Bacterial Colonization and Th17 Immunity are Shaped by Intestinal Sialylation in Neonatal Mice

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Research

Keywords: Sialylation, ST6GAL1, microbiome

Posted Date: December 31st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-136577/v1

Abstract

Background: Interactions between the neonate host and its gut microbiome are central to the development of a healthy immune system. However, the mechanisms by which animals alter early colonization of microbiota for their benefit remain unclear. Host-derived carbohydrates, which can serve as metabolic substrates for the expansion of specific commensal and pathogenic bacteria, are one method by which the host may influence interspecies competition in the microbiome. Here, we investigated the role of early-life expression of the α2,6-sialyltransferase ST6GAL1 in microbiome phylogeny and mucosal immunity.

Methods: Intestinal sialylation was characterized by RT-qPCR, immunoblot, microscopy, and sialyltransferase enzyme assays in genetic mouse models at rest or with glucocorticoid receptor modulators. The fecal, upper respiratory, and oral microbiomes of pups expressing or lacking St6gal1 were analyzed by 16S rRNA sequencing. Pooled fecal microbiomes from syngeneic donors were transferred to antibiotic-treated wild-type mice, before analysis of recipient mucosal immune responses by flow cytometry, RT-qPCR, microscopy, and ELISA.

Results: ST6GAL1 was highly expressed in duodenal enterocytes between birth and weaning, driving temporary sialylation of the associated glycocalyx and secretion of active ST6GAL1 into the intestinal lumen, with subsequent uptake by colonic goblet cells. Expression was mediated by the P1 promoter of the St6gal1 gene, which was efficiently inhibited by intraperitoneal dexamethasone. At weaning, the fecal microbiome of St6gal1-KO pups was unchanged in diversity but exhibited reductions in Clostridium, Coprobacillus, and Adlercreutzia, along with increased Helicobacter and Bilophila. Transfer of St6gal1-KO microbiome induced an AhR-dependent Th17 response, with expression of T-bet and IL-17, and IL-22-dependent gut lengthening.

Conclusions: Intestinal sialylation by the sialyltransferase ST6GAL1 in the neonatal period is a developmentally regulated host mechanism coordinating bacterial colonization in the early gut microbiome. The inability to produce α2,6-sialyl ligands results in microbiome-dependent Th17 inflammation, highlighting a pathway by which intestinal epithelium regulates mucosal immunity. Considering the prevalence of intestinal fucosylation in adult animals, sialic acid may promote an early stage of ecological succession in the developing gut. Trial registration: N/A

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and accessed as a PDF.

