Can we elucidate the specific probiotic mechanisms of action of lactobacilli by studying their persistence and dynamics in the gut ?

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Společnost pro probiotika a prebiotika

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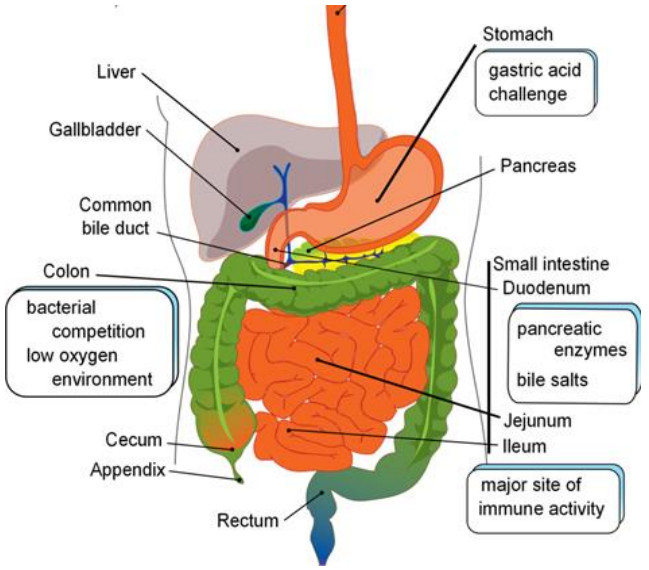
Université de Lille · cnrs · CIIL CENTER FOR INFECTION & IMMUNITY OF LILLE · Inserm La science pour la santé From science to health · CHU LILLE · Institut Pasteur de Lille Vivre mieux, plus longtemps

New experimental strategies to visualize bacteria in the intestine

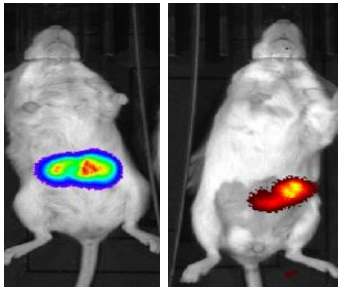
- **Intestine**: complex and heterogeneous environment
- Capacity to **survive and persistence** of LAB
- Invasive actions in humans
- Animal model

- Bioluminescence and fluorescence **imaging in living mice**
  - ✓ detection
  - ✓ follow-up and localization



Adapted from Daniel et al. 2011
Trends in Biotechnology

IVIS Lumina, Perkin Elmer

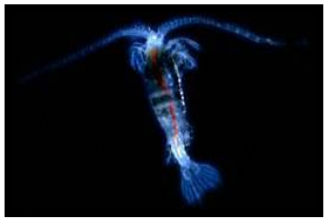


Interaction of administered probiotic microorganisms with host intestinal cells and microbiota ?

In vivo Bioluminescence imaging

- ✓ Initially developed for intestinal pathogens which replicate
- ✓ Production of various luciferases by genetic engineering

- Luc from the firefly/click beetle: protein of 60 kDa



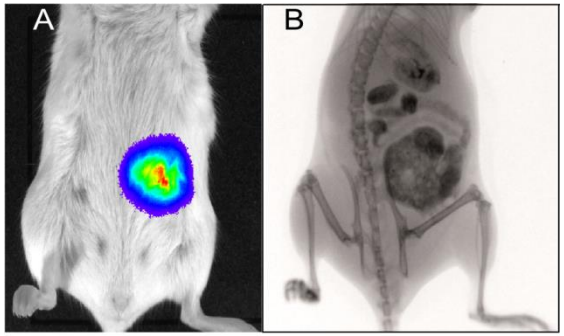
- Luc from marine organisms *Gaussia princeps* and *Renilla* (20 kDa)
- Luc from bacteria (heterodimeric proteins)

- ✓ Technically…

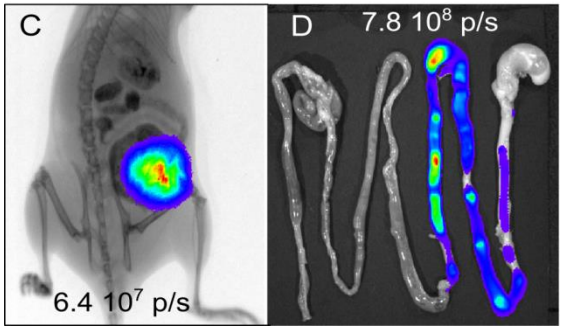
IVIS Lumina, Perkin Elmer, Lille
Small animal imaging system

In vivo imaging: persistence of LAB in mice

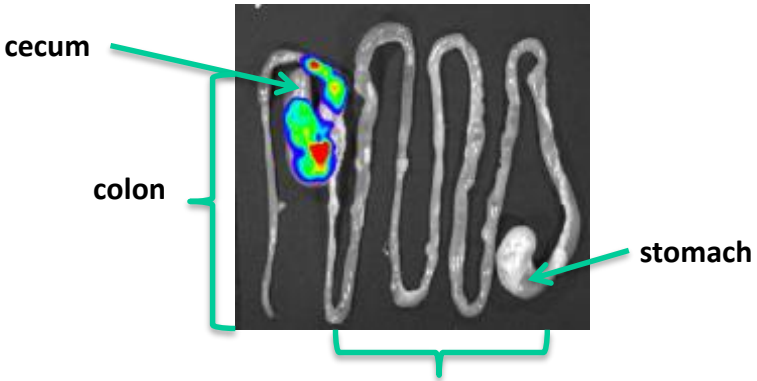
- ✓ Production of 3 luciferases in *L. lactis* MG1363 and *L. plantarum* WCSF1
- ✓ Detection of bioluminescent bacteria after **oral administration** (red luciferase)
- ✓ Bacteria transit in the DT and a small proportion of ***L. plantarum*** **persists** in the **colon and cecum**



Bioluminescence Barium sulfate (X-ray)



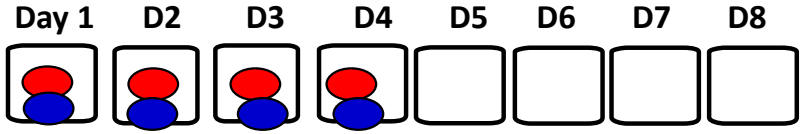
Bioluminescence +X-ray Dissection of GIT

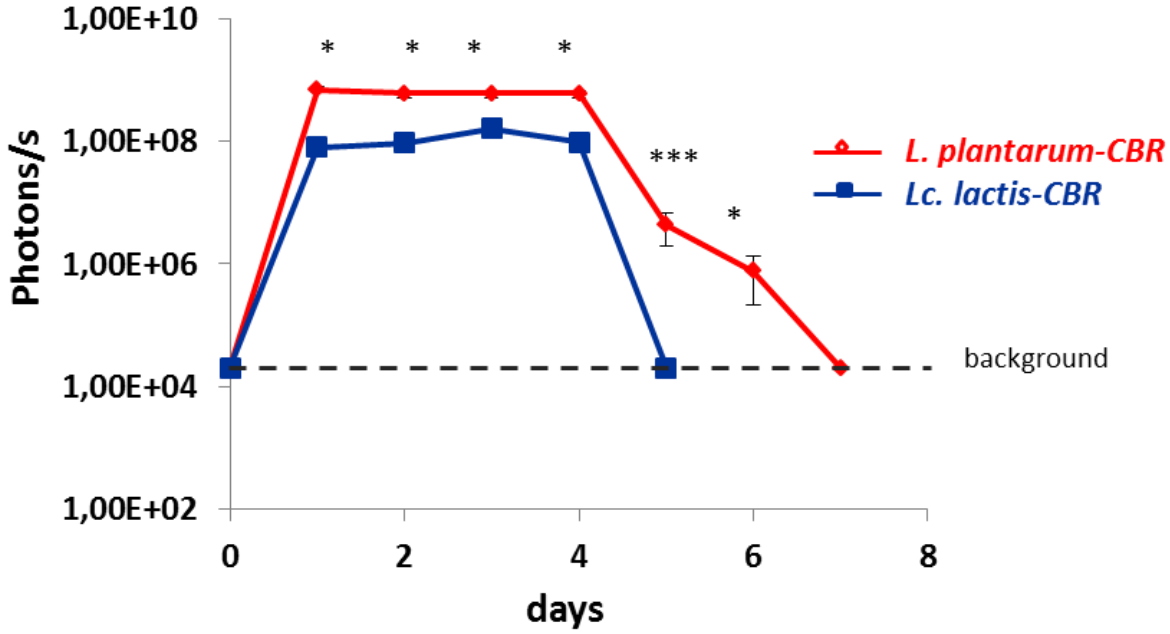


Daniel et al. 2013 ***Appl Environ Microbiol*** *and 2015* ***Appl Environ Microbiol***

In vivo imaging: persistence of LAB in mice

- ✓ Production of red luciferase in *L. lactis* MG1363 and *L. plantarum* WCSF1
- ✓ **High signal** for both strains day 1- day 4
- ✓ No signal for *L. lactis* on day 5
- ✓ *L. plantarum* till **day 7, colon and cecum**

