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ORIGINAL ARTICLE

Lipoteichoic acid from the cell wall of a heat killed *Lactobacillus paracasei* D3-5 ameliorates aging-related leaky gut, inflammation and improves physical and cognitive functions: from *C. elegans* to mice

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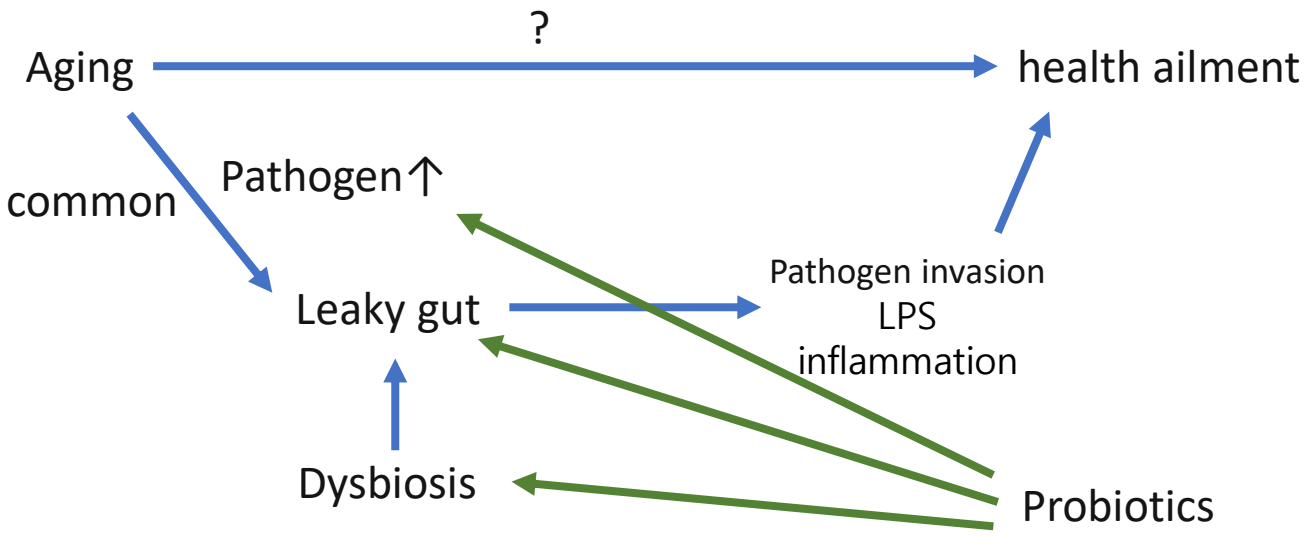
박재한

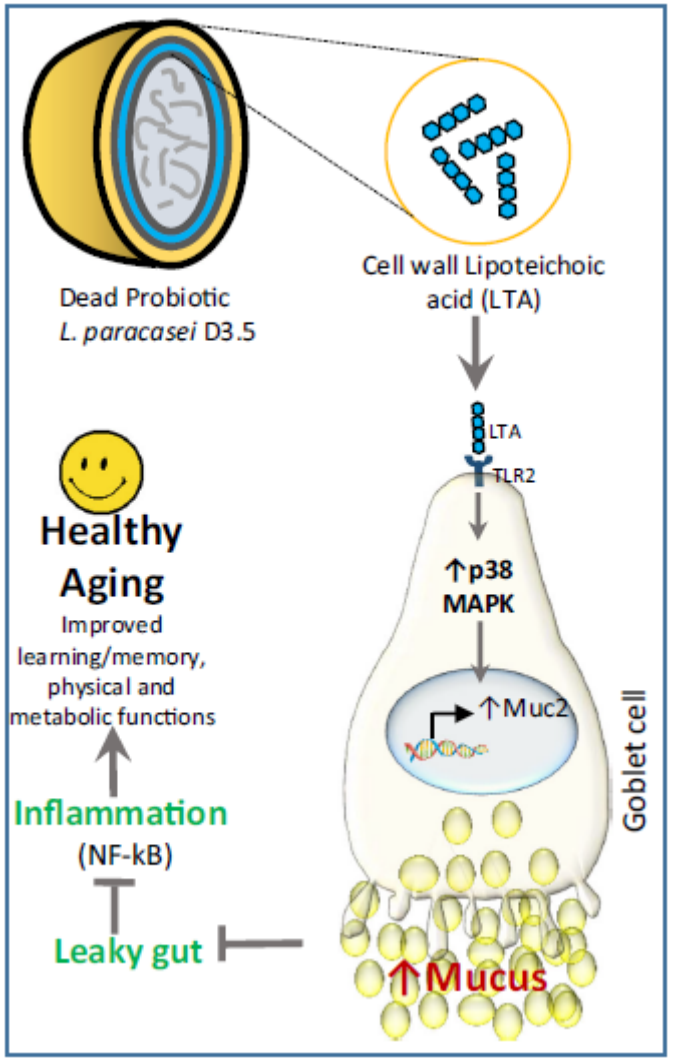
Introduction

The world's population is rapidly **aging**

-> Aging-related comorbidities

obesity, diabetes, cardiovascular diseases, cancer, infections, cognitive decline





