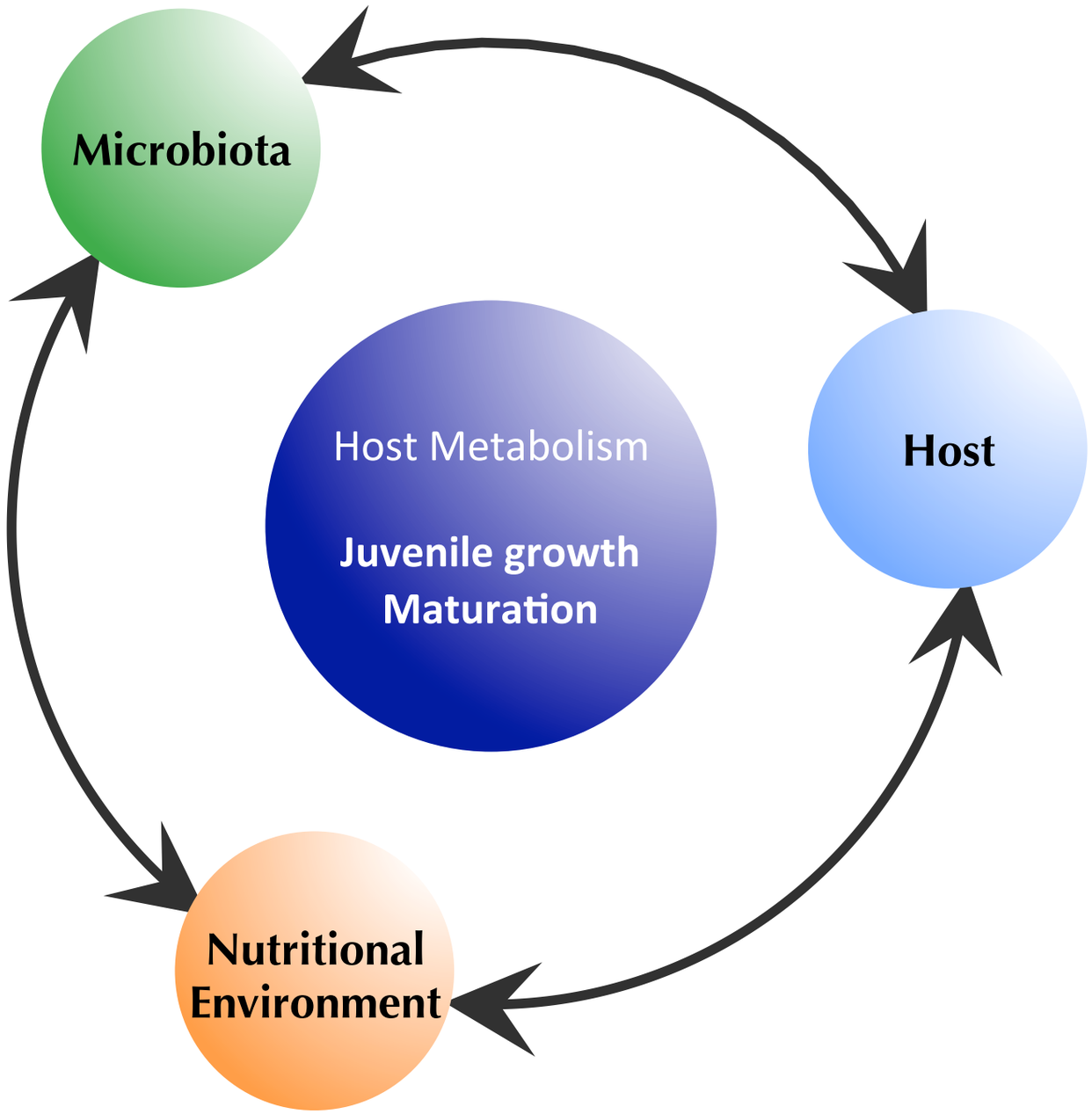
Institut de Génomique Fonctionnelle de Lyon

UNIVERSITÉ DE LYON

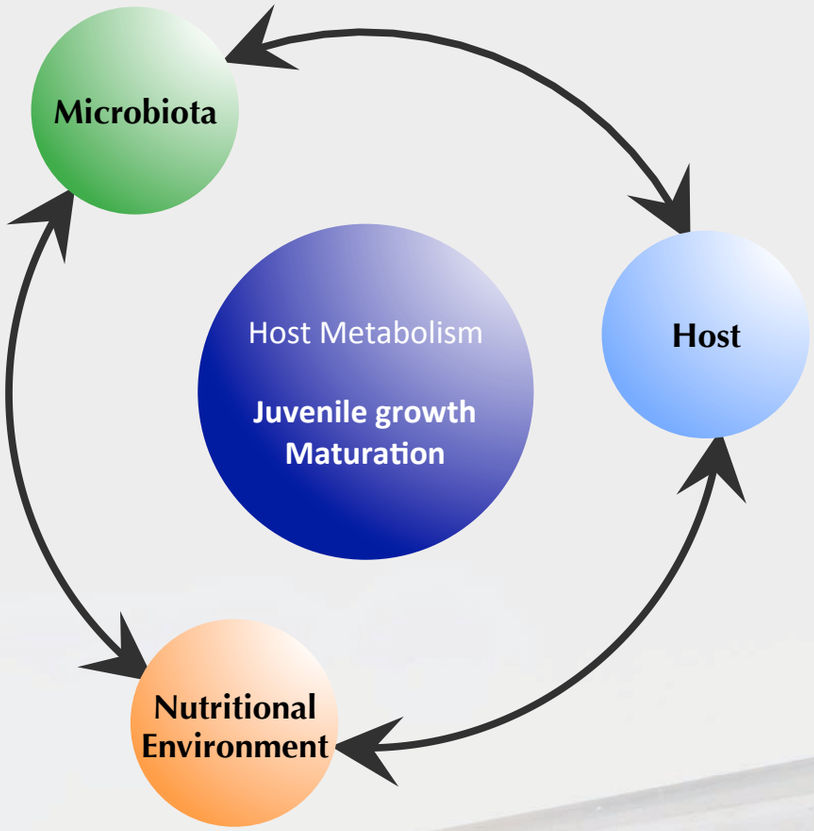
Host-microbiota mutualism in development and metabolism: lessons from gnotobiotic animal models

François Leulier

Integrative physiology



Integrative physiology

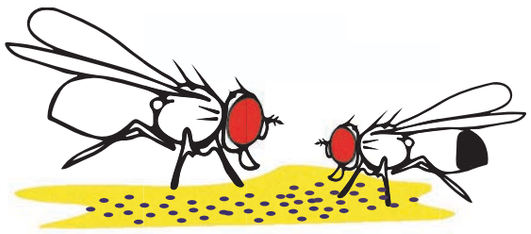


Drosophila melanogaster a host model to study host-commensals interactions

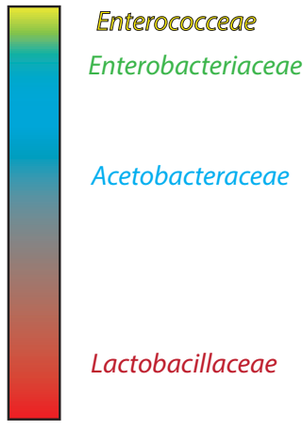
Live and feed on fermenting fruits:
microbe rich environments

Drosophila melanogaster a host model to study host-commensals interactions

Live and feed on fermenting fruits:
microbe rich environments



Drosophila feed on bacteria-rich food



Gut gets colonized

The progeny gets colonized by eating the bacteria-rich food and/or its bacteria-seeded eggshell

Colonized fly seeds its (new) environment and the surface of newly laid eggs with fecal microbiota

Simple commensal bacterial communities:

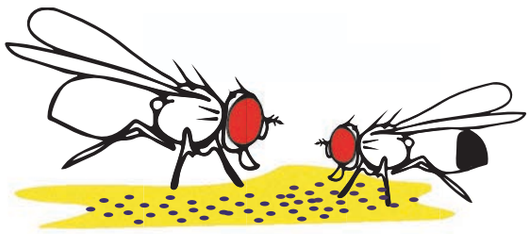
Lactobacillales

Acetobacteraceae

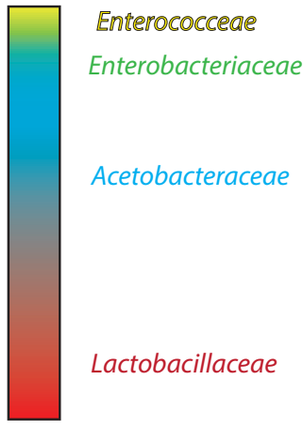
3-5 dominant species

Drosophila melanogaster a host model to study host-commensals interactions

Live and feed on fermenting fruits:
microbe rich environments



Drosophila feed on bacteria-rich food



Gut gets colonized

The progeny gets colonized by eating the bacteria-rich food and/or its bacteria-seeded eggshell

Colonized fly seeds its (new) environment and the surface of newly laid eggs with fecal microbiota

Simple commensal bacterial communities:

Lactobacillales

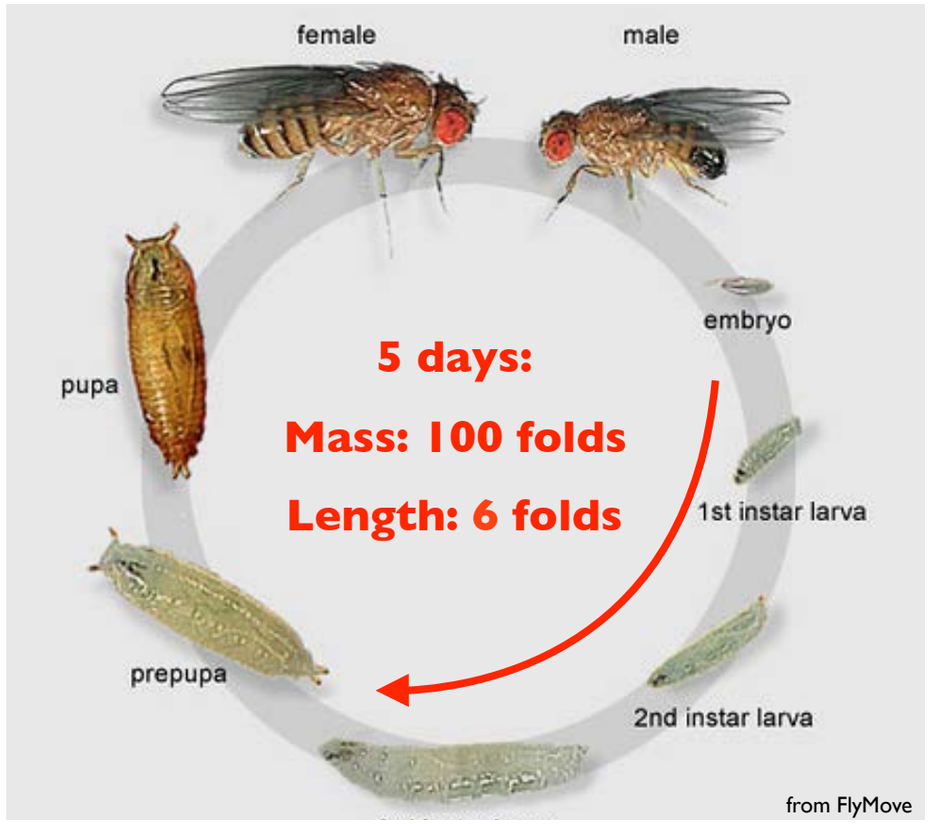
Acetobacteraceae

3-5 dominant species

Are there mutualists among commensals?

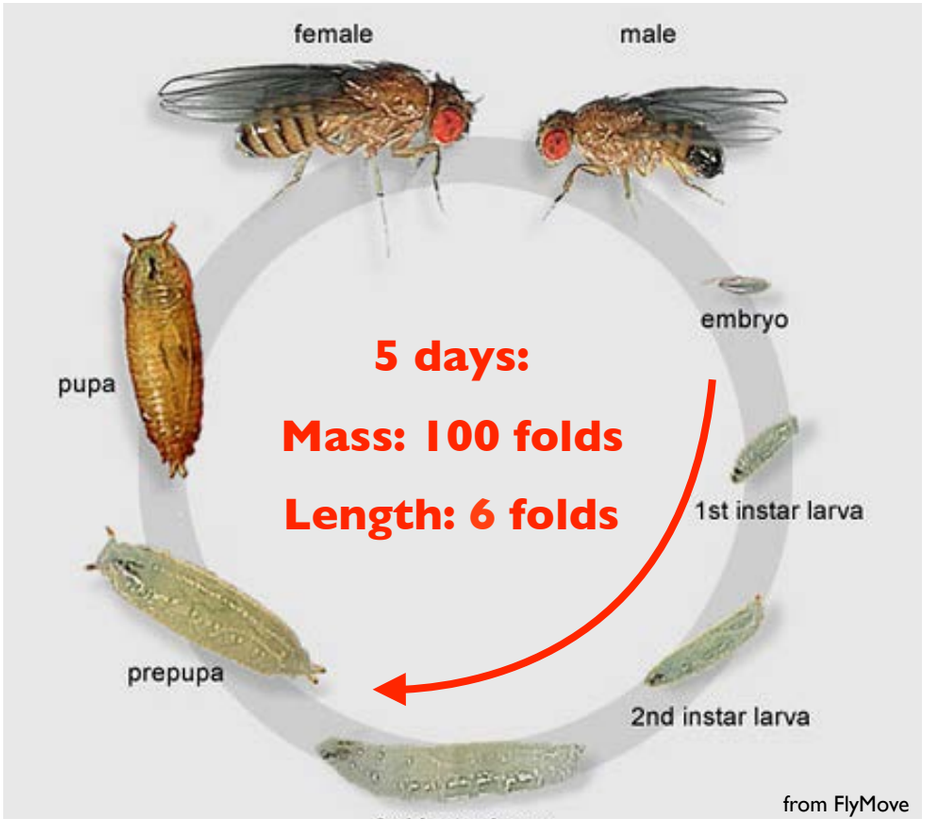
Does microbiota influence *Drosophila* juvenile growth?

Laboratory breeding diet
=
Optimized nutrition

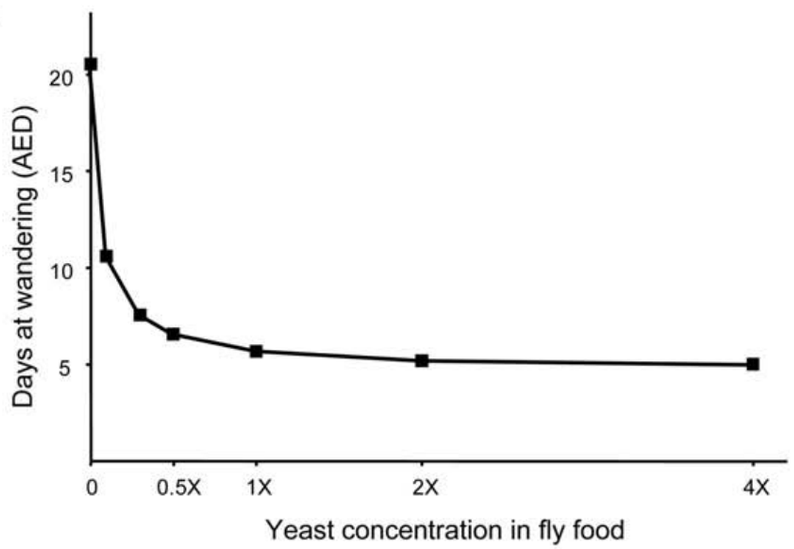


Does microbiota influence *Drosophila* juvenile growth?

Laboratory breeding diet
=
Optimized nutrition



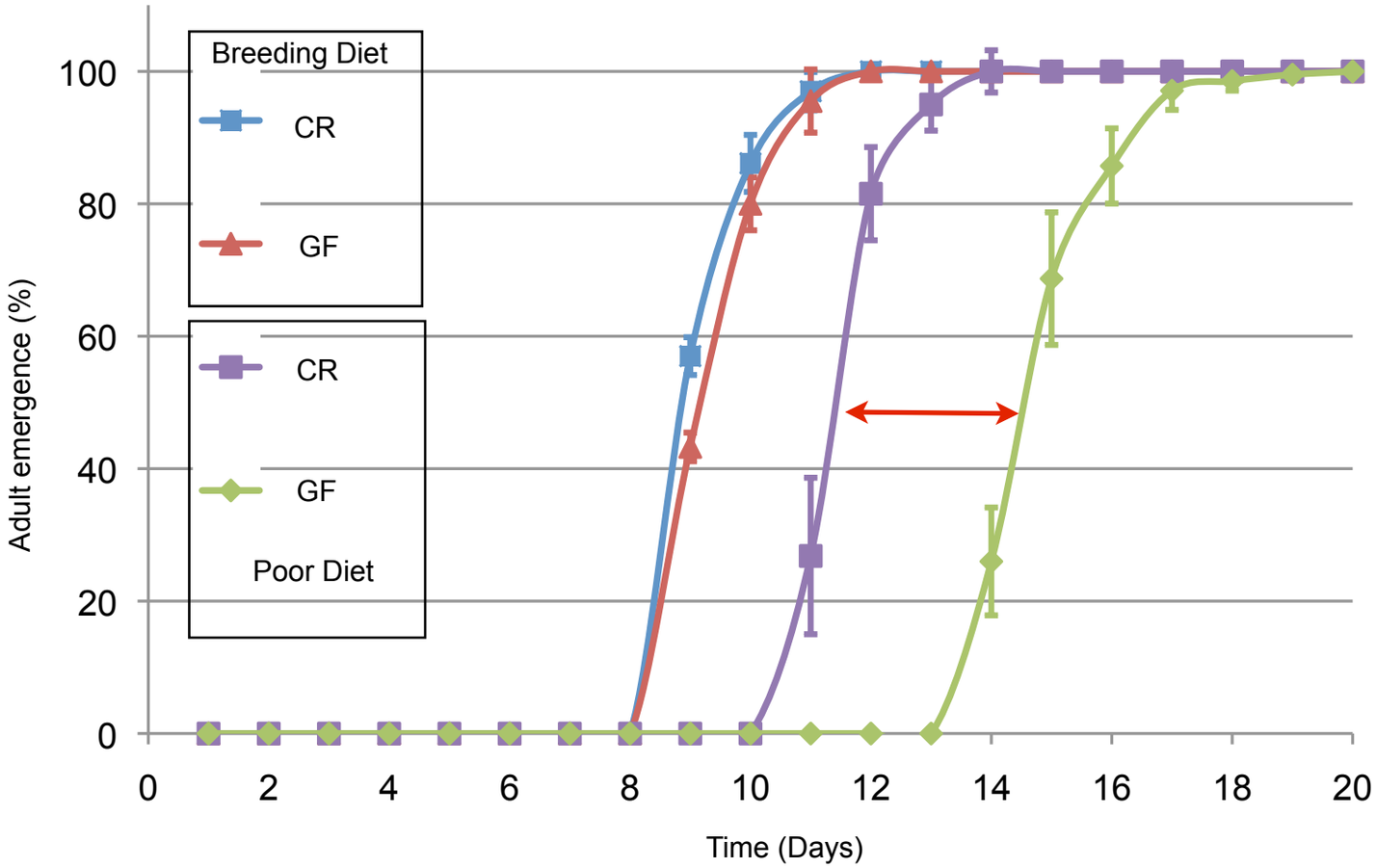
Undernutrition (reduced nutrient intake) triggers stunting: developmental delay and small size



Undernutrition

Normal nutrition

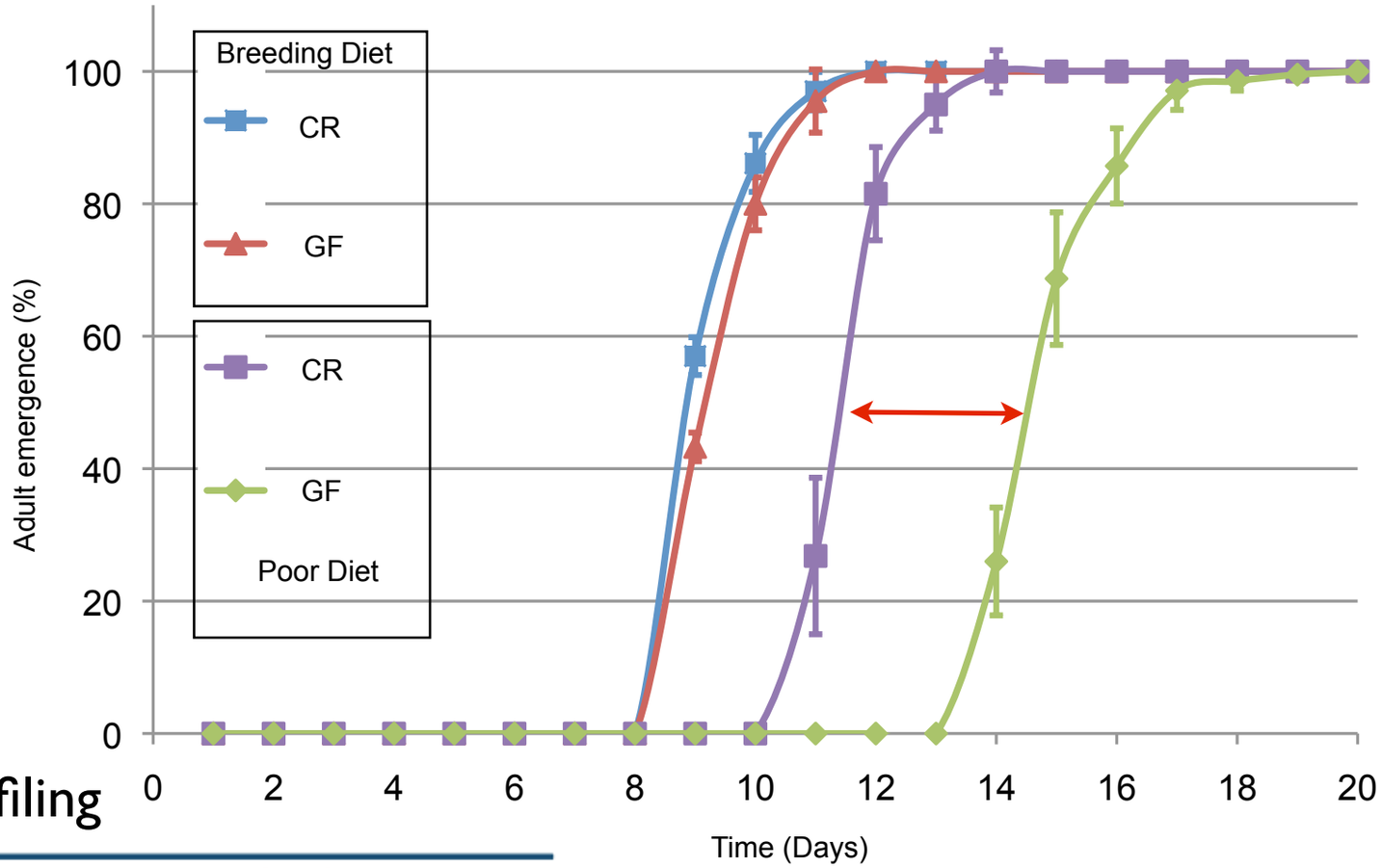
Drosophila microbiota promotes juvenile growth upon undernutrition



CR: Conventionally reared (+commensals)

GF: Germ-Free

Drosophila microbiota promotes juvenile growth upon undernutrition



16S rDNA gene profiling

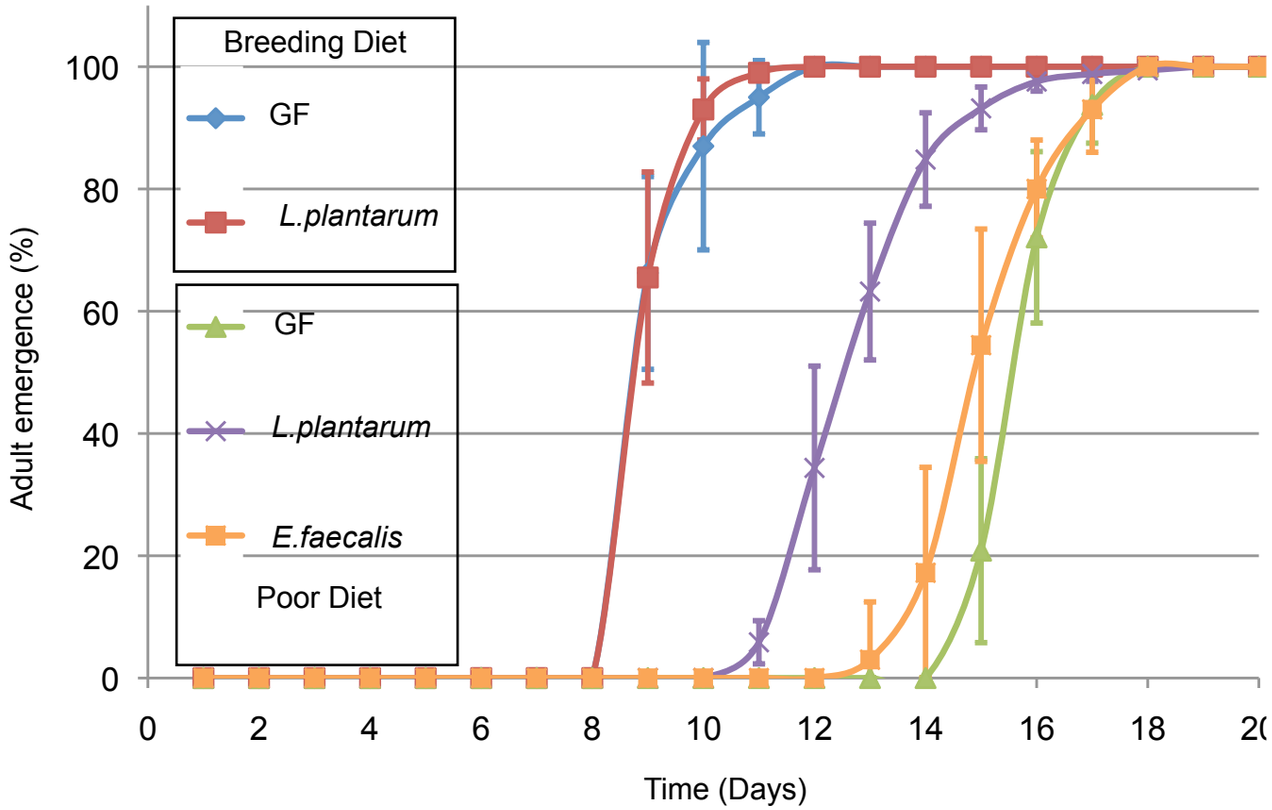
Table 1. Bacterial Species Associated with Our Conventionally Reared Wild-Type Fly Strain

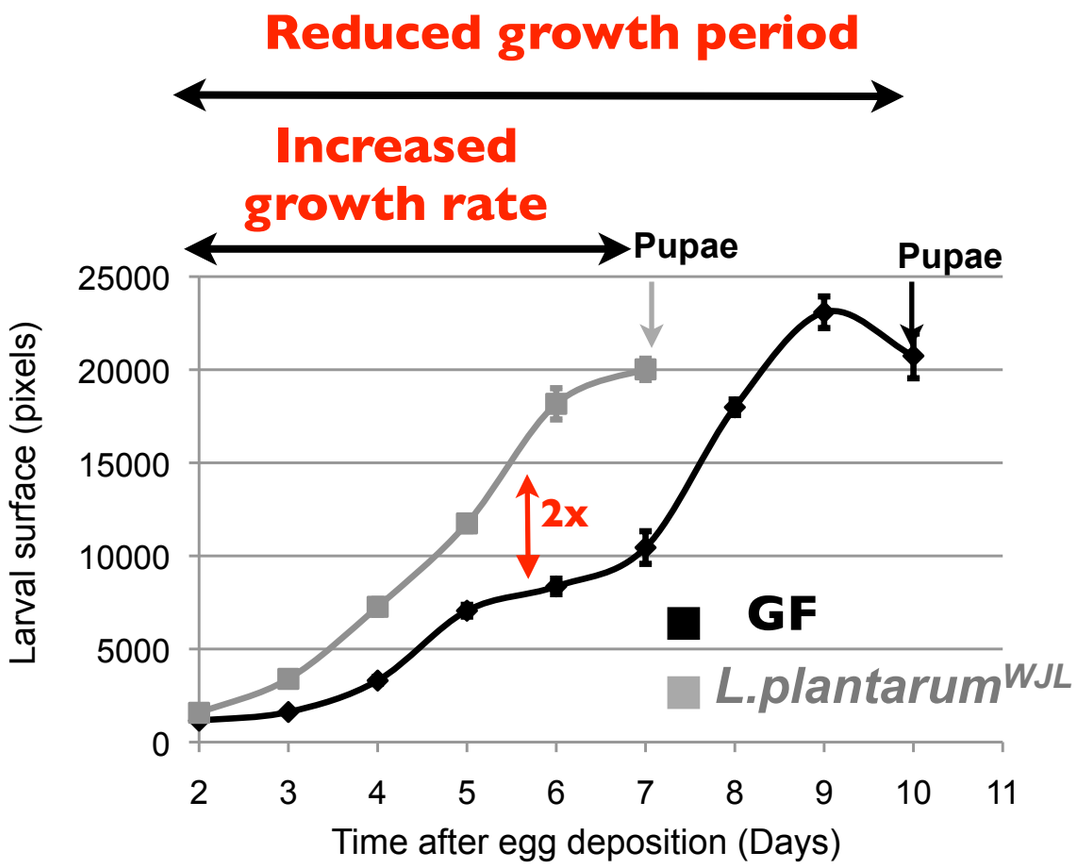
CRyw Whole Body Library		
Phylotype	Closest strain	% identity
Enterococcus faecalis	*Enterococcus faecalis* V583	99%
Lactobacillus plantarum	*Lactobacillus plantarum* WCFS1	99%
Aerococcus spp.	*Aerococcus viridans* ATCC11563	97%
CRyw Adult Midgut Library		
Phylotype	Closest strain	% identity
Enterococcus faecalis	*Enterococcus faecalis* V583	99%
Lactobacillus plantarum	*Lactobacillus plantarum* WCFS1	99%
Corynebacterium variabile	*Corynebacterium variabile* DSM20132	98%

CR: Conventionally reared (+commensals)

GF: Germ-Free

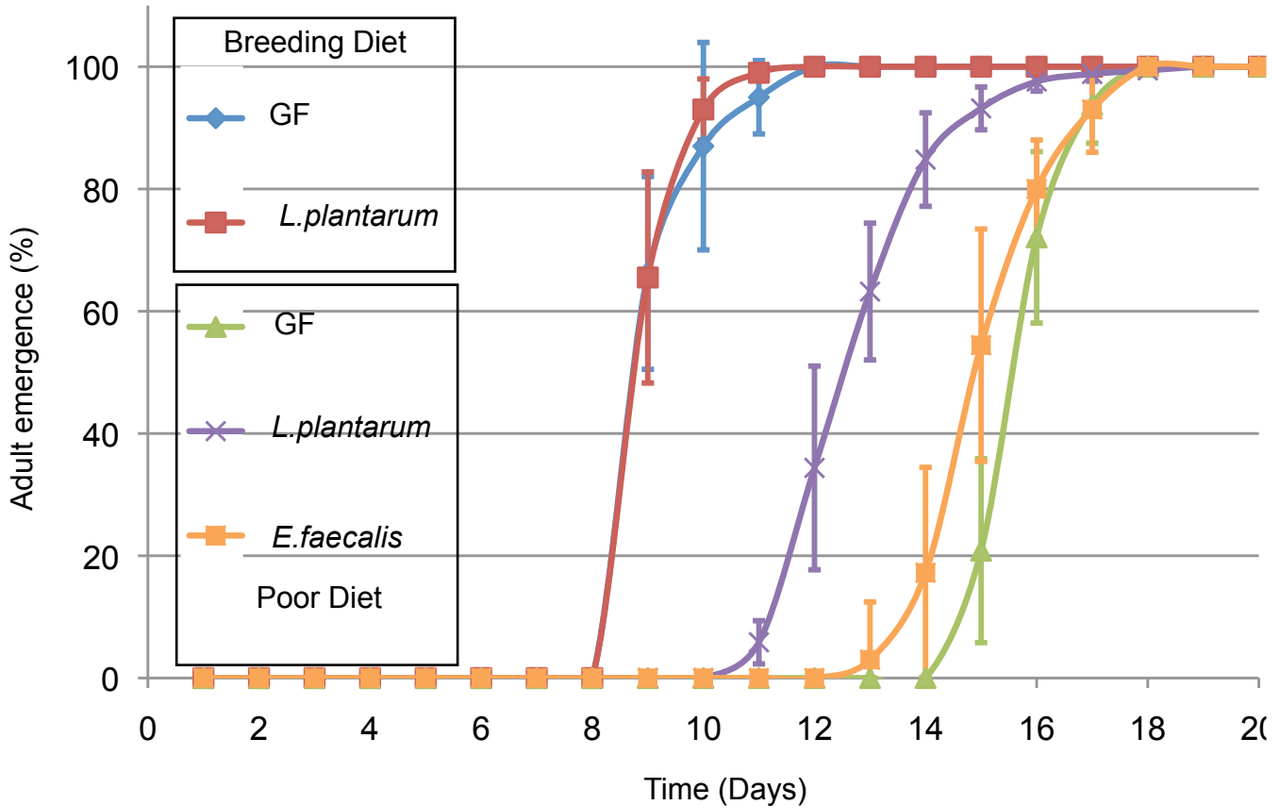
Mono-association of GF animals with one commensal: *Lactobacillus plantarum* promotes *Drosophila* juvenile growth

