



Supplementary Material for

Recurrent infection progressively disables host protection against intestinal inflammation

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Figs. S1 to S10
References

Supplementary Figure S1

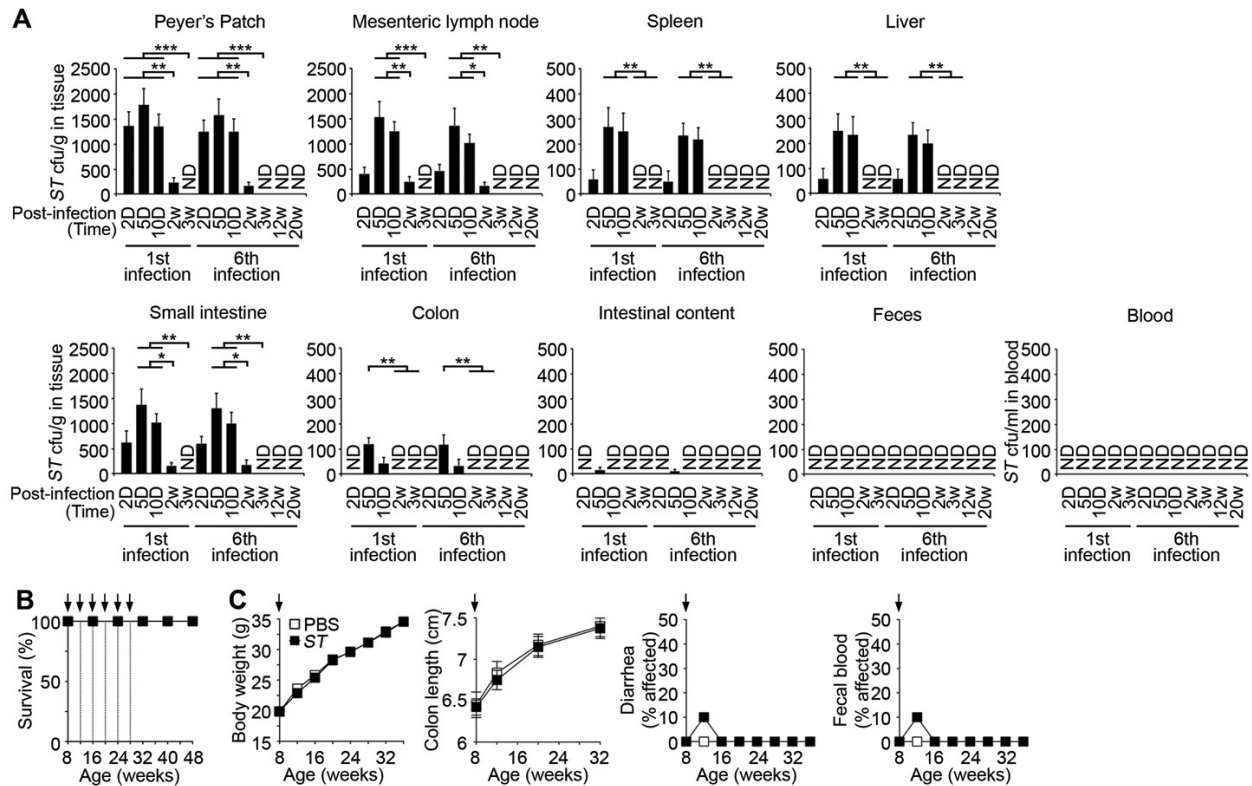


Fig. S1. Bacterial burden and host survival following recurrent *Salmonella* infection. (A) C57BL/6J wild-type mice were re-infected orally (2×10^3 cfu) with *ST* as in Fig. 1A. Host site colonization of *ST* was assessed on 2 days, 5 days, 10 days, 2 weeks, and 3 weeks after the first infection and 2 days, 5 days, 10 days, 2 weeks, 3 weeks, 12 weeks and 20 weeks after the sixth infection in different tissues following *ST* recurrent infections ($n = 72$). D, days; W, weeks; ND, not detected. Limits of detection: Peyer's Patch, mesenteric lymph node, spleen, small intestine, and colon < 50 cfu; liver < 25 cfu; intestinal content, feces, and blood < 10 cfu. (B) Survival of C57BL/6J wild-type mice following *ST* recurrent infections ($n = 40$). (C) Analyses of C57BL/6J wild-type mice during single oral *ST* infection (2×10^3 cfu) or sham infected with PBS (arrows). Measurements with age included body weight ($n = 8$ per condition), colon length ($n = 32$ per condition), frequency of diarrhea (*ST*, $n = 10$; PBS, $n = 8$) and frequency of fecal blood (*ST*, $n = 10$; PBS, $n = 8$) immediately prior to infection at each time point. Error bars, means \pm SEM. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; one-way ANOVA with Tukey's multiple comparisons test (A).

