Supplementary Fig. S1. Synthesis process of mucus *O*-glycans.

Supplementary Fig. S2. The timeline of the experiment in rats.

Note: All 30 SD rats were randomly divided into 3 groups (CON group, ABX group and ABX+GOS group) with 10 rats each group. The CON group was fed the basal diet, the ABX group was fed the basal diet supplemented with antibiotics added to the water. Lastly, the ABX+GOS group was fed the basal diet supplemented with both antibiotics and GOS. After 2 weeks, ileal microbiota was analyzed by 16S rRNA MiSeq sequencing (n=10 per group). Composition and distribution of *O*-glycans in the ileal mucus were detected by UPLC-MALDI-TOF-MS (n=3 per group). The abundance of sialic acid in the ileal mucus was determined by lectin staining (n=6 per group). Gene expressions of Glycosyltransferases (Including sialyltransferases) were evaluated by RT-qPCR (n=10 per group).

Supplementary Fig. S3. Effects of ABX and ABX+GOS on diversity of intestinal microbiota in the intestine of rats.

(A) Alpha diversity analysis. (B) PCoA analysis. (C) Venn analysis. (D&E) Ileal microbiota composition at the phylum level. n = 10. Graphs represent mean ± SEM. The one-way ANOVA was applied for statistical analysis. $*P \leq 0.05$, $**P \leq 0.01$.

Supplementary Fig. S4. Effects of ABX and ABX+GOS on the pathways and factors related to glycosyltransferase expression.

(A) The relative expression levels of TLR4/MyD88/NF-κB pathway in the intestine mucosal tissue. (B) The relative expression levels of inflammatory factors in the intestinal tissue.

Supplementary Fig. S5. Intestinal alkaline phosphatase activity (IAP) and its colocalization with different types of sialic acids

(A) Immunofluorescence staining of intestinal alkaline phosphatase. (B) The average fluorescence intensity of intestine alkaline phosphatase. (C) Immunofluorescence colocalization of intestinal alkaline phosphatase with α 2, 3 sialic acid and α 2, 6 sialic acid. (D) The average fluorescence intensity of intestine alkaline phosphatase with α 2, 3 sialic acid. (E) The average fluorescence intensity of intestine alkaline phosphatase with α 2, 6 sialic acid. Scale bar = 50 μm.

Supplementary Fig. S6. Correlation analysis between microbial community and *O*-glycans and sialyltransferases.

(A) Correlation analysis between microbial community and *O*-glycans. The cells are colored based on the Spearman's correlation coefficient. Red represents positive correlation, blue represents negative correlation. The color depth indicates the strength of the correlation. (B) Correlation analysis between microbial community and sialyltransferases. The arrow represents sialyltransferases, the circle represents microbial community, the quadrant where the arrow is located represents the positive or negative correlation between sialyltransferases and microbial community, and the length of the arrow represents the degree of correlation between sialyltransferases and microbial community distribution. *$P \leq 0.05$, **$P \leq 0.01$.

Supplementary Table S1 The sialyltransferases family.

Supplementary Table S2 Primers used for RT-qPCR in this study.

Supplementary Table S3 UPLC elution program applied to the separation of *O*-glycans