

# Humoral Immunity in the Gut Selectively Targets Phenotypically Virulent Attaching-and-Effacing Bacteria for Intraluminal Elimination

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# Background

- Pathogenicity
- > nutrients
  - > exploit the host machinery
  - > circumvent host defense

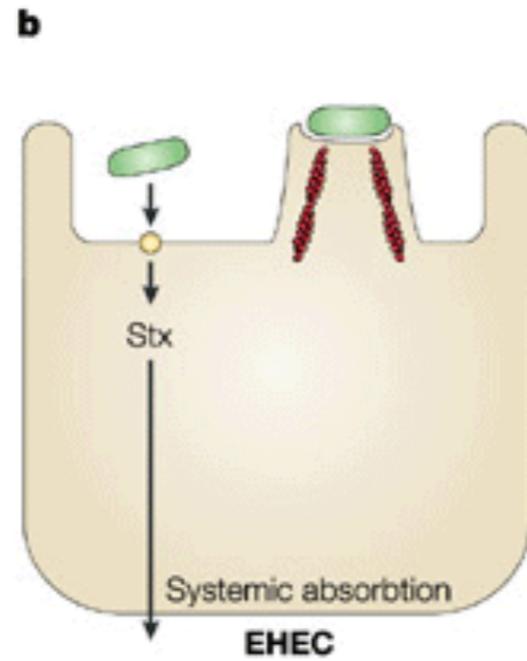
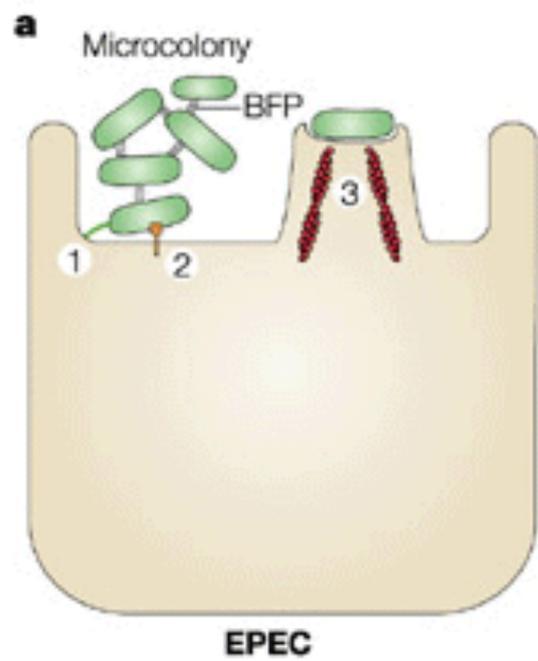
**Colonization**

**Invasion**

*Expression of bacterial virulence?*

# Background

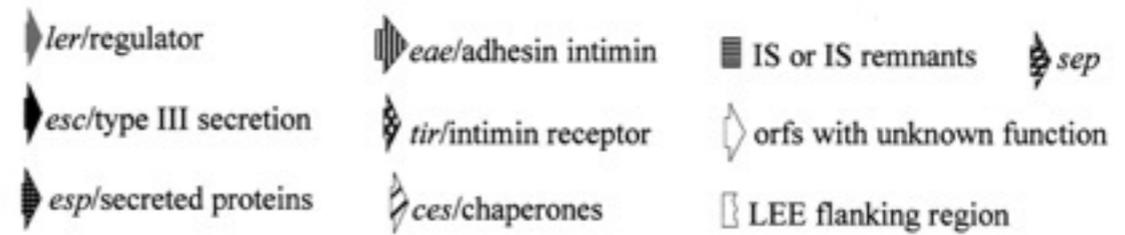
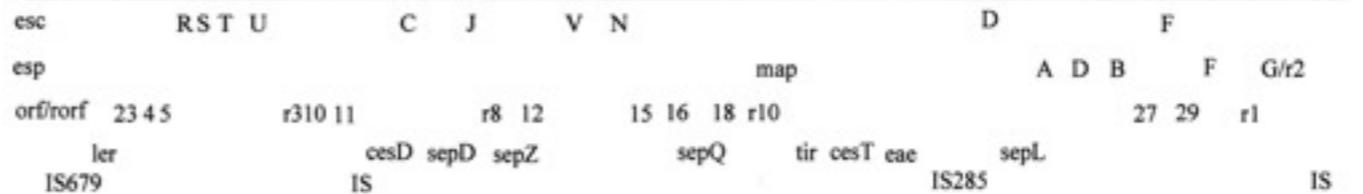
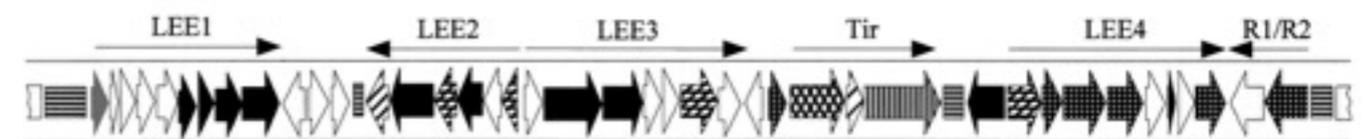
EHEC  
EPEC  
C. rodentium



## A. EPEC LEE



## B. Citrobacter rodentium LEE

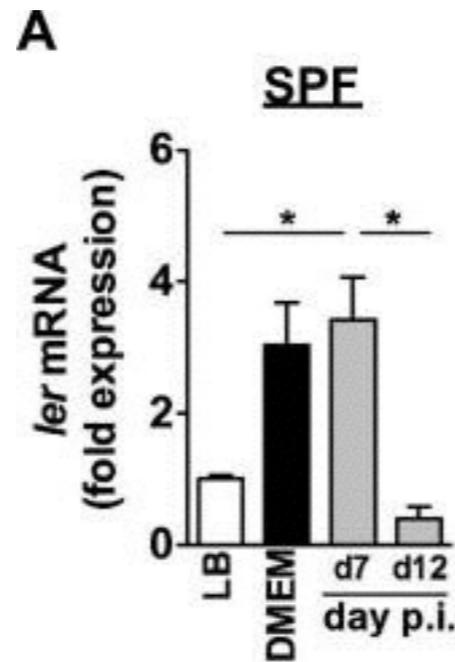


# Background

## *C. rodentium*

- TLR signaling
- IL-22
- humoral immunity

clearance + damage reduction  
prevention of systemic spreading  
clearance + limitation of systemic spread



Kamada et al, 2012

## Aim

- how is the elimination of LEE virulence mediated?

# Adaptive immunity is required for the clearance of *C. rodentium*

Aim To address the role of the adaptive immunity in *C. rodentium* infection

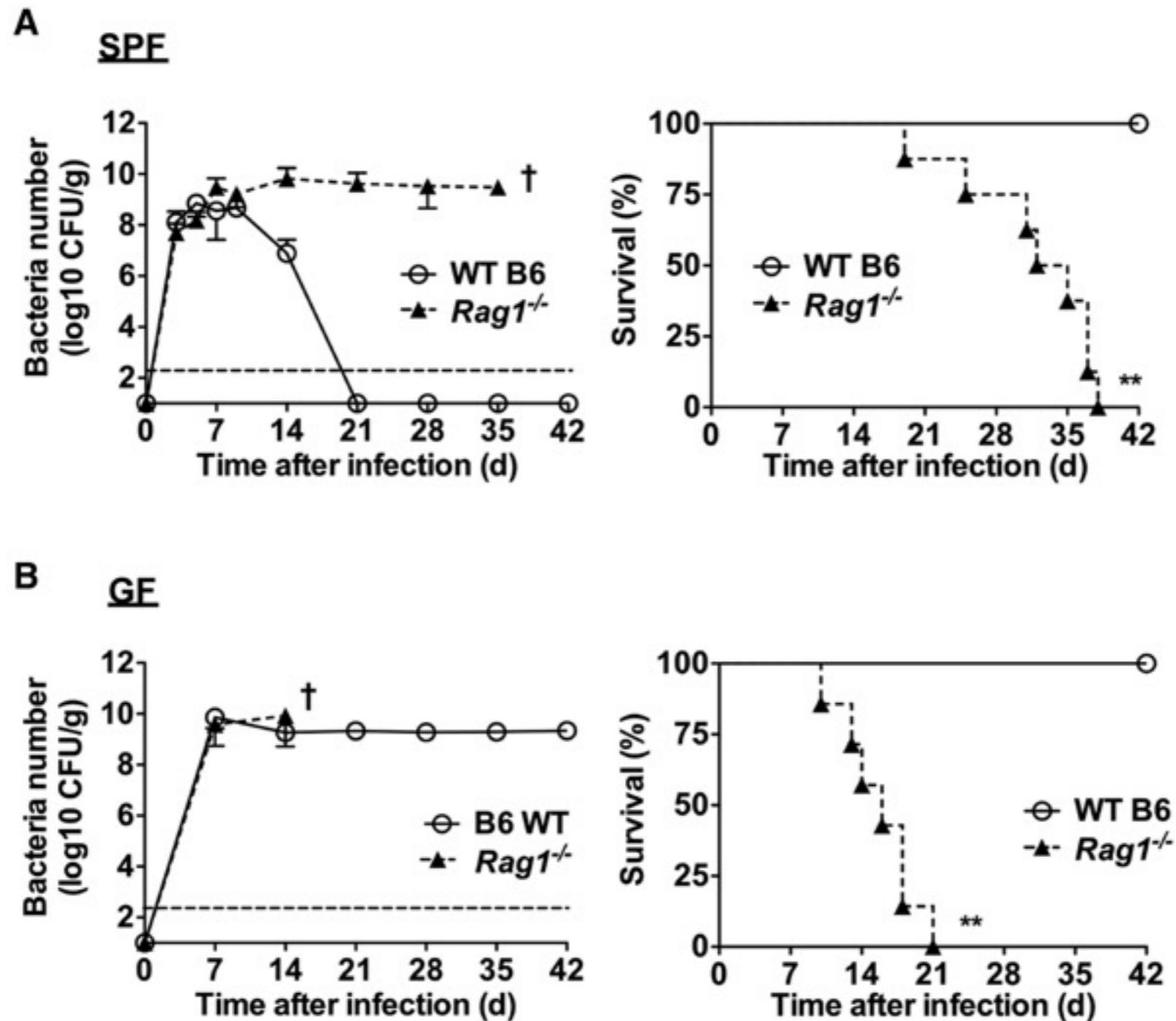


Figure 1

# Adaptive immunity is required for LEE virulence downregulation during *C. rodentium* infection

Aim To assess whether the adaptive immunity regulates LEE virulence

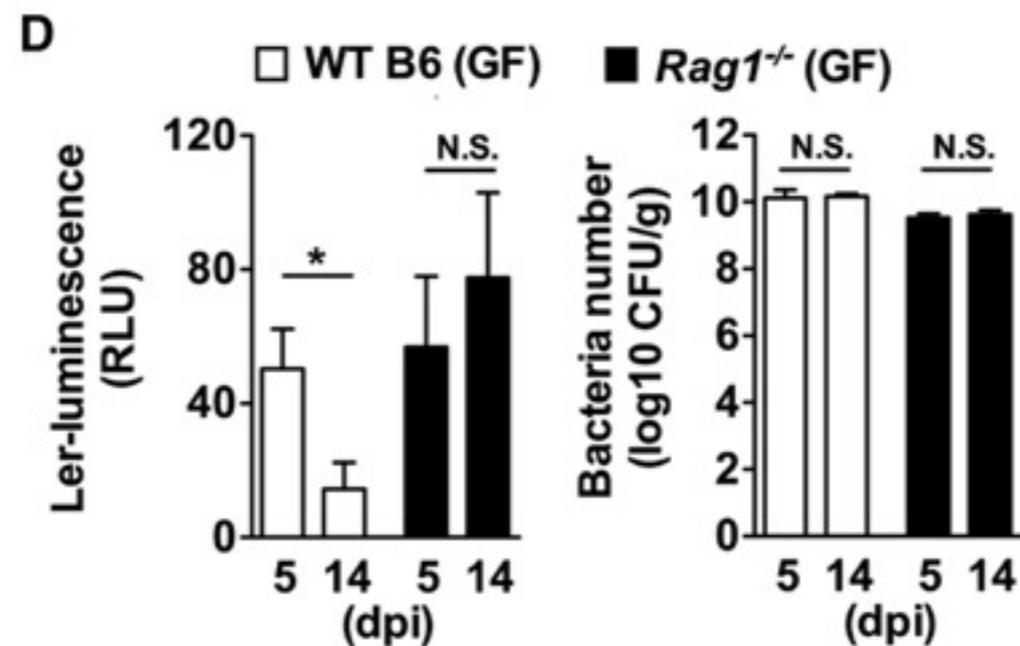
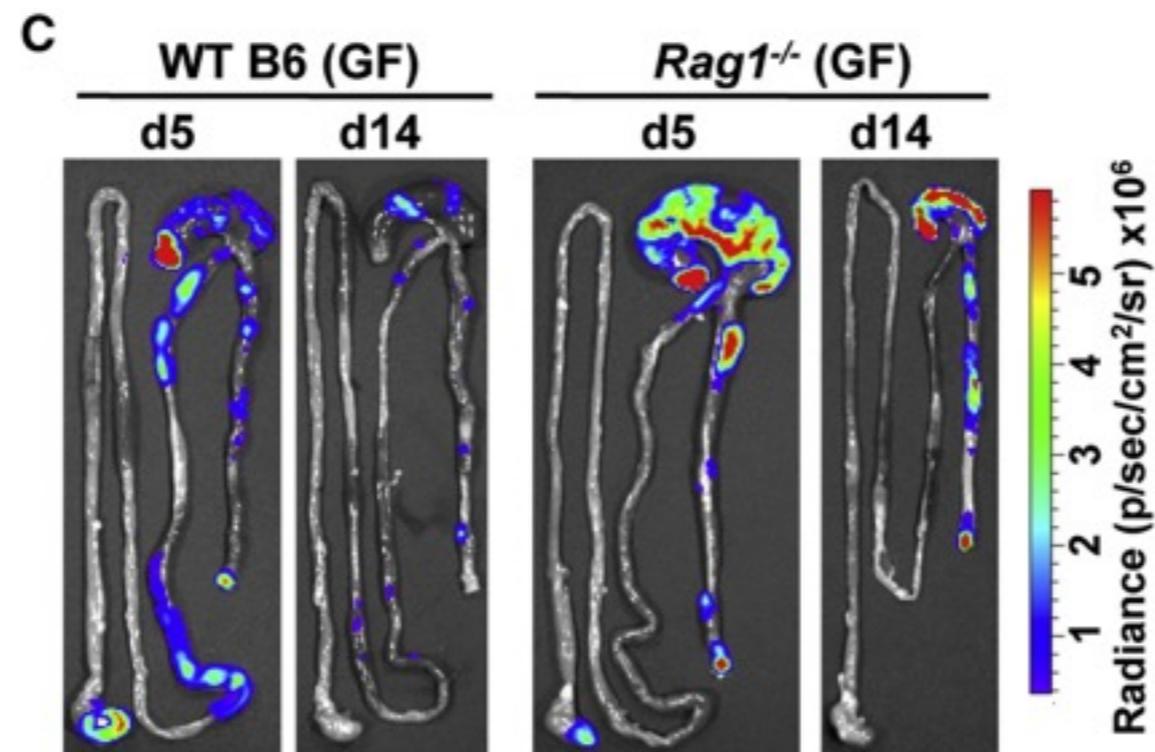