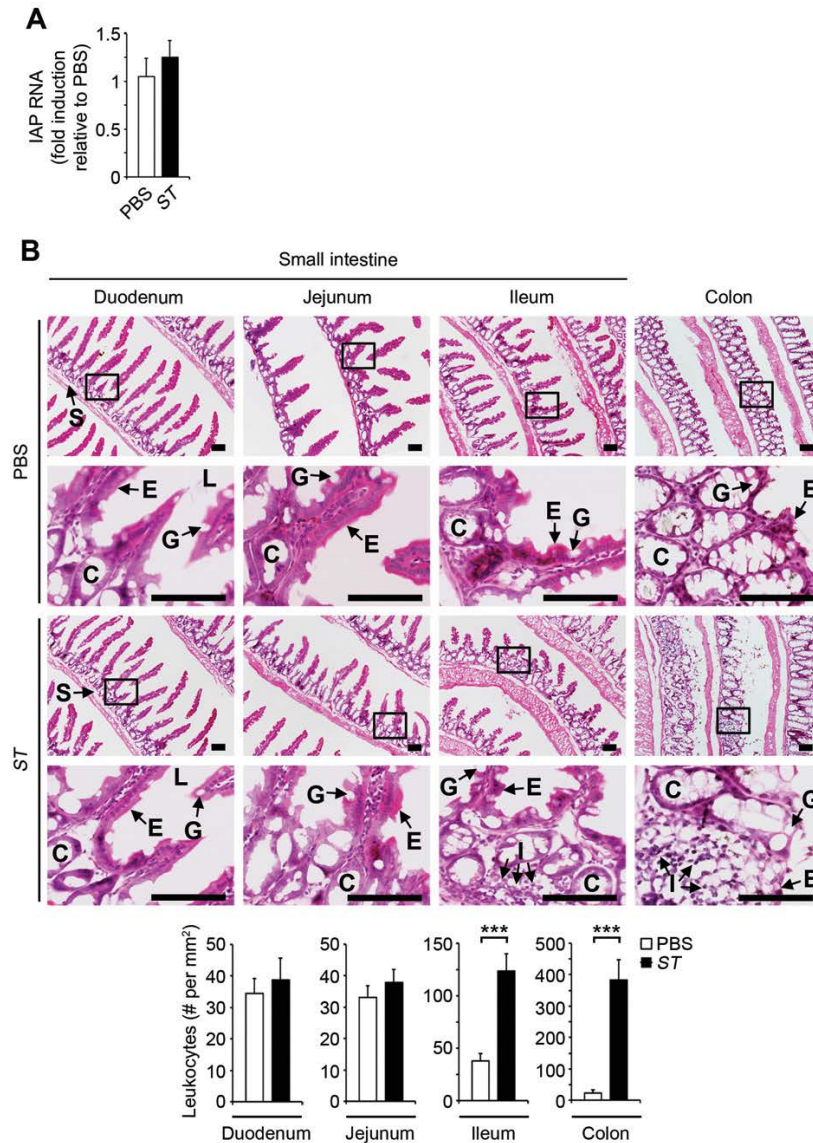


Fig. S2. IAP mRNA expression and gut histology following recurrent *Salmonella* infection.

(A) IAP mRNA expression in the small intestine (duodenum) at 20 weeks of age ($n = 8$ per condition) following *ST* re-infection as in Fig. 1. (B) Duodenum, jejunum, ileum and colon sections from *ST* re-infected wild-type mice at 48 weeks of age were stained with H & E to evaluate histological differences. The graphs shown are representative of 16 fields of view obtained from four mice from each group. L, intestinal lumen; E, epithelial layer; C, crypt; G, goblet cell; S, submucosa; I, infiltration of leukocytes. All scale bars: 100 μm . Error bars, means \pm SEM. *** $P < 0.001$; Student's t test.