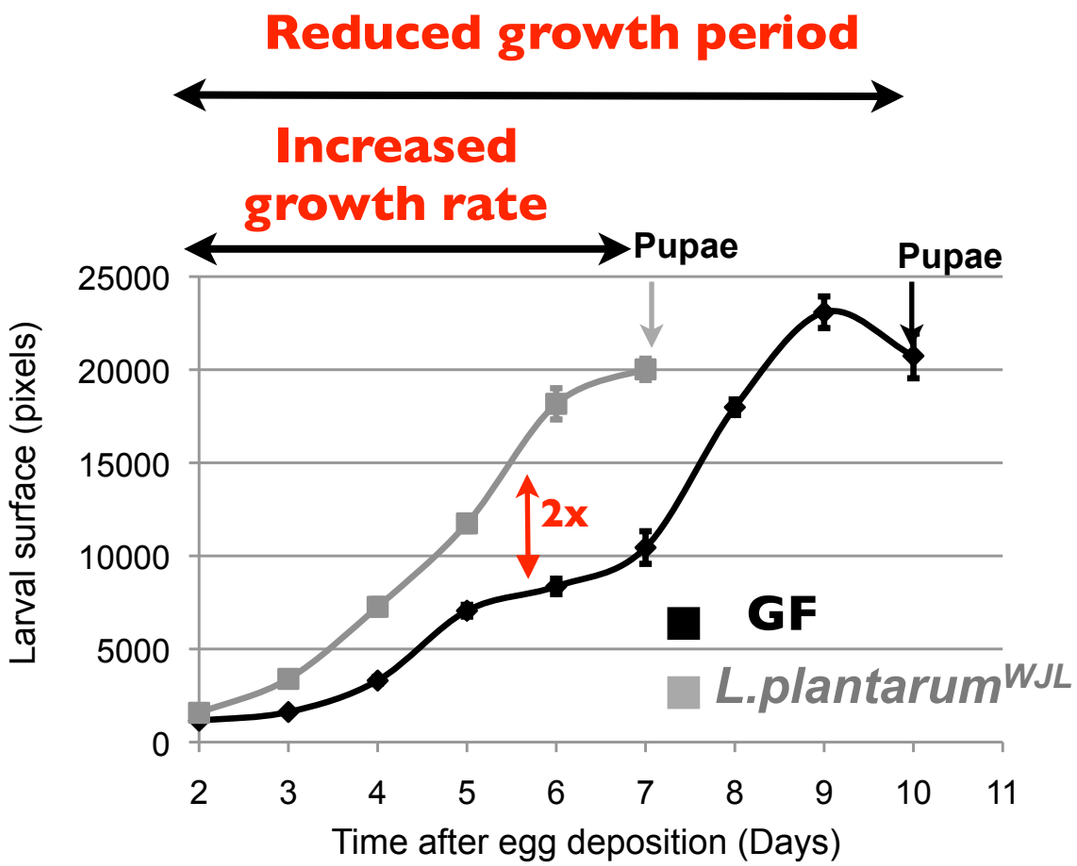




Storelli *et al.* Cell Metabolism 2011

Mono-association of GF animals with one commensal: *Lactobacillus plantarum* promotes *Drosophila* juvenile growth





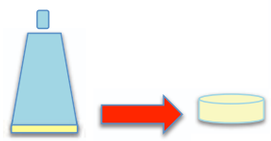
Lactobacillus plantarum^{WJL} = *Drosophila* mutualist

(some strains of *Acetobacter* too)

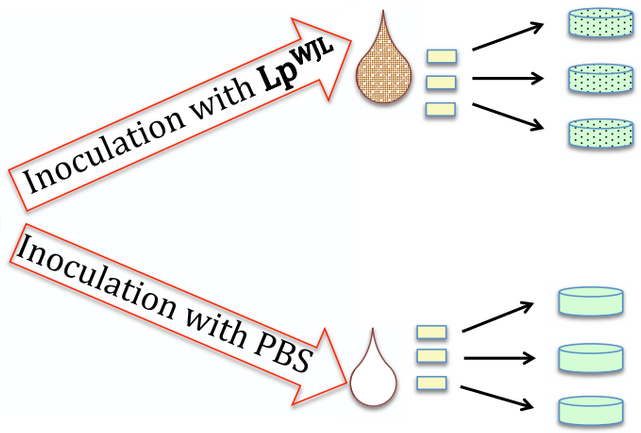
Storelli *et al.* Cell Metabolism 2011

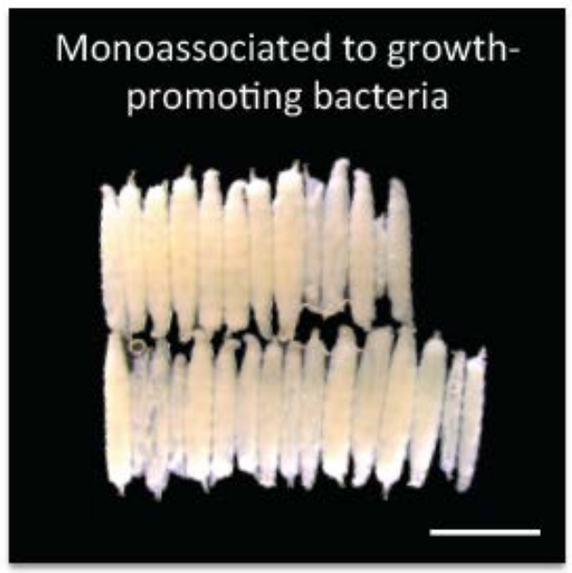
A bacterial strain-dependent phenomena

Make germ-free flies

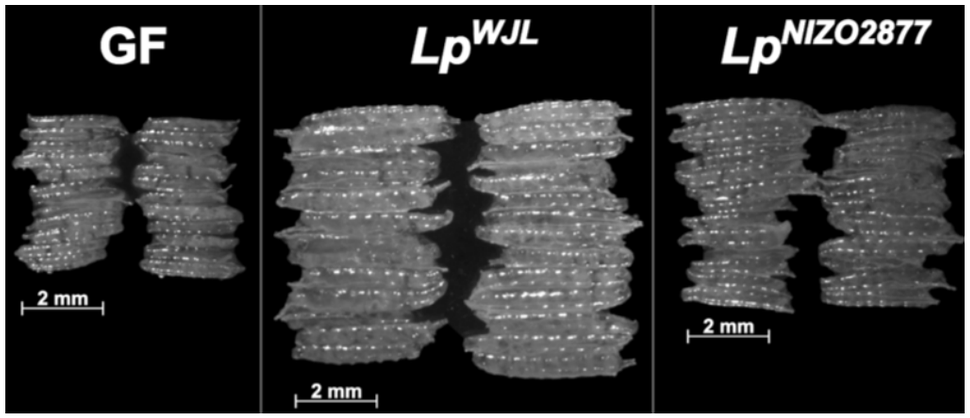


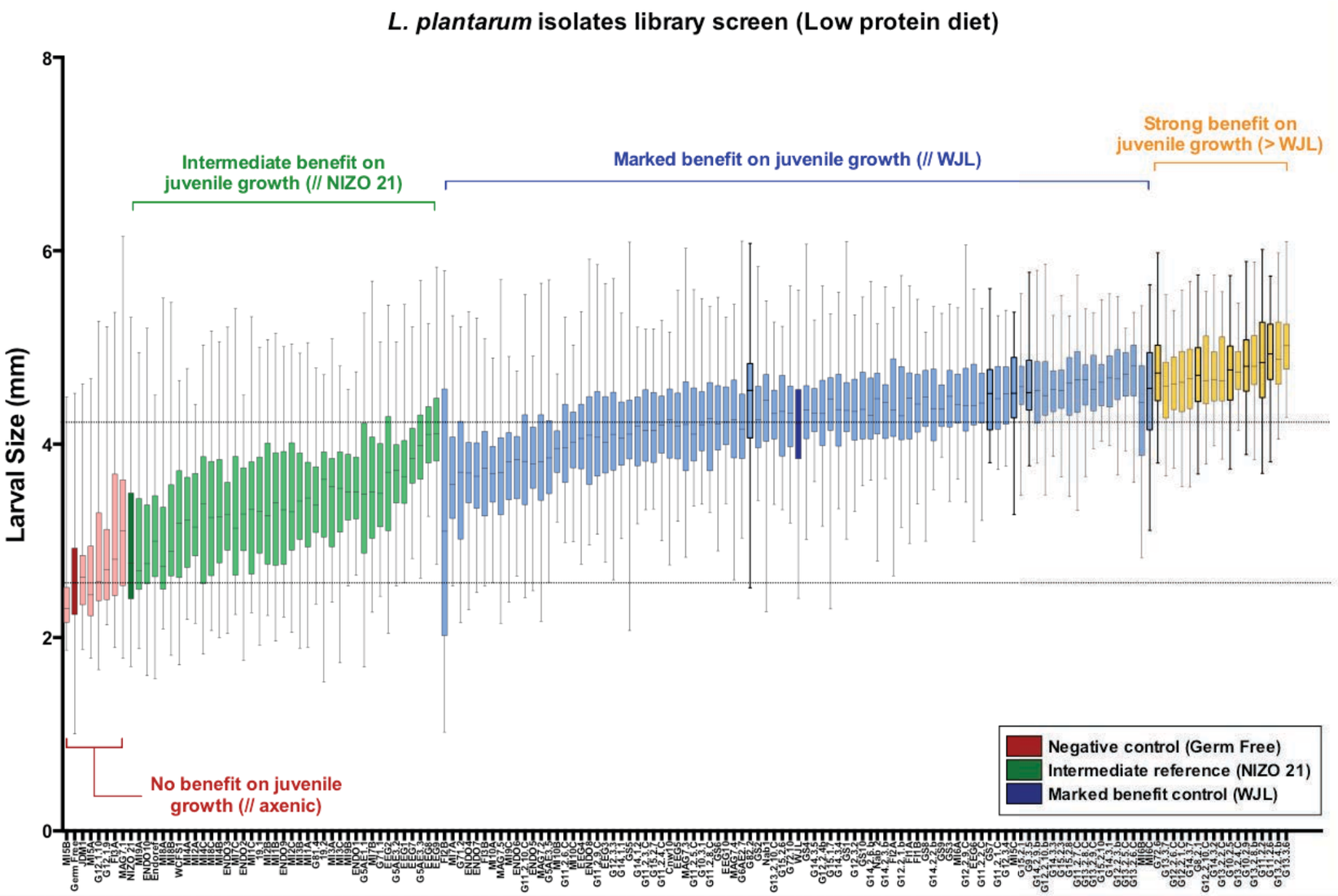
Collect axenic embryos

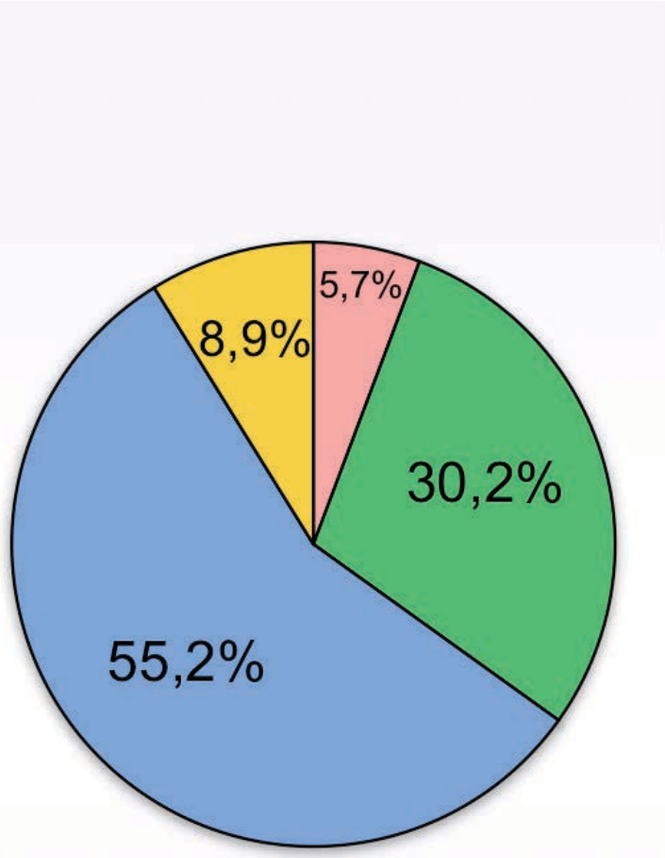




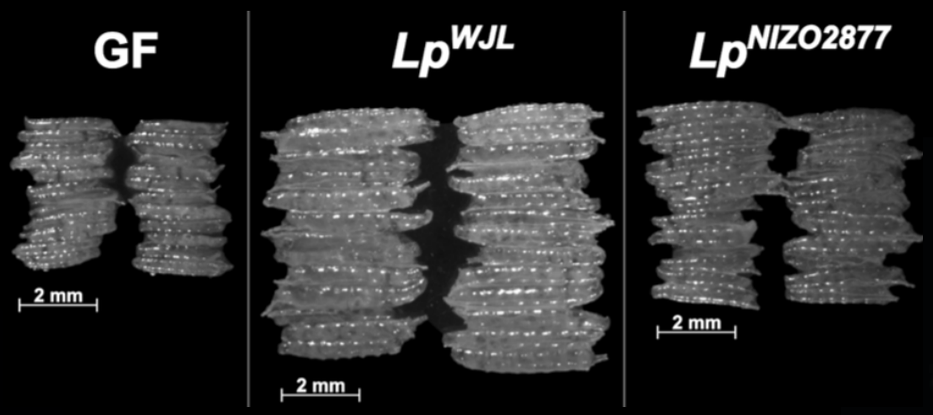
A bacterial strain-dependent phenomena

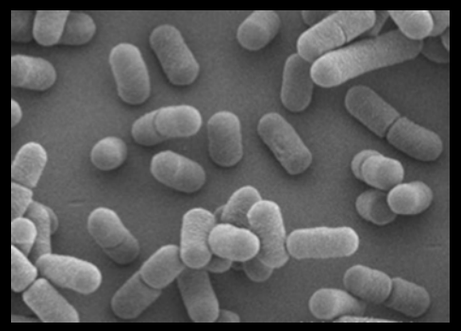






Ideal experimental model to probe the molecular underpinnings of Lactobacilli-mediated juvenile growth performance





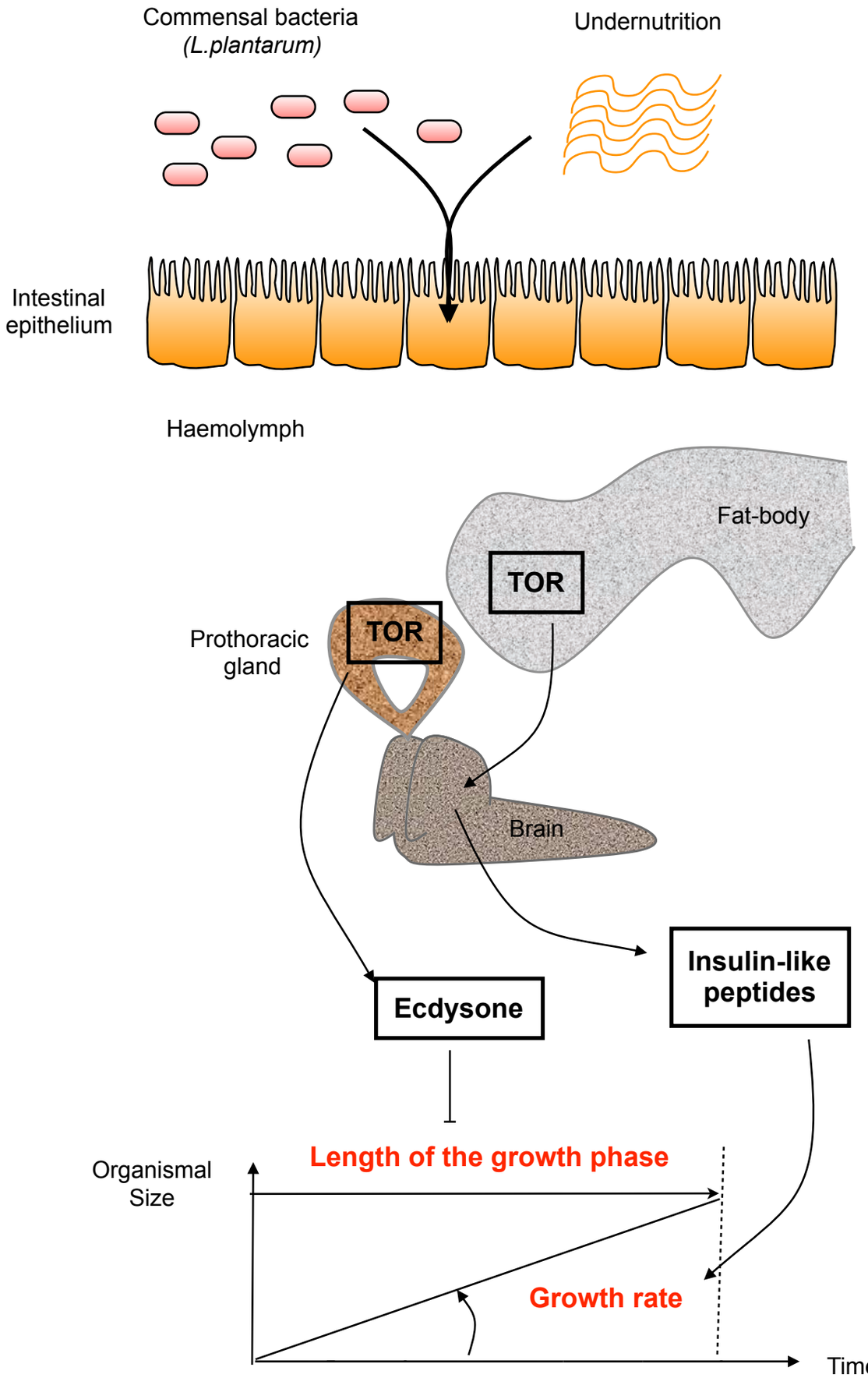
Simple and controlled experimental model
one host - one commensal

Genetically tractable:
Drosophila and *Lactobacilli* genetics

A robust commensal mediated host phenotype

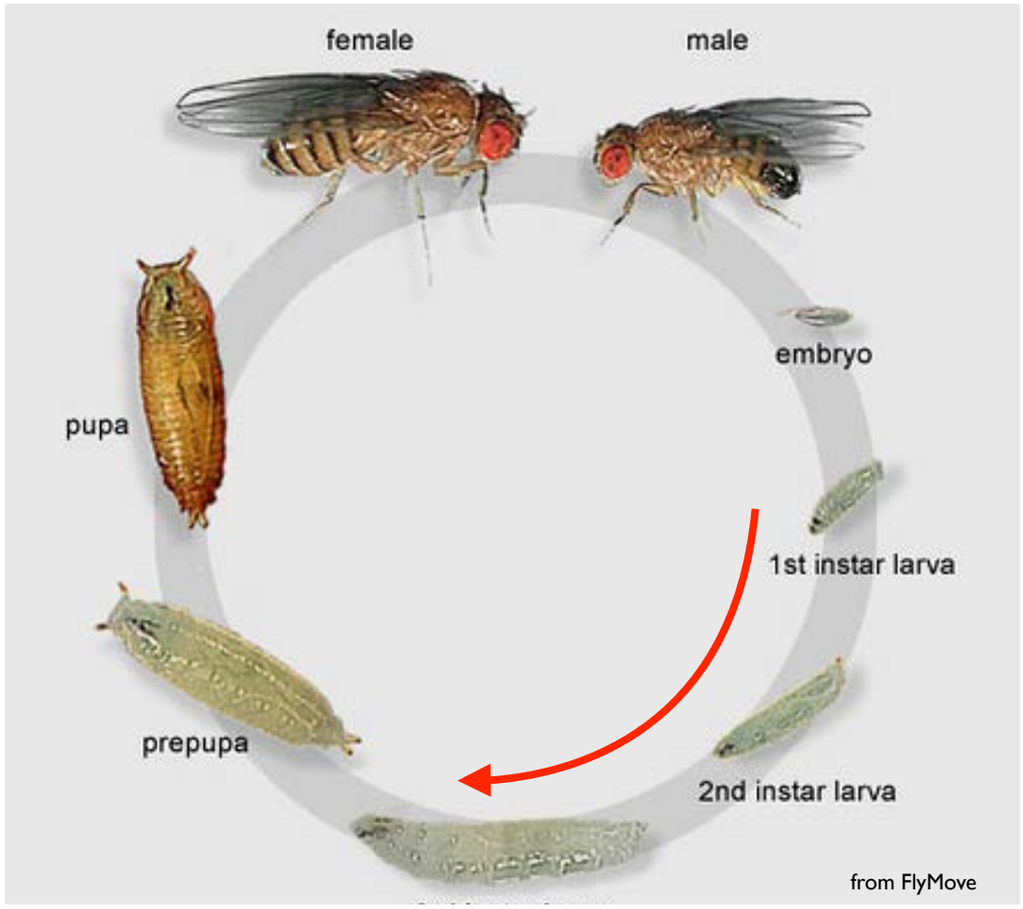
Two sided functional approach (in bacteria - in host)

Upon undernutrition, microbiota in general and *L.plantarum* in particular promote juvenile growth...

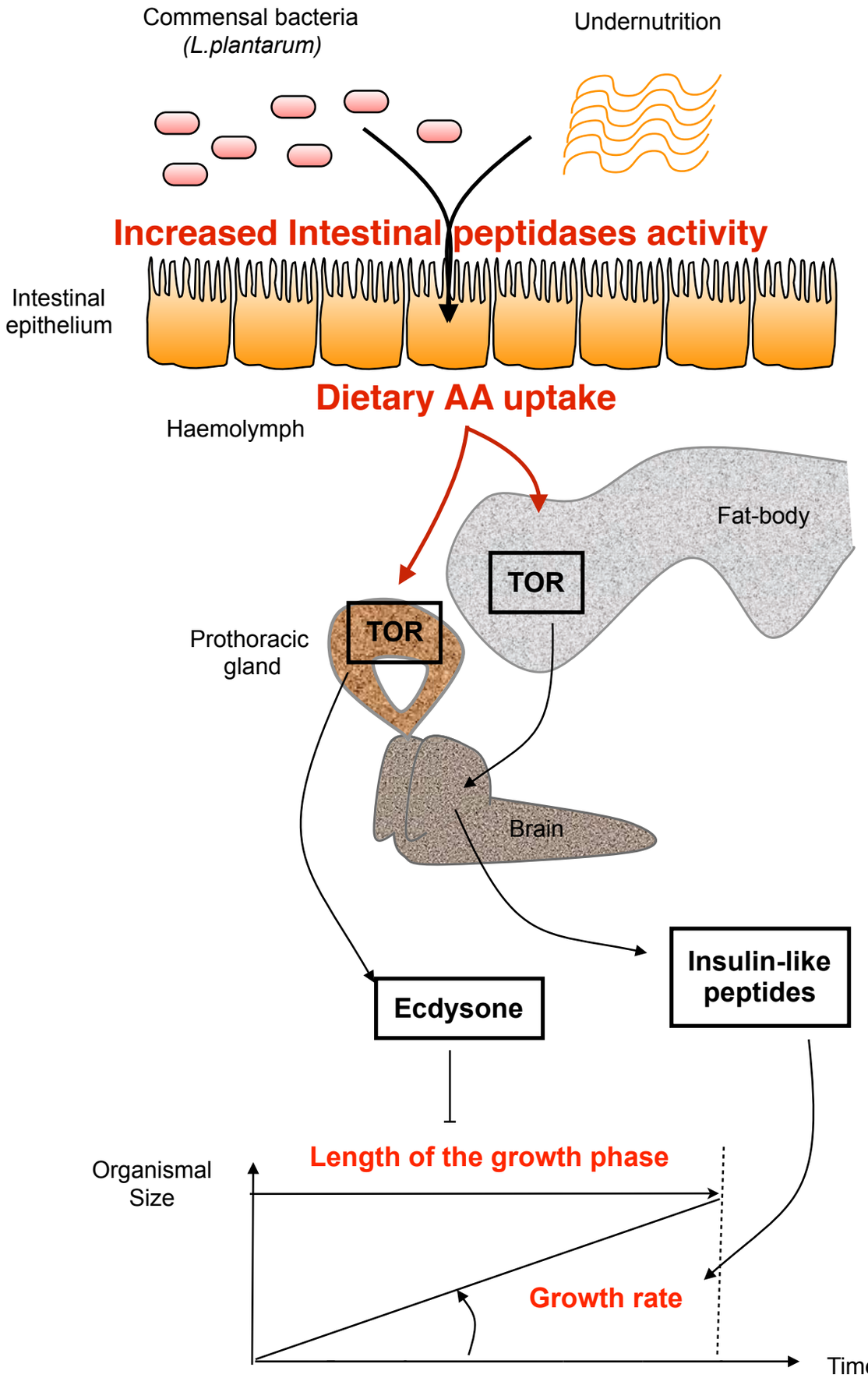


...via enhanced maturation hormone and growth factors activity...

...in a strain dependent manner



Storelli et al. (2011) *Cell Metabolism*

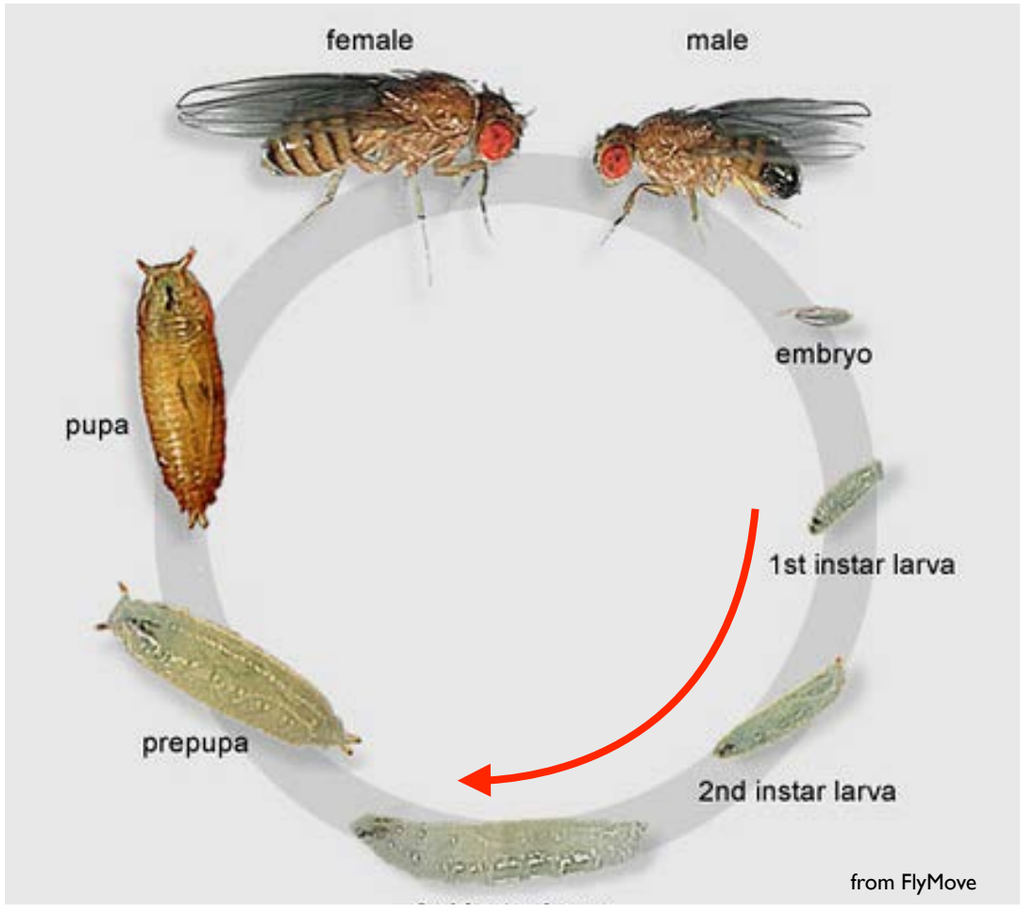


Upon undernutrition, microbiota in general and *L.plantarum* in particular promote juvenile growth...

...via enhanced dietary AA uptake...

...via enhanced maturation hormone and growth factors activity...

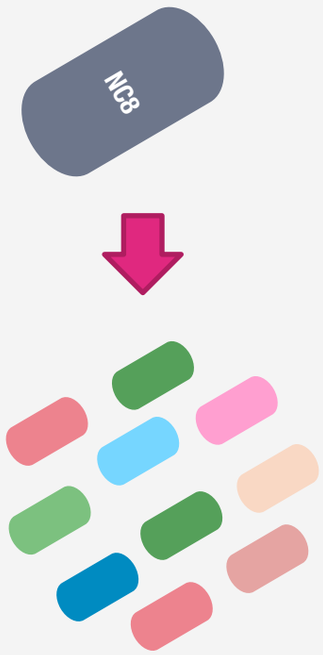
...in a strain dependent manner



Storelli et al. (2011) *Cell Metabolism*
Erkosar et al. (2014) *PLoS ONE*
Erkosar*, Storelli* et al. (2015) *Cell Host and Microbe*

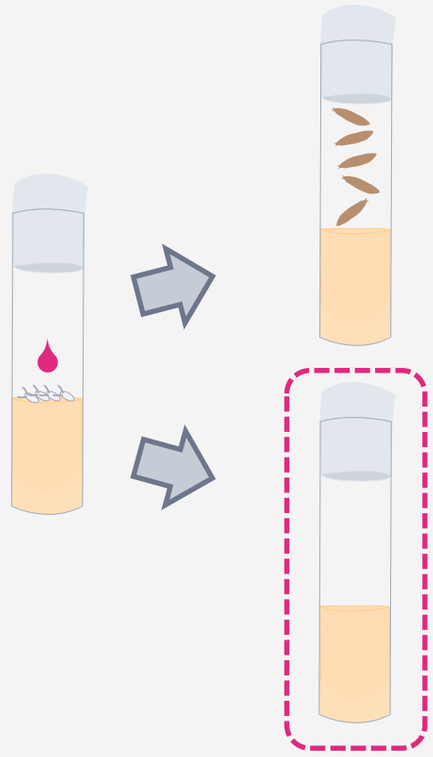
How does *L. plantarum* promote *Drosophila's* growth?

tn insertion mutant library using P_{junc}-TpaseIS$_{1223}$ system
(Licandro-Seraut et al. AEM. 2012)



(2091 mutants)

Screening for loss-of-growth promotion phenotype



Candidates

Mobilome

Carbohydrate transport and metabolism

Post-translational modification, protein turnover, and chaperones

Cell wall/membrane/ envelope biogenesis

PbpX2
D-Ala-D-Ala Carboxipeptidase

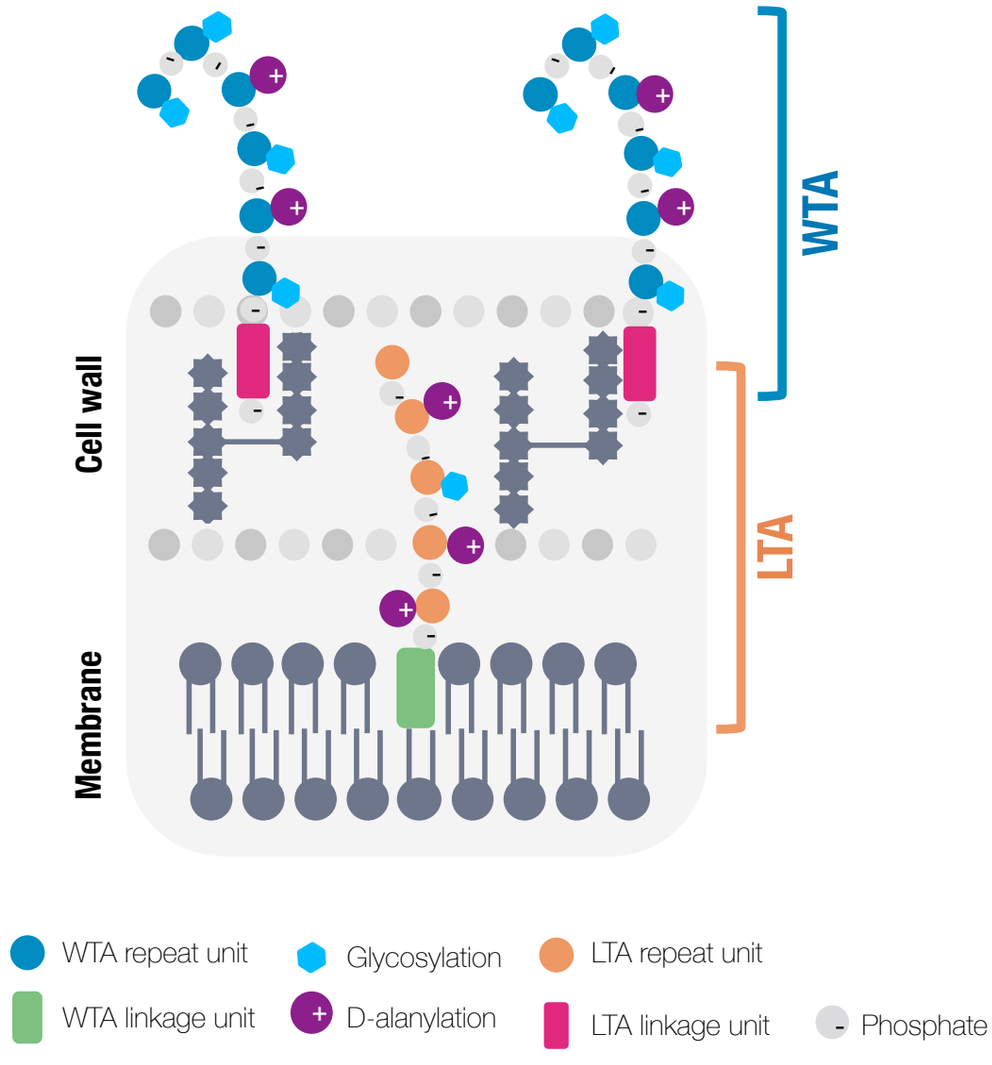
Matos *et al.* (2017) Nature Microbiology

PbpX2: D-Ala-D-Ala carboxipeptidase

PbpX2 predicted to be involved in peptidoglycan biosynthesis

***dlt* operon is responsible to the addition of D-Ala substitutions to the polyol repeats of teichoic acids:**

Cell surface glycopolymers located within the gram positive bacteria cell wall.



