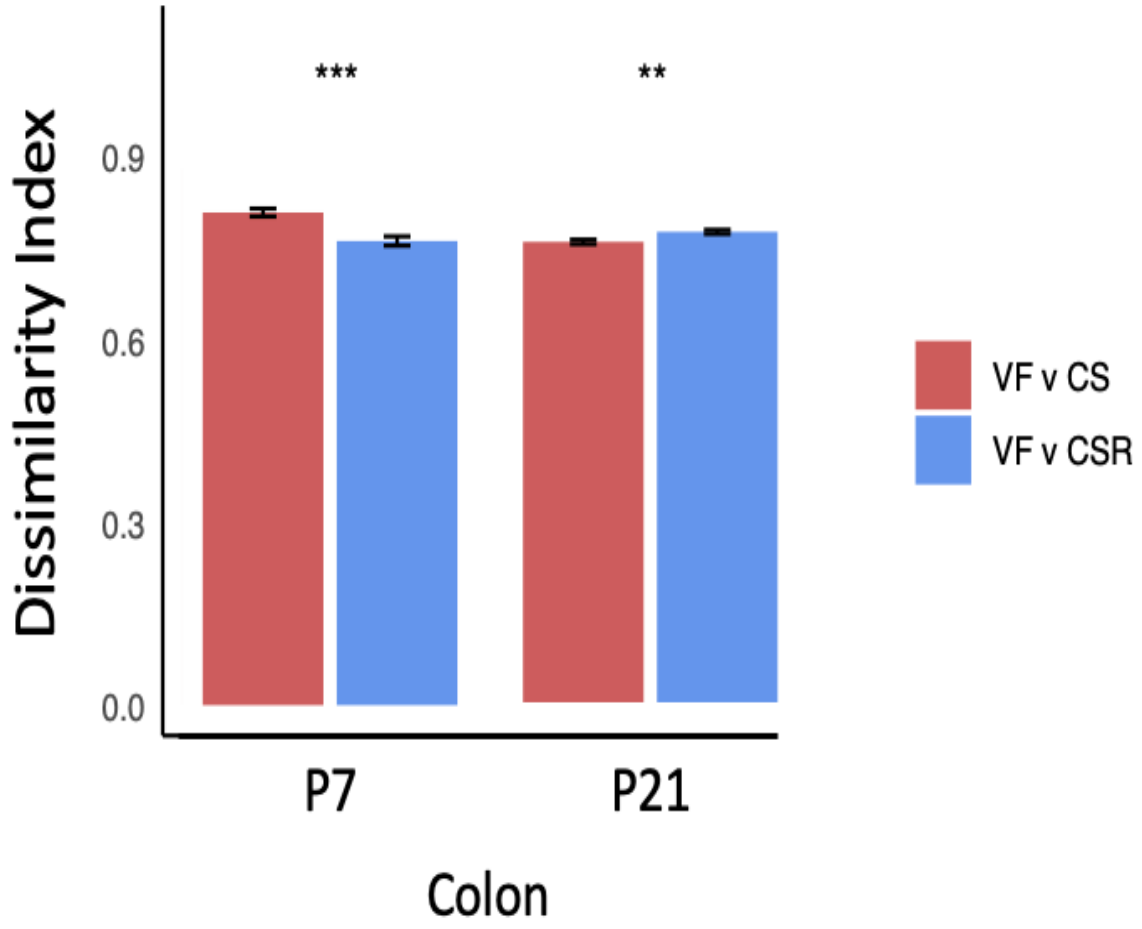


Beta Diversity

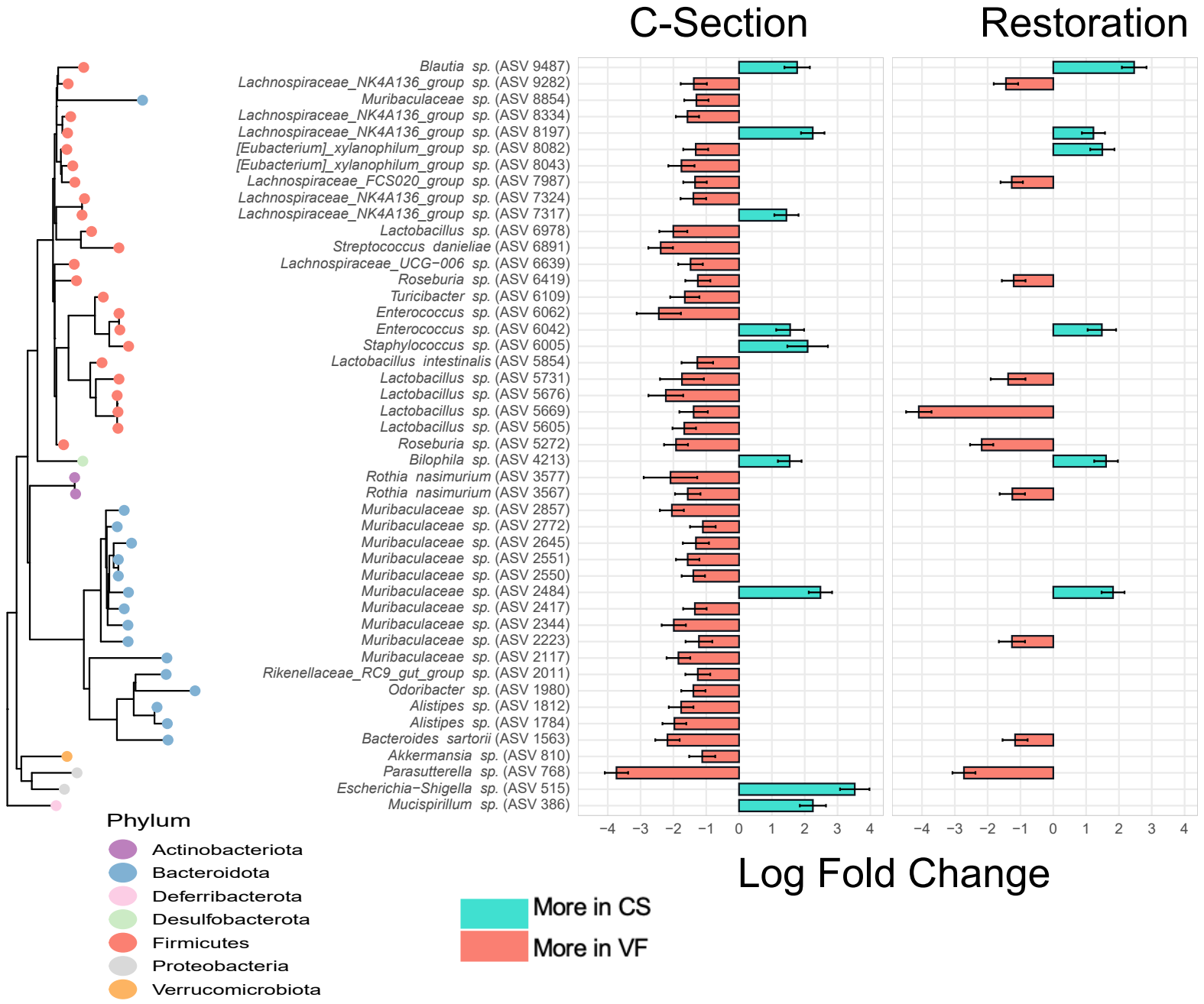




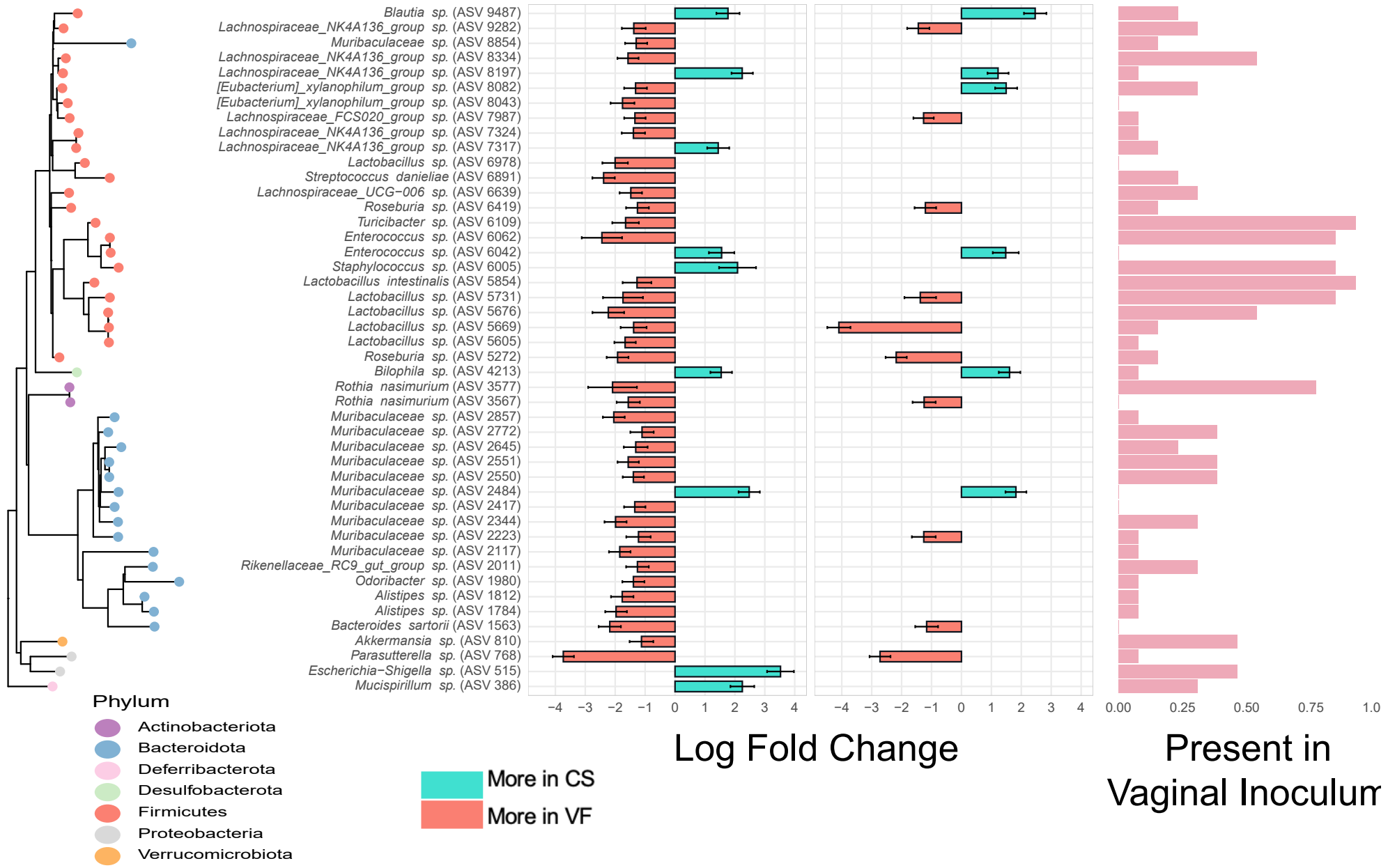
Restoration decreases Bray Curtis distance between C-section and –vaginal birth



