

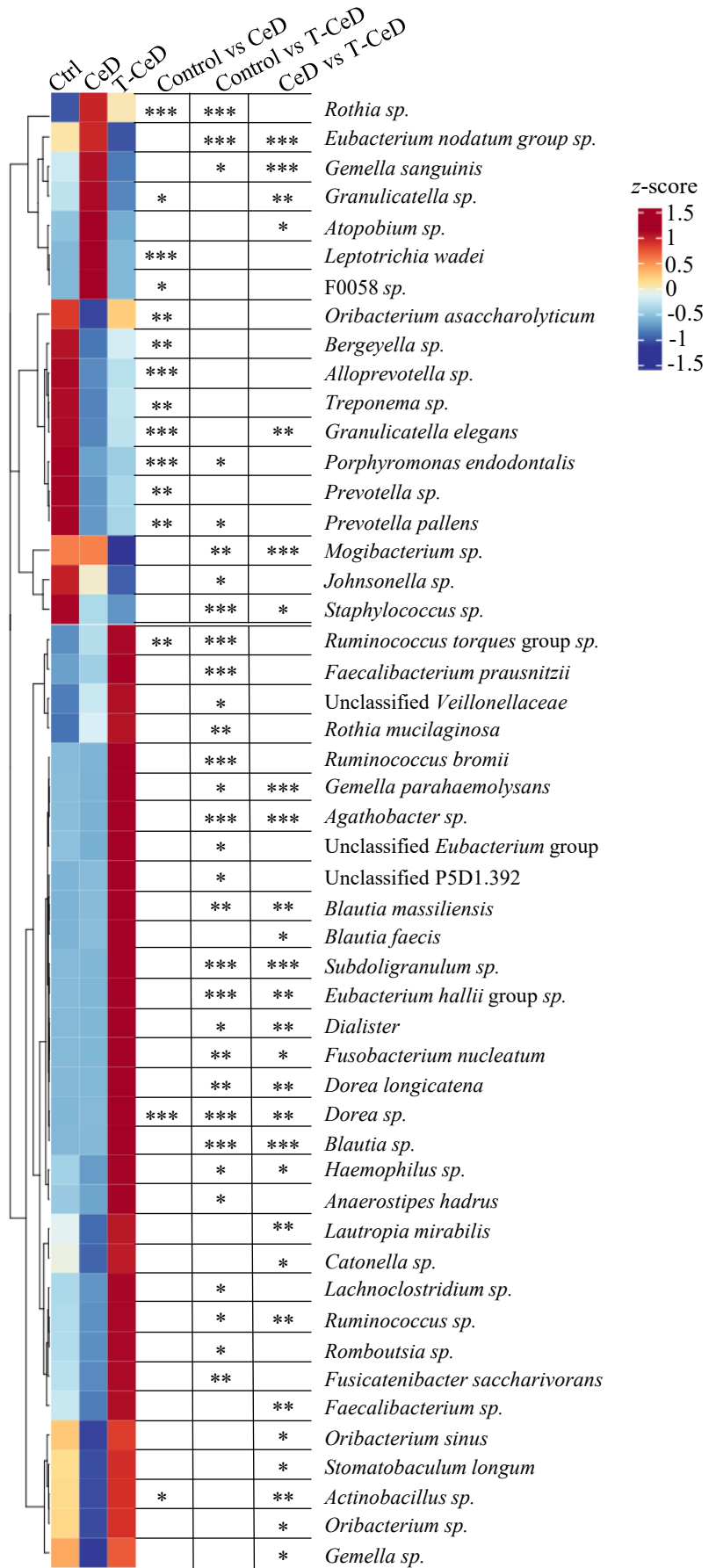
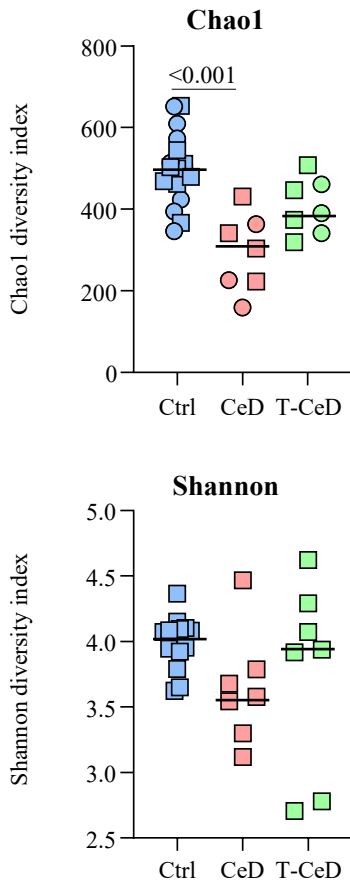
Supplemental information for