Figure 1

# Adaptive immunity is required for the clearance of the *C. rodentium*

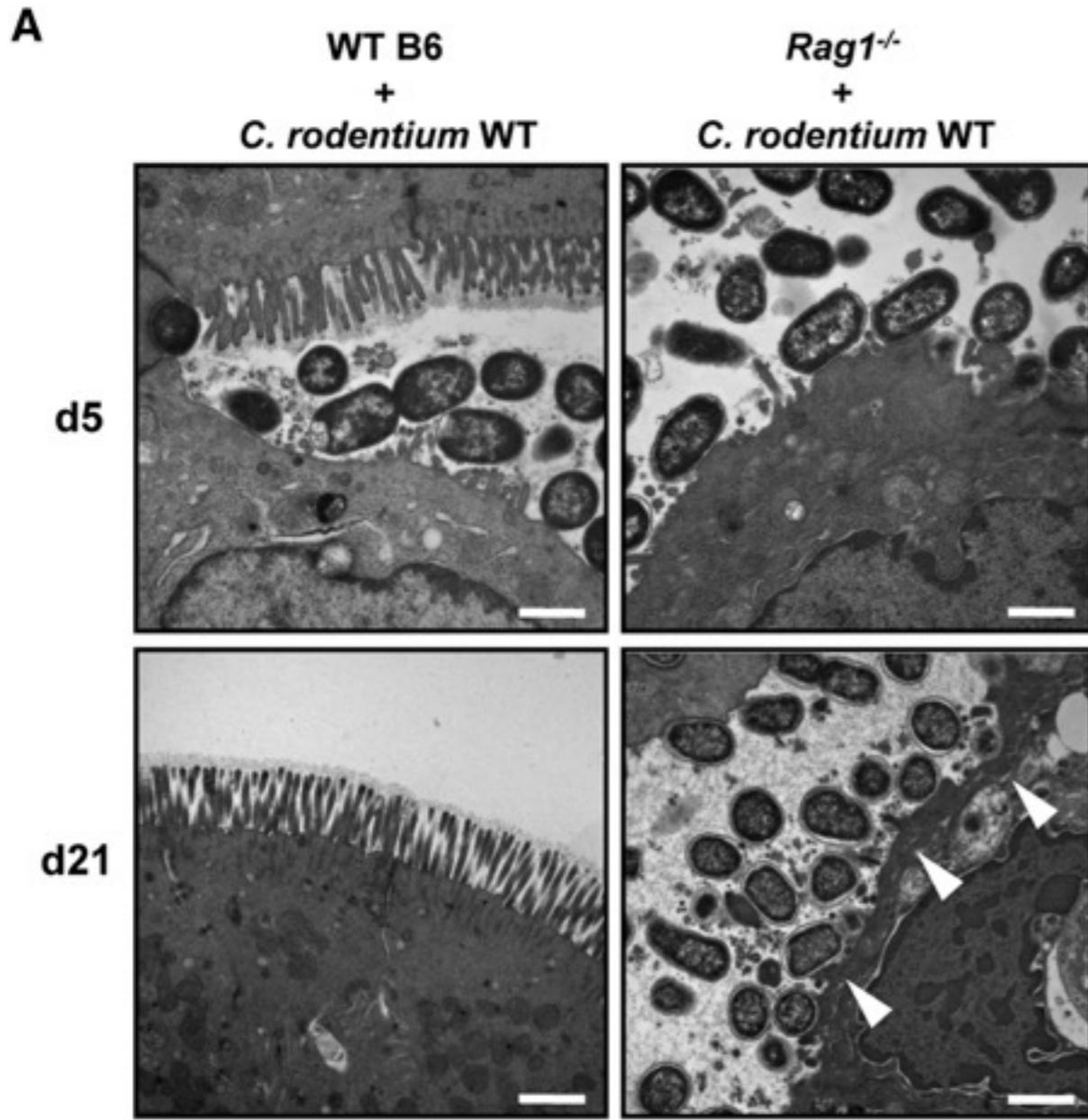


Figure 2

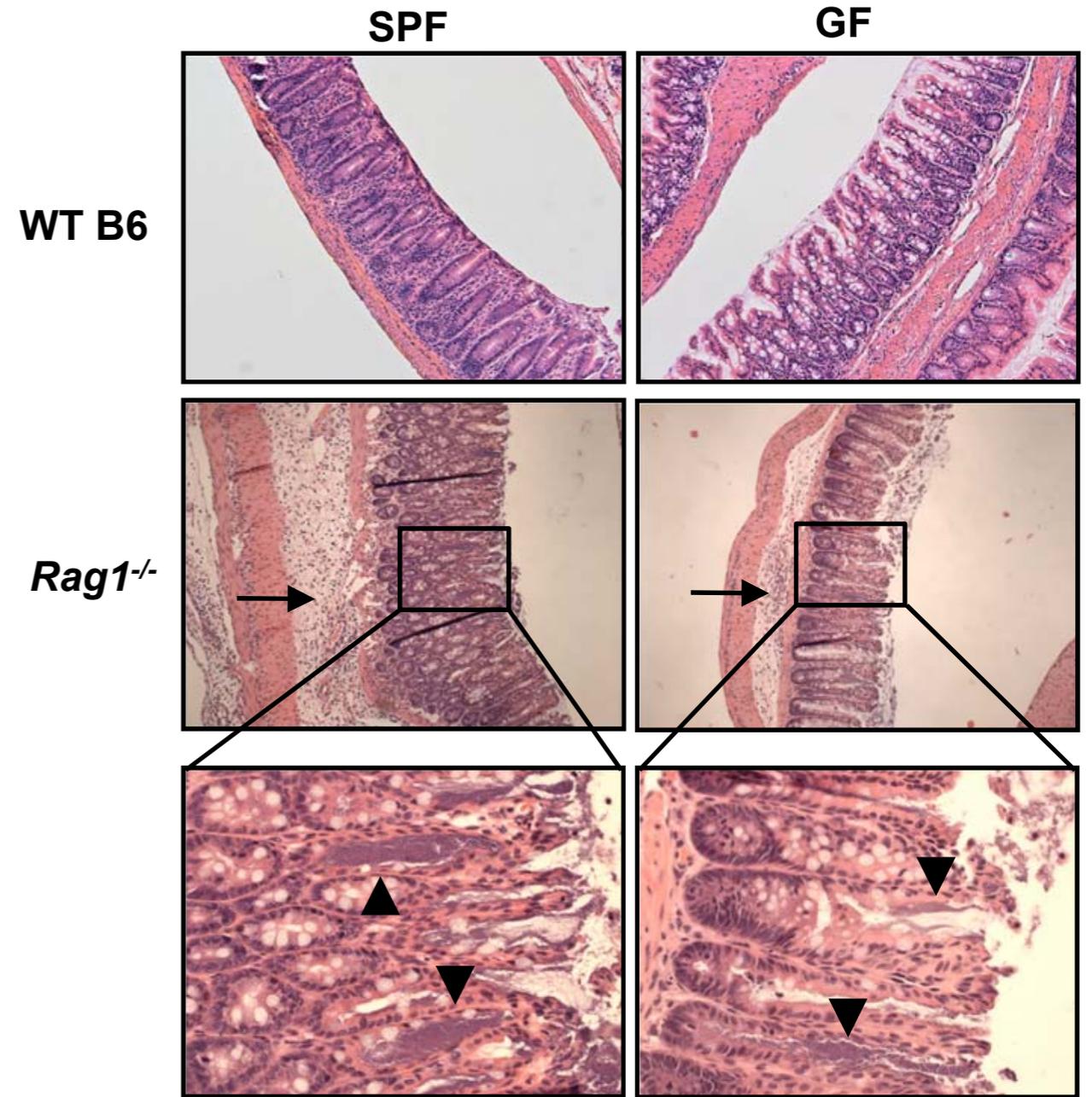


Figure S1

# LEE virulence is required for the demise of the *Rag1*<sup>-/-</sup> mice

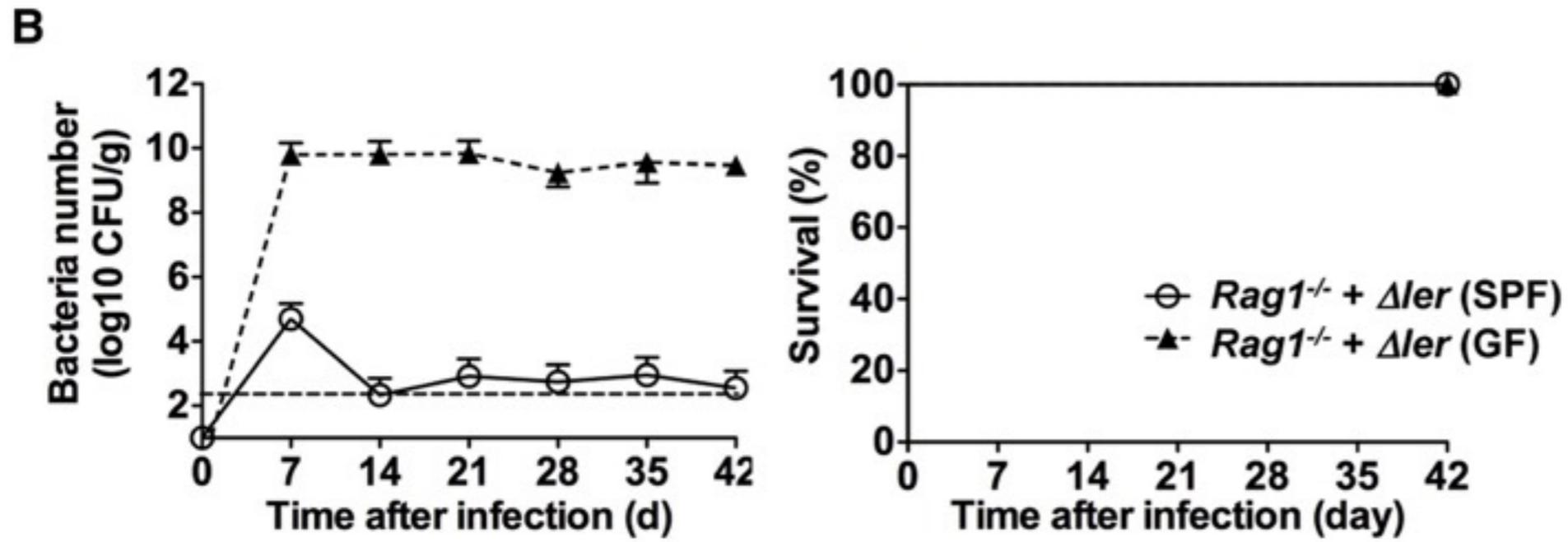


Figure 2

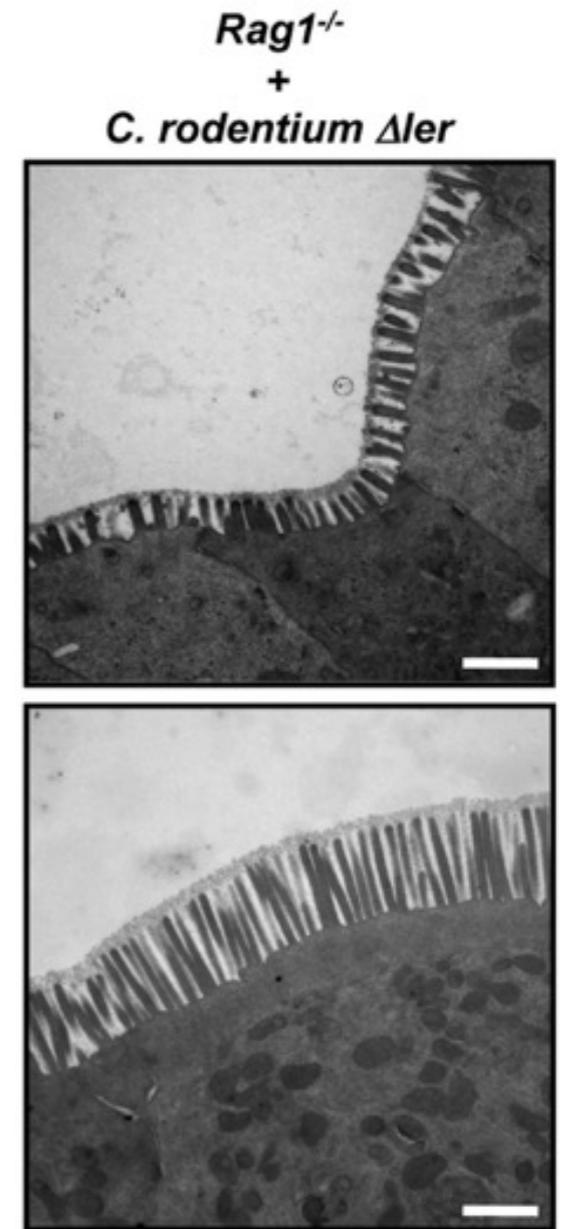
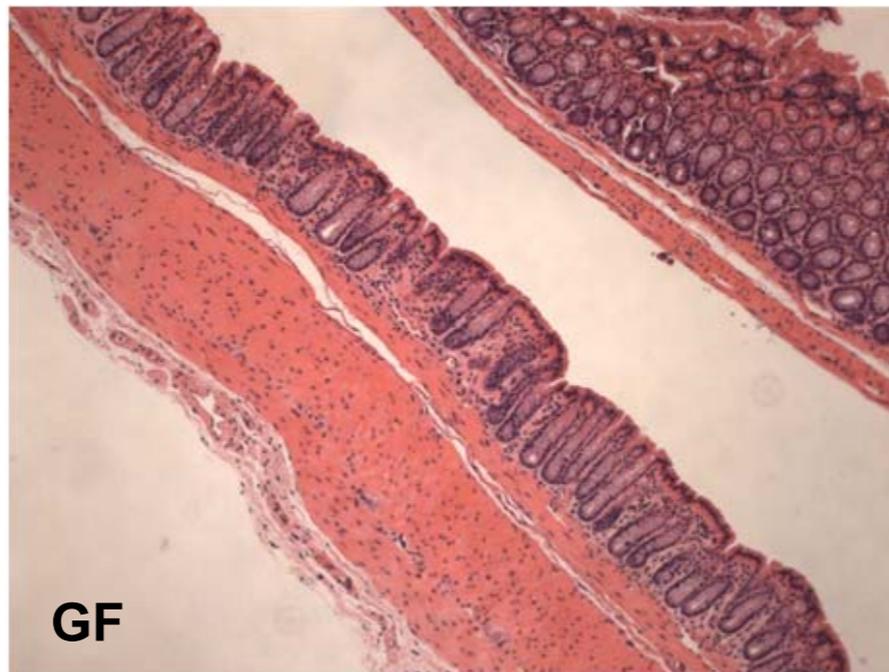
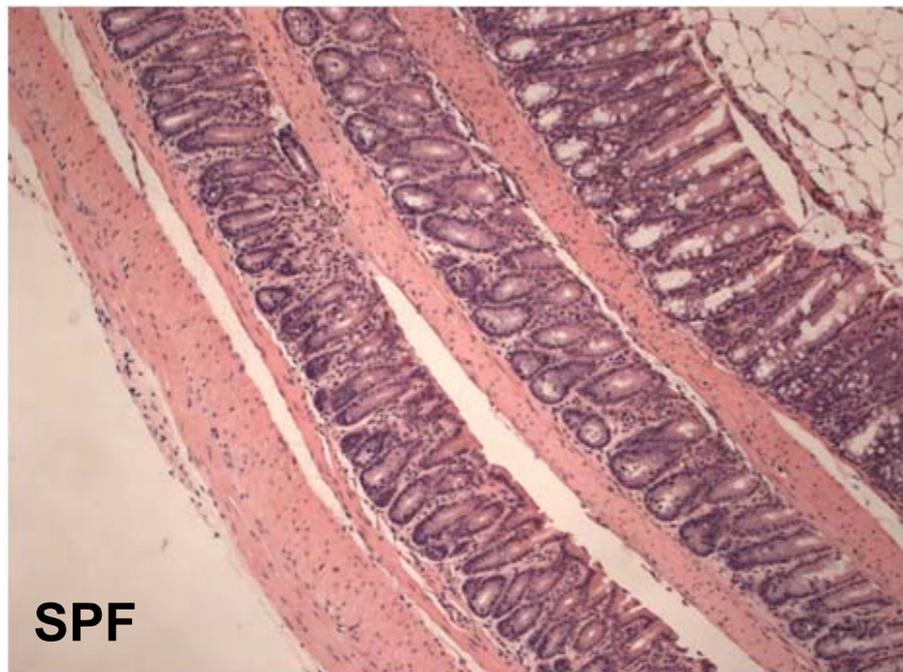


