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Estradiol modulates gut microbiota in female *ob/ob* mice fed a high fat diet

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Abstract

Estrogens protect against diet-induced obesity in females. For example, loss of estrogens in postmenopausal women is associated with an increased risk of weight gain. Furthermore, ovariectomized rodents on a high-fat diet (HFD) become obese, which can be prevented by estrogen treatment. Estrogens act with other hormones, including leptin, to regulate energy homeostasis in females. Leptin, a hormone encoded by the *ob* gene, is produced by adipocytes and acts in brain to signal satiety. Leptin-deficient mice (*ob/ob*) exhibit morbid obesity and insulin resistance. In addition to estrogens and leptin, the gut microbiome (gut microbes and their metabolites), is critical in regulating metabolism. The present study investigates whether estrogens and leptin modulate gut microbiota in ovariectomized *ob/ob* (obese) or heterozygote (Het; lean) control mice fed a HFD that received either 17β-Estradiol or vehicle implants. E2 attenuated weight gain in both genotypes compared to their vehicle counterparts. Moreover, both obesity (in *ob/ob* mice) and E2 reduced gut microbial alpha diversity. *ob/ob* mice exhibited lower species richness than control mice, while E2-treated mice had reduced evenness compared to vehicle mice. At the taxa level, E2 treatment was associated with higher abundances of the *S24-7* family. Leptin was associated with higher abundances of *Coriobacteriaceae* and the *Clostridium and Lactobacillus* spp. The present findings suggest that E2 and leptin profoundly

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alter the gut microbiota of HFD-fed female mice. Understanding the role of E2 and leptin in modulating gut microbiota will allow the development of therapeutic options targeting the gut microbiome for hormone-dependent metabolic disorders in women.

Keywords: Estrogen, microbiome, obesity, leptin, diabetes, insulin resistance

Introduction

Estrogens profoundly influence energy homeostasis [1-3], as well as reproductive physiology and behavior [4-6]. Estrogens reduce food intake, prevent body weight gain and adiposity, and increase physical activity in both humans and rodents [1,2]. Postmenopausal women have lower levels of circulating estrogens and an increased tendency to gain fat weight, which increases their risk for obesity, cardiovascular disease, stroke, and type 2 diabetes [7-9]. In support of the anorectic effects of estrogens, ovariectomized mice display increased energy intake and become obese when fed a HFD, while E2 treatment prevents weight gain [2,10-13], indicating that E2 protects against HFD-induced obesity.

Leptin is a peptide hormone secreted primarily by adipocytes, which acts in the brain to stimulate metabolism, promote satiety, and regulate fat storage [14-16]. A mutation in the *ob* gene that encodes leptin results in mice lacking the hormone (*ob/ob)* [17]. While phenotypically normal at birth, *ob/ob* mice quickly develop obesity and diabetes [18]. Additionally, *ob/ob* mice exhibit increased food intake and decreased physical activity, energy metabolism, and body temperature compared to lean controls, making *ob/ob* mice an excellent genetic model of obesity [19-21]. Administering leptin to adult *ob/ob* mice reverses these effects by decreasing food intake, increasing energy output and decreasing circulating levels of glucose and insulin [22,23].

Leptin and estrogen signaling pathways interact to influence reproduction and energy metabolism. High levels of E2 are associated with increased leptin sensitivity in both male and female rodents [24]. In mice, ovariectomy decreases leptin sensitivity, but can be restored by E2 administration [25]. While female *ob/ob* mice do not have an estrous cycle, leptin administration restores fertility, including successful ovulation, pregnancy, and birth, indicating the profound effects of leptin on reproduction [26]. While these findings indicate functional interactions

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between E2 and leptin, mechanisms by which these hormones interact to regulate energy metabolism are not well understood.