Supplementary Figure S3

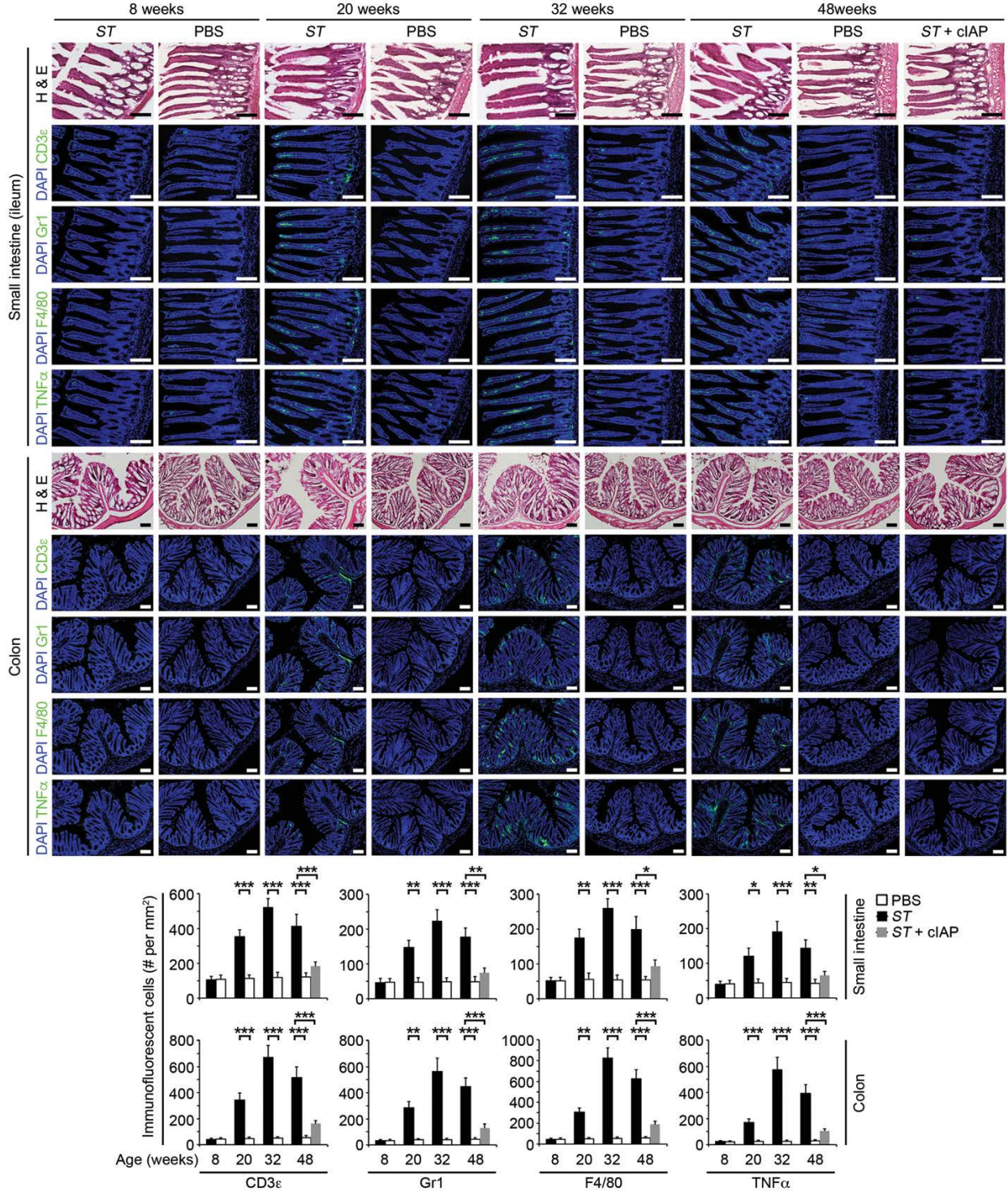


Fig. S3. Immune infiltrates in the intestinal mucosa following recurrent *Salmonella* infection. C57BL/6J wild-type mice were re-infected orally (2×10^3 cfu) with *ST* as in Fig. 1A

in the presence or absence of cIAP. Colon and small intestine serial sections were stained with H&E or fluorescence using antibodies specific for CD3 ϵ , Gr1, F4/80, or TNF α at 8 (prior to the first infection), 20 (prior to 4th infection), 32 weeks (4 weeks after the sixth infection) and 48 weeks age. DNA is stained with DAPI. The graphs shown indicate the abundance of immunofluorescent cells and are representative of ten fields of view obtained from four mice of each condition and time point. Error bars, means \pm SEM. ***P < 0.001; **P < 0.01; *P < 0.05; one-way ANOVA with Tukey's multiple comparisons test. All scale bars: 100 μ m.