Figure S2

The presence of commensals is critical for the elimination of *C. rodentium*

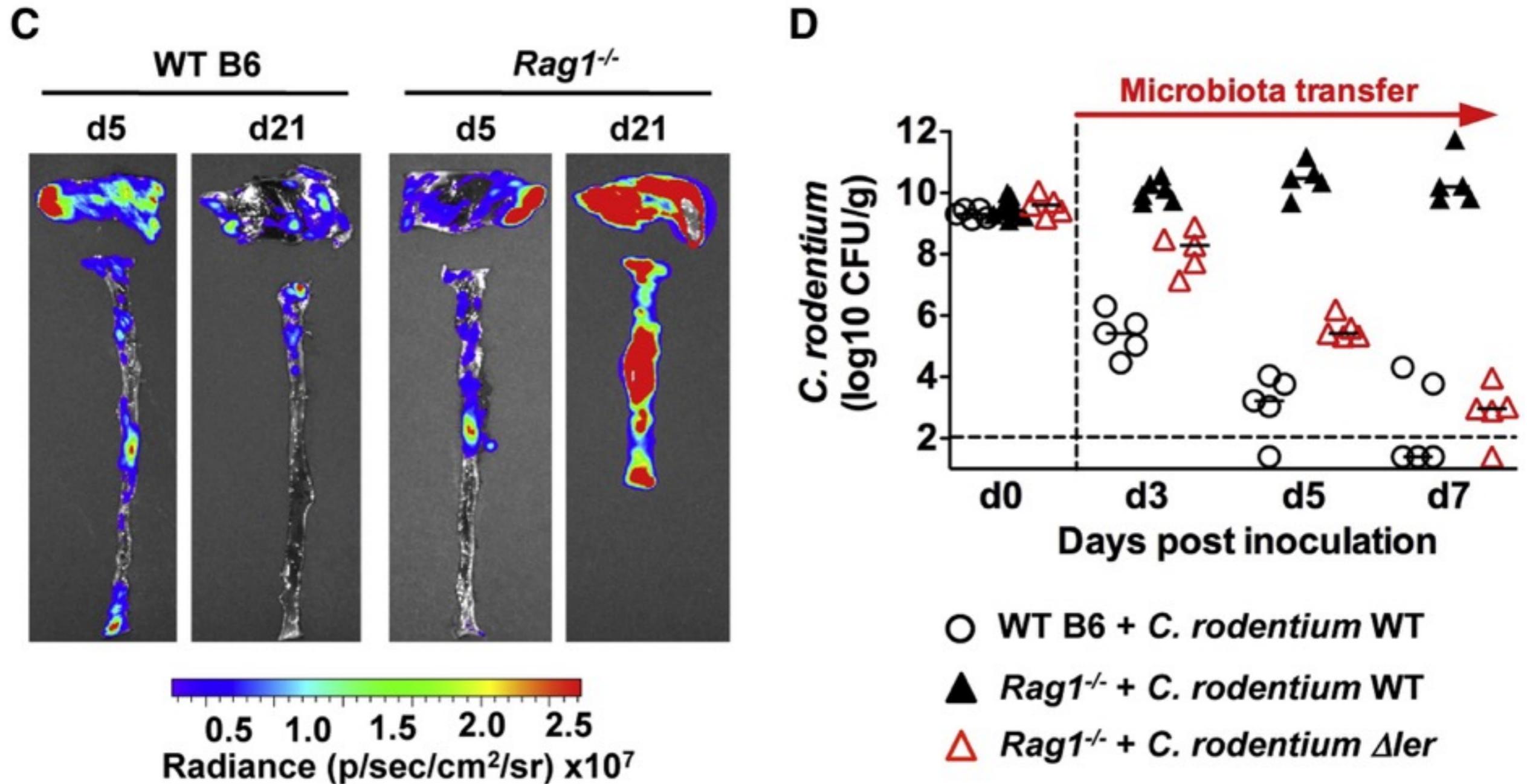


Figure 2

# Use of a QM-mouse to avoid the production of specific antibodies

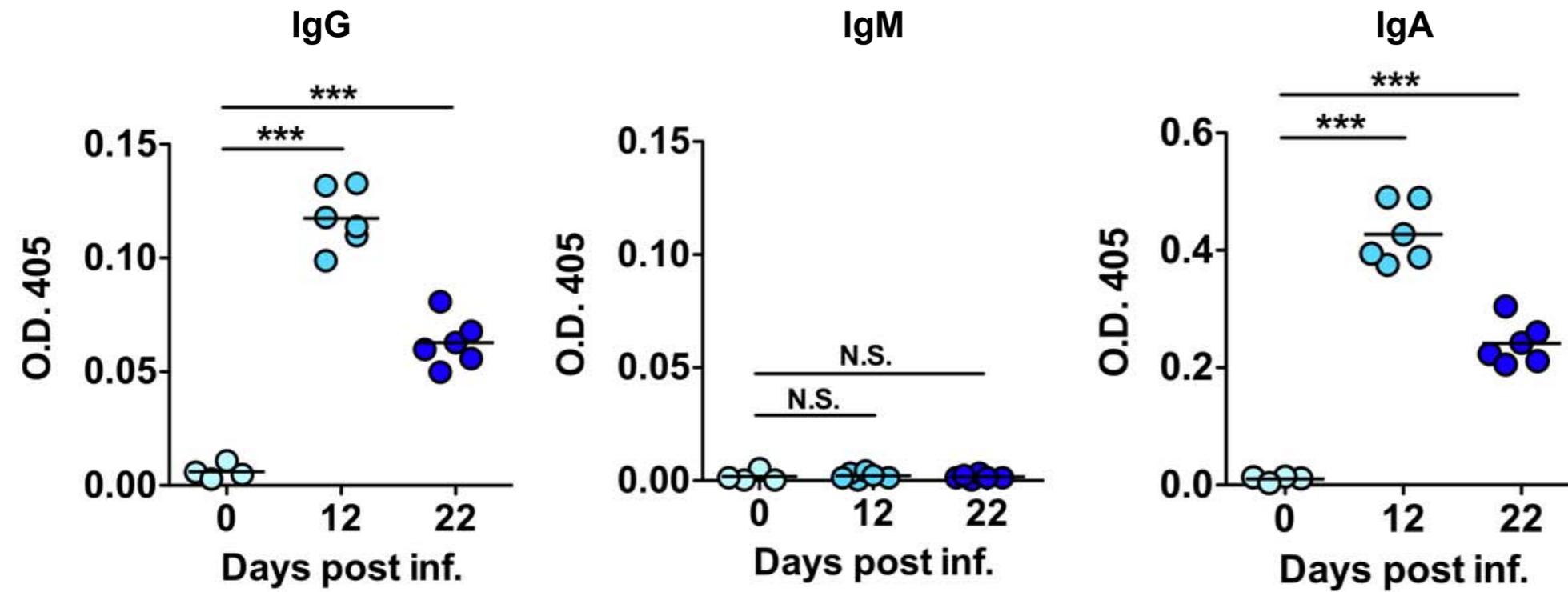


Figure S3

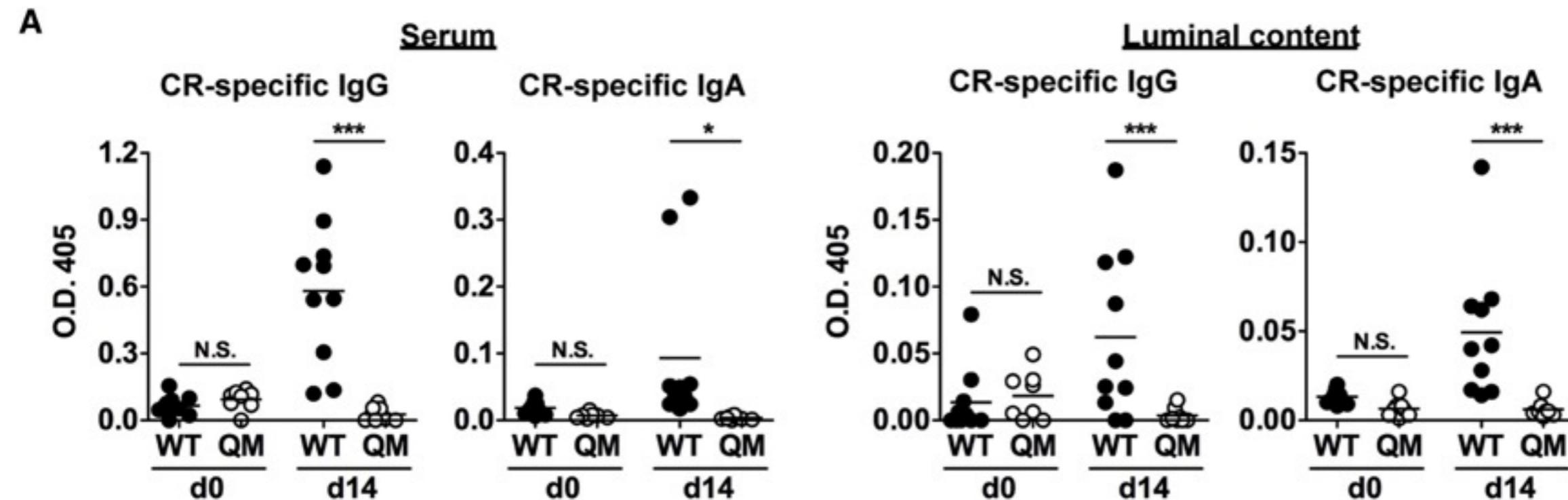


Figure 3

# Pathogen-specific IgG is required for downregulation of LEE expression

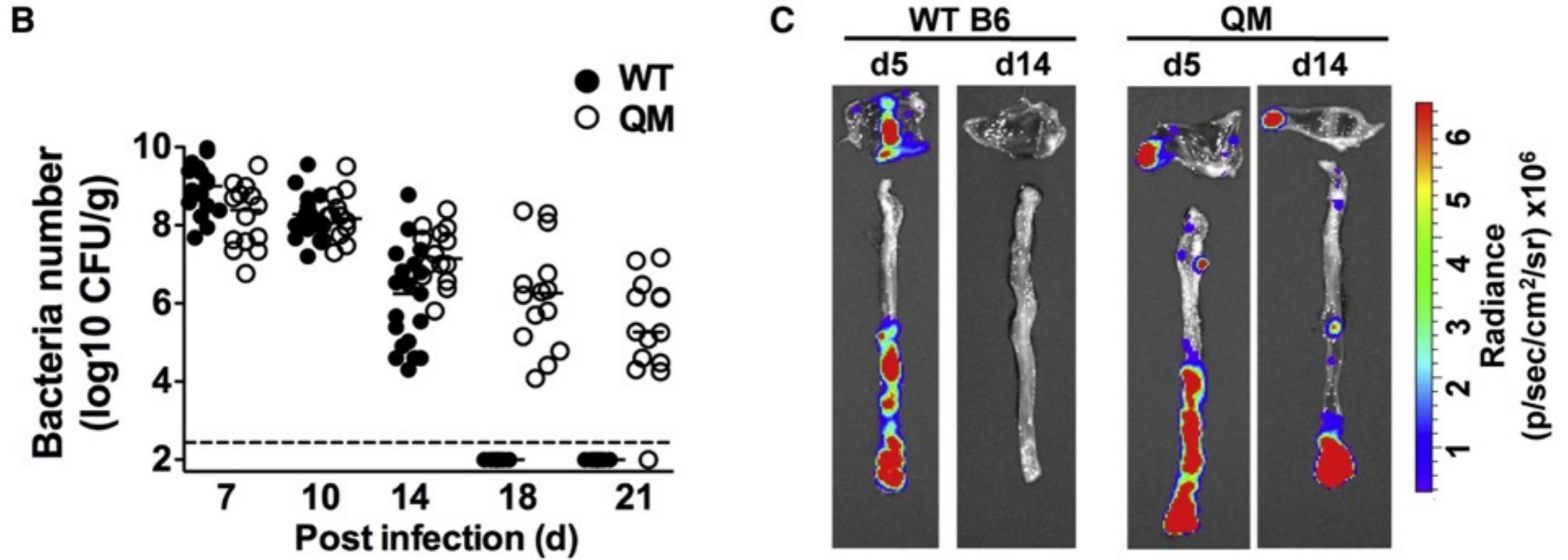


Figure 3

# Pathogen-specific IgG selectively bind virulent bacteria

**A**

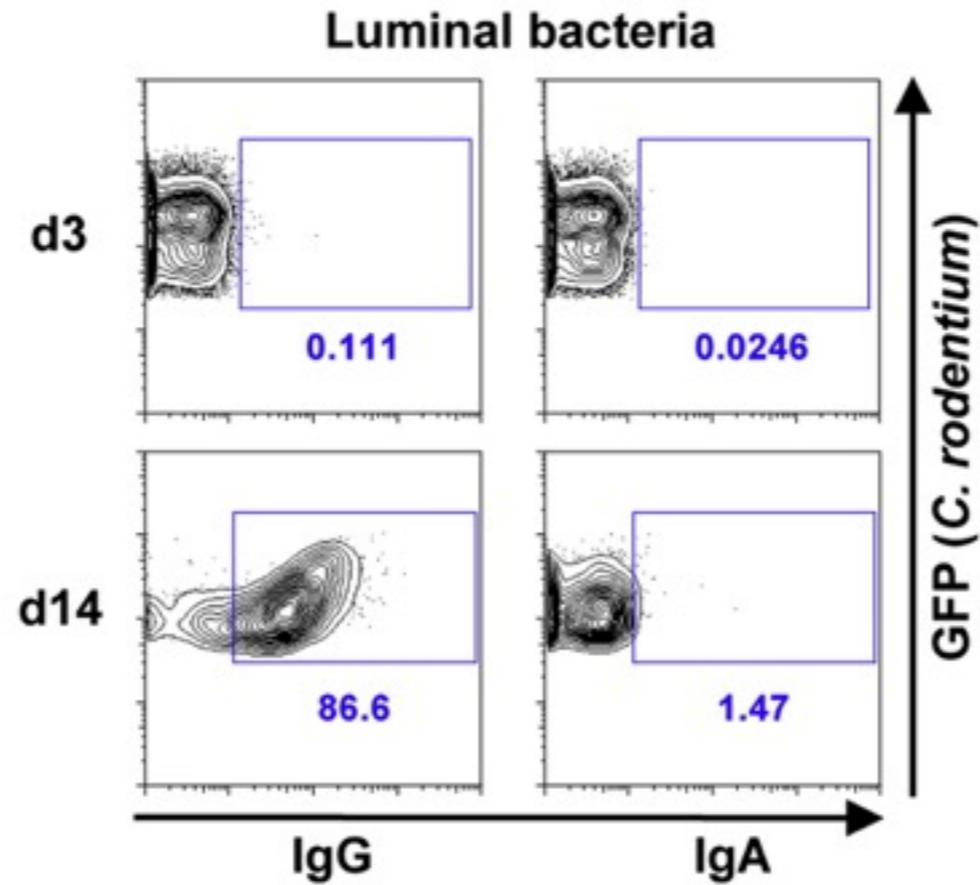


Figure 4

**A**

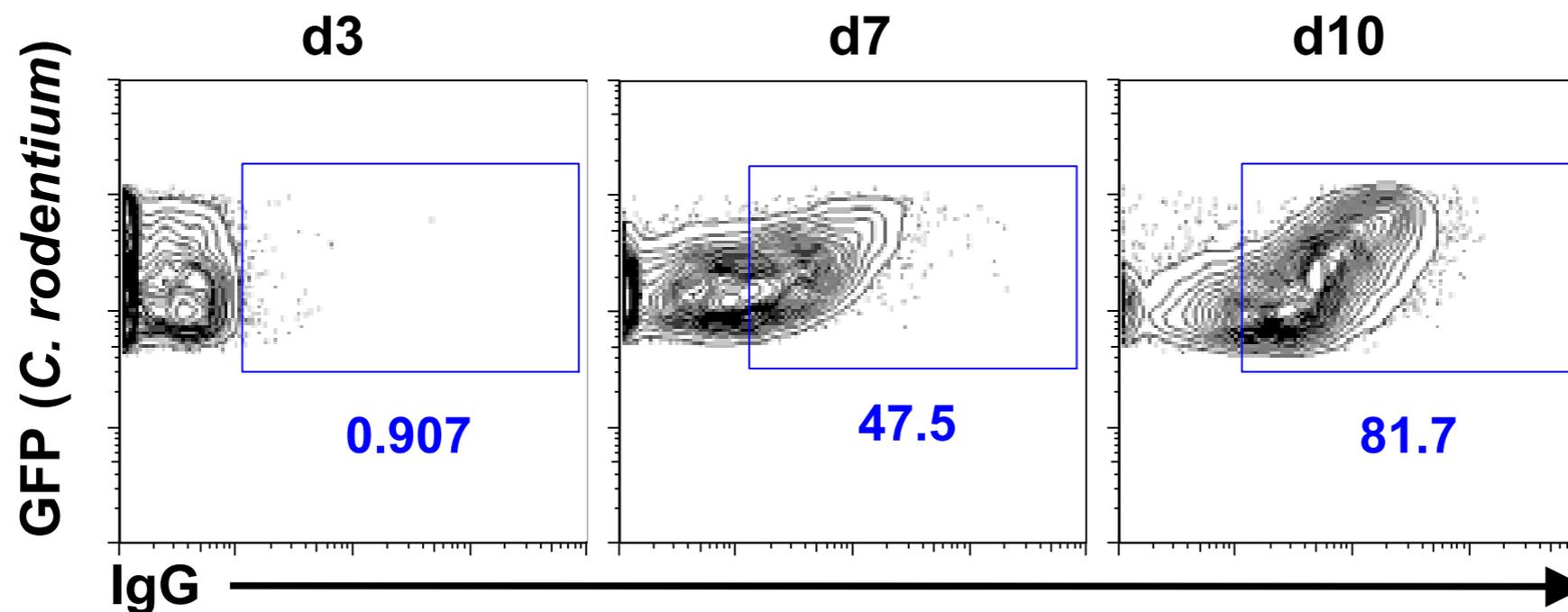
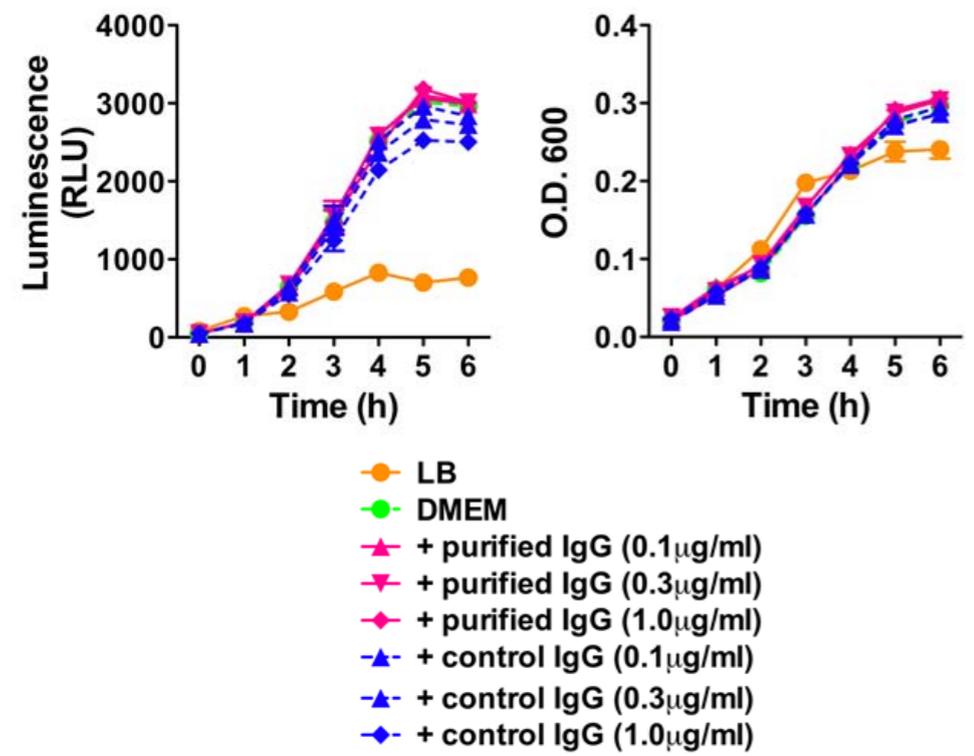


