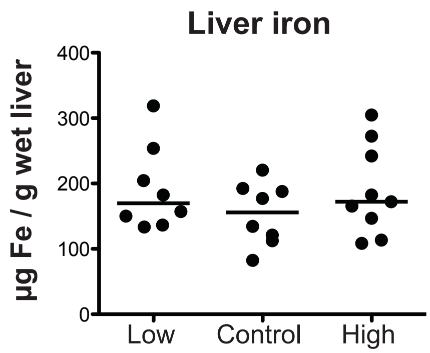

Figure S1: Liver iron stores in WT mice. Liver iron concentrations were measured in ex-GF WT mice 4 weeks following conventionalization and administration of an iron deficient (low), control or iron supplemented (high) diet. Each symbol represents an individual mouse, n = 8-9 mice per group. Lines are at the medians. *P*-values were determined by pairwise comparisons by the Kruskal-Wallis test.

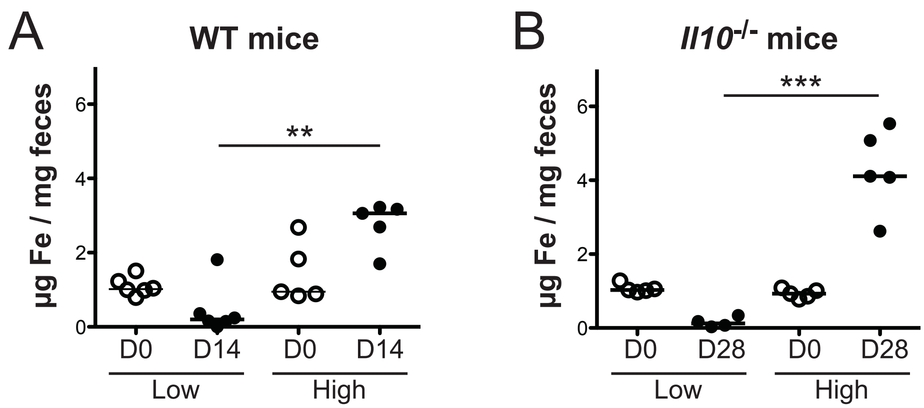

Figure S2: Dietary iron restriction or supplementation alters fecal iron concentrations. Fecal iron concentrations were measured in A) ex-GF WT mice after 14 days and B) ex-GF *Il10*$^{-/-}$ mice after 28 days following conventionalization and administration of an iron deficient (low), control or iron supplemented (high) diet. Prior to the dietary interventions, mice were maintained on a typical mouse diet. Each symbol represents an individual mouse, n = 5-6 mice per group. Data are represented as μg Fe per mg dried feces. Lines are at the medians. *P*-values were determined by pairwise comparisons by the Kruskal-Wallis test. ** $p < 0.01$, *** $p < 0.001$

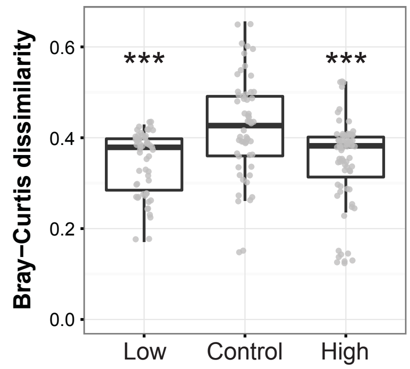

Figure S3: Dissimilarity distances in fecal communities between WT mice within each diet group. Ex-GF WT mice were maintained on an iron deficient (low), control or iron supplemented (high) diet. Data are represented as pair-wise Bray-Curtis distances between samples within each diet group. Box and whisker plots show the minimum, first quartile, median, third quartile and maximum distance. *P*-values were determined using a Student's *t*-test. *** $p < 0.001$ relative to the control diet group.