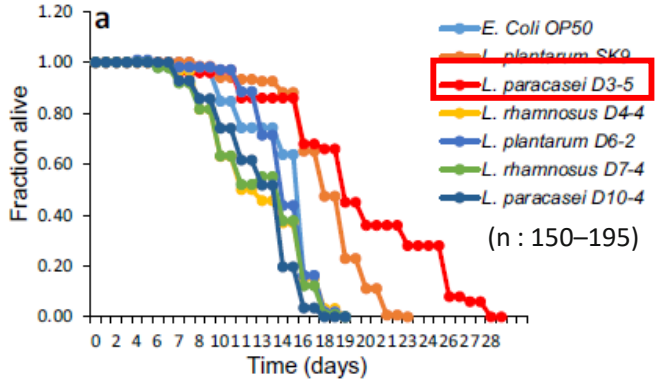
Few studies on the effect of probiotics in longevity

No studies using HK probiotics

Figure 1. Feeding dead probiotics extends lifespan, improves physical function muscle mass in C. elegans.

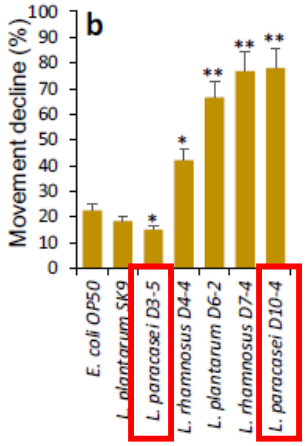
A : longevity assay, C. elegans N2, n : 150–195
B : picked in to M9 buffer, stroke number count
C : imageJ
D : muscle mass, muscle-specific GFP-labelled strain MAH19

longevity assay, C. elegans N2

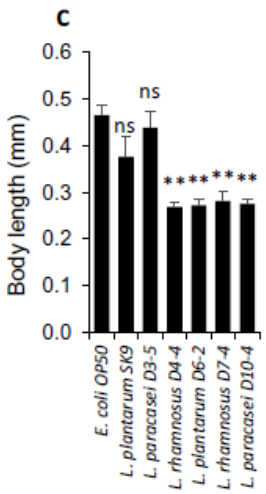


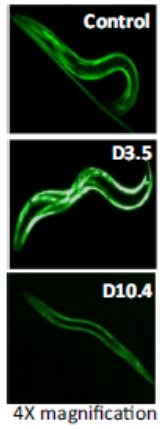
➡ Certain dead probiotics are beneficial on **extending life-span**

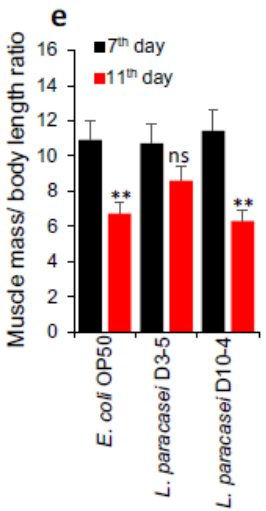
Movement decline

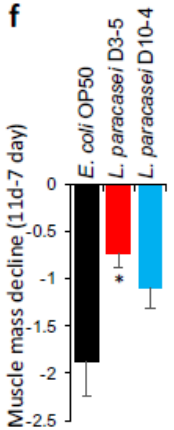


Body length & muscle mass







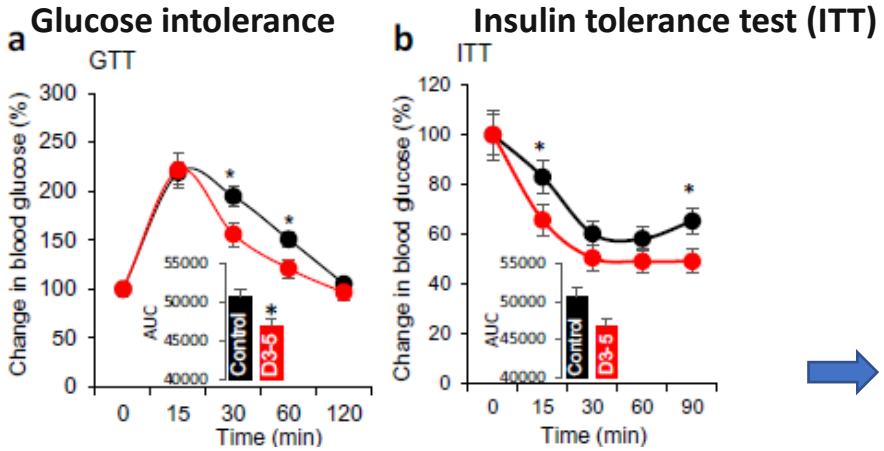


➡ Certain dead probiotics are beneficial to aging related ailments **preserving better physical function & muscle mass**