**Small intestinal microbial fiber metabolism
dysfunction in celiac disease**

Wulczynski *et al.*

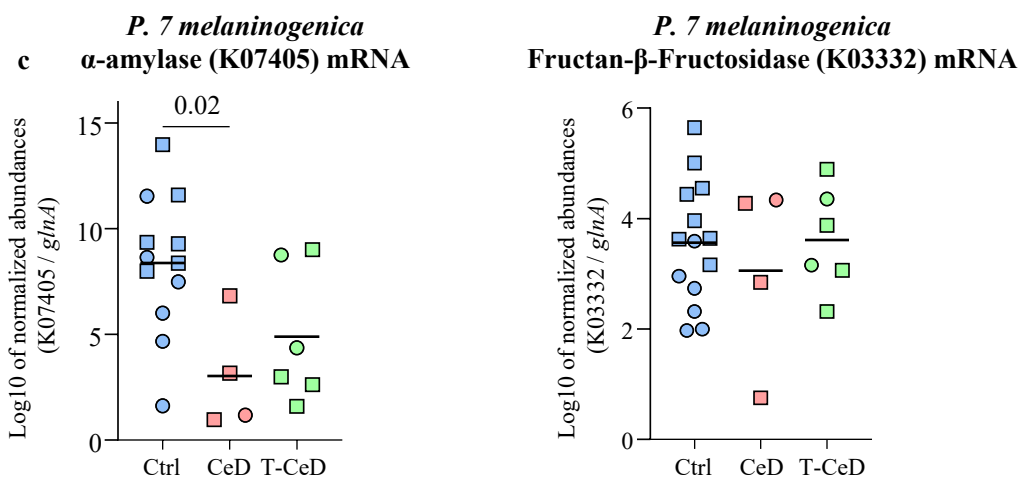
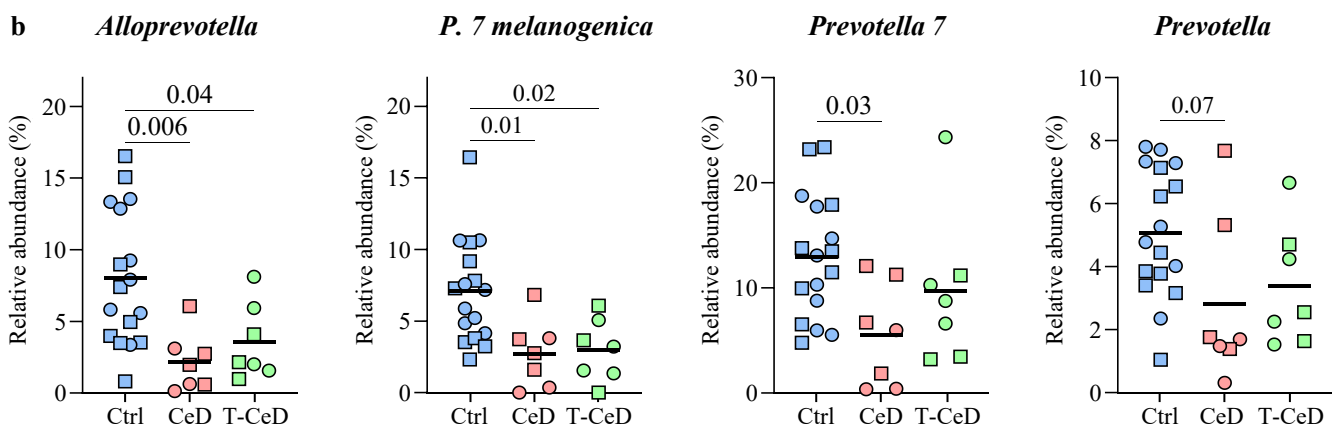
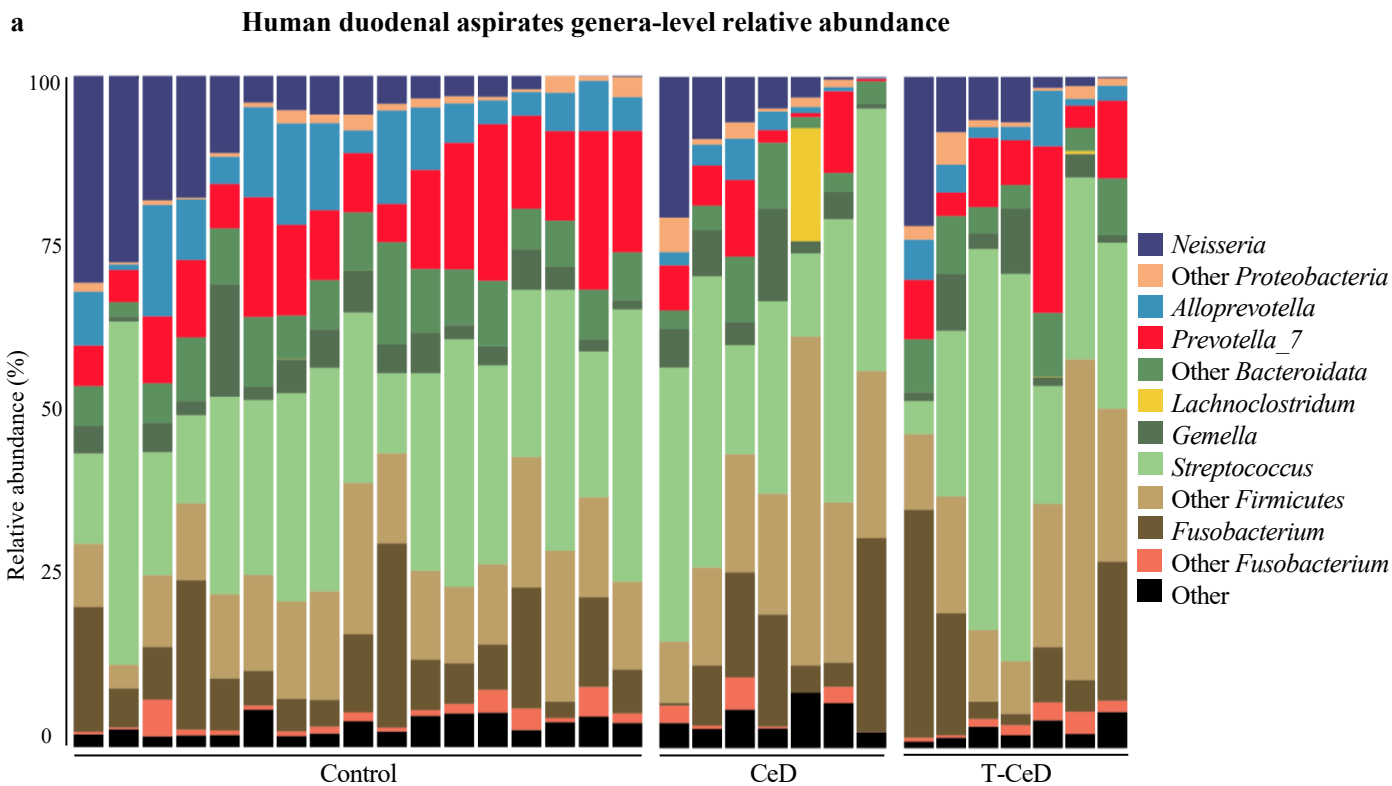
b Differences in the avg. relative abundance of duodenal bacteria

a Duodenal Alpha-diversity



Supplementary Figure 1: Duodenal microbiota composition in controls compared to untreated and treated celiac disease patients.