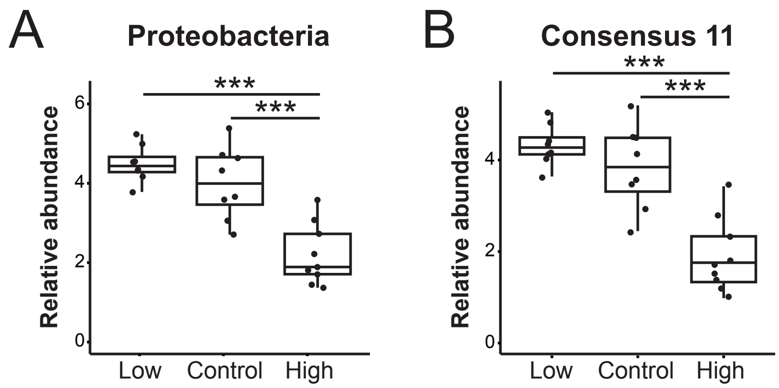

Figure S4: Dietary iron restriction restricts luminal expansion of Proteobacteria and Enterobacterioaceae. Relative fecal abundances of A) Proteobacteria and B) Enterobacteriaceae (OTU Consensus 11) in WT mice. Each symbol represents an individual mouse, n = 8-9 mice per group. Box and whisker plots show the minimum, first quartile, median, third quartile and maximum relative abundance. FDR-corrected p-values were determined using a mixed linear model. *** $p < 0.001$