Figures

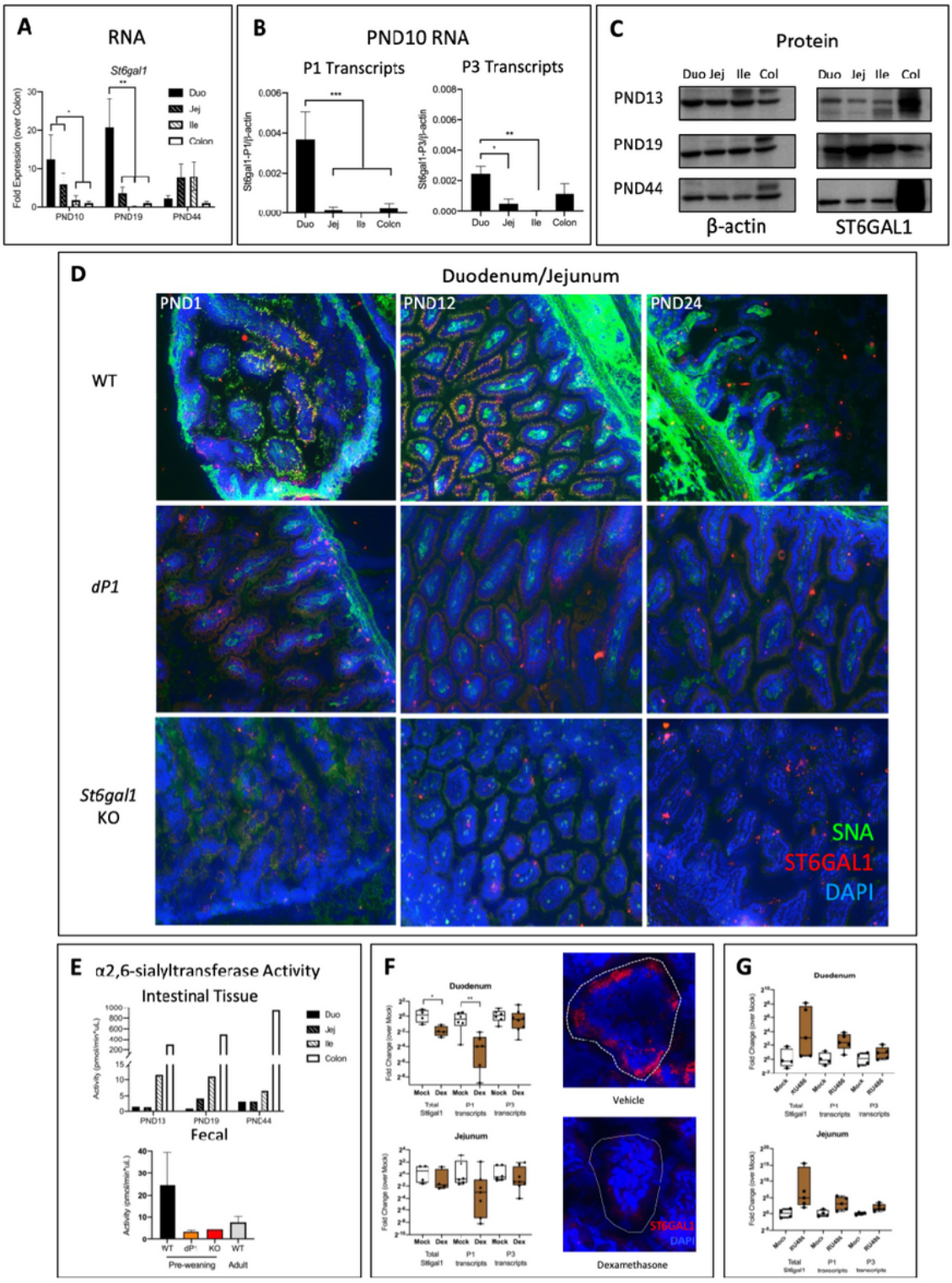

Figure 1

ST6GAL1 Expression in the Neonatal Duodenum is Mediated by the P1 Promoter and Inhibited by Glucocorticoids. (A) Relative expression of St6gal1 transcripts within the duodenum, jejnum, ileum, and colon on postnatal days (PND) 10, 19, and 44. (B) Relative abundance of P1-dependent and P3-dependent St6gal1 transcripts on postnatal day 10. (C) Immunoblot for ST6GAL1 from total tissue of duodenum, jejunum, ileum, and colon on postnatal days 13, 19, and 44. (D) Frozen sections of mouse

total small intestine tissue at postnatal days 1, 12, and 24 were stained for ST6GAL1 (red) and with Sambucus nigra lectin (green). Comparison of wild-type (WT), P1 promoter conditional knockout (dP1), and global St6gal1 KO mice is shown. (E) α2,6-sialyltransferase activity in WT intestinal tissues at indicated ages (above) and in fecal pellets at 10d of age (below). (F) Postnatal day 10 mice were given a single bolus of intraperitoneal dexamethasone, then sacrificed after 24 hours and total RNA levels of total, P1-specific and P3-specific St6gal1 transcripts quantified in duodenum and jejunum (left). Proximal small intestine was stained for ST6GAL1 protein in vehicle and dexamethasone-treated PND15 mice (right). (G) Adult WT mice were treated with intraperitoneal RU-486 or vehicle control for 3 days, then abundance total, P1-specific and P3-specific St6gal1 transcripts quantified in duodenum and jejunum.