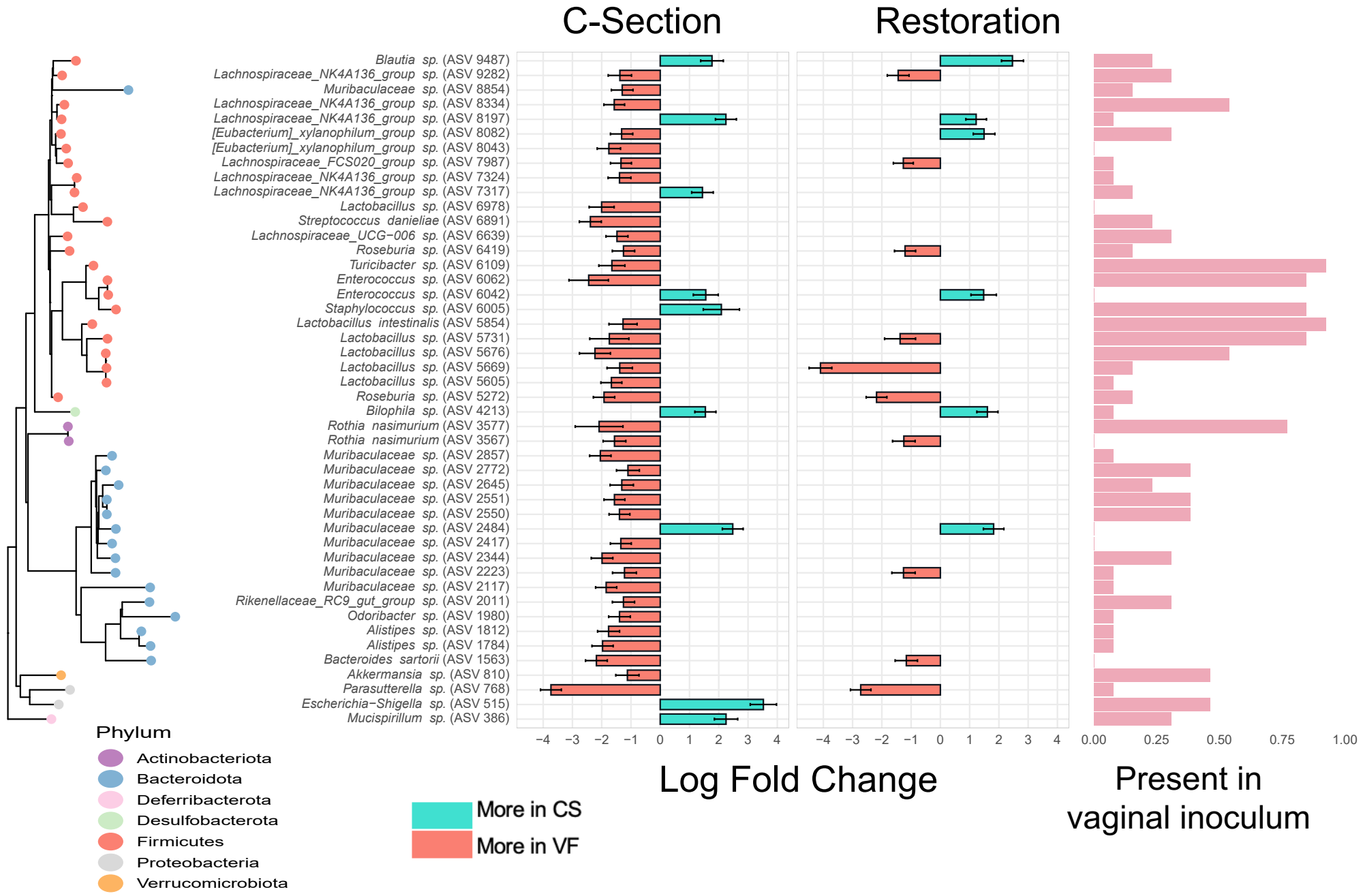
Restoration in the mouse colon at P7

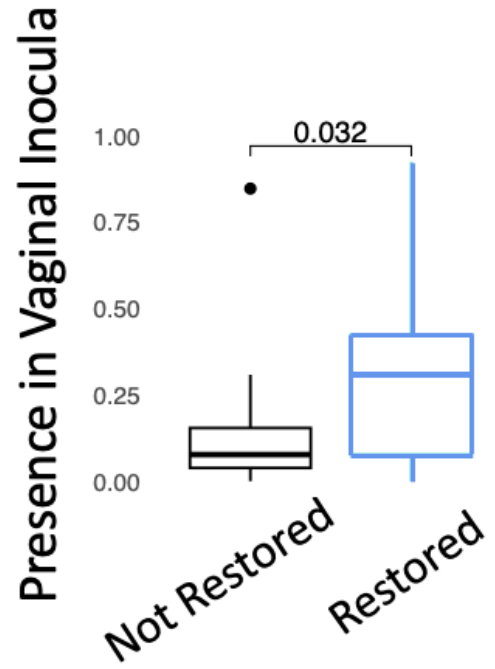


Restoration in the mouse colon at P7



Vaginal inocula is responsible for colon restoration at P7





(Mann Whitney U Test)

Brain protein profile effects induced by C-section

Proliferation

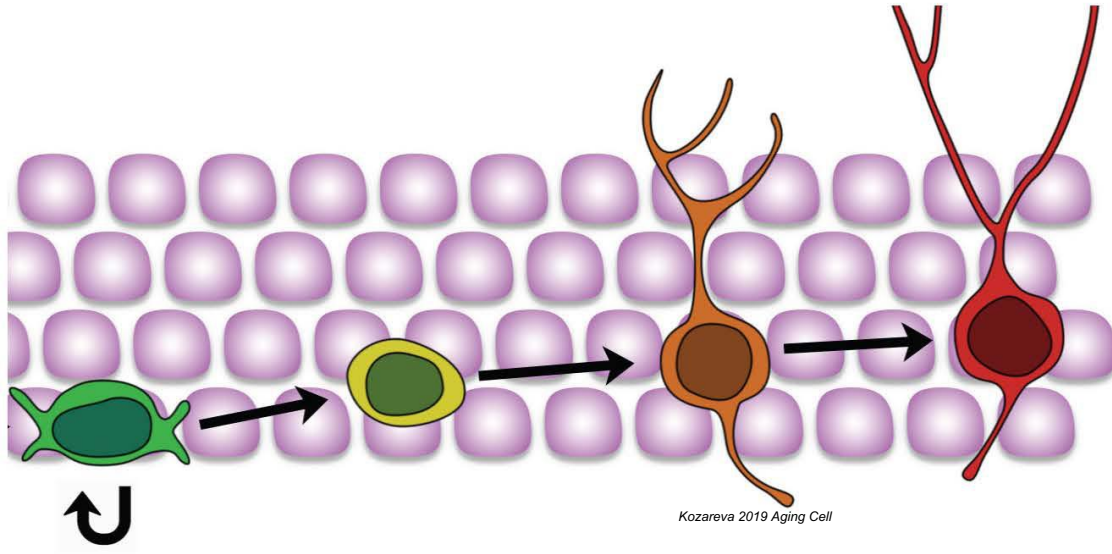
- Cyclin E G_1 -> S phase transition
- Sox2 SRY Box 2: Pluripotent neural stem-cells

Post-mitotic cytoskeleton

- Tuj1 B3 Tubulin: Neuron cytoskeleton
- Dcx Doublecortin: Developing brain

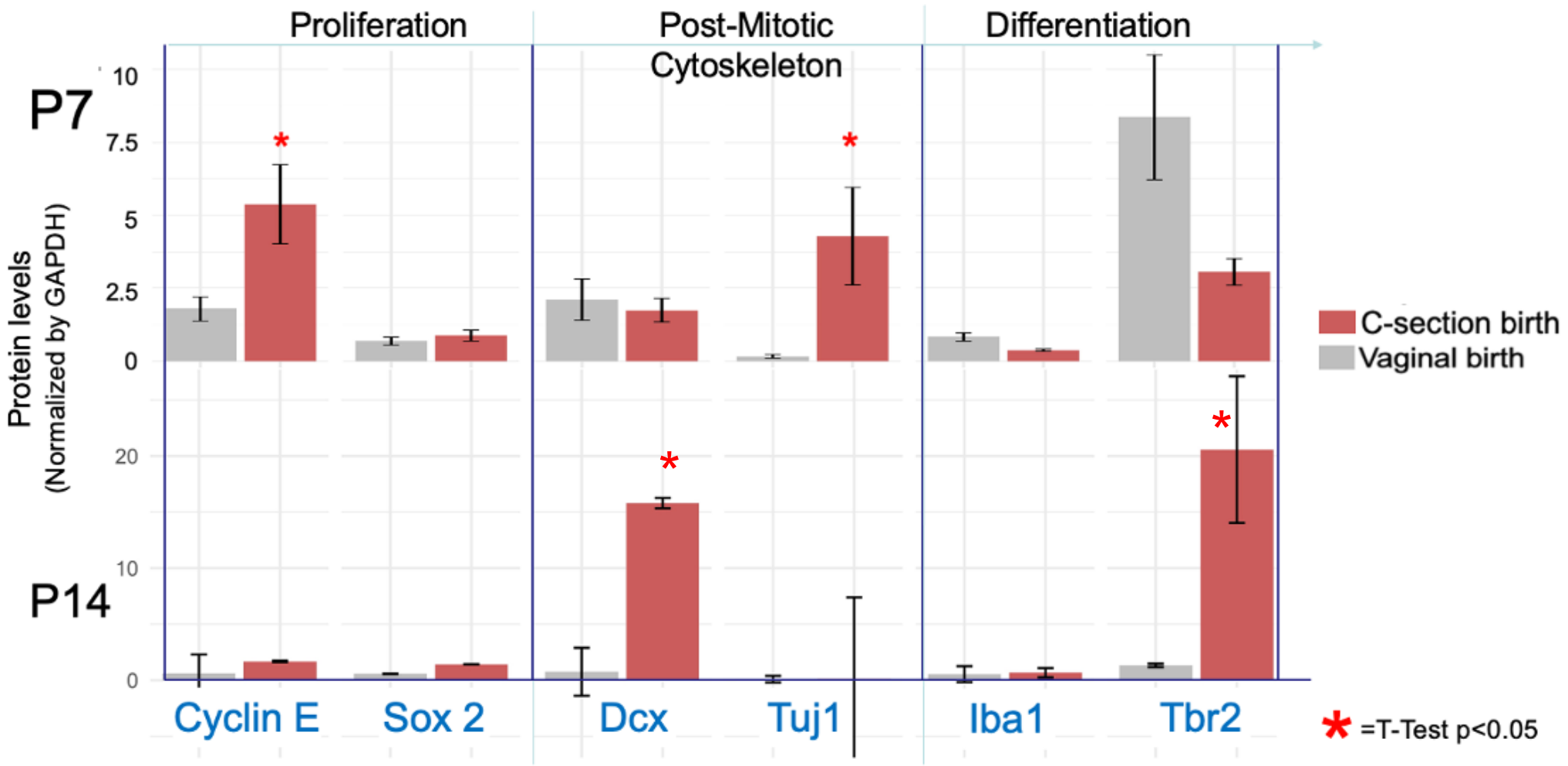
Differentiation

- GFAP Glial Fibrillary Acidic Protein: Astrocytes
- Tbr2 T-box Brain 2: Developing brain
- Iba1 Ionized Ca^{2+} Binding Adapter 1: Microglia