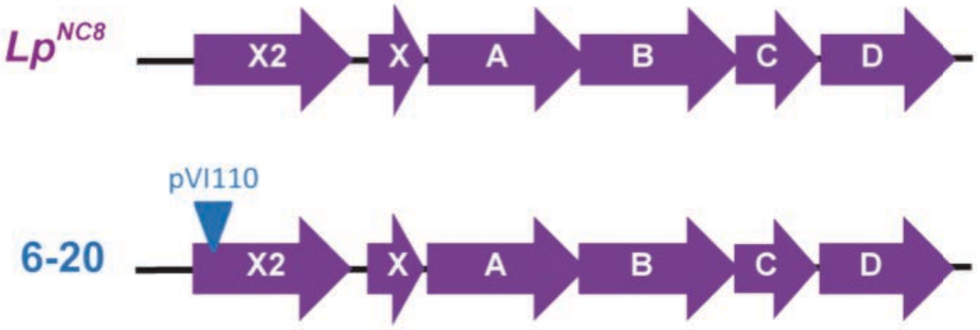
D-Alanyl substitutions strongly contribute to the functions of TAs.

- Regulation of cell division and morphology
- Antimicrobial peptides resistance
- Define the mechanical properties of the cell wall
- Cation homeostasis
- Adhesion and colonization

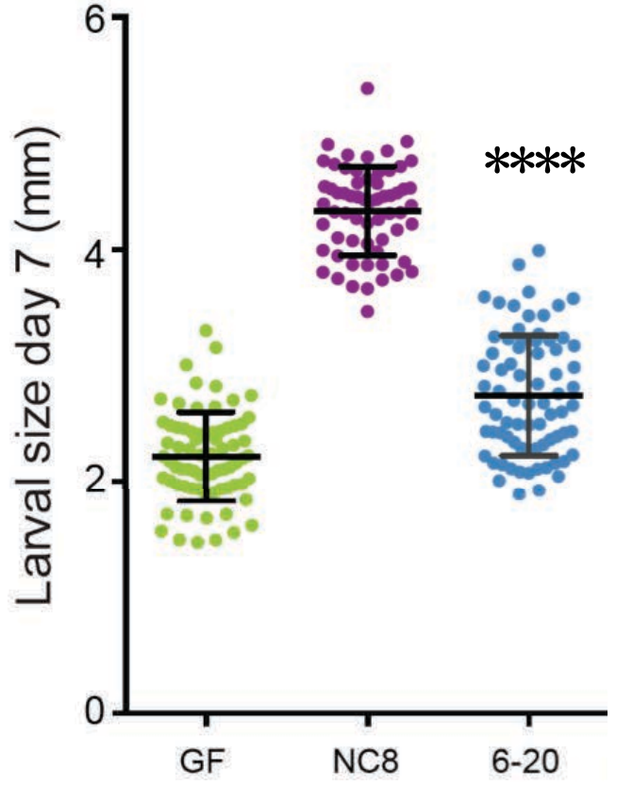
Palumbo et al. 2006 / Neuhaus and Baddiley 2003

Impact on *Drosophila* growth promotion

Gene deletion by homology-based recombination

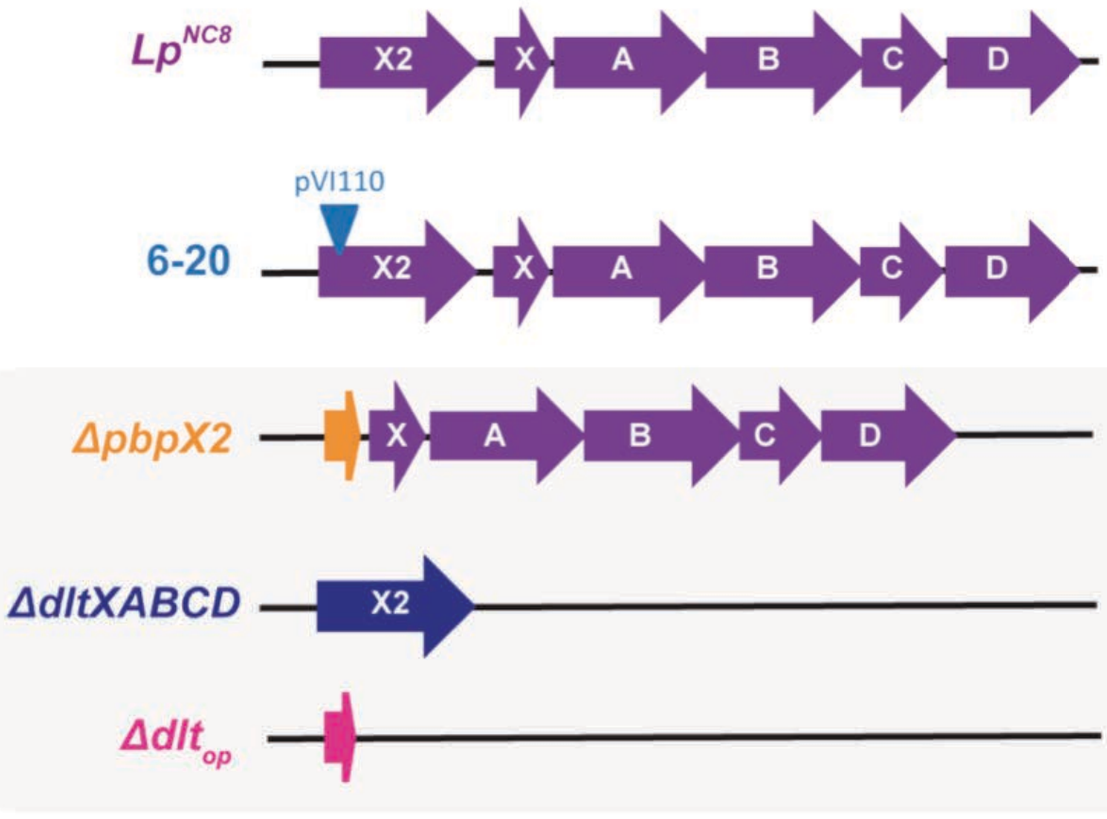


Larval Size

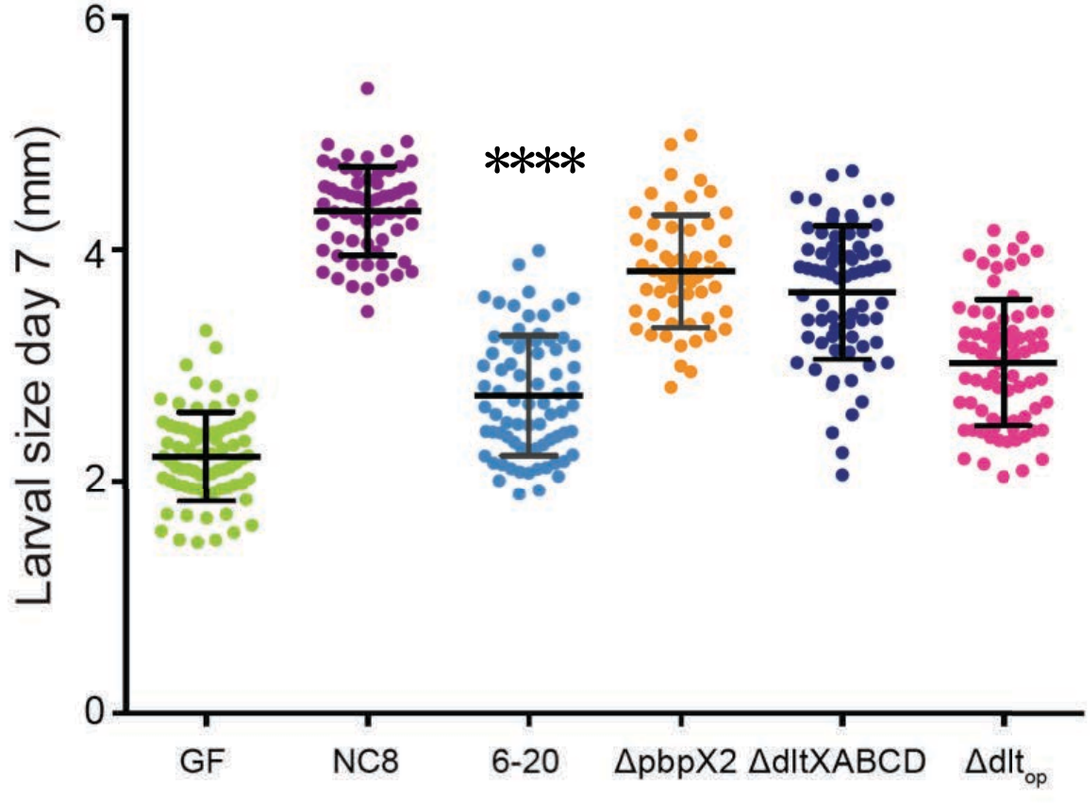


Impact on *Drosophila* growth promotion

Gene deletion by homology-based recombination

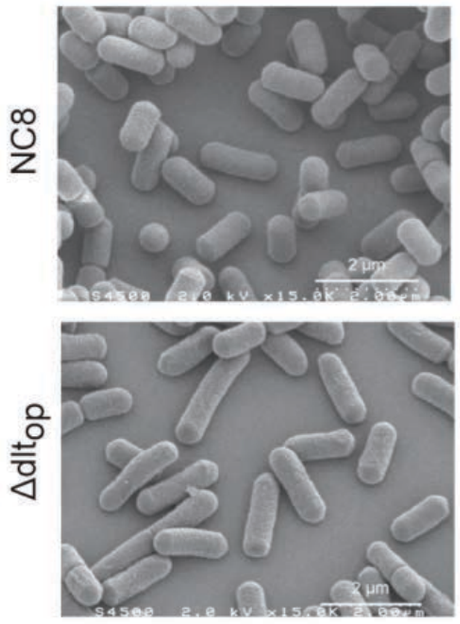


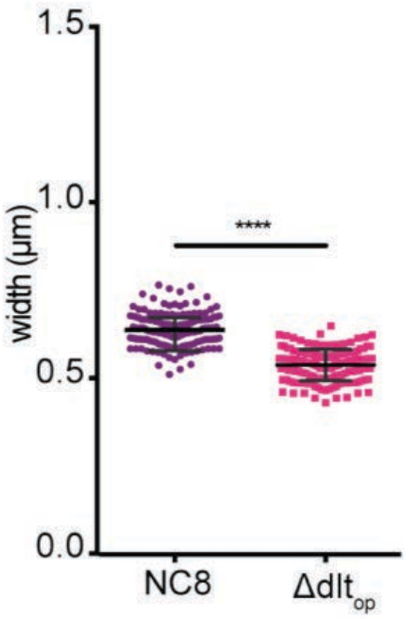
Larval Size



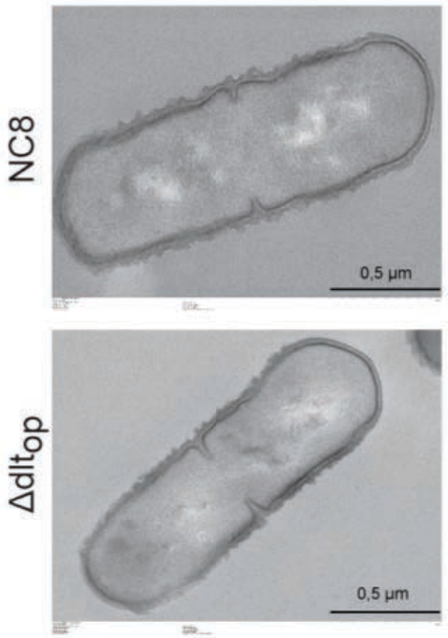
Impact on bacterial cell shape and composition

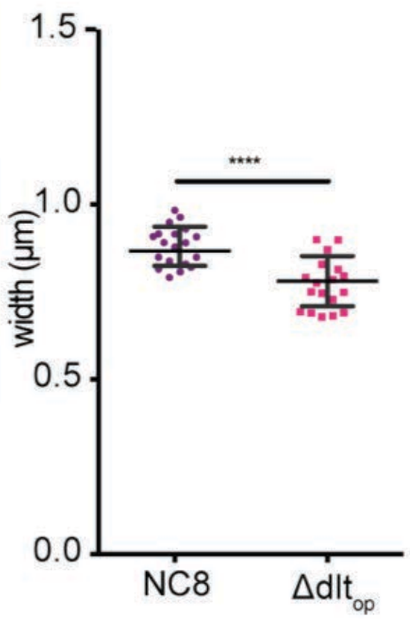
SEM



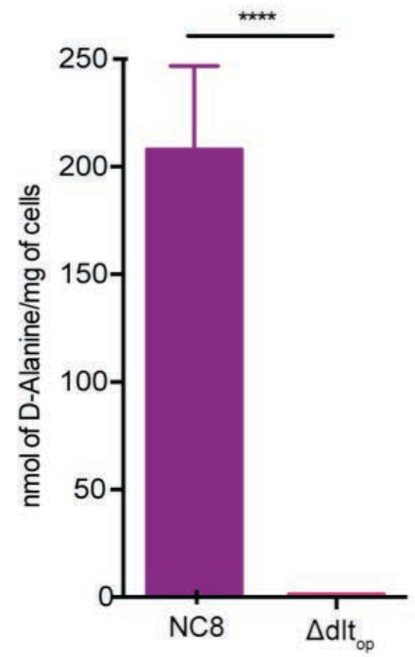


TEM





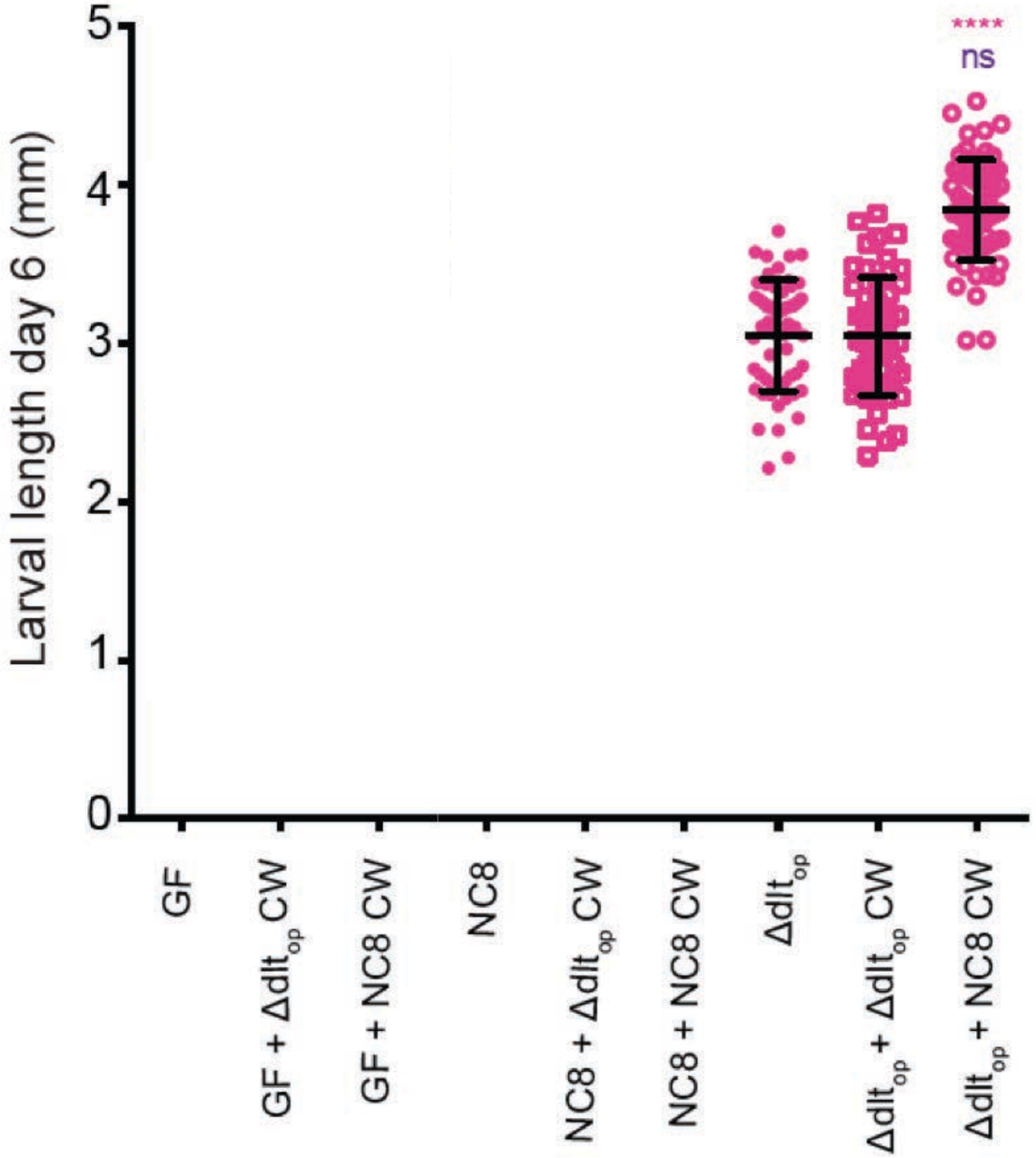
HPLC detection of D-alanine released from whole cells



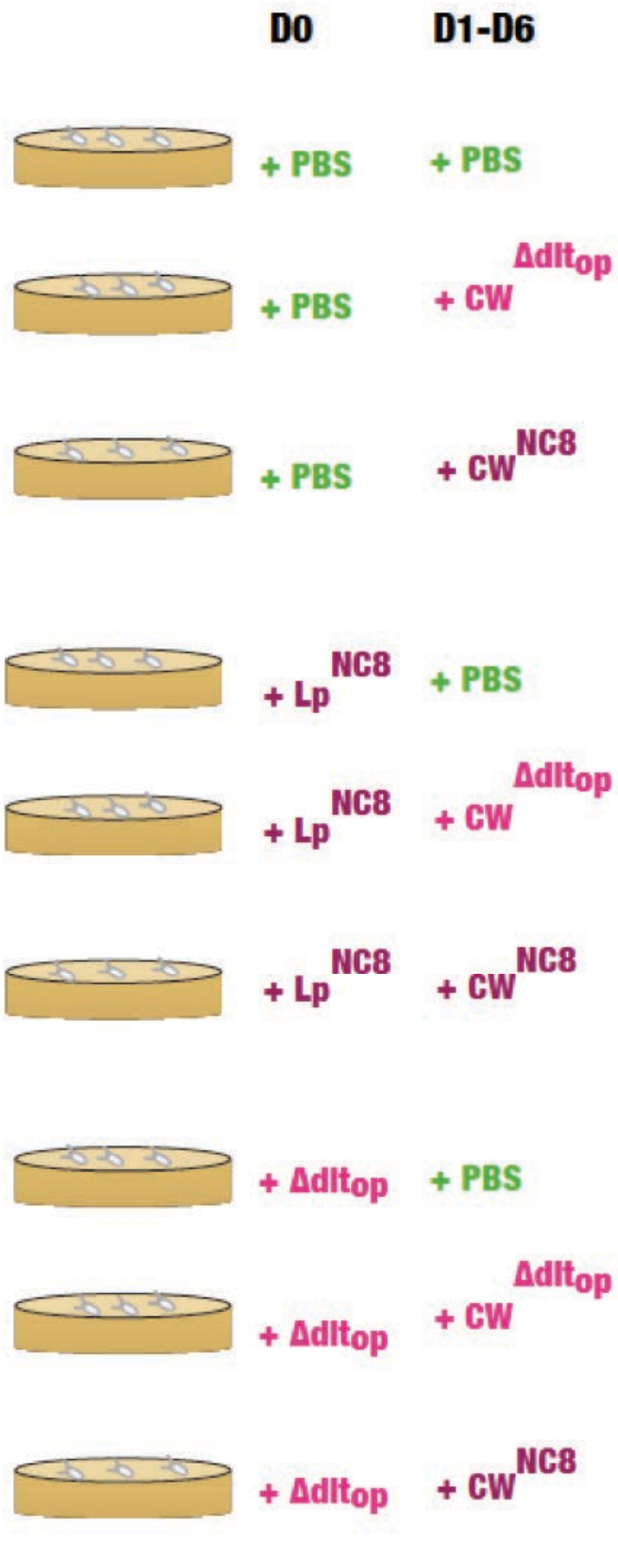
Deletion of pbpX2-dltXABCD genes leads to a **loss of D-alanine esterification** on teichoic acids and **size reduction** in the mutant cells.

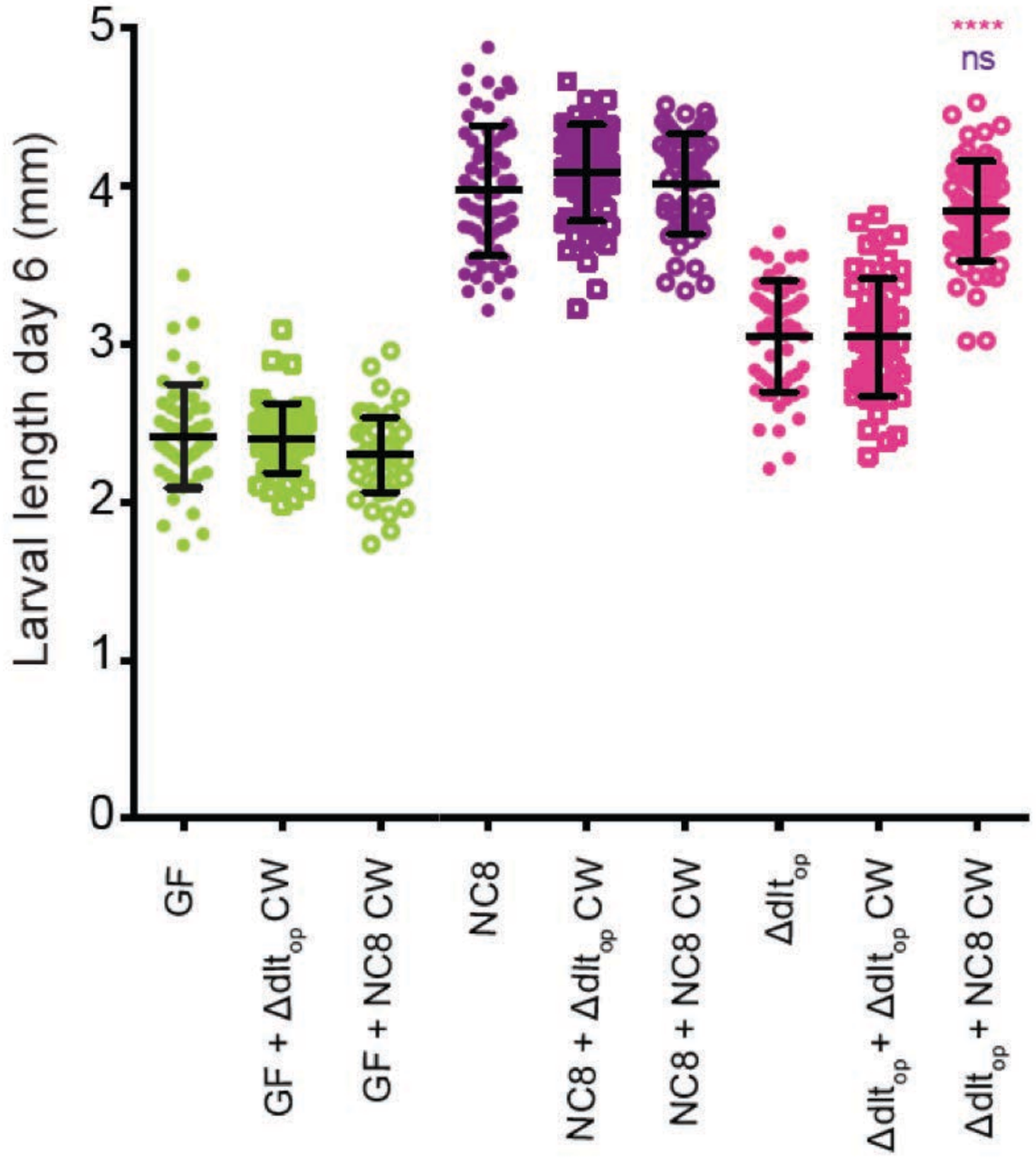
D-alanine esterification of teichoic acids (TA) is required for Lp^{NC8} mediated Drosophila growth promotion upon chronic undernutrition.

Cell wall modification directly influence larval growth

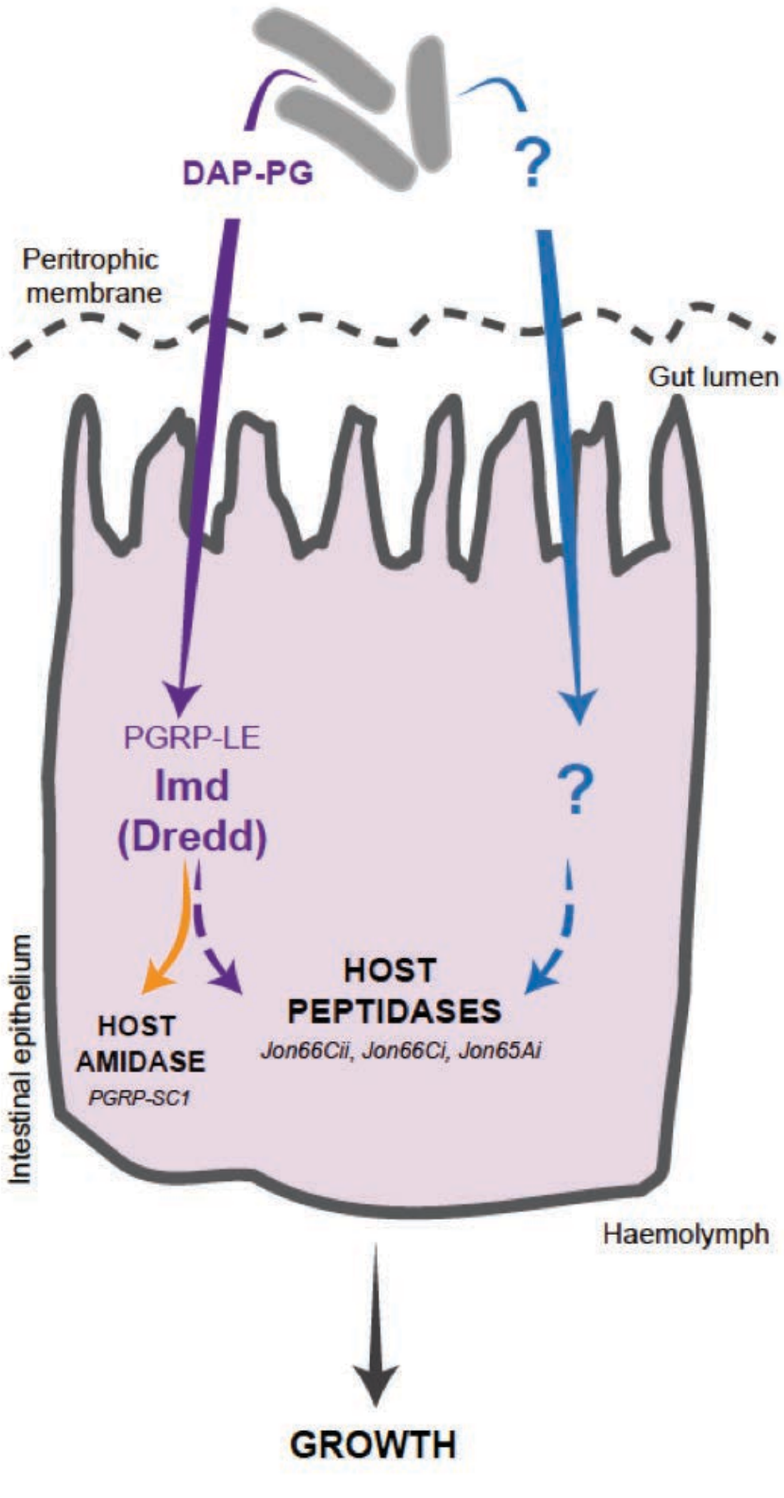


Cell wall modification directly influence larval growth



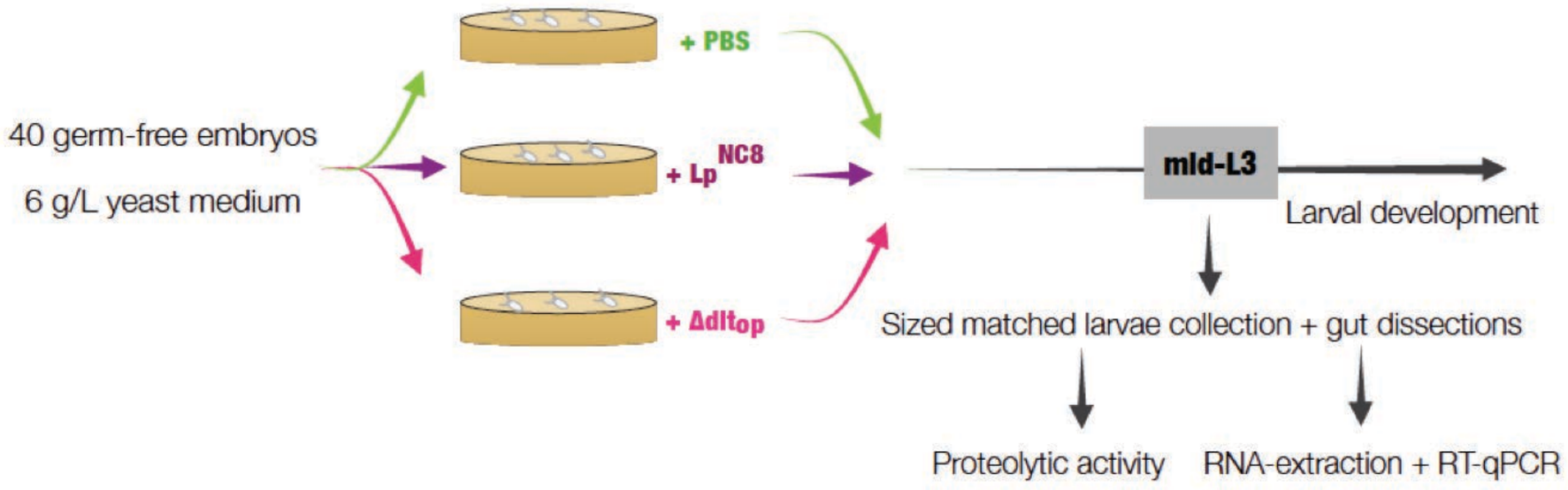


Impact on Drosophila proteases expression

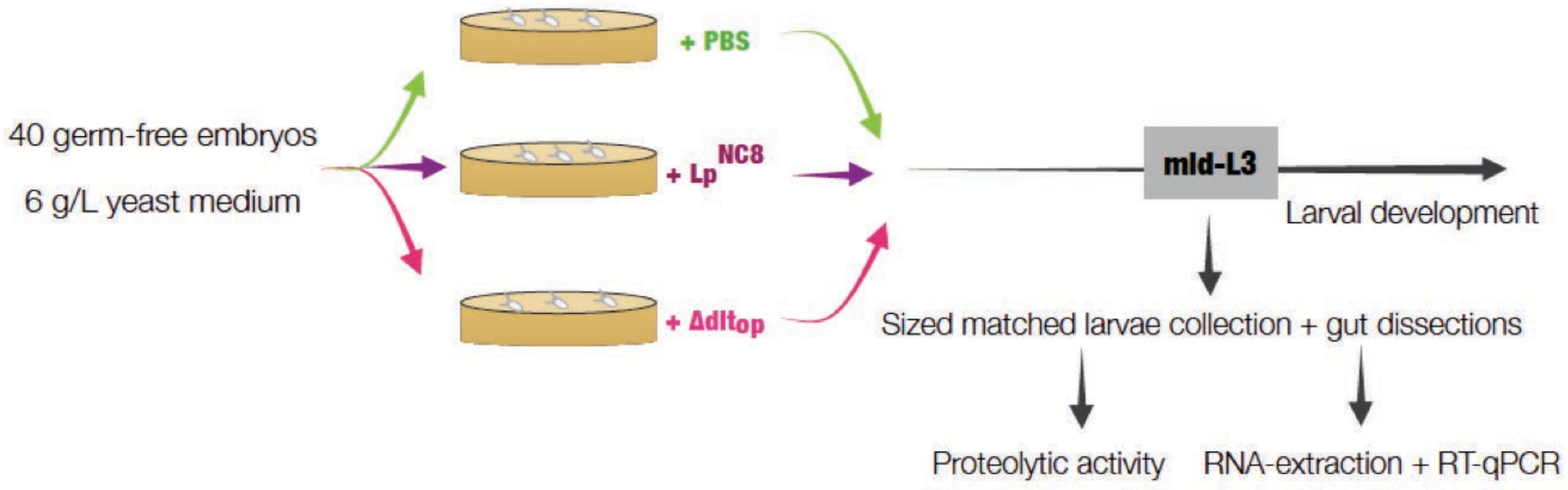


Erkosar*, Storelli* *et al.* Cell Host and Microbe 2015

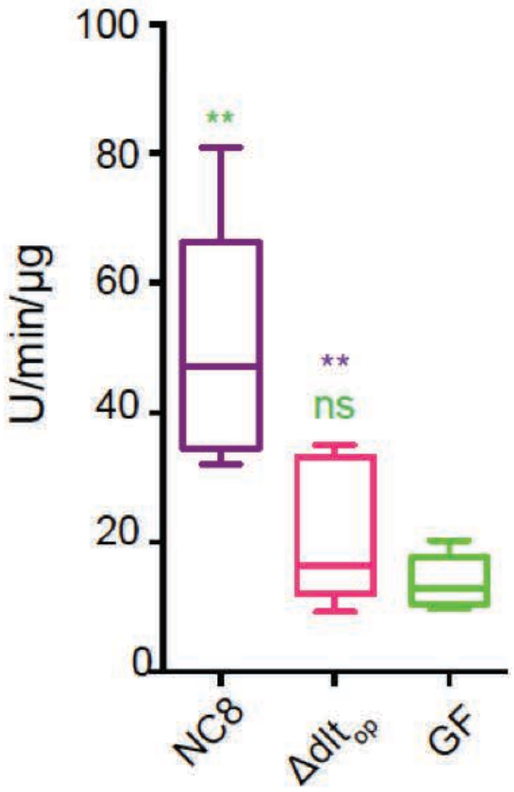
Impact on *Drosophila* proteases expression