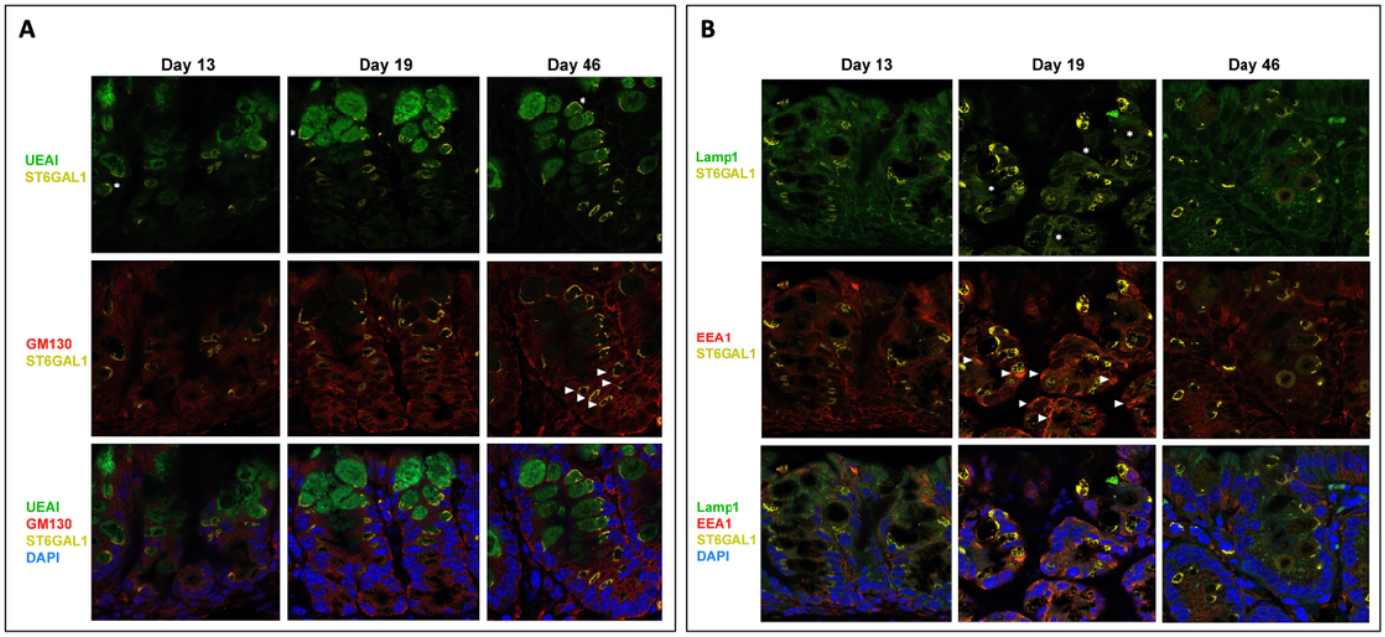

Figure 2

Neonatal Colonic Goblet Cells Endocytose Luminal ST6GAL1. Confocal microscopy analysis of ST6GAL1 localization within the colon of WT mice in relation to organellar markers at indicated ages is shown. (A) Co-staining of ST6GAL1 (yellow), goblet cell mucins (UEA-I, green), Golgi apparatus (GM130, red), and nuclei (blue) reveals presence of ST6GAL1 within goblet cell mucin granules at all ages (white asterisks) and presence of Golgi-associated ST6GAL1 only in the adult mouse (white arrowheads). (B) Co-staining of ST6GAL1 (yellow), lysosomes (LAMP-1, green), early endosomes (EEA-1, red), and nuclei (blue) demonstrates diffuse, low-intensity staining of ST6GAL1 (white asterisks) and its presence within endosomes on day 19 (orange staining, indicated by white arrowheads).

