Housing of laboratory mice in a natural habitat

influence on immunity, gut microbiota and colorectal cancer development

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DVO Årsmøde, Københavns Universitet 13 juni 2023

Background

Laboratory mice in research

- Dominating model to study human biology in an organismal setting
- Housed under strictly hygienic conditions
- Derived from the common house mouse (*Mus musculus* spp.)

wild

lab

Background

- Germ-free mice – no microbiota – underdeveloped immune system[1]
- Wild mice – diverse microbiota – immunological steady state different from lab mice[2-4]

wild **lab**

high Microbial exposure **low**

high Immunological experience **low**

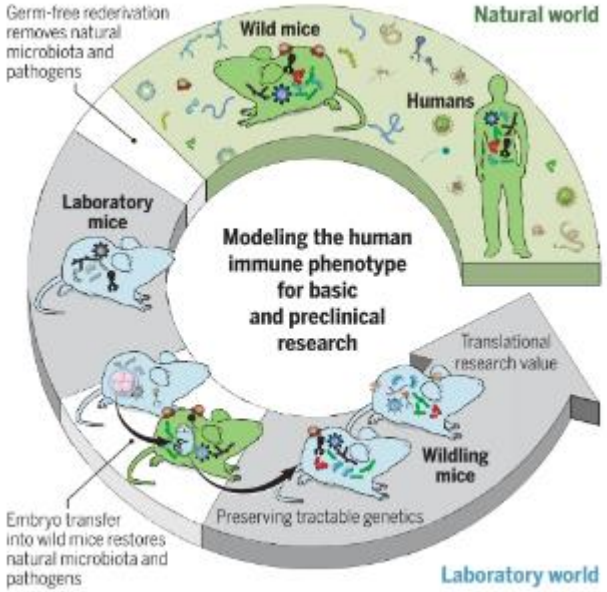
high Disease resistance? **low**

[1]Round JL *et al.* Nat Rev Immunol 2009
[2]Boysen P et al, Mol Ecol. 2011
[3]Weldon L *et al.* PLOS ONE 2015
[4]Abolins *et al.* PLoS Biol 2018
[5]Abolins S *et al.* Nat Commun. 2017

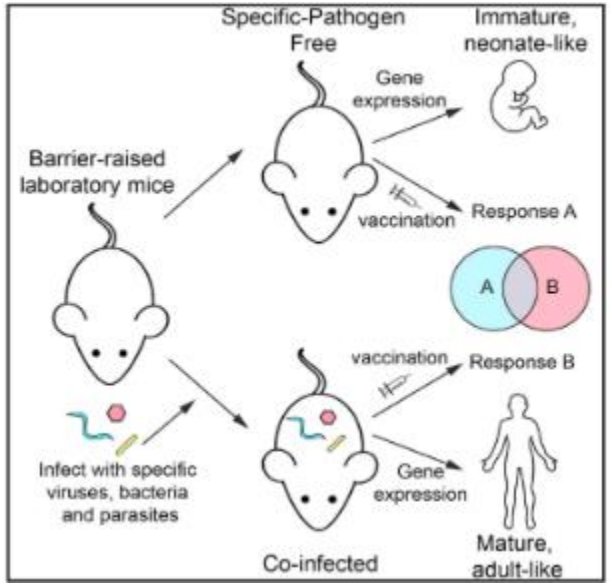
Background

“Naturalization” of laboratory mice

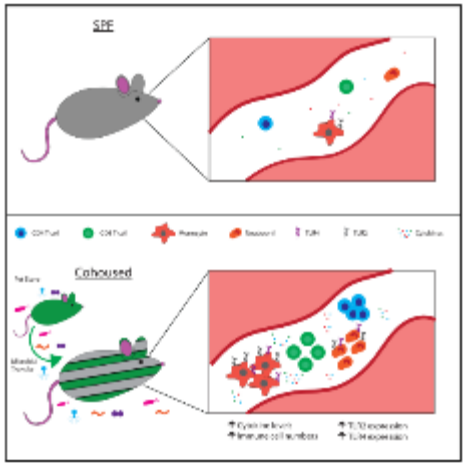
- Microbiota transfer from wild mice - “Wildlings”
- Infection history
- The whole animal by alternative housing:
  - Co-housing with pet store mice – “Dirty mice”
  - Outdoor housing in large enclosures - “Re-wilding”
  - **Our approach: Housing in farmyard-like habitat - “Feralization”**



Rosshart SP *et al*. Science 2019



Reese TA *et al*. Cell Host Microbe 2016



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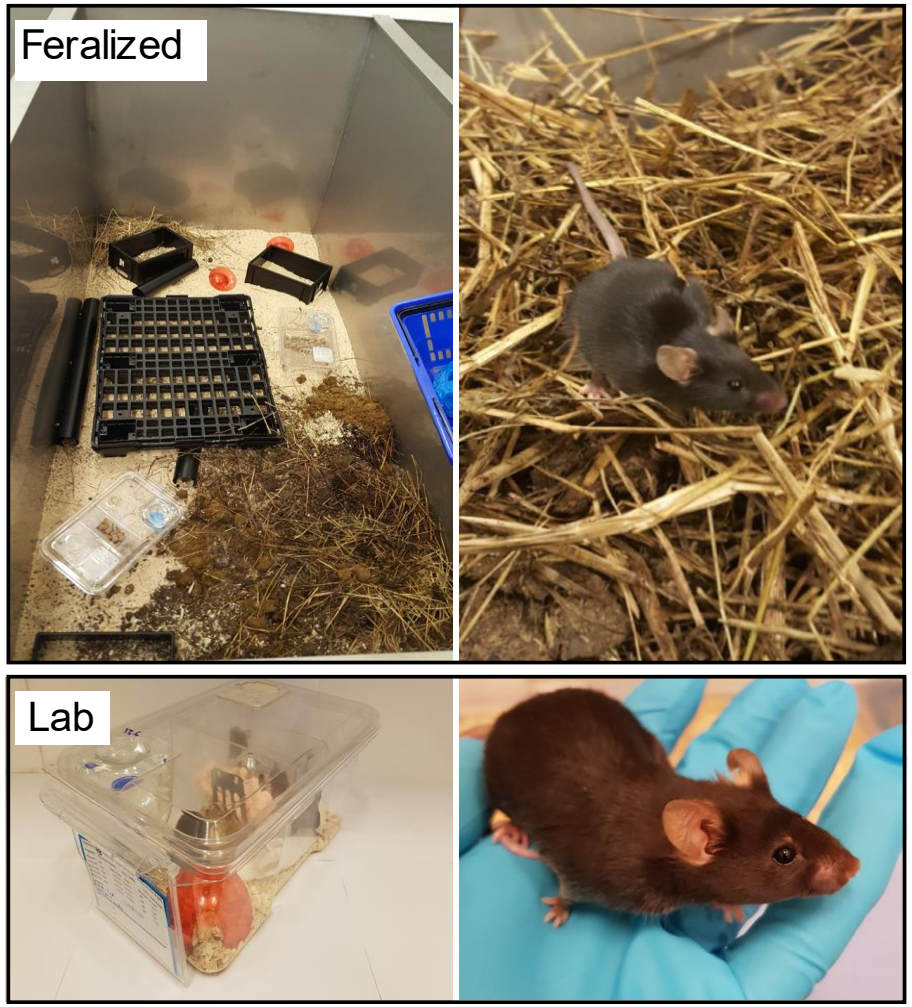
Yeung F *et al*. Cell Host Microbe 2020