Figure S4

# Specific IgG are produced in response to the virulence factors

B



B

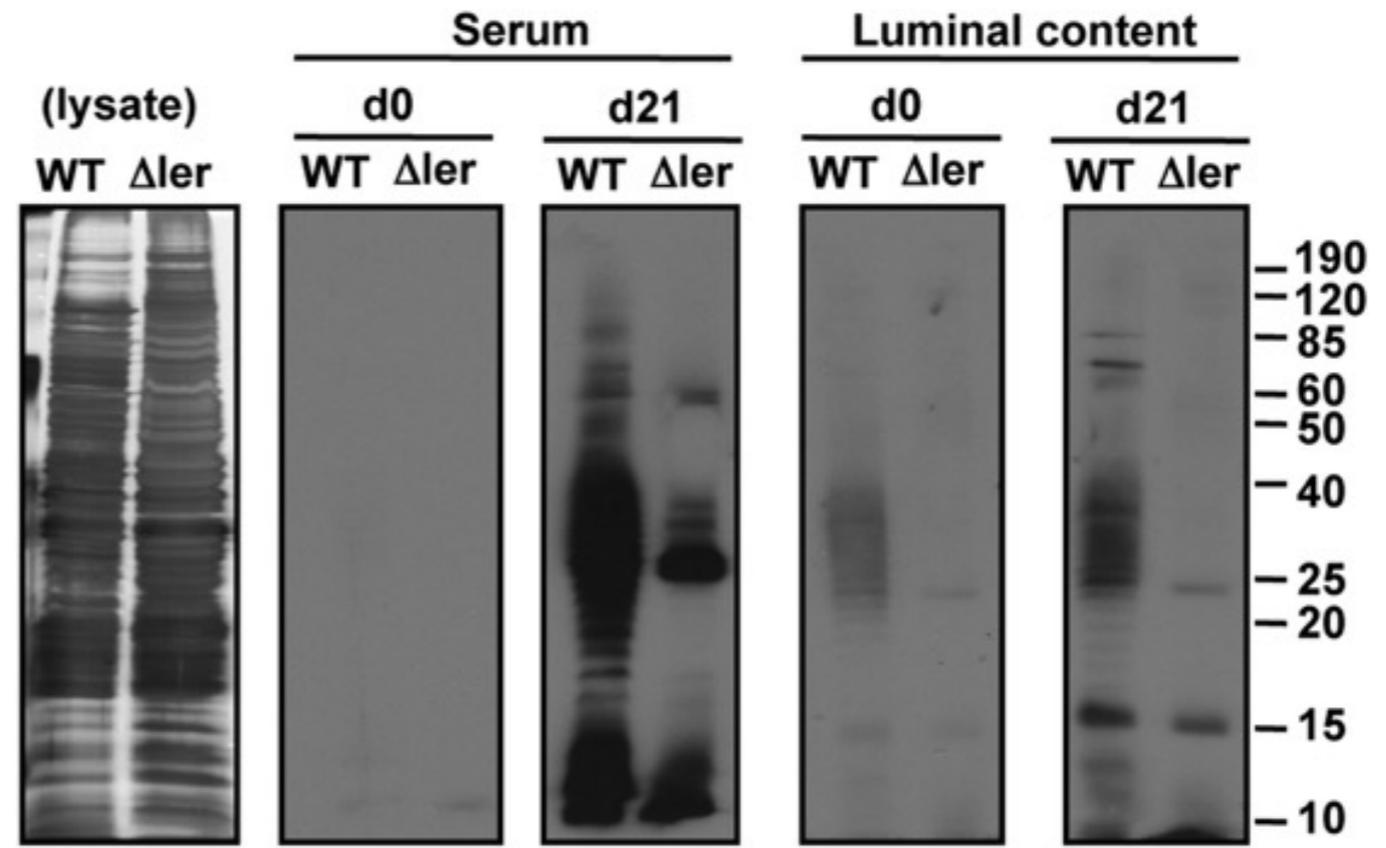


Figure S4

Figure 4

C

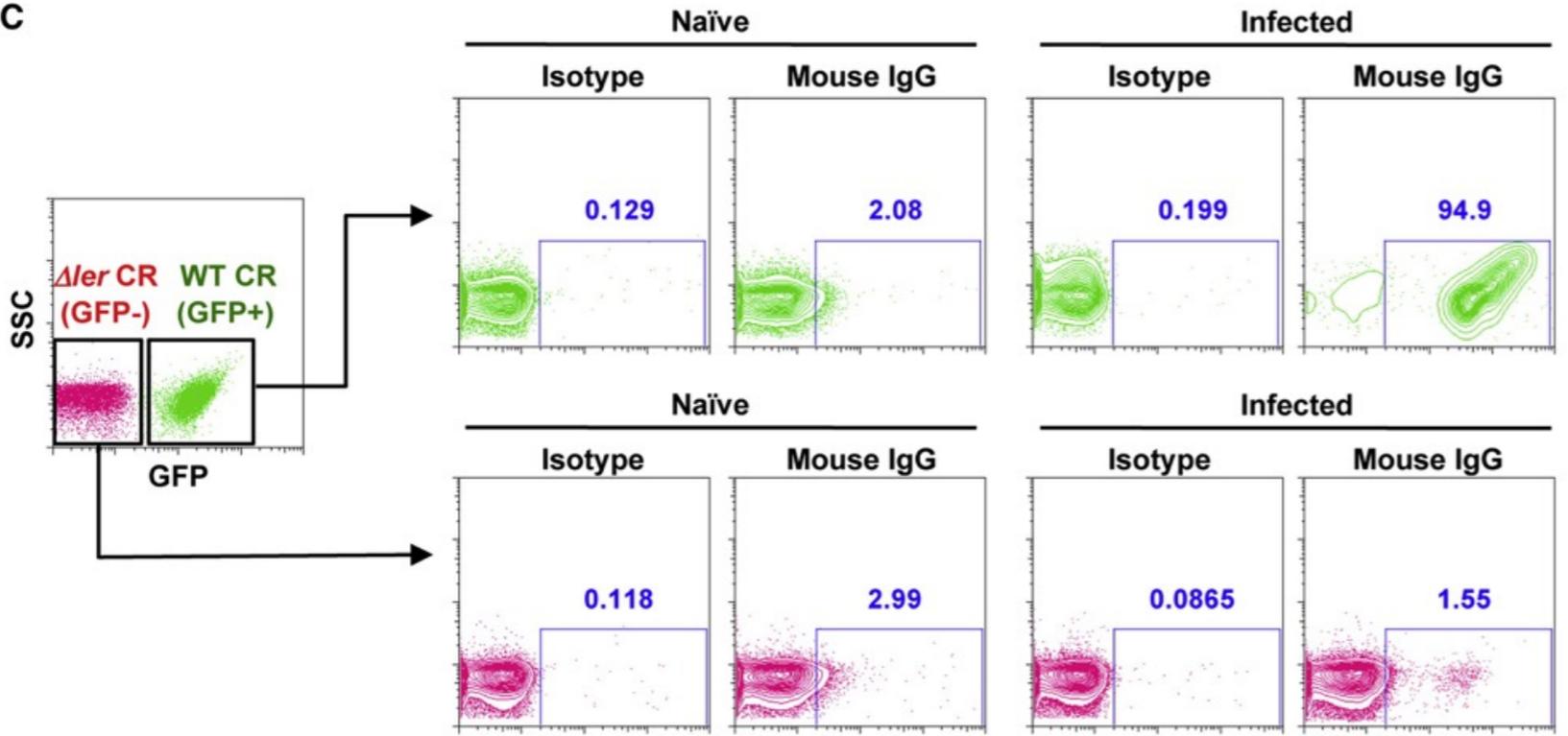


Figure 4

There are 2 phenotypically different *C. rodentium* populations

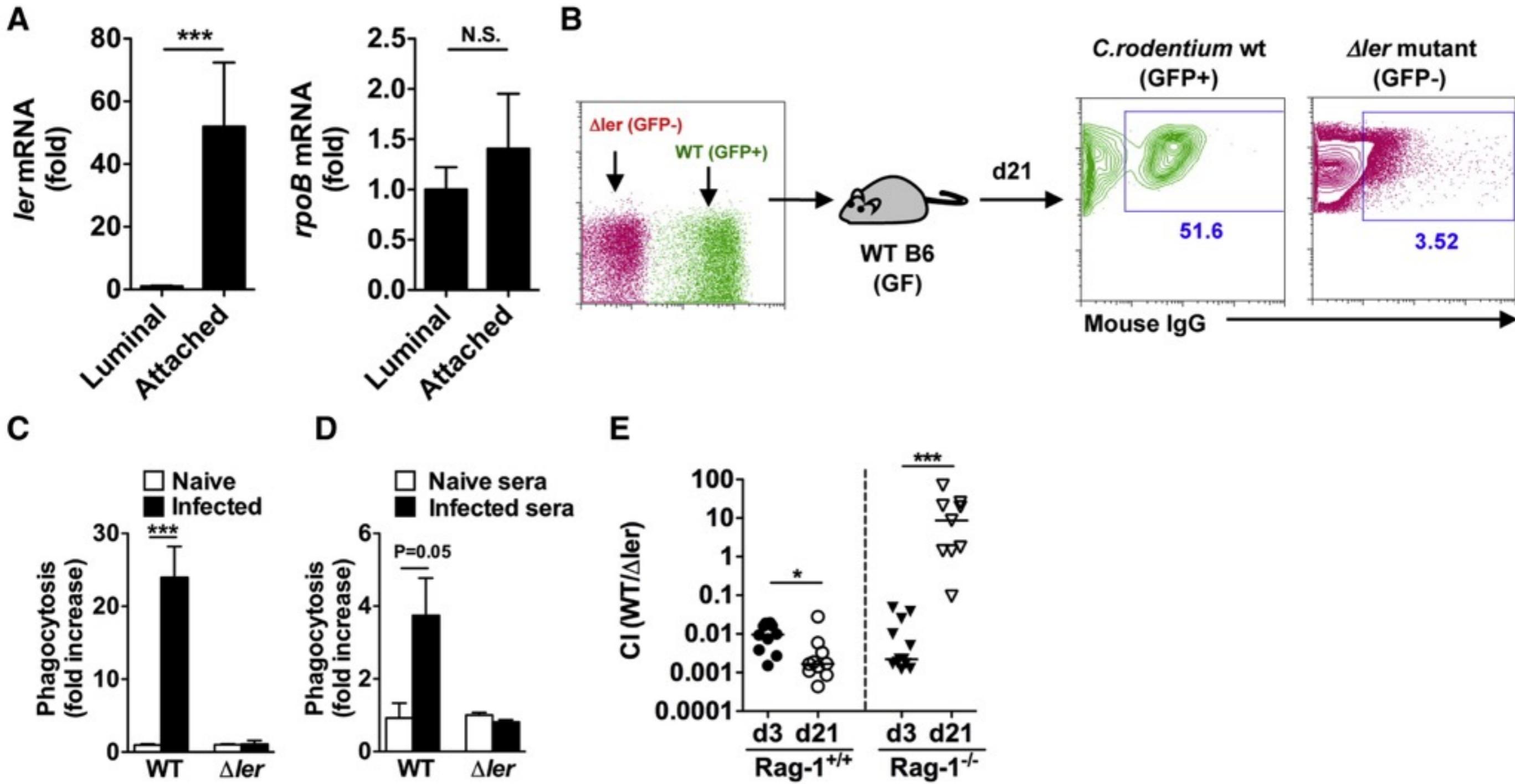


Figure 5

# Neutrophils are required for the elimination of phenotypically virulent *C. rodentium*

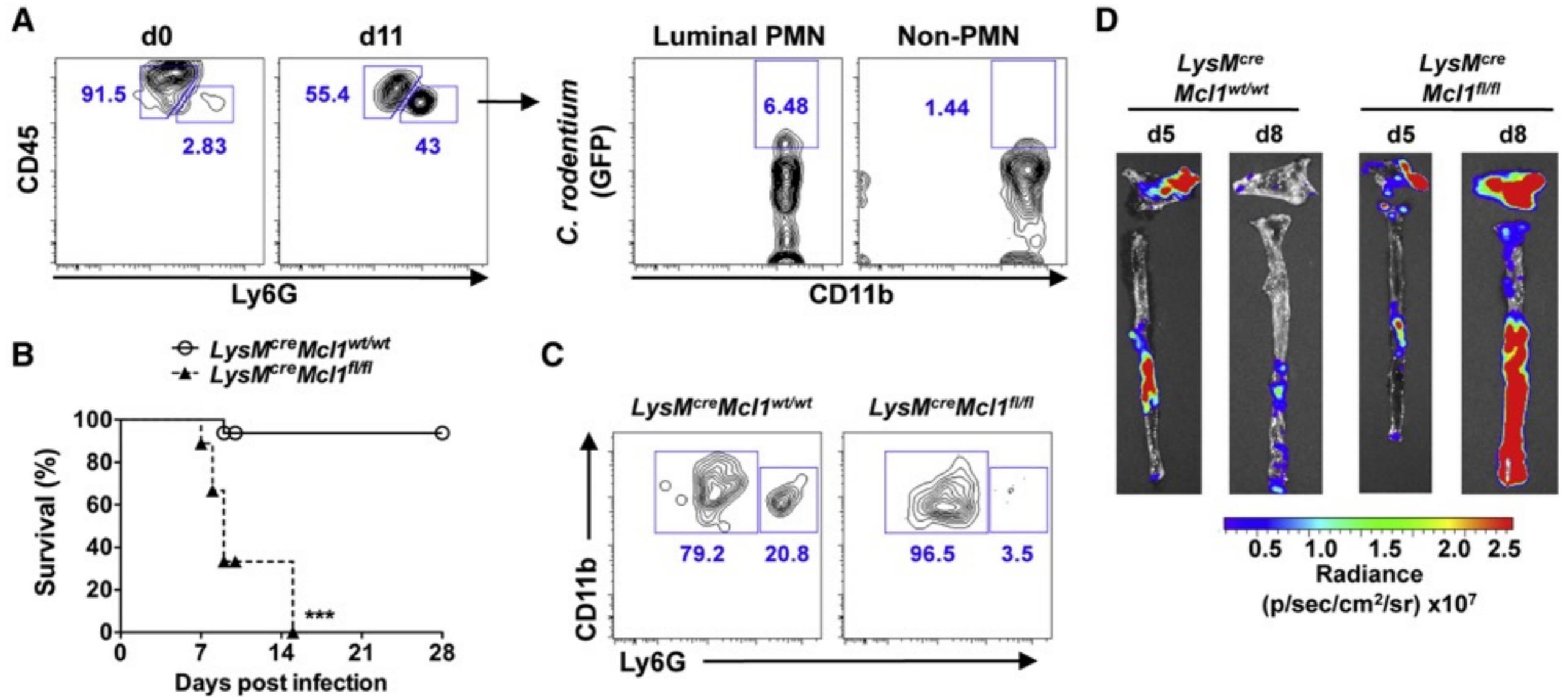


Figure 6

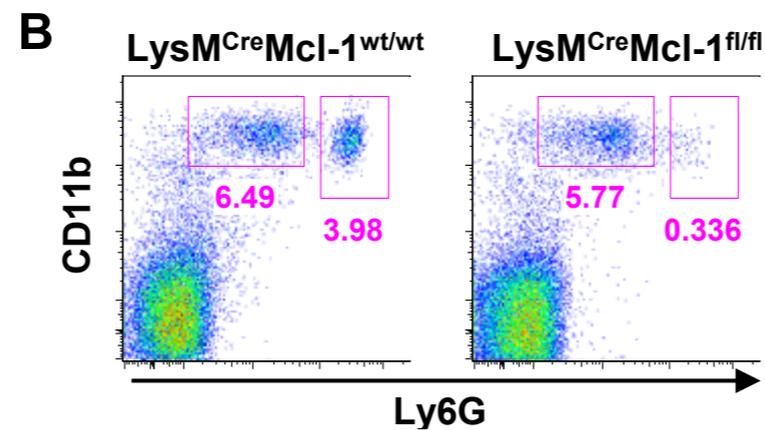
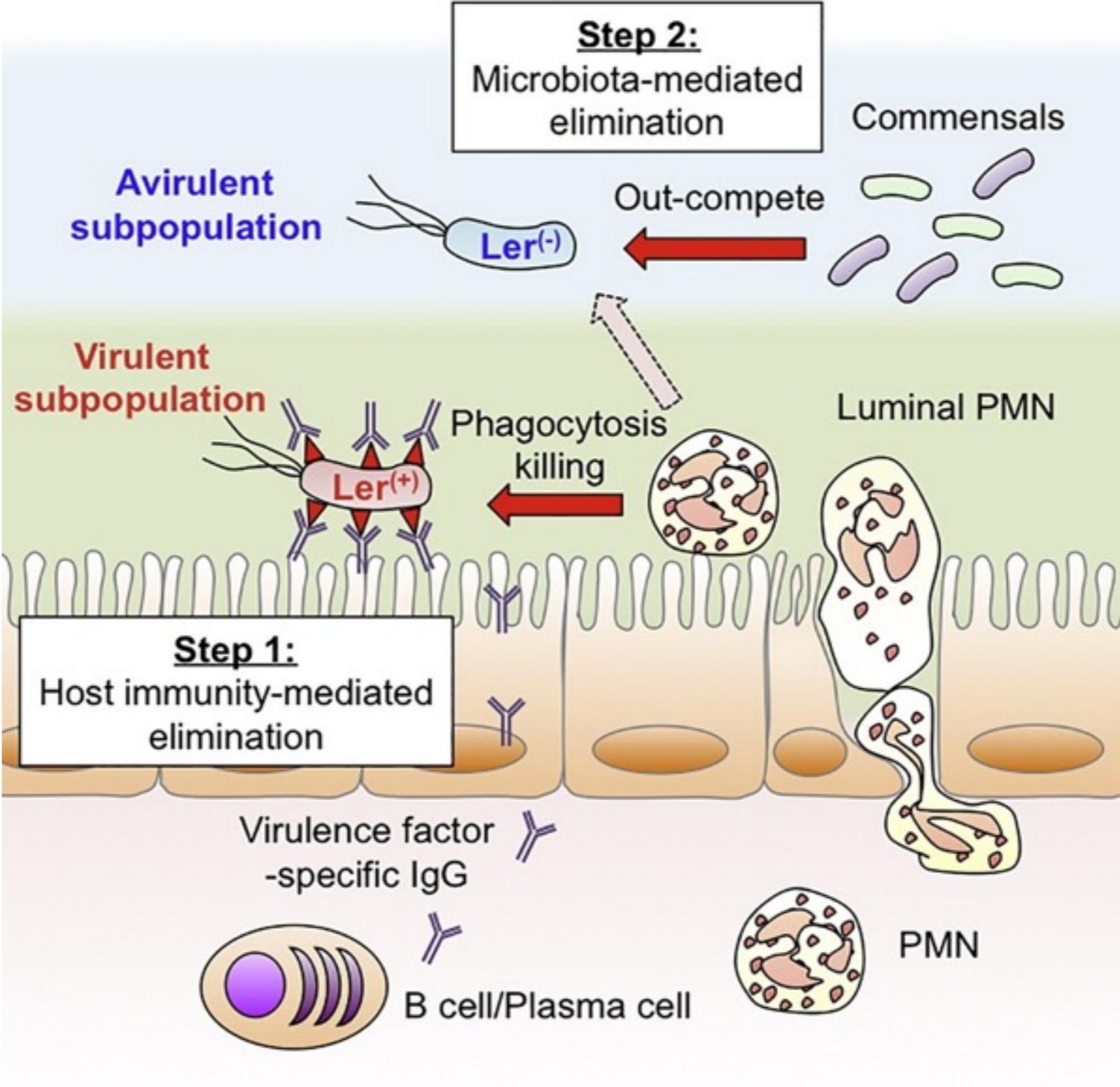


Figure S5

# Discussion



# Discussion

- Adaptive immune system is necessary for the survival of the animal in absence of other competing bacteria
- The presence of virulent and avirulent subpopulations contributes to the virulence stability
- LEE virulence is microbiota independent but its activation mechanism is not known
- IgG are generated against LEE virulence factors but not against other avirulent surface antigens: the mechanisms are not known
- Intimin is a highly antigenic protein
- The way of IgG to the lumen is not known
- Other component from the adaptive immune system may be critical for the outcome

# The Bamboo-Eating Giant Panda Harbors a Carnivore-Like Gut Microbiota, with Excessive Seasonal Variations

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# Background

- belong to the family of the Ursidae
- started to eat bamboo 7 million years ago (MYA)
- exclusively bamboo-eating species 2-2.4 MYA



- genetic: pseudogenization of umami taste receptor gene
  - anatomic: powerful jaws and teeth, enlarged pseudthumb
- But harbor a carnivore-like gut!



symbiotic gut microbes?

# Background

Herbivores bacteria

Bacteroidales, Clostridiales,  
Fibrobacterales, Spirochaetales

Carnivores

Enterobacteriaceae, Enterococcus

# Study design

Sampling	121 fecal samples from giant pandas 24 adults, 16 juveniles, 5 unweaned cubs 12 males, 21 females completed the whole trial
Timecourse	3 seasons: Spring (T1), Summer (T2), late autumn (T3)
Sequencing	barcoded pyrosequencing of V3 region from 16S rRNA genes
Dataset	92'819 usable reads corresponding to 781 OTUs