Supplementary Figure S4

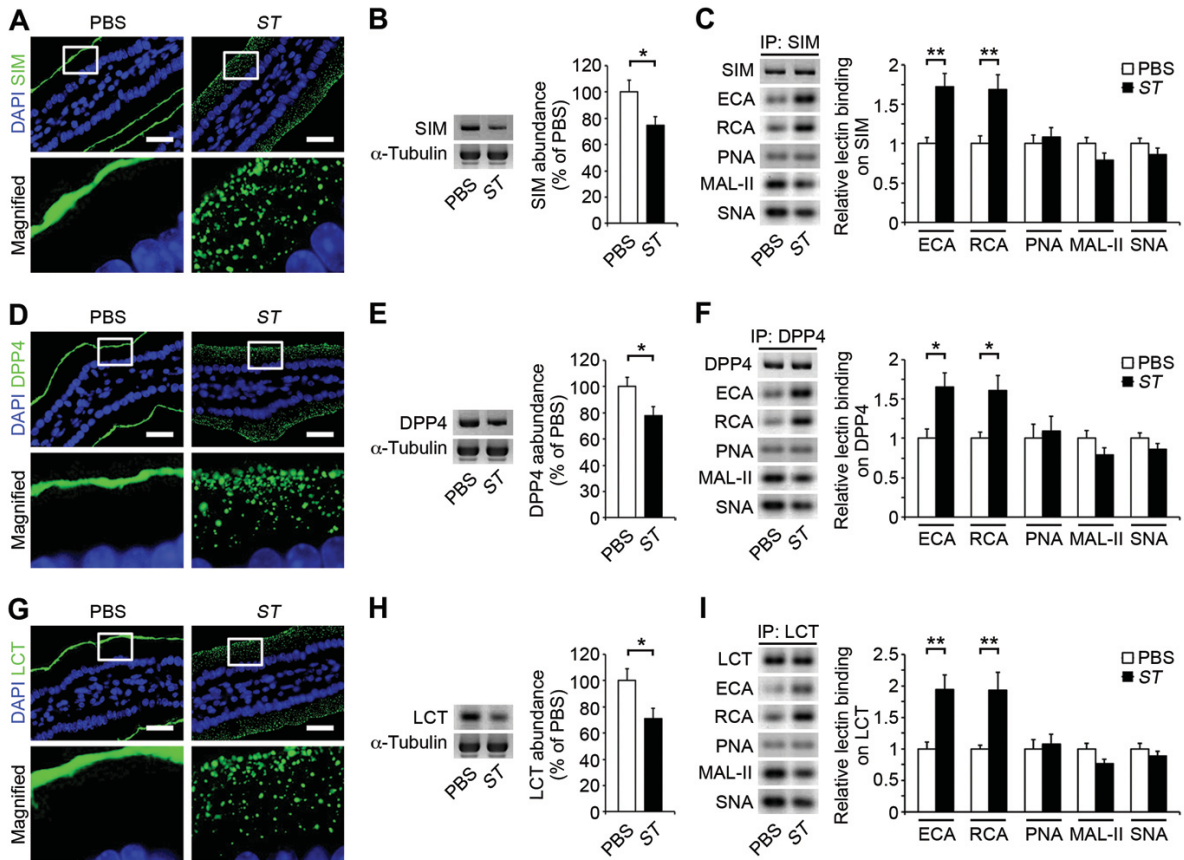


Fig. S4. Endocytosis and altered glycosylation of enterocyte membrane proteins following recurrent *Salmonella* infection. (A–C) In situ localization, abundance and lectin binding of sucrase-isomaltase (SIM) at the cell surface among enterocytes of the small intestine (duodenum) at 20 weeks of age (immediately before the fourth *ST* infection) (n = 8 per condition). (D–F) In situ localization, abundance and lectin binding of dipeptidyl peptidase 4 (DPP4) at the cell surface among enterocytes of the small intestine (duodenum) at 20 weeks of age (immediately before the fourth *ST* infection) (n = 8 per condition). (G–I) In situ localization, abundance and lectin binding of lactase (LCT) at the cell surface among enterocytes of the small intestine (duodenum) at 20 weeks of age (immediately before the fourth *ST* infection) (n = 8 per condition). Error bars, means ± SEM. **P < 0.01; *P < 0.05; Student's *t* test.