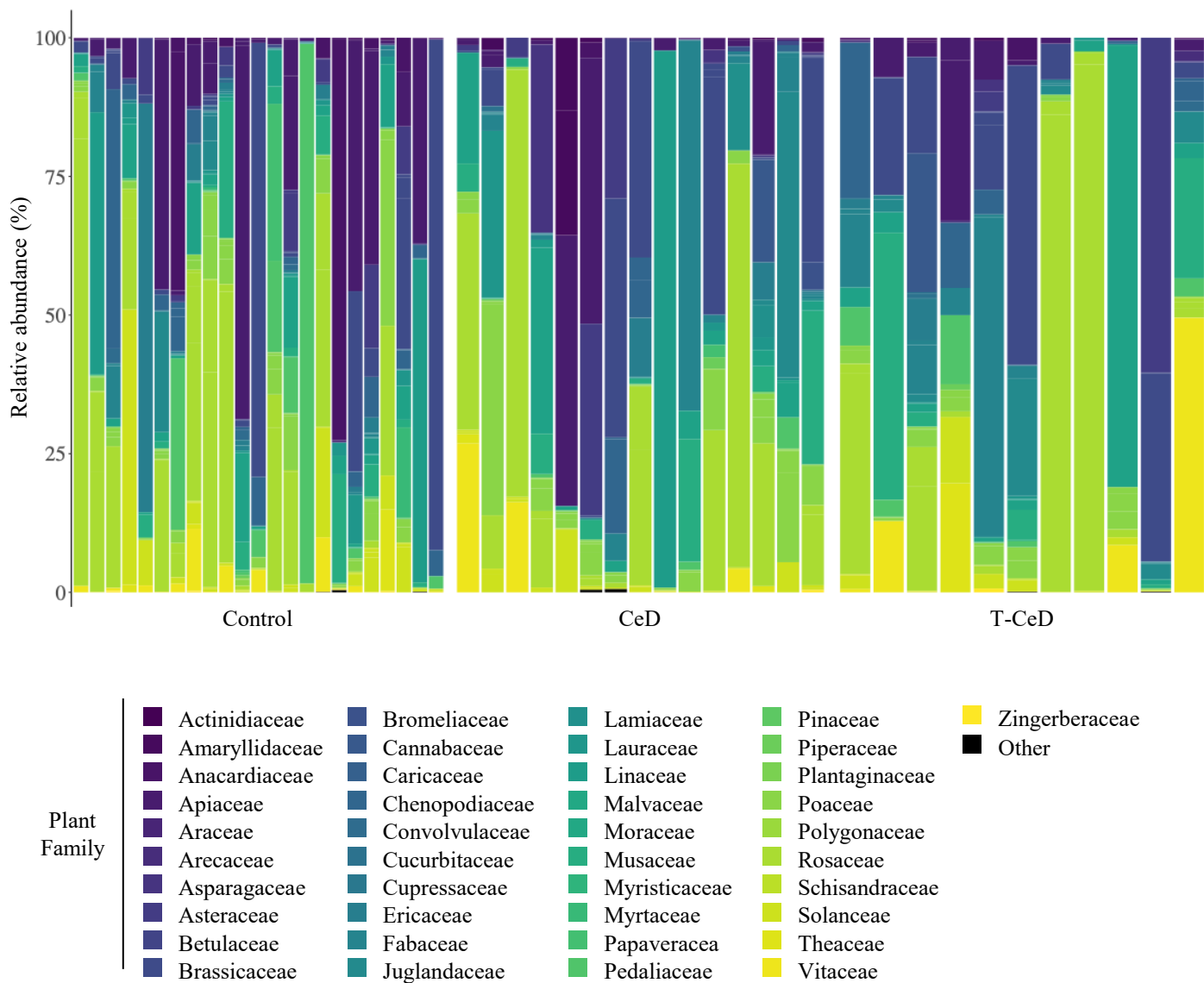
a Human duodenal microbiota α -diversity measures (Chao1 diversity and Shannon diversity). Multiple groups were compared using one-way ANOVA followed by Tukey's test, and sex was represented by females (squares) and males (circles). **b** Heatmap summary of differentially abundant bacteria between controls (n=17), CeD (n=7), T-CeD (n=7). Each column represents the average z-score for each bacterium.