The gut microbiome, which is composed primarily of the bacteria in the intestinal tract and their genomes (collectively referred to as gut microbiota) and metabolites, has profound effects on energy metabolism [27]. Changes in body weight have been associated with changes in gut microbiota diversity in humans and rodents [28]. For example, the transfer of gut microbiota from obese human donors results in an obese phenotype in recipient mice [29,30]. The gut microbiome aids in digestion and absorption of macro- and micro-nutrients from food [31]. Gut microbiota are also essential modulators of host immune homeostasis. Protective polysaccharides produced from the break-down of dietary fibers attenuate infection and inflammation [32,33]. Finally, gut microbiota can synthesize and metabolize neurotransmitters and hormones to alter host physiology [34-36].

A variety of factors, including host genetics, diet, and stress can alter the gut microbiome [35-39]. There is mounting evidence that gonadal hormones influence gut microbiota [36]. Sex differences in gut microbiota have been reported in humans and rodents [40]. In a European population, higher levels of *Bacteroidetes* and *Prevotella* were observed in men compared to women [41]. Male mice exhibited higher abundances of *Lachnospiraceae (*phylum *Firmicutes*) and *Parabacteroides* spp. (phylum *Bacteroidetes*) and *Proteobacteria* than female mice [35,42]. Additionally, testosterone administration to female neonatal rats decreased gut microbial diversity during adulthood, and increased the ratio of the two most abundant phyla, *Firmicutes and Bacteroidetes* [37]. The hormone-dependent changes in gut microbiota were more robust compared to the diet-induced changes in these rats [37]. Ovariectomy also alters gut microbial diversity in adult mice [35,42,43]. While obesity and *ob/ob* genotype are associated with a reduction in *Bacteroidetes/Firmicutes* ratio in humans and male mice, respectively [44-46], it is not known whether leptin affects gut microbiota in females. In addition, it is essential to understand the potential effects of the interaction of leptin and E2 on gut microbiota. Thus, using a mouse model of obesity (*ob/ob*), the present study tested the hypothesis that E2 and leptin alter gut microbiota and energy homeostasis in female mice.

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Results

Estradiol and genotype altered weight gain and food intake in female mice on a HFD

Body weight and food intake data were collected every four days from the four experimental groups: control E2, control Veh, *ob/ob* E2, and *ob/ob* Veh. Longitudinal analysis of weight change showed that E2 decreased weight gain (F=15.67, p<0.001; ANOVA) in female mice fed a HFD. E2 treatment attenuated weight gain from day 11 through the end of the study (Figures 1A and 1B). Within-genotype comparison showed that E2 attenuated weight gain in Het mice from days 11-35, compared to Veh mice. For *ob/ob* mice, E2 reduced weight gain on days 11, 15 and 27-35, and showed strong trends on days 19 (p=0.059) and 23 (p=0.063). Because the Het and *ob/ob* mice had different weights at the beginning of the study, the % weight gain was also analyzed to remove the bias due to existing differences in weights prior to hormone treatment and diet change. Similar to the effects on body weight, E2 treatment reduced % weight gain from days 11-35. In particular, E2-treated Het mice gained less % weight than Veh Het mice on days 11- 35 (Figure 1B). Within the *ob/ob* mice, E2 decreased % weight gain on days 7-15 and 27-35.

Genotype also affected weight gain (F=1119.79, p<0.001; ANOVA) and % weight gain on longitudinal measures (F=4.23, p=0.047; ANOVA). *ob/ob* mice weighed more than control Het mice at all time points regardless of the hormone treatment. At the start of the study, *ob/ob* mice weighed approximately twice that of the Het mice, which persisted throughout the study. Finally, there was an interaction of E2 and genotype on body weight (F=4.21, p=0.048; ANOVA), with E2-treated Het mice gaining more % weight than E2-treated *ob/ob* mice on days 4 and 7 (Figure 1B). The Veh mice did not differ in % weight gain between the two genotypes.