Supplementary Figure S5

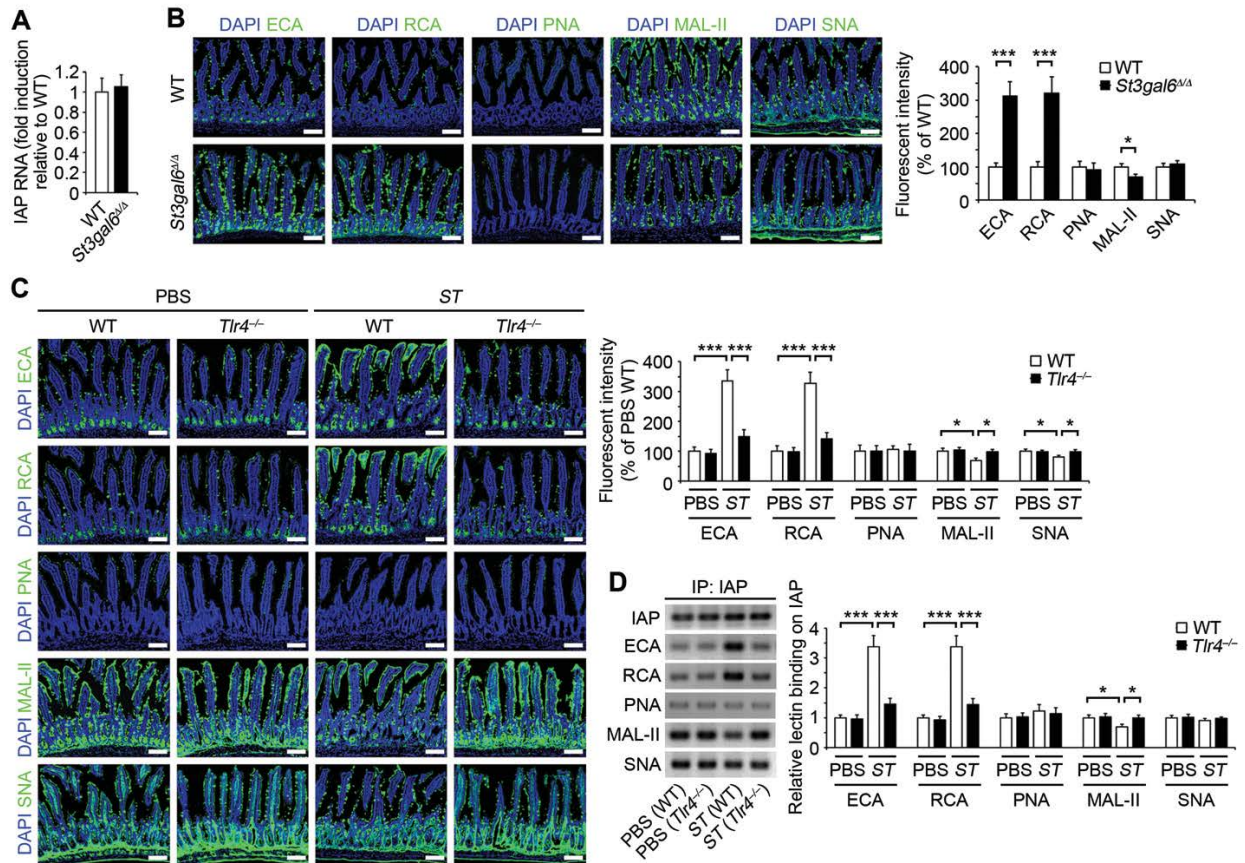


Fig. S5. IAP mRNA expression and lectin binding in the small intestine of ST3Gal6-deficient and TLR4-deficient mice following recurrent *Salmonella* infection. (A) IAP mRNA expression in the small intestine of mice lacking the ST3Gal6 sialyltransferase at 8-10 weeks of age (n = 8 per condition). (B and C) Serial sections of small intestine (duodenum) from (B) mice lacking ST3Gal6 and wild-type littermates at 8–10 weeks of age or (C) indicated genotypes at 20 weeks of age (before the fourth *ST* infection) stained with FITC-conjugated ECA, RCA, PNA, MAL-II, and SNA lectins. DNA is stained with DAPI. Histograms denote fluorescent intensity and are representative of 10 fields of view obtained from 4 mice of each genotype or condition. (D) Lectin blotting of IAP protein from small intestine from indicated genotypes (n = 6 per condition) at 20 weeks of age (before the fourth *ST* infection). Error bars, means \pm SEM. ***P < 0.001; **P < 0.01; *P < 0.05; Student's *t* test (B) or one-way ANOVA with Tukey's multiple comparisons test (C) and (D). Scale bars: 100 μ m.

Supplementary Figure S6

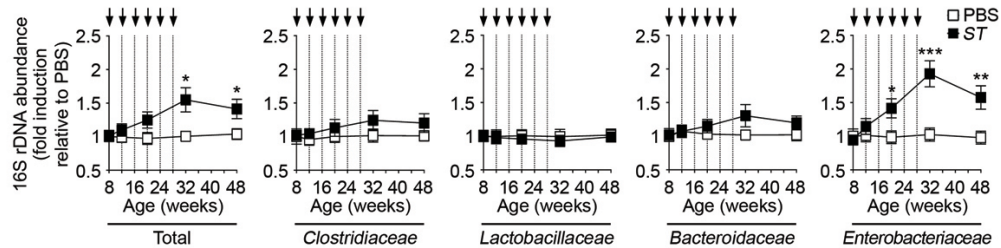


Fig. S6. Abundance of total microbiota in intestinal content during recurrent *Salmonella* infection. The distribution of total and four families of commensal microbiome (Gram-positive *Clostridiaceae* and *Lactobacillaceae*, Gram-negative *Bacteroidaceae* and *Enterobacteriaceae*) 16S rDNA in intestinal content of indicated genotypes was measured by quantitative real-time PCR (n = 40 per condition). A total 10 μ g of DNA was isolated from 100 mg of intestinal contents and 0.1 μ g of isolated DNA was used for the template in 50 μ l of quantitative real-time PCR mixture. Error bars, means \pm SEM. ***P < 0.001; **P < 0.01; *P < 0.05; Student's *t* test.