beneficial effects are **strain-specific**

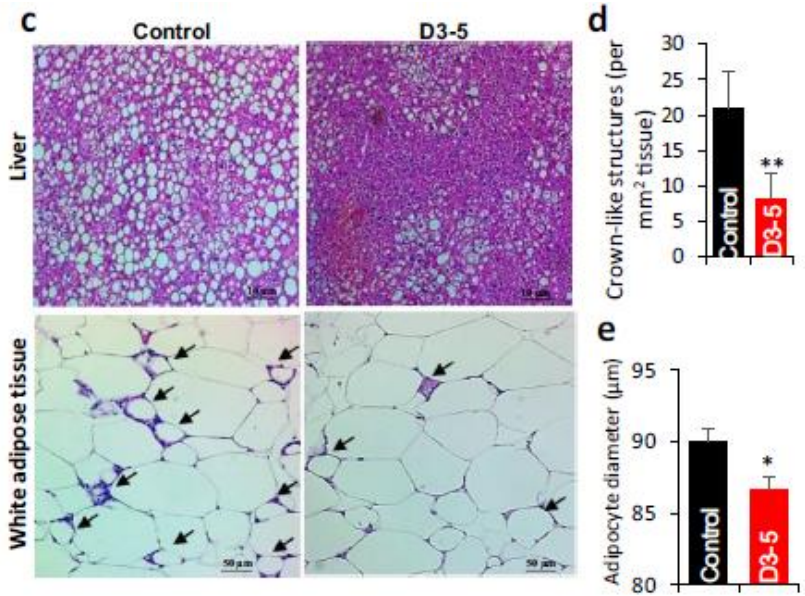
Figure 2. Feeding dead probiotics prevents HFD-induced metabolic dysfunctions in older mice

78–80-week-old;equivalent to > 65 years of human age, fed with HK D3-5 in drinking water, High fat diet
A : Glucose tolerance test, fasted 10–12 h, gavage of glucose (2.5 g/kg bodyweight)
B : Insulin tolerance test (ITT) fasted 4–6 h, intraperitoneal injection with insulin
C : H&E staining



→ D3-5 feeding prevented HFD-induced **glucose intolerance**, **insulin resistance**

Fat accumulation in liver



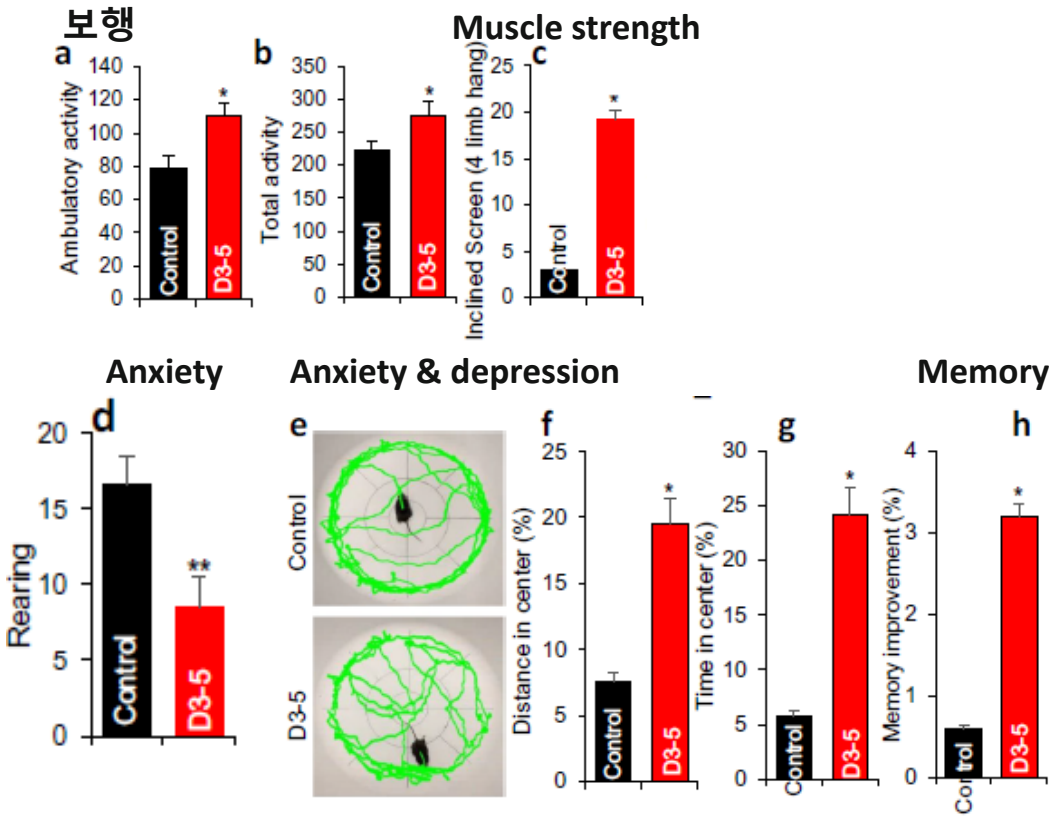
→ D3-5 feeding prevented **hepatic steatosis**, **inflammation** in WAT

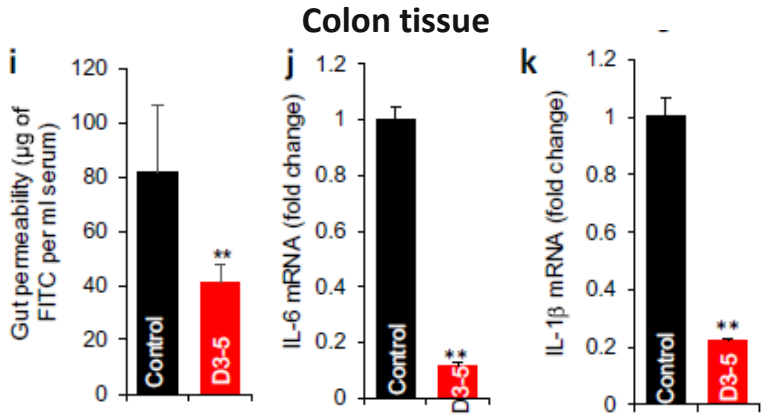
*Crownlike structures (indicator of inflammation) in the white adipose tissue