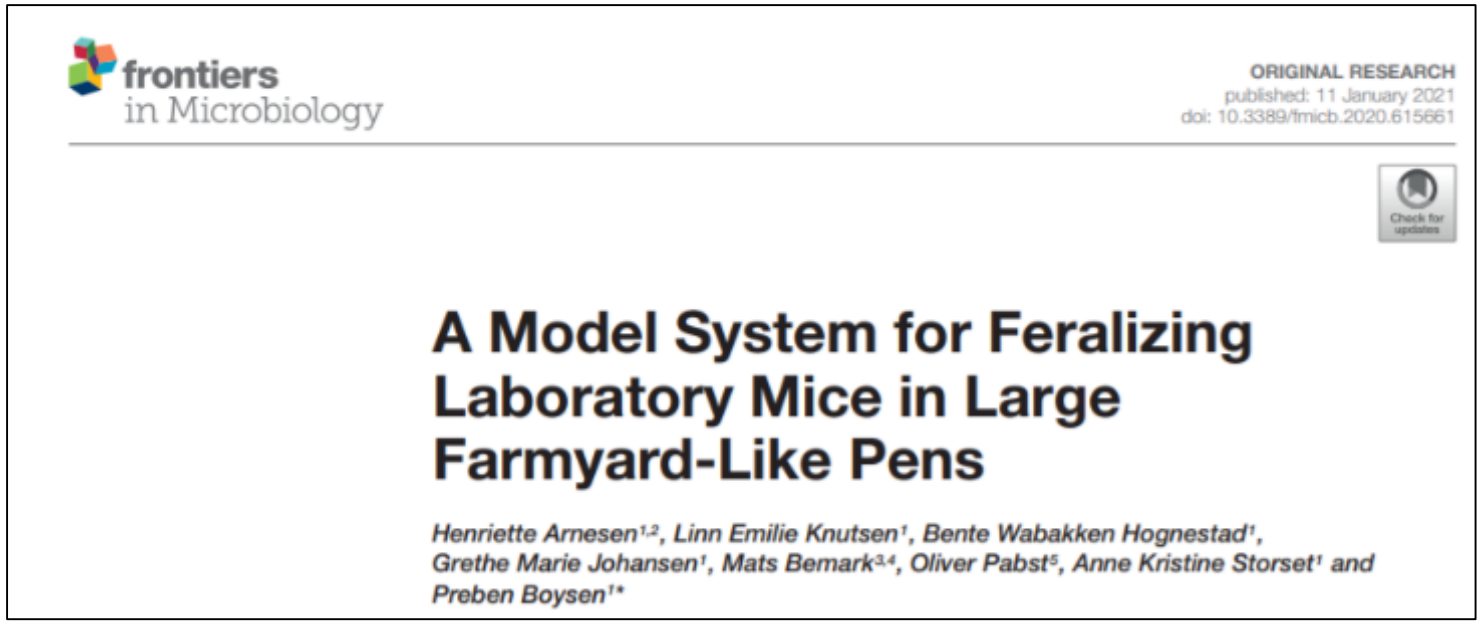
Background



The “feralization” model

- “Back to the roots”: *Mus musculus* typically dwell near domestic animals and people.
- Housing in large “mouse pens” enriched with material representing such a habitat.
- Feralization = Passive transfer and colonization of “natural” microbes (undefined consortia) to laboratory mice

Model establishment



frontiers in Microbiology

ORIGINAL RESEARCH
published: 11 January 2021
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Check for updates

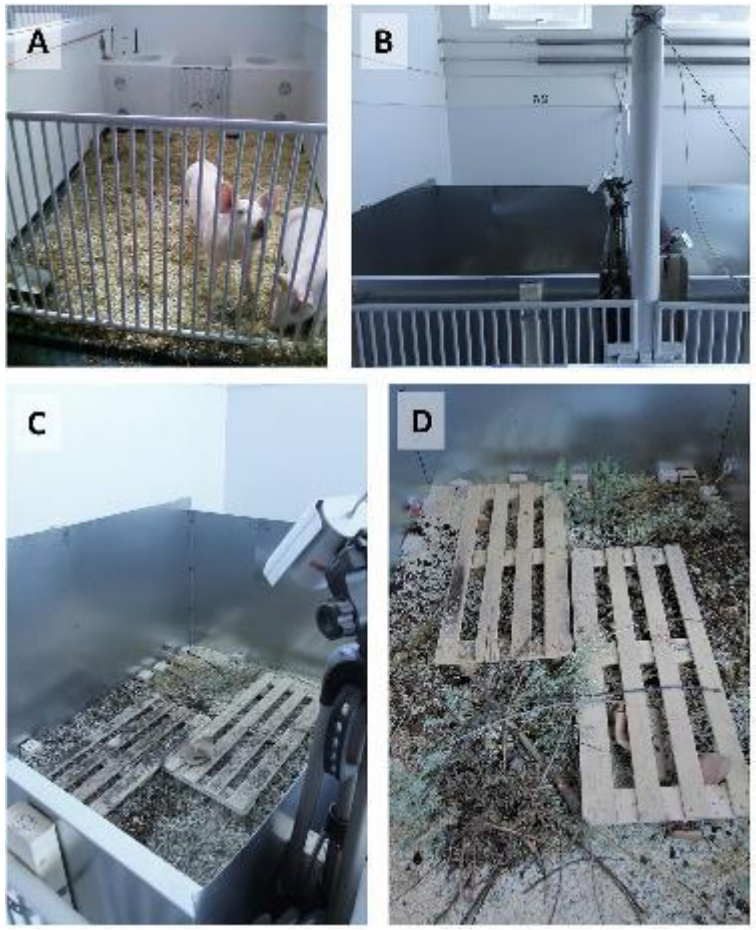
A Model System for Feralizing Laboratory Mice in Large Farmyard-Like Pens

*Henriette Arnesen[1,2], Linn Emilie Knutsen[1], Bente Wabakken Hognestad[1], Grethe Marie Johansen[1], Mats Bemark[3,4], Oliver Pabst[5], Anne Kristine Storset[1] and Preben Boysen[1]**

- Was this doable?
- How would feralization influence general immune phenotypes and gut microbiota?
- First: "All-in" approach: Farmyard-like habitat and co-housing with wild-caught mice.

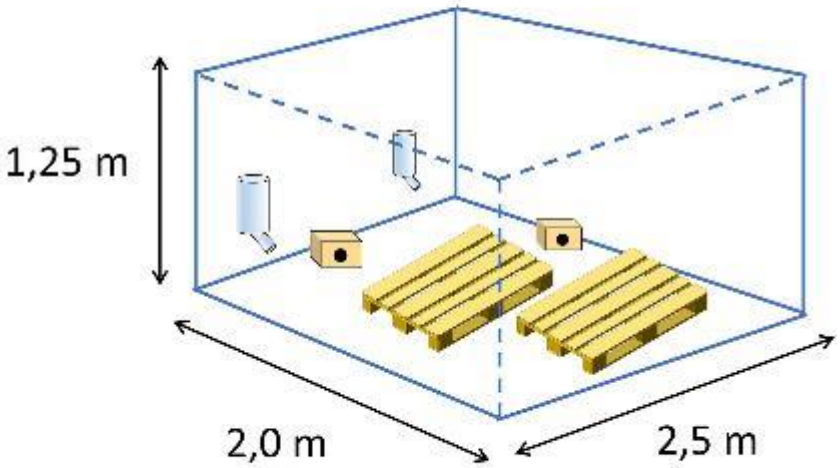
Model establishment

Experimental setup



- Feral mice = wild mice caught at farms around eastern Norway

E



Arnesen H et al. *Front Microbiol* 2021

Model establishment

Feralized mice approached a feral-like immunophenotype

Feral Feralized

B6 feralized with feral females in pen, seed diet

Feral ♀ *n*=8

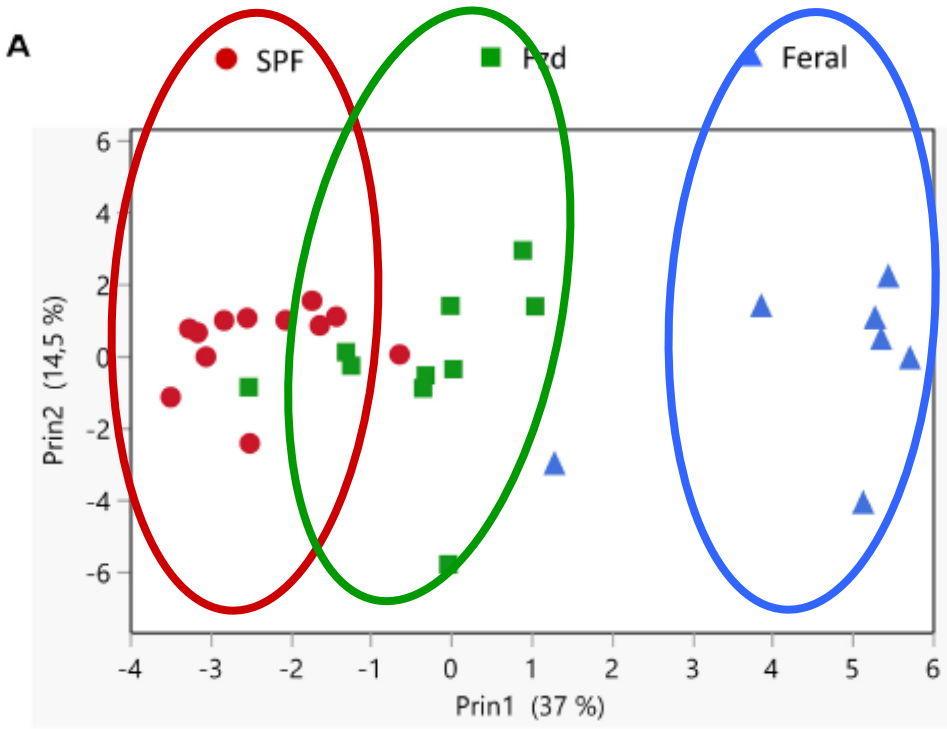
Fzd ♀ *n*=15 start age ≤ 28d.