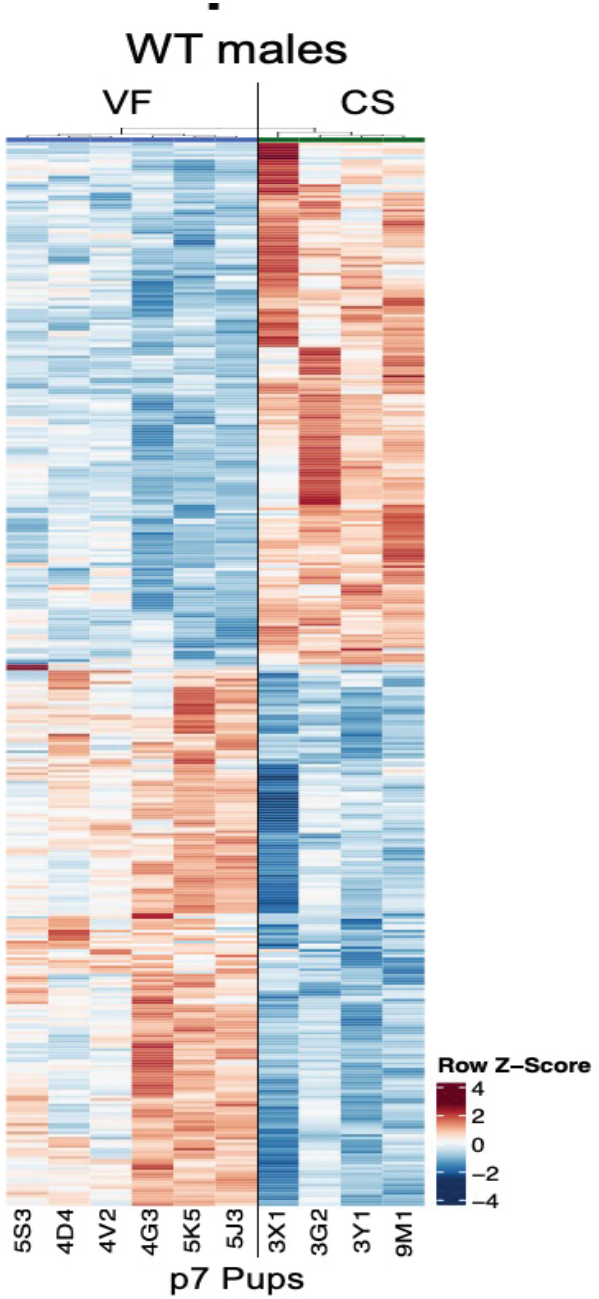
Kozareva 2019 Aging Cell

Cerebellum protein profile effects induced by C-section



Changes also in hippocampus proteins

Unsupervised Heatmap: CS affects Hyppocampus transcriptome



189 Genes Up in CS

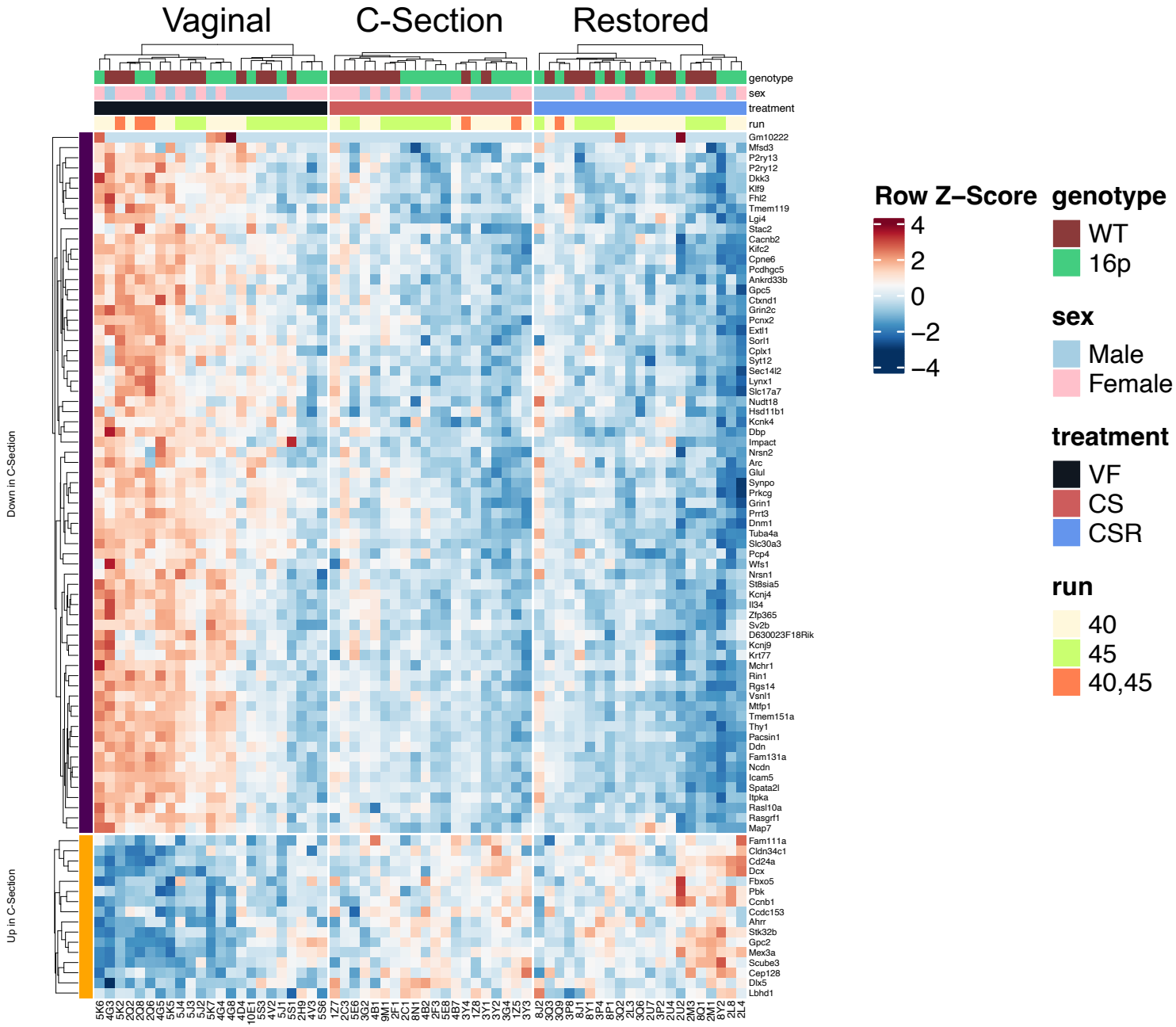
	Selected GO Biological Process	p Value
CS Upregulated	neural plate development	0.002
	ventricular system development	0.004
	central nervous system development	0.023
	brain development	0.024
	neural tube development	0.029
	neural plate morphogenesis	0.029

196 Genes Down in CS

	Selected GO Biological Process	p Value
CS Downregulated	synaptic vesicle recycling	< 0.001
	synapse organization	< 0.001
	regulation of neuronal synaptic plasticity	< 0.001
	trans-synaptic signaling	0.001
	synaptic vesicle endocytosis	0.001
	neurogenesis	0.001
	presynaptic endocytosis	0.001
	synaptic signaling	0.001
	synapse assembly	0.001
	neuron projection morphogenesis	0.001
	neuron projection development	0.001
	synaptic vesicle maturation	0.001
	postsynapse organization	0.002
	regulation of trans-synaptic signaling	0.003
	postsynaptic endocytosis	0.004
	postsynaptic neurotransmitter receptor internalization	0.004
	regulation of synapse organization	0.005
	neuron development	0.005
	regulation of synapse structure or activity	0.006
	neurotransmitter receptor internalization	0.006
	synaptic vesicle lumen acidification	0.006
	oligodendrocyte differentiation	0.006
	regulation of synaptic plasticity	0.007
	gliogenesis	0.008
	learning or memory	0.009
	axonogenesis	0.011
	cell morphogenesis involved in neuron differentiation	0.011
	glial cell differentiation	0.015
	regulation of synaptic vesicle endocytosis	0.016
	neuron differentiation	0.018
	axon development	0.023
	regulation of nervous system development	0.026
	myelination	0.027
	regulation of postsynaptic membrane neurotransmitter receptor levels	0.028
	generation of neurons	0.029
	postsynaptic specialization assembly	0.029
	regulation of postsynapse organization	0.029
	excitatory synapse assembly	0.029
	peripheral nervous system development	0.029
	synaptic vesicle transport	0.038
	postsynapse assembly	0.047
	Schwann cell differentiation	0.05

CS-altered hippocampus transcriptomic not restored by vaginal seeding

P7 Hippocampus



Summary

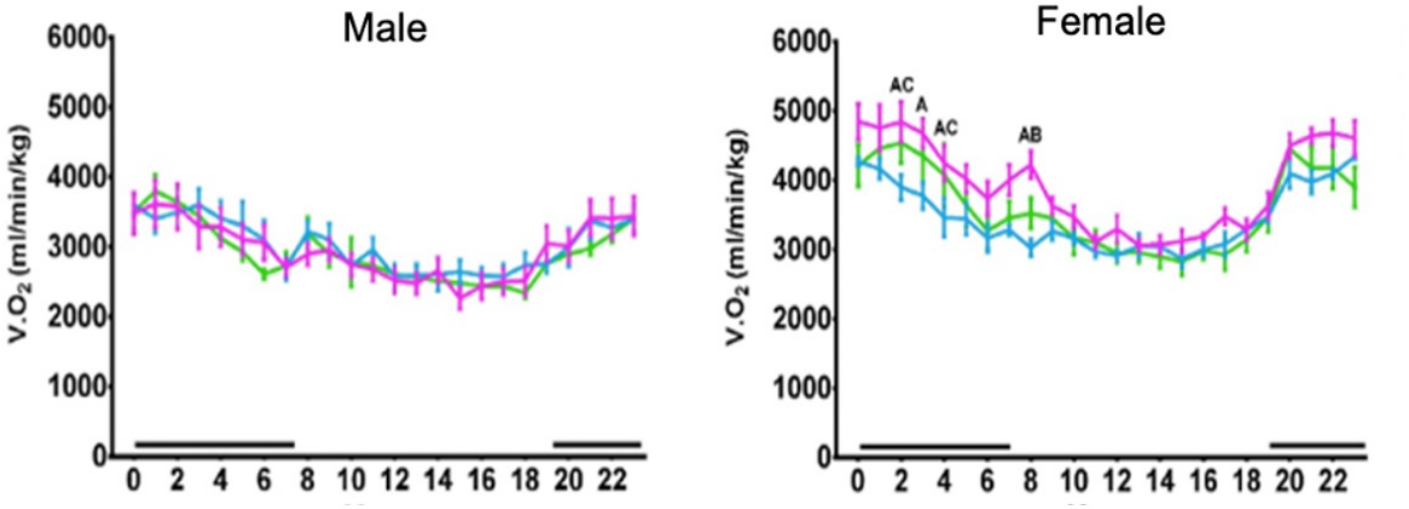
C-section causes early alterations in brain development

Microbial restoration after C-section partially restored the microbiome

Microbial restoration did not fully rescue brain gene expression at P7

Metabolic/Behavioral effects induced by C-section birth in 11w old Swiss Webster mice

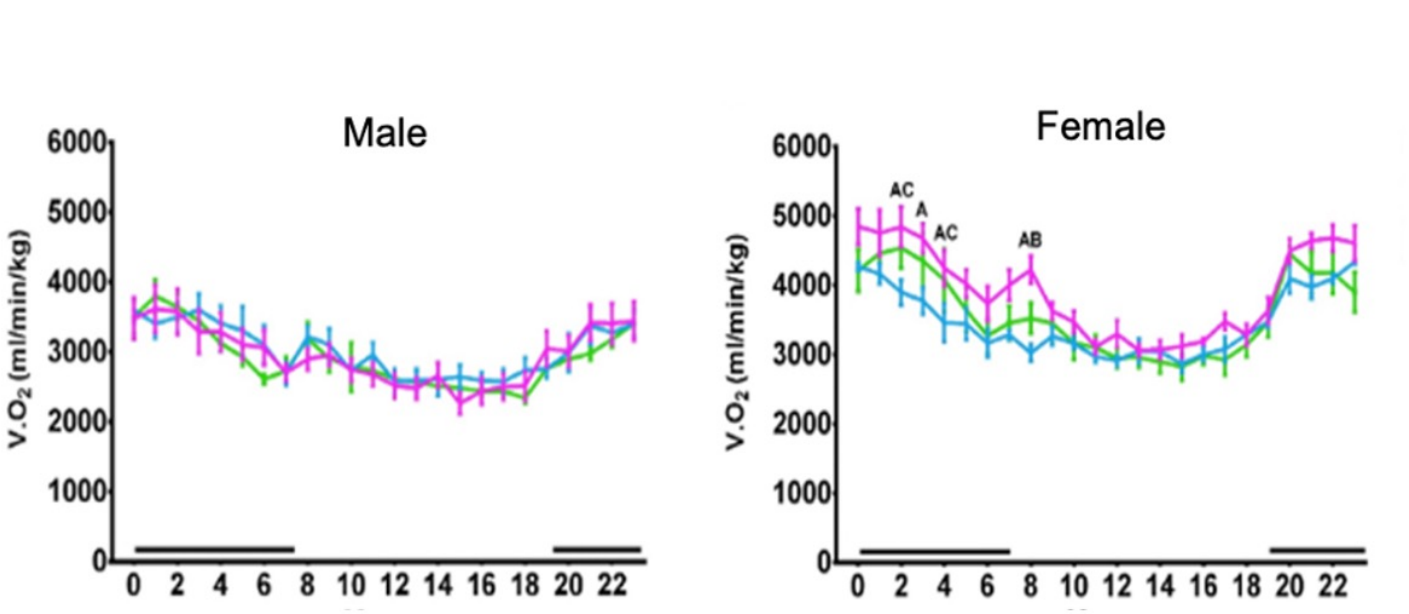
5-6 individuals from different litters per group



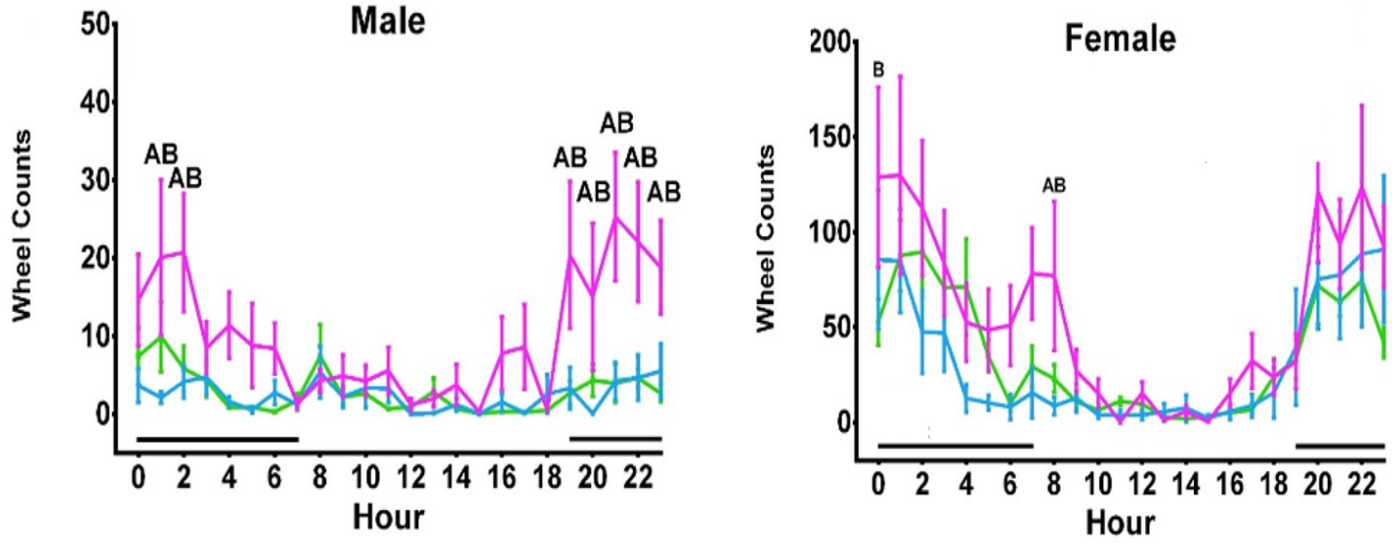
Vaginal birth
C-section birth
C-section + vaginal restoration

