Supplementary Materials and Methods

Intestinal epithelial cell isolation. The small intestinal and colonic tissues were incubated with 0.5 mM dithiothreitol and 3 mM EDTA at room temperature for 1.5 hr without shaking. Villi and crypts were released from the tissues by vigorous shaking and the suspension was filtered through 70 μm cell filter strainer. Epithelial cells were sorted using a biotin-labeled E-cadherin antibody (BD Biosciences) and streptavidin magnetic beads (BD Biosciences).

Real-time PCR assay. Total RNA was isolated from small intestinal and colonic epithelial cells, and colonic tissues using an RNA isolation kit (Qiagen) and was treated with RNase-free DNase. Reverse transcription was performed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Real-time PCR was performed using Taqman Gene Expression Master Mix and primers, MUC2 (Mm 00439306), CLDN3 (Mm00515499), APRIL (Mm03809849-s1), TNF (Mm00443259), IL-6 (Mm00446190), and KC (Mm00433859). The relative abundance of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used to normalize levels of the mRNAs of targeted genes. All cDNA samples were analyzed in triplicate.

Immunhistochemistry. Paraffin-embedded tissue sections were deparaffinized. To unmask antigens, tissue sections were boiled for 15 min in Antigen Unmasking Solution. Then tissue sections were blocked using 5% goat serum in PBS. For Ki67 staining, tissue sections were incubated with a rabbit anti-ki67 monoclonal antibody (Biocare Medical, LLC) overnight at 4°C and followed by MACH 2 Universal HRP Polymer Detection reagents (Biocare Medical, LLC) for 1 h at room temperature. For MUC2 staining, tissue sections and cultured cell slides were incubated with an rabbit anti-MUC2 antibody (Santa Cruz Biotechnology, INC) for 48 hr at 4°C, followed by a horseradish peroxidase (Dako)-labeled goat anti-rabbit IgG antibodies at room temperature for 1 hr. The sections were developed using the ImmPACT™ DAB substrate (Vector laboratories, Inc.), counterstained with hematoxylin, and observed using light

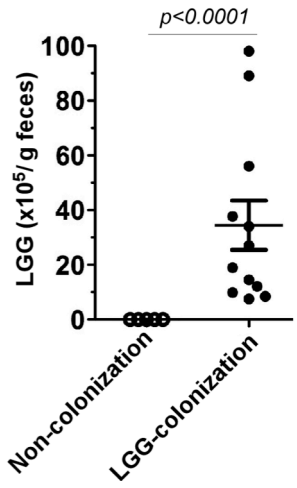
microscopy. The number of Ki67 and MUC2 positive cells were determined by counting the absolute number of positive stained cells in at least 300 villi or crypts for each mouse.

For ZO-1 and IgA staining, sections were incubated with a rabbit anti ZO-1 antibody (Invitrogen Corporation, Carlsbad, CA) and a rat-anti-IgA antibody (Cell Signaling Technology), respectively, overnight at 4°C, followed by FITC-labeled anti-rabbit (Cell Signaling Technology) or anti-rat (Life Technologies) secondary antibodies for 1 hr at room temperature. Slides were mounted using Mounting Medium with DAPI and observed using fluorescence microscopy. FITC and DAPI images were taken from the same field.

Detection of LGG in feces by quantitative PCR (qPCR). DNA was extracted from feces using ZR Fecal DNA Miniprep™ kit (ZYMO research Corporation). qPCR was performed using LGG strain specific primers, 5'-CGCCCTTAACAGCAGTCTTC-3' and 5'-GCCCTCCGTATGCTTAAACC-3', which have been previously published (Ahlroos, T. and Tynkkynen, S. *J Appl Microbiol*, 2009, 106:506-514), and IQ™SYBR® Green Supermix (Bio-Rad Laboratories), according to the manufacturer's instruction. To generate a feces-based LGG concentration curve, LGG (10^4, 10^5, 10^6, 10^7, and 10^8 / g feces) was mixed with feces from non-colonized mice (there was no detectable LGG in feces from mice detected by DNA fingerprint analysis and conventional PCR analysis using LGG specific primers). DNA was extracted from feces for qPCR analysis.