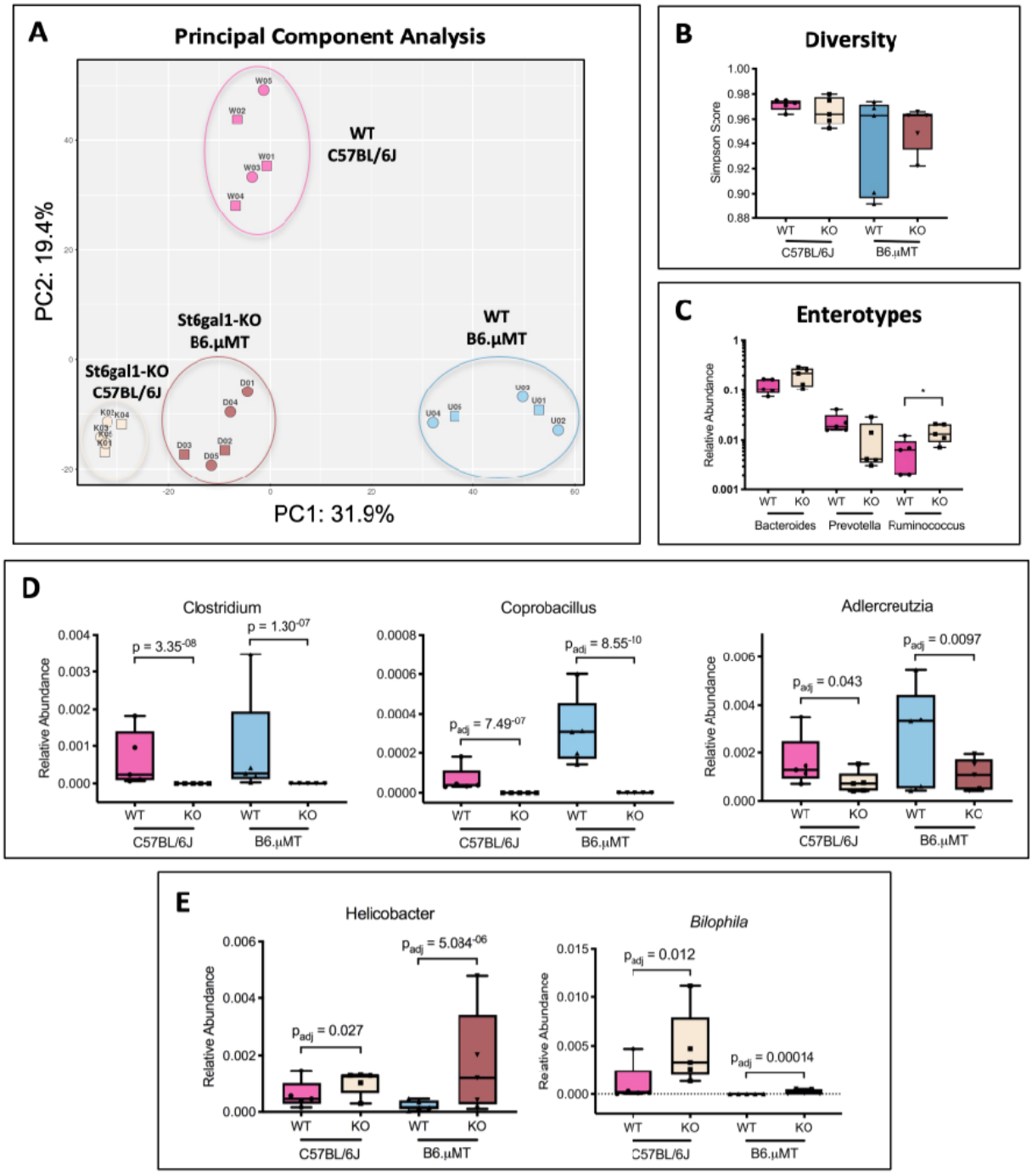

Figure 3

ST6GAL1 Influences Neonatal Fecal Microbiome Composition. Fecal pellets were collected from mice of indicated genotypes upon weaning and extracted DNA subjected to 16S sequencing. (A) Principal components analysis of 16S sequencing data for ST6GAL1-sufficient and deficient mice on C57BL/6J and B6.µMT backgrounds. (B) Simpson diversity score for microbiomes derived from indicated genotypes. (C) Relative abundances of Bacteroides, Prevotella, and Ruminococcus genera, indicative of enterotypes observed in the human microbiome. (D) OTUs assigned to Clostridium, Coprobacillus, and

Adlercreutzia genera were enriched in ST6GAL1-expressing animals.(E) OTUs assigned to Helicobacter and Bilophila were depleted in ST6GAL1-expressing animals.