Supplementary Figure S7

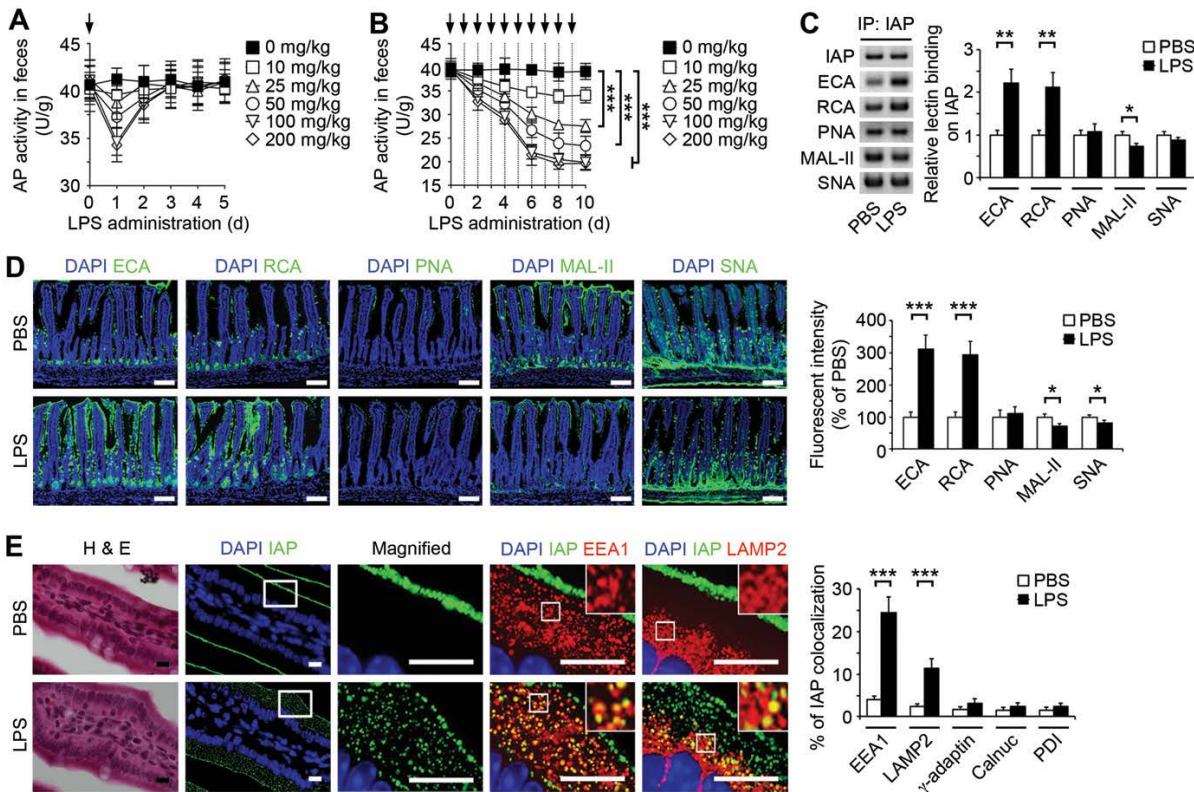


Fig. S7. Altered IAP abundance and glycosylation in the gut following oral LPS administration. (A) Total AP activity in feces following single oral LPS administration of multiple doses (*E. coli* 0111:B4) (n = 8 per condition). (B) Total AP activity in feces following repeated oral LPS administrations (24-h intervals for 10 days, arrows) of multiple doses (n = 8 per condition). (C) Lectin blot analyses of identical amounts of IAP isolated from the small intestine of 8-week-old wild-type mice on day 6 following repeated LPS administrations (n = 8 per condition). (D) Serial tissue sections of small intestine (duodenum) of 8-week-old wild-type mice on day 6 following repeated LPS administrations (100 mg/kg; *E. coli* 0111:B4, 24-h intervals for 10 days) stained with fluorescence using FITC-conjugated ECA, RCA, PNA, MAL-II, and SNA lectins. DNA is stained with DAPI. Plots indicate the fluorescent intensity and are representative of ten fields of view obtained from four mice from each group. Scale bars, 100 μ m. (E) In situ localization and co-localization (yellow) of IAP with various intracellular compartments in the small intestine (duodenum) of 8-week-old wild-type mice on day 6 following LPS administrations. Small intestine (duodenum) serial sections were stained with H

& E or by fluorescence using antibodies as in Fig. 3D. DNA was stained with DAPI. The graphs indicate the percentage of IAP co-localized (yellow) with the above intracellular compartments and IAP abundance at the cell surface and are representative of ten fields of view obtained from four mice of each condition. Scale bars: 10 μ m. Error bars, means \pm SEM. ***P < 0.001; **P < 0.01; *P < 0.05; one-way ANOVA with Tukey's multiple comparisons test (B) or Student's *t* test (C) to (E).