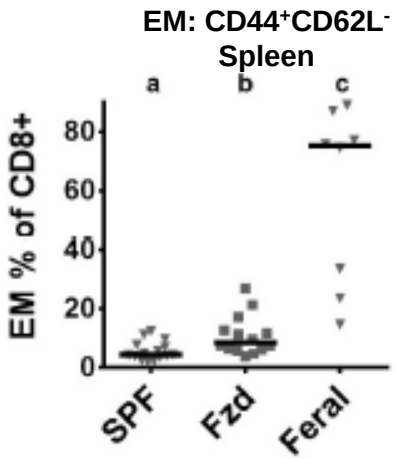
SPF (Lab)

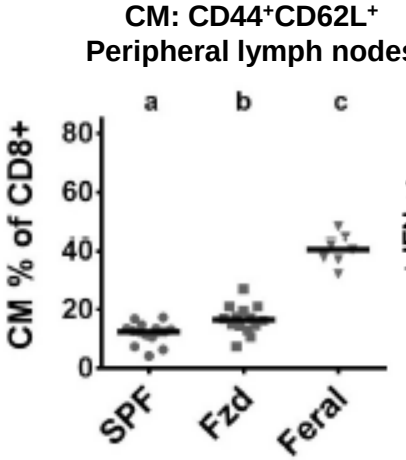
B6 females in SPF cages, chow diet

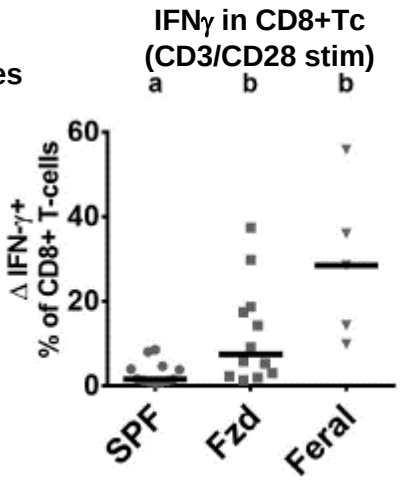
SPF ♀ *n*=15 start age ≤ 28d.

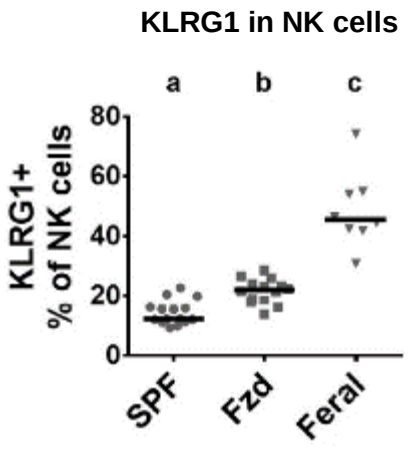
Combined set of immune parameters:







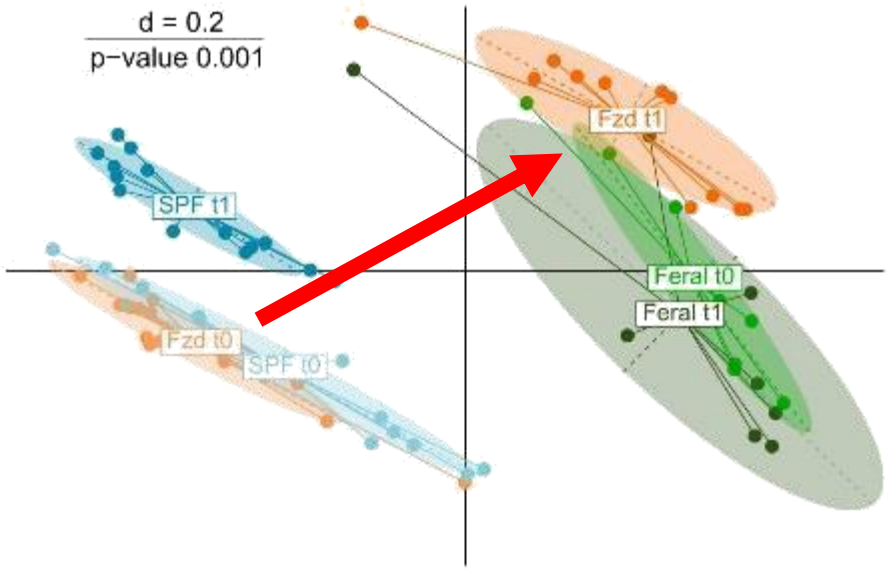


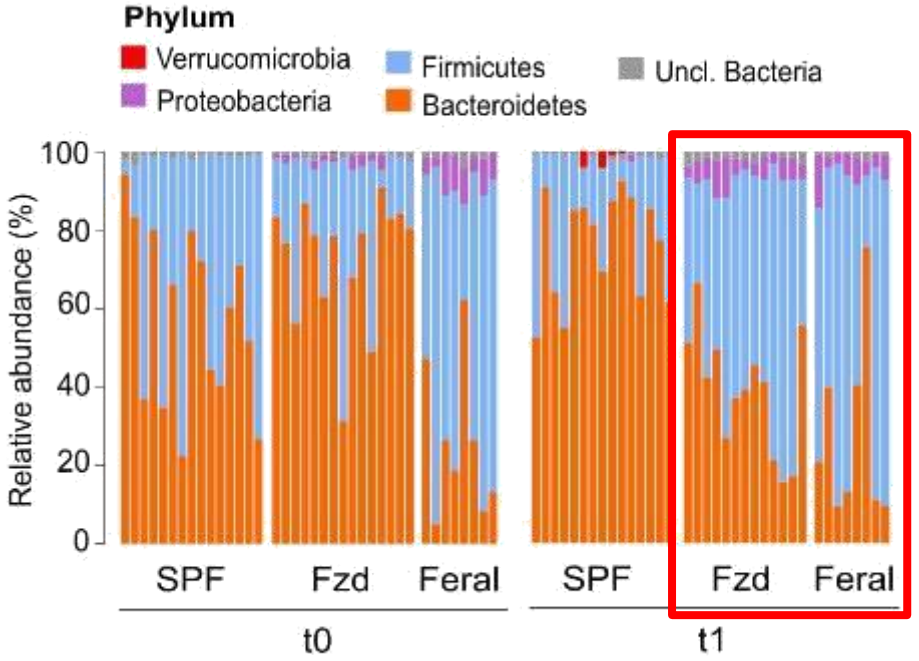


Arnesen H et al. *Front Microbiol* 2021

Model establishment

- Feralized mice approached a feral-like microbiota (Fzd = feralized)





Next step: Performance in disease models?

Colon cancer experiment

GUT MICROBES
2021, VOL. 13, NO. 1, e1993581 (22 pages)
https://doi.org/10.1080/19490976.2021.1993581

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RESEARCH PAPER

OPEN ACCESS Check for updates

Naturalizing laboratory mice by housing in a farmyard-type habitat confers protection against colorectal carcinogenesis

Henriette Arnesen[a,b], Thomas C. A. Hitch[c], Christina Steppeler[d], Mette Helen Bjørge Müller[d], Linn Emilie Knutsen[a], Gjermund Gunnes[a], Inga Leena Angell[b], Ida Ormaasen[b], Knut Rudi[b], Jan Erik Paulsen[d], Thomas Clavel[c], Harald Carlsen[b], and Preben Boysen[a]

- Characterize how feralization may influence immune and gut microbiota profiles.
- This time: farmyard-type habitat without feral mice.
- Assess if feralization influences neoplastic development in the mouse colon.
- Evaluate the role of feralization timing and thus microbial encounter.