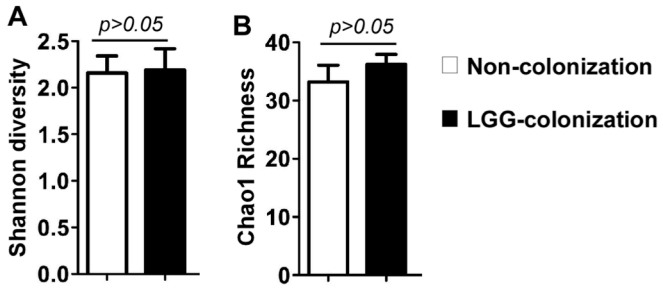
Supplemental Data

Supplemental Figure 1

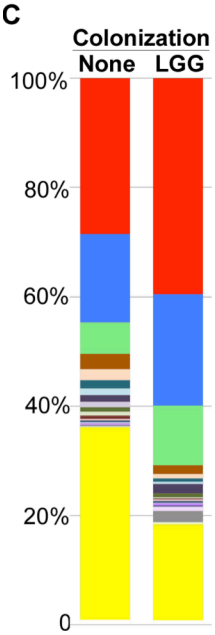


Supplemental Figure 1. Quantitative detection of LGG in feces using qPCR analysis. DNA was isolated from feces of 8-week old mice with and without LGG colonization, as detected by DNA fingerprint analysis, for qPCR analysis. The expression levels from qPCR were compared to the feces-based LGG concentration curve to obtain the amount of LGG in feces.

Supplemental Figure 2



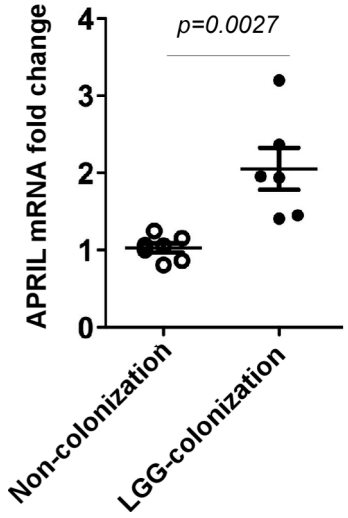
C



Abundance (%):	Fix-LGG	Live-LGG	p value
Lactobacillus	28.5	39.4	0.047
Clostridium	16.1	20.4	0.332
Bacteroides	5.8	10.9	0.044
Alistipes	2.8	1.8	0.164
Parasutteralla	2.1	0.8	0.045
Eubacterium	1.5	0.6	0.054
Candidatus Arthromitus	1.2	0.4	0.081
Clostridials unknown	1.2	1.7	0.079
Adlercreutzia	1.0	0.1	0.043
Bacteeroidales unknown	0.8	0.7	0.608
Helicobacter	0.8	0.3	0.256
Lachnospiraceae unknown	0.6	0.1	0.116
Roseburia	0.3	0.4	0.574
Prevotella	0.3	0.2	0.699
Enterorhabdus	0.3	0.1	0.299
Parabacteroides	0.2	0.4	0.267
Coriobacteriaceae unknown	0.1	0.1	0.503
Anaerotruncus	0.1	0.1	0.831
Oscillospira	0.1	0.7	0.026
Blautia	0.1	2.0	0.025
Oscillibacter	<0.1	0.2	0.203
Lachnospira	<0.1	0.3	0.259
No hit	35.2	17.6	0.004

Supplemental Figure 2. The effects LGG colonization of on intestinal microbiota in 8-week old conventionally raised mice. Mice were treated with live LGG from postnatal day 1 to day 5 and colonization was confirmed by detecting LGG in feces, as described in Figure 1. Fixed LGG was used to treat pups as non-colonization control. Genomic DNA was extracted from caecal feces to assess the community composition of the microbiota using 454-based 16S rRNA amplicon library sequencing. Shannon diversity (A), Chao1 richness (B), and detailed relative abundance of bacterial taxa at the genus level (C) are shown. n=5 in each group.

Supplemental Figure 3



Supplemental Figure 3. LGG colonization enhances APRIL expression in small intestinal epithelial cells in 2-week old mice. Mice were treated as described in Figure 2. mRNA was isolated from small intestinal epithelial cells for Real-time PCR analysis of APRIL. The average of mRNA expression levels in the non-colonized mice was set as 100%, and the mRNA expression level of each mouse was compared to this average.