-Lower nocturnal metabolic rate in CS females- partially restored

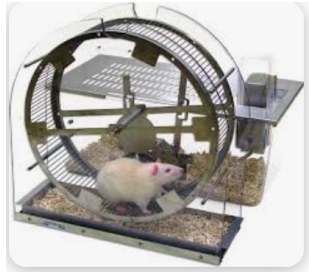
Metabolic/Behavioral effects induced by C-section birth in 11w old Swiss Webster mice



-Lower nocturnal metabolic rate in CS females- partially restored



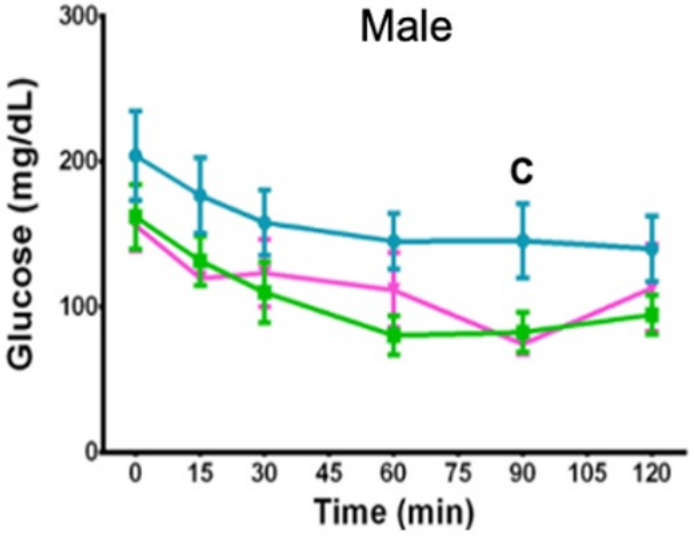
-Lower nocturnal activity in CS- restored

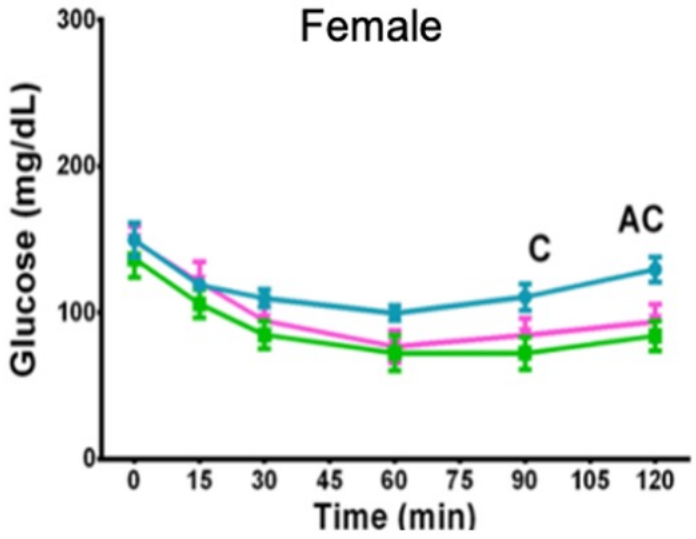


Metabolic effects induced by C-section birth in 11w old Swiss Webster mice

5-6 individuals from different litters per group

Vaginal birth
C-section birth
C-section + vaginal restoration





-Glucose intolerance in CS restored

Lower energy expenditure in babies born to germ-free mice fed HFD

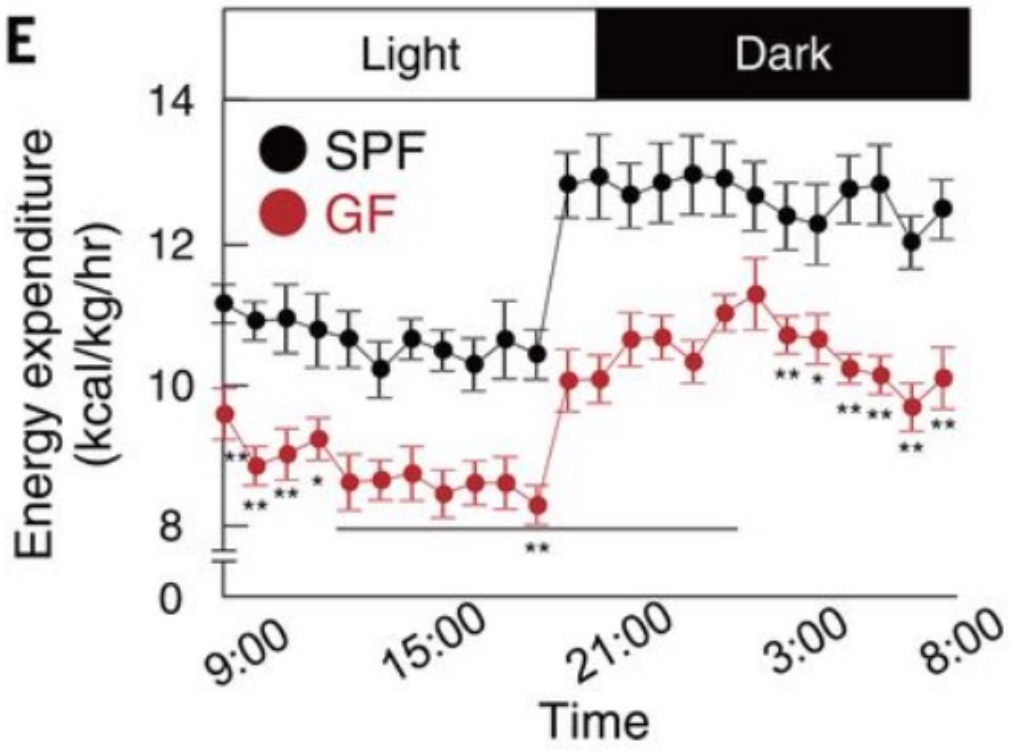


Fig. 1 Offspring from GF mothers exhibit severe obese phenotype when fed a HFD.

Kimura et al, 2020, Science Vol 367

Lower energy expenditure in babies born to germ-free mice fed HFD

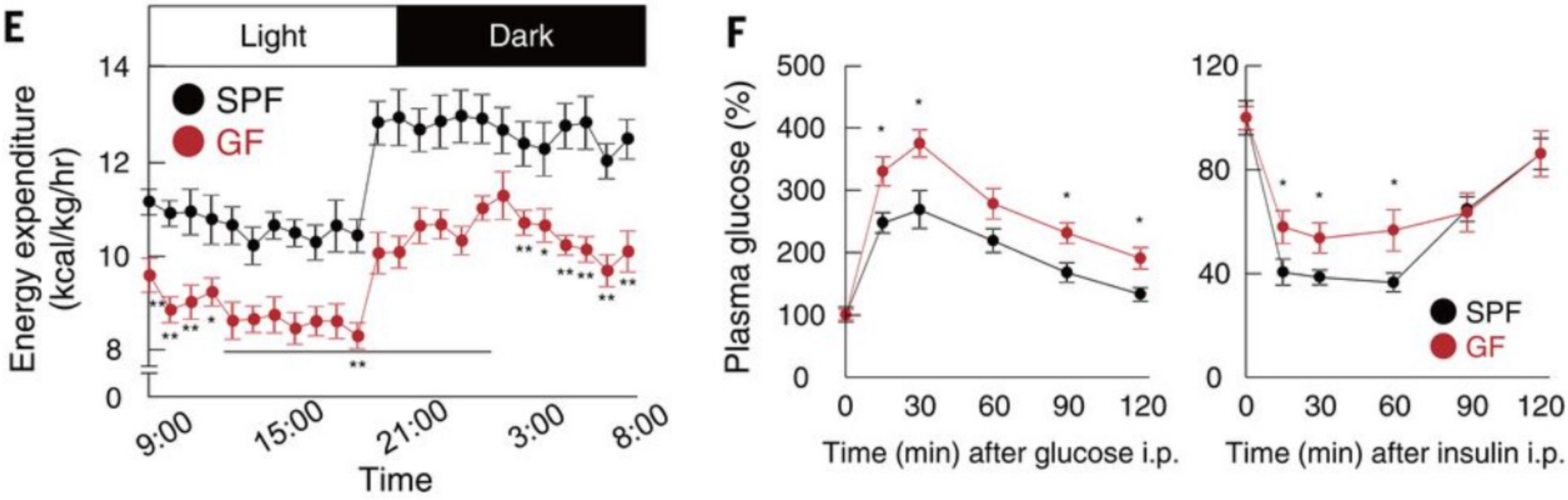
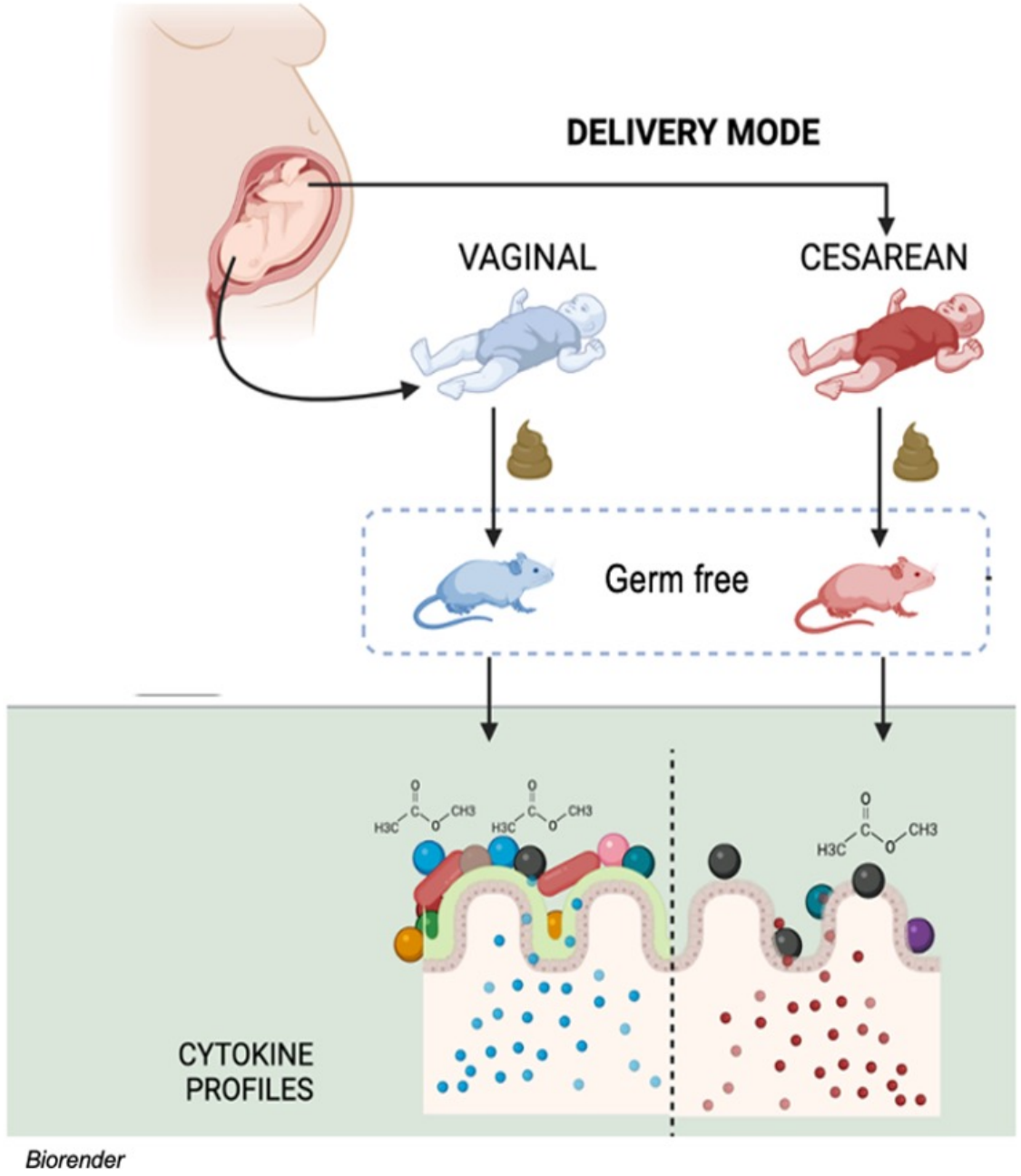