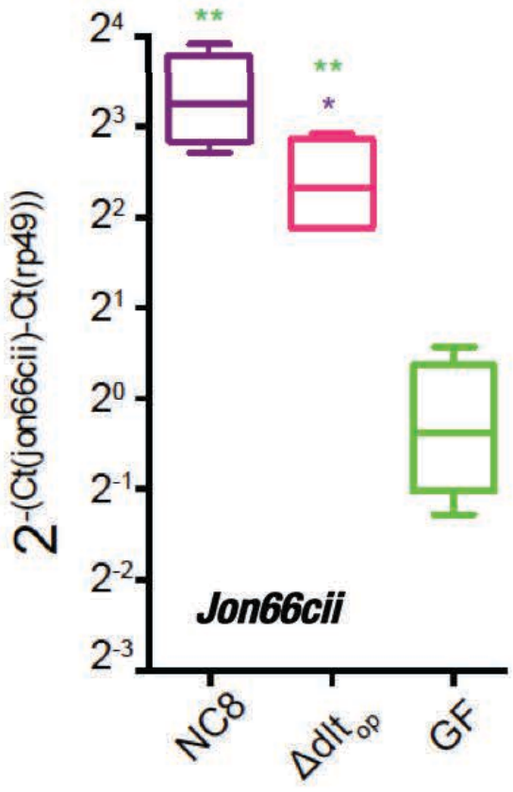
Impact on *Drosophila* proteases expression

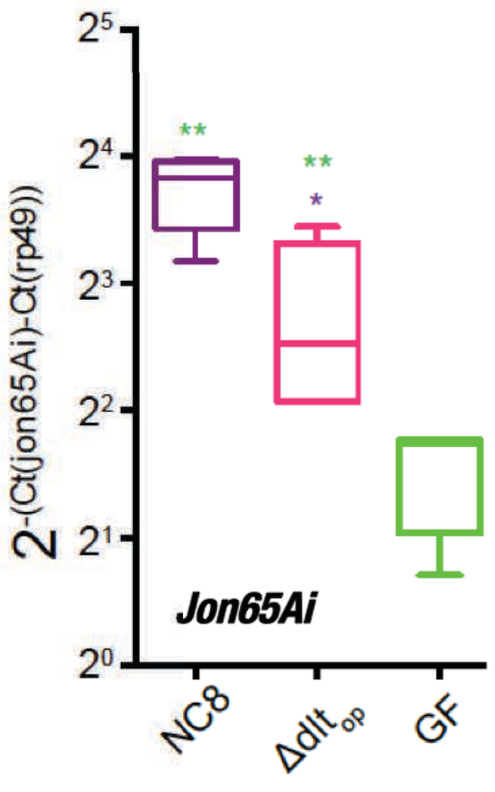


Proteolytic activity

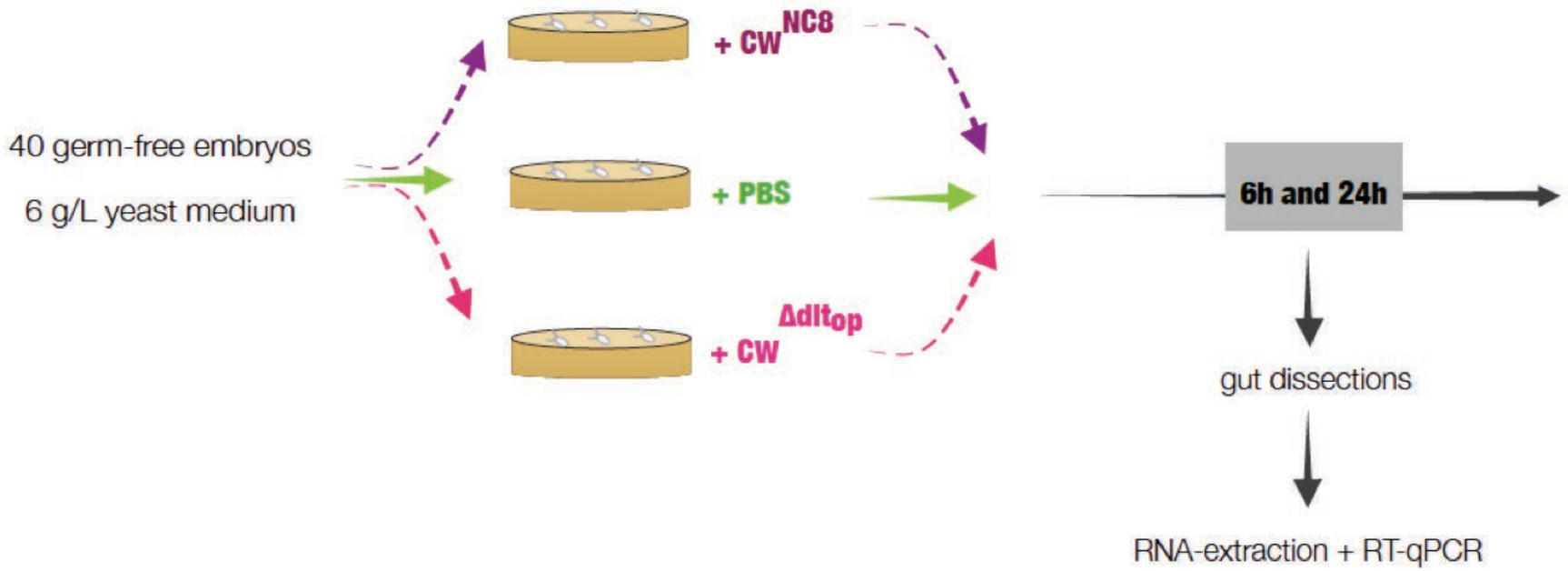


Expression

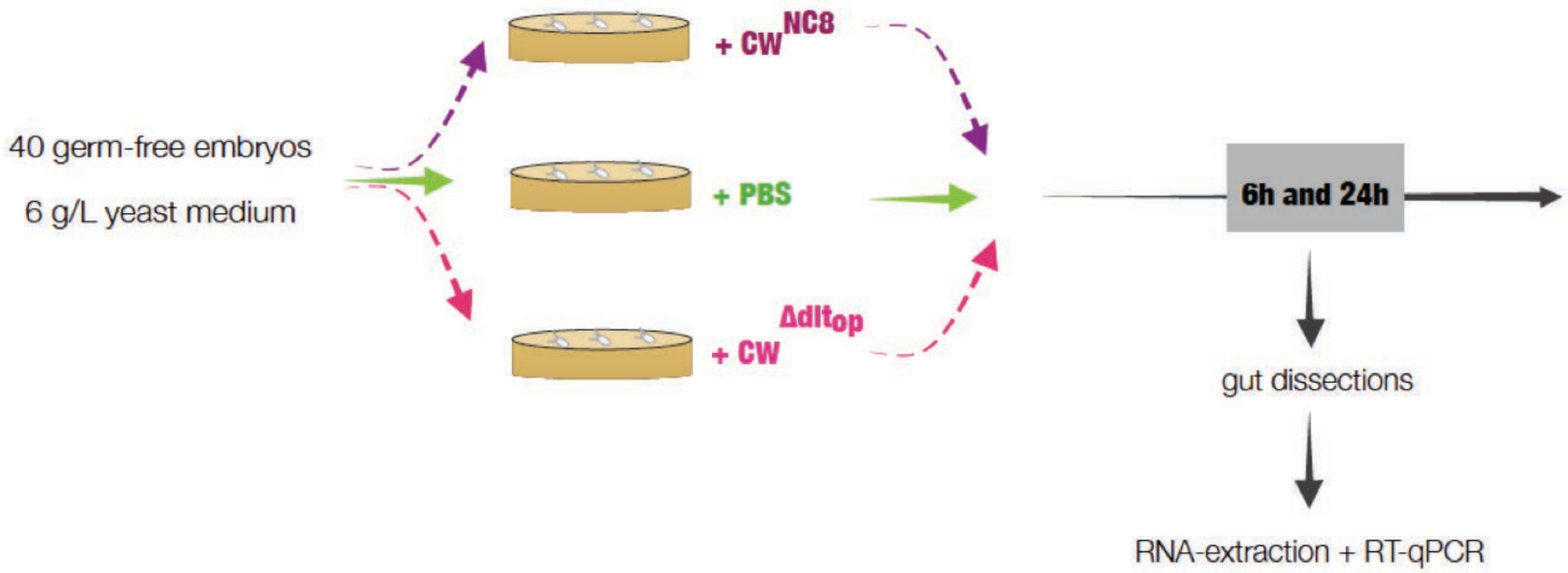




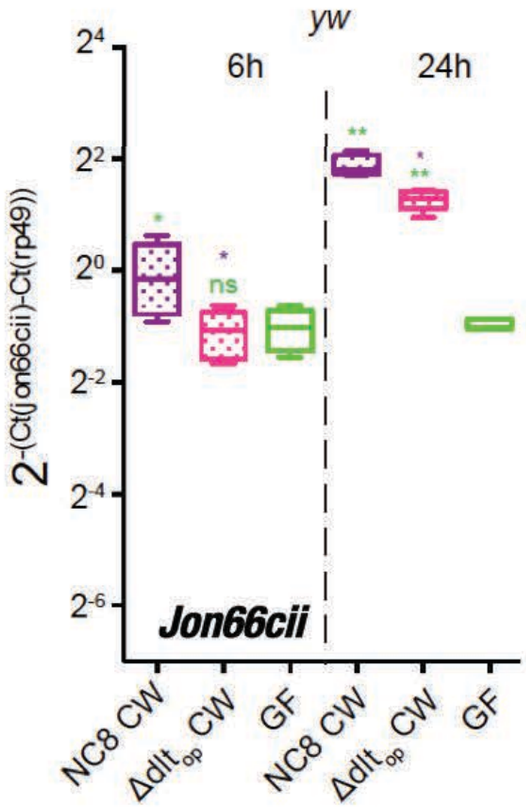
Purified cell wall triggers *Drosophila* intestinal peptidases

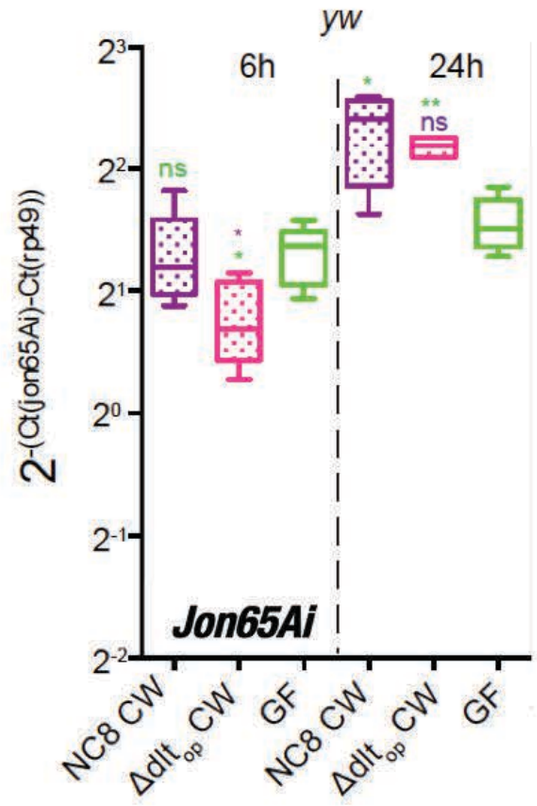


Purified cell wall triggers *Drosophila* intestinal peptidases

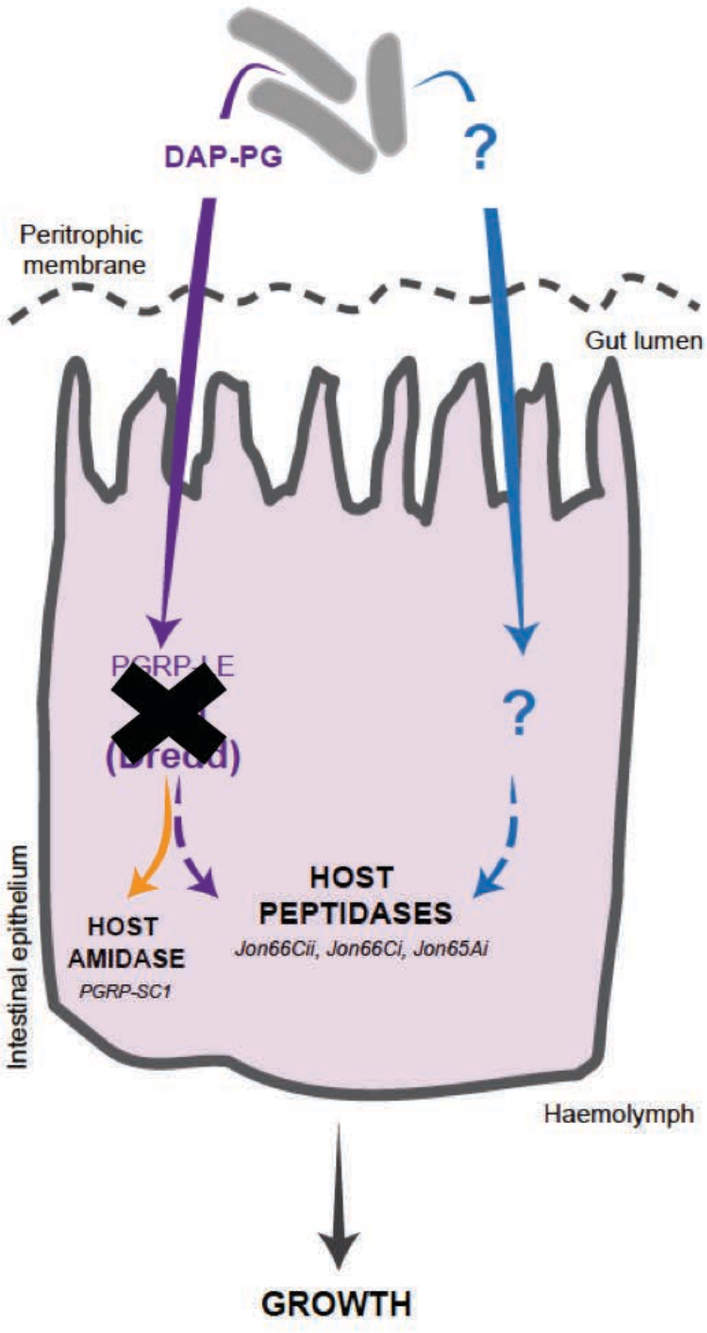


Expression

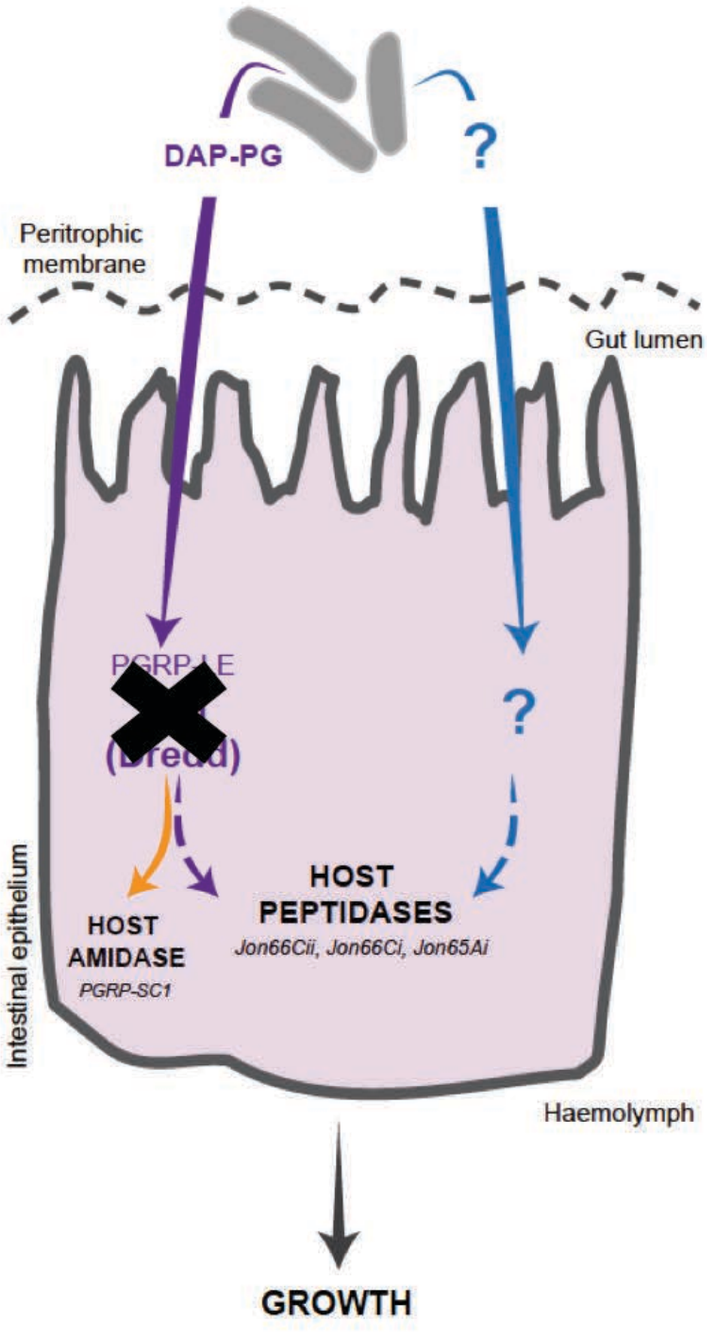




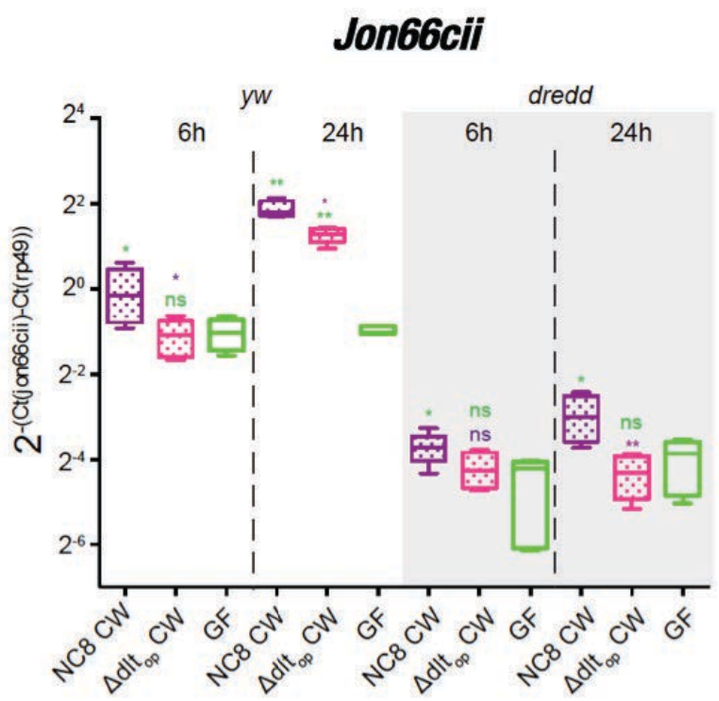
Impact on Drosophila proteases expression

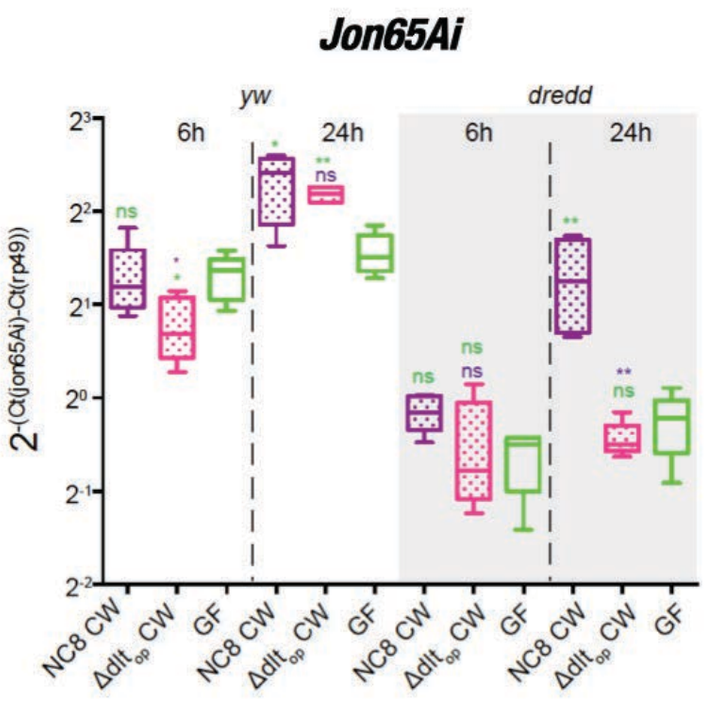


Impact on Drosophila proteases expression



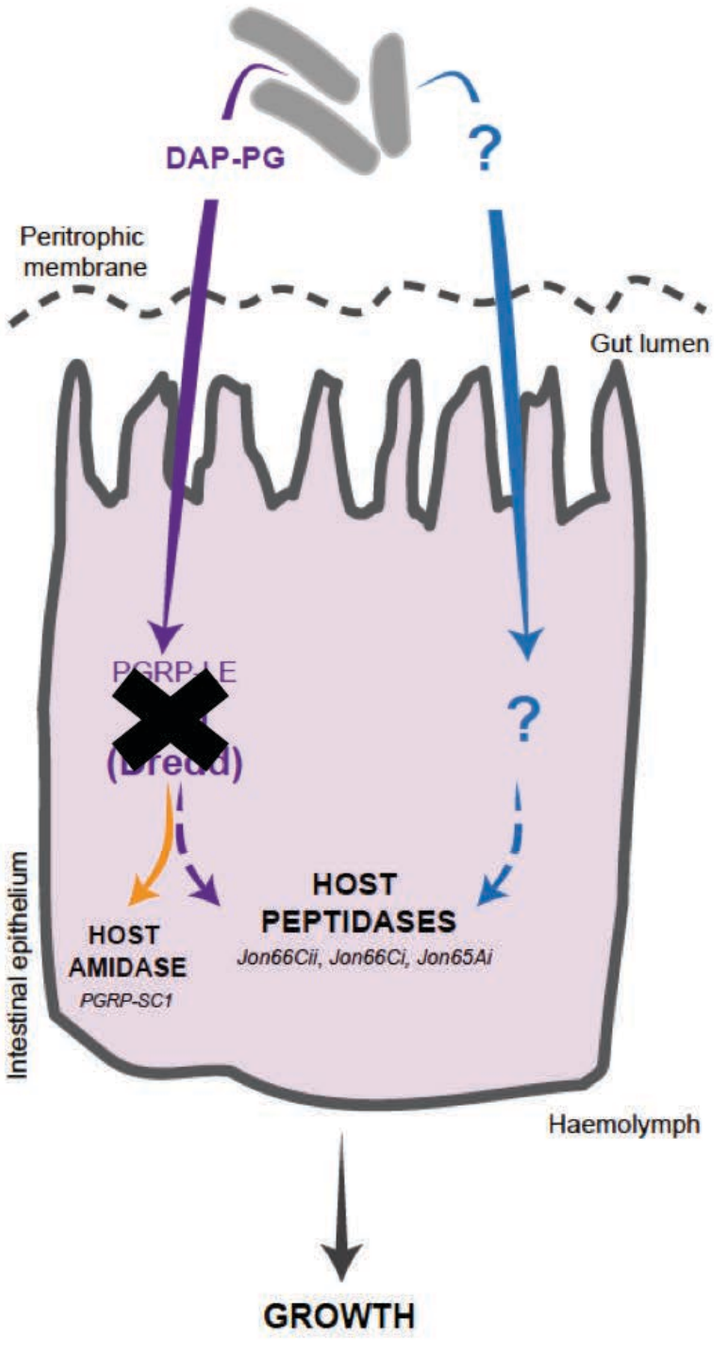
Expression



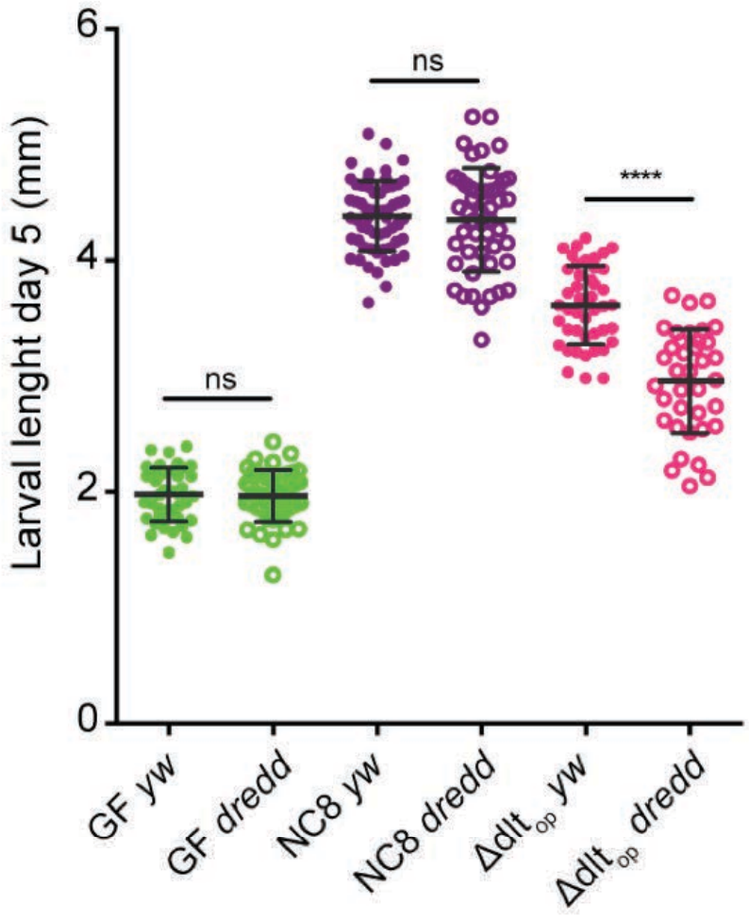


The mechanism sensing bacteria bearing **D-alanylated** teichoic acids acts in **concert with Dredd-dependent peptidoglycan sensing** to elicit **optimal expression** response of the intestinal proteases.

Impact on Drosophila growth in *Dredd* mutants

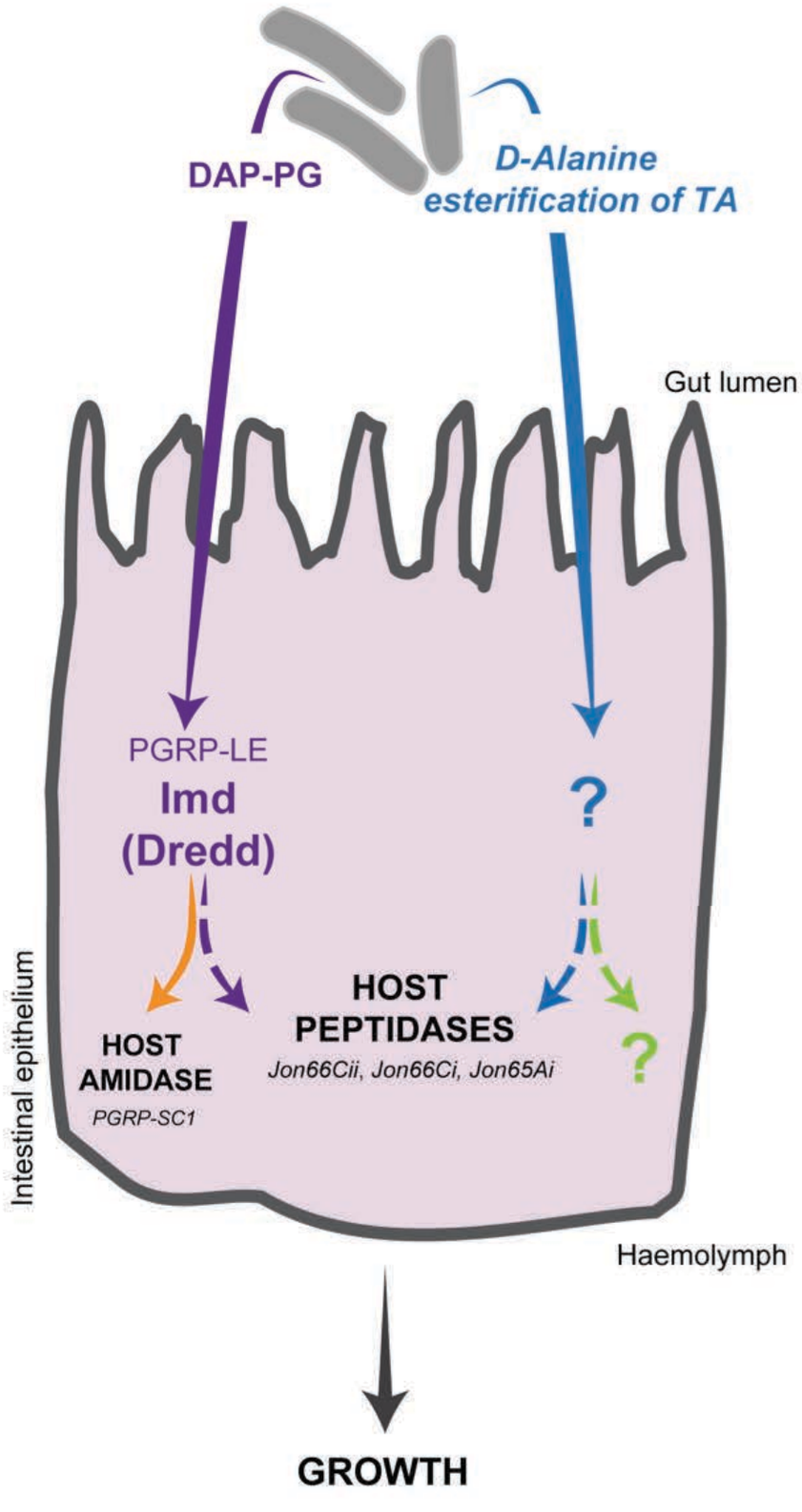


Larval Size



Both bacterial **peptidoglycan** and **additional signals** related to **D-alanine** esterification of teichoic acids are **required** for optimal Lp mediated **Drosophila growth and maturation** upon chronic undernutrition.

Working model



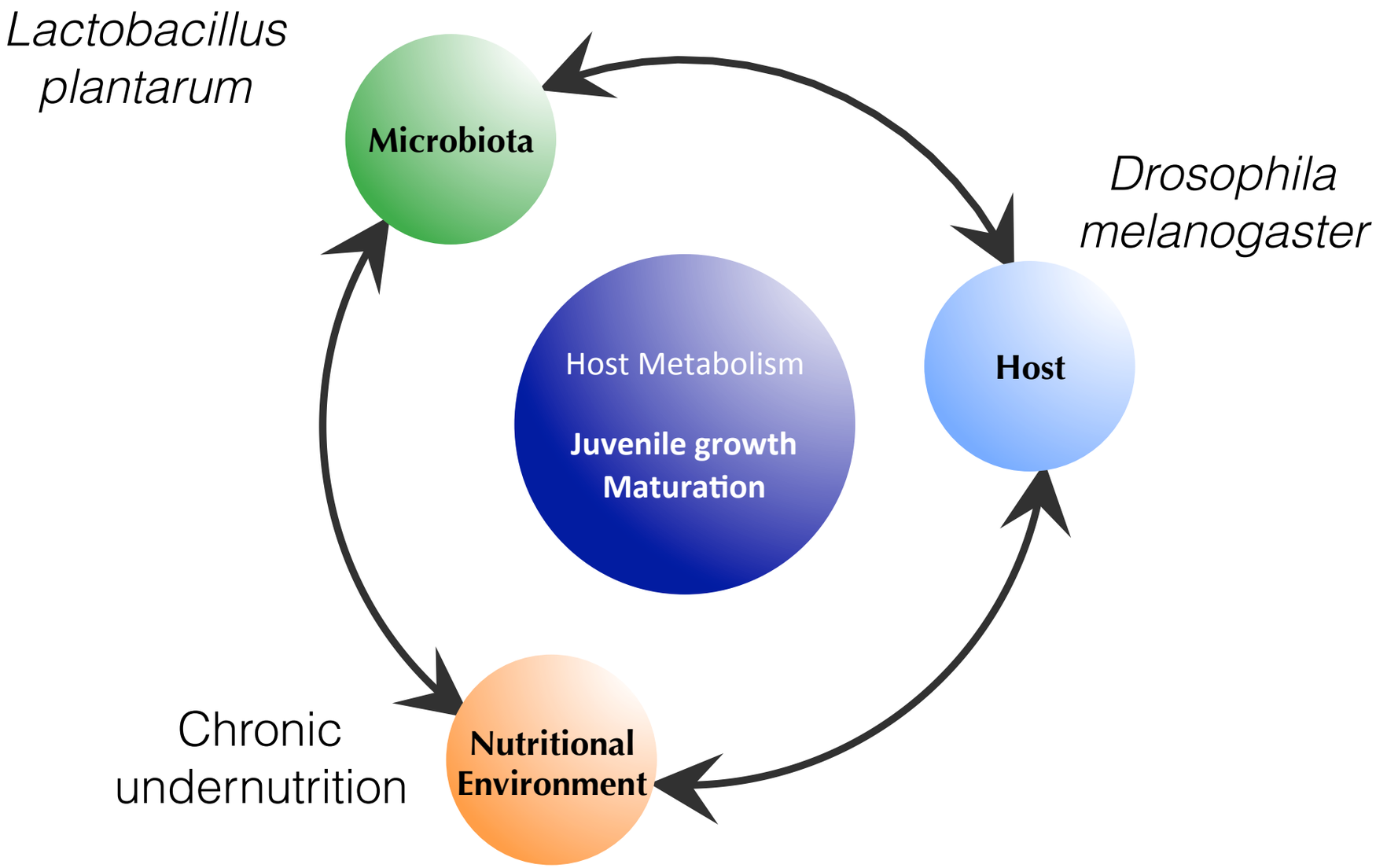
Host enterocytes sense and signal the presence of Lp cells by at least two mechanisms:

- through PGRP-LE-mediated **peptidoglycan** fragment recognition and Imd/Relish signalling and,

- sensing of bacteria bearing **D-alanylated teichoic acids** in their cell wall and signalling by yet to discover host mechanisms.