Figure 3. D3-5 feeding improves aging-related ailments in older obese mice

78–80-week-old;equivalent to > 65 years of human age, fed with HK D3-5 in drinking water, High fat diet
A, B : Behavior measure
E : Open field test
H : Water maze test
I : FITC-dextran (3–5 kDa) from gut to the blood
J, K : qRT-PCR

Aging related ailment : **physical function**, **increased anxiety**, and **decreased cognition**





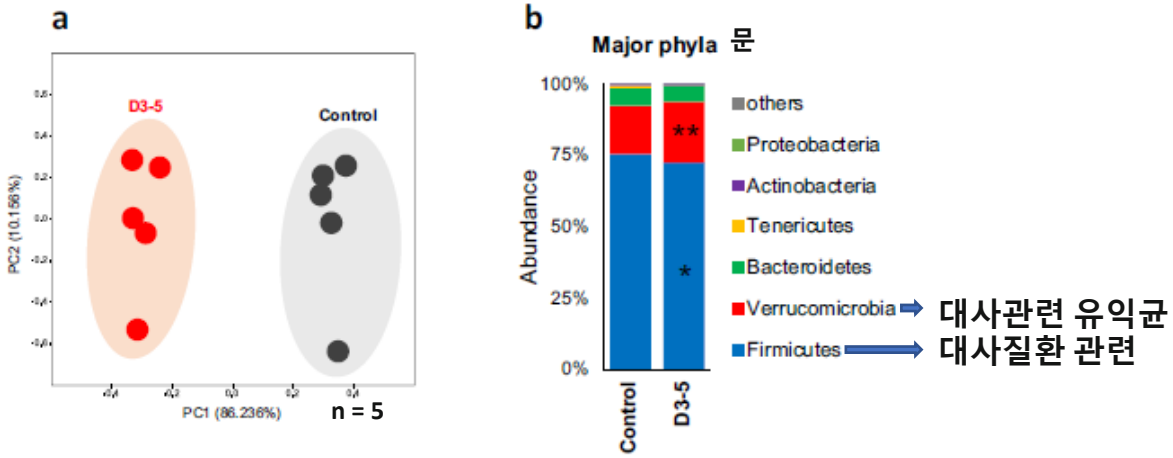
➡ **Decreased gut permeability** and **inflammation** in older HFD-fed obese mice.

➡ D3-5 feeding led to
Better physical and **cognitive functions, reduced anxiety/depression**

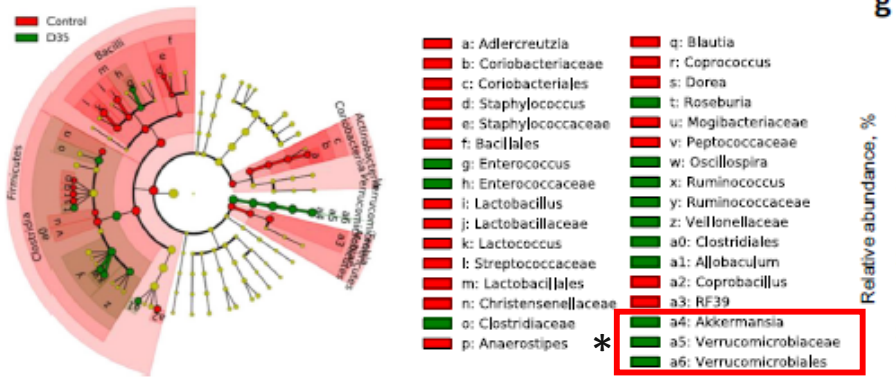
Figure 4. D3-5 feeding results in favorable changes in gut microbiome

78–80-week-old;equivalent to > 65 years of human age, fed with HK D3-5 in drinking water, High fat diet
Gut microbiome analyses
A : Principal coordinate analysis (PCoA)
F : linear discrimination analysis