Arnesen H et al. *Gut Microbes* 2021

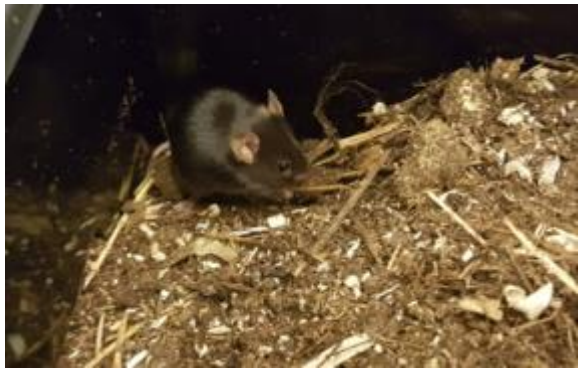
Colon cancer experiment

Experimental setup

- Two trials employing different mouse models of CRC

A/J Min/+ mice: Spontaneous (genetic) induction

AOM/DSS in B6 mice: Chemical induction



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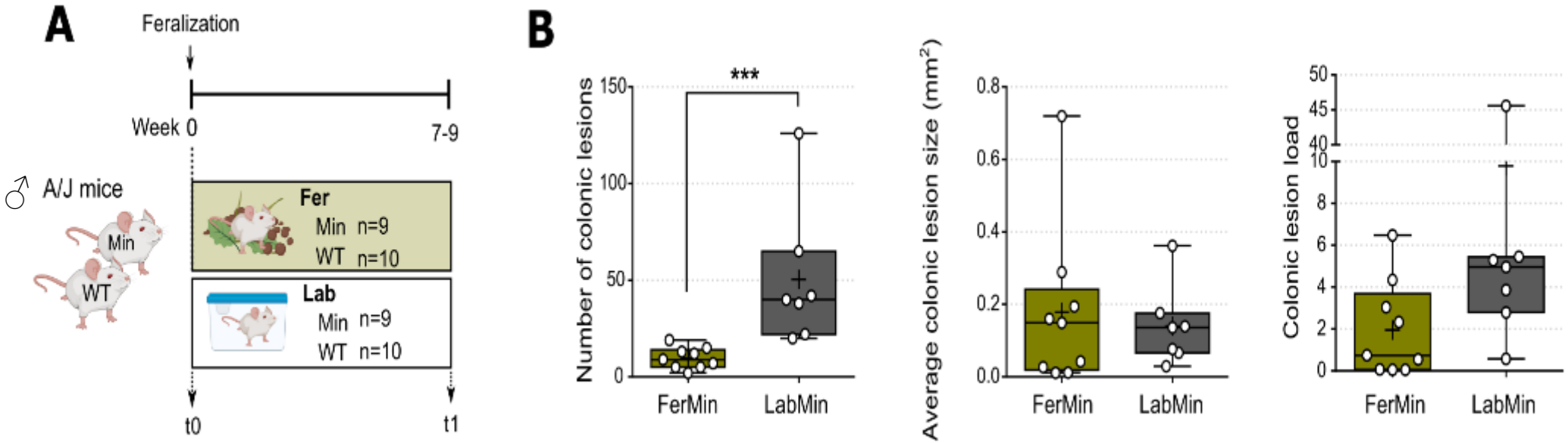
Colon cancer experiment

A/J Min/+trial



- Feralization of A/J Min/+ mice led to diminished spontaneous colonic lesion formation

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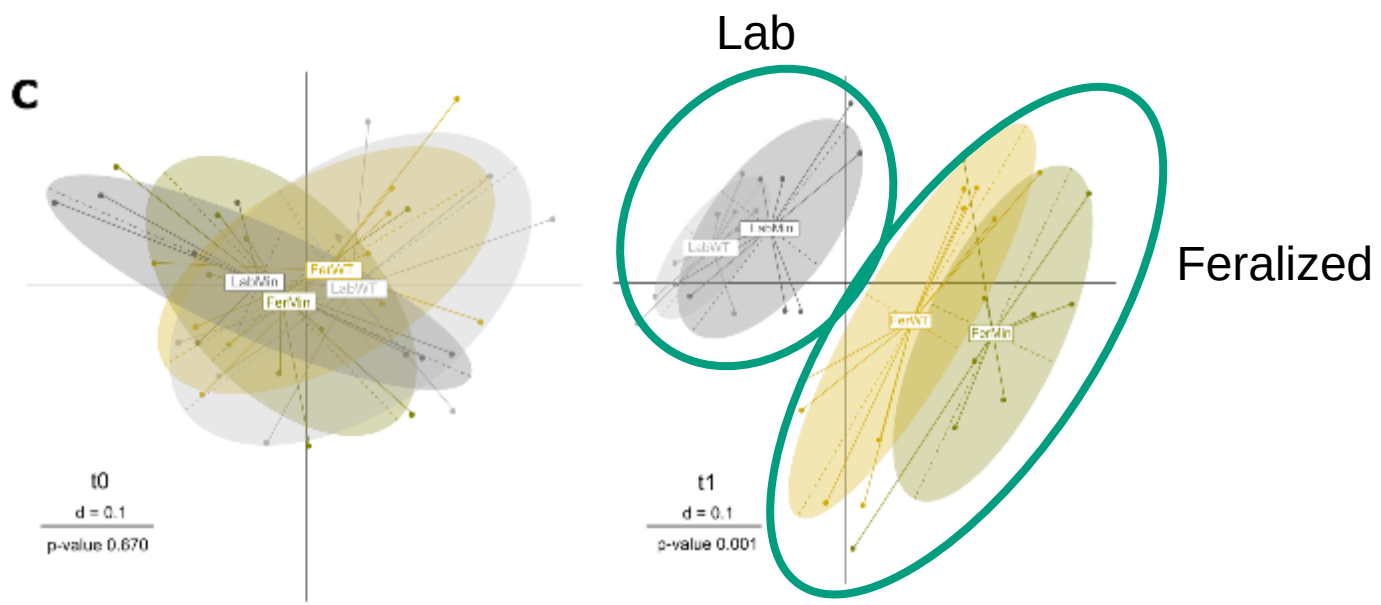
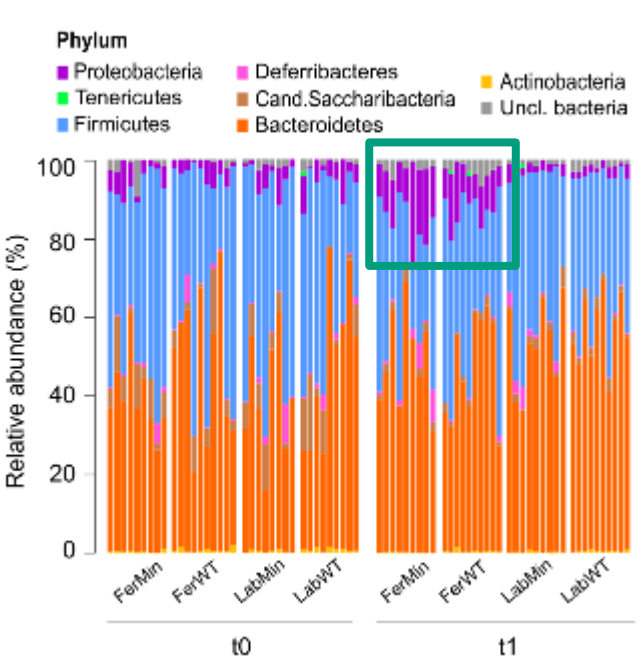
Colon cancer experiment **A/J Min/+trial**

