METHODS

DNA extraction and PCR amplifications:

Ileal contents were used for genomic DNA extraction using bead-beating system (Matrix E and Bio101 FastPrep cell disrupter, MP Biomedicals; Solon, OH) and a commercial kit (QIAamp DNA Stool Mini Kit, Qiagen, Valencia, CA) following the manufacturer recommendations. Extracted genomic DNA was used as a template for PCR amplification and quantification (ng of DNA/μl) of different bacterial groups. Bacterial DNA concentrations were estimated by quantitative PCR (qPCR) assays using previously validated primers [1]. Total Bacteria was estimated based on amplification of the RNA polymerase beta subunit (*rpoB*). This gene is highly conserved in Bacteria and appears to exist as a single copy gene across different phylogenetic groups[2, 3, 4]. Importantly, quantification total Bacteria by means of *rpoB* gene overcomes the inherent problem of multiple 16S rRNA copies in many species [2, 3, 4].

Family-specific primers targeting 16S rRNA genes included Bacteroidaceae-Porphyromonadaceae-Prevotellaceae, Lachnospiraceae-Ruminococcaceae, Enterococcaceae [5] and Lactobacillaceae [6]. Analyses were carried out using the qPCR protocols described elsewhere [1].

For quantification of *Lactobacillus rhamnosus* GG, a qPCR assay was standardized using well validated strain-specific primers: strain-specific primers Lrhamn1 (5'-CAATCTGAATGAACAGTTGTC-3') and Lrhamn2 (5'-TATCTTGACCAAACTTGACG-3') [7, 8]. Specificity of this primer set was verified by using BLAST analysis [9], *in silico* PCR amplification [10] and by PCR assays using different species of *Lactobacillus* laboratory-type strains (*L. casei and L. acidophilus*) and intestinal contents (ileum and cecum) of mice in which *L. rhamnosus* GG is not present. For *L. rhamnosus GG* specific PCR amplification, annealing temperature was determined empirically by temperature gradient PCR. The cycling protocol for this primer sets was as follows: initial denaturation at 94°C (3 min), 33 cycles of 94°C (45 sec), 53.9°C (30 sec),

72°C (45 sec), followed by one cycle of 72°C (7 min). Specificity of the PCR method was confirmed by visualizing single and expected-size PCR amplicons via agarose gel electrophoresis using ethidium bromide fluorescent dye.

All qPCR assays were carried out in a Mastercycler ep realplex real-time PCR machine (Eppendorf, Westbury, NY) and using SYBR Green PCR technology (Clontech Laboratories, Mountain View, CA). Each 17 µl qPCR mixture consisted of 8.5 ul of 2X SYBR Green Master Mix (Clontech Laboratories), 1.7 ul of BSA (100 µg/ml), 0.5 ul of each primer (10 µM), 2 ul of extracted genomic DNA and 3.10 ul PCR-grade water. Denaturation curves were determined from 60°C to 95°C for all products for quality assurance. DNA concentrations (*rpoB* and 16S rRNA genes) in each fecal sample were determined using the absolute quantification method. Standard curves were constructed with 5-fold dilutions of genomic DNA templates of known concentration. DNA extracted from mouse intestinal contents was used as a template for total Bacteria and family-specific qPCR, whereas DNA extracted from *L. rhamnosus GG* laboratory-type strain was used for species-specific qPCR. Concentrations of DNA used in the standard curves ranged from 100 ng to 6.4 pg/µl. For each qPCR assay, standard curves were amplified at the same time as fecal samples. PCR amplifications were performed in triplicate. Bacterial group-specific qPCR signals were normalized (divided) to total Bacteria (*rpoB* gene) qPCR signal. Then, bacterial DNA densities were estimated by gram of fecal pellet. Detection limits for family-specific and *L. rhamnosus GG* qPCR assays were 6.4 pg/µl and 1.38 pg/µl, respectively.