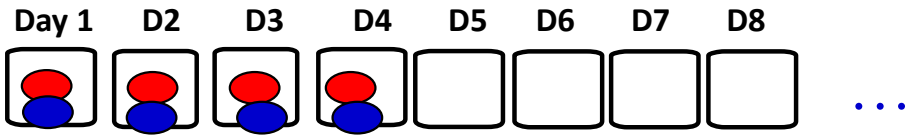


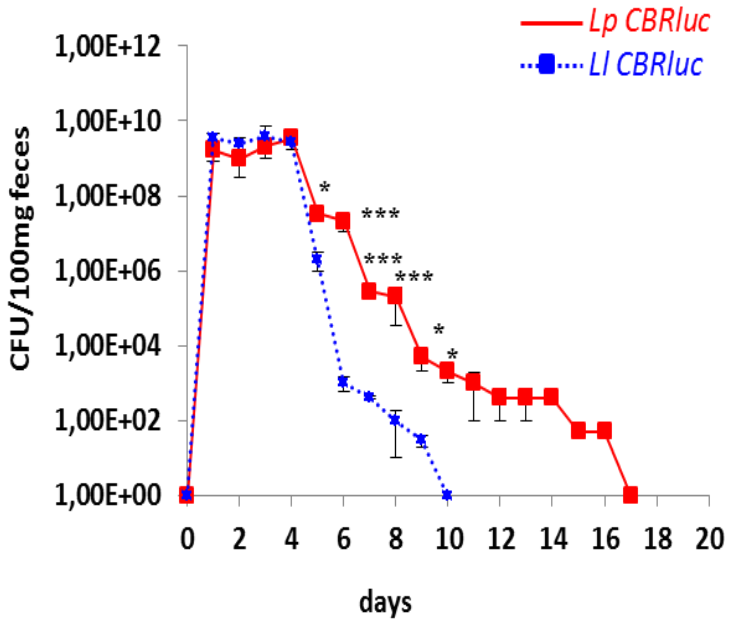


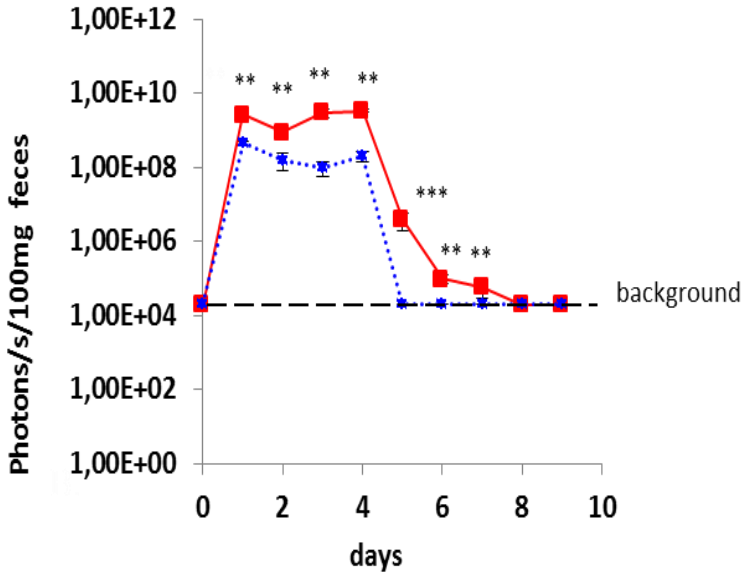
Daniel et al. 2013 ***Appl Environ Microbiol*** *and 2015* ***Appl Environ Microbiol***

Enumeration of *L. lactis* and *L. plantarum* in feces

- ✓ *L. plantarum* WCSF1 persists for 12 days after the last inoculation
- ✓ *L. lactis* detected at lower levels after day 4 and fewer days than *L. plantarum*
- ✓ Bioluminescent signal in feces similar to whole-body signal



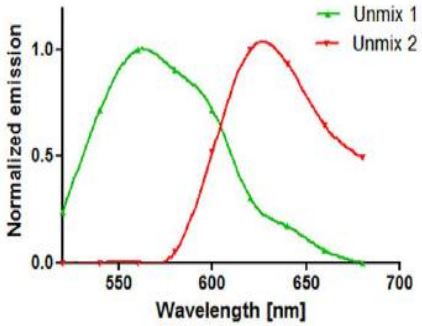




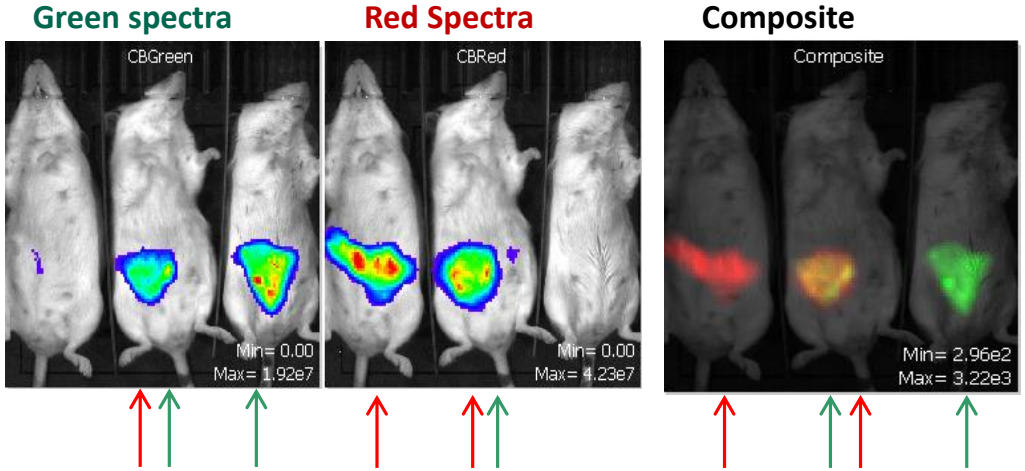
Daniel et al. 2013 ***Appl Environ Microbiol*** *and 2015* ***Appl Environ Microbiol***

Dual color bioluminescence imaging

- ✓ *L. lactis* and *L. plantarum* strains producing two luciferases from
  - **CBRLuc which emits in the red (620 nm)**
  - **CBGLuc emitting in the green (540 nm)**



- ✓ Follow-up of ***L. plantarum*** and ***L. lactis*** by double colour imaging with spectral unmixing



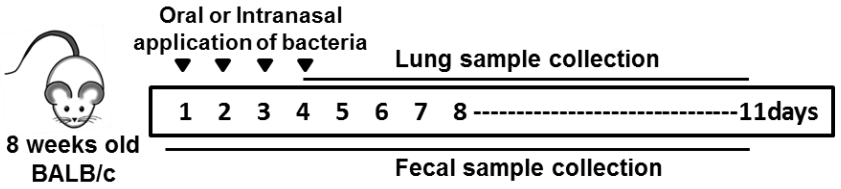
Daniel *et al*. 2015 ***Appl Environ Microbiol***

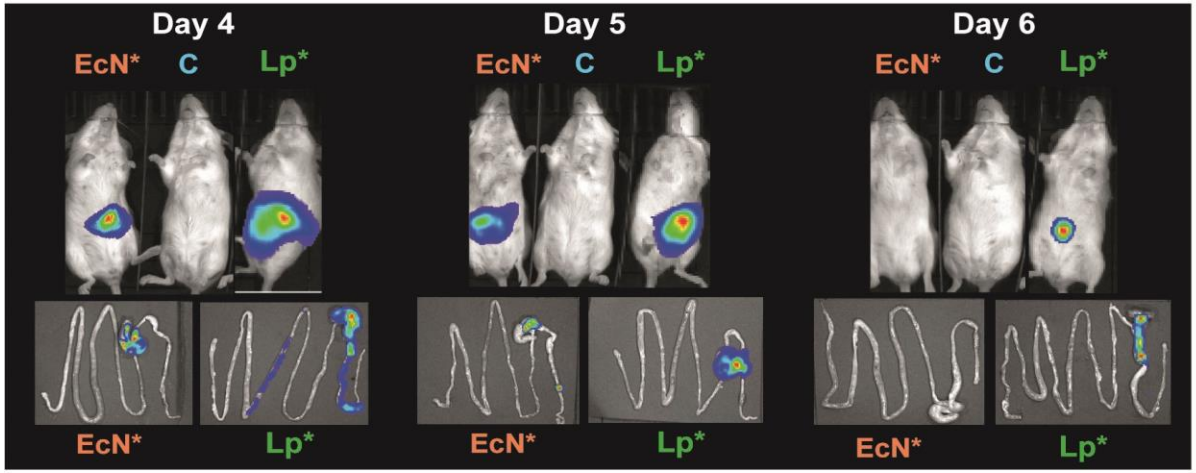
Persistence of LABs : intranasal versus oral application

- ✓ Production of red luciferase in *L. plantarum* WCSF1 and *E. coli* Nissle
- ✓ Oral/intranasal administration
- ✓ **Oral**: gut
- ✓ **Intranasal**: nose, lungs and gut (not the brain)
- ✓ Transient persistence along intestinal and respiratory tracts