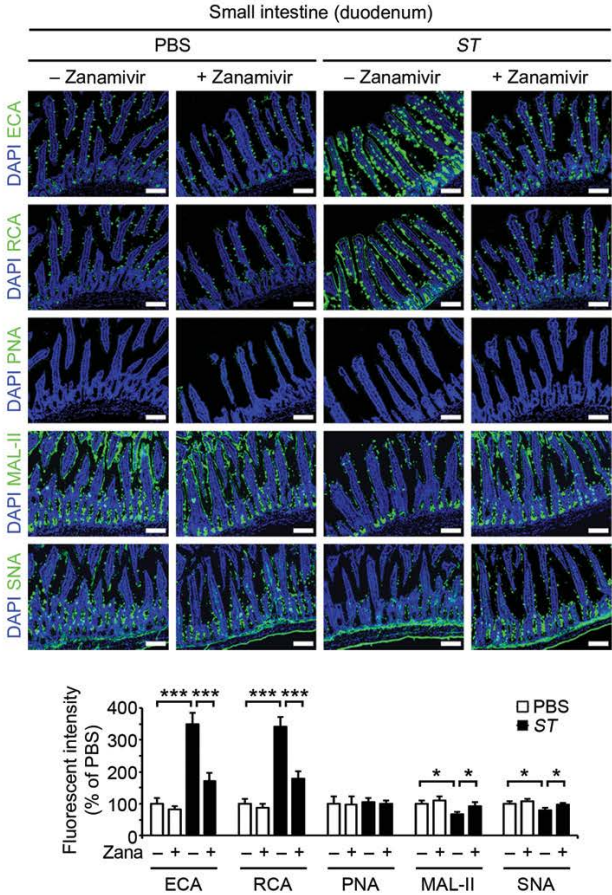


Fig. S8. Lectin binding in the small intestine following recurrent *Salmonella* infection and Zanamivir treatment. Serial sections of small intestine (duodenum) tissues from wild-type mice at 20 weeks of age (prior to 4th *ST* infection) in the absence (PBS) or presence of Zanamivir treatment from the time of the initial infection. Tissues were visualized following FITC-conjugated ECA, RCA, PNA, MAL-II, and SNA lectin binding. DNA is stained with DAPI. The graphs shown indicate the fluorescent intensity and are representative of ten fields of view obtained from four mice of each condition. Error bars, means \pm SEM. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; one-way ANOVA with Tukey’s multiple comparisons test. Scale bars: 100 μ m.

Supplementary Figure S9

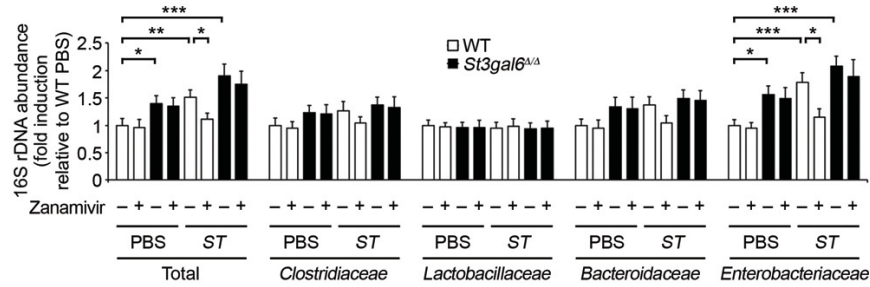


Fig. S9. Effect of Zanamivir on total microbiota abundance in intestinal content following recurrent *Salmonella* infection. The distribution of total and four families of commensal microbiome 16S rDNA in intestinal content of indicated genotypes was measured by quantitative real-time PCR at 32 weeks of age (4 weeks after the sixth infection with *ST*) in the presence or absence of Zanamivir (n = 10 per condition). Error bars, means \pm SEM. ***P < 0.001; **P < 0.01; *P < 0.05; one-way ANOVA with Tukey's multiple comparisons test.