E2 treatment reduced HFD consumption (F=11.04, p=0.002; ANOVA) from days 7 to 19 (Figure 1C). Analysis within each genotype showed that E2 attenuated food intake in *ob/ob* mice, but not in Hets, on days 7, 11 and 15. Comparison between lean Hets and *ob/ob* mice showed that obesity profoundly increased food intake (F=110.5, p<0.001, ANOVA). Het mice consumed less calories than *ob/ob* mice throughout the study. Within the E2-treated groups, an increase in food intake in *ob/ob* groups compared to E2 Het mice was observed only at the beginning (days 7 and 11) and end (days 27, 31 and 35) of the study. There was an interaction between genotype

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and treatment on food intake (F=5.52, p=0.029; ANOVA). We also observed a decrease in food intake on day 19 in *ob/ob* Veh mice, but not other groups, following surgery for removal of BrdU osmotic pumps on day 16.

Estradiol and genotype alter gut microbial diversity during HFD

To assess the impact of E2 treatment and obesity on α-diversity of the gut microbiota, 16S rDNA from fecal samples from *ob/ob* and Het mice were analyzed. The data set contained 137 samples after removing samples with less than 2,000 reads. The 16S rDNA targeted sequencing yielded 25399 reads/sample on average (range 11,154 - 82,733). Clustering of these 16S sequence tags produced 473 OTUs at 97% similarity level. The identified OTUs belonged to 10 phyla, 42 families and 62 genera based on the Greengenes database. During HFD, *ob/ob* mice had a lower number of total identified OTUs than Het mice, indicating that *ob/ob* mice had lower species richness than control mice (Figure 2A; p=0.002, linear regression). In addition, E2 treatment was associated with lower species evenness in both genotypes, suggesting that E2-treated mice have a more heterogeneous distribution of gut microbial communities than Veh mice (Figure 2B; p=0.0008).

The ordination plot based on Bray-Curtis Distance, which measures differences due to relative abundances and composition, showed that the four experimental groups differ from each other in microbial communities as shown by their distinct clustering (Figure 3; p=0.004, PERMANOVA). The first and second principal coordinates (PCo1 and PCo2) explain 29.4% and 17.5% of the variation in the microbial communities, respectively. Genotype, most likely through obesity, accounted for most of the difference observed as reflected by PCo1 (p=4E-8, t-test, Figure 3). There was also a clear effect of E2 treatment, represented by PCo2 (p=0.001, t-test, Figure 3).

Similar analyses were performed using Bray-Curtis Distance measures on each day to characterize the temporal changes in gut microbiota across groups. The effect of E2 on β-diversity was robust and continuously observed from the 2nd week of OVX (days 15-35). The effect of genotype was also observed on days 7,15, 23 and 35, and a trend was observed on the remaining two days (4 and 31) (Table 1).

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Estradiol treatment alters relative abundances of intestinal microbiota

Gut microbial community composition was analyzed further on aggregated data from all days to identify the relative abundances at the phylum, family and genus levels (Figure 4). E2-mediated shifts in relative abundances was evident on many of these taxa levels. In all four experimental groups, the most prevalent phyla (>90%) were *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Tenericutes* (Figure 4A). The two most abundant families were *S24-7* and *Lachnospiraceae*, within *Bacteroidetes* and *Firmicutes*, respectively (Figure 4B). At the genus level, *Coprococcus* and *Ruminococcus* (both within phylum *Firmicutes*) were the most abundant (Figure 4C).

To identify whether the differences in community structure were driven by differences in the relative abundances of particular microbial taxa, a differential abundance analysis based on overdispersed Poisson regression model was run on aggregate data to uncover the effects of treatment (Figure 5A) and genotype (Figure 5B). In the cladogram, the nodes in the first circle represent phyla, and the extending outer nodes in each level represent lower taxa within each phylum. A total of 26 taxa were differentially associated between E2 and Veh groups (Figure 6A). At the phylum level, *Firmicutes* were more associated with Veh treatment, while *Bacteroidetes* were associated with E2 treatment (Figures 5A and 6A). Within *Firmicutes*, the class *Erysipelotrichi*, including *Allobaculum* and *Coprobacillus* spp., were more abundant in Veh than E2 mice. Similarly, the relative abundances of the class *Bacilli*, including its families *Lactobacillaceae* and *Peptostreptococcaceae*, were greater in Veh than E2 groups. Another family, *Streptococcaceae* (phylum *Firmicutes*), including *Lactococcus* spp., were also more associated with Veh mice. In contrast, the family *Ruminococcaceae* (phylum *Firmicutes*) was more abundant in E2 mice. Within *Bacteroidetes*, the class *Bacteroidia*, and its lower taxa *Bacteroidales* and *S24-7*, were more abundant in E2 than Veh mice (Figures 5A and 6A).