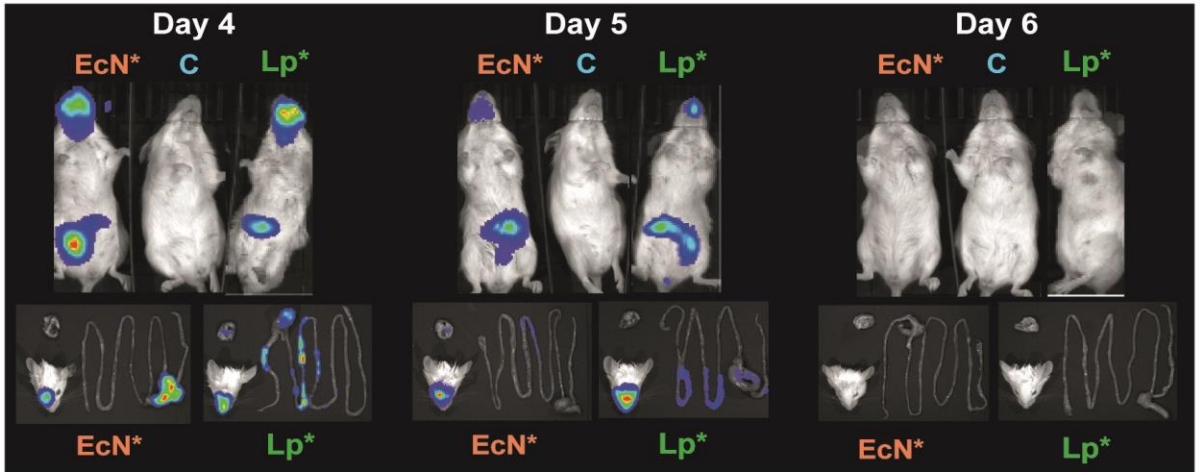
Effective immunomodulation ?

Oral





Intranasal



Sarate P *et al*. 2019 *Mucosal Immunol*.

Link with probiotic mechanisms ?

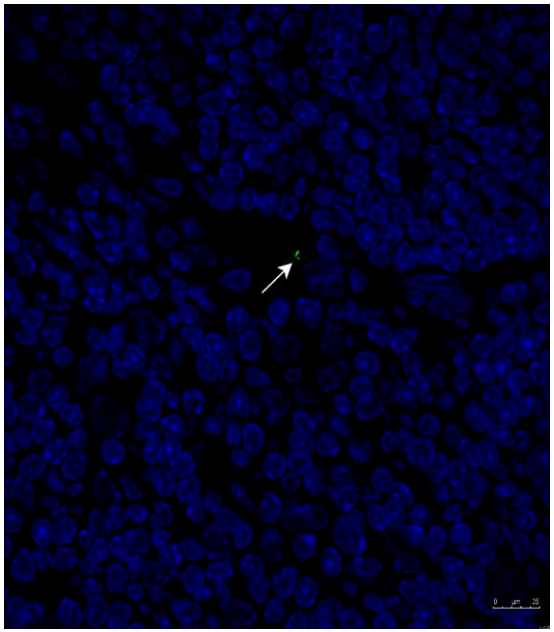
- ✓ Intranasal route more effective to prevent polysensitization (birch and grass pollens) *Sarate P et al. 2019 Mucosal Immunol*
- ✓ Transient persistence / intestinal and respiratory tracts
- ✓ Activation of the immune system at two mucosal compartments ?

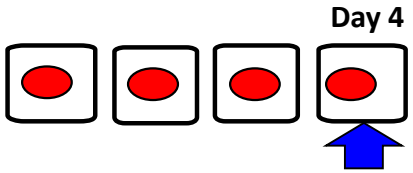
- ✓ **Collaborative project**:

  Is immune sampling of *L. plantarum* WCSF1 in the Peyer patches part of probiotic mechanism to induce immune changes in healthy mucosa ?

- ✓ True sampling of bacteria by M cells in PP is rare
- ✓ But DCs in the PPs are activated
- ✓ **Sensing via DCs in the PPs (by PRRs) enough to induce immunomodulation of DCs and T-cells (spleen + intestinal)**

Bermudez-Brito M *et al.*, 2018, *Scientific Report*

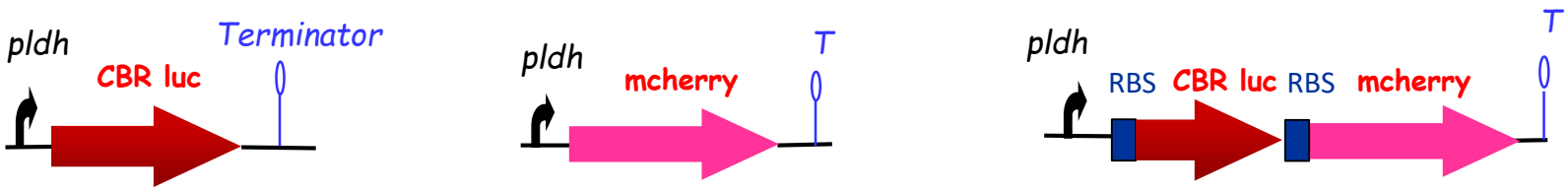




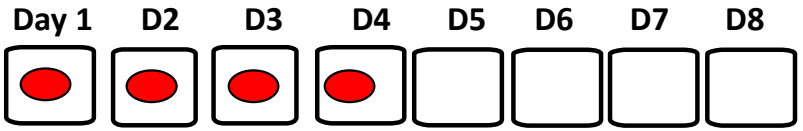
L. plantarum WCSF1

In vivo Fluorescence imaging

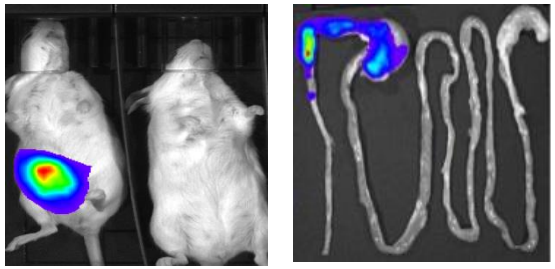
- ✓ Fluorescent proteins for imaging in the red/far red spectra → Ideal for small animal imaging/spectral unmixing
- ✓ Genetic engineering of LAB producing red/far red fluorescent proteins: *L. plantarum*, *L. lactis* (Berlec *et al*. 2015) and *L. reuteri* (Karimi *et al.* 2016)
- ✓ Genetic engineering of new *L. plantarum* strains producing fluorescent proteins and dual luciferase/fluorescent to combine bioluminescence imaging and fluorescence (microscopy)
- ✓ **Cellular level, link with probiotic mechanisms ?**

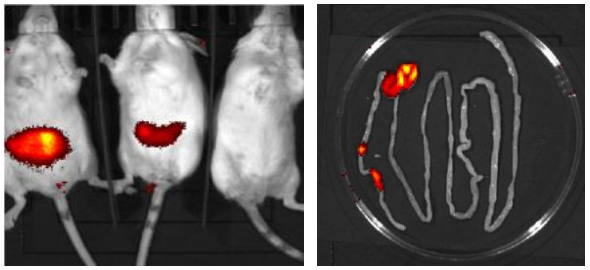


Bioluminescence versus fluorescence imaging

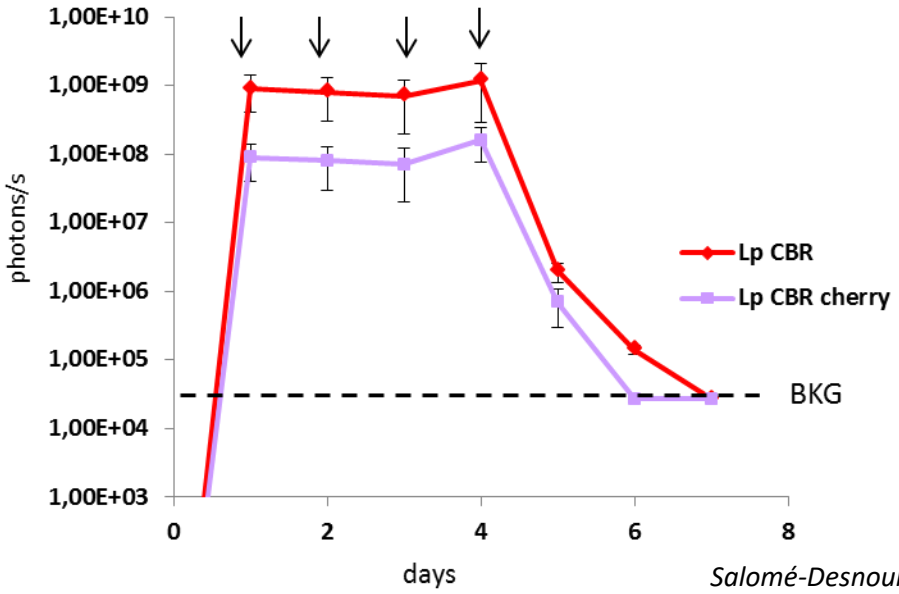


L. plantarum CBRluc
L. plantarum mCherry daily gavage
L. plantarum CBRluc-mCherry

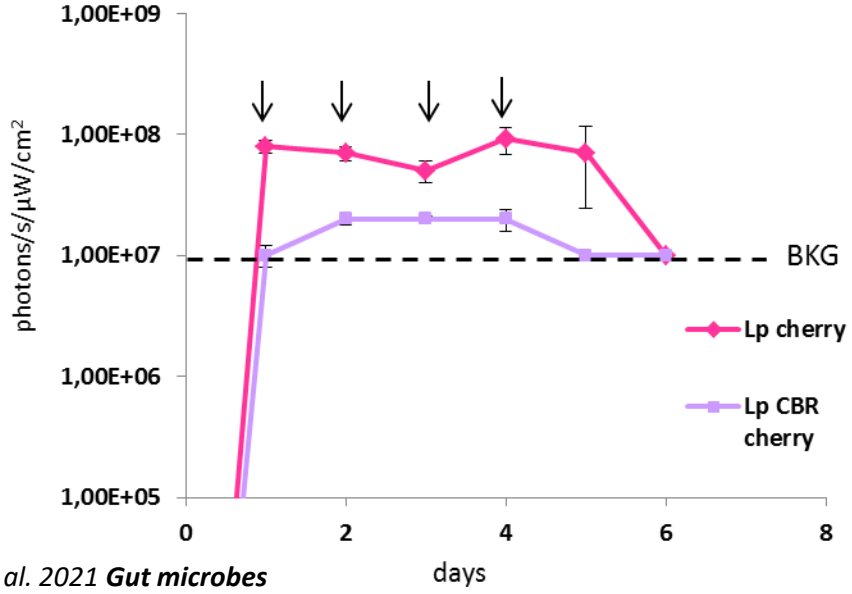




In vivo Bioluminescence



In vivo Fluorescence



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Bioluminescence versus fluorescence imaging

