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Supplemental information

Microbiome-encoded bile acid metabolism modulates colonic transit times

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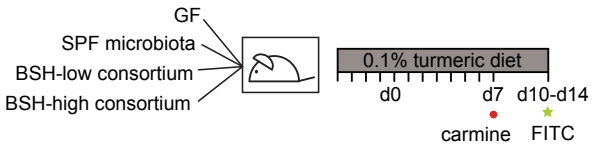
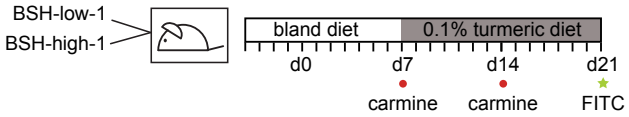
A *Gnotobiotic study design for majority of experiments presented:*



B *Gnotobiotic study design for experiment testing effect of oscillating diet exposure (±turmeric):*



C *Gnotobiotic study design for experiment testing bland diet:*

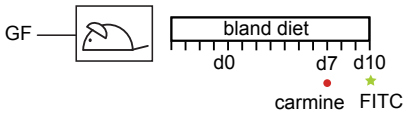
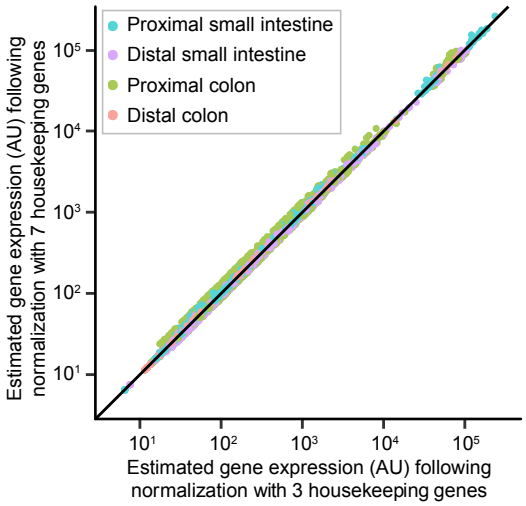


Figure S1. Gnotobiotic mouse experimental schematics (Related to Figures 2-6). A. Study design of majority of experiments presented: a monotonous diet experiment in which mice were fed a turmeric-containing diet and were subjected to the FITC and carmine gut transit assays. Day 0 was defined as the day of fecal microbiota transplantation via oral gavage in colonized mice. **B.** An oscillating diet experiment in which mice were fed a bland diet and a turmeric-containing diet serially. The carmine motility assay was performed at the ends of both diet phases. **C.** Schematic of monotonous diet experiment involving bland diet administration.

A



B

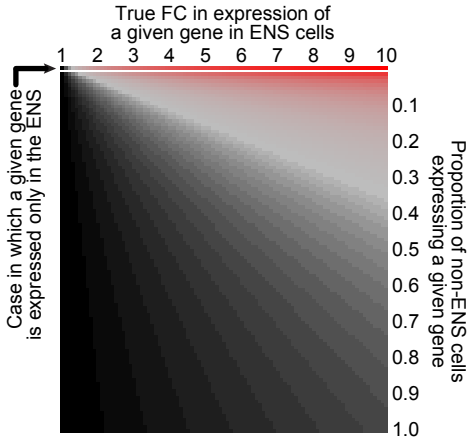


Figure S2. Effects of normalization strategy and ENS specificity on readouts in a NanoString gene expression panel (Related to STAR Methods and Figure 6). A. Estimated gene expression values were highly concordant when normalizing with 7 housekeeping genes or with a subset of 3 of these genes. Values are color-coded by gut segment. The solid line represents y=x, and the slope of the line of best fit is 1.01. **B.** Simulation of the effect of non-ENS-specific gene expression on estimation of gene expression changes in ENS cells using our NanoString gene expression panel.