- Feralization in absence of feral mice altered the mouse gut microbiota profile





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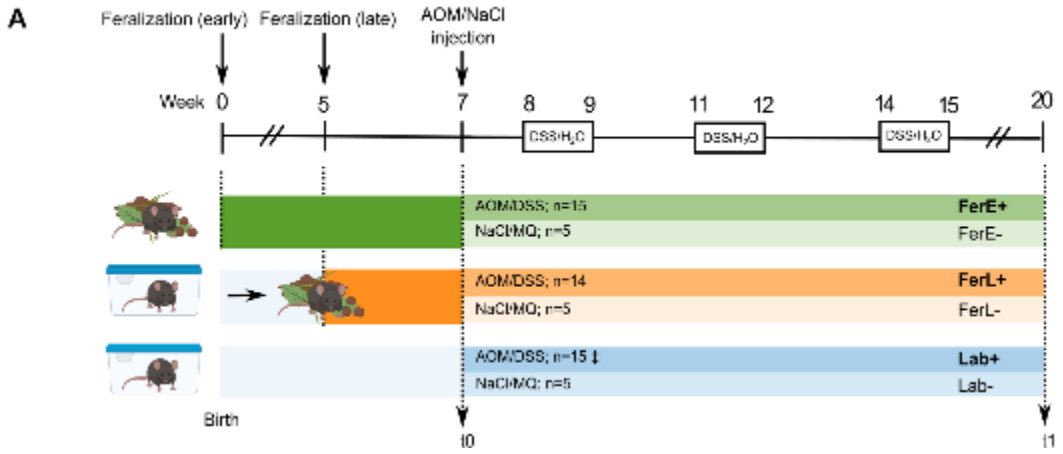
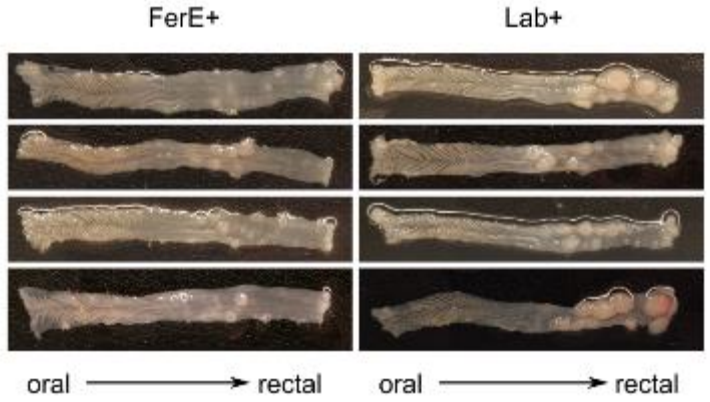
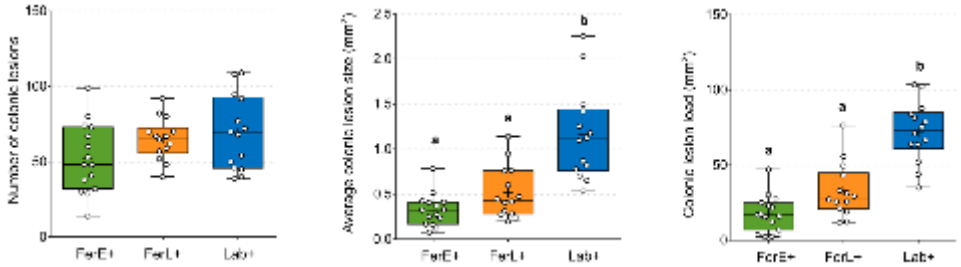
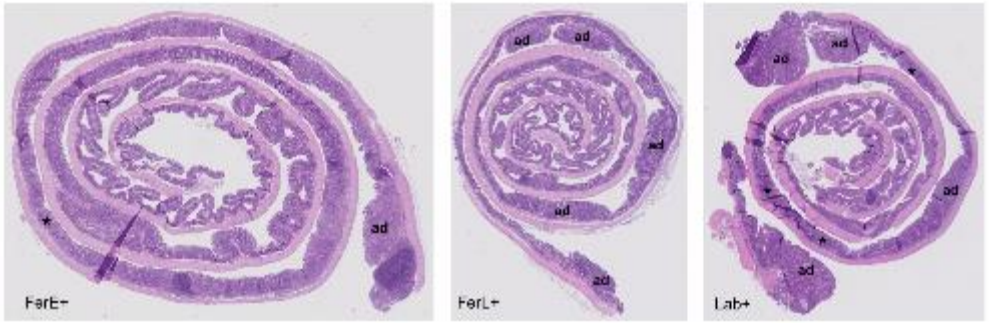
Colon cancer experiment **AOM/DSS trial**









- Feralization conferred protection against mutagen- and colitis-induced carcinogenesis in B6 mice
- Early microbial encounter was not decisive in mitigating the CRC outcome

	FerE+		FerL+		Lab+	
Hyperplasia	5/6	1.33 (1.03)	6/6	2.17 (1.17)	6/6	3.83 (3.06)
Adenoma	6/6	1.50 (0.84)	5/6	4.50 (3.62)	6/6	5.00 (3.46)
Carcinoma	0/6	-	0/6	-	0/6	-
Total	6/6	2.83 (1.33)*	6/6	6.67 (3.67)	6/6	8.83 (5.04)*

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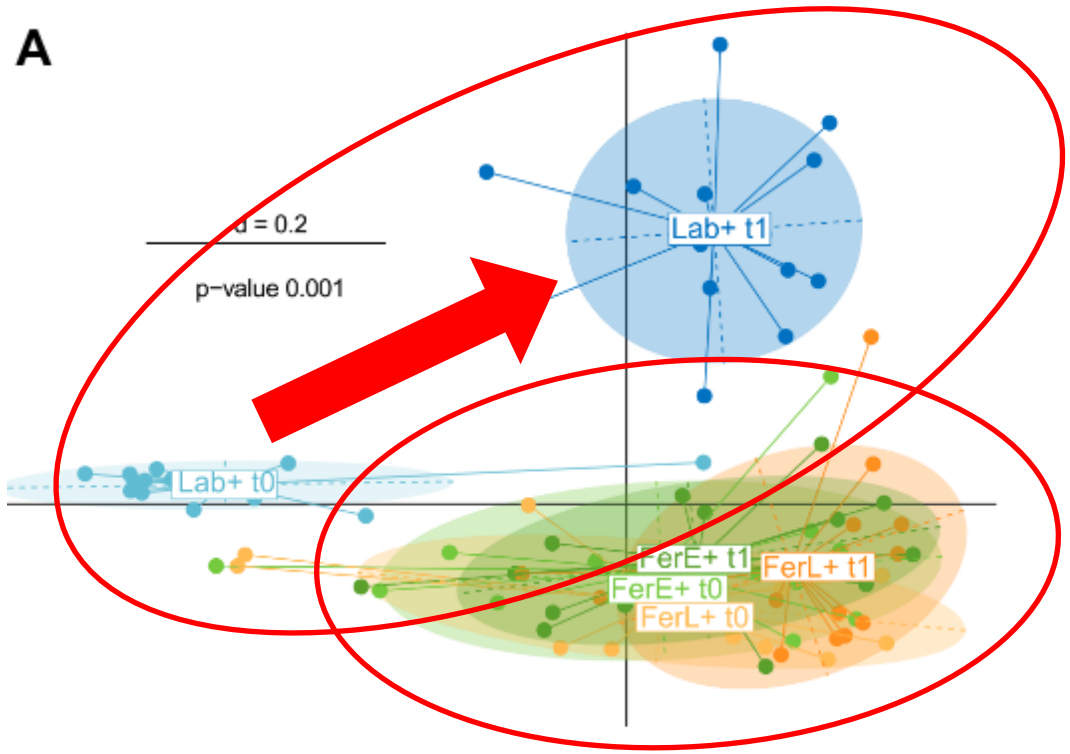
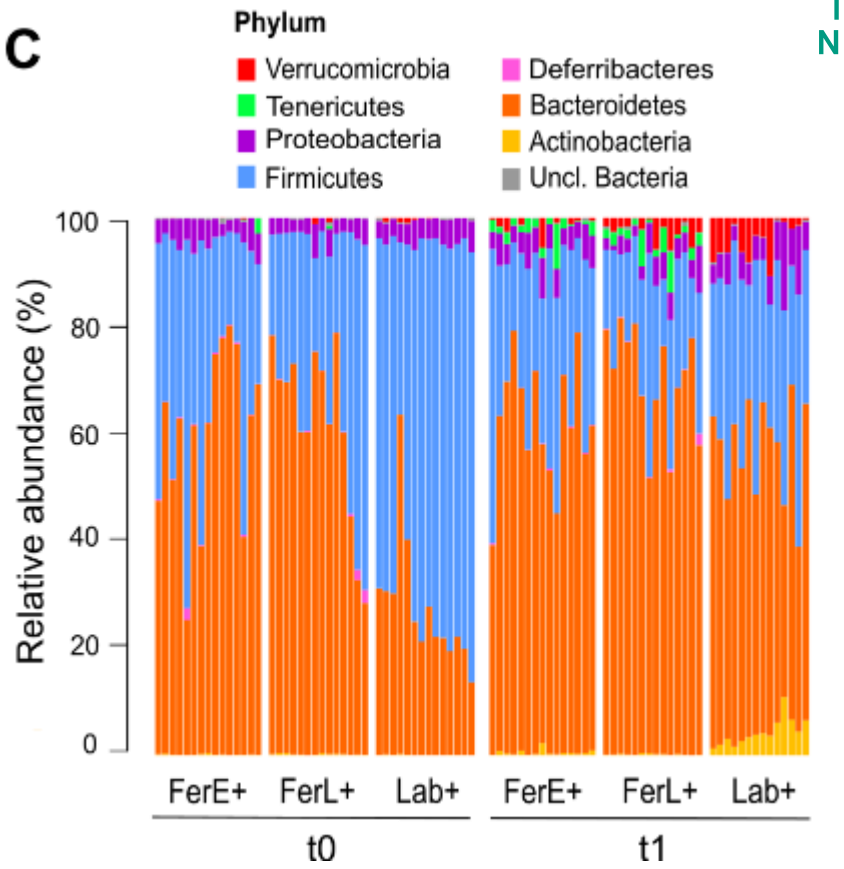
Colon cancer experiment **AOM/DSS trial**