Supplementary Figure S10

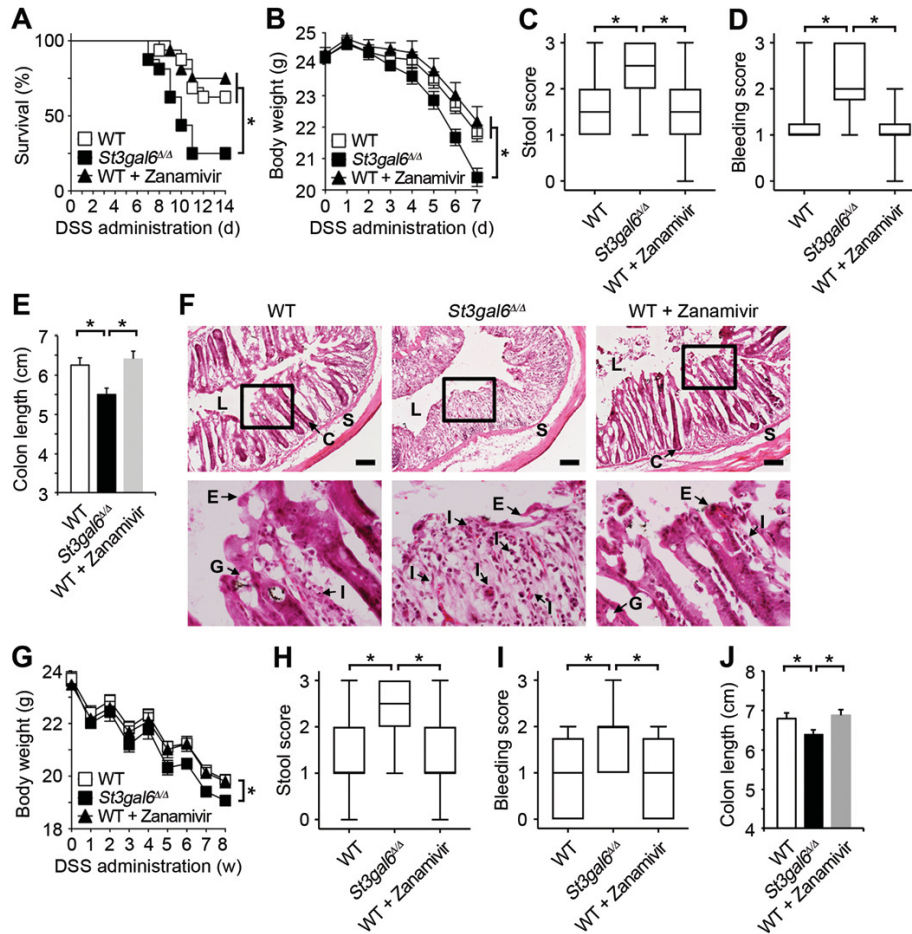


Fig. S10. DSS-induced acute and chronic colitis in ST3Gal6-deficient and Zanamivir-treated mice. (A–F) DSS-induced experimental acute colitis. (A) ST3Gal6-deficient mice and wild-type littermates at 12 weeks of age were fed 4% DSS solution in drinking water for 5 days, followed by normal drinking water. Survival was monitored until day 14 post DSS administration. Zanamivir was provided in both DSS drinking water and normal drinking water until the end of the experiment (n = 16 per condition). (B–D) ST3Gal6-deficient mice and wild-type littermates were fed 2% DSS solution in drinking water for 5 days in the presence or absence of Zanamivir. (B) Body weight, (C) Box and whisker plots on day 7 post DSS administration showing stool consistency score (0, well-formed pellets; 1, semiformed stools that did not adhere to the anus; 2, semiformed stools that adhered to the anus; 3, liquid stools that adhered to the anus) and (D) Box and whisker plots on day 7 post DSS administration showing rectal bleeding score (0, no blood as tested with hemocult (Beckman Coulter); 1, positive

hemocult; 2, blood traces in stool visible; 3, gross rectal bleeding) were checked daily (n = 10 per condition). (E) Colon length was measured on day 7 post DSS administration (n = 8 per condition). (F) Histopathological analysis in colon cross sections were examined by H&E staining. Images shown are representative of ten fields of view obtained from four mice of each condition. L, intestinal lumen; E, epithelial layer; C, crypt; G, goblet cell; S, submucosa; I, infiltration of leukocytes. All scale bars: 100 μ m. (G–J) DSS-induced experimental chronic colitis. (G) Body weight, (H) Box and whisker plots on week 8 post DSS administration showing stool consistency score, and (I) Box and whisker plots on week 8 post DSS administration showing rectal bleeding score (n = 12 per condition). (J) Colon length was measured on week 8 post DSS administration (n = 8 per condition). Error bars, means \pm SEM. *P < 0.05; log-rank test (A), one-way ANOVA with Tukey's multiple comparisons test (B), (E), (G), and (J), or Kruskal-Wallis test with Dunn's multiple comparisons test (C), (D), (H), and (I).

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