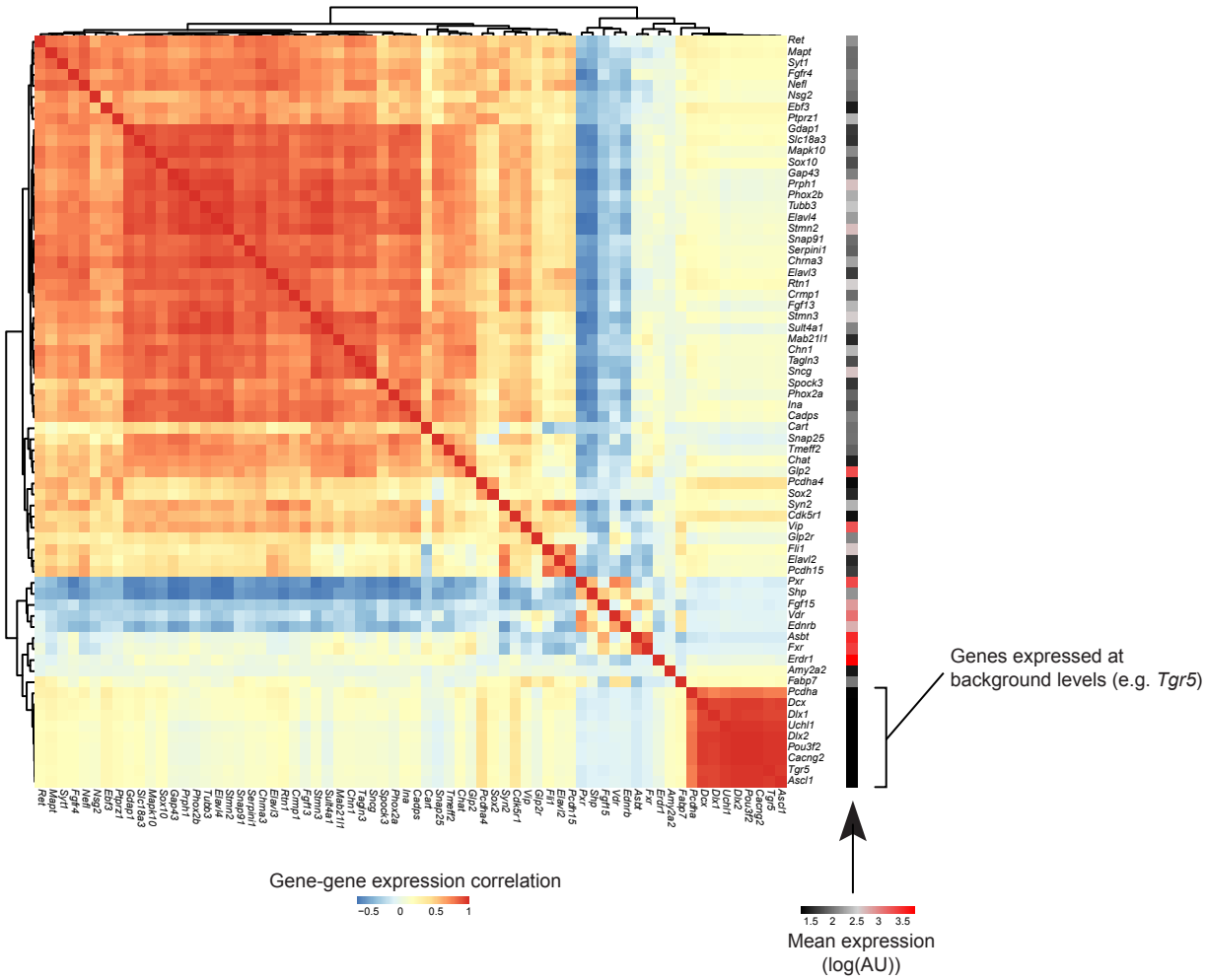


Figure S3. Heatmap of Pearson correlation matrix of log-transformed gene expression values in the gene expression dataset (Related to Figure 6). Hierarchical clustering was applied across rows and columns. The right-sided panel represents the mean log-transformed gene expression. Genes not profiled in all samples were removed for this calculation.