- Feralized gut microbiota this time characterized by high bacteroidetes – low firmicutes.
- Feralized mice demonstrated a highly stable gut microbiota structure as opposed to lab mice.

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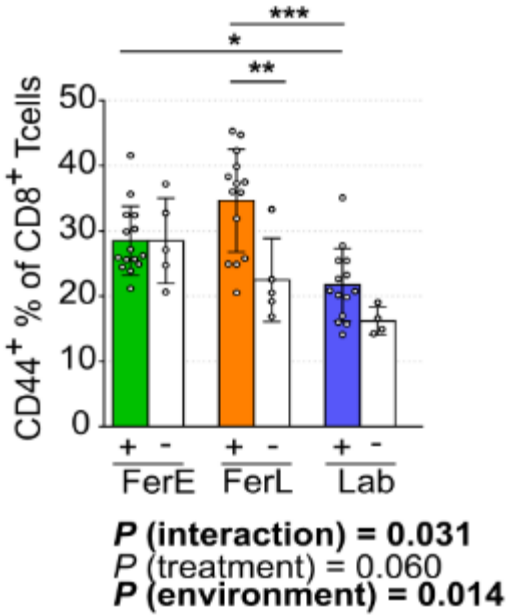
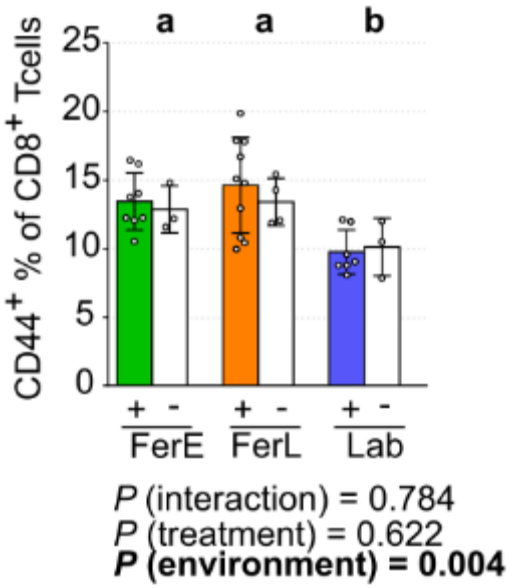
Colon cancer experiment **AOM/DSS trial**

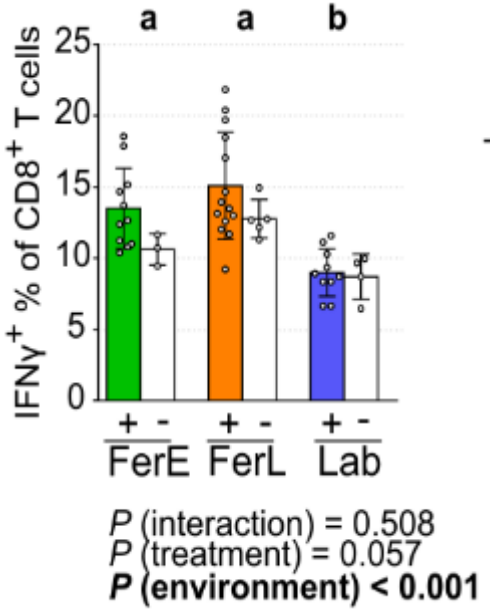
- Feralized mice immune cells showed increased expression of maturation markers
- CD8⁺ T cells had increased IFNγ response to *ex vivo* stimuli