For the taxa that differed across groups as detected by the aggregated data, the effect of E2 treatment on relative abundances was analyzed for each day. A comprehensive list of the taxa and associated q-values and fold changes between E2 and Veh mice for each day is provided in Supplemental Table 1. At the phylum level, E2-treated mice resisted a decrease in relative abundances of *Bacteroidetes* compared to Veh mice on days 23 (q=0.007) and 31 (q=0.09)

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(Figure 7A). A main driver of the shifts in *Bacteroidetes* was the family *S24-7*. E2-treated mice resisted a decrease in *S24-7* abundances compared to Veh mice on day 23 (q=0.015) (Figure 7B).

Compared to Veh mice, E2-treated animals resisted changes in relative abundances of the phylum *Firmicutes*, with lower relative abundances of *Firmicutes* on days 23 (q=0.01), 31 (q=0.06) and 35 (q=0.04) compared to Veh mice (Figure 8A). Within *Firmicutes*, *Lactobacillus* spp. showed a significant reduction on day 31 (q=0.05) in E2-treated mice (Figure 8D).

Estradiol also altered the relative abundance of the phylum *Actinobacteria* from days 15 to 35. On these days, E2 mice resisted increases in the relative abundances of *Actinobacteria* compared to Veh mice (Figure 9A). The E2-mediated effect on *Actinobacteria* was due mostly to the family *Coriobacteriaceae* which resisted this increase also on days 15 (q=0.001), 23 (q<0.001) and 35 (q=0.03) (Figure 9B).

Obesity modifies relative abundances of gut microbiota

As the gut microbial communities of the female mice clustered separately based on obesity (in *ob/ob* genotype) (Figure 3), we further analyzed the effects of obesity on relative abundances at the taxa levels (Figure 5B). A total of 26 identified taxa showed a differential association between *ob/ob* and Het mice (Figure 6B). Comparison of the gut microbial communities between genotypes revealed that the phylum *Firmicutes*, including its families *Lactobacillaceae*, *Turibacteraceae*, *Peptostreptococcaceae*, *Dehalobacteriaceae*, and *Ruminococcaceae* and the genera *Lactobacillus*, *Turibacter*, *Dehalobacterium*, *Dorea*, *Ruminococcus*, and *Oscillospira* were more abundant in control Het mice than *ob/ob* mice. Similarly, the phylum *Actinobacteria* and its family *Coriobacteriaceae* showed a greater abundance in control Het mice. In contrast, *Enterococcus*, *Coprococcus*, *Lachnospira*, *Anaerotruncus* and *Coprobacillus* spp. of the phylum *Firmicutes* were more abundant in *ob/ob* mice compared to Het mice (Figures 5B and 6B).

Differential abundance analysis across days showed that genotype affected the relative abundances of multiple taxa (Supplemental Table 2). Leptin increased the relative abundances of *Bacteroidetes* and its family *S24-7* in Het mice at the beginning (day 4; q=0.04), but by day 23 (after 19 days on HFD; q=0.03), levels of these microbes were lower than in *ob/ob* mice (Figure

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7). Within the phylum *Firmicutes*, *Clostridium*, *Lactobacillus* and *Lactococcus* spp. were altered by genotype. *Lactococcus* was more abundant in *ob/ob* mice on days 7 (q=0.01), 15 (q=0.002) and 35 (q=0.007) (Figure 8D). In contrast, *Clostridium* and *Lactobacillus* spp. were more abundant in Het mice. *Clostridium* was higher in Het mice on days 4 (q=0.04), 7 (q=0.01) and 15 (q<0.001) than *ob/ob* mice (Figure 8B). Similarly, the relative abundance of *Lactobacillus* increased in Het mice compared to *ob/ob* mice on days 4 (q=0.01), 7 (q<0.001) and 23 (q=0.001) (Figure 8D).