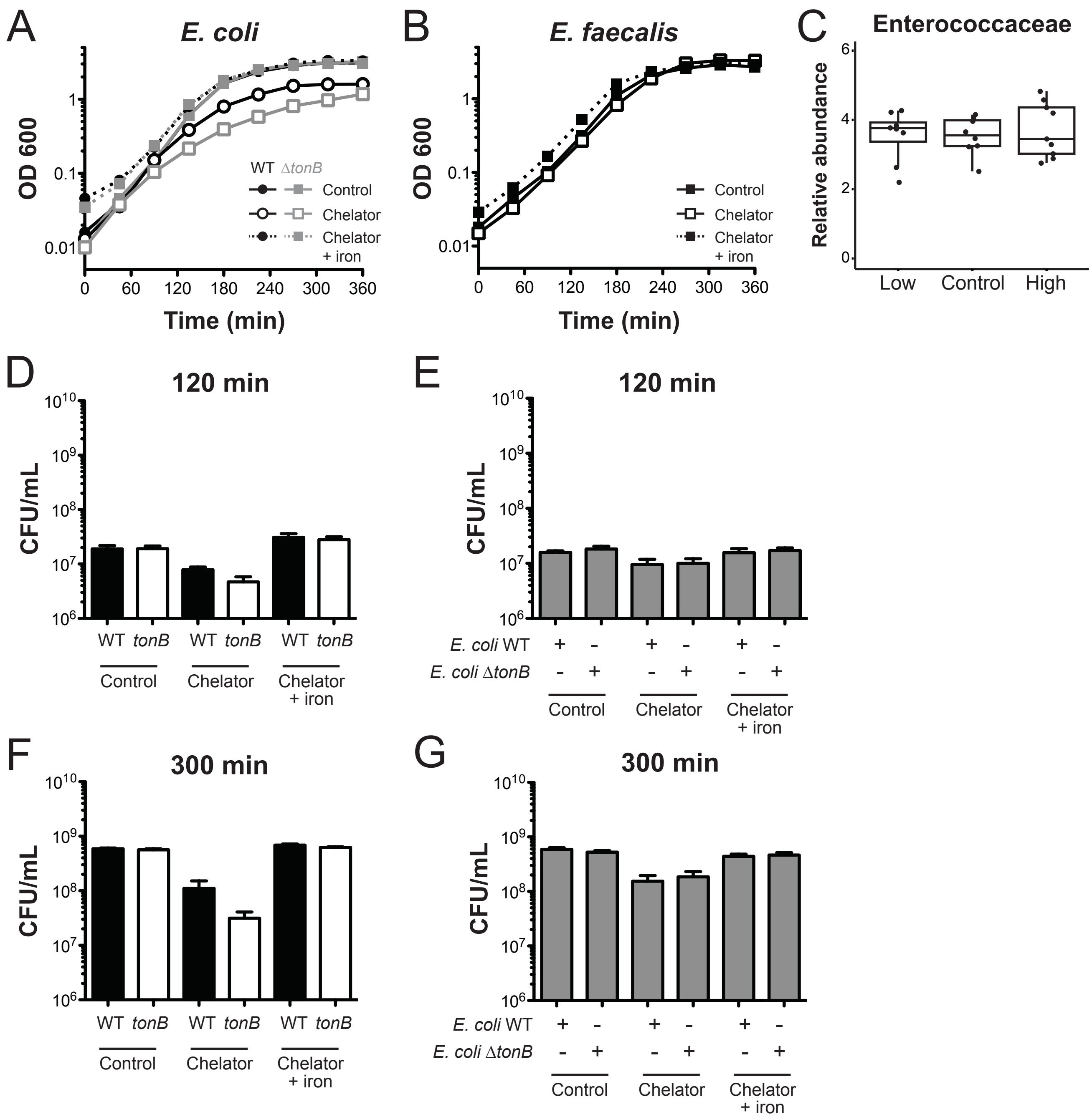

Figure S5: *In vitro* growth of *E. faecalis* and the parental and *tonB*-deficient *E. coli* strains in the presence or absence of an iron chelator. A, B) Representative growth curves of A) *E. coli* WT (circles + black lines) or the *tonB*-deficient mutant (squares + grey lines) and B) *E. faecalis* grown in rich medium (control), in rich medium with an iron chelator (chelator) or in rich medium with an iron chelator and additional ferrous iron (chelator + iron). D) Relative fecal abundance of Enterococcaceae in WT mice. Each symbol represents an individual mouse, n = 8-9 mice per group. Box and whisker plots show the minimum, first quartile, median, third quartile and maximum relative abundance. FDR-corrected p-values were determined using a mixed linear model. D-G) *E. coli* WT or the *tonB*-deficient mutant was grown in the presence of *E. faecalis*. Abundance of bacteria was determined by quantitative selective plating. The abundance of D, F) *E. coli* WT or the *tonB* mutant in the presence of *E. faecalis* and E, G) the abundance of *E. faecalis* in the presence of *E. coli* WT or the *tonB* mutant after 120 or 300 min of growth. Data are represented as the mean ± SEM of at least three independent experiments.