- ✓ Click beetle red luciferase best-performing system for *in vivo* detection of LAB/mcherry

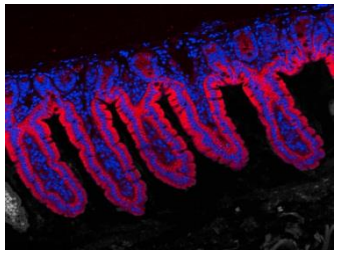
- ✓ New *Lactobacillus* producing both bioluminescent and fluorescent proteins to combine bioluminescence imaging and fluorescence

  - ➢ *L. plantarum* producing CBR/mcherry not as performant as individual producing strains

- ✓ Bioluminescence imaging (BLI) more efficient than fluorescence imaging (FI) to visualize *L. plantarum* (Karimi *et al.* 2016):

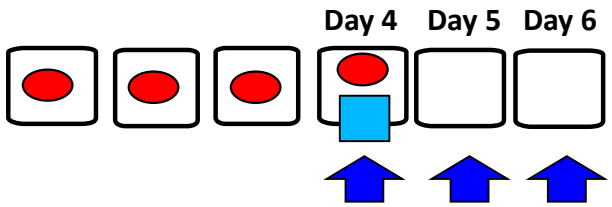
  - ➢ More background with FI
  - ➢ More sensitivity and higher signals with BLI
  - ➢ Only fluorescence can be used in microscopic studies

- ✓ Limits to BLI and FLI: high bacterial concentration + localization



Earle *et al.*, 2015, Cell Host & Microbe

Follow-up of *L. plantarum* in healthy vs colitis mice

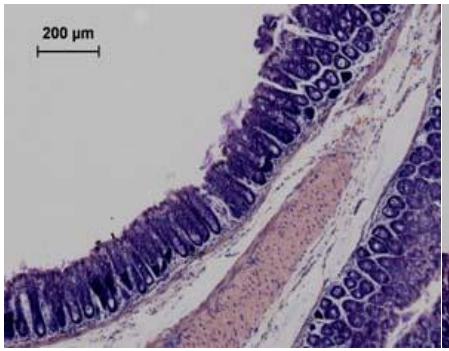


Chemical colitis induced by TNBS (dinitrobenzene sulfonic acid)

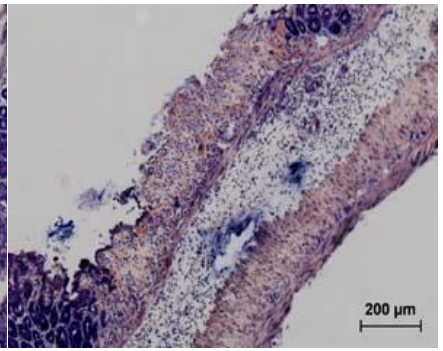
L. plantarum mCherry daily gavage for 4 days

- ✓ Follow-up of *L. plantarum*/mCherry daily in healthy vs colitis mice
- ✓ Day 4: colitis, days 5 and 6: sacrifice of mice, fluorescence signal and scoring of inflammation
- ✓ Inhibition of inflammation with ***L. plantarum*** **score: 4** (bact + colitis) in comparison **TNBS control: 5.2** (PBS buffer + colitis)

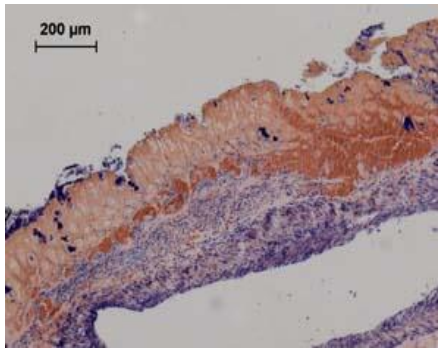
Score 0 Score 4 Score 8



Score : 0 : healthy



Score : 4: *L. plantarum*



Score : 5.5: TNBS control

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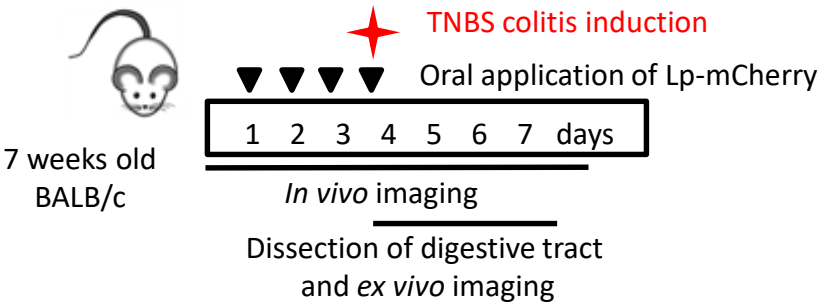
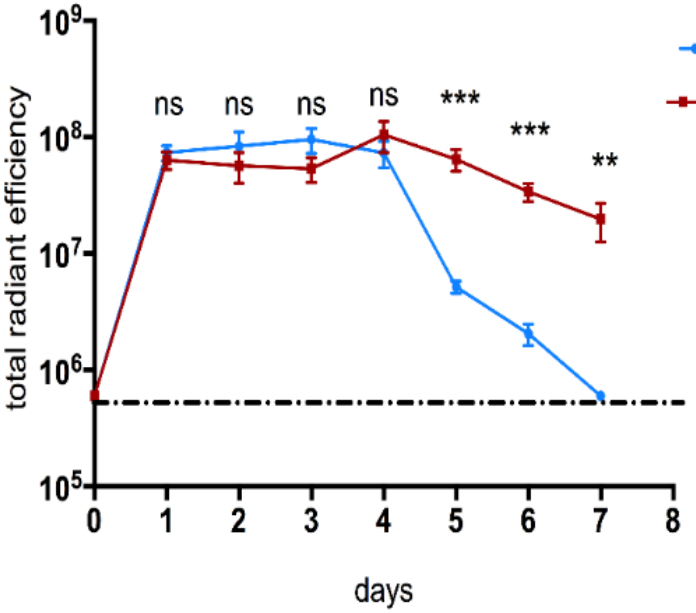
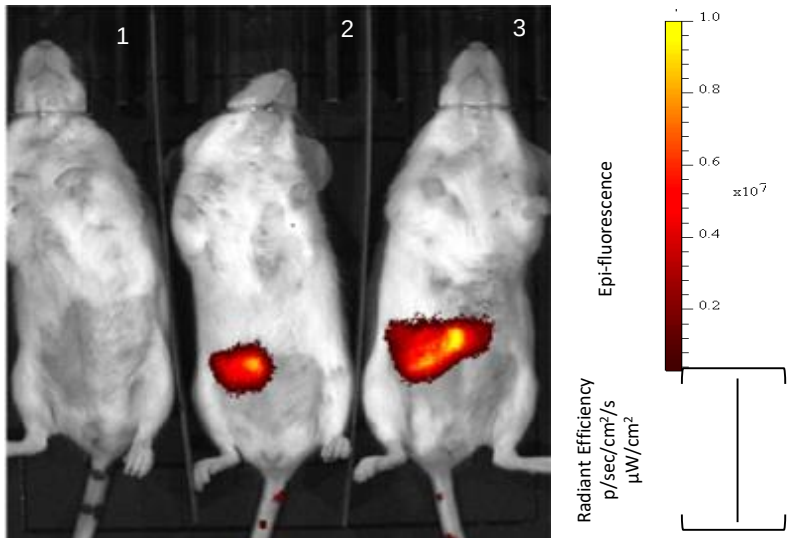
L. plantarum persists for longer in colitis vs healthy mice







- ✓ High fluorescence signal and then decreases more rapidly in healthy versus colitis mice
- ✓ Bacteria persist in caecum/colon and are not eliminated in the feces (inflammation)

