Electronic Supplementary Material (ESI) for Food & Function.

Supplementary file

Core Fucosylation of Maternal Milk N-Glycans Imparts Early-life Immune Tolerance through Gut Microbiota-dependent Regulation on RORγt$^+$ Treg Cells

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MATERIAL AND METHODS

Milk protein extraction and lectin blotting analysis

Each milk sample was defatted via centrifugation at 8000 *g* at 4°C for 30 min. Then the whey protein of the skim milk was quantitated by a bicinchoninic acid protein assay (Takara Bio, Otsu, Japan). Then, the whey proteins were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by transfer to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% bovine serum albumin in TBST overnight at 4°C and then incubated with 0.5 g/mL biotinylated Aleuria aurantia lectin (AAL), which preferentially recognizes fucosylated glycans, in TBST for 1 h at room temperature. After washing with TBST four times, lectin-reactive protein bands were then visualized by using an ECL kit (Beyotime).

Figure S1

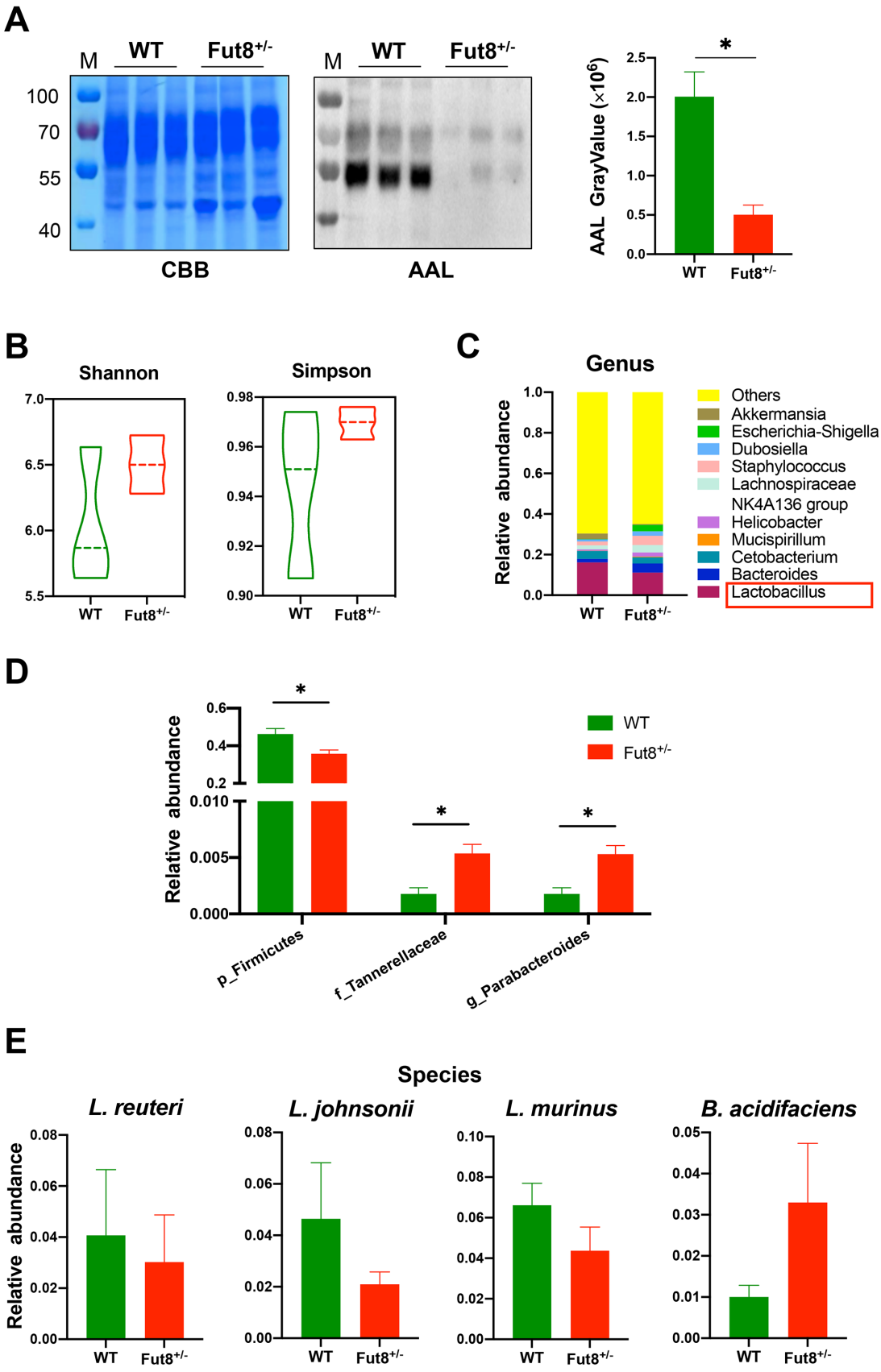
A
M
WT
Fut8+/-
100
70
55
40
CBB
AAL
AAL GrayValue (×10^6)
B
Shannon
Simpson
C
Genus
Relative abundance
Others
Akkermansia
Escherichia-Shigella
Dubosiella
Staphylococcus
Lachnospiraceae
NK4A136 group
Helicobacter
Mucispirillum
Cetobacterium
Bacteroides
Lactobacillus
D
Relative abundance
p_Firmicutes
f_Tannerellaceae
g_Parabacteroides
E
Species
L. reuteri
L. johnsonii
L. murinus
B. acidifaciens
Relative abundance

Fig. 1. Comparison of core fucosylation levels of milk N-glycans and the gut microbiota between Fut8+/- and WT maternal mice. (A) Comparison of AAL binding levels of milk N-glycans between Fut8+/- and WT maternal mice. (B) Analysis of α diversity of the gut microbiota in maternal mice. (C) Relative abundance of the gut microbiota between Fut8+/- and WT maternal mice at the genus level. (D) Comparison between Fut8+/- and WT maternal mice based on the T test. (E) The comparison based on the T test at the species level. AAL: Aleuria aurantia lectin; CBB: Coomassie brilliant blue. *, $p < 0.05$. Mean and SEM of one independent experiment with n = 3 mice per group is displayed.