Supplementary Figure 2: Individual study participant duodenal microbiota composition.

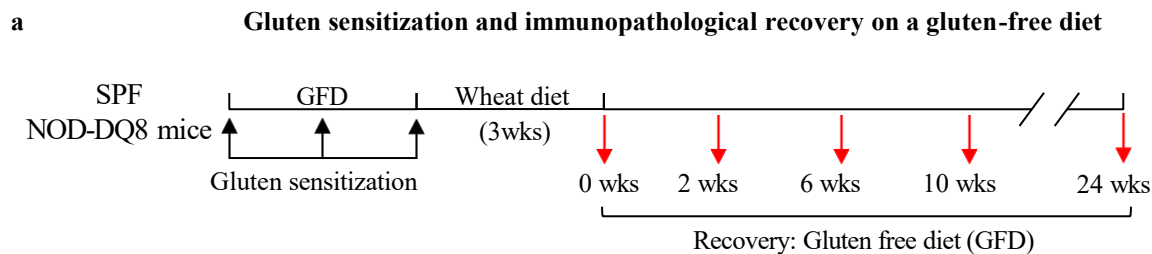
a Abundance plot of duodenal genera in each individual participant, with legend. Healthy controls (n= 17), CeD (n= 7), and T-CeD (n= 7). **b** Relative abundance of *Prevotellaceae* members predicted to have the largest metagenomic contribution to microbial saccharolytic enzymes. **c** Duodenal microbial mRNA transcript expression of α -amylase (K07405) and fructan- β -fructosidase (K03332) normalized to *glnA* transcript levels in healthy controls (blue, n = 12-14), CeD (red, n = 4) and T-CeD (green, n = 6). Samples with low RNA integrity were excluded from analysis. Multiple differences were compared using one-way ANOVA followed by Tukey's test, and sex was represented by females (squares) and males (circles).

Relative abundance of dietary plant taxa in individual fecal samples

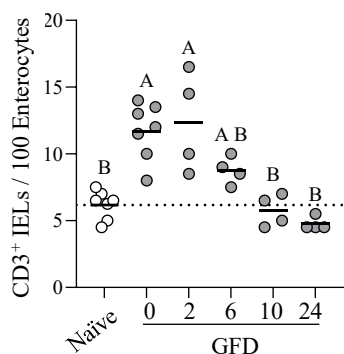


Supplementary Figure 3: Individual heterogeneity of dietary plant DNA in feces.

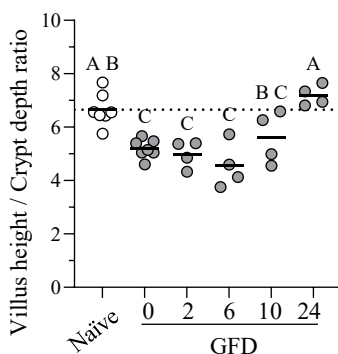
Abundance plot of plant taxa in stool samples from each individual participant, with legend.