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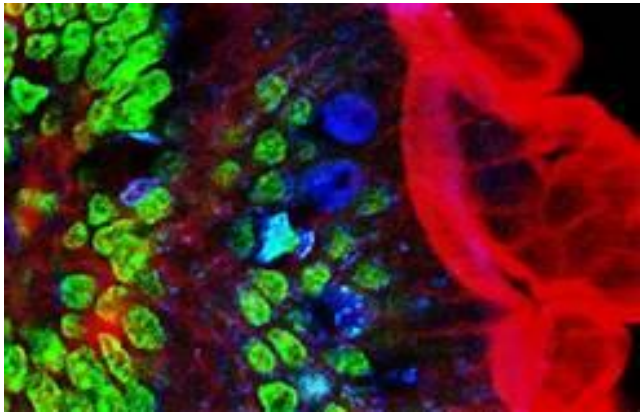
Multi-photons microscopy

- ✓ Multi-photons microscopy is a method of choice for imaging living, intact biological tissues on length scales from the molecular level through the whole organism

- ✓ Uniquely suited to perform experimental measurements with minimal invasion over long periods of time (explants or intravital)

- ✓ MPM holds inherent advantages for imaging living tissues by improving depth penetration (100-400 nm) and reducing photodamage



Diaspro *et al*., 2010, Biomedical bioengineering online

MP microscope animal facility

Multiphoton microscope
Leica TCS SP8 MP
Imaris software
3D view

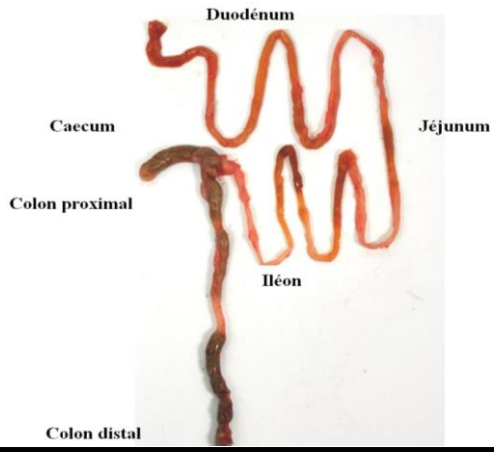
Day 4 Healthy Colon

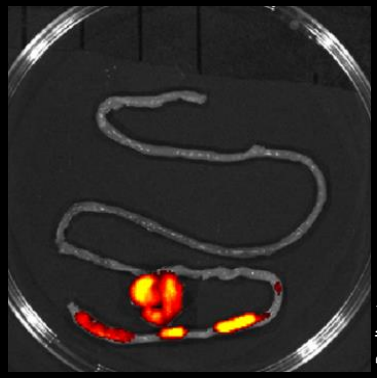
7 weeks old
BALB/c

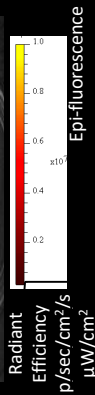
Oral application of Lp-mCherry

TNBS colitis induction

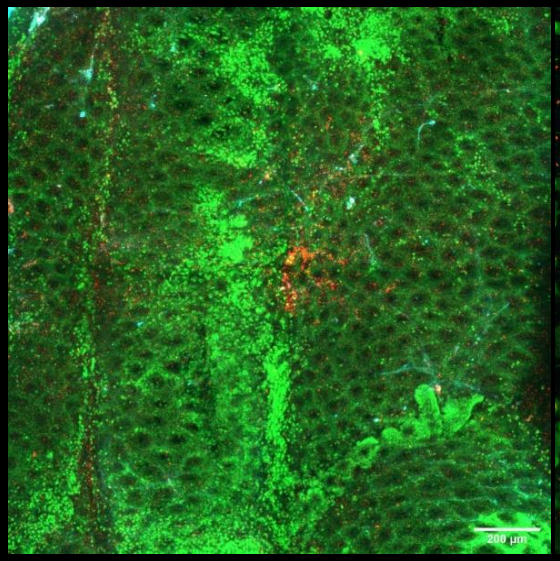
Dissection of digestive tract
and *ex vivo* imaging



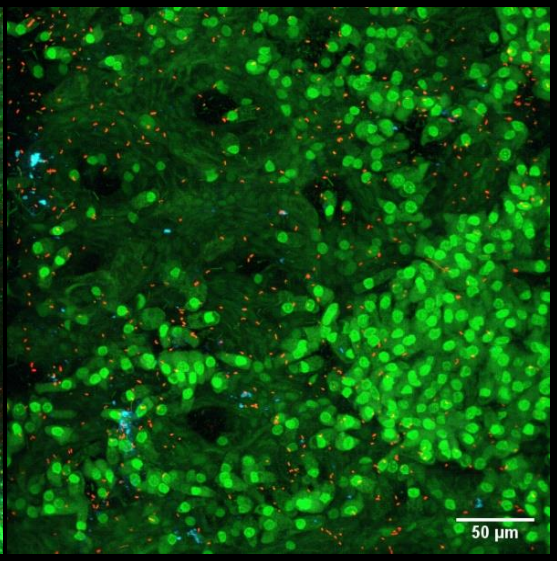




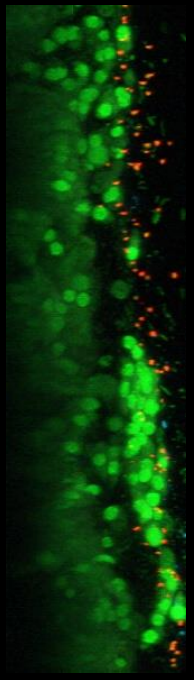
DNA Bacteria Mucus



10x



25x



3D

- ✓ **Strong** *ex vivo* fluorescence signal predominantly found in **cecum and colon**
- ✓ Confocal microscopy on **fresh tissues**: **large numbers** present in the proximal colon
- ✓ Zoomed 3D rendered image: most of the bacteria **far from the intestinal cells**

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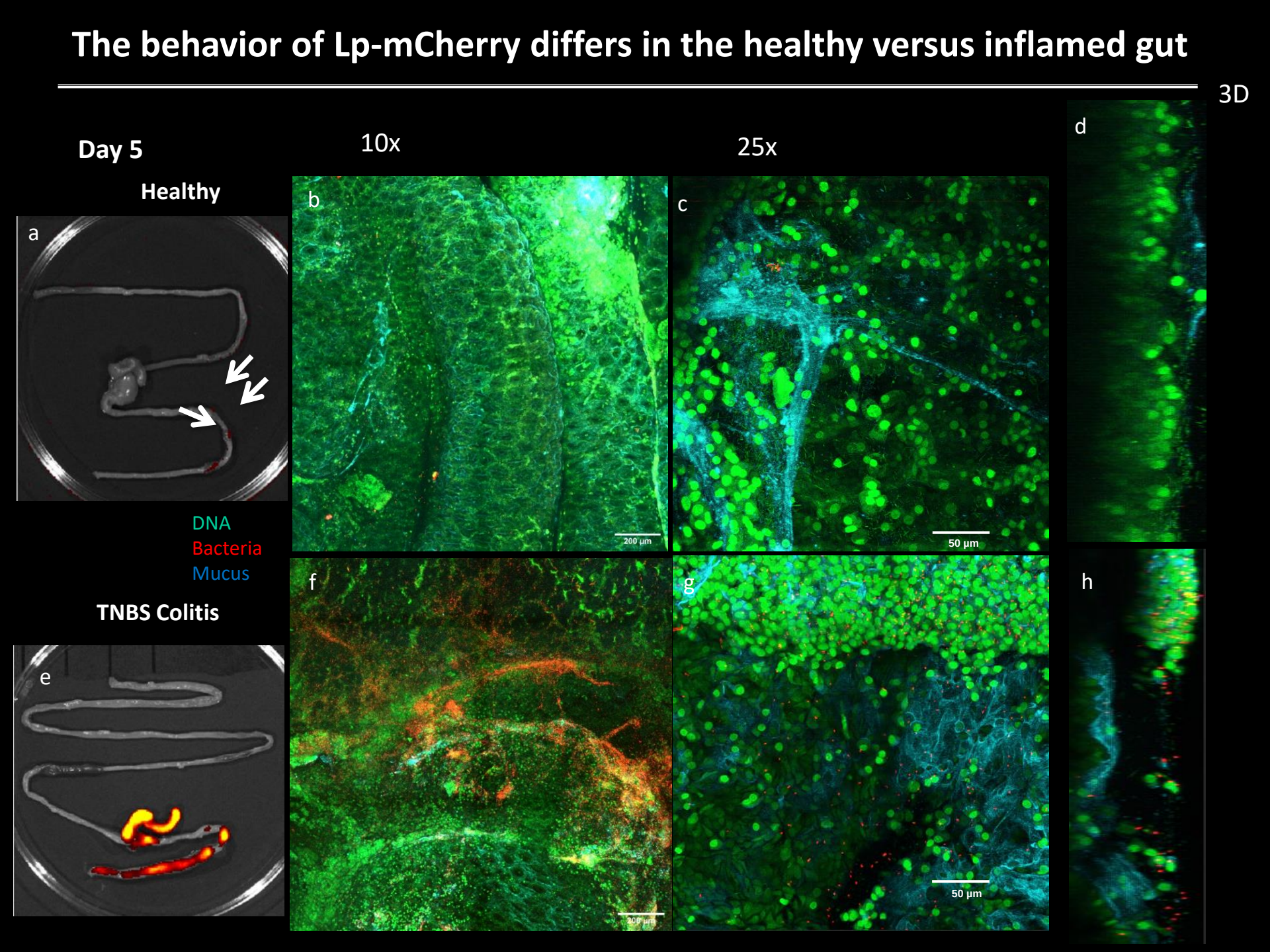
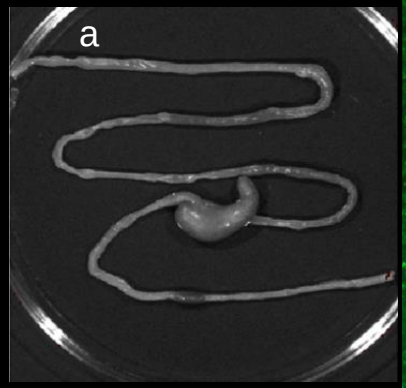
The behavior of Lp-mCherry differs in the healthy versus inflamed gut