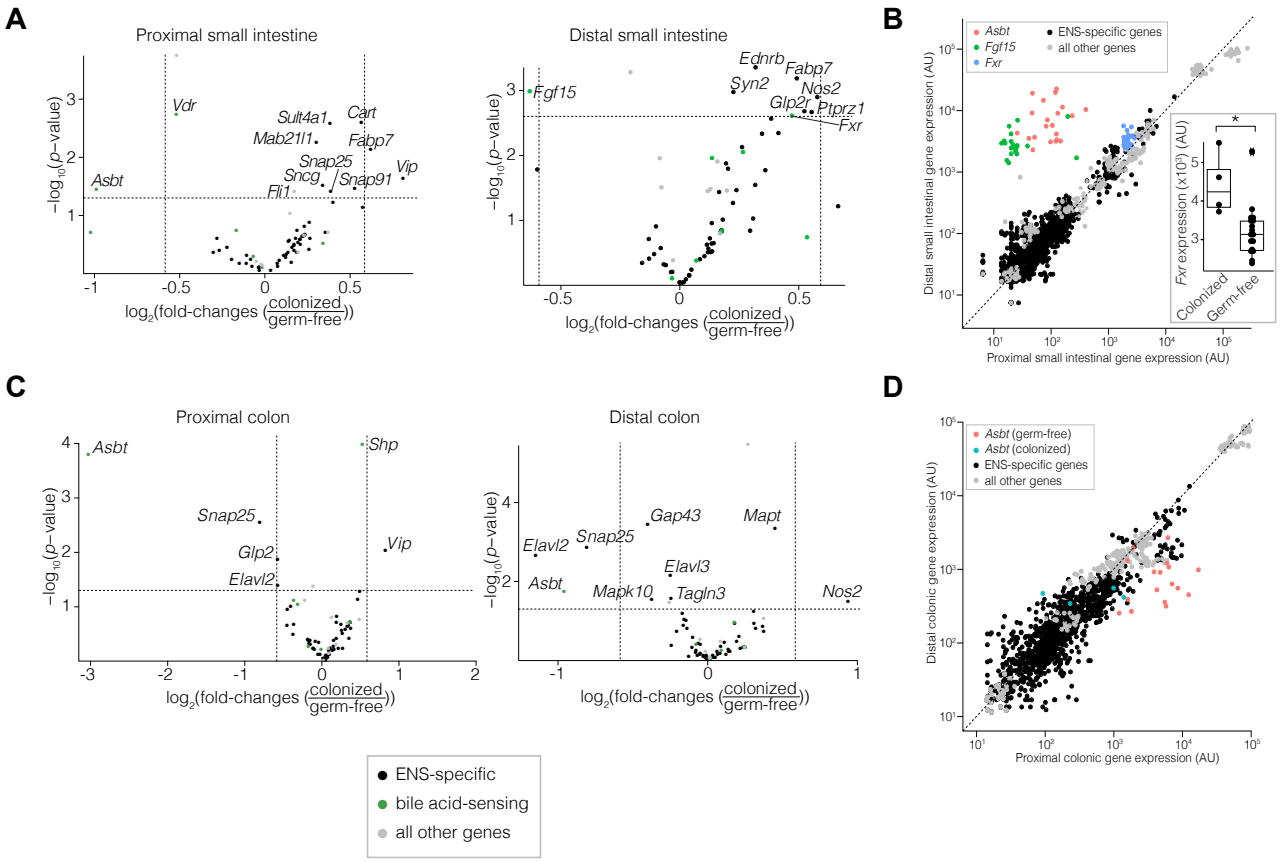


Figure S4. Differential gene expression in a comparison of gnotobiotic mice colonized with a complete mouse microbiota and GF controls (Related to Figure 6). Volcano plots showing differential expression in the **(A)** small intestine and **(C)** colon in a comparison of SPF-microbiota-colonized and germ-free mice. The dark green dots indicate genes involved in bile acid sensing; black dots indicate ENS-specific genes; and light gray dots indicate other genes. ENS-specific genes and bile acid sensing genes passing either the *p*-value significance threshold or FC>1.5 threshold are labeled. **B.** Comparison of gene expression in distal versus proximal aspects of the small intestine. Inset: Effect of colonization on *Fxr* expression. **D.** Comparison of gene expression in distal versus proximal aspects of the colon.