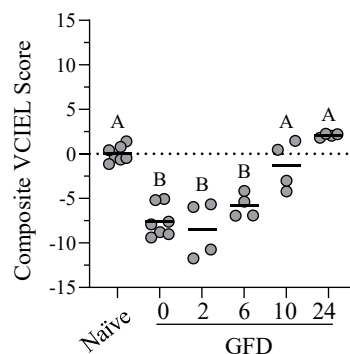
b **CD3⁺ IEL counts**



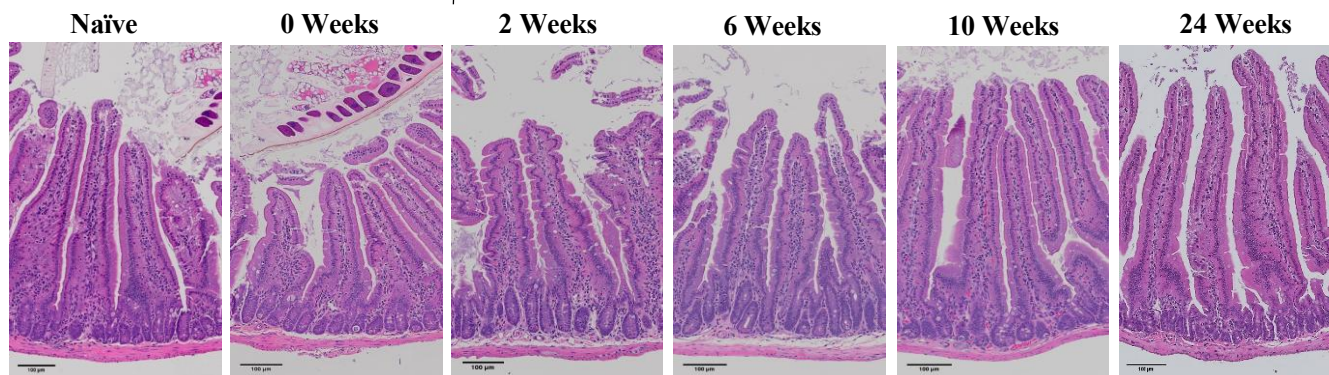
c **Villus-to-crypt ratio**



d **Composite VCIEL score**



e **Recovery on GFD**

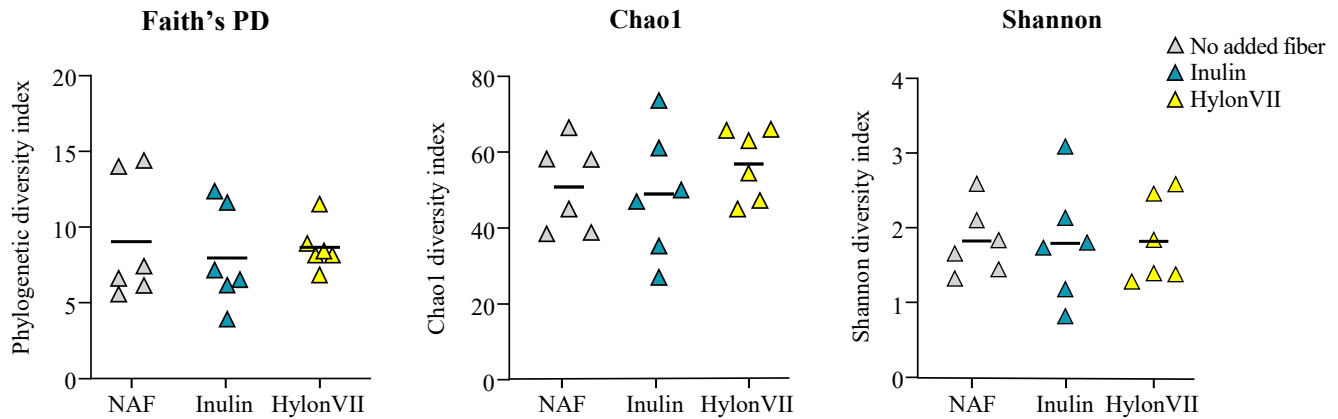


Supplementary Figure 4: Gluten-immunopathology resolves by week 10 of GFD.