Spleen



mLNs



mLNs

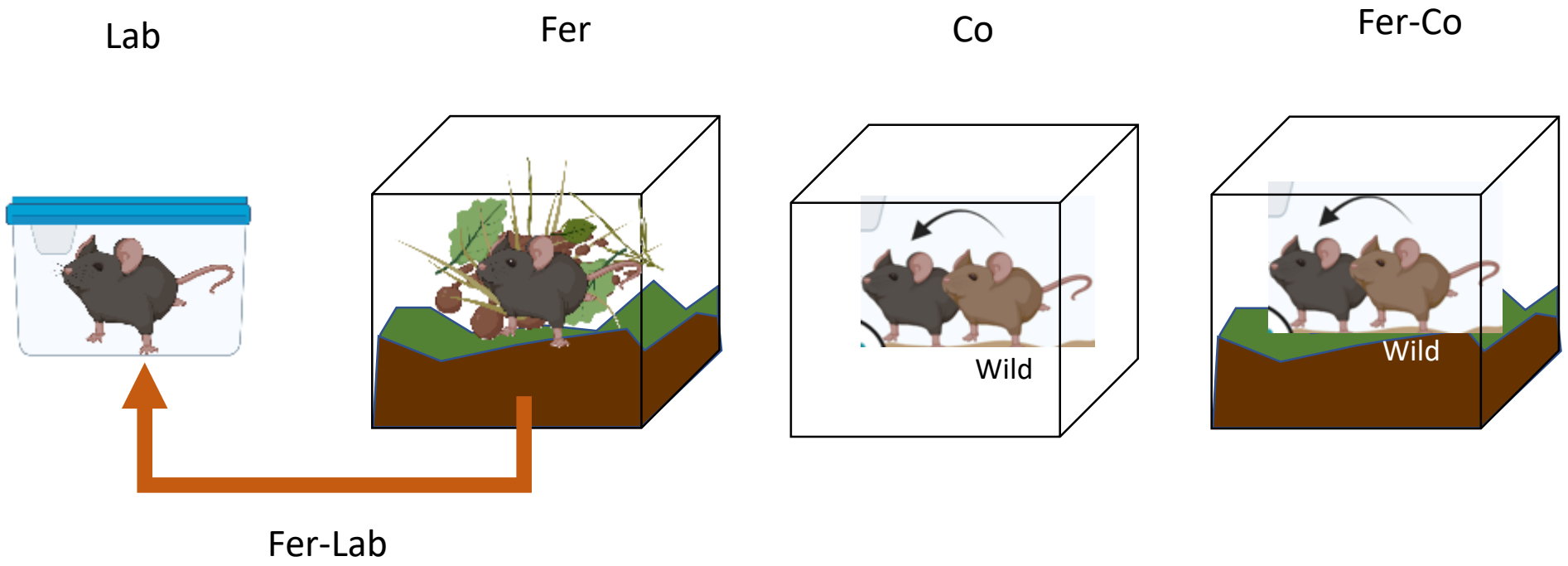
Arnesen H et al. *Gut Microbes* 2021

Mucus quality and mucosal transcriptome

Results to be published

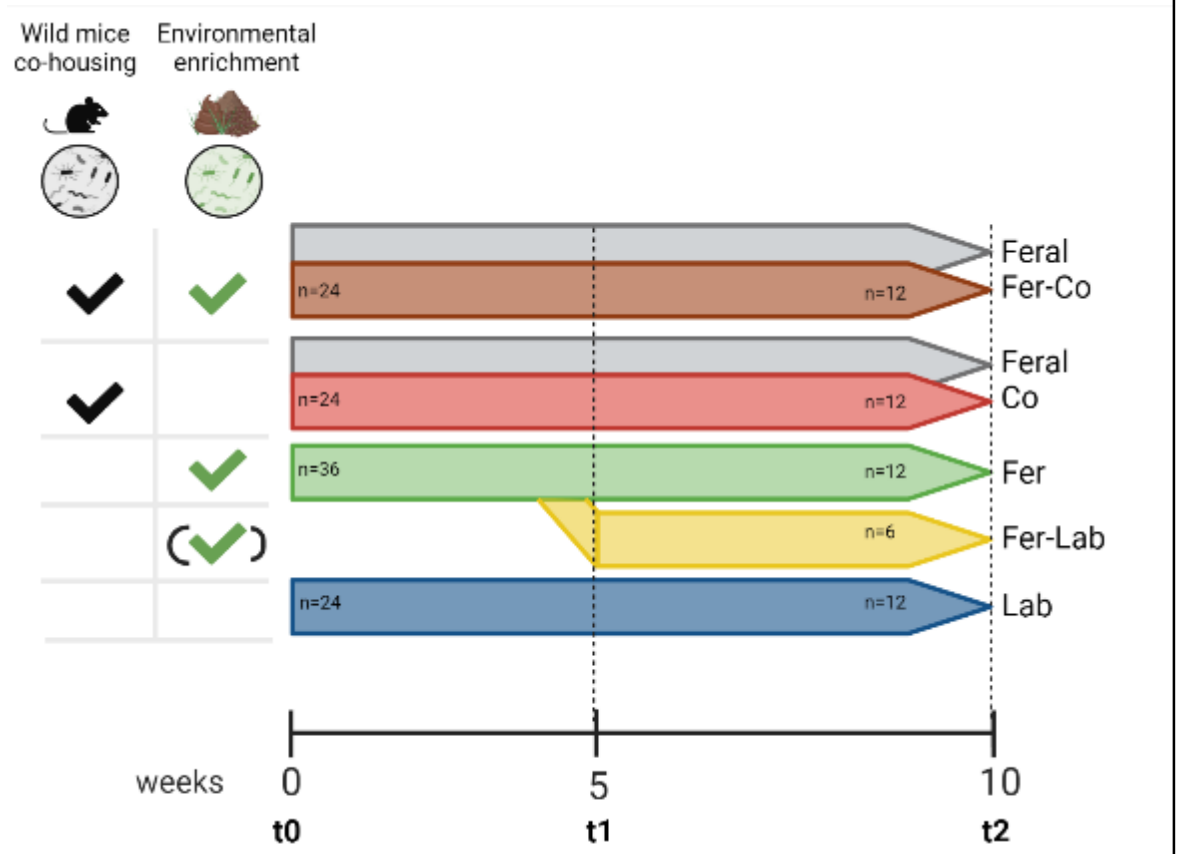
Modes of Feralization (unpublished)

- What is the relative contribution of farmyard environment and co-housing with wild mice on immunophenotype/microbiota/intestinal barrier functions?



Modes of Feralization

Experimental setup



Hypotheses

1. The housing environment and feral mice contributes differentially to the composition of the feralized mice gut microbiota