β-diversity clustering

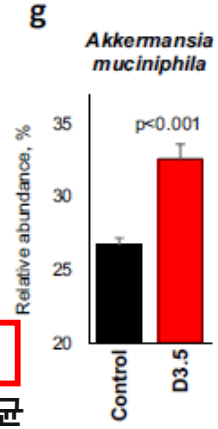


f Clustering of gut microbiome



대사관련 유익균

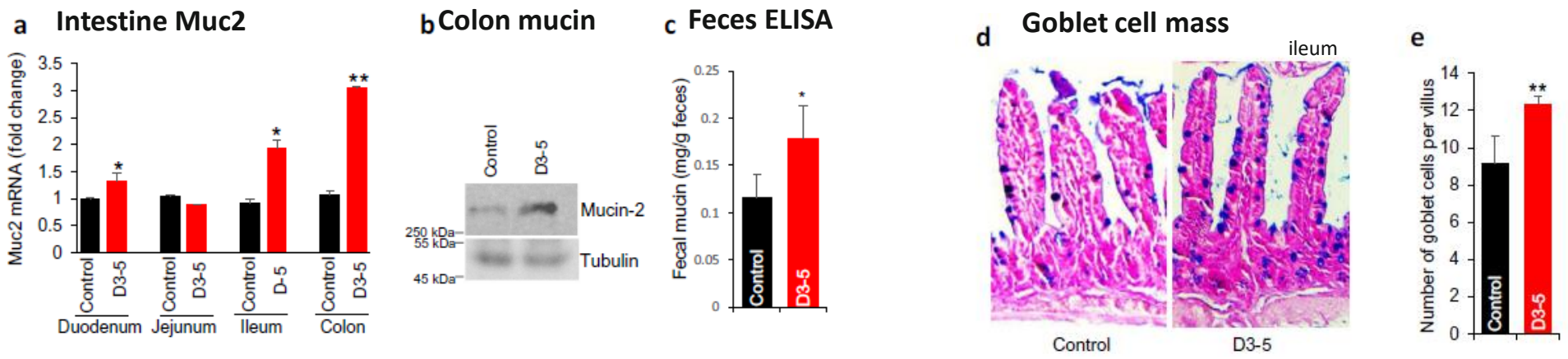
Mucin degrader



➡ D3-5 feeding results in **favorable changes** in gut microbiome

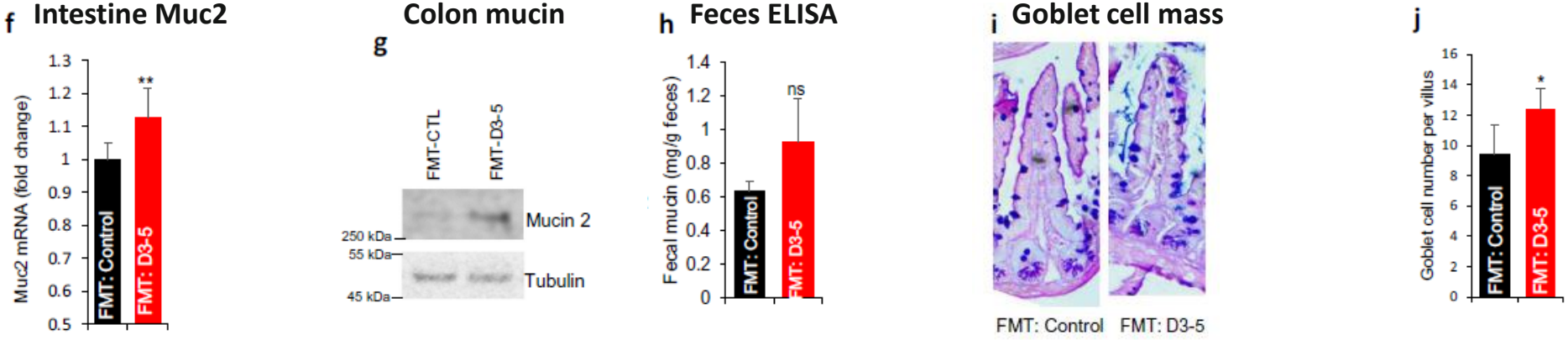
Figure 5. D3-5 treatment enhances mucin production and goblet cell mass in the gut of older obese mice

78–80-week-old;equivalent to > 65 years of human age, fed with HK D3-5 in drinking water, High fat diet
A : qRT-PCR
B : Western blot colon tissue
C, H : Feces ELISA
D, I : AB/PAS staining
F-J : Fecal microbiota transplantation, transplanted D3-5 fed & control mice to gut cleaned (GC; using antibiotics and polyethylene glycol [PEG] protocol) mice



➡ D3-5 treatment enhanced **mucin production** and **goblet cell**

FMT



➡ **D3-5-induced gut microbiome** enhanced **goblet cell mass** and **mucin production**

Figure 6. Cell wall-derived lipoteichoic (LTA) of D3-5 enhances mucin production

CMT93 cells (a mouse goblet cell line)
A : qRT-PCR
B, D : PAS staining

D3-5 cultivated in MRS at 37 ℃
↓
Cell pellets were disrupted with a high pressure homogenizer in citrate buffer → Cell homogenates stirred with an equal volume of butanol 30min
↓
Remove cell debris
↓
centrifugation at 21,000g, 15 min
↓
Supernatants ultra-centrifuged 70,000g, 30 min
↓
↓ → supernatant : **cytoplasm**
Aqueous phage collected
↓
Pellet : **Cell wall** (peptidoglycan, Lipoteichoic acid)
↓
Dialyzed against water for 5 days
↓
4% SDS, incubated at 100 ℃, 30min stirring overnight RT
↓
centrifuged (30,000g, 15 min),
↓
Wash with citrate buffer x5
Ethanol x5
Lyophilize
Peptidoglycan
↓
Lyophilized
LTA