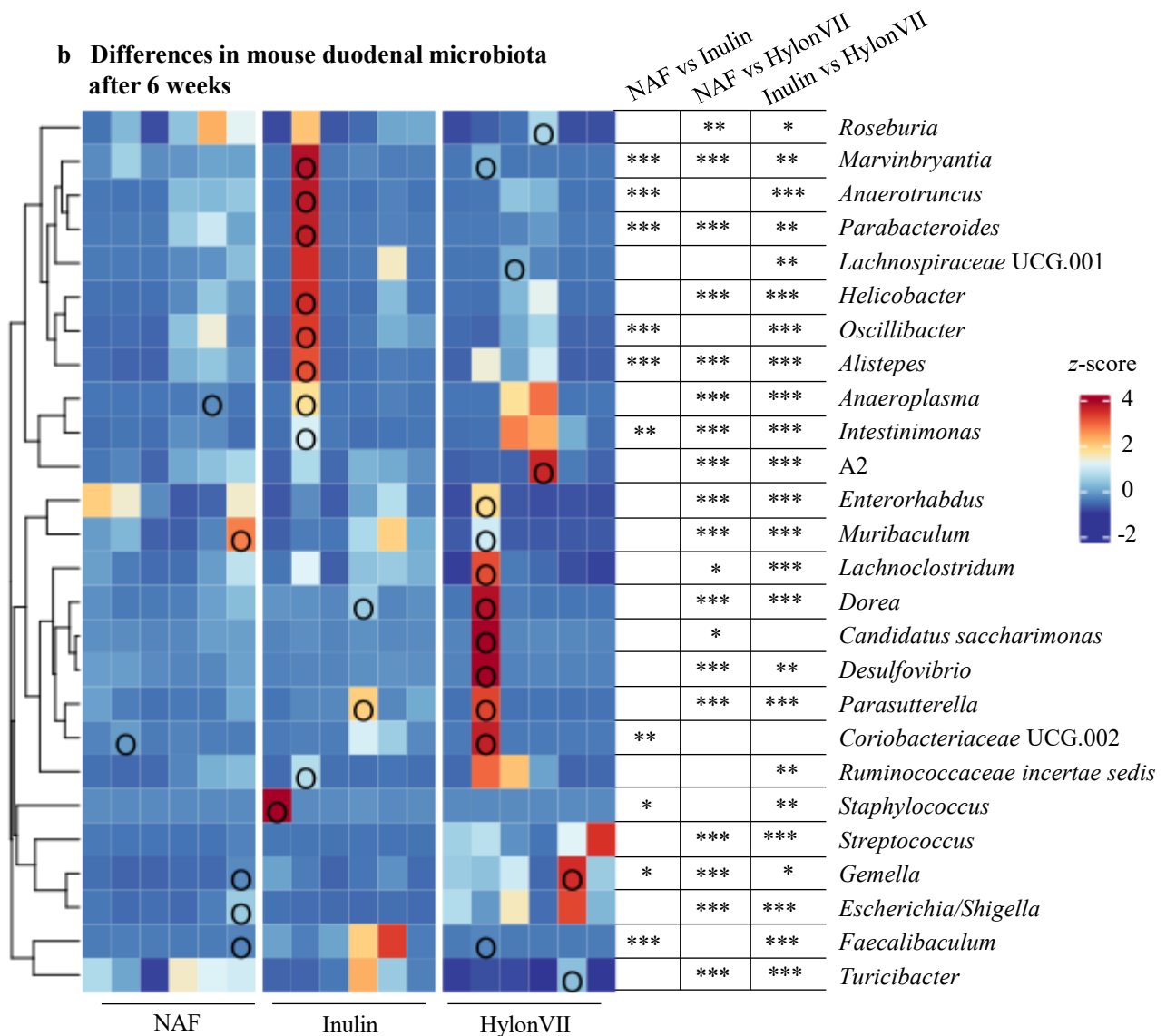
a Protocol for gluten sensitization, challenge, and GFD-recovery. 8–10-week-old specific pathogen free (SPF) NOD/DQ8 mice were sensitized to gluten by weekly oral gavage with 25 μ g cholera toxin and 1 mg of pepsin/trypsin digested gliadin. Mice were then given gluten containing wheat diet for 3 weeks before being returned to GFD for recovery. Mice ($n = 4-8$ per group) were sacrificed after 0, 2, 6, 10, and 24 weeks of recovery and compared to naïve controls (non-sensitized and without gluten exposure). **b** CD3⁺ intraepithelial lymphocytes numbers over time during GFD recovery. **c** Villus-to-crypt ratio over time during GFD recovery. **d**, Composite VCIEL scores over time during GFD recovery. Dashed line represents the baseline determined from naïve controls. **e** Representative histological images of jejunal tissue over time during GFD recovery. For all data, multiple groups were compared using one-way ANOVA followed by Tukey's test, significant differences ($p < 0.05$) between groups were indicated with a compact letter display. (where A indicates statistical difference from B, and AB indicates a group is not different from A or B, etc).