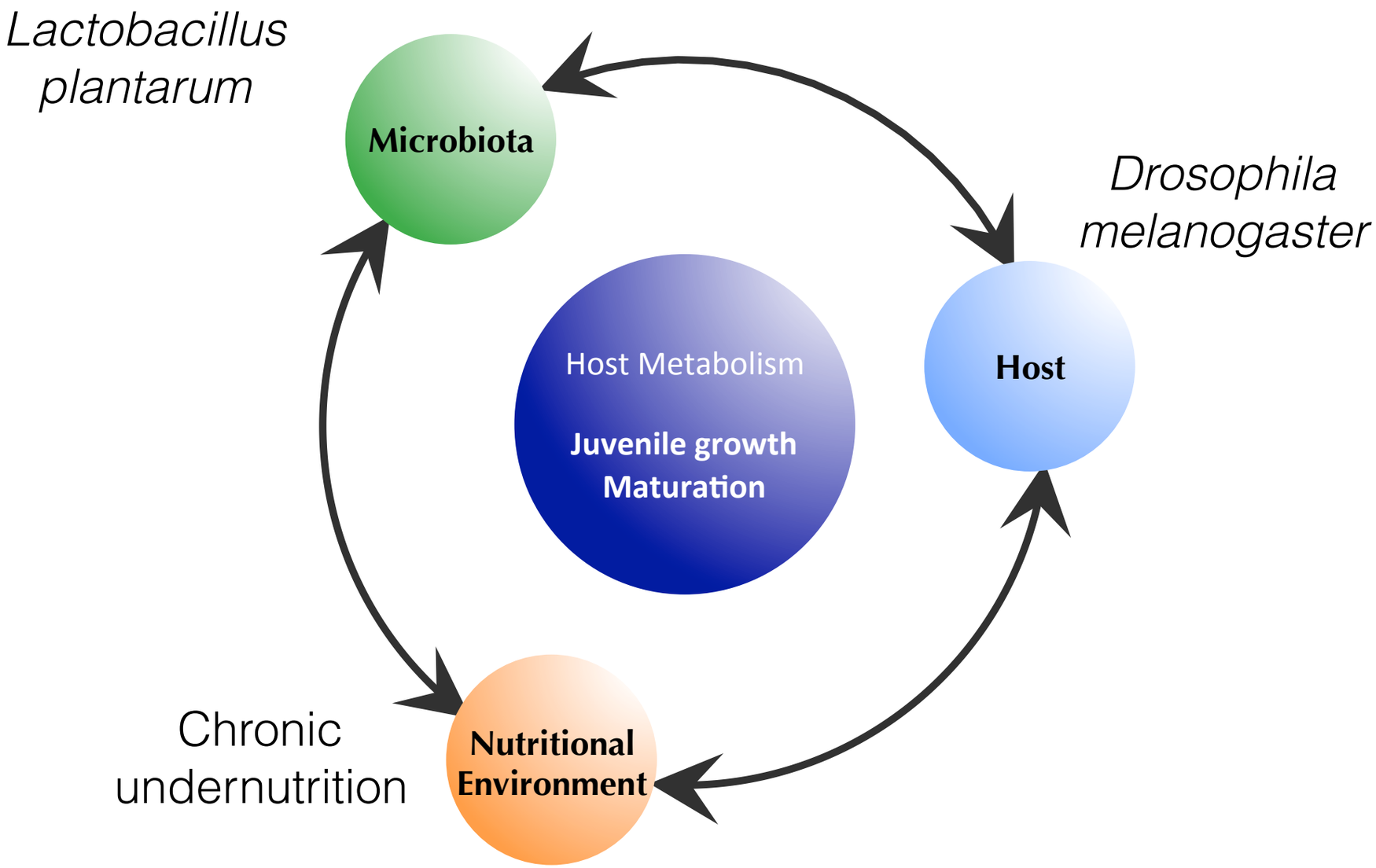
Matos *et al.* (2017) Nature Microbiology

Take home messages - I



Take home messages - I

Genetically tractable model to decipher the molecular underpinnings of host-microbiota mutualism upon chronic undernutrition



Role of microbial environment (and Lactobacilli) on mammalian juvenile growth upon undernutrition?

MALNUTRITION

The Human Gut Microbiota and Undernutrition

Jeffrey I. Gordon,[1*] Kathryn G. Dewey,[2] David A. Mills,[3,4] Ruslan M. Medzhitov[5]

Childhood malnutrition is a global health problem that cannot be attributed to food insecurity alone. The gut microbiota may contribute to this devastating health disorder. In this Perspective, we call for the application of tools and concepts emerging from studies of the human gut microbiota to better understand the nutritional needs of infants and children and the role of the microbiota in the pathogenesis and treatment of undernutrition. This effort will require elucidation of the interrelationships between breast milk composition and the development of the microbiota and immune system in the context of the maternal-infant dyad.

Undernutrition in mammals

Acute undernutrition or Protein-energy undernutrition

Chronic undernutrition or prolonged nutritional deficit

Undernutrition in mammals

Acute undernutrition or Protein-energy undernutrition

Wasting: weight loss diagnosed by a reduction in weight-for-height index

Chronic undernutrition or prolonged nutritional deficit

Stunting: growth failure leading to short stature

Undernutrition in mammals

Acute undernutrition or Protein-energy undernutrition

Wasting: weight loss diagnosed by a reduction in weight-for-height index

Gut Microbiomes of Malawian Twin Pairs Discordant for Kwashiorkor

Michelle I. Smith,[1*] Tanya Yatsunenko,[1*] Mark J. Manary,[2,3,4] Indi Trehan,[2,3] Rajhab Mkakosya,[5] Jiye Cheng,[1] Andrew L. Kau,[1] Stephen S. Rich,[6] Patrick Concannon,[6] Josyf C. Mychaleckyj,[6] Jie Liu,[7] Eric Houpt,[7] Jia V. Li,[8] Elaine Holmes,[8] Jeremy Nicholson,[8] Dan Knights,[9,10†] Luke K. Ursell,[11] Rob Knight,[9,10,11,12] Jeffrey I. Gordon[1‡]

1 FEBRUARY 2013 VOL 339 **SCIENCE** www.sciencemag.org

Persistent gut microbiota immaturity in malnourished Bangladeshi children

Sathish Subramanian[1], Sayeeda Huq[2], Tanya Yatsunenko[1], Rashidul Haque[2], Mustafa Mahfuz[2], Mohammed A. Alam[2], Amber Benezra[1,3], Joseph DeStefano[1], Martin F. Meier[1], Brian D. Muegge[1], Michael J. Barratt[1], Laura G. VanArendonk[1], Qunyuan Zhang[4], Michael A. Province[4], William A. Petri Jr[5], Tahmeed Ahmed[2] & Jeffrey I. Gordon[1]

19 JUNE 2014 | VOL 510 | NATURE | 417

Immature dysbiotic microbiota triggers weight loss

Chronic undernutrition or prolonged nutritional deficit

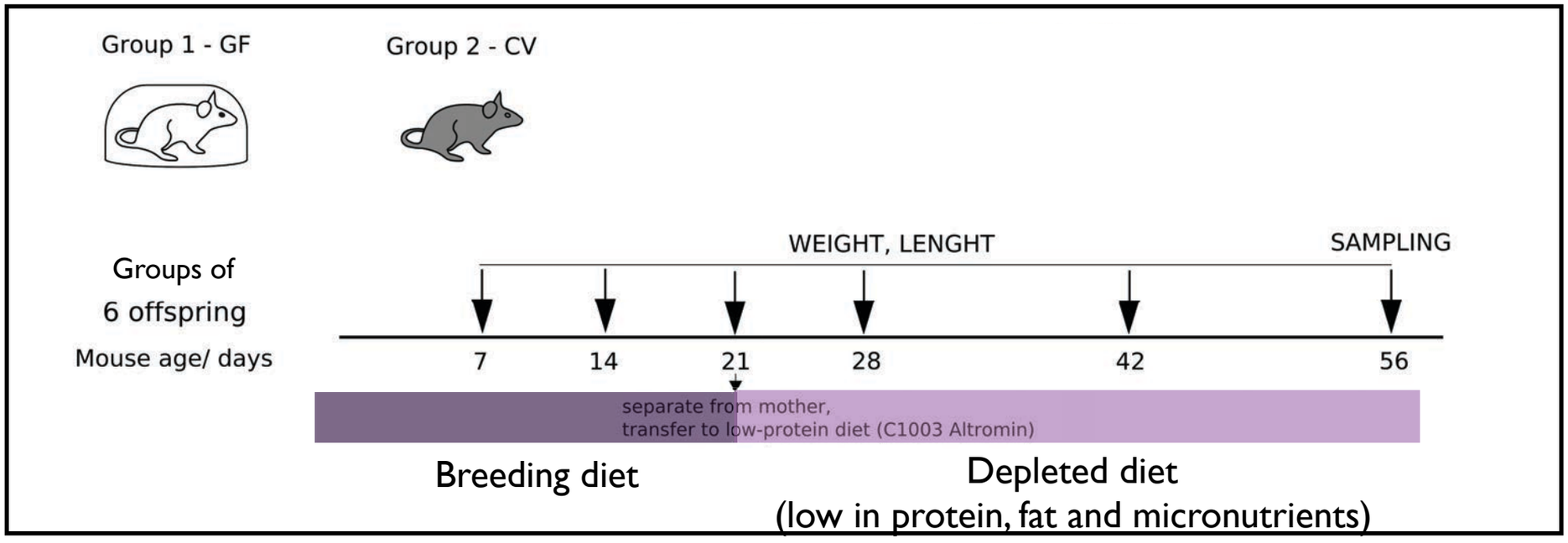
Stunting: growth failure leading to short stature

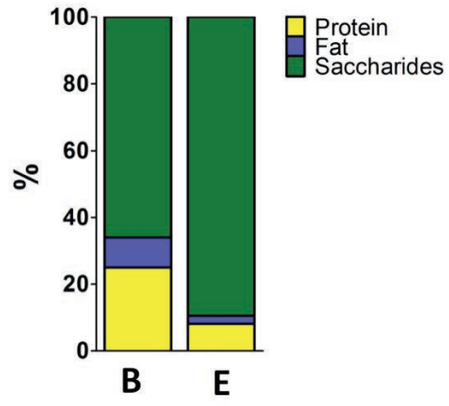
?

How microbiota contributes to mouse juvenile growth upon chronic undernutrition?

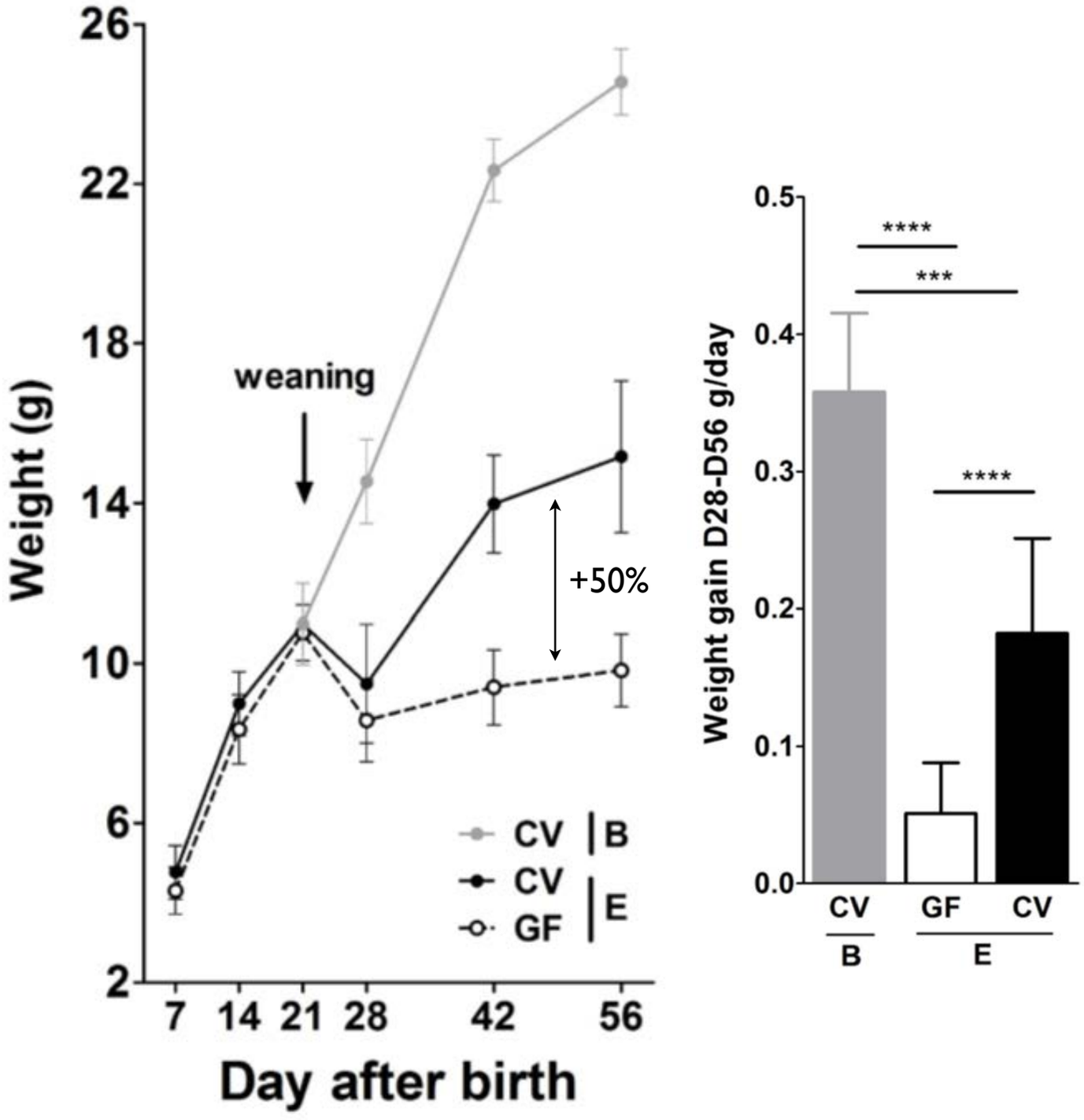
Collab: Dr M.Schwarzer & Dr H.Kozakova
Laboratory of Gnotobiology
Institut of Microbiology
Science Academy of Czech Republic

Gnotobiotic
Balb/c line

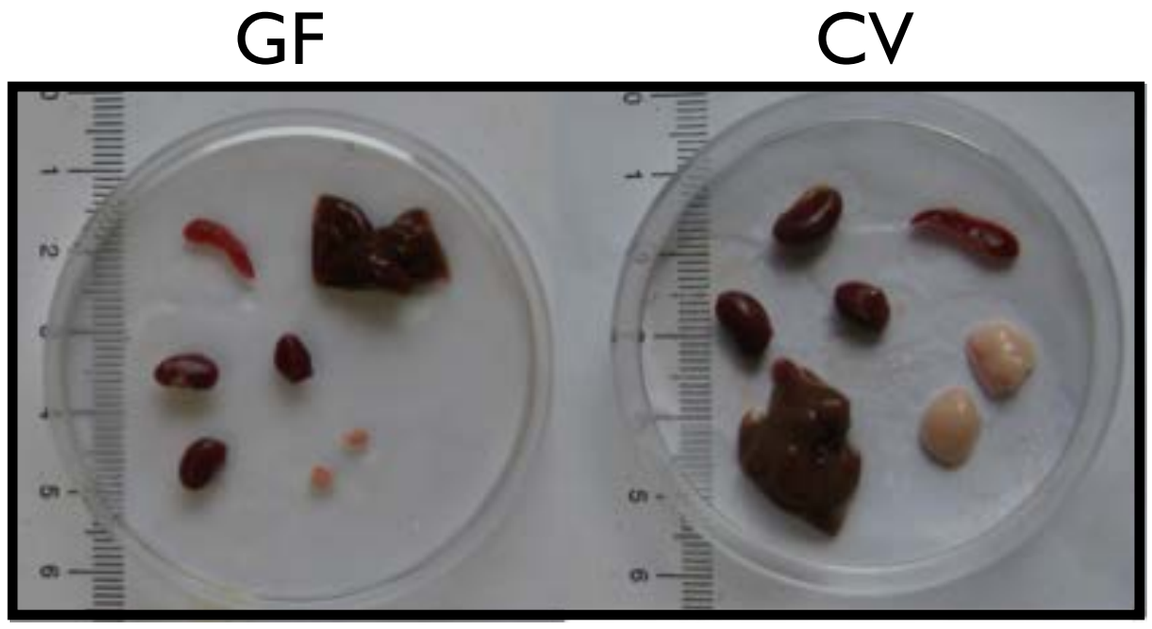


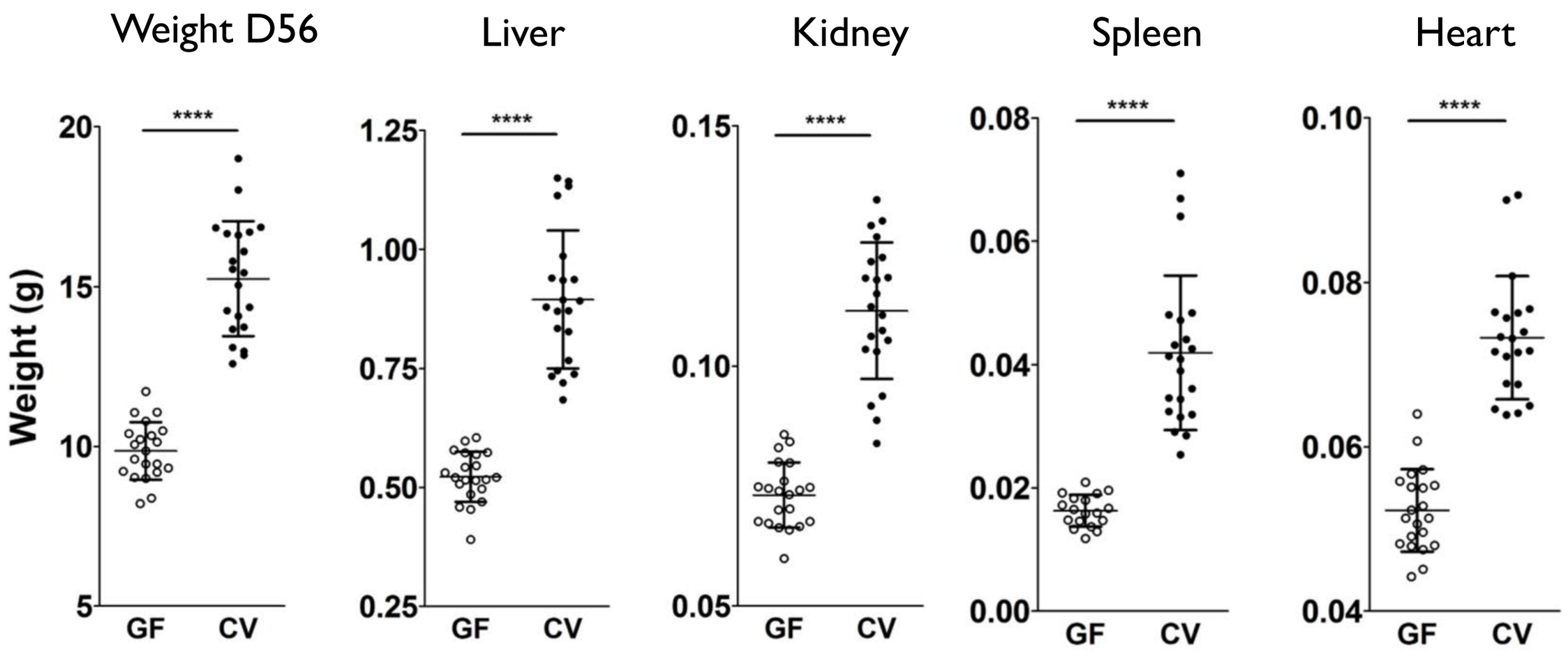


Microbiota maintains juvenile growth upon chronic undernutrition

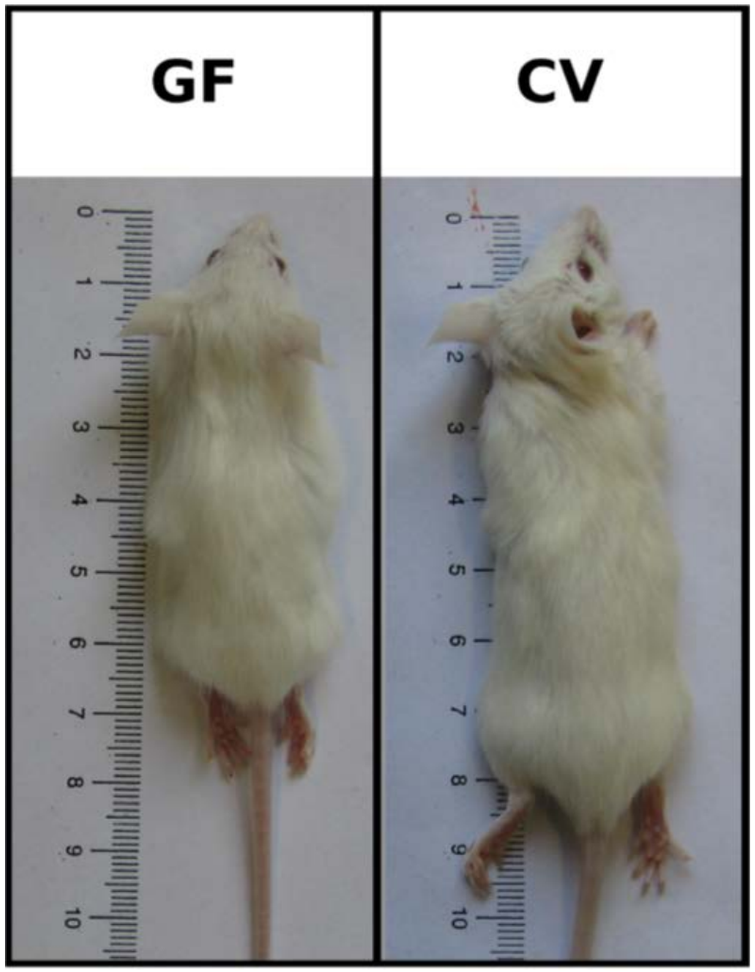


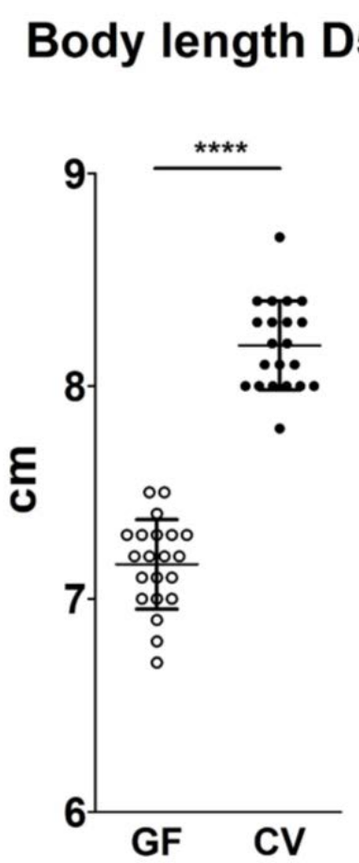
Microbiota maintains systemic growth upon chronic undernutrition



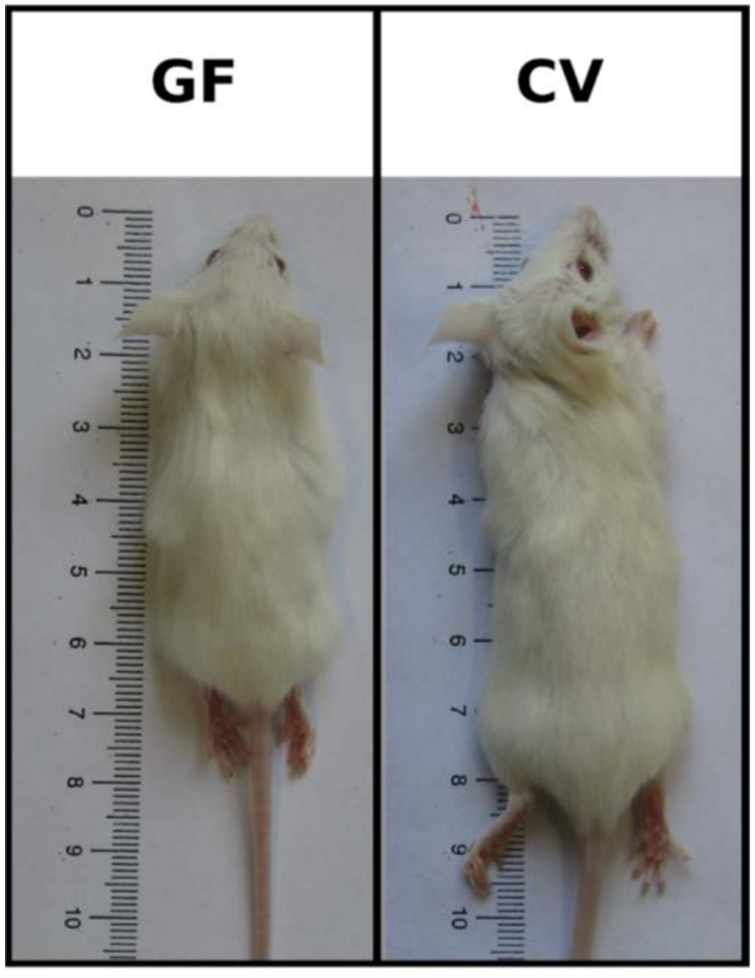


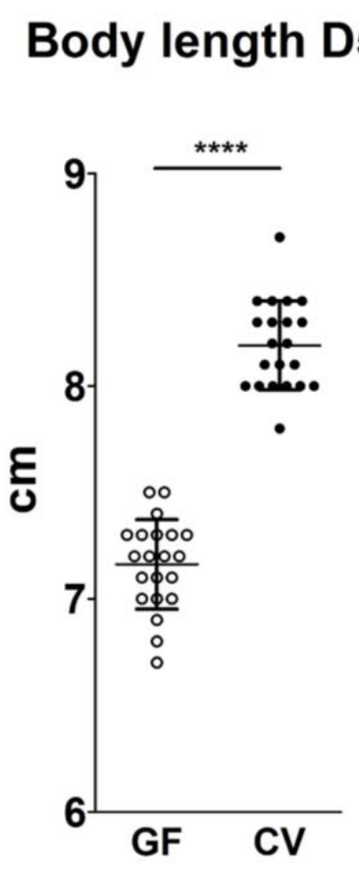
Microbiota maintains longitudinal growth upon chronic undernutrition

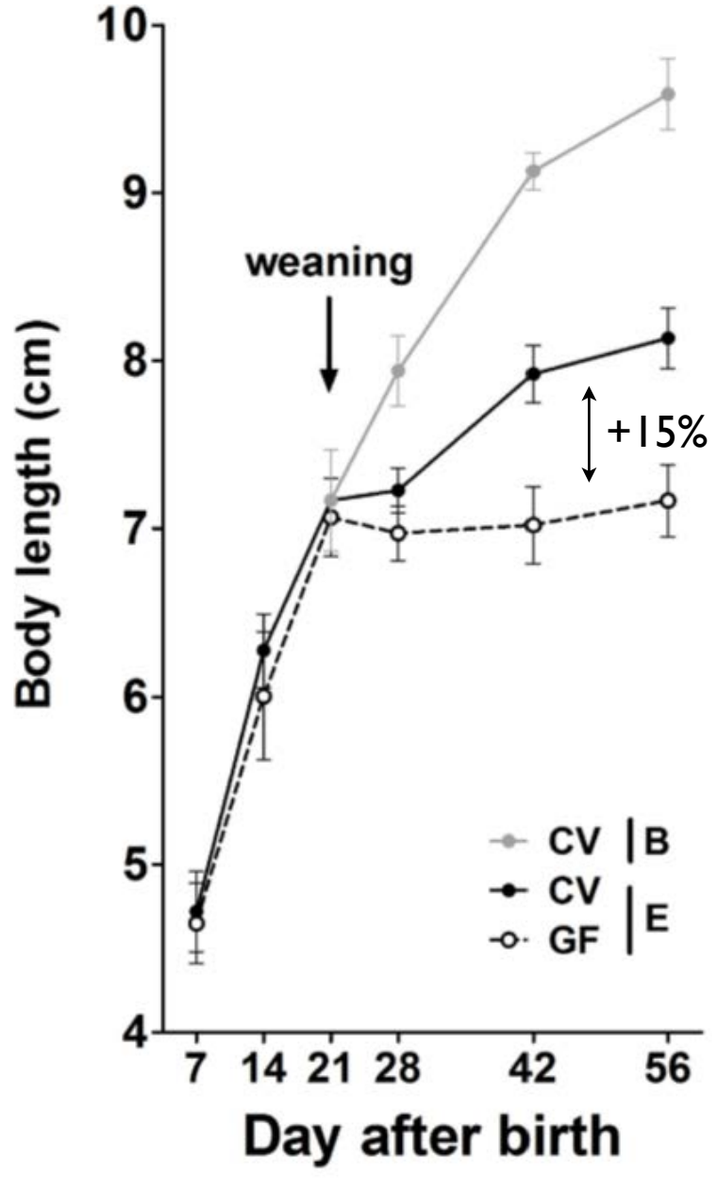


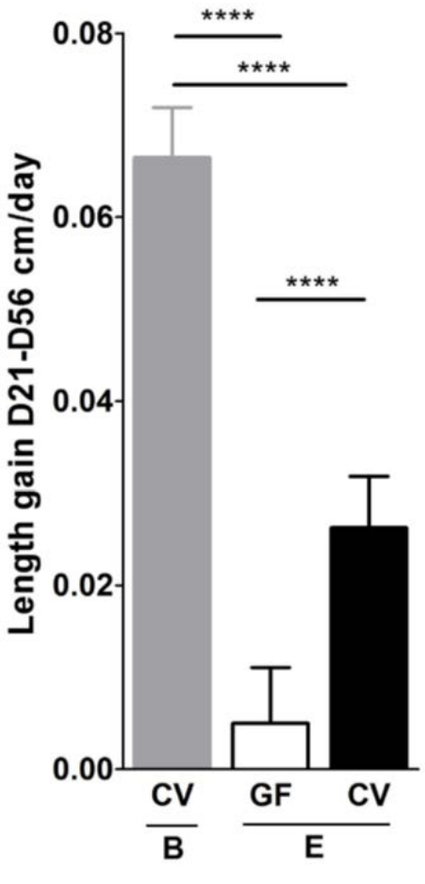


Microbiota maintains longitudinal growth upon chronic undernutrition

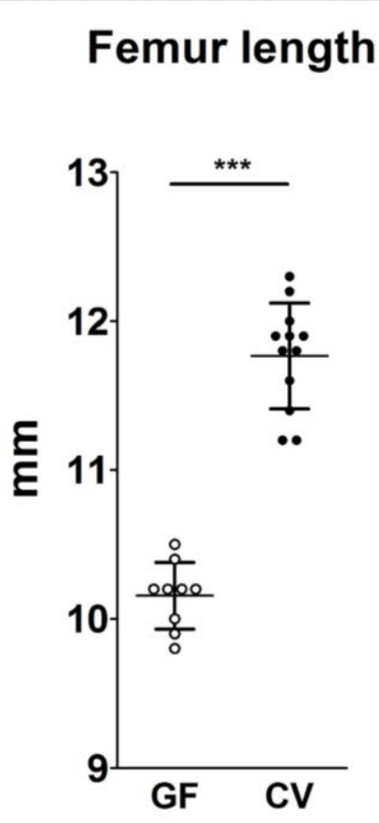






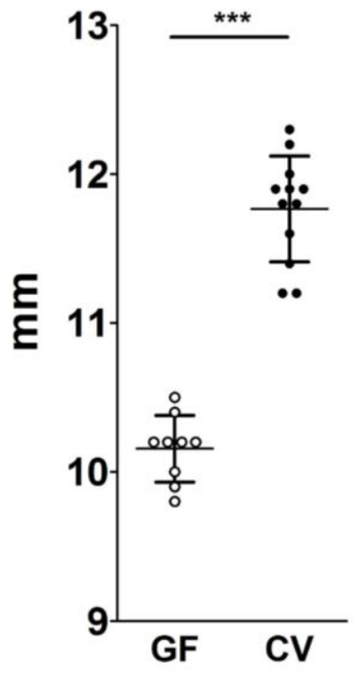


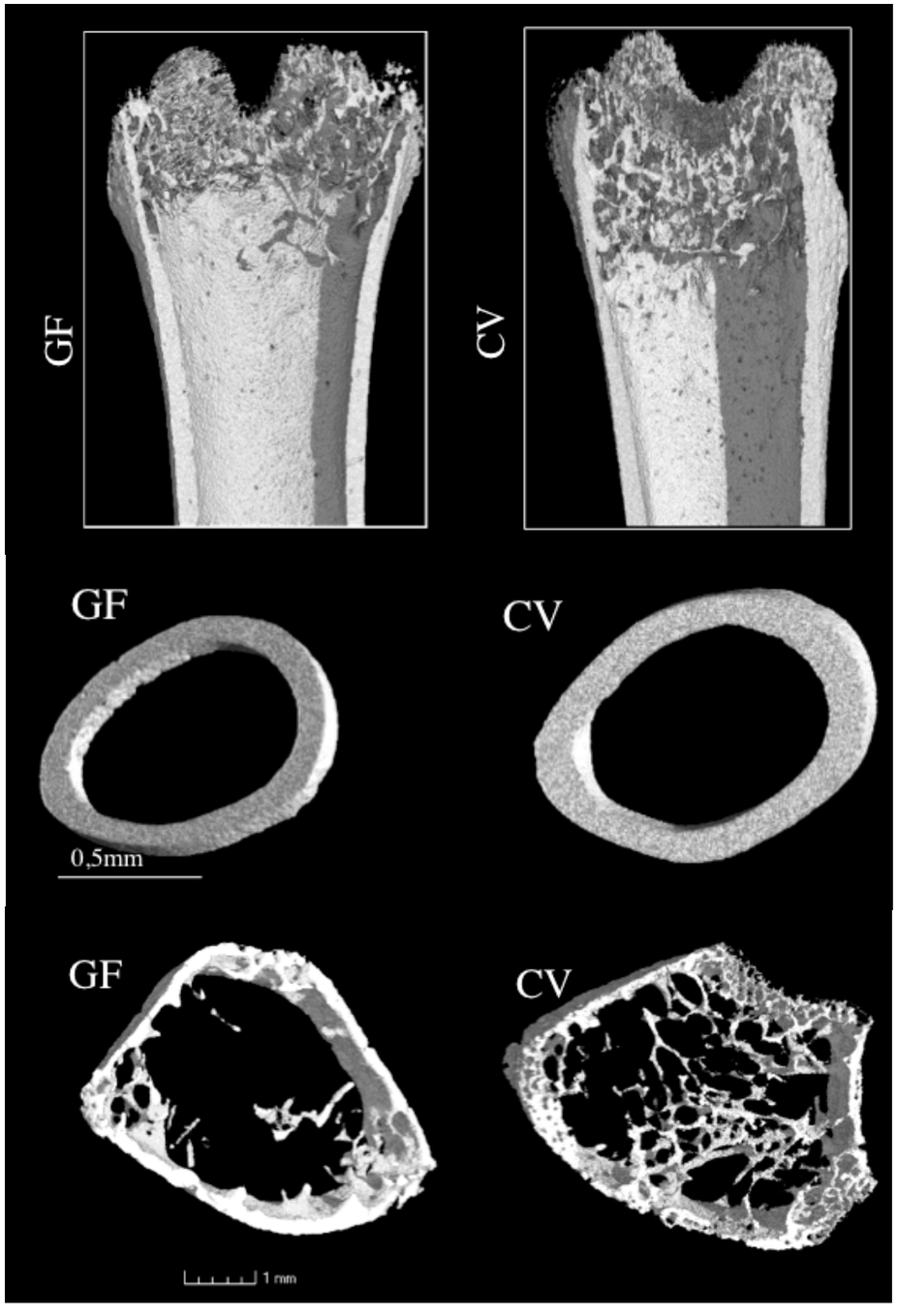
Microbiota maintains bone growth upon chronic undernutrition