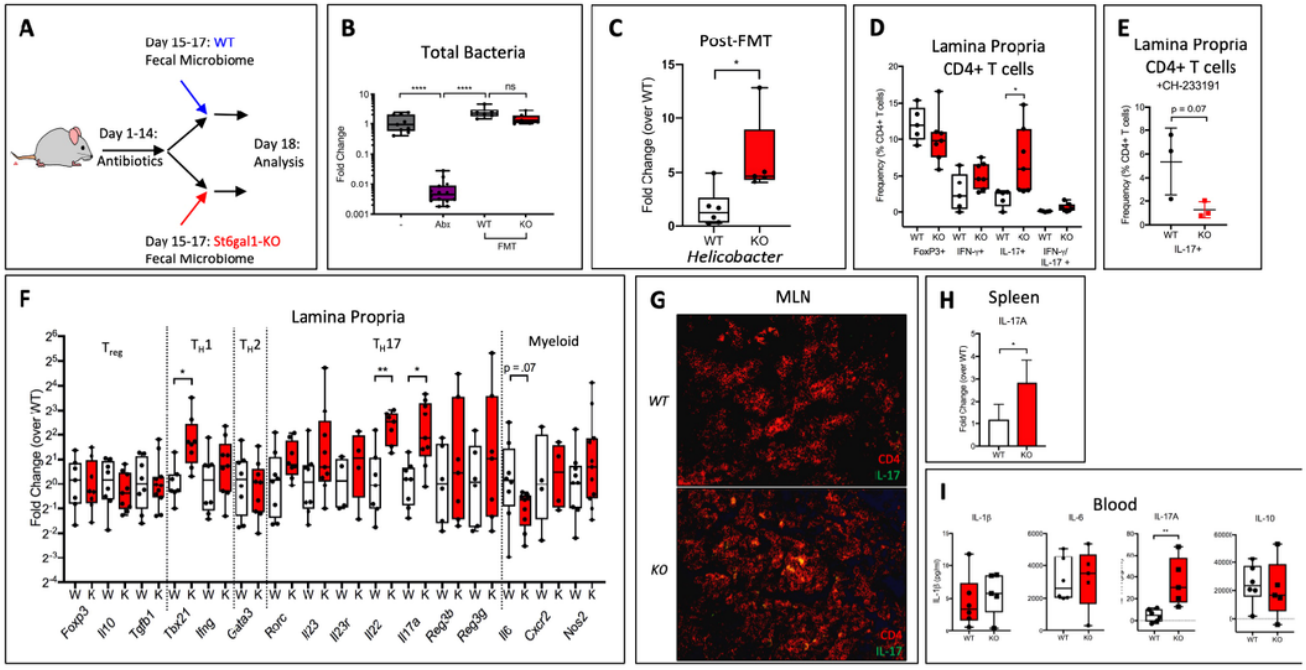

Figure 4

ST6GAL1 Deficiency Promotes Microbiome-dependent Local and Systemic Th17 Responses. (A) Wild-type mice were treated for 7 days with antibiotics (ampicillin, vancomycin, metronidazole, neomycin) in drinking water, then given fecal microbiome transplants (FMT) with either WT or St6gal1-KO feces from postnatal day 20-35 donor mice. After 4-8 days, mice were sacrificed for analysis. (B) Depletion of fecal microbiome by antibiotics and restoration by FMT. (C) Reconstitution of elevated Helicobacter prevalence in mice receiving St6gal1-KO microbiome. (D) Quantitation of frequency of CD4+ T cell subsets with indicated fecal microbiome transfer donor genotype. (E) Treatment with CH-233191 depletes Th17 cell increase induced by St6gal1-KO microbiome