The behavior of Lp-mCherry differs in the healthy versus inflamed gut

Day 6

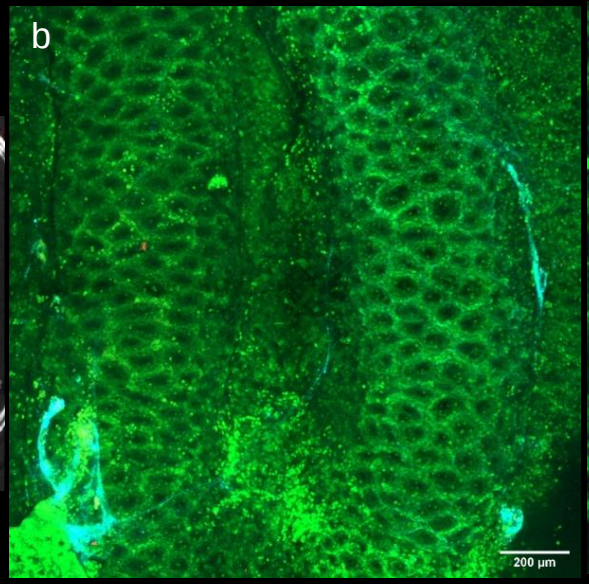
Healthy



DNA
Bacteria
Mucus

TNBS Colitis

10x



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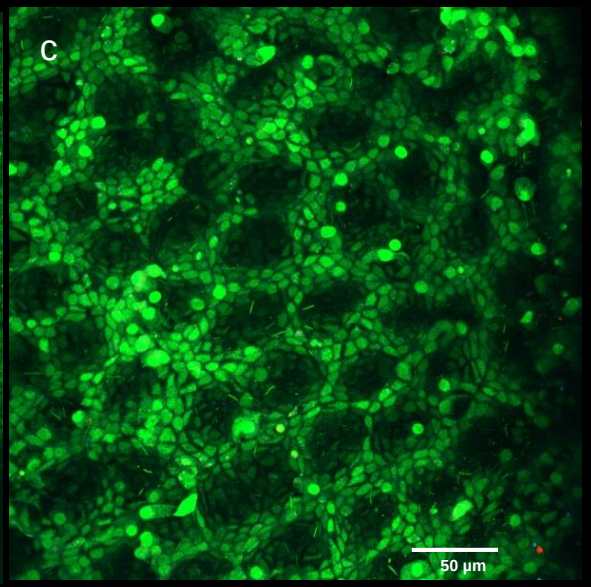
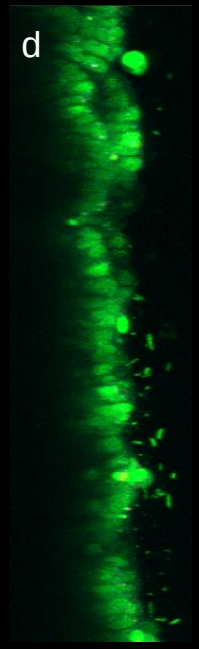
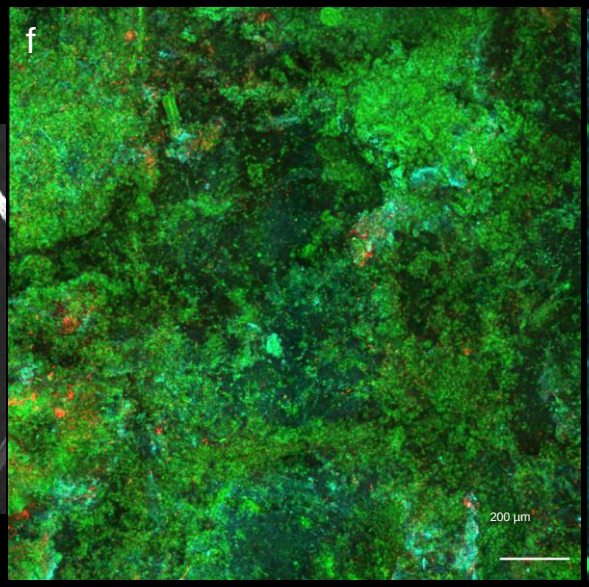
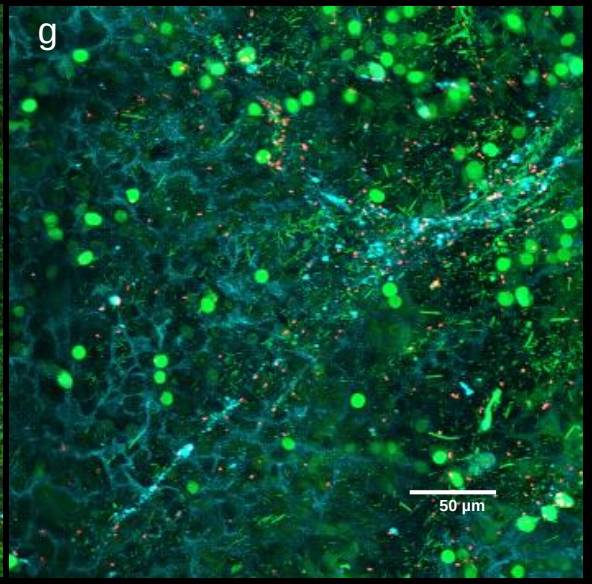
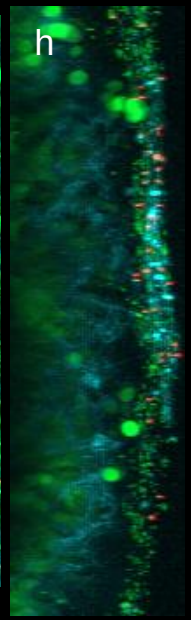