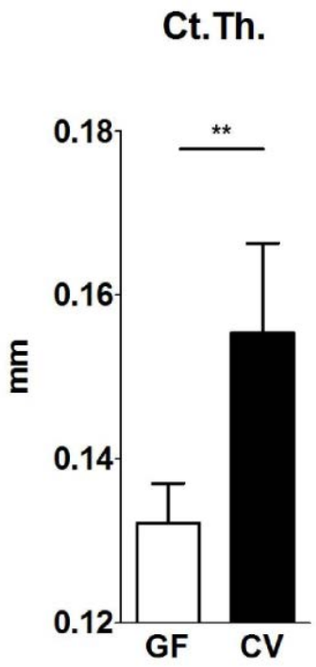


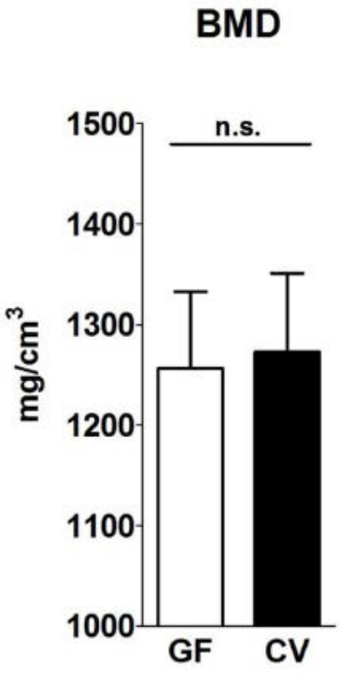
Microbiota maintains bone growth upon chronic undernutrition

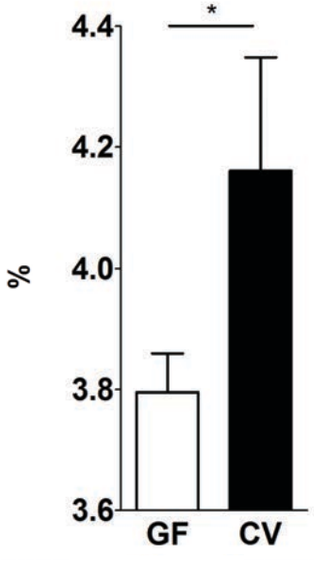
Femur length









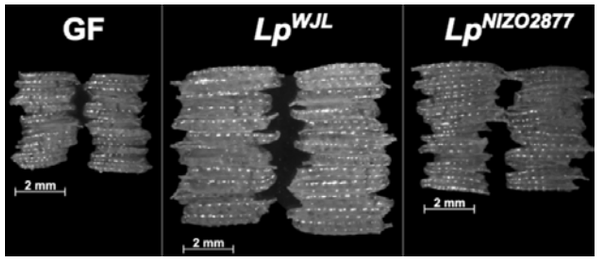


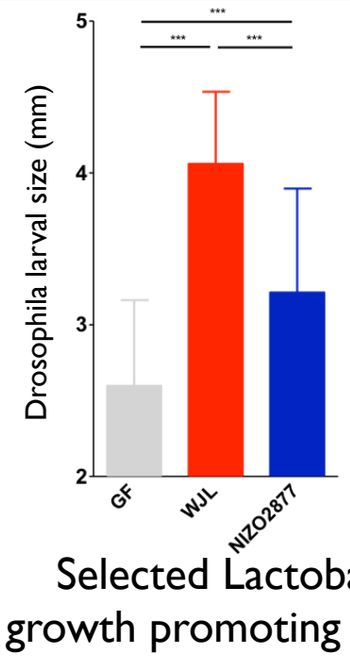
Dr I.Machuca, IGFL

Microbiota in necessary to maintain juvenile growth (tissue and skeletal growth) upon chronic undernutrition

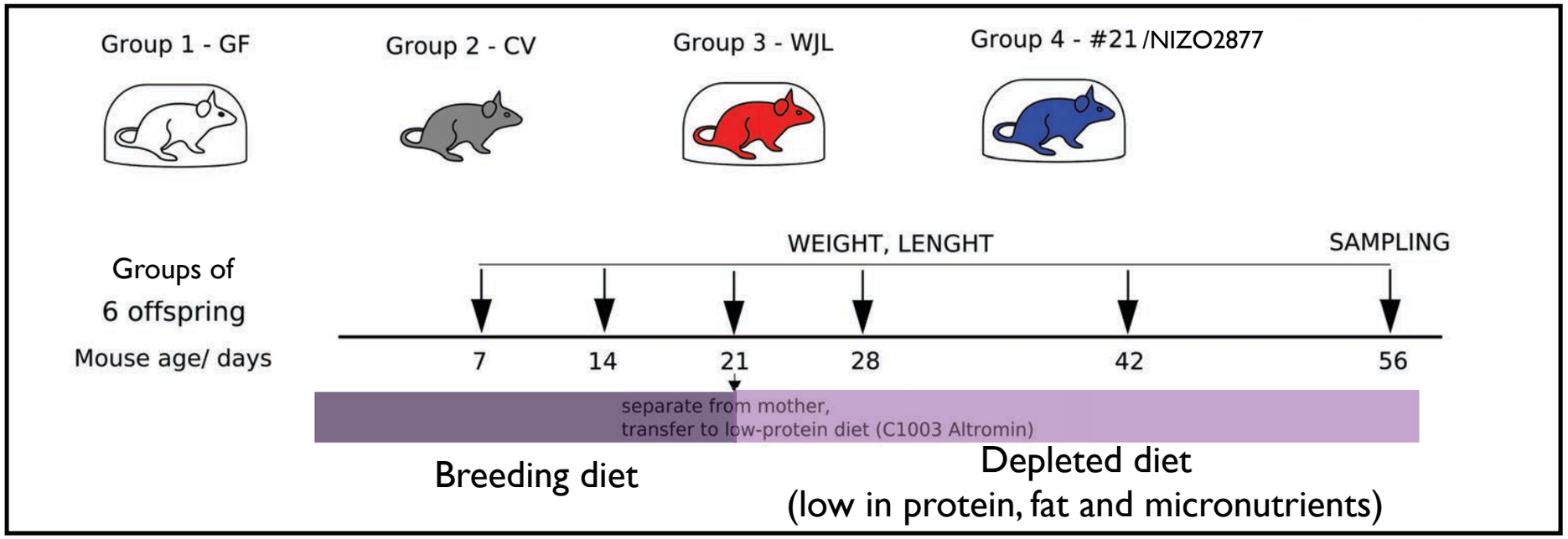
Microbiota in necessary to maintain juvenile growth (tissue and skeletal growth) upon chronic undernutrition

What about Lactobacilli strains?

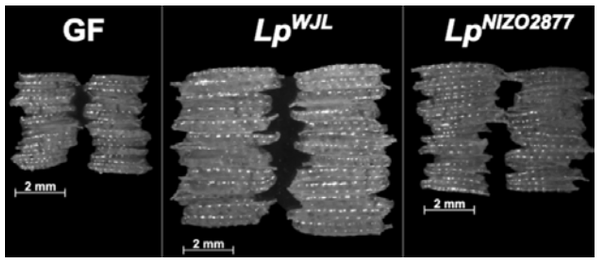


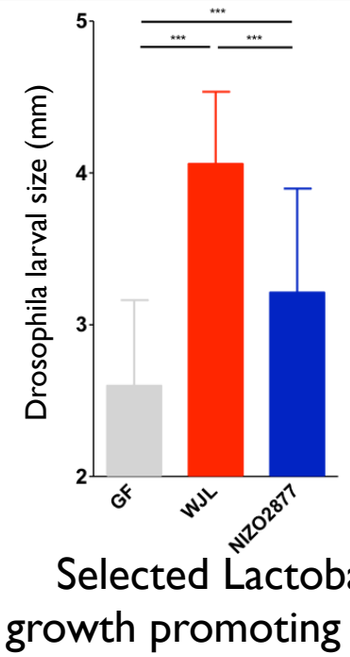


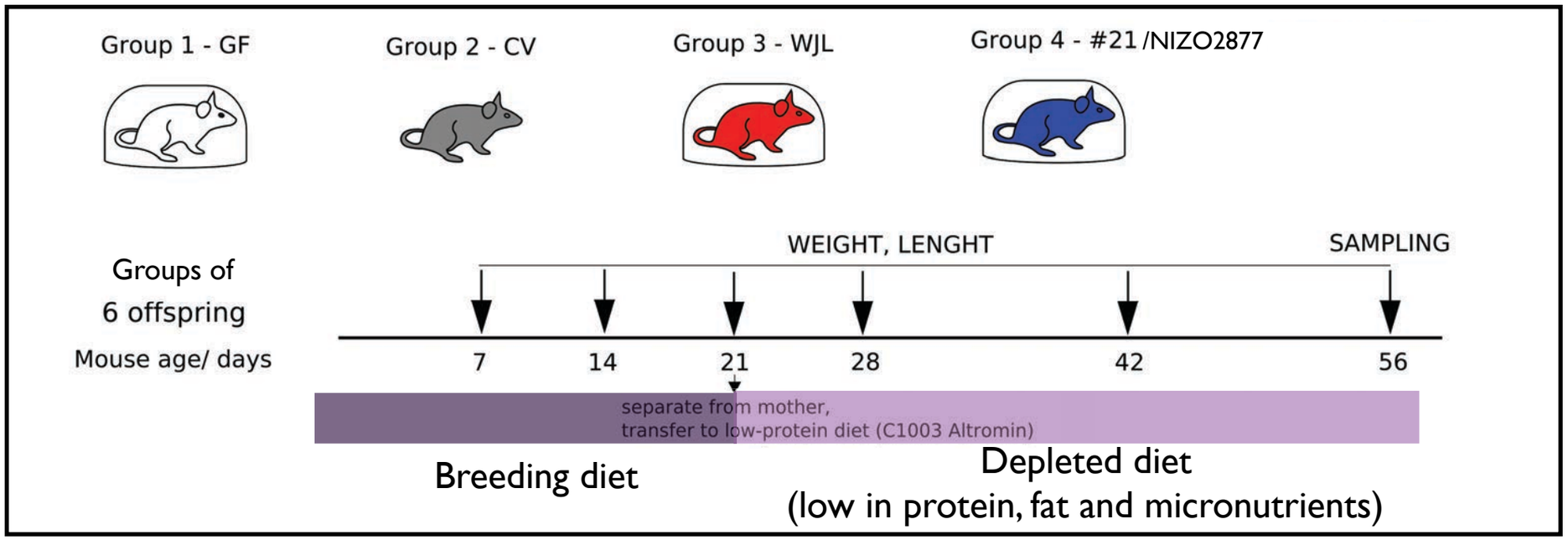
Selected Lactobacilli
growth promoting strains



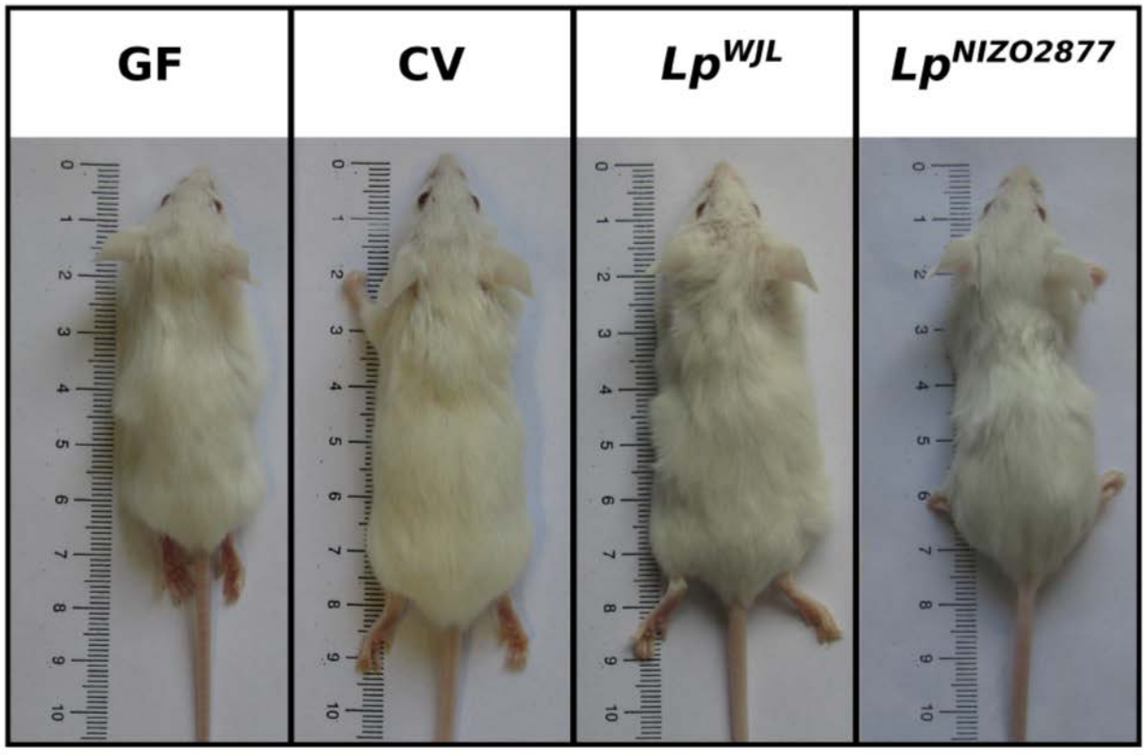
Selected Lactobacilli strains are sufficient to maintain post-natal growth upon chronic undernutrition

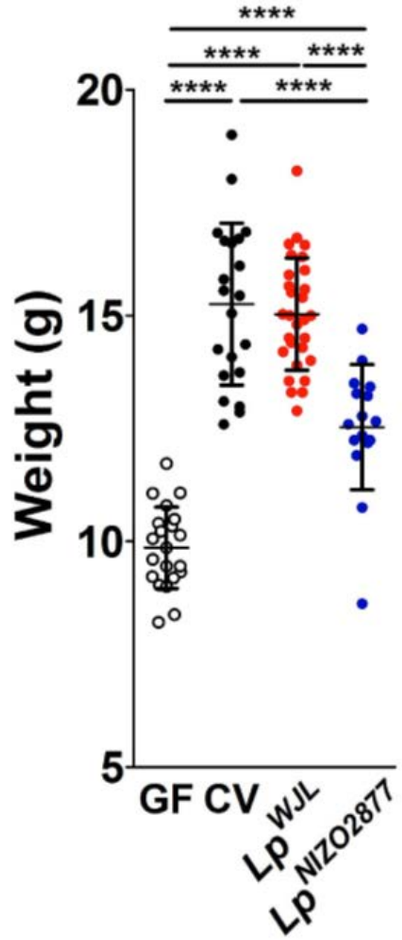


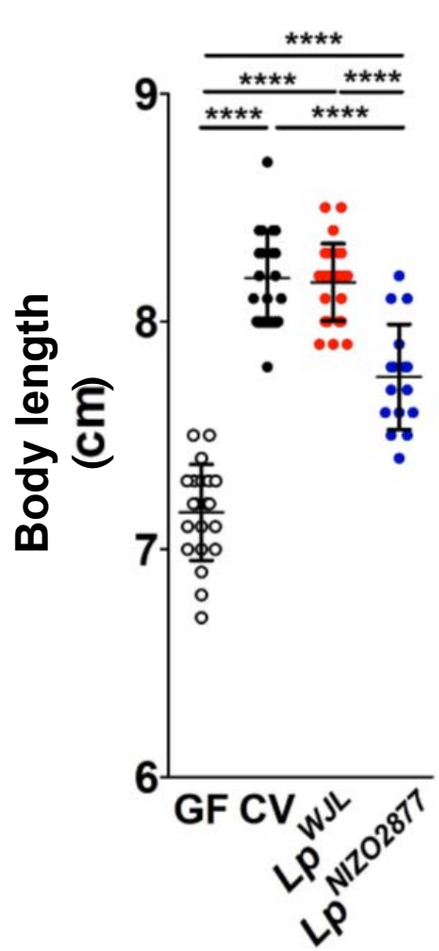




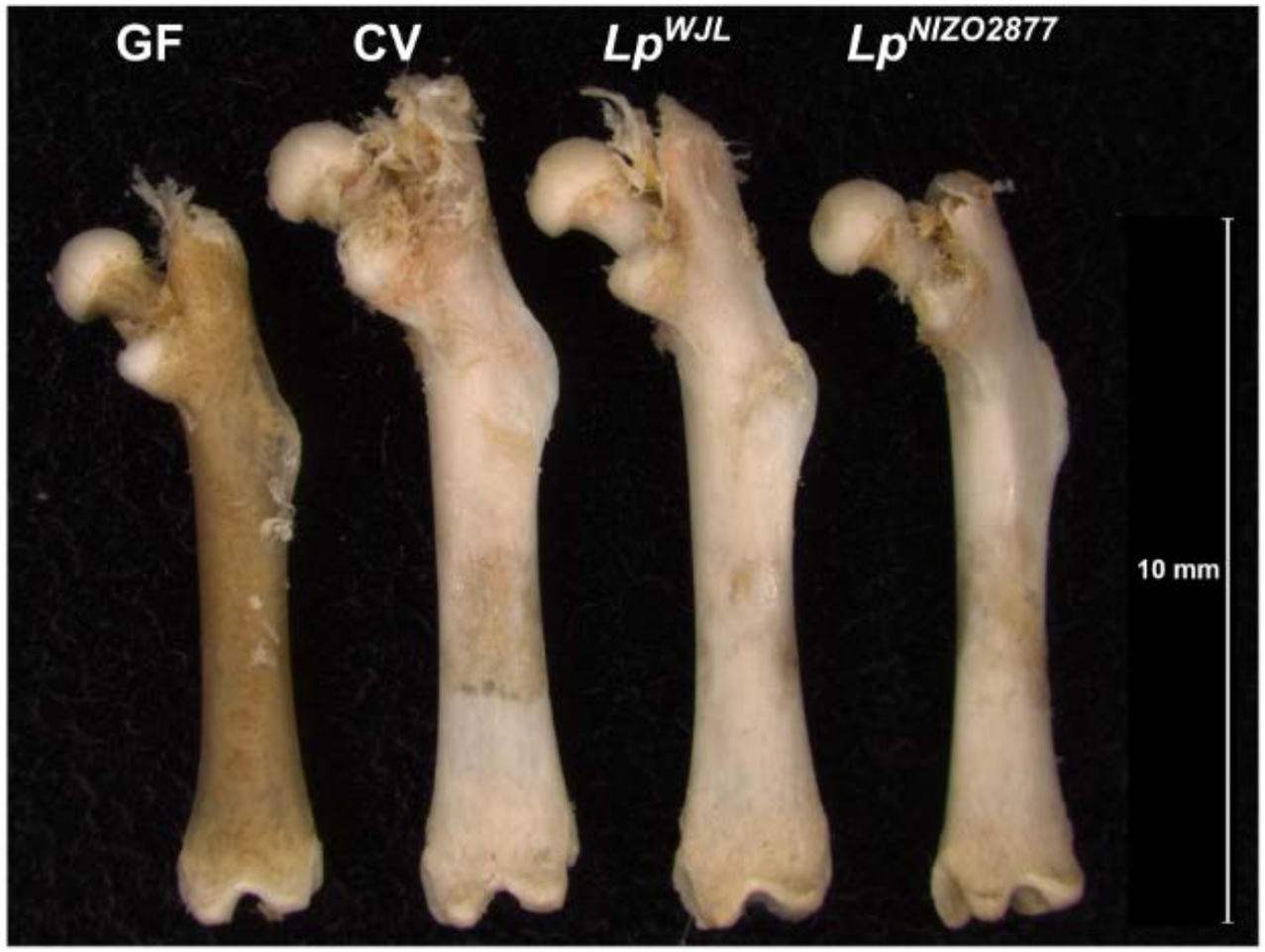
Selected Lactobacilli growth promoting strains

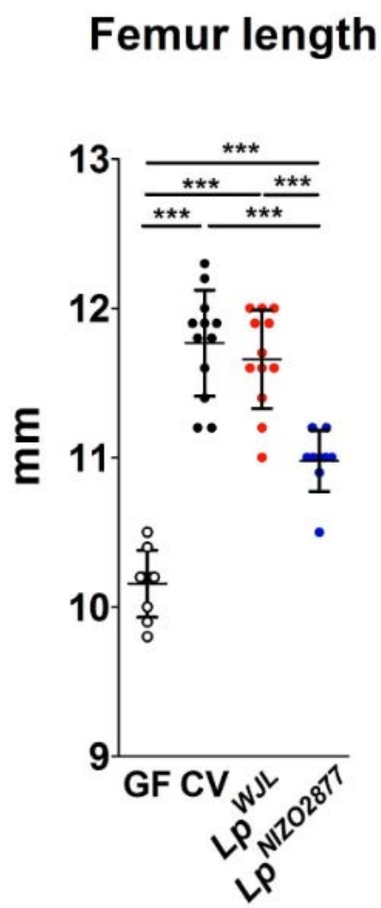






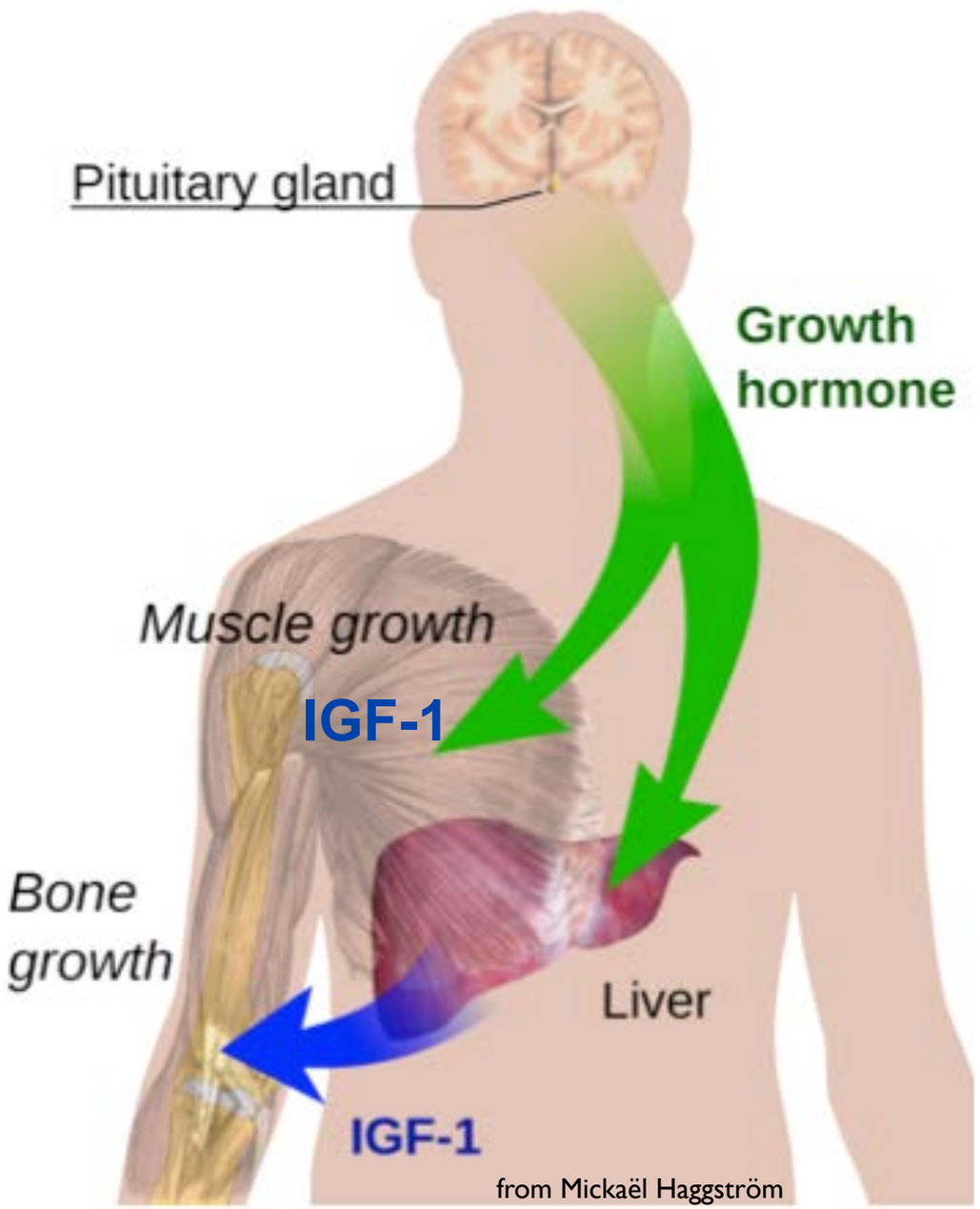
Selected Lactobacilli strains are sufficient to maintain bone growth upon chronic undernutrition





How is post-natal growth regulated in mammals?

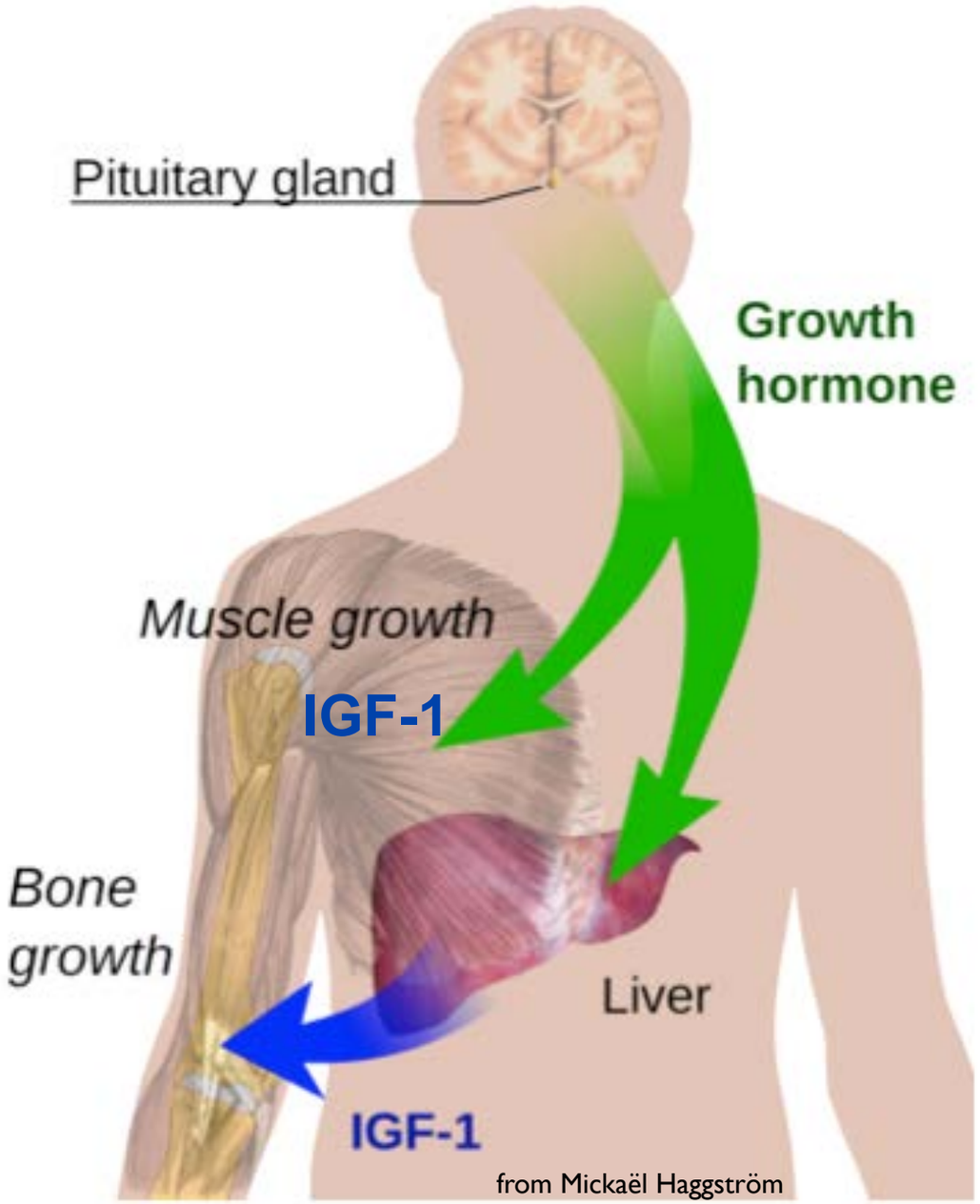
Somatotropic axis regulates post-natal growth...



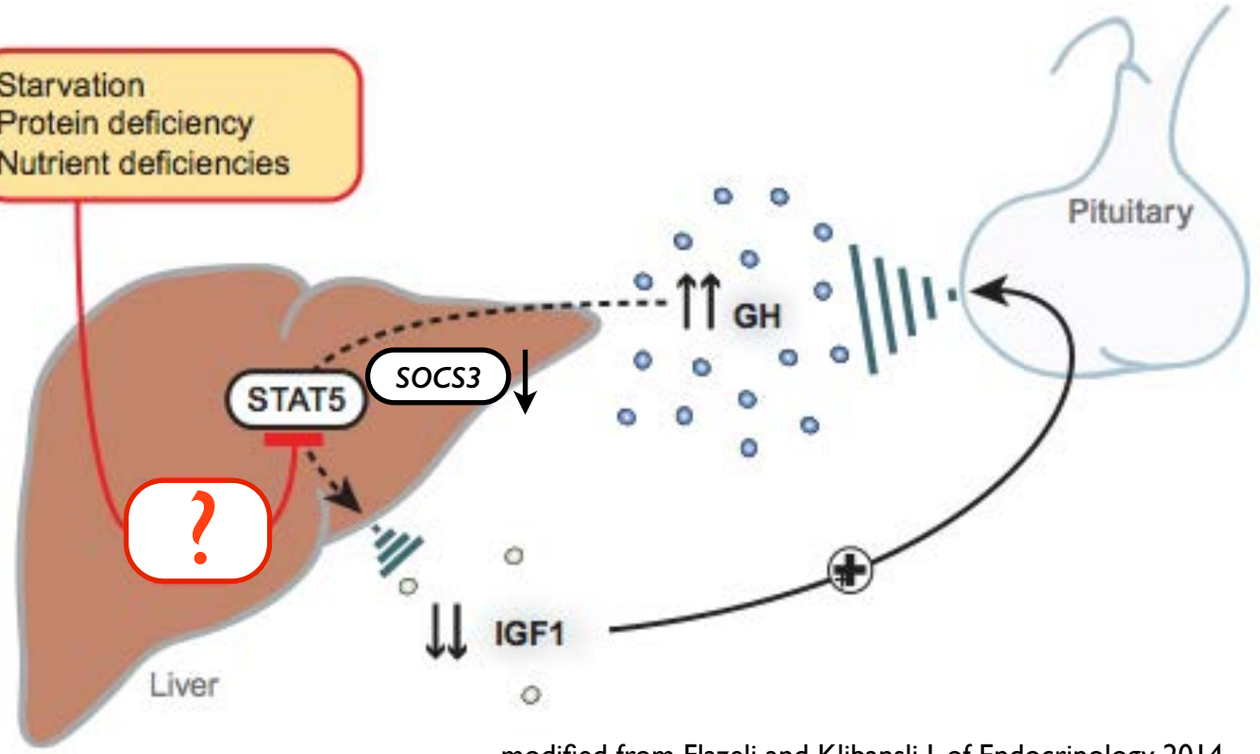
Dr H.Vidal,
Dr J.Rieusset
CARMEN
INSERM/Lyon-sud Hospital

Somatotropic axis regulates post-natal growth...