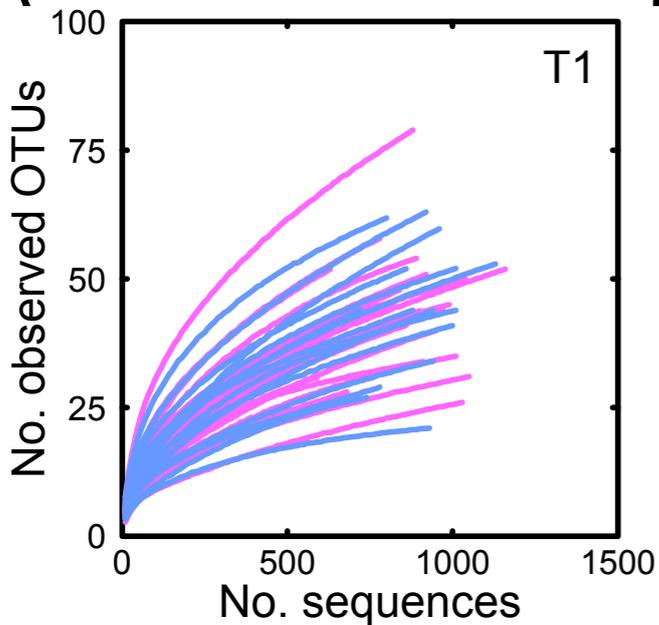
# Technical features of the dataset

T1

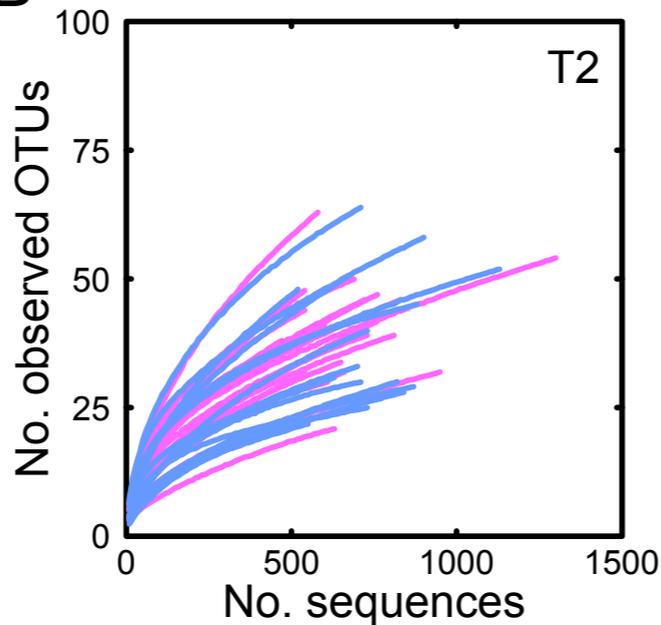
T2

T3

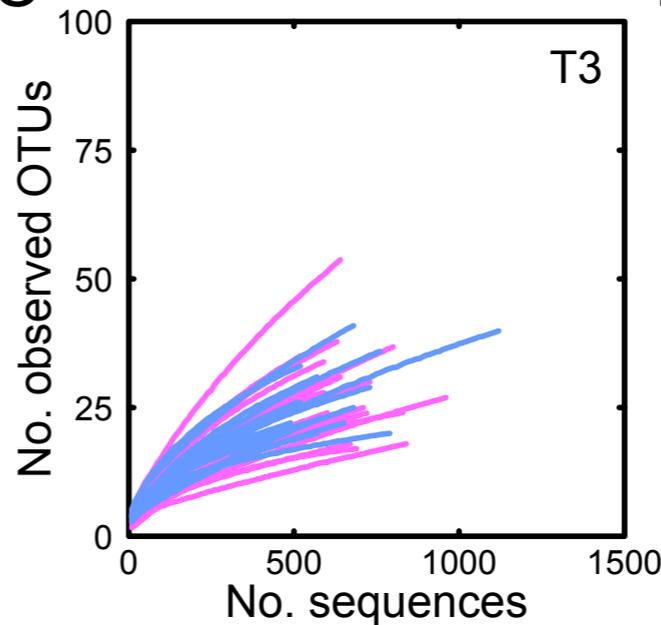
A



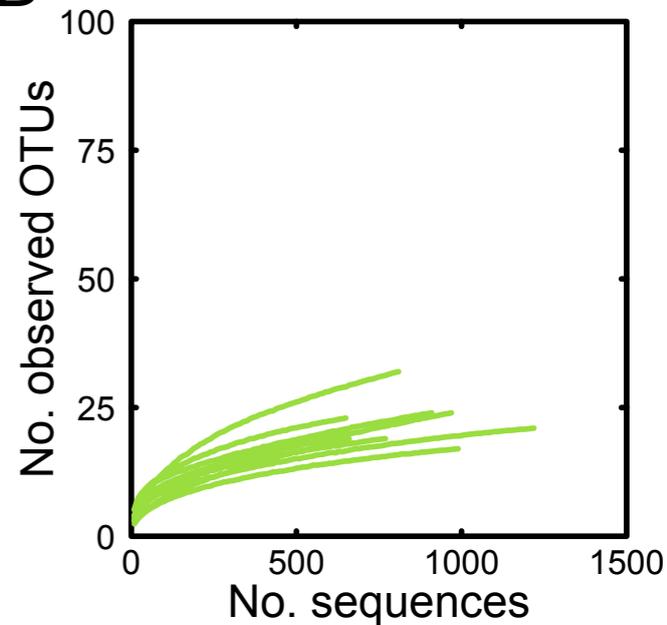
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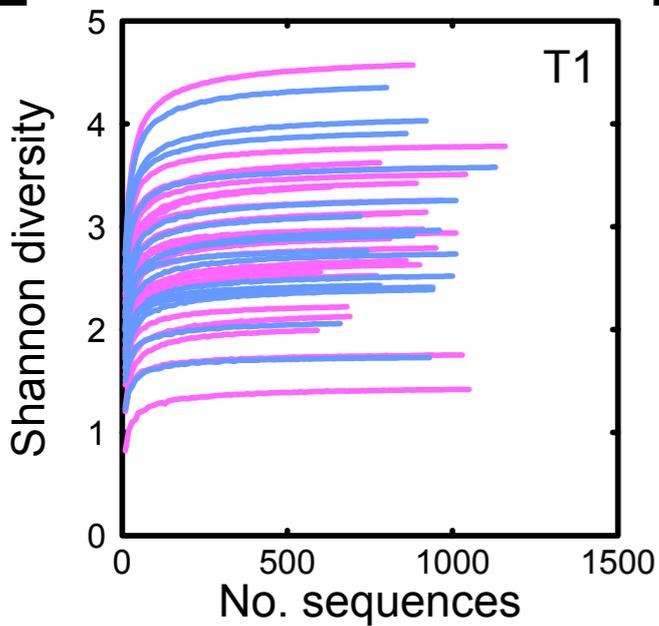
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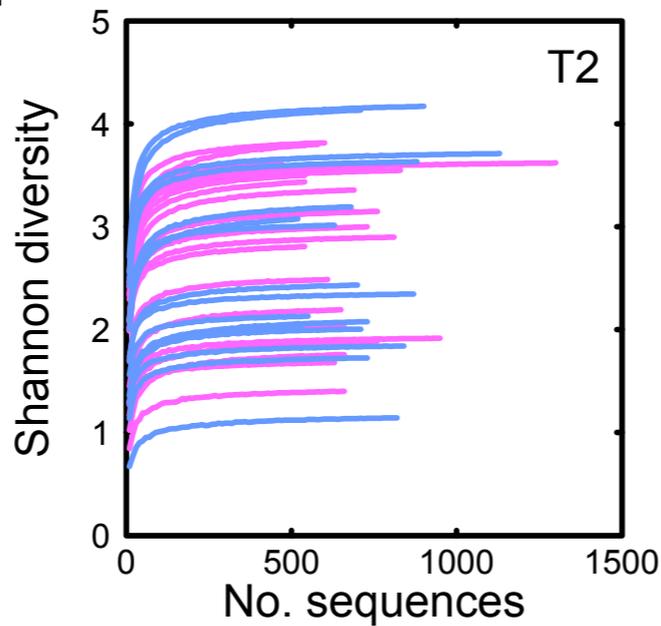
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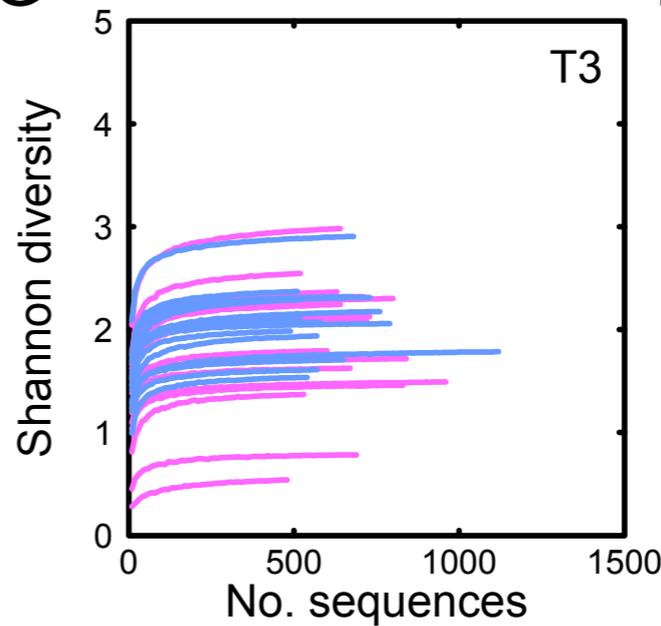
E