3D

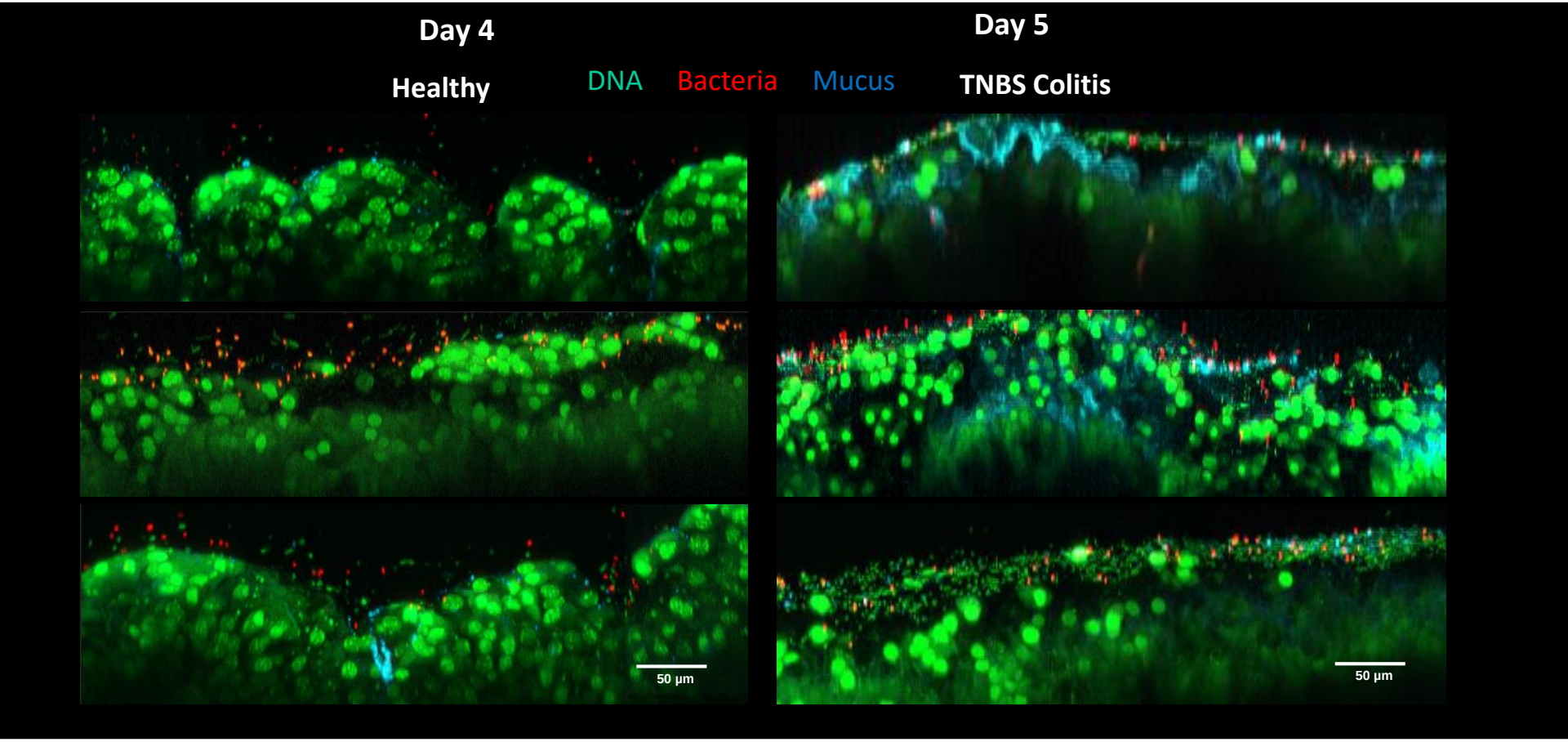








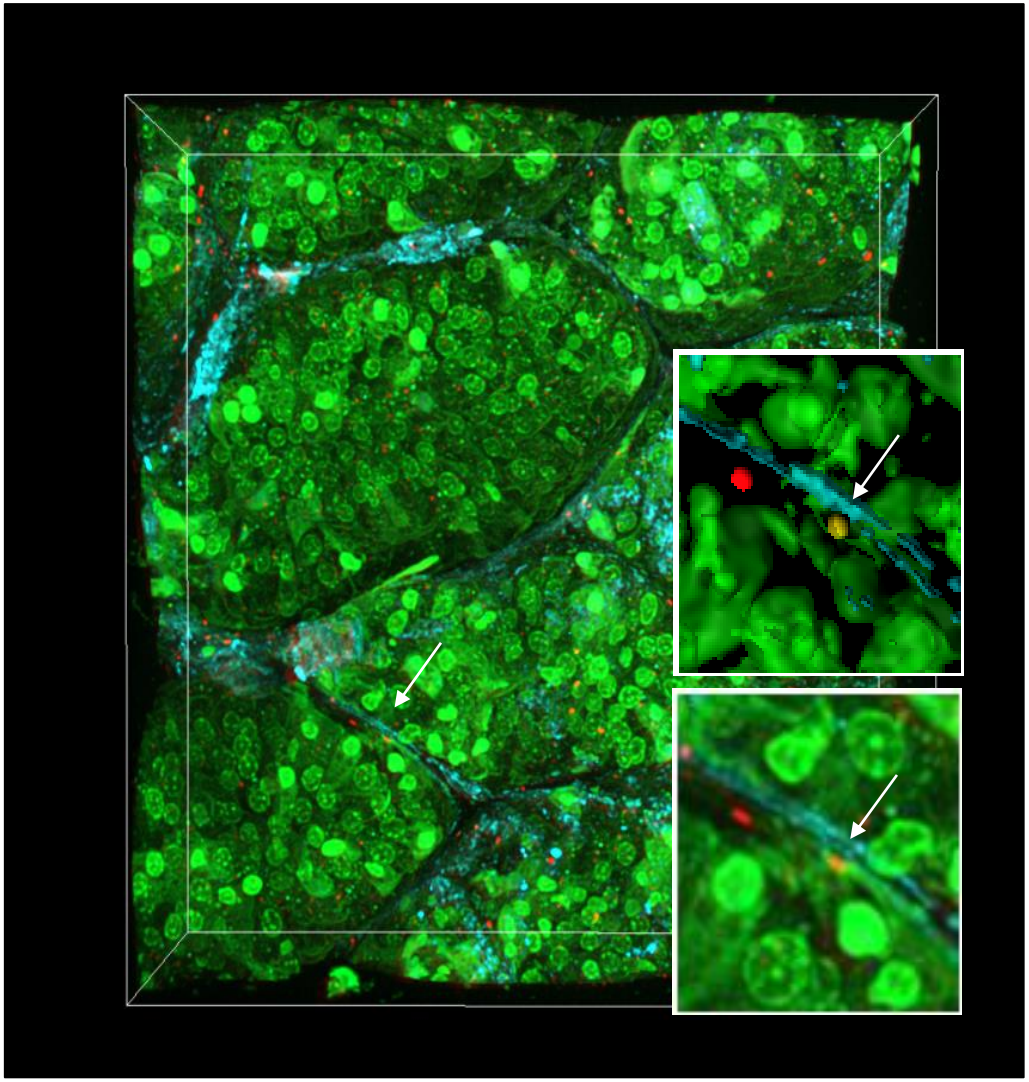
Direct contact between Lp-mCherry and intestinal cells is rare in the healthy colon and frequent in the inflamed colon



- **Healthy:** in lumen and crypts, proximity to endogenous bacteria gut microbiota, in and outside the mucus layer **but rarely** in contact with intestinal cells
- Large number of individual *L. plantarum* in the lumen and mucus layer in **direct contact with damaged or dead** cells in inflamed areas of the colon

Direct contact between Lp-mCherry and intestinal cells is rare in the healthy colon

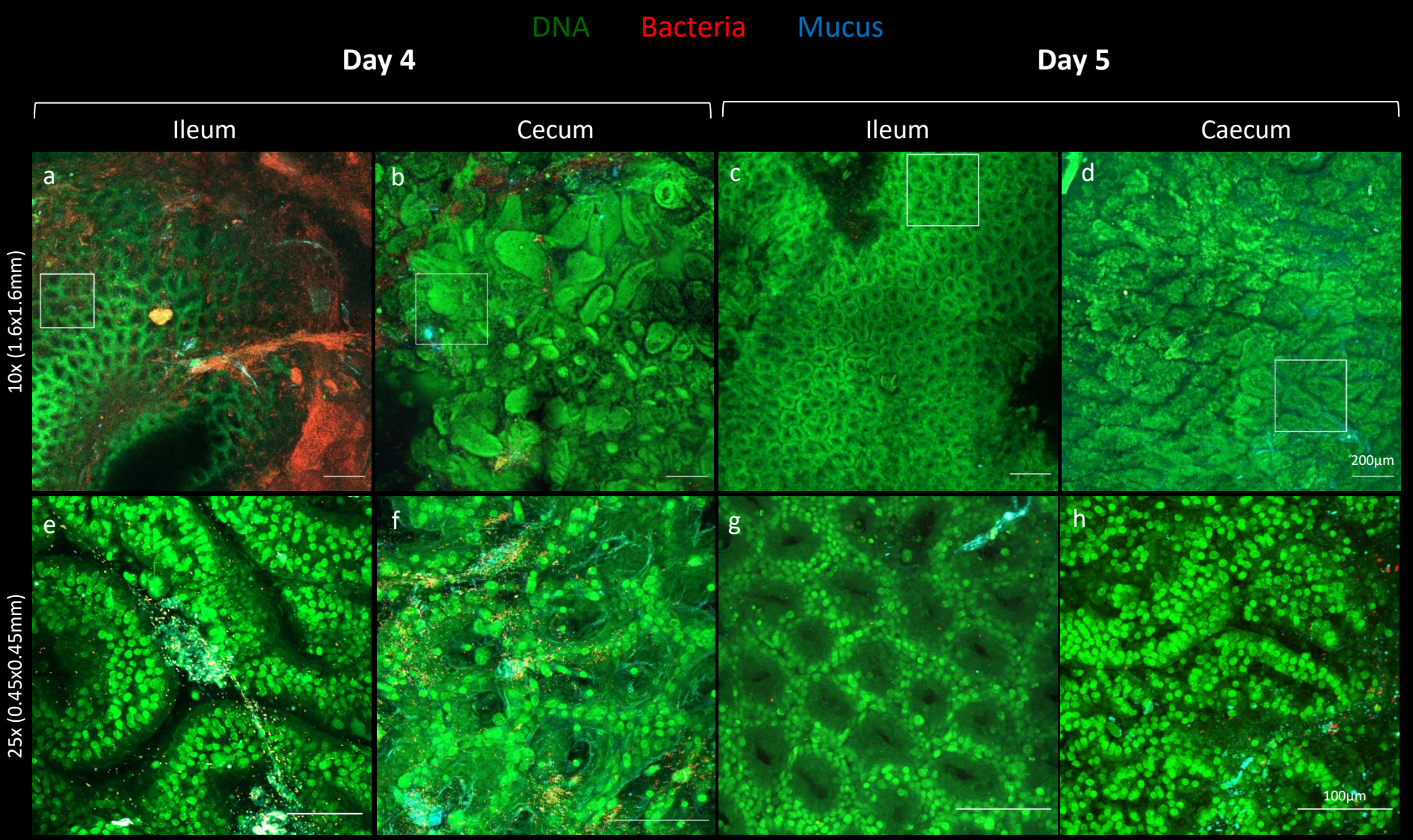
DNA Bacteria Mucus



- ✓ **3% approximately of the bacteria** are at a distance < 4µm of a nucleus (Quantification done with Imaris software on spots) in colon, terminal ileum, and caecum

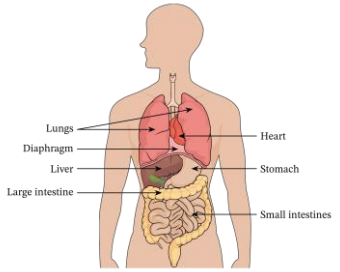
Salomé-Desnoulez et al. 2021 ***Gut microbes***

L. plantarum also present in healthy ileum and caecum



Conclusions

✓ **New bioluminescent tools for live imaging LAB**



- Detection and semi-quantification in the GIT (O) and nose/lungs (I)
- Activation of the immune system in two mucosal compartments ?