a

Mouse duodenal microbiota α -diversity

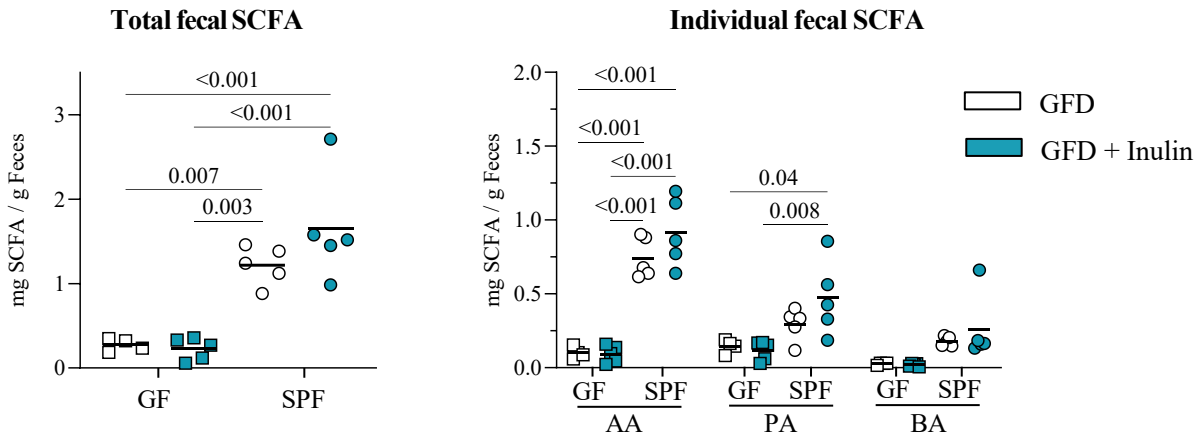


b Differences in mouse duodenal microbiota after 6 weeks



Supplementary Figure 6: NOD/DQ8 mouse small intestinal microbiota composition after 6 weeks of recovery

a Mouse duodenal microbiota α -diversity measures (Faith's Phylogenetic Diversity, Chao1 diversity, Shannon diversity) after six weeks of recovery on either no added fiber GFD (grey), inulin (blue), or HylonVII (yellow). Multiple groups were compared using one-way ANOVA followed by Tukey's test. **b** Heatmap summary of differentially abundant bacterial taxa grouped by diet. Each row represents the z-score for one bacterium and **O** indicates statistical outlier defined as a value more than three times the interquartile range.



Supplementary Figure 7: Effect of microbiota and fiber on fecal SCFA and expression of intestinal SCFA receptors

Fecal SCFA after 3 weeks of no added fiber GFD (white) or inulin diet (blue) in GF (squares) and SPF (circles) mice (n= 5 / group). Multiple groups were compared using one-way ANOVA followed by Tukey's test.