Supplementary Table 1: qRT-PCR primer sequences

Gene	Primer Sequences	
GAPDH	F 5'-TGC ACC ACC AAC TGC TTA G-3'	R 5'-GAT GCA GGG ATG ATG TTC-3'
COX-2	F 5'-ACA CAC TCT ATC ACT GGC ACC-3'	R 5'-TTC AGG GCG AAG CGT TTG C-3'
TNFα	F 5'-ACT TCG GGG TGA TCG GTC CCC	R 5'- TGG TTT GCT ACG ACG TGG GCT AC
L.rhamn	F 5'-CAA TCT GAA TGA ACA GTT GTC-3'	R 5'-TAT CTT GAC CAA ACT TGA CG-3'
TLR1	F 5' CCG TGA TGC ACA GCT CCT TGG T 3'	R 5' TGC CAC ATG GGT ATA GGA CGT TTC T 3'
TLR2	F 5' TCC GAA TTG CAT CAC CGG TCA GA 3'	R 5' GTG AAA GAC CTG GAG CGG CCA 3'
TLR3	F 5' TGG GAA CGG GGG TCC AAC TGG 3'	R 5' GGG GGT TCA GTT GGG CGT TGT3'
TLR4	F 5' ACC AGG AAG CTT GAA TCC CTG CA 3'	R 5' AGG TGG TGT AAG CCA TGC CAT GC 3'

mTLR5	F 5' CGG TCC CGC CAG CCA TTT CA 3'	R 5' GGT GCG TGG GGG AAC TCA GC 3'
TLR6	F 5' AGA AAA TGG TAC CGT CAG TGC TGG A 3'	R 5' AGG CCA GGG CGC AAA CAA AG 3'
TLR7	F 5' AGG CTC TGC GAG TCT CGG TT 3'	R 5' CCG TGT CCA CAT CGA AAA CAC CAT T 3'
TLR8	F 5' GAA CAT GGA AAA CAT GCC CCC TCA 3'	R 5' GGC ACT GGT TCC AGA GGA CAG C 3'
TLR9	F 5' ATC TCC CAA CAT GGT TCT CCG TCG A 3'	R 5' ATG CTG CCG CAG AGA AAC GGG 3'
TLR11	F 5' TCC TTC CTC TGA TTA GCT GTC CTA A 3'	R 5' TCC ACA TAA TTT CCA CCA ACA AGT 3'
TLR12	F 5' GCC GCC ATT CCA AGC TAT C 3'	R 5' CTC CAC AGT CCG AGG TAC AAC TT 3'
TLR13	F 5' GTG CTA GGA GCT TCT GAG AGA ACC T 3'	R 5' CCC TTC TCA TGT TCA AAG GCA CGG 3'

FIGURE LEGENDS

Supplementary Figure 1: Crypt survival in LGG treated WT mice radiated concurrently with knockout mouse experiments. N ≥ 4 for all experiments.

REFERENCES

1. Nava GM, Friedrichsen HJ, Stappenbeck TS. Spatial organization of intestinal microbiota in the mouse ascending colon. The ISME journal. 2010;5:627-638
2. Case RJ, Boucher Y, Dahllof I, et al. Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. Appl Environ Microbiol. 2007;73:278-288
3. Adekambi T, Shinnick TM, Raoult D, et al. Complete rpoB gene sequencing as a suitable supplement to DNA-DNA hybridization for bacterial species and genus delineation. Int J Syst Evol Microbiol. 2008;58:1807-1814
4. Dahllof I, Baillie H, Kjelleberg S. rpoB-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. Appl Environ Microbiol. 2000;66:3376-3380
5. Rinttila T, Kassinen A, Malinen E, et al. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol. 2004;97:1166-1177
6. Barman M, Unold D, Shifley K, et al. Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. Infect Immun. 2008;76:907-915

7. Brandt K, Alatossava T. Specific identification of certain probiotic Lactobacillus rhamnosus strains with PCR primers based on phage-related sequences. International journal of food microbiology. 2003;84:189-196
8. Dommels YE, Kemperman RA, Zebregs YE, et al. Survival of Lactobacillus reuteri DSM 17938 and Lactobacillus rhamnosus GG in the human gastrointestinal tract with daily consumption of a low-fat probiotic spread. Appl Environ Microbiol. 2009;75:6198-6204
9. Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research. 1997;25:3389-3402
10. Bikandi J, San Millan R, Rementeria A, et al. In silico analysis of complete bacterial genomes: PCR, AFLP-PCR and endonuclease restriction. Bioinformatics (Oxford, England). 2004;20:798-799