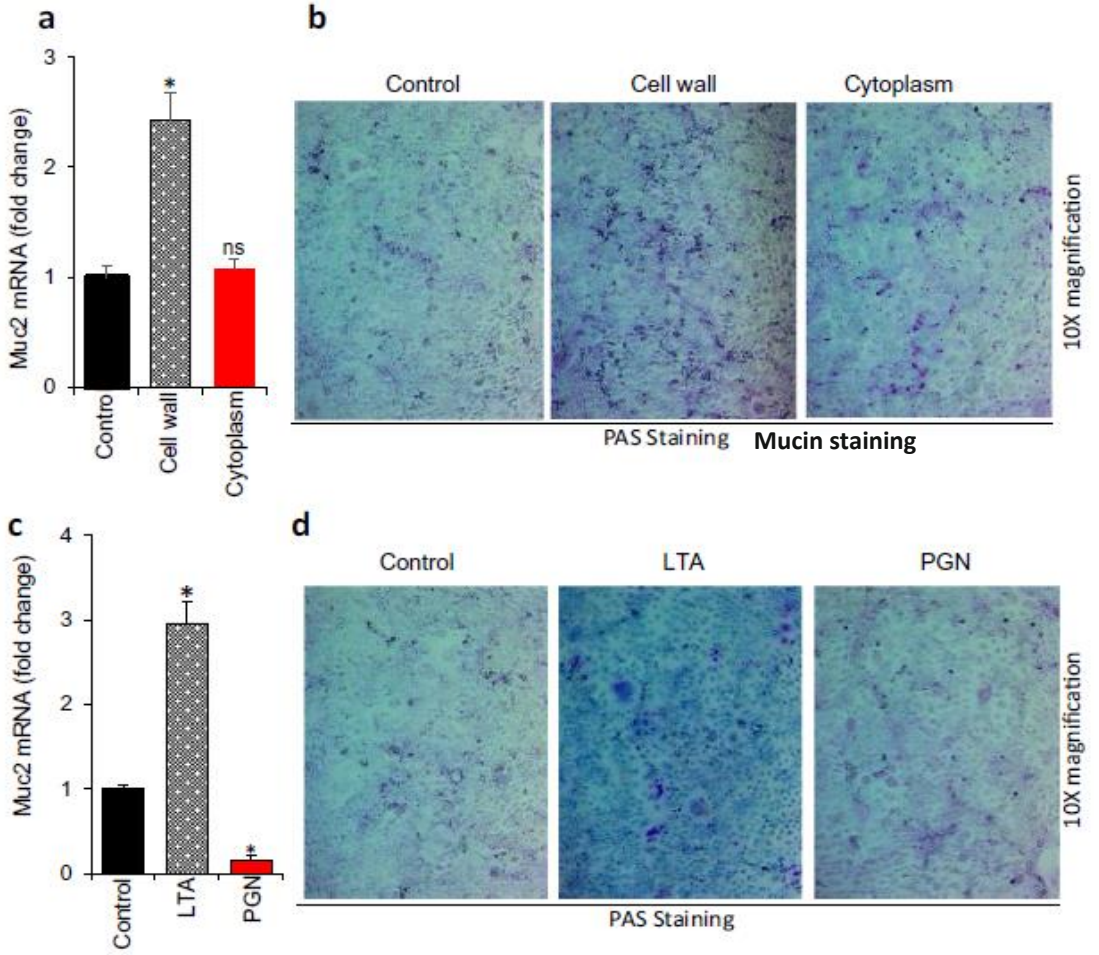
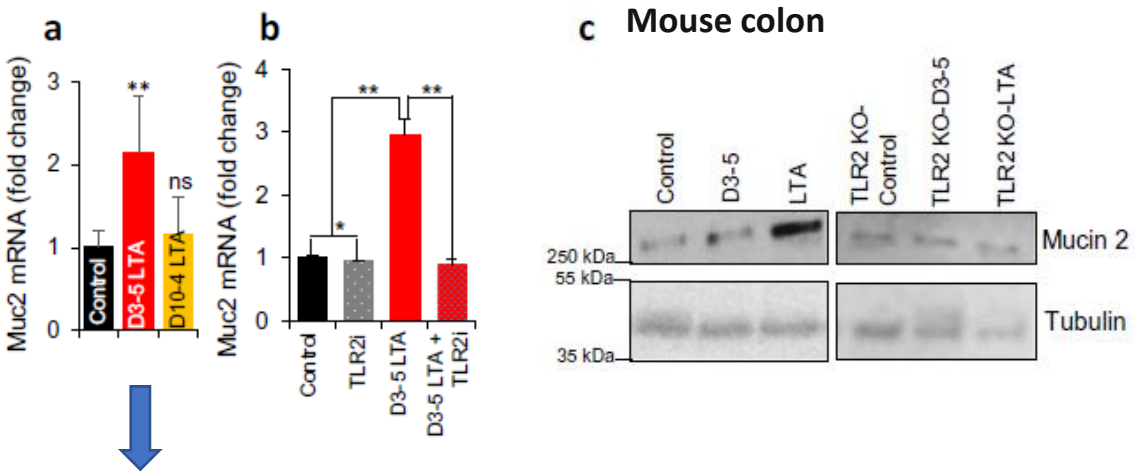
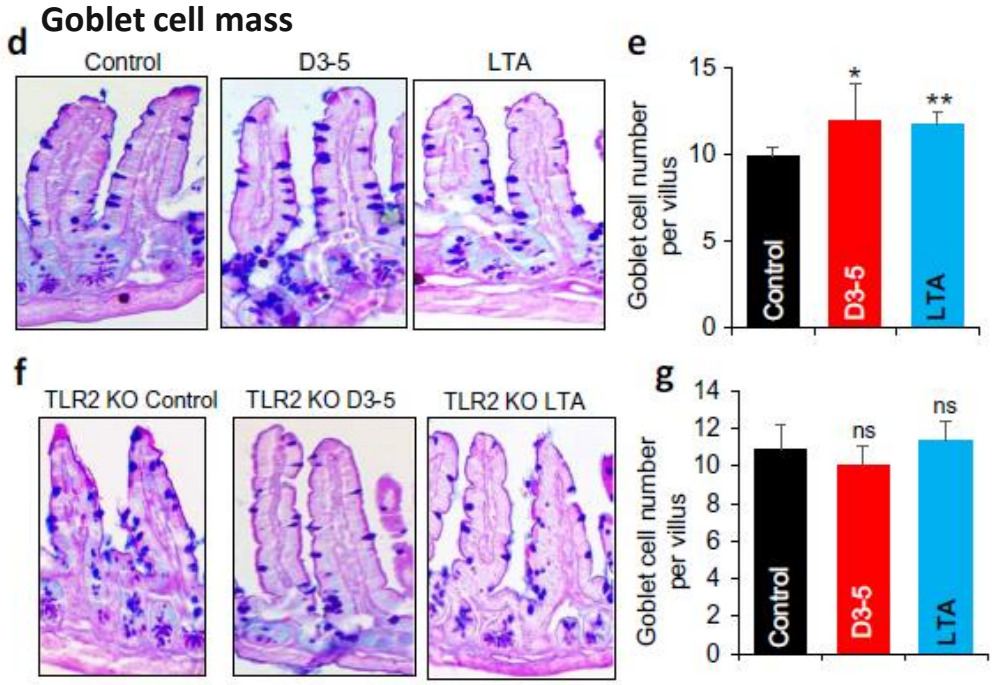