2. Feralized mice establish a gut microbiota that remains stable after feralization is discontinued

Modes of Feralization

Catching wild house mice

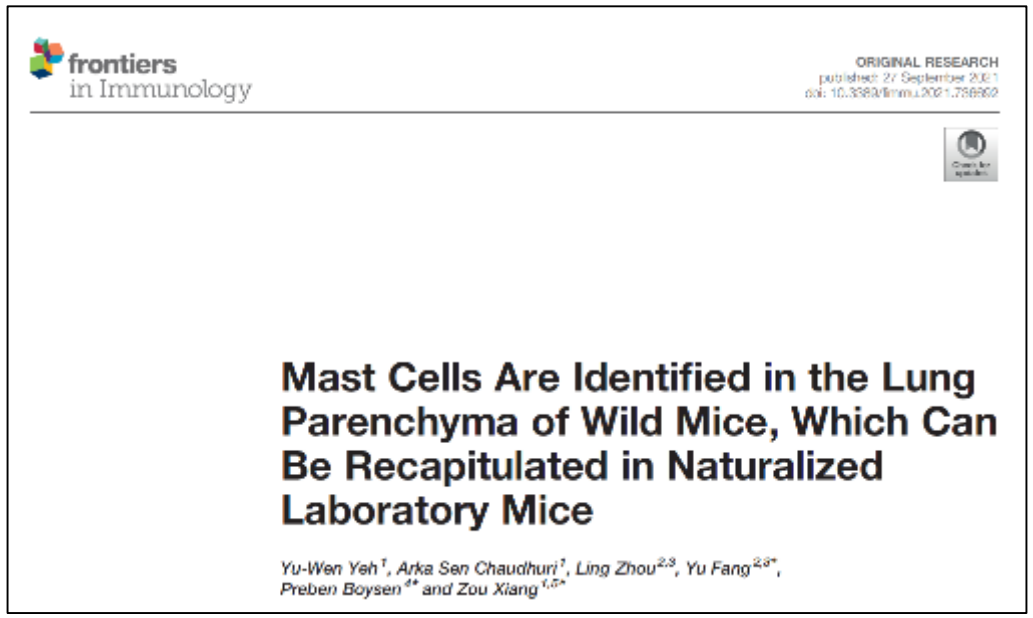
Modes of Feralization

Results to be published

Modes of Feralization

Results to be published

Mast cells emerge in lungs in wild and feralized mice

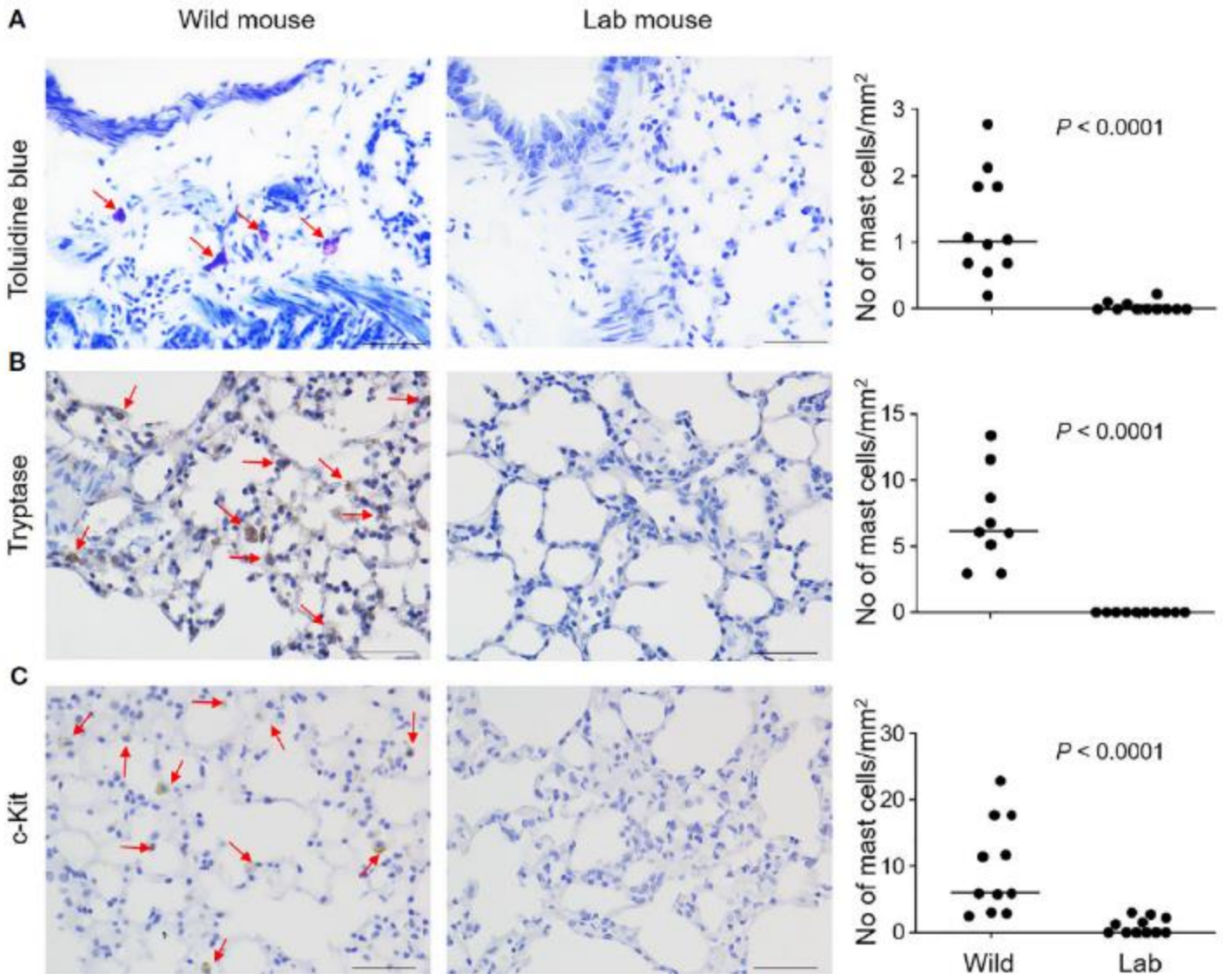

frontiers in Immunology

ORIGINAL RESEARCH

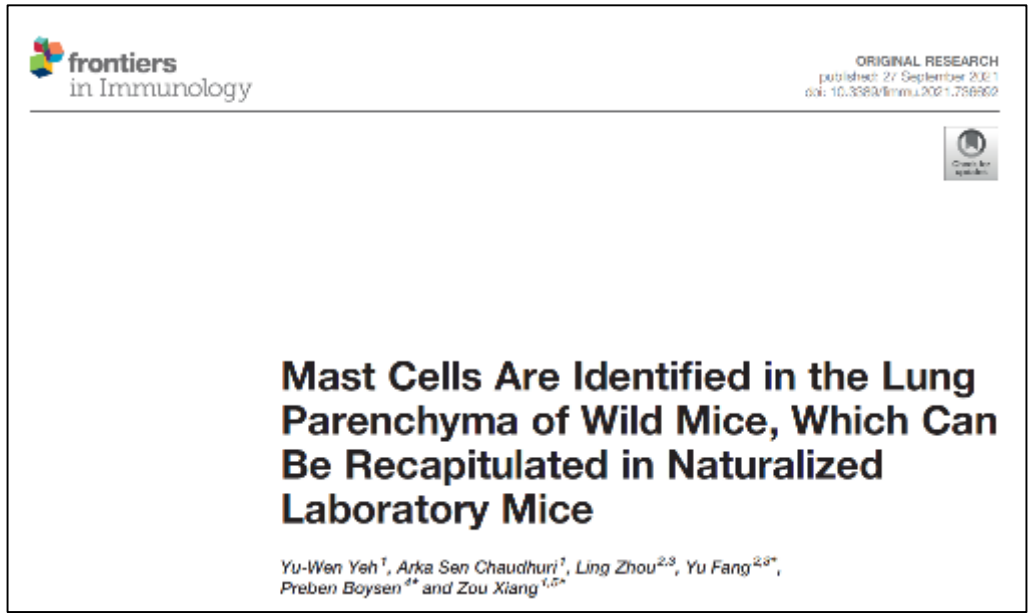
Mast Cells Are Identified in the Lung Parenchyma of Wild Mice, Which Can Be Recapitulated in Naturalized Laboratory Mice

Yu-Wen Yeh[†], Arka Sen Chaudhuri[†], Ling Zhou[2,3], Yu Fang[2,3], Preben Boysen[4]* and Zou Xiang[1,5]**

- Laboratory mice lack lung parenchymal mast cells and make poor asthma models.
- Wild mice have lung mast cells, like humans do.



Mast cells emerge in lungs in wild and feralized mice


frontiers in Immunology

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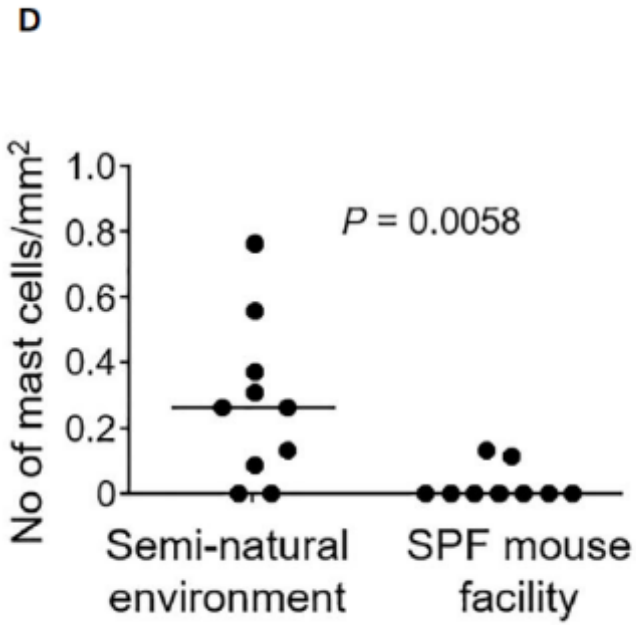
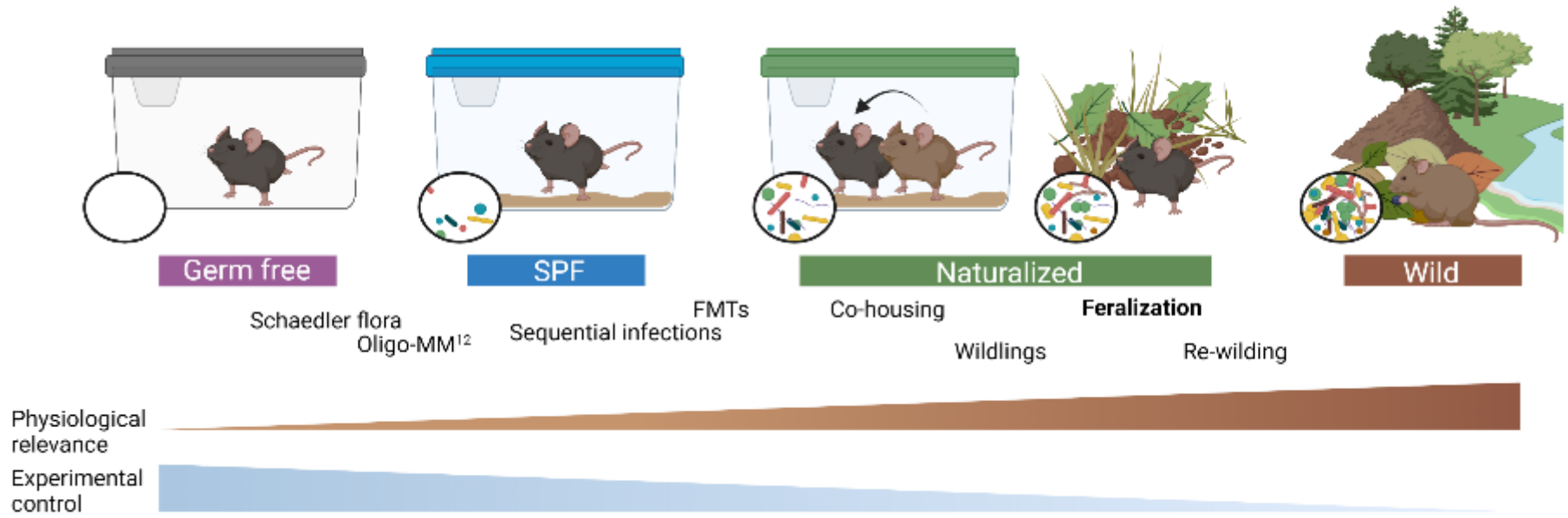


- Laboratory mice lack lung parenchymal mast cells and make poor asthma models.
- Wild mice have lung mast cells, like humans do.
- **Feralization leads to a similar presence.**

- **Restitution as asthma model?**

- Naturalized mice may be used to
  - Enhance the translational value of mouse experiments
  - Study the effect of the outer environment on complex processes
- Relevance at the expense of control and vice versa



People

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Bente Wabakken Hognestad (former student)
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The Livestock Production Research Centre (SHF)

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Oliver Pabst

Mats Bemark
Gunnar Hansson
George Birchenough

David Masopust
Ryan Langlois
& teams

NOCC grant

Leticia Monin
Adrian Hayday

Ramme gård (fecal donor)

& others!

Thank you!