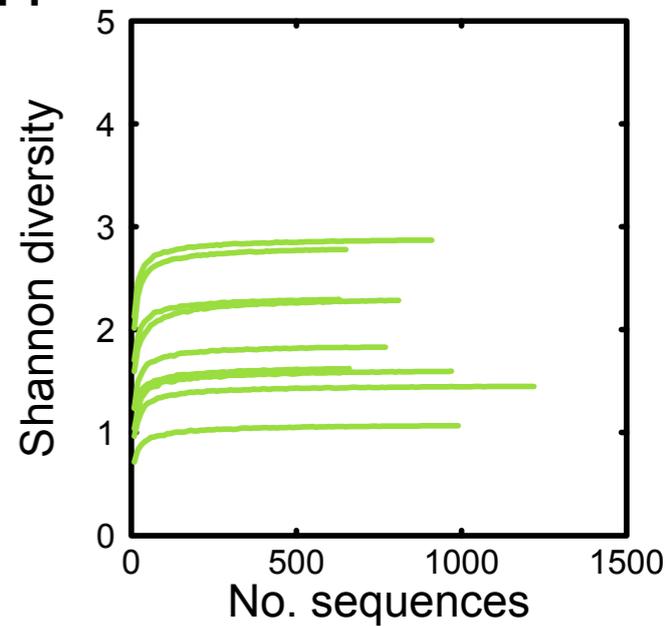
F



G



H



— Adults — Juveniles — Cubs

Figure S1

# Overall gut microbiota structure in giant pandas

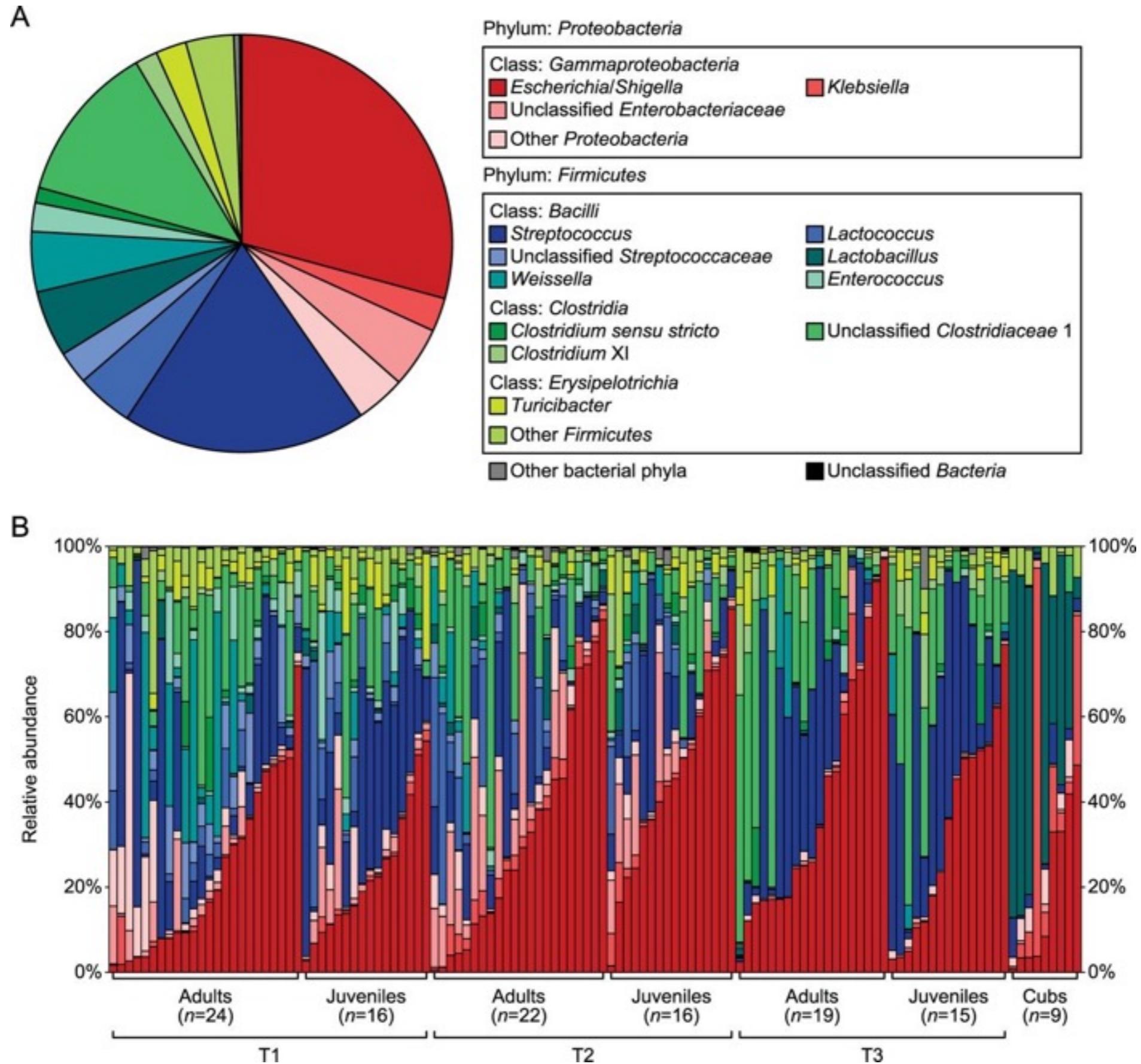


Figure 1

# Seasonal and age-dependent variation of the alpha diversity

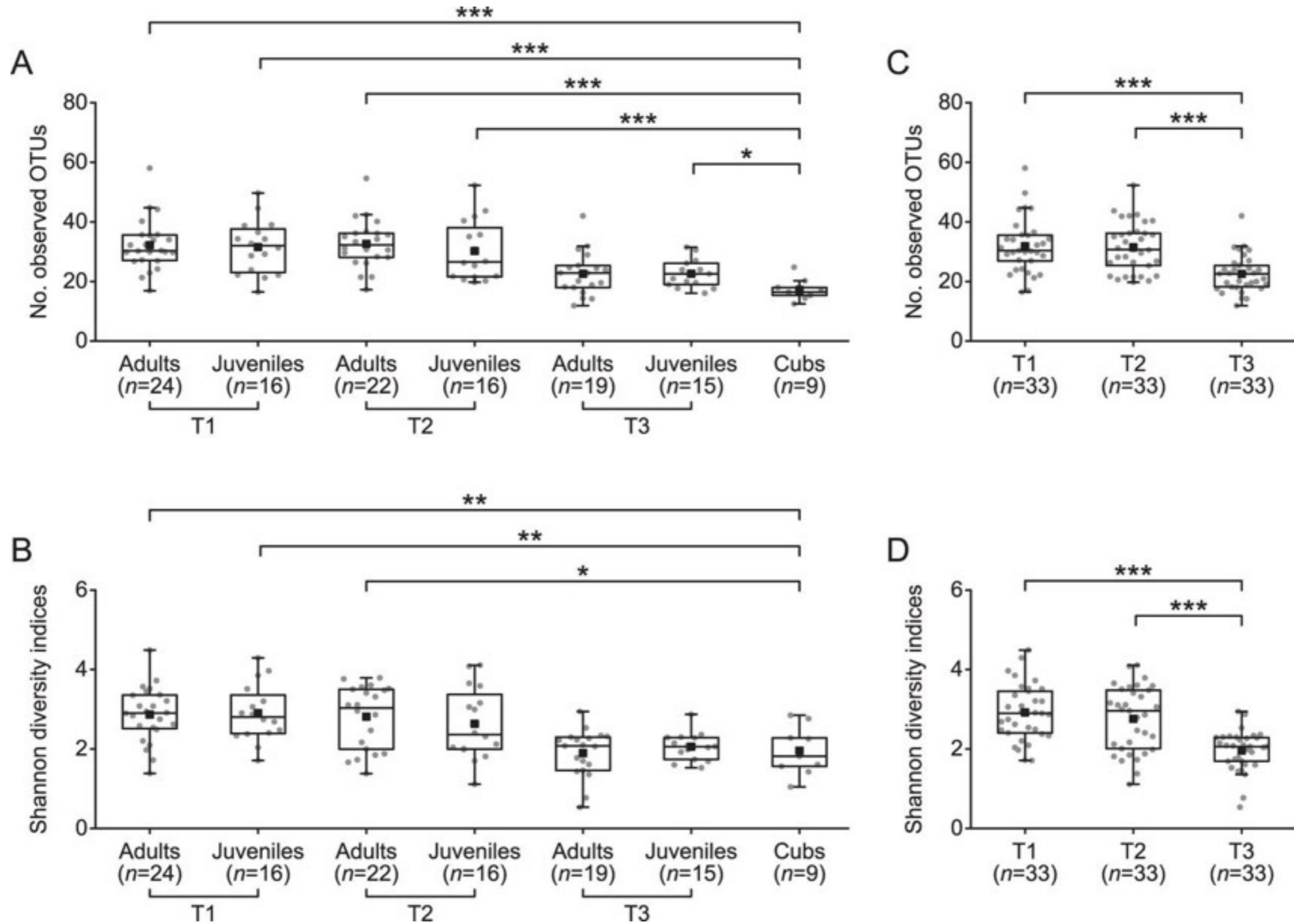


Figure 2

# Interseasonal variation in species abundance

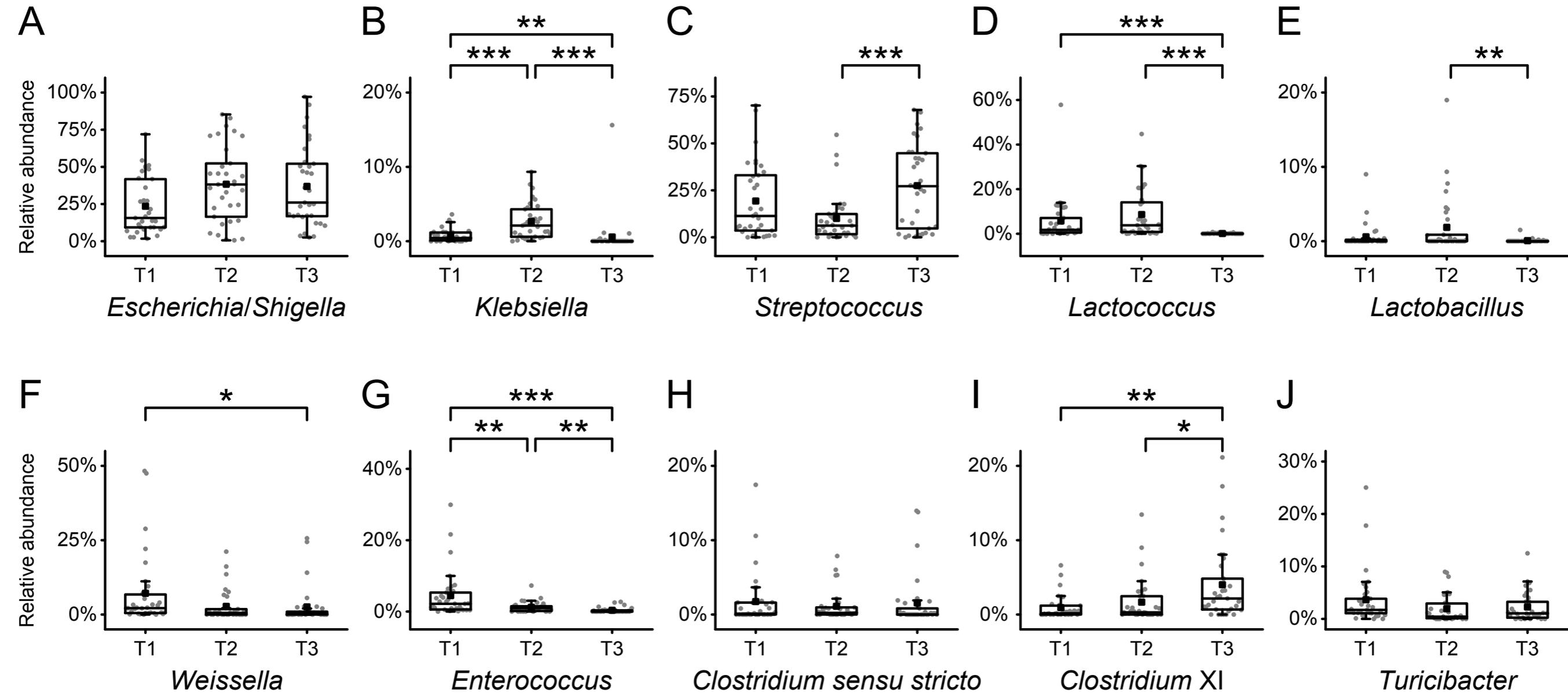


Figure S2

# Inter- and intraindividual variation

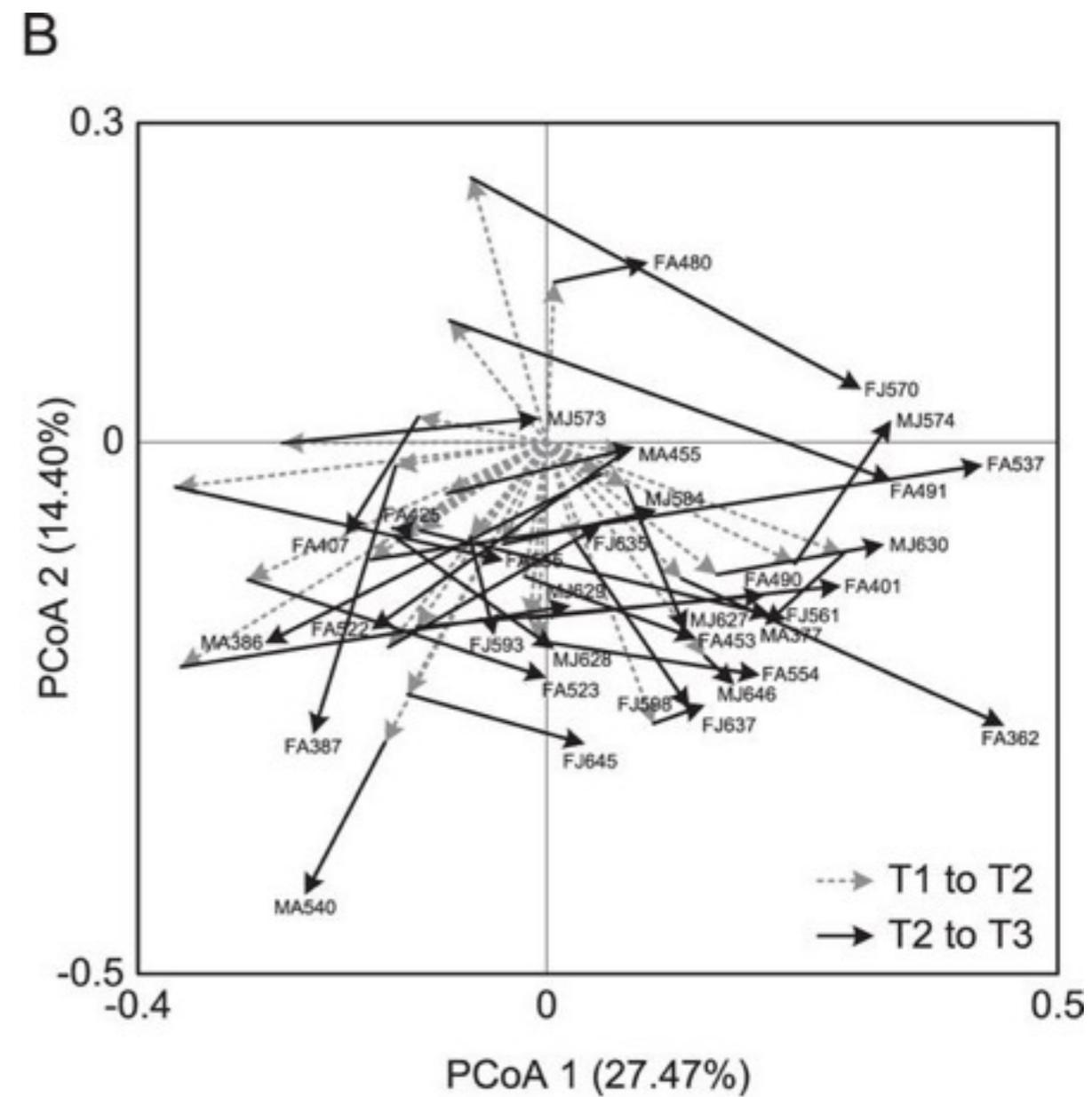
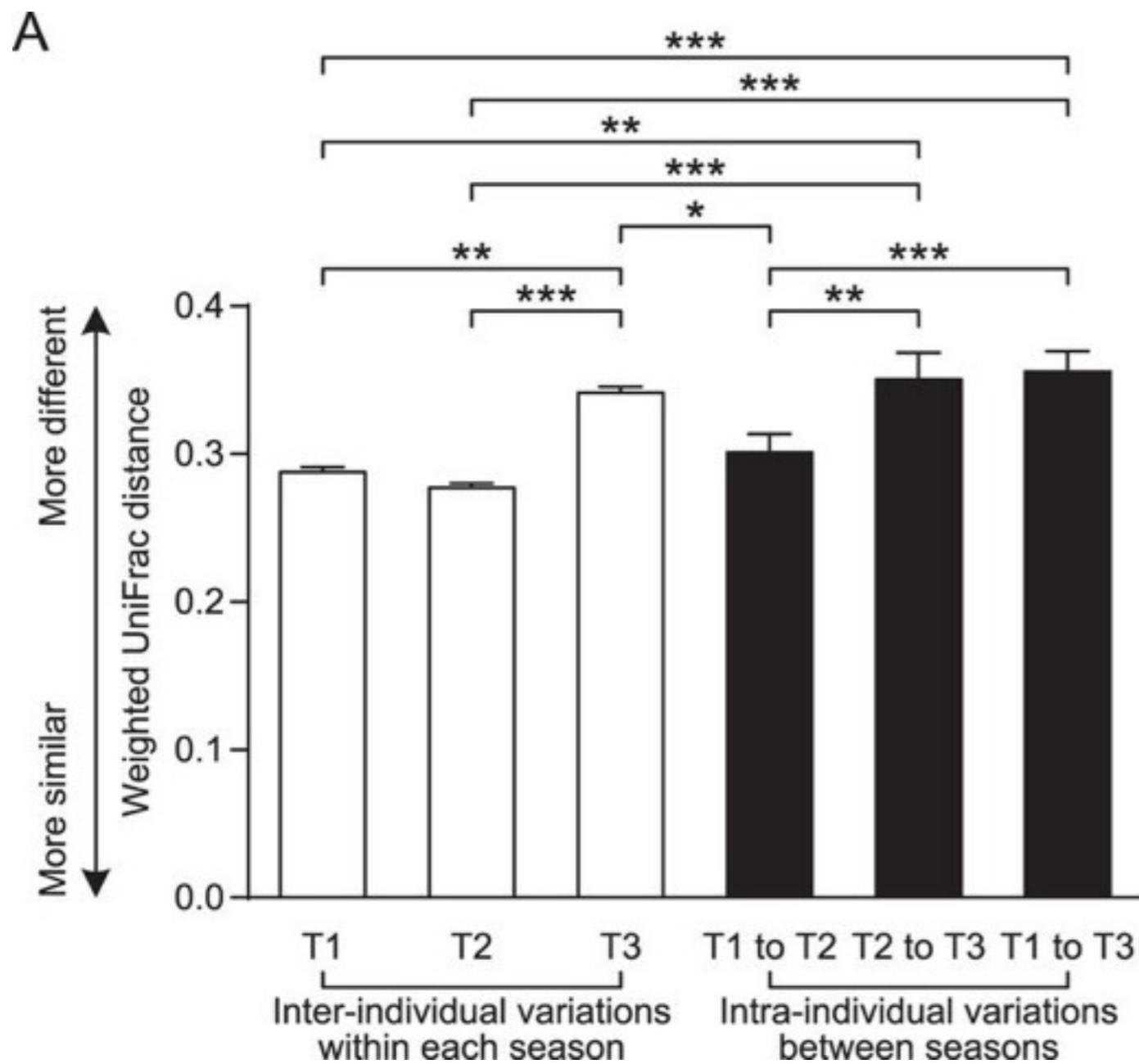