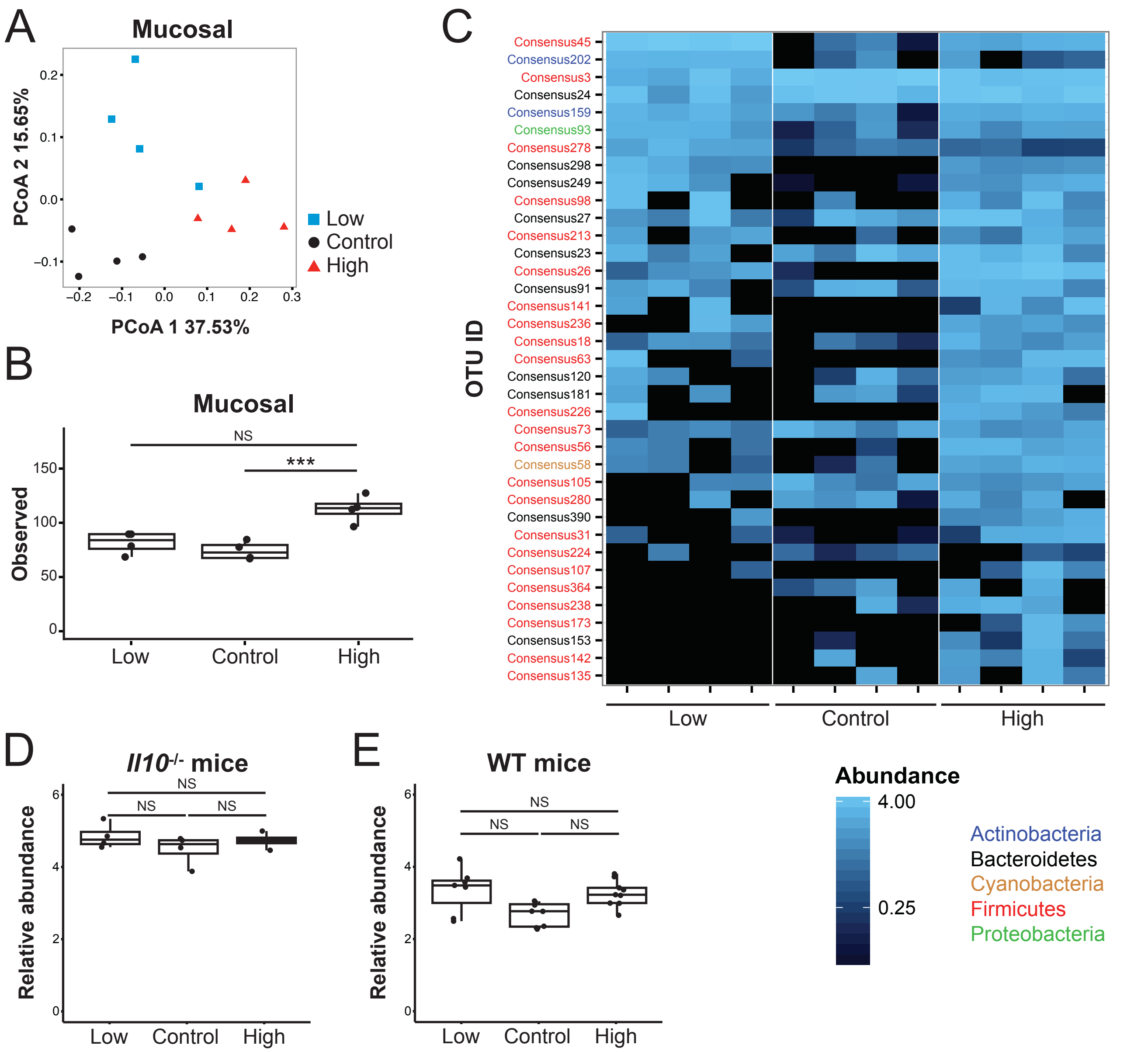

Figure S6: Compositional changes to the mucosal microbiota in *Il10$^{-/-}$* mice on variable iron diets. A) Principal coordinate analysis based on Bray-Curtis metrics for the mucosal microbiota in ex-GF *Il10$^{-/-}$* mice maintained on an iron deficient (low), control or iron supplemented (high) diet. B) Microbial richness as measured by observed OTUs. C) Heat map of OTUs that are significantly different in abundance (FDR-corrected $p < 0.05$) as determined using a mixed linear model. Each column represents an individual *Il10$^{-/-}$* mouse. Each row represents individual OTUs, color coded by phylum. The colors of the heat map represent the mean relative abundance (normalized and log transformed) of each OTU. D, E) Relative mucosal abundance of Enterobacteriaceae in D) *Il10$^{-/-}$* mice and E) WT mice. Each symbol represents an individual mouse, n = 7-9 mice per group. Box and whisker plots show the minimum, first quartile, median, third quartile and maximum values. Comparisons and FDR-corrected p-values were determined using a mixed linear model. *** $p < 0.001$

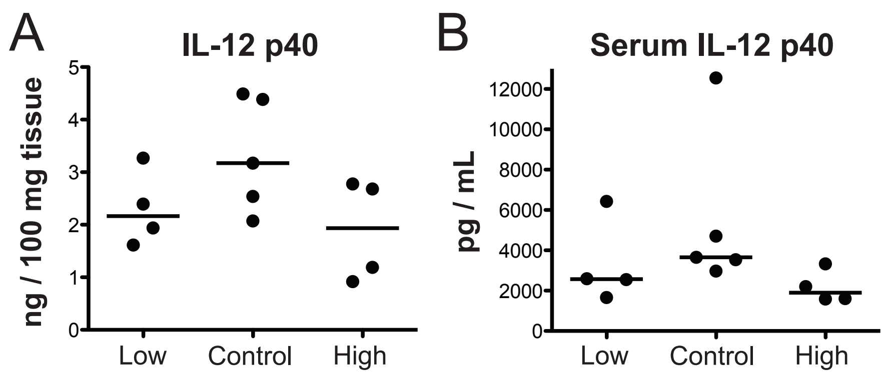

Figure S7: IL-12 p40 levels in colitic *Il10$^{-/-}$* mice with variable iron diets. Ex-GF *Il10$^{-/-}$* mice were maintained on an iron deficient (low), control or iron supplemented (high) diet for 4 weeks. A) Spontaneous secretion of IL-12 p40 by colonic explant cultures. B) Serum IL-12 p40 levels. Each symbol represents an individual mouse, n = 4-5 mice per group. Lines are at the medians. *P*-values were determined by pairwise comparisons by the Kruskal-Wallis test.