...its activity is altered upon undernutrition (state of GH-resistance)



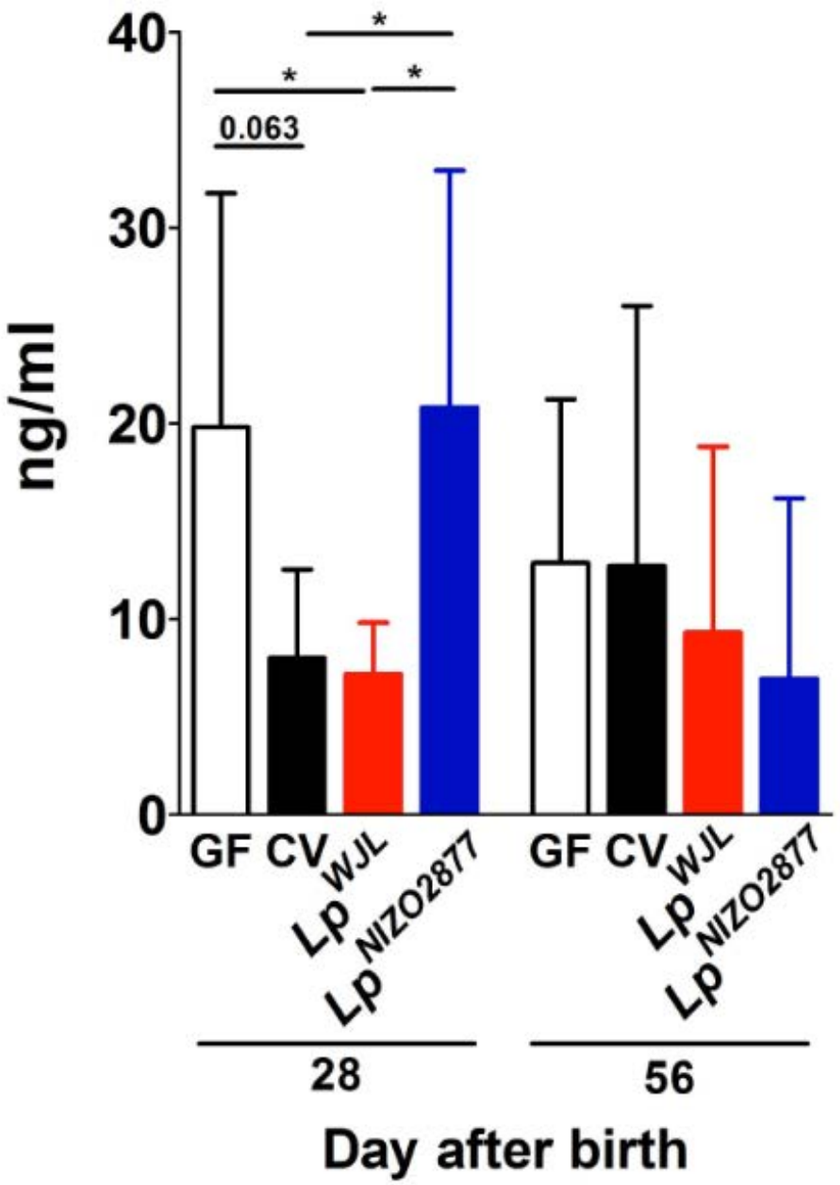
from Mickaël Haggström



modified from Flazeli and Klibansli J. of Endocrinology 2014

Dr H.Vidal,
Dr J.Rieusset
CARMEN
INSERM/Lyon-sud Hospital

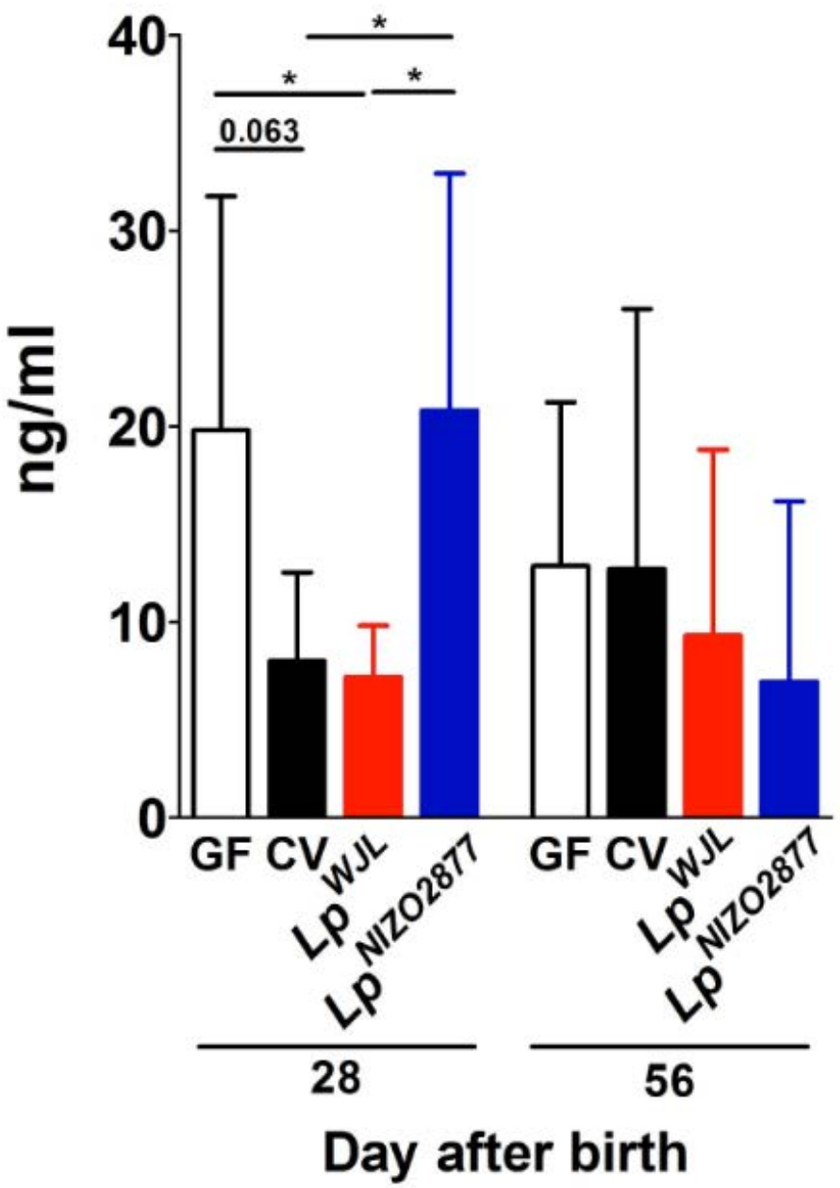
Serum titres GH



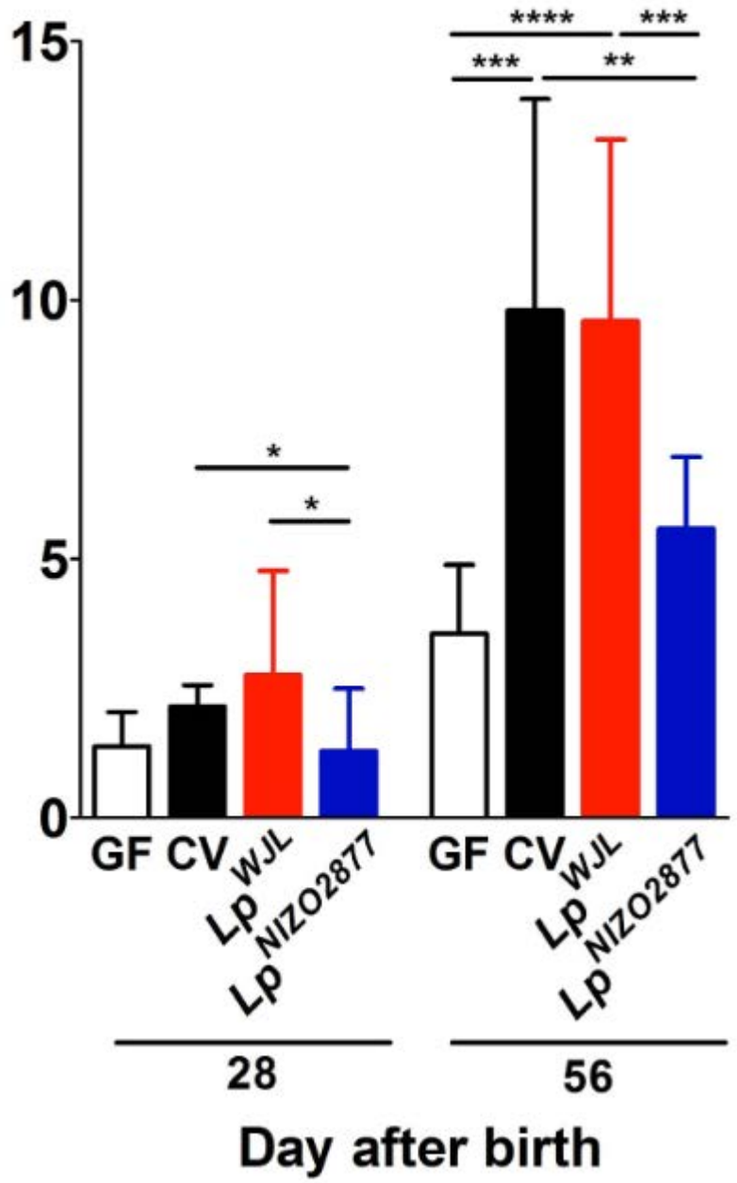
Microbiota is necessary and selected Lactobacilli strains are sufficient to maintain somatotropic axis activity upon chronic undernutrition

Serum titres

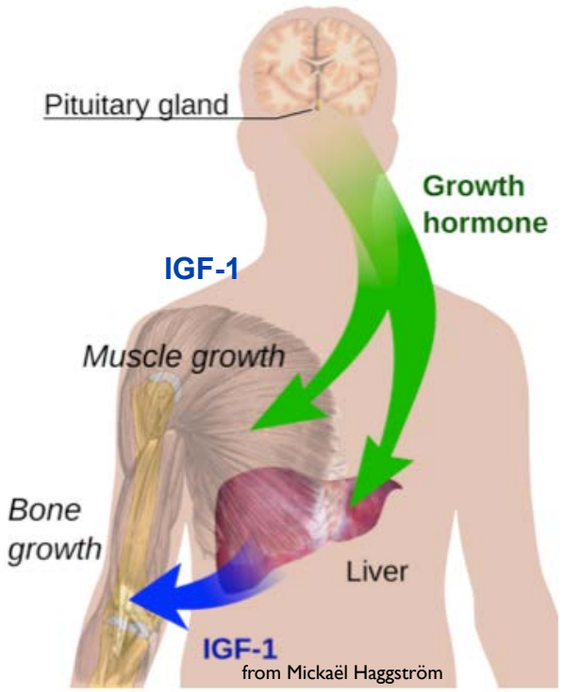
GH



IGF-1



Microbiota is necessary and selected Lactobacilli strains are sufficient to maintain somatotropic axis activity upon chronic undernutrition



GH

↓

GHR

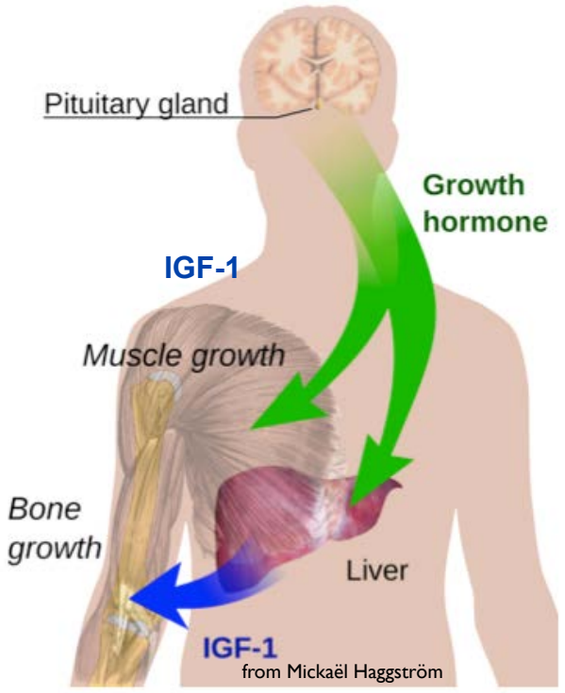
↓

STAT5

↓

SOCS3/IGF1

Microbiota is necessary and selected Lactobacilli strains are sufficient to maintain somatotropic axis activity upon chronic undernutrition



GH

↓

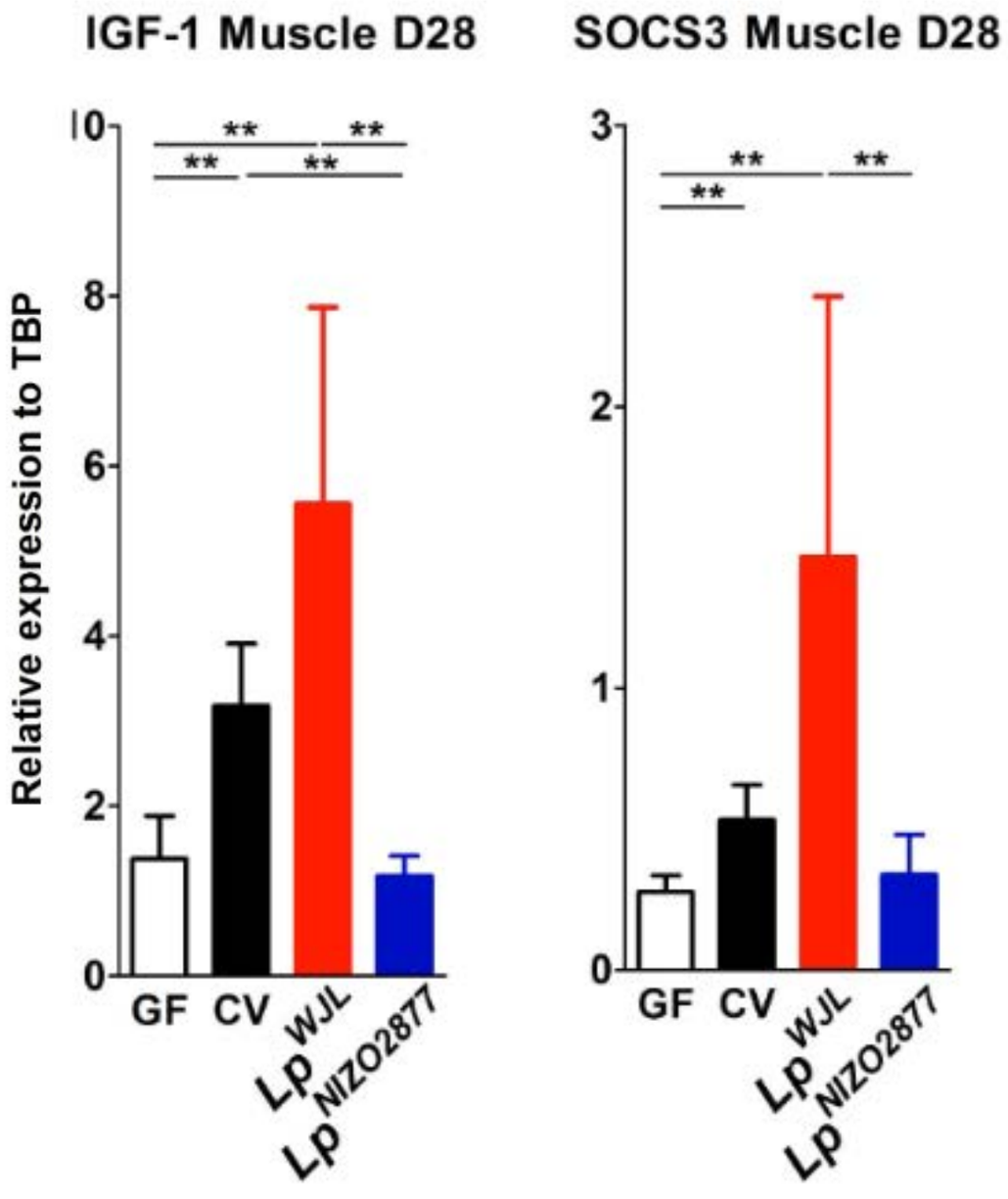
GHR

↓

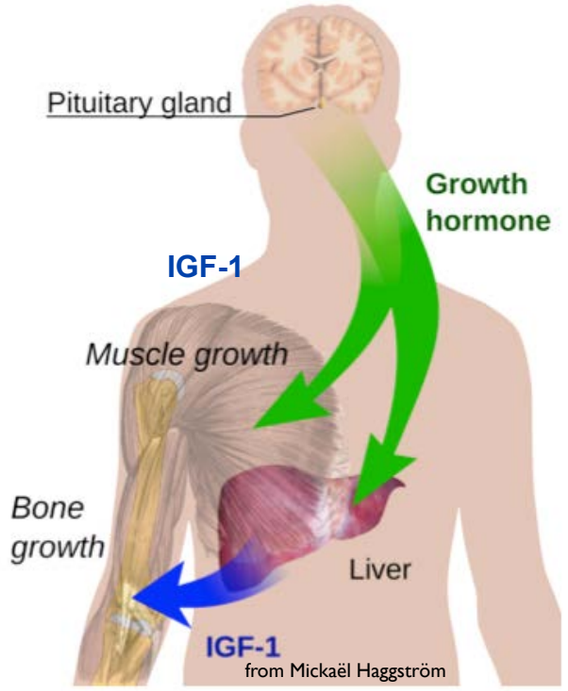
STAT5

↓

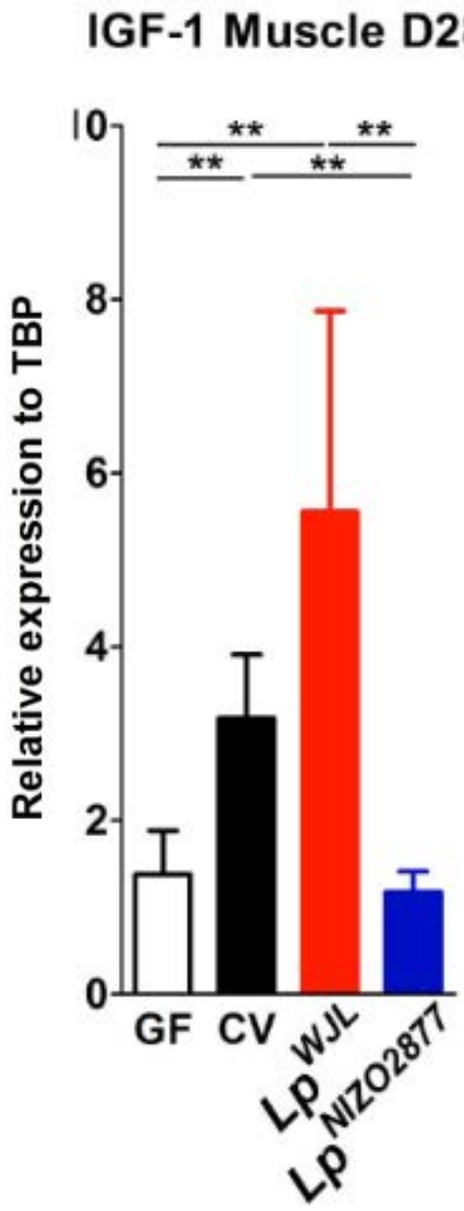
SOCS3/IGF1

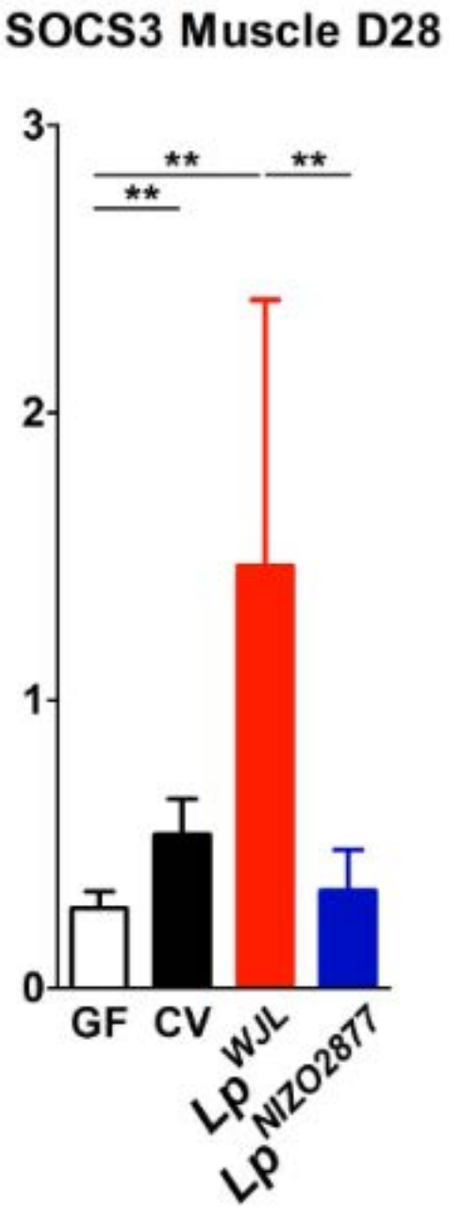


Microbiota is necessary and selected Lactobacilli strains are sufficient to maintain somatotropic axis activity upon chronic undernutrition

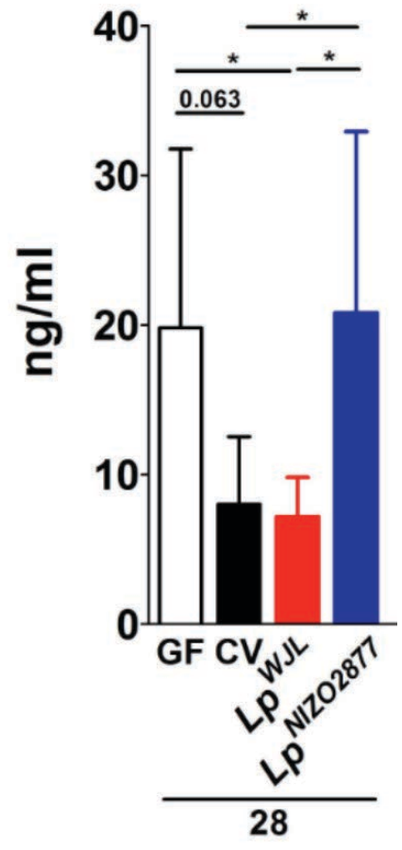


GH
↓
GHR
↓
STAT5
↓
SOCS3/IGF1





Serum titres GH

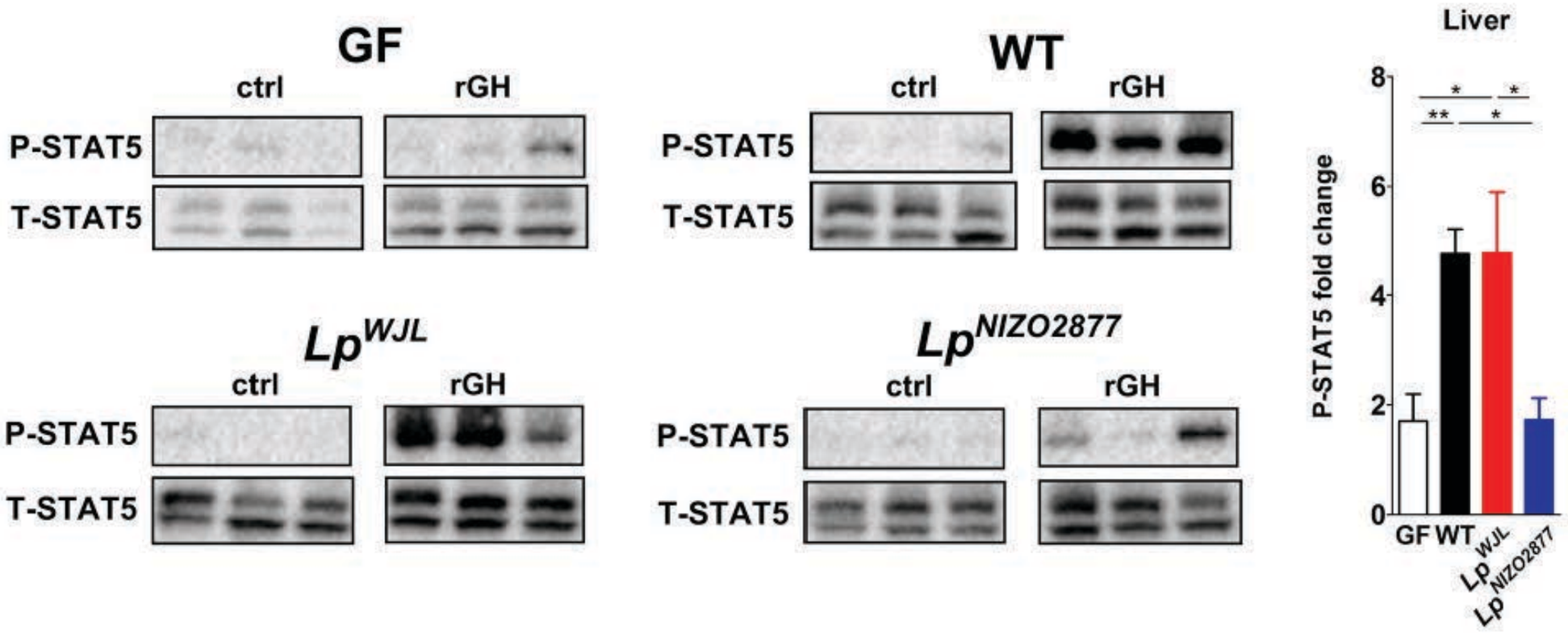


Microbiota and selected Lactobacilli strains maintain GH-sensitivity in peripheral tissue upon chronic undernutrition

Liver harvested from D28 animals (weaned for one week on the experimental diet), 15’ post-GH or PBS injection

Microbiota and selected Lactobacilli strains maintain GH-sensitivity in peripheral tissue upon chronic undernutrition

Liver harvested from D28 animals (weaned for one week on the experimental diet), 15' post-GH or PBS injection



Microbiota and Undernutrition in mammals

Chronic undernutrition or prolonged nutritional deficit

Stunting: growth failure leading to short stature

MICROBIOME

Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition

Martin Schwarzer,[1,2]* Kassem Makki,[1,3] Gilles Storelli,[1] Irma Machuca-Gayet,[1]†
Dagmar Srutkova,[2] Petra Hermanova,[2] Maria Elena Martino,[1] Severine Balmand,[4]
Tomas Hudcovic,[2] Abdelaziz Heddi,[4] Jennifer Rieusset,[3] Hana Kozakova,[2]
Hubert Vidal,[3] François Leulier[1]*

Microbiota and Undernutrition in mammals

Acute undernutrition or Protein-energy undernutrition

Wasting: weight loss diagnosed by a reduction in weight-for-height index

Ruminococcus gnavus

Clostridium symbiosum

MICROBIOME

Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children

Laura V. Blanton,[1] Mark R. Charbonneau,[1] Tarek Salih,[1] Michael J. Barratt,[1] Siddarth Venkatesh,[1] Olga Ilkaveya,[2] Sathish Subramanian,[1] Mark J. Manary,[3,4] Indi Trehan,[3,5] Josh M. Jorgensen,[6] Yue-mei Fan,[7] Bernard Henrissat,[8,9] Semen A. Leyn,[10] Dmitry A. Rodionov,[10,11] Andrei L. Osterman,[11] Kenneth M. Maleta,[4] Christopher B. Newgard,[2,12] Per Ashorn,[7,13] Kathryn G. Dewey,[6] Jeffrey Gordon[1]*

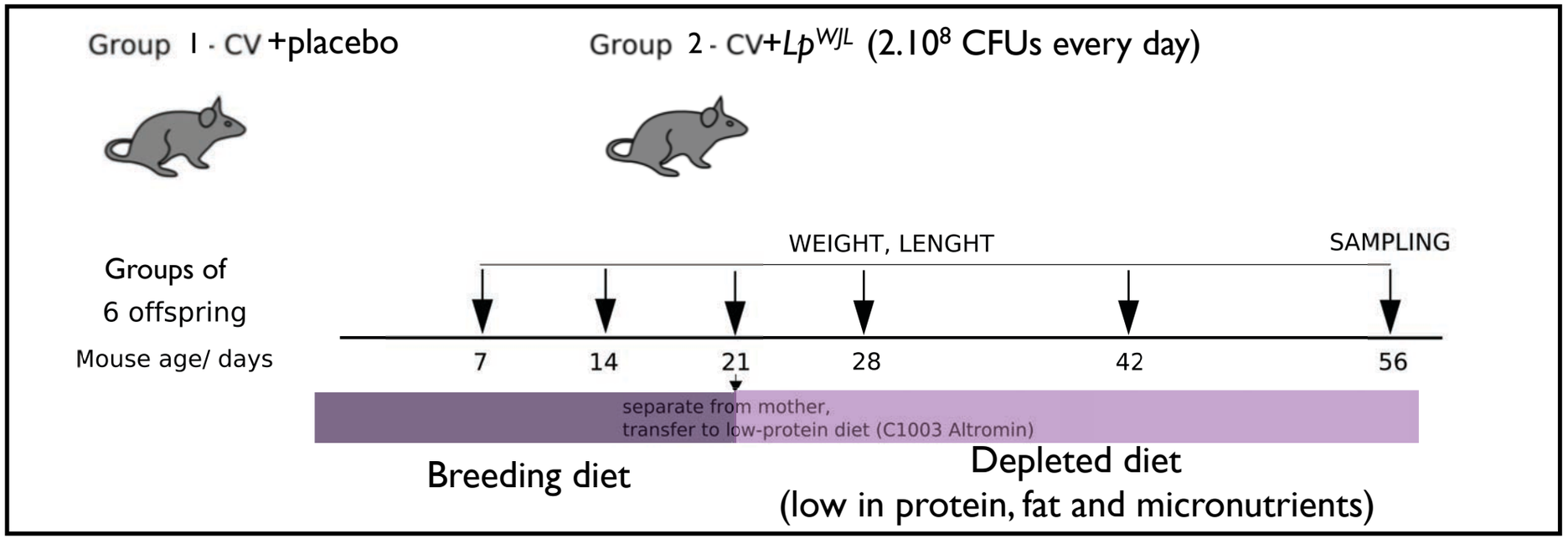
Chronic undernutrition or prolonged nutritional deficit

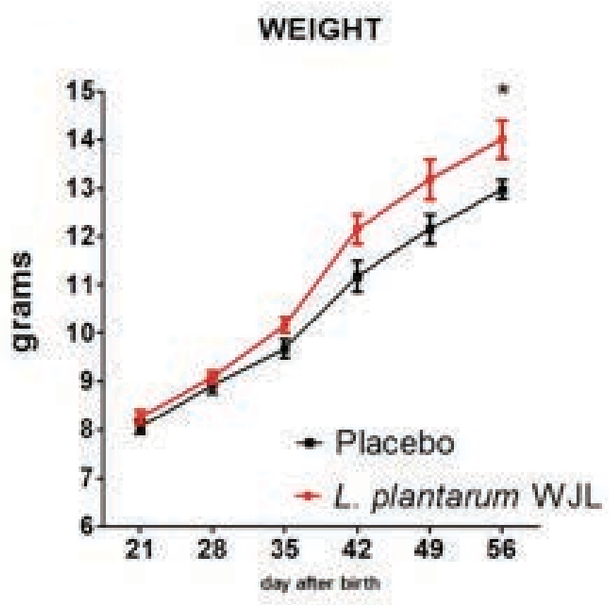
Stunting: growth failure leading to short stature

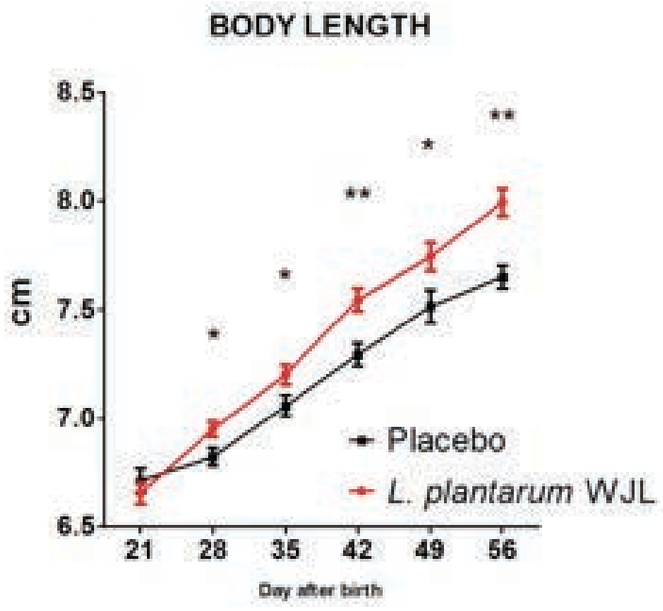
MICROBIOME

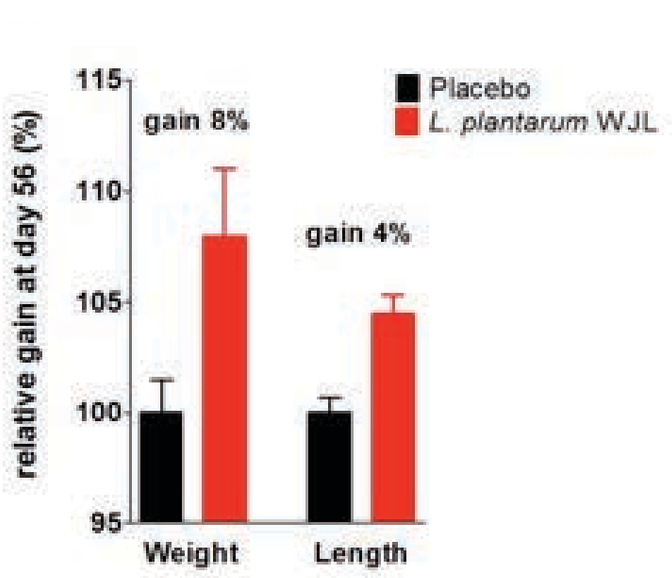
Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition