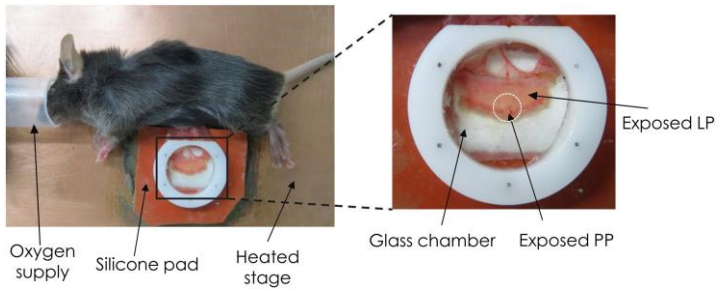
✓ **Bioluminescence more efficient than fluorescence** imaging to visualize *L. plantarum*

✓ **New fluorescent tools developed for imaging LAB and intravital microscopy**

- Oral administration / fresh tissues observed after sacrifice
- Bacteria persist longer in DT and not eliminated in the feces (inflammation vs healthy)
- *L. plantarum* in the lumen with endogenous microbiota (H)
- **Rare event**: contact bacteria-intestinal cells (H)
- Mislocalization of *L. plantarum* as well as endogenous bacteria in murine colitis models (I)
- Mode of actions of *L. plantarum* and probiotics in the host's mucosa ?
- Interaction with microbiota, mucus, EC, IC/ metabolites secreted/ increase thickness mucus ?

Perspectives

Intravital two-photon imaging of the gastrointestinal tract, J Imunol methods, 2015



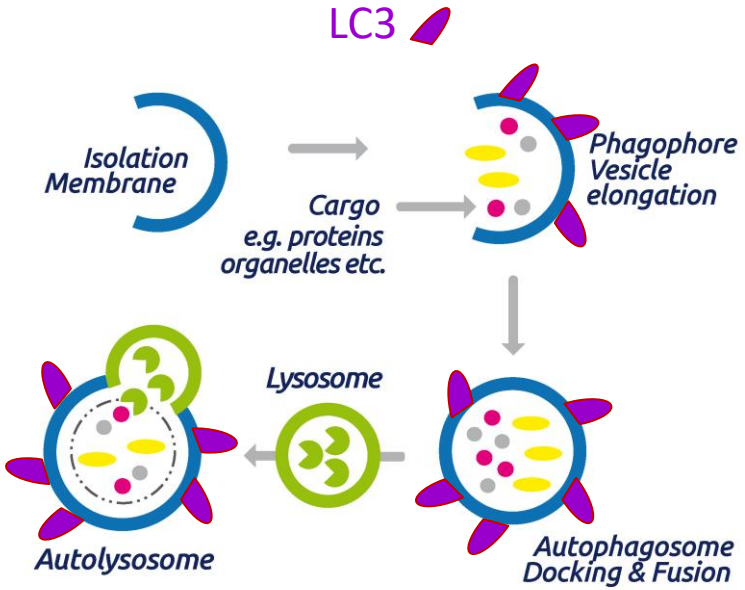
✓ **Next steps:**

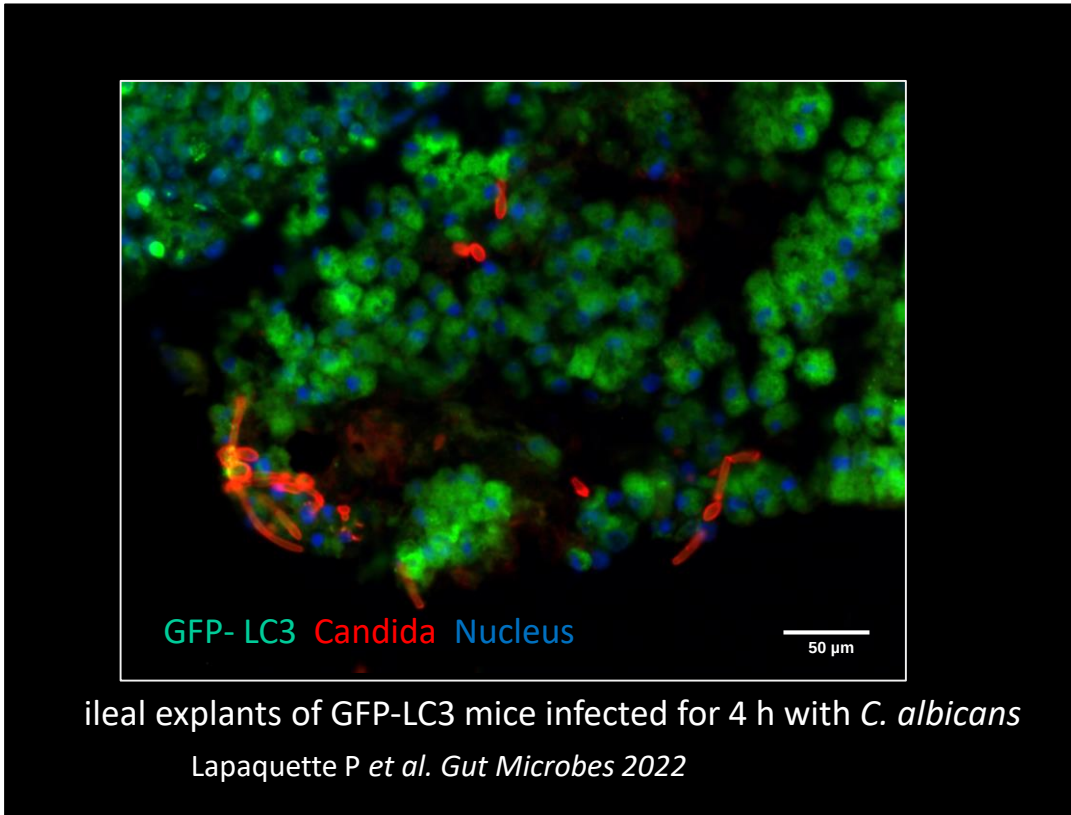
- *real time imaging*: organoids, intestinal loop, animal with imaging window…
- *more visualization tools*: chemical biology to simultaneously label bacteria and metabolite
- *label a community of different LAB*: color-coded bacteria
- Label a community of different probiotics : *Yarrowia lipolytica, Bifidobacterium longum*

Perspectives

✓ **Next steps:**

- label specific cells or use fluorescent transgenic mice
- Autophagy inducing capacity: mechanisms explaining gut probiotic effects ?
- Use GFP-LC3 mice and *in situ* imaging of the autophagic response in the gut (*ex vivo*)





ileal explants of GFP-LC3 mice infected for 4 h with *C. albicans*

Lapaquette P *et al. Gut Microbes 2022*

Thank you for your attention

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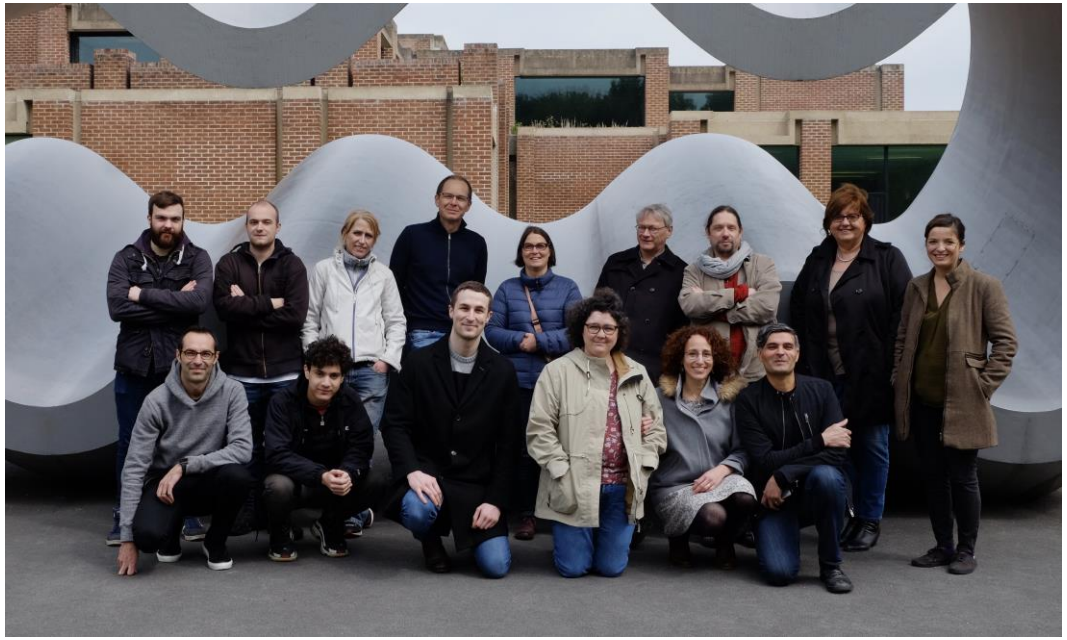
+ Sabine !



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