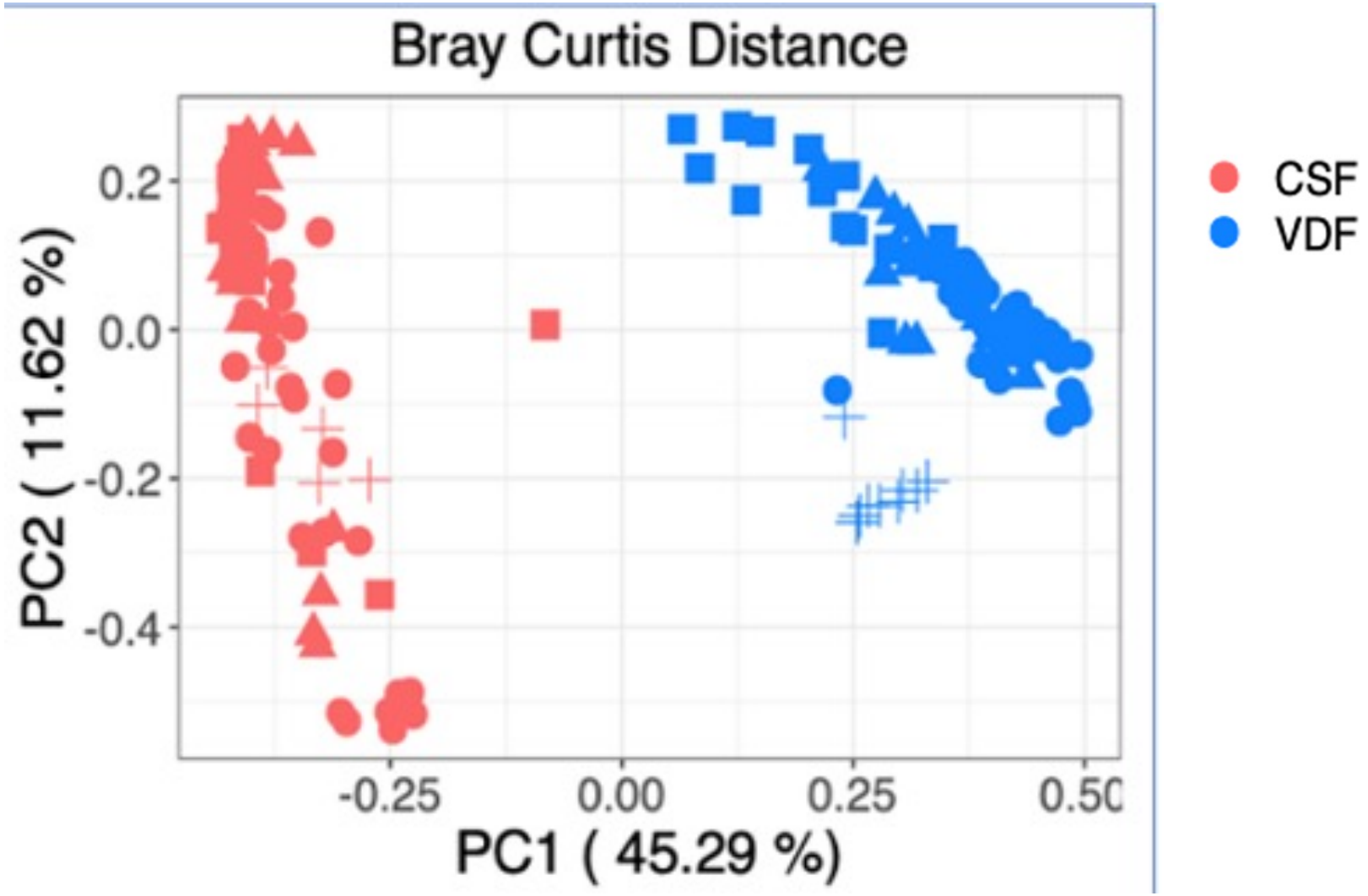
Fig. 1 Offspring from GF mothers exhibit severe obese phenotype when fed a HFD.

Kimura et al, 2020, Science Vol 367

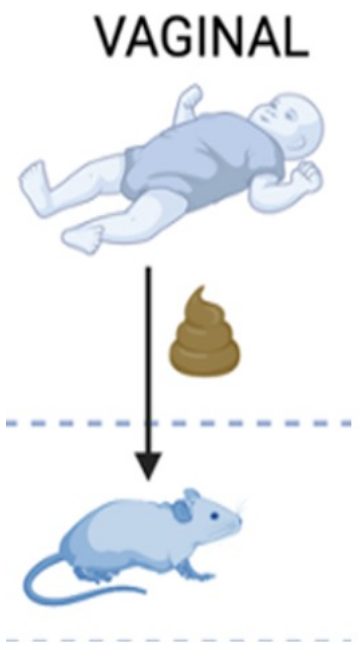
Experimental Design

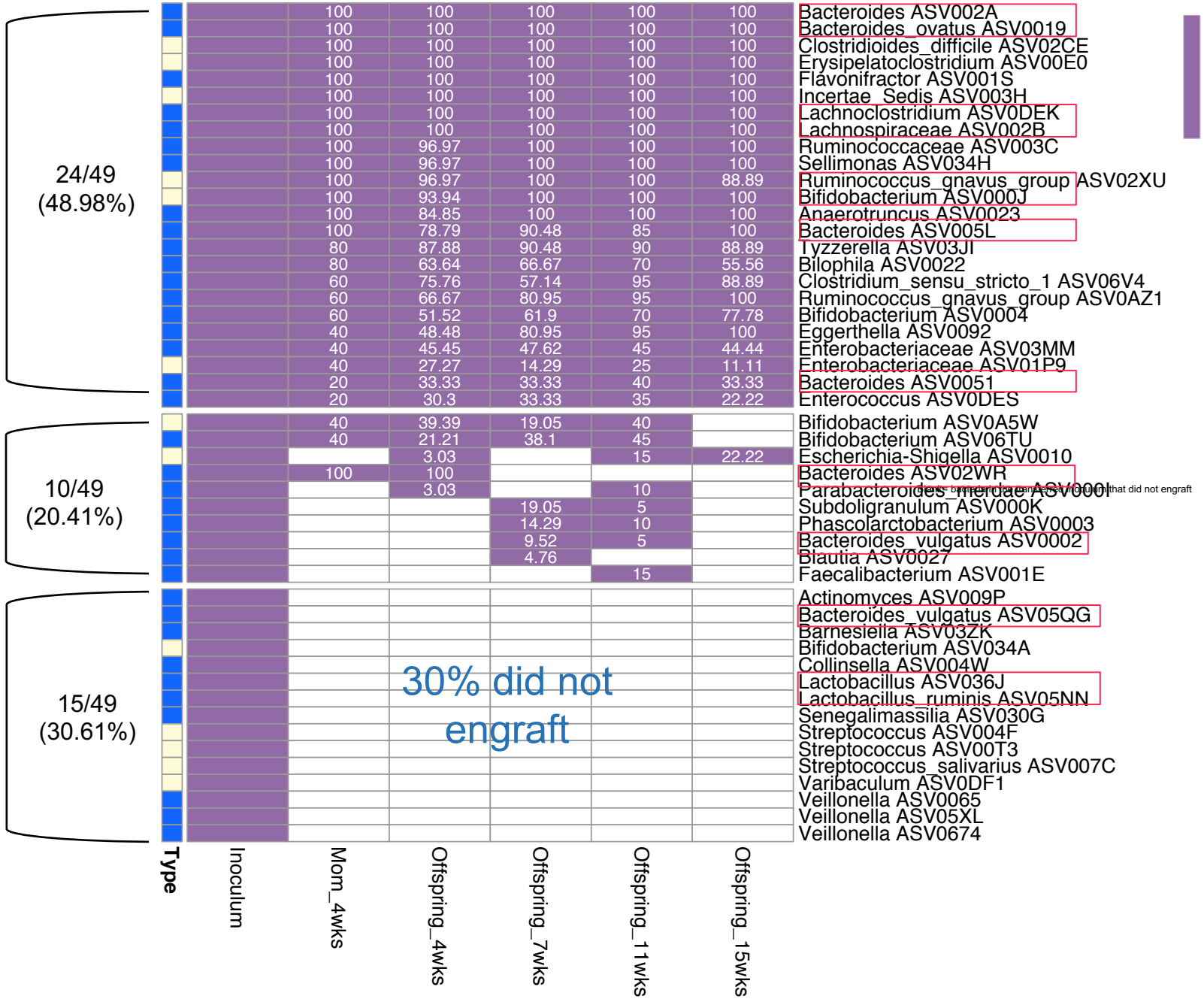


Fecal diversity in 4 week-old offspring mice transplanted with human baby feces

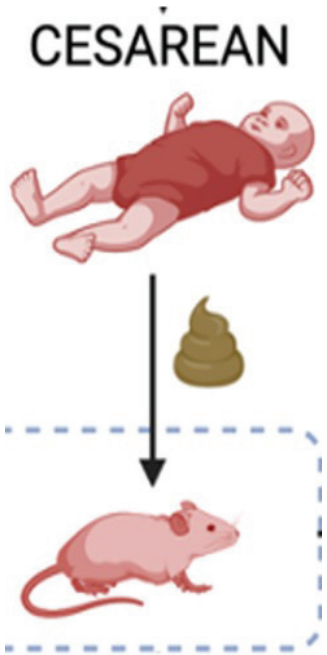


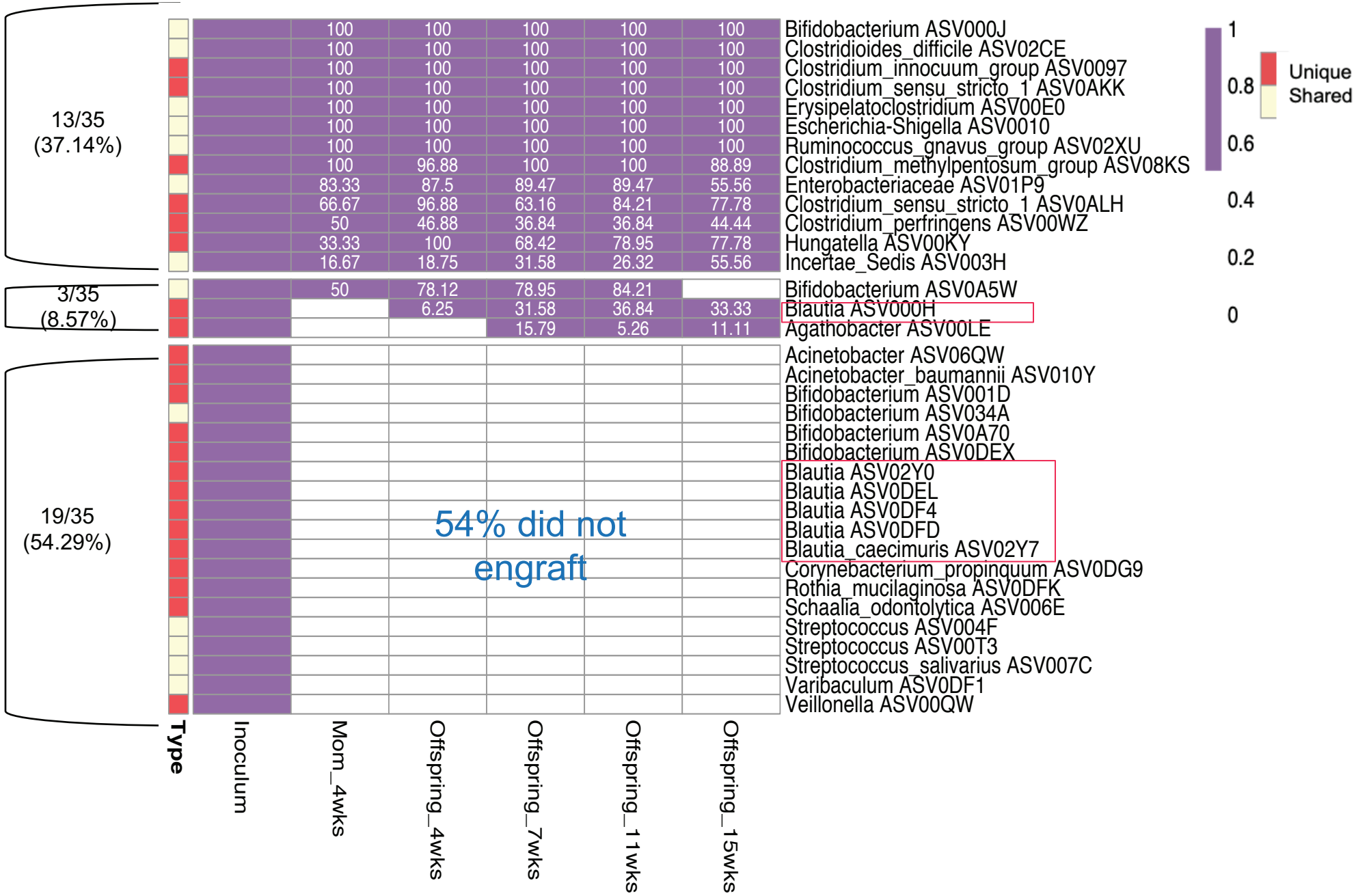
70% mouse bacterial engraftment from vaginally-born babies