Martin Schwarzer,[1,2]* Kassem Makki,[1,3] Gilles Storelli,[1] Irma Machuca-Gayet,[1]† Dagmar Srutkova,[2] Petra Hermanova,[2] Maria Elena Martino,[1] Severine Balmand,[4] Tomas Hudcovic,[2] Abdelaziz Heddi,[4] Jennifer Rieusset,[3] Hana Kozakova,[2] Hubert Vidal,[3] François Leulier[1]*

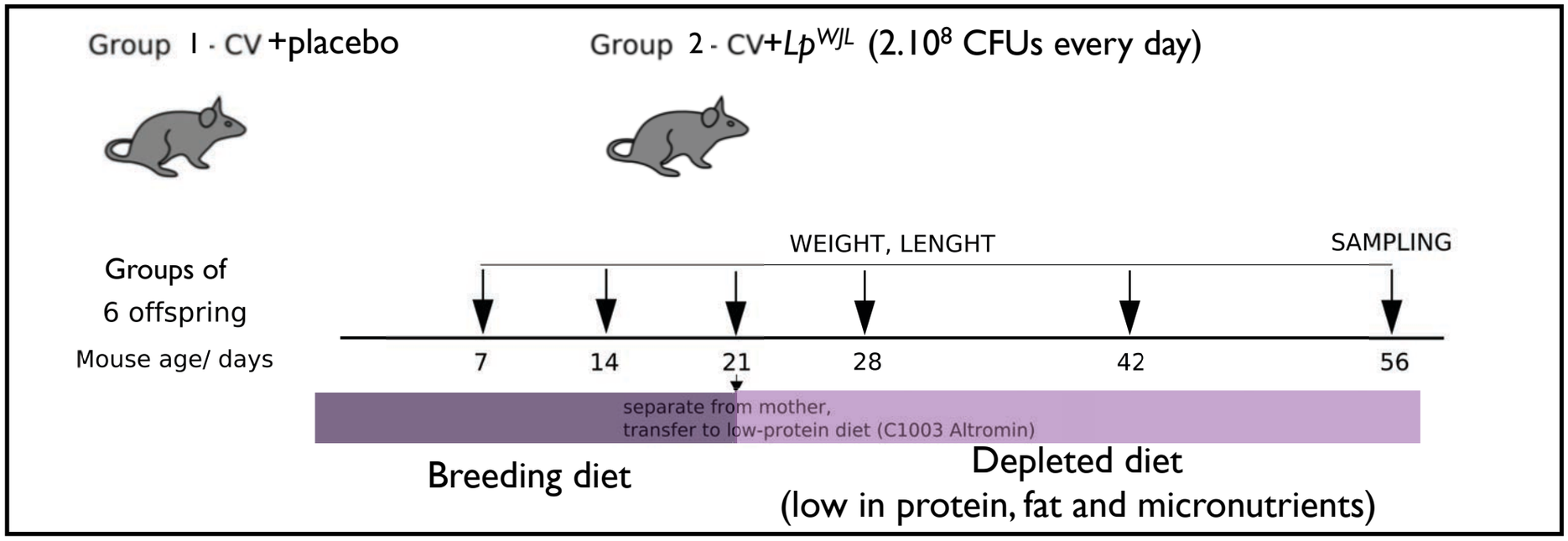

Group 1 - CV +placebo
Group 2 - CV+Lp^WJL (2.10^8 CFUs every day)
Groups of
6 offspring
Mouse age/ days
WEIGHT, LENGHT
SAMPLING
7
14
21
28
42
56
separate from mother,
transfer to low-protein diet (C1003 Altromin)
Breeding diet
Depleted diet
(low in protein, fat and micronutrients)

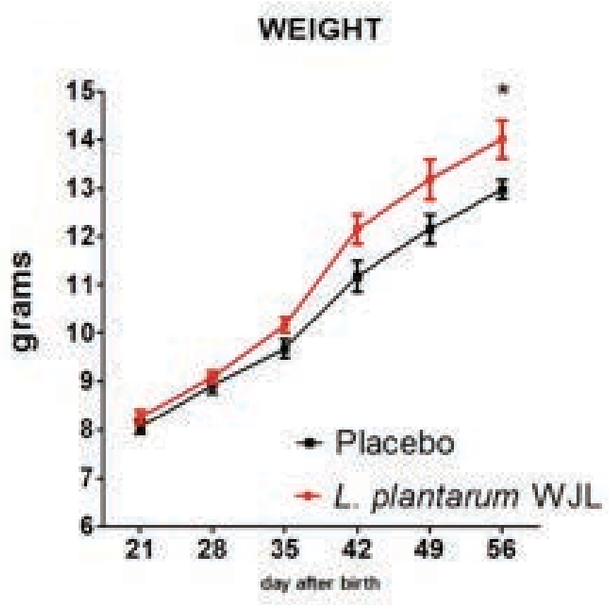

WEIGHT
grams
day after birth
Placebo
L. plantarum WJL

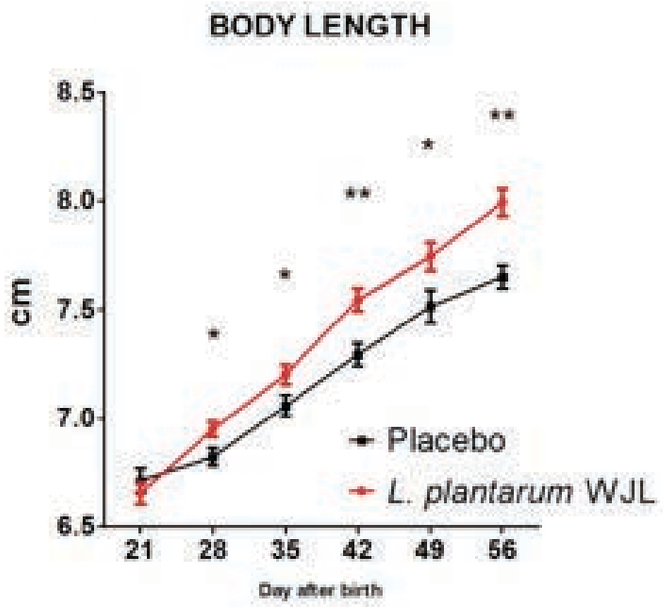

BODY LENGTH
cm
Day after birth
Placebo
L. plantarum WJL

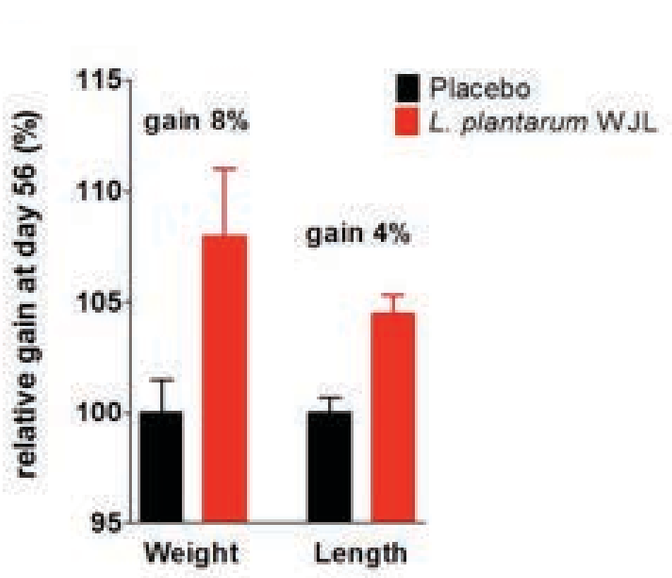

relative gain at day 56 (%)
gain 8%
gain 4%
Placebo
L. plantarum WJL
Weight
Length

Effect of *Lp*^{WJL} on CONV mice linear growth upon chronic undernutrition



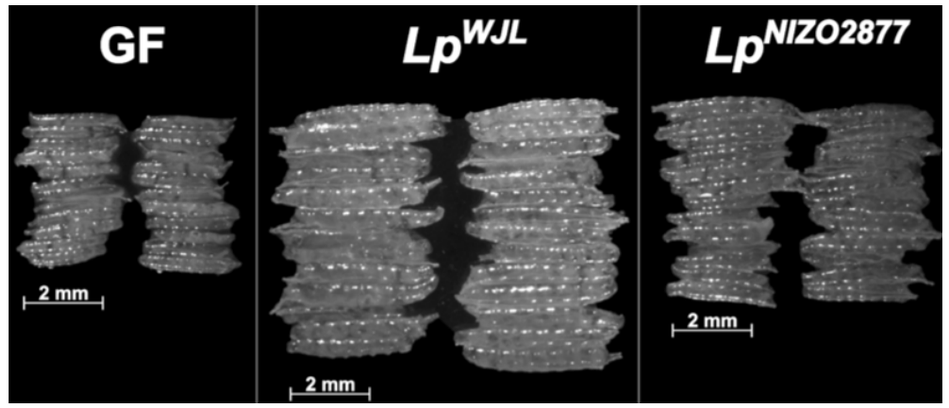


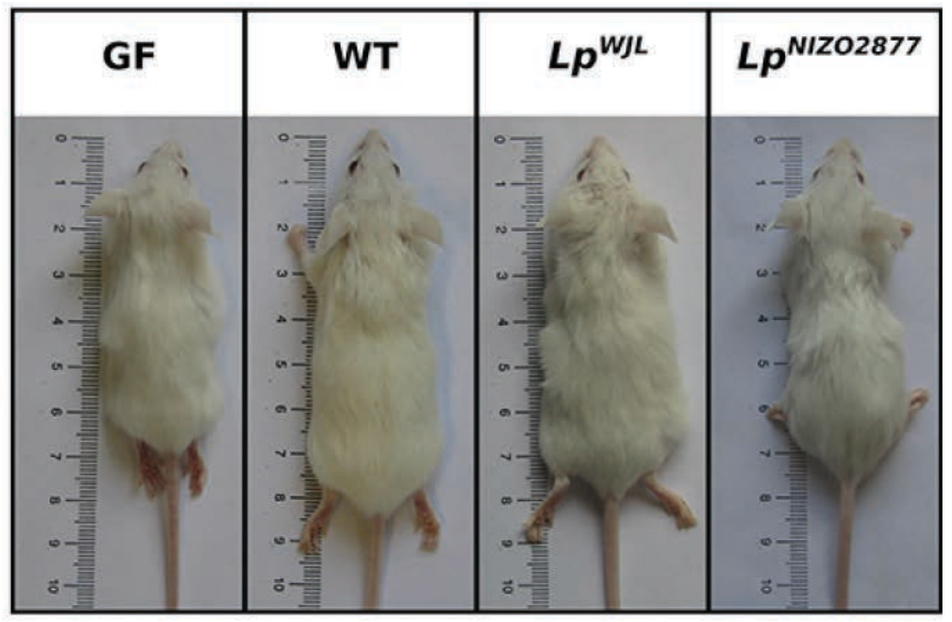




Take home messages - II

The microbiota acts as a buffer to the adverse effects of chronic undernutrition on linear growth

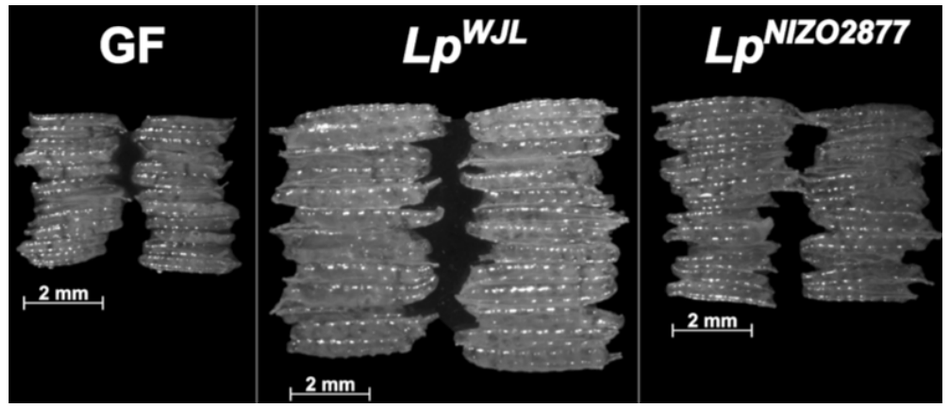


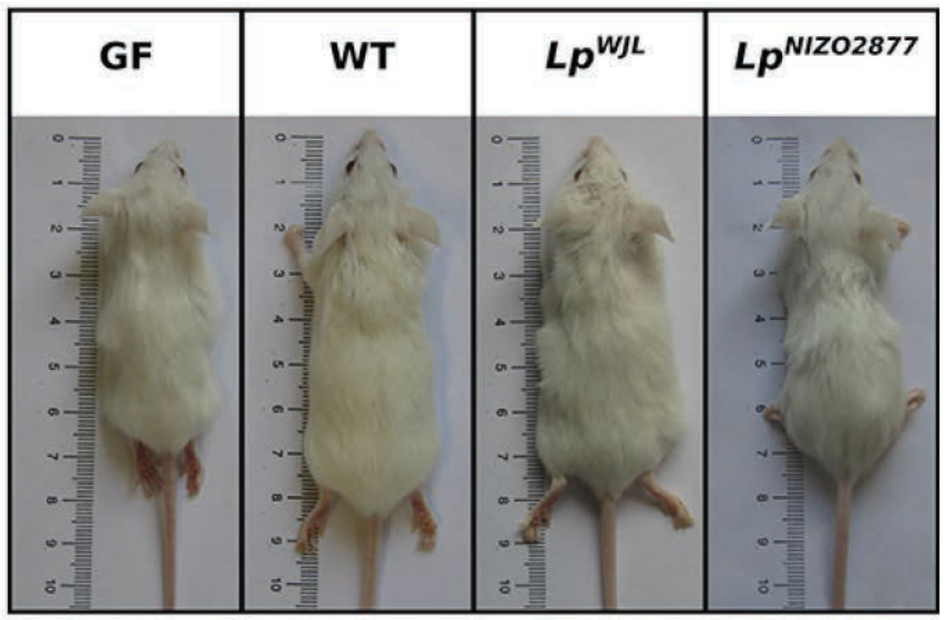


Take home messages - II

The microbiota acts as a buffer to the adverse effects of chronic undernutrition on linear growth

The microbiota maintains somatotropic axis activity (improved GH-sensitivity)



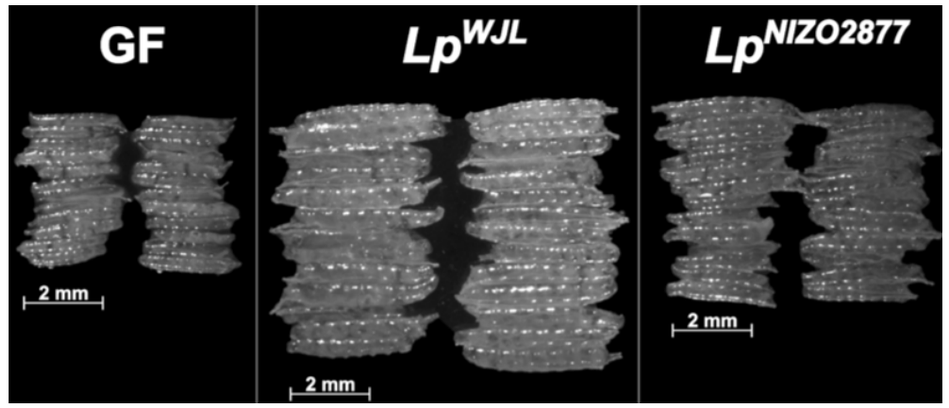


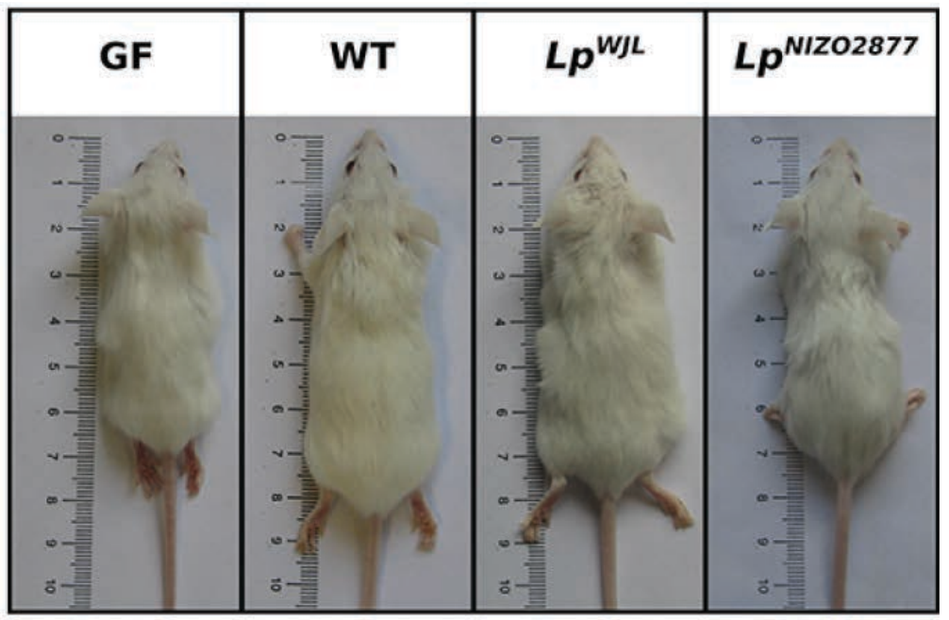
Take home messages - II

The microbiota acts as a buffer to the adverse effects of chronic undernutrition on linear growth

The microbiota maintains somatotropic axis activity (improved GH-sensitivity)

Selected Lactobacilli strains recapitulate the microbiota effect in a strain dependent manner





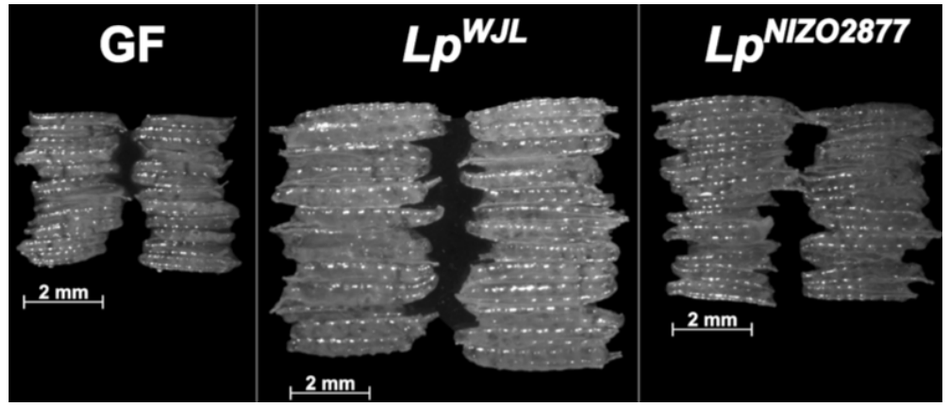
Take home messages - II

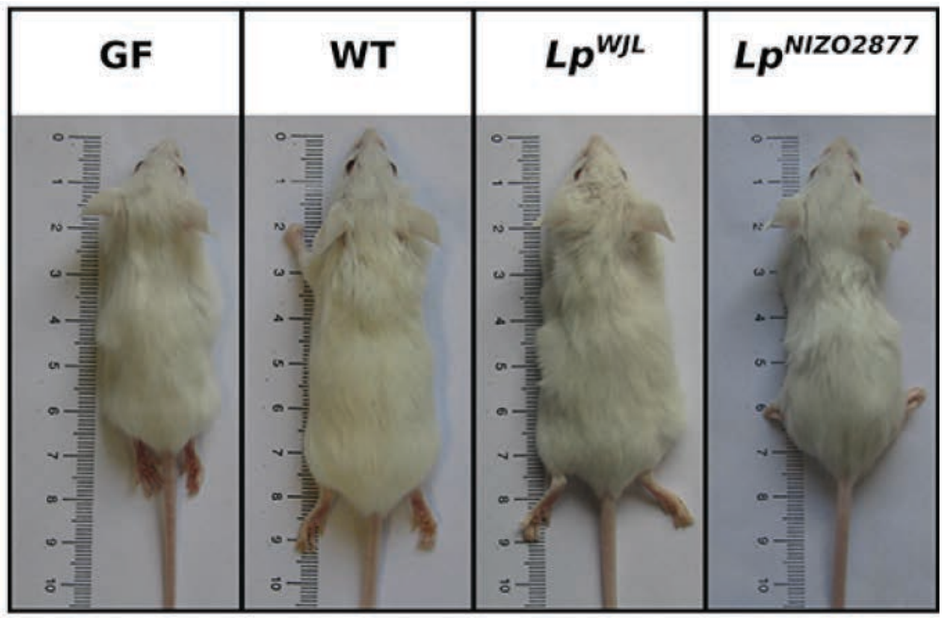
The microbiota acts as a buffer to the adverse effects of chronic undernutrition on linear growth

The microbiota maintains somatotropic axis activity (improved GH-sensitivity)

Selected Lactobacilli strains recapitulate the microbiota effect in a strain dependent manner

Evolutionary conserved functionality of selected Lactobacilli strains to maintain juvenile growth upon chronic undernutrition





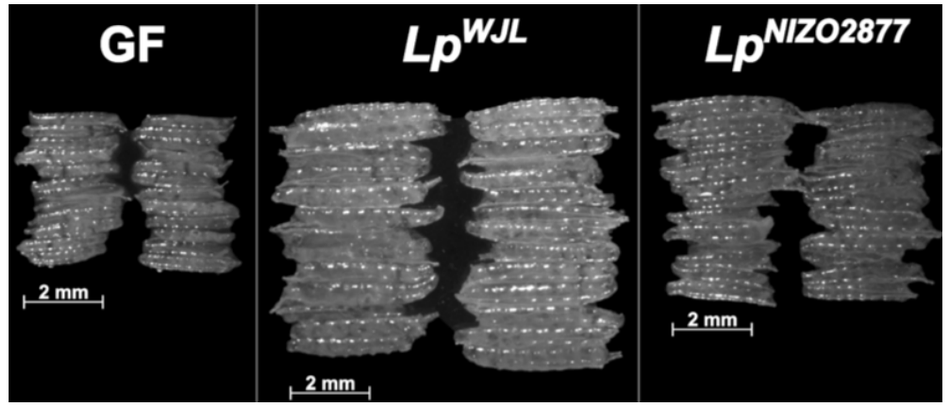
Take home messages - II

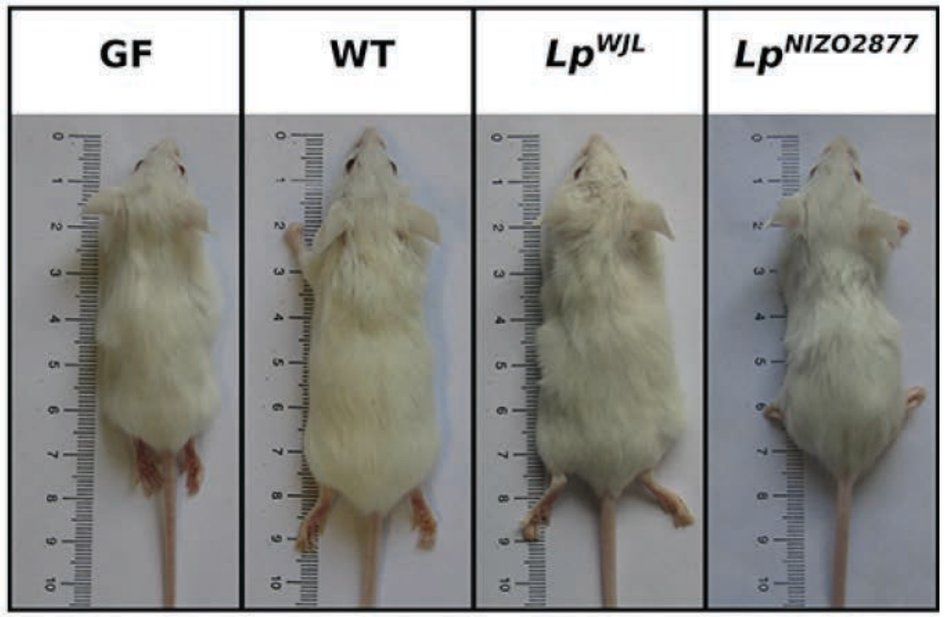
The microbiota acts as a buffer to the adverse effects of chronic undernutrition on linear growth

The microbiota maintains somatotropic axis activity (improved GH-sensitivity)

Selected Lactobacilli strains recapitulate the microbiota effect in a strain dependent manner

Evolutionary conserved functionality of selected Lactobacilli strains to maintain juvenile growth upon chronic undernutrition





Current work

Effect of *Lp*^{WJL} on CONV mice linear growth upon chronic undernutrition

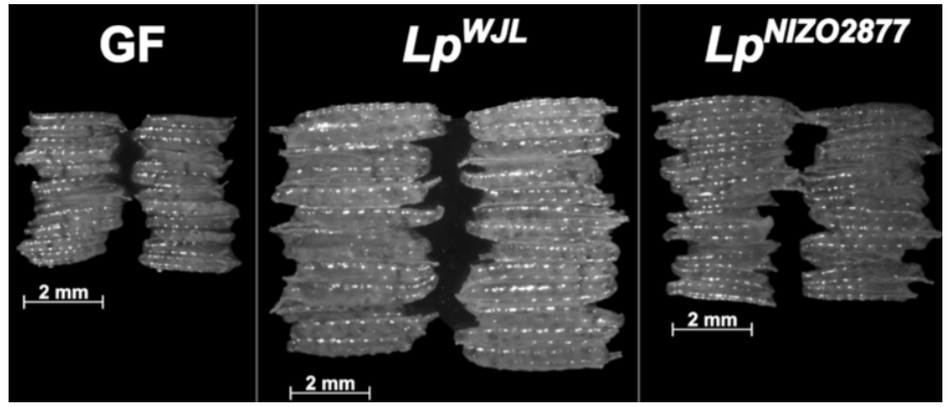
Take home messages - II

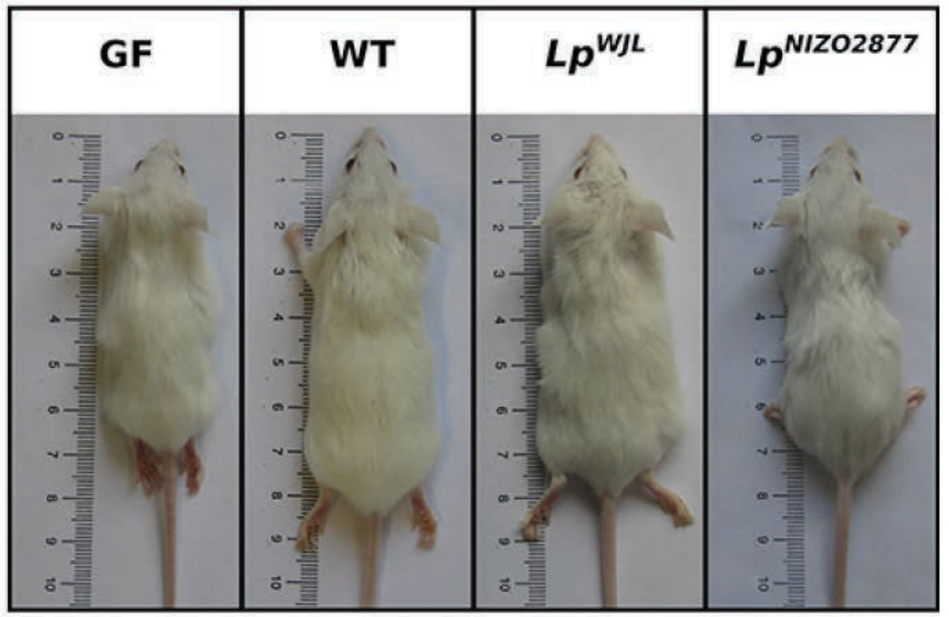
The microbiota acts as a buffer to the adverse effects of chronic undernutrition on linear growth

The microbiota maintains somatotropic axis activity (improved GH-sensitivity)

Selected Lactobacilli strains recapitulate the microbiota effect in a strain dependent manner

Evolutionary conserved functionality of selected Lactobacilli strains to maintain juvenile growth upon chronic undernutrition





Current work

Effect of *Lp*^{WJL} on CONV mice linear growth upon chronic undernutrition

Perspectives

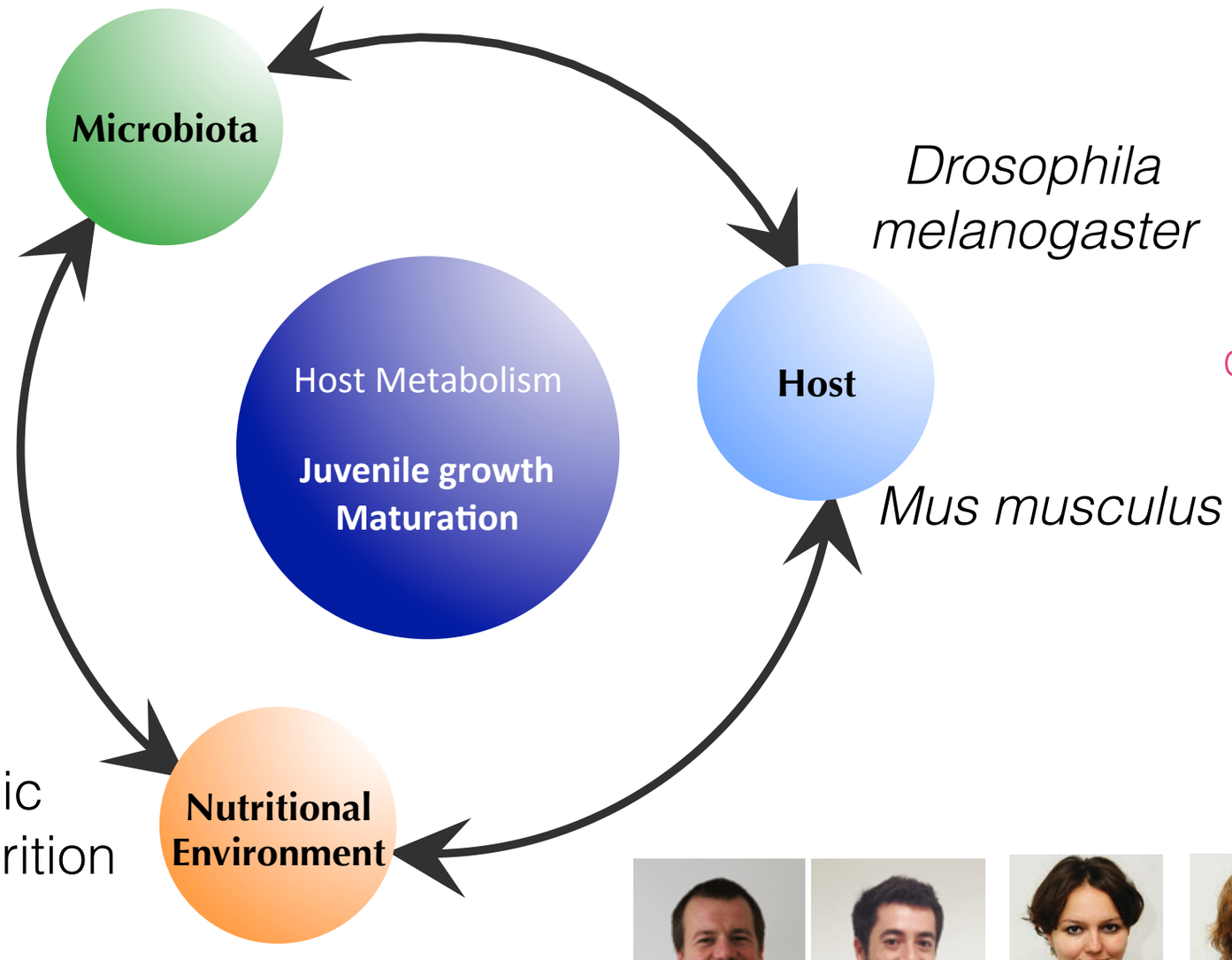
How *Lp*^{WJL} impinges on the somatotropic axis activity

Translate our results into products for animal breeding industry and the human probiotic market

« Functional Genomics of Host-Intestinal Bacteria interactions »

PI: François Leulier @LeulierLab

Lactobacillus plantarum



Drosophila melanogaster

Mus musculus

Chronic undernutrition

Lacto side

Renata Matos (post-doc)

Maria-Elena Martino (post-doc)

Pauline Joncour (BioInfo)

Fly side

Cathy Ramos (lecturer)

Dali Ma (post-doc)

Théodore Grenier (Ph'D)

Claire-Emmanuelle Indelicato (Ph'D)

Mouse side

Pierre Poinsot (Ph'D)

Anne Lambert (Tech)

Stéphanie Geoffroy (Tech)

Anne-Laure Bulteau (Staff Scientist)

Past members

Martin Schwarzer (post-doc)

Kassem Makki (post-doc)

Berra Erkosar (post-doc)

Gilles Storelli (Ph'D)