Figure 3

# Comparison of gut microbiota structure of pandas with other mammals

Studies

This study

8 captives, 8 wild giant pandas

3 other data sets with mammals

Animals

128 animals, 57 species from 13 different taxonomic orders

Sequences

229'288 sequences

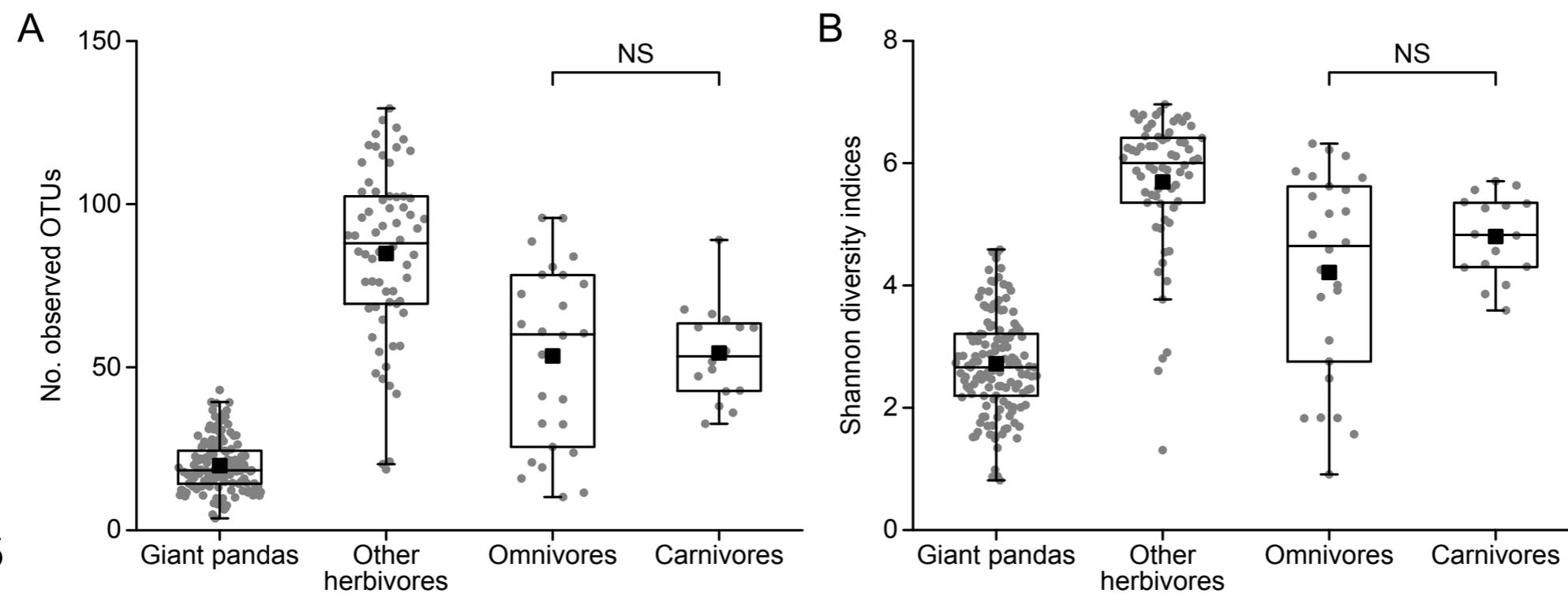


Figure S5

# Comparison of gut microbiota structure of pandas with other mammals

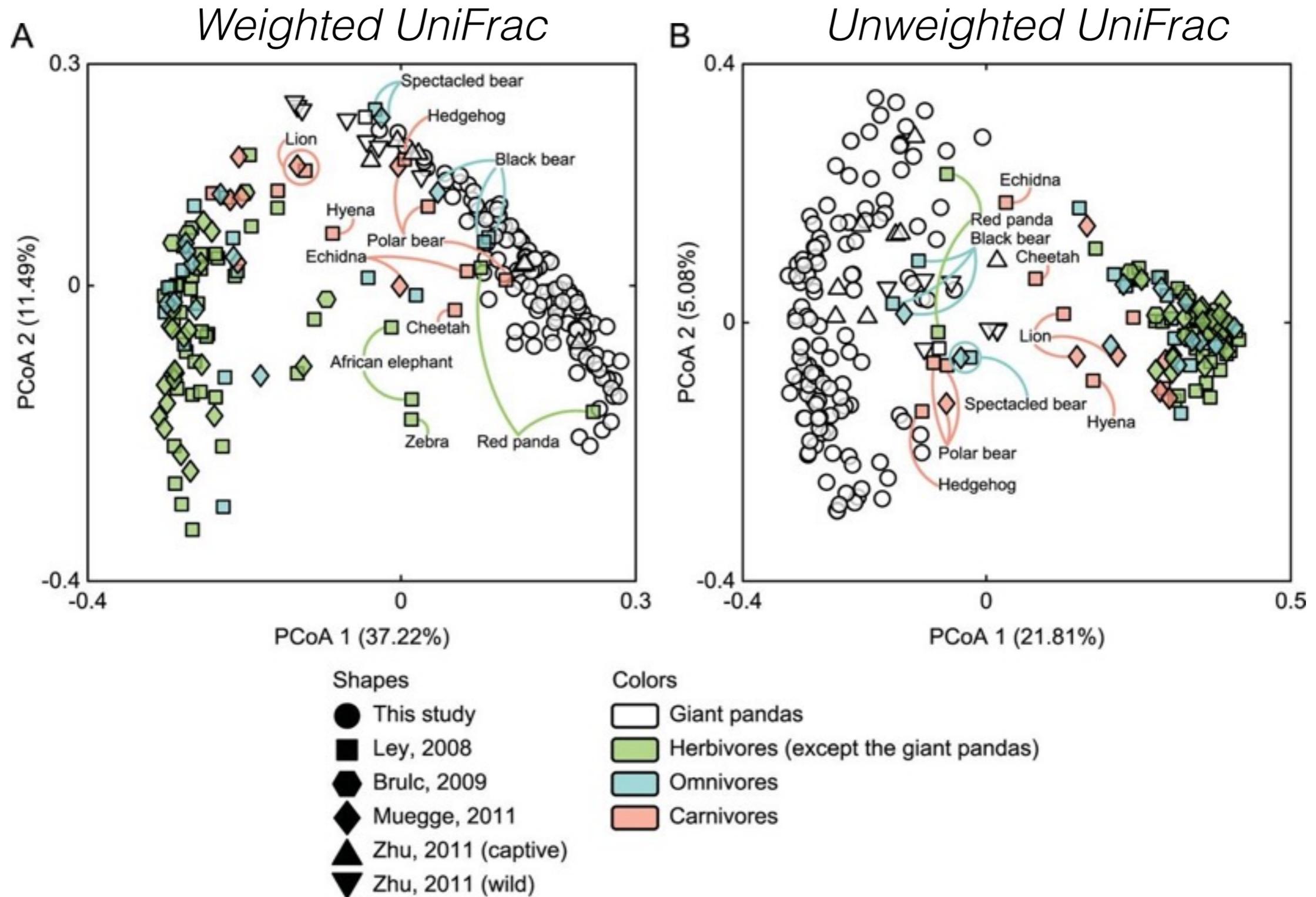


Figure 4

# Comparison of gut microbiota structure of pandas with other mammals: new samples

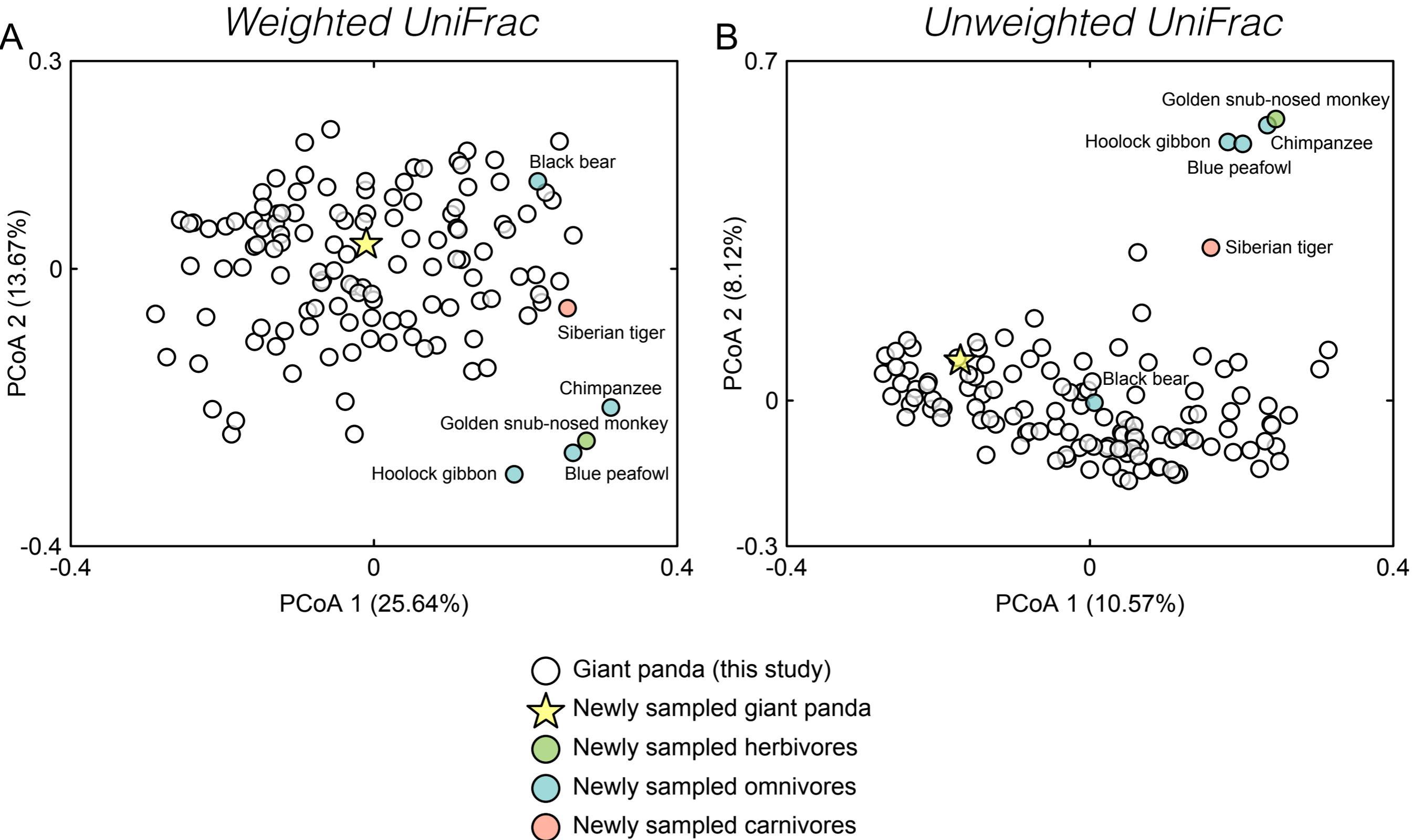


Figure S7

# Phylotypes involved in the deviation of panda gut microbiota from the non-panda herbivores

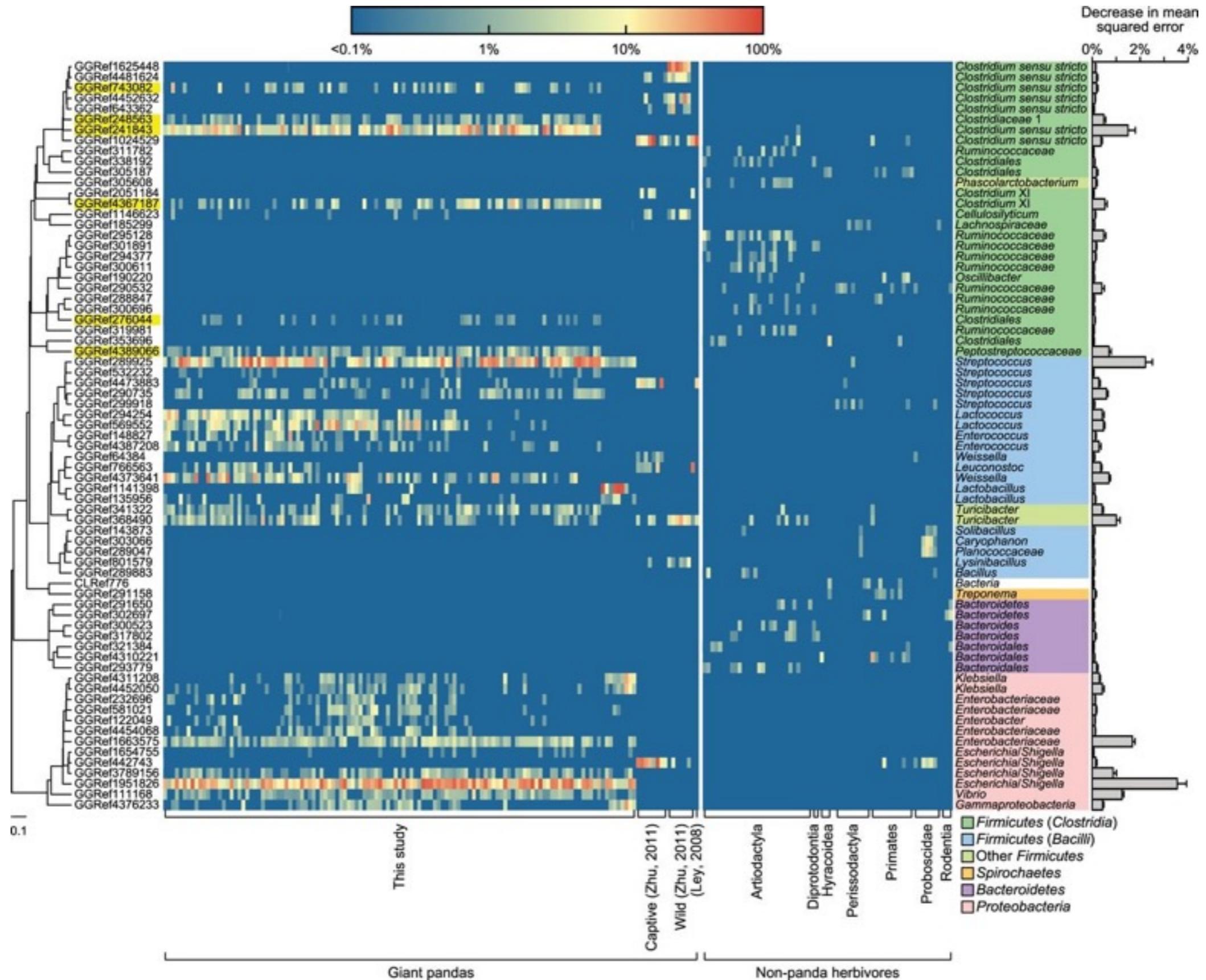


Figure 5

# Identification of putatively cellulolytic bacteria

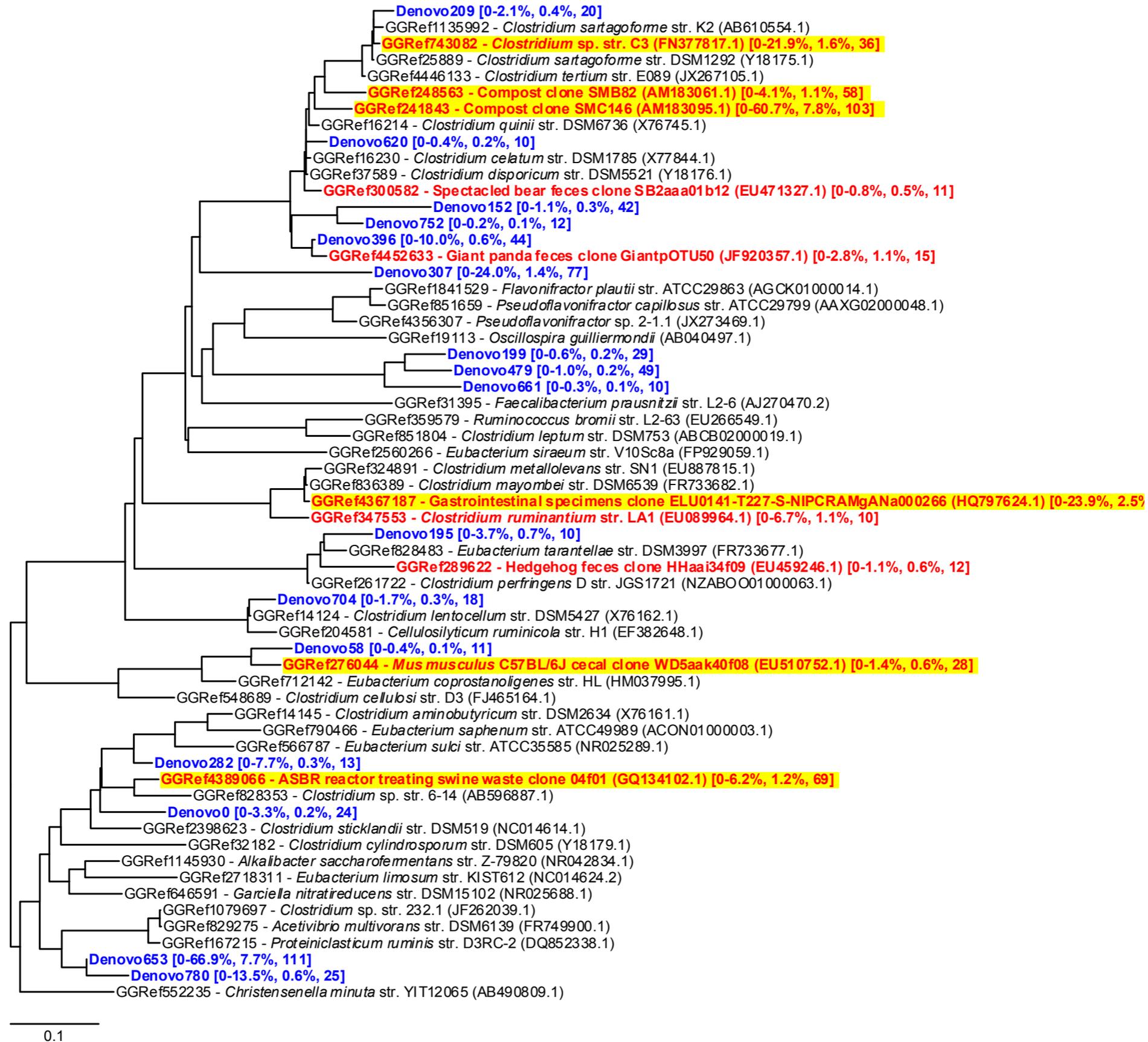


Figure S8

# Discussion

- No adaptation of the microbiota to the lifestyle, and this over 2 Mio years
- low microbial diversity <-> fragile lifestyle?
- intra- >> interindividual variation reflects the stability of the ecosystem
- physiological mechanisms remain to be explained