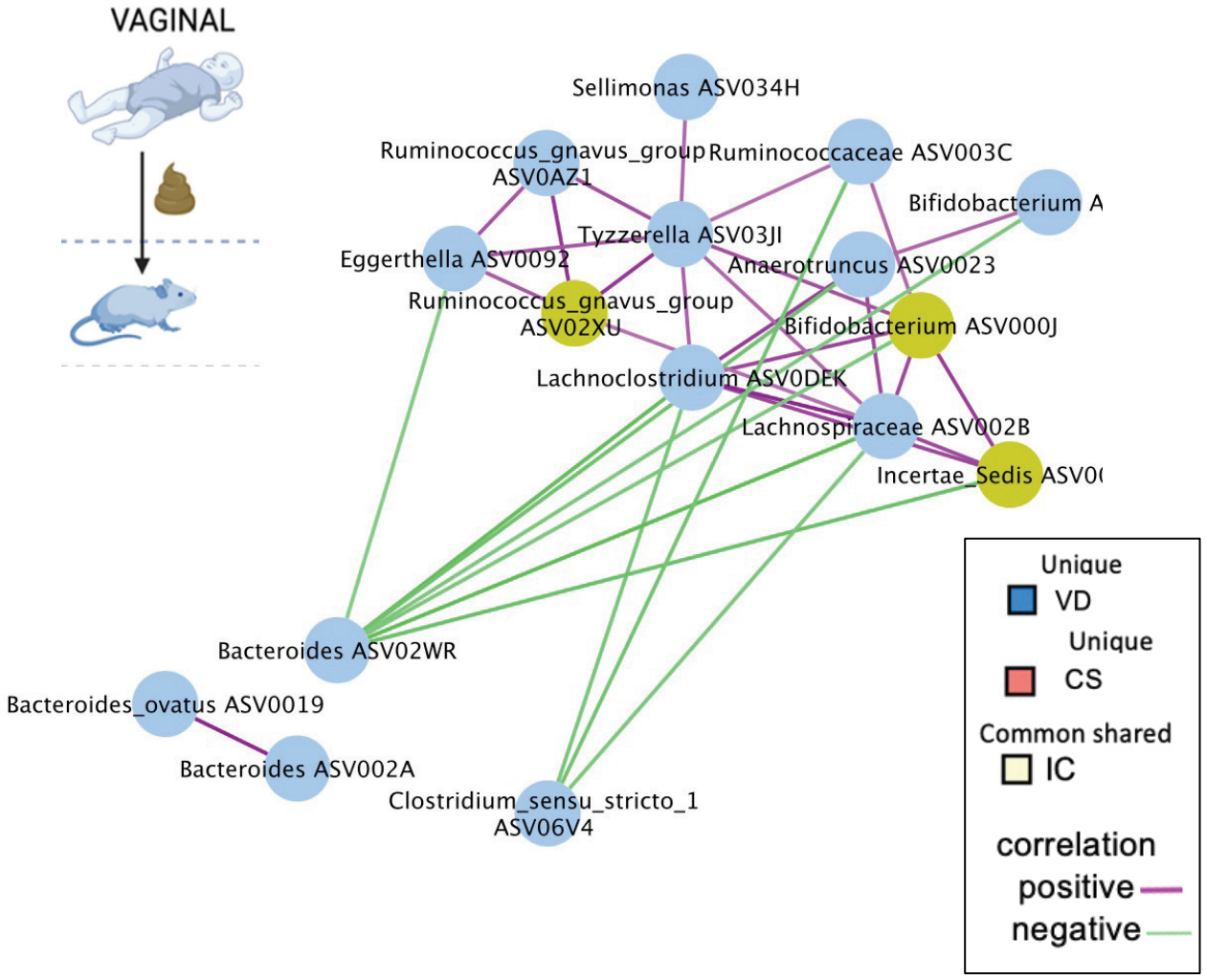
46% mouse bacterial engraftment from C-section-born babies



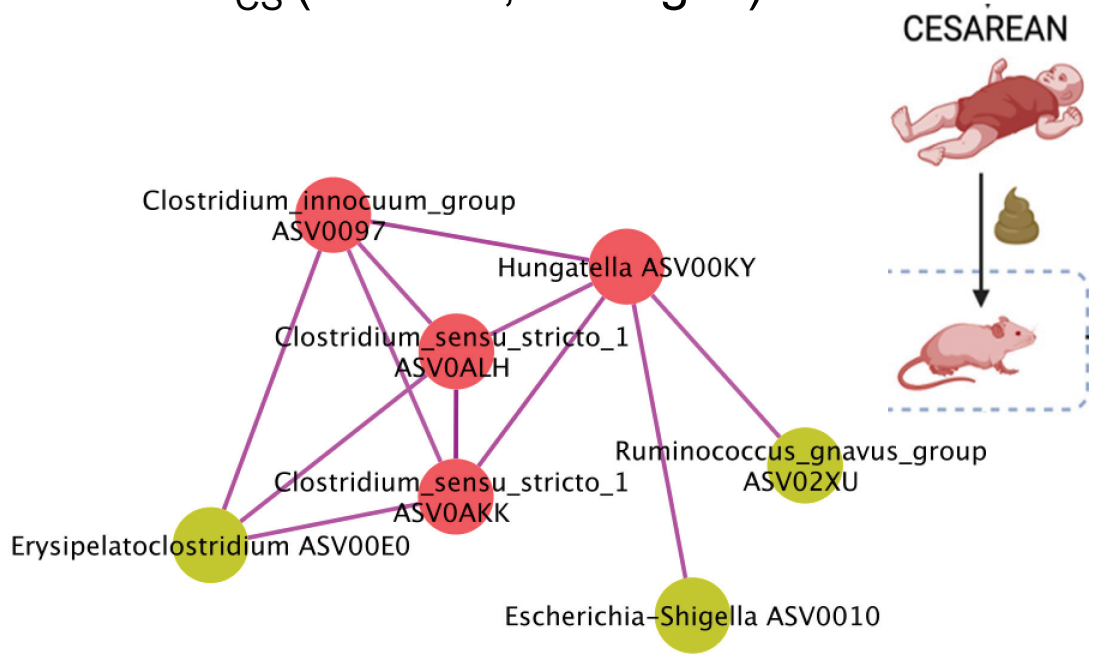


Simpler co-abundance network of human bacterial ASVs from the C-section feces mouse group

G_{VD} (16 nodes, 33 edges)

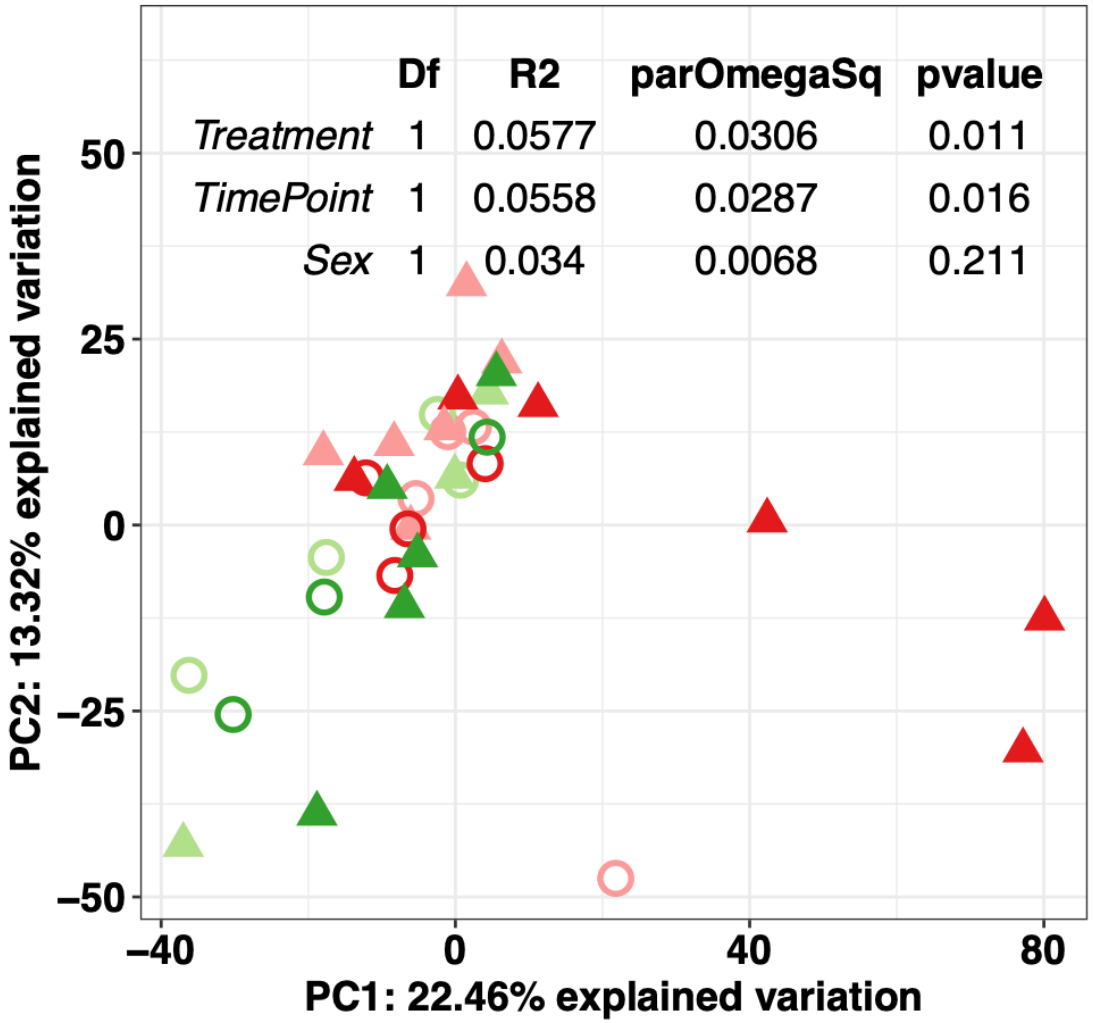


G_{CS} (7 nodes, 11 edges)



Repeated measurement correlation based on log-transformed ASV abundance.
Correlations with BH adjusted $p<0.05$ were kept in the network.

Ileum gene expression in mice receiving feces from vaginally or C-section born human babies