transfer. (F) qPCR analysis of total lamina propria cells between mice receiving WT or St6gal1-KO fecal microbiome. (G) Immunofluorescence staining of mesenteric lymph nodes for CD4 (red) and IL-17 (green). (H) qPCR analysis of IL-17A expression within the spleen. (I) Serum analysis of indicated cytokines after FMT.

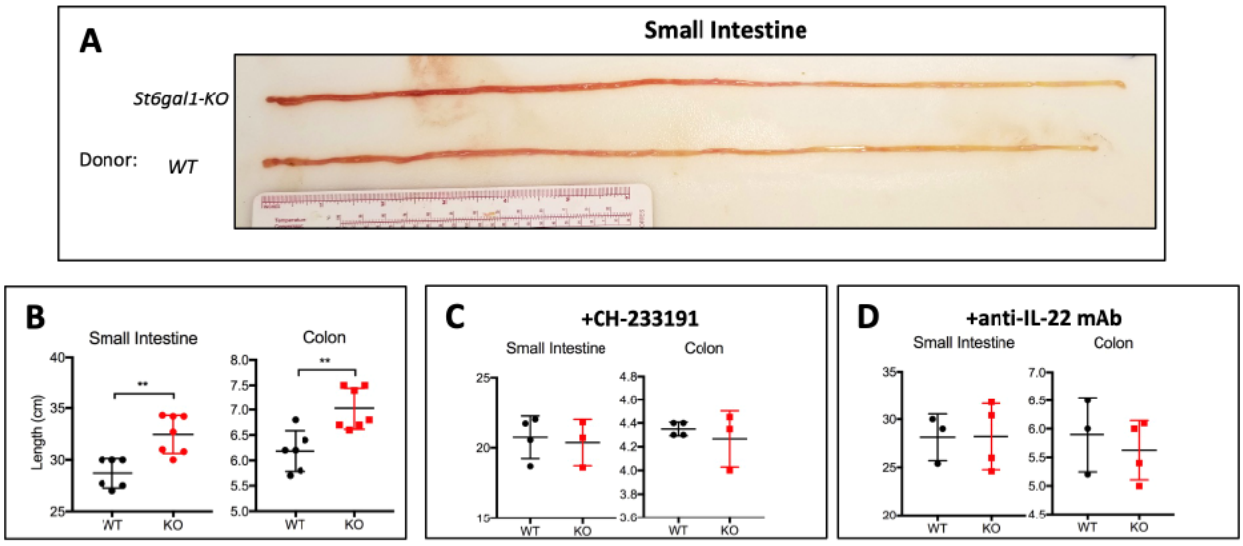

Figure 5

ST6GAL1 Deficient Microbiome Promotes Gut Lengthening via an AhR/IL-22 Pathway. (A) Representative image of small intestine from FMT recipients at time of sacrifice. (B) Length of small intestine and colon between mice receiving WT or KO FMT, with or without concurrent administration of (C) CH-233191 or (D) neutralizing anti-IL-22 mAb.

Supplementary Files

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- EI04SupFigsv1.pdf