Figure 7. LTA induces mucin via TLR2 signaling

A, B : CMT93 cells (a mouse goblet cell line), qRT-PCR
C-G : C57BL/6 wild-type and TLR2 KO mice (6–8week-old)
C : mouse colon tissue western blot
D, F : AB/PAS staining



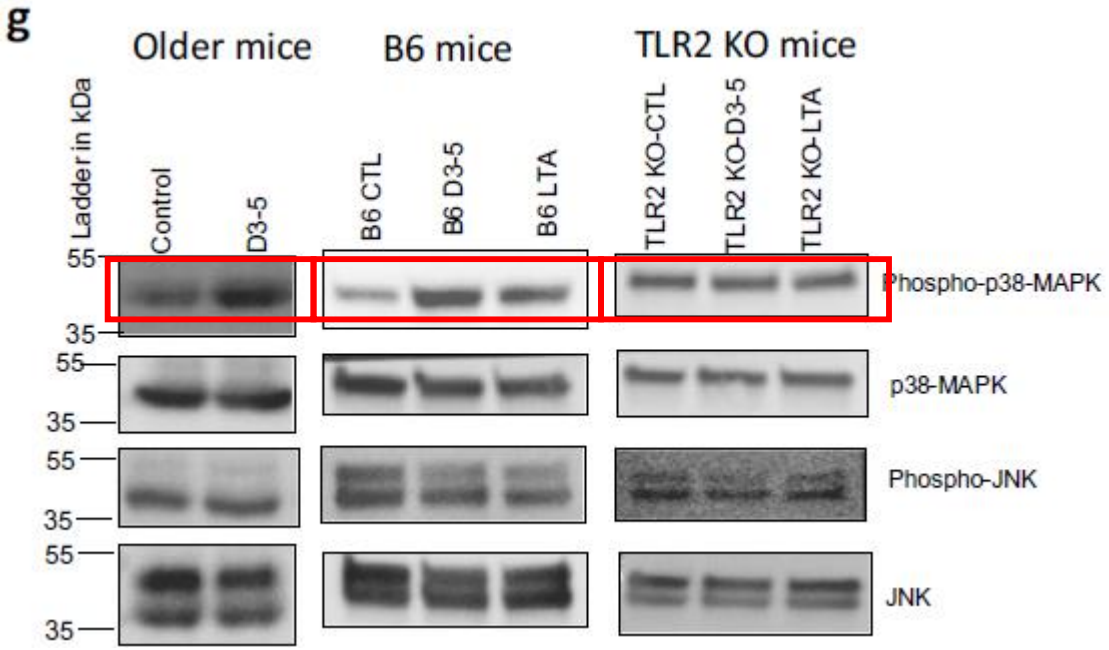
Strain-specific effect

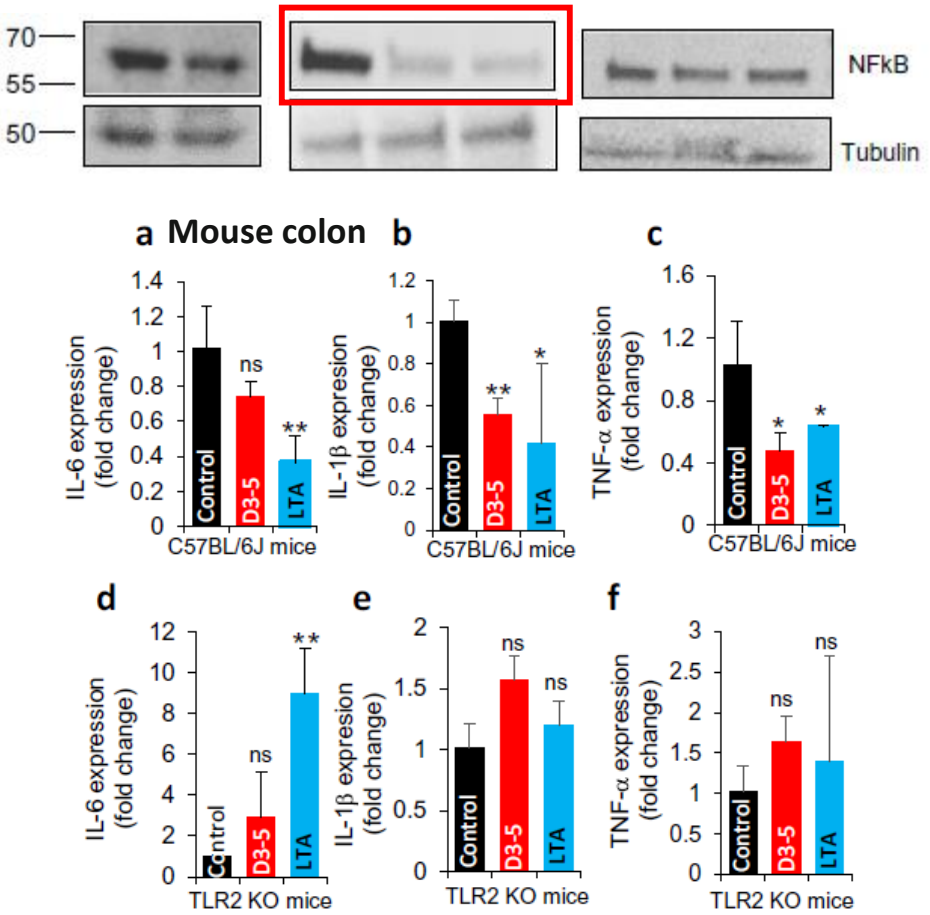


LTA stimulated TLR2 signaling
→ Enhanced goblet cell mass & mucin production

Figure 8. D3-5 cell wall-derived LTA activates TLR2-p38 MAPK signaling and suppresses NF-kB signaling

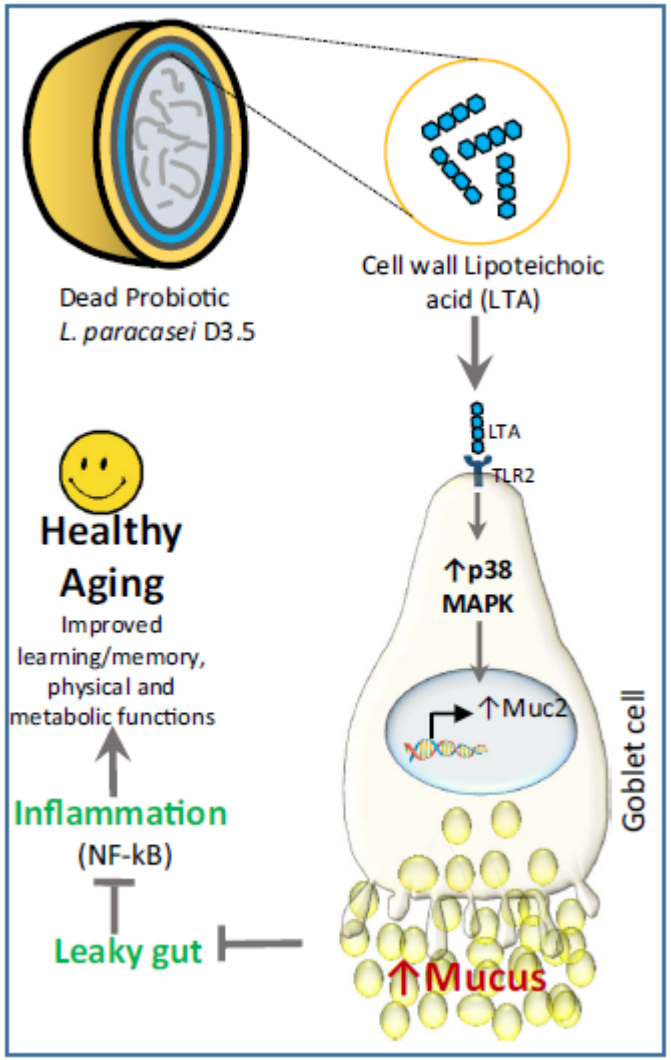
A-/f : mouse colon tissue qRT-PCR
D, F : AB/PAS staining





➡ LTA → TLR2 → p38 MAPK signaling → (improve mucin, prevent leaky gut)
→ suppressed NF-κB signaling → reduced inflammatory cytokines

Summary



- Feeding Specific dead probiotics extends lifespan in C. elegans
- D3-5 In mouse
  - Prevents metabolic dysfunctions
  - Improves aging-related ailments
  - Favorable changes in gut microbiome
  - Enhances mucin production and goblet cell mass

- Cell wall-derived lipoteichoic (LTA) of D3-5 enhances mucin production
- LTA induces mucin via TLR2 signaling
- D3-5 cell wall-derived LTA activates TLR2-p38 MAPK signaling and suppresses NF-kB signaling

➡ D3-5 and its LTA could be used as a biotherapy
To treat age-related gut microbiome dysbiosis, leaky gut, and inflammation in older adults.