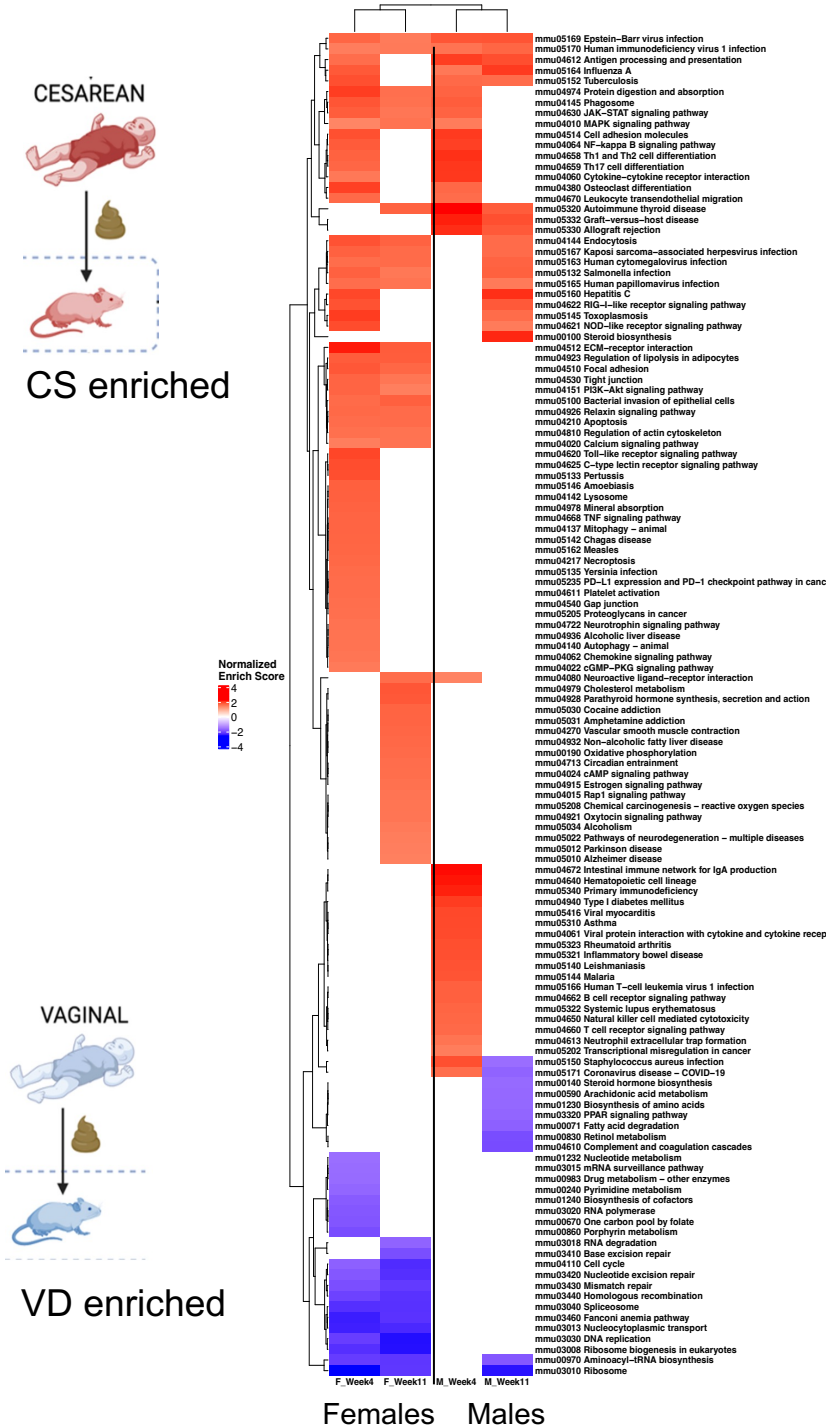
CS

VD

N= 35 mice

Inoculum has a significant effect

KEGG ileal gene pathways in mice at week 4 post- gavage



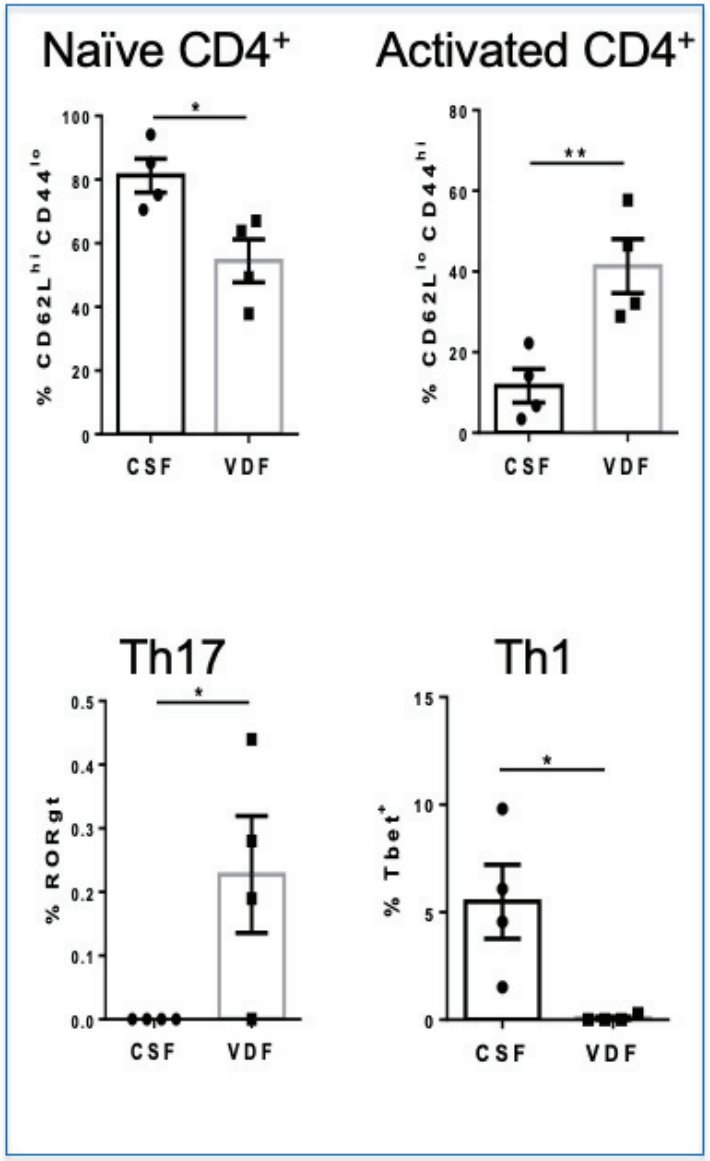
Tuberculosis, Influenza A, Antigen processing and presentation, Endocytosis, Salmonella infection, Human papillomavirus infection, Kaposi sarcoma-associated herpesvirus infection, Human cytomegalovirus infection, Epstein-Barr virus infection, Human immunodeficiency virus 1 infection, Phagosome, Protein digestion and absorption, JAK-STAT signaling pathway, MAPK signaling pathway, Autoimmune thyroid disease

Cell cycle, DNA replication, Nucleocytoplasmic transport, Spliceosome, Fanconi anemia pathway, Ribosome biogenesis in eukaryotes, Homologous recombination, Mismatch repair, Nucleotide excision repair, Ribosome, Aminoacyl-tRNA biosynthesis

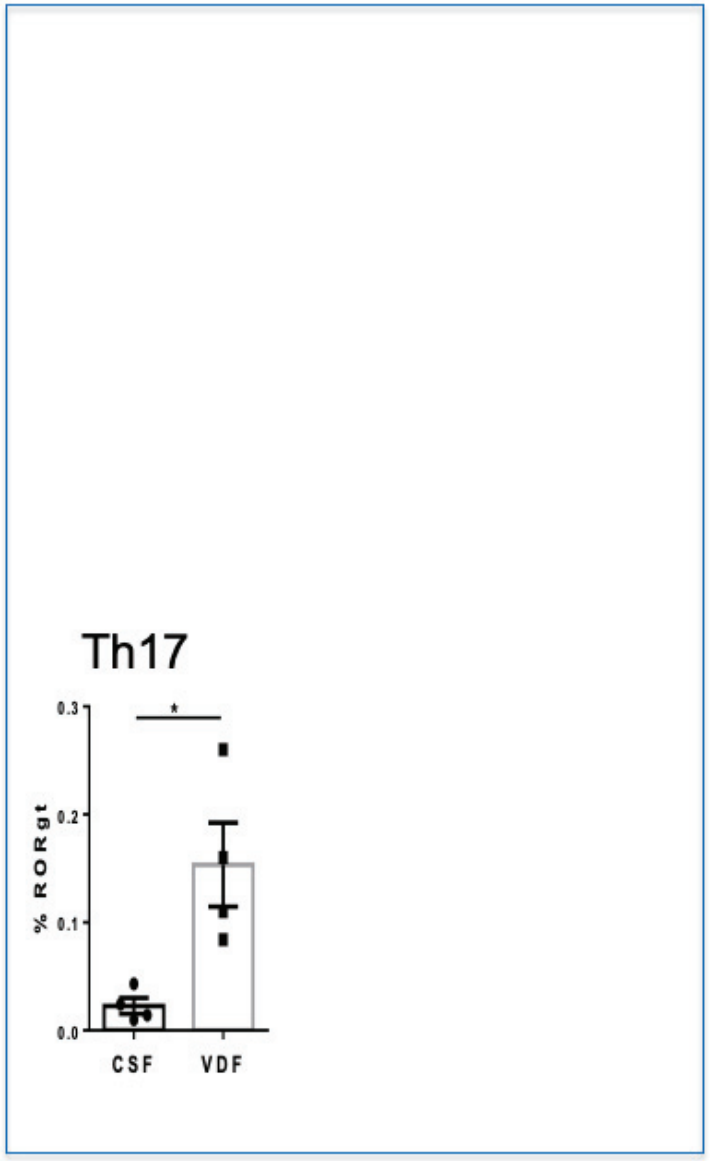
T cell profiles in mice transplanted baby microbiotas at w6

Derek Sant'Antgelo

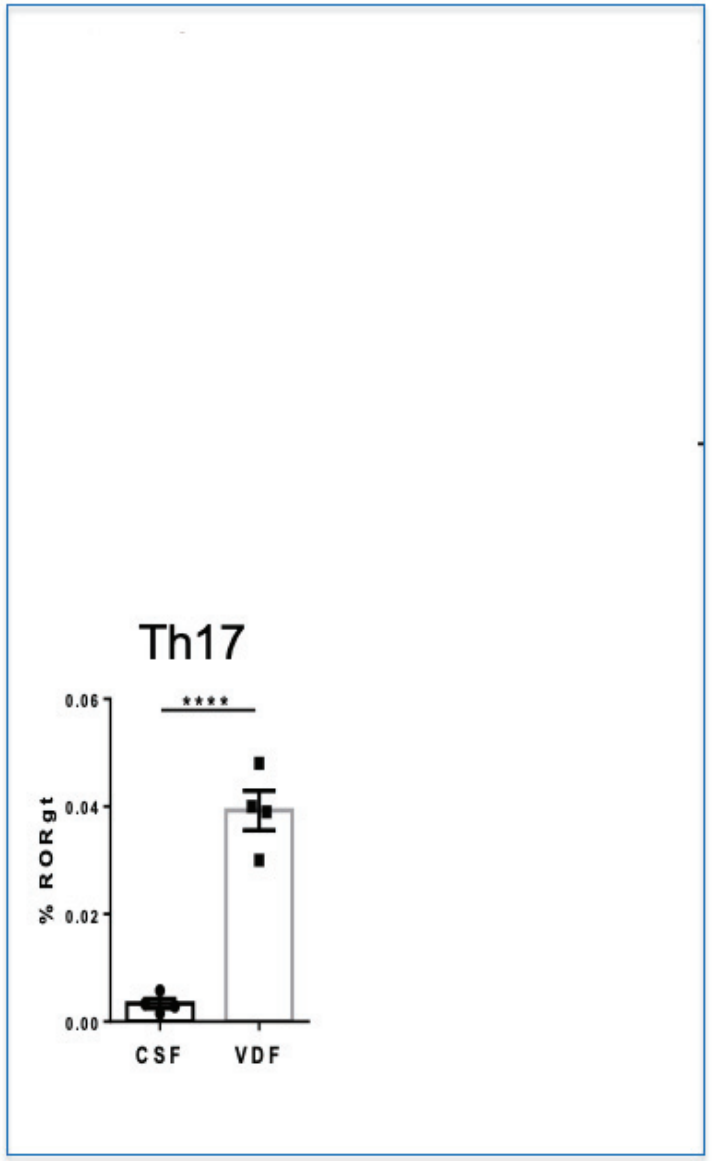
Colonic lamina propria

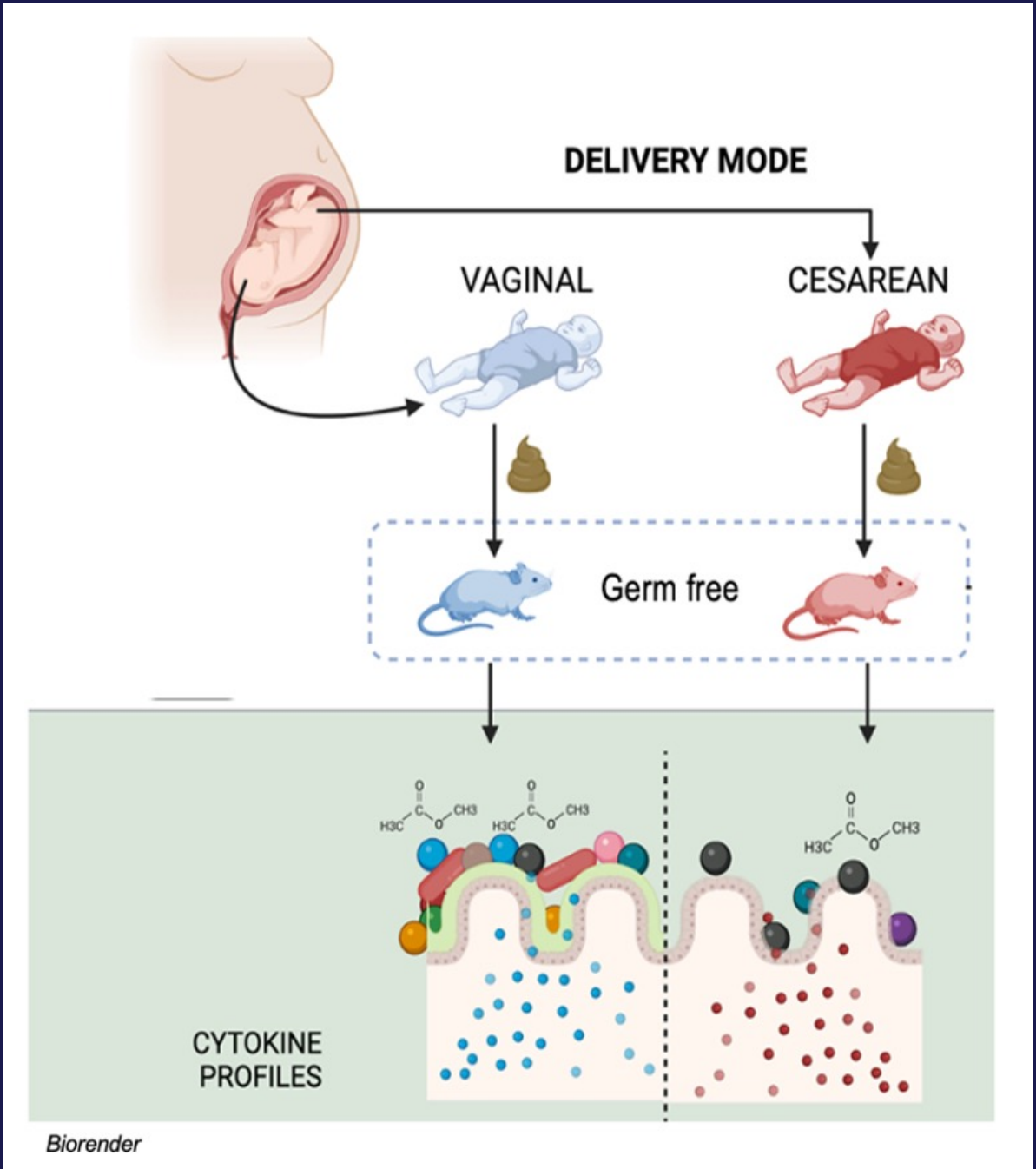


Inguinal lymph nodes



Spleen





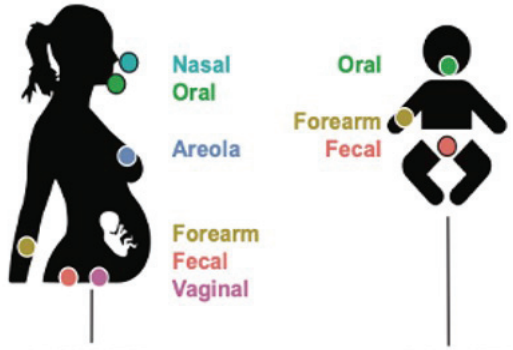
Summary

In relation to feces from vaginally born, feces from C-section human babies caused in transplanted mice:

- Different bacterial engraftment
- Altered intestinal gene expression
- Altered immune responses towards low tolerance/high inflammation

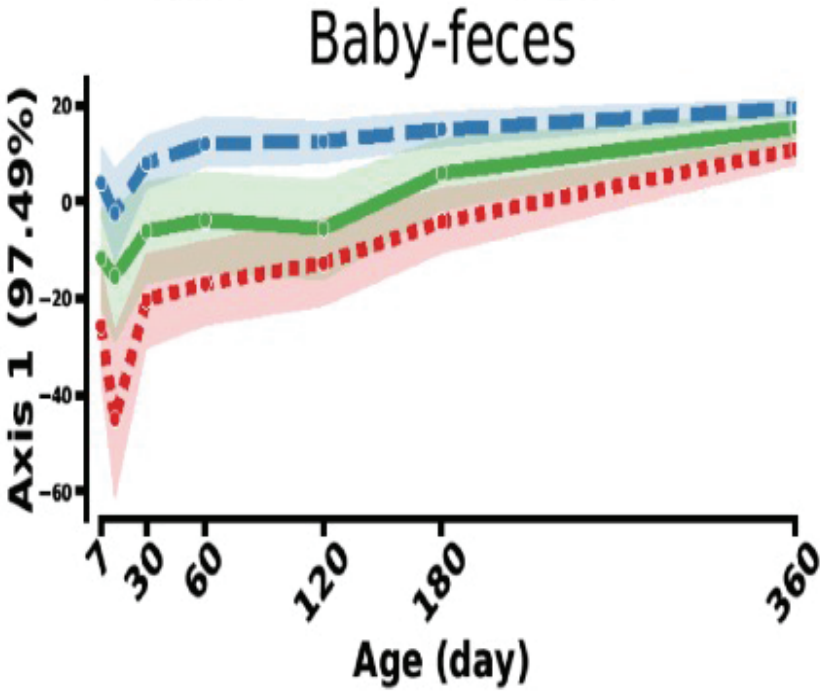
Vaginal seeding after C-section

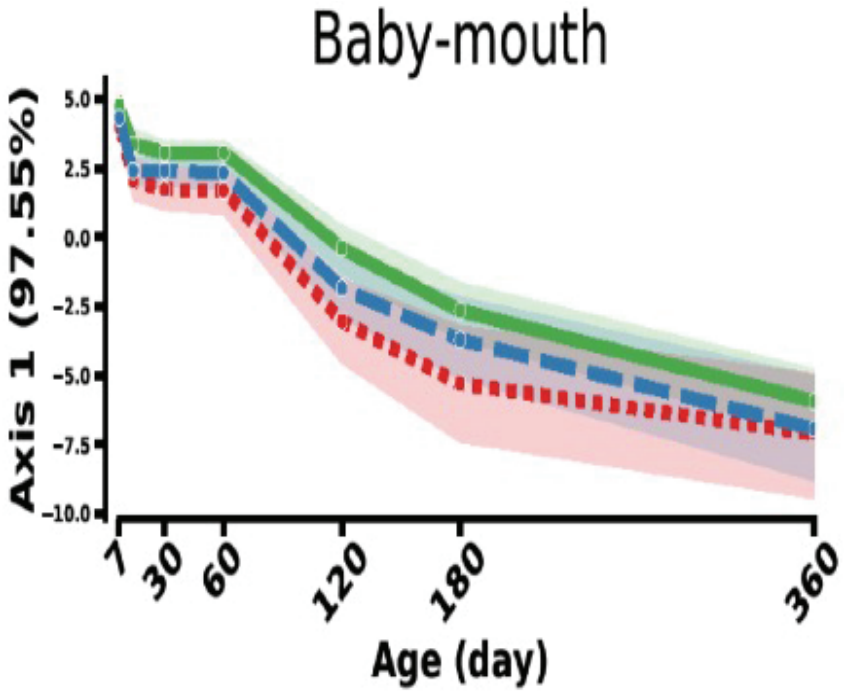
Microbiome trajectories by birth mode and exposure

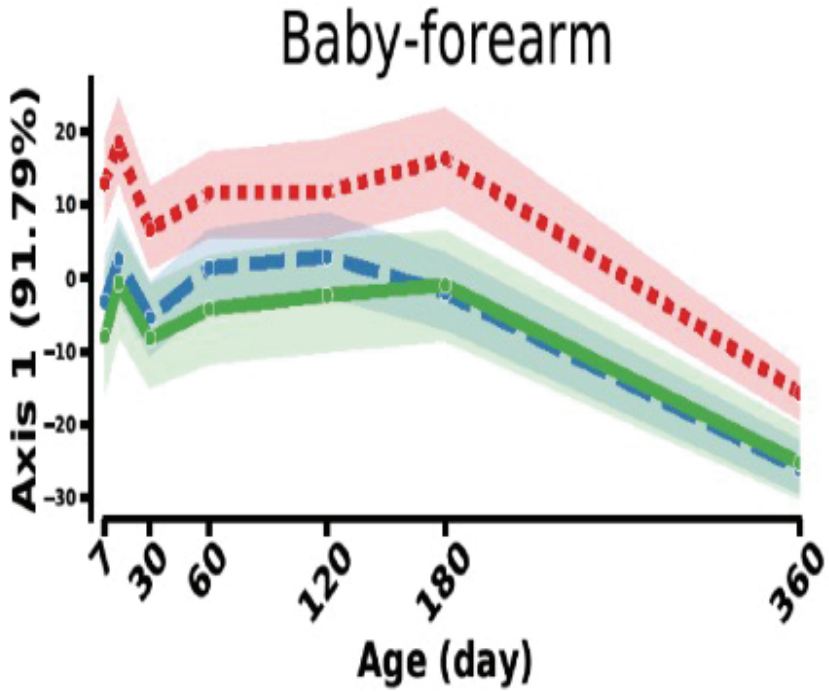


VD
CS
CSR

Compositional Tensor Factorization (CFT) analyses







Song, Martino, Wang, et al. Med 2021

Randomized clinical trials

Primordial Restoration of Infant Microbiome and Development

PRIMED

Effect of vaginal seeding in Cesarean-delivered infants BMI-for-age z score over the first 3 years of life.

Randomized trial, 600 infants

PIs: Larry Appel (PI)

ClinicalTrials.gov Identifier: NCT03298334

Johns Hopkins Bloomberg School of Public Health

INOVA

Rutgers University | New Brunswick

Allergy Prevention in **C**-sec**T**ion **I**nfants **ViA** Microbiome **T**ransf**E**r

ACTIVATE

Effect of vaginal seeding in Cesarean born infants on the microbiome and allergic sensitization to food allergens

120 infants, 80 born by C-section with randomized exposure (pilot)

PIs: Hugh Sampson and Jose Clemente

ClinicalTrials.gov Identifier: NCT03567707

Icahn School of Medicine at Mount Sinai

Restoration of Microbiota in Newborns

RoMaNs

Karolinska Institutet Anno 1810

Vaginal-fecal exposure at birth in CS born babies, microbiota and systemic / mucosal immune responses during infancy (up to 4 years).

Randomized trial, 200 infants

PIs: Lars Engstrand (PI)

ClinicalTrials.gov Identifier: NCT03928431


THE FUTURE IS
Restoration
222
220
Created by AI

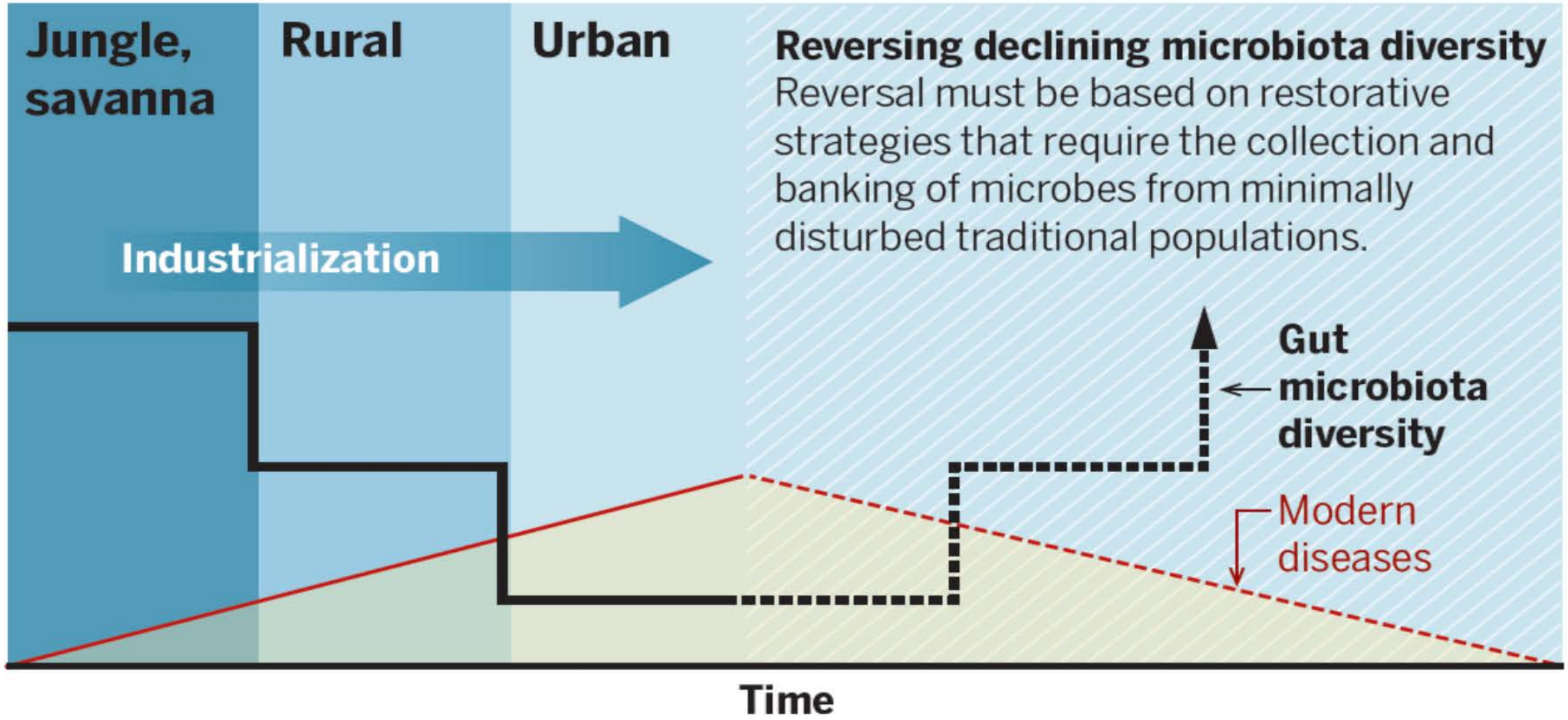
MICROBIOLOGY

Preserving microbial diversity

Science 2018

Microbiota from humans of all cultures are needed to ensure the health of future generations

By **Maria G. Dominguez Bello[1], Rob Knight[2], Jack A. Gilbert[3], Martin J. Blaser[4]**



Thank you!

THE MICROBIOTA VAULT

CIFAR

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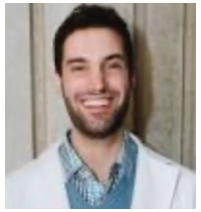
THE EMCH FOUNDATION

MTM Foundation


Haipeng Sun

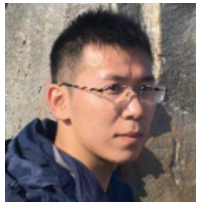

Jeremy Lessing

Jincheng Wang

Melissa Woortman


Wessam Mohamed


Emanuel DiCicco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Liping Zhao


Martin Blaser


Troy Roepke


Pieter Dorrestein
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Cameron Martino
UCSD


Se Jin Song
Univ Colorado


Rob Knight
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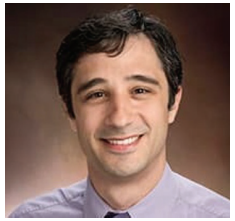
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