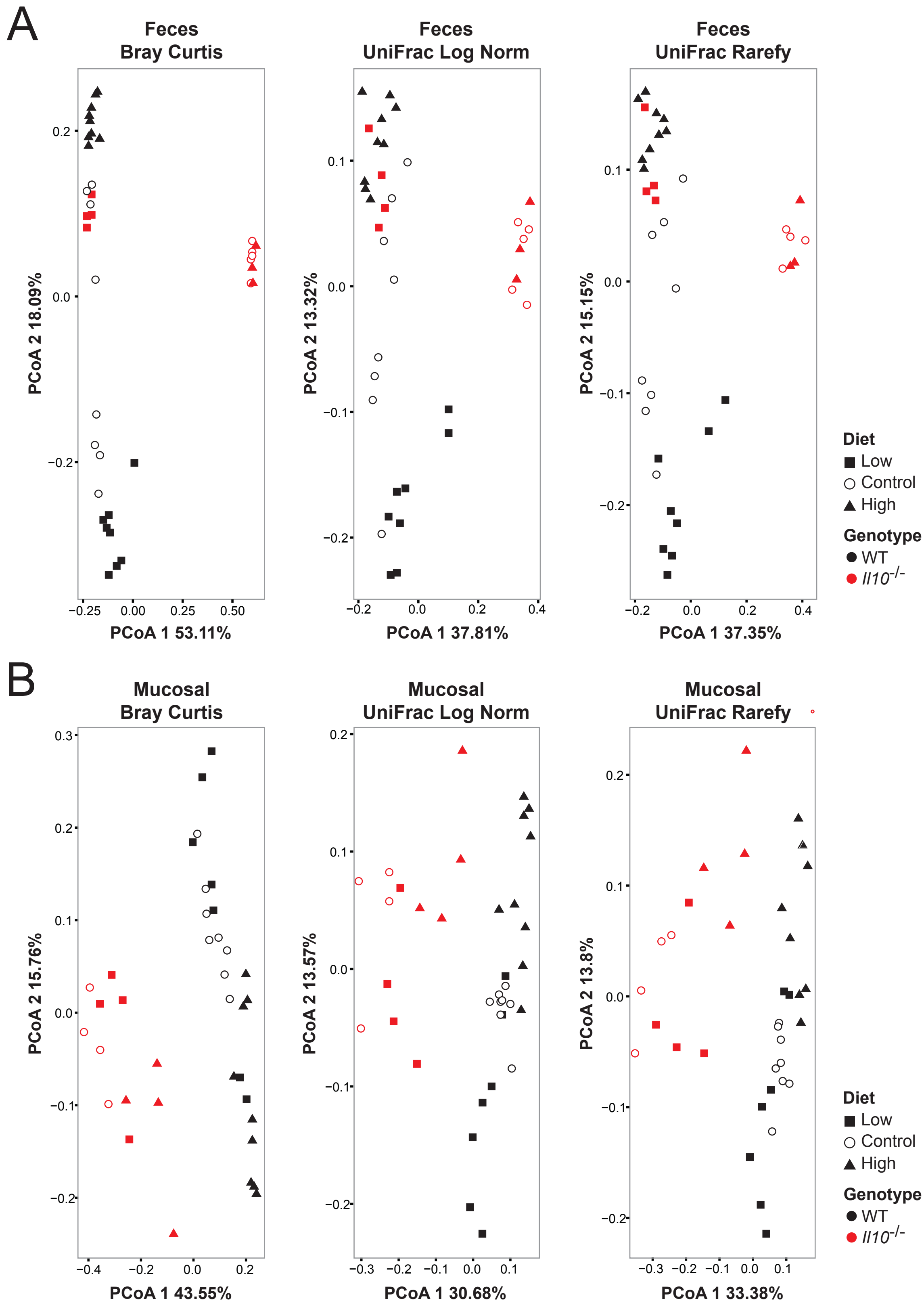

Figure S8: Dietary iron modulation differentially impacts the WT and *Il10*$^{-/-}$ fecal microbiotas. Principal coordinate analysis for the A) fecal and B) mucosal microbiota based on either Bray-Curtis metrics using QIIME close reference OTUs ($\log_{10}$ normalized counts) and UniFrac metrics after normalizing and $\log_{10}$ transforming the OTU counts or after rarefying the OTU counts. Each symbol represents an individual mouse, *n* = 3-9 mice per group.