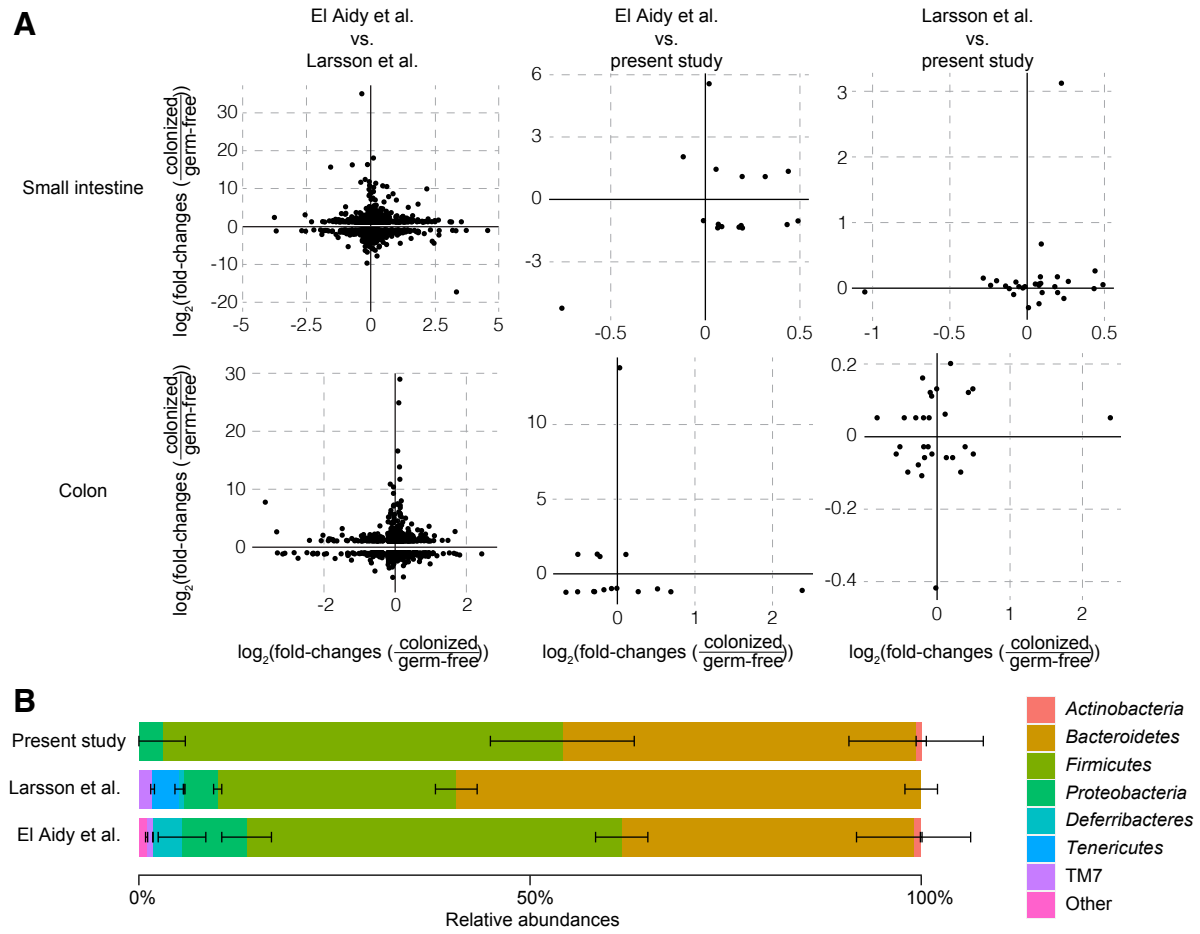


Figure S5. A comparison between studies that have profiled intestinal gene expression in GF mice compared to mice harboring an SPF microbiota (Related to Figure 6). A. Pairwise correlations of gene expression fold-changes between colonized mice and GF mice in small intestine and colon comparing El Aidy et al., Larsson et al., and our findings. The first listed study in each column is represented along the y-axis. **B.** Phylum-level community structure of gut microbiota in two other studies and this study.