Obesity also altered the relative abundance of the phylum *Actinobacteria* over time. Compared to *ob/ob* mice, Het mice had an increase in relative abundance of *Actinobacteria* from days 7 to 35 (Figure 9A). This leptin-dependent increase in *Actinobacteria* in Hets was due mostly to *Coriobacteriaceae* (family). Similar to *Actinobacteria*, *Coriobacteriaceae* abundance increased in Het mice from days 7 to 35 (Figure 9B), suggesting this microbial family is influenced by leptin or obesity status during HFD.

Discussion

Using leptin-deficient *ob/ob* female mice fed a HFD, we investigated the effects of estradiol and obesity (due to leptin deficiency) on body weight, energy intake, and gut microbiota in the present study. We found that E2 treatment decreases food intake and protects against HFD-induced weight gain in both *ob/ob* (obese) and Het (lean) females. *ob/ob* mice had increased HFD intake and greater body weight, compared to the Het controls. These differences in food intake and body weight between the two genotypes are reflected in their changes in gut microbiota. Both E2 treatment and genotype altered gut microbiota diversity. o*b/ob* female mice exhibited lower species richness, which supports previous studies that link obesity with a reduced gut microbiota diversity in humans and male mice [47-49]. Interestingly, E2 treatment was associated with lower species evenness compared to Veh mice. At the phylum level, E2 treatment slowed down a HFD-induced decrease in *Bacteroidetes* and increase in *Firmicutes* and *Actinobacteria* compared to Veh controls. Leptin also altered the relative abundances of *Bacteroidetes* and *Firmicutes* and profoundly increased *Actinobacteria*. These findings suggest that E2 and leptin modulate gut microbiota to mediate energy regulation during HFD intake in females.

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E2 alters gut microbial community diversity in ob/ob female mice on a HFD

We and others have shown that E2 protects female mice from HFD-induced obesity [10,11,13,50-53]. Estrogens exert protective effects by acting directly on brain [10-12,54], pancreas [55], liver [50,51,53,56], adipose tissue [53], and muscle [57,58] to regulate energy production and utilization. Moreover, long-term E2 treatment improves glucose tolerance and insulin sensitivity and attenuates lipid biosynthesis in liver in *ob/ob* female mice [59]. The present findings provide evidence that the modulation of gut microbiota is another mechanism by which E2 mediates energy homeostasis. While E2 was associated with a decrease in the gut microbial evenness in the current study, an increase in microbial diversity has been reported in cycling rats and E2-treated female mice [37,60]. These differences across studies could be due to fluctuating estrogens and other ovarian hormones in the cycling rats and/or species differences [37], and a much higher dose of estradiol (2.5 mg/day, consistent with levels at pregnancy) used in the mice [60]. Alternatively, while a lower microbial diversity is usually associated with obesity and metabolic disorders [61,62], a change in relative abundance without any changes in microbial richness or evenness can also alter microbial homeostasis [44].

Potential implications of E2-mediated alterations in gut microbiota composition in ob/ob female mice on HFD

E2 attenuated a longitudinal shift in the two major phyla *Bacteroidetes* and *Firmicutes*, compared to the Veh controls. In particular, the order *Bacteroidales*, and its families *S24-7* and *Ruminococcaceae* were positively associated with E2 treatment, whereas relative abundances of *Allobaculum* spp. were decreased. The current and previous studies suggest that various gut microbial communities collectively exert E2-mediated protection against HFD-induced weight gain [37,42].

Functional predictions of microbial taxa in a population are mostly derived from the gene families they express. *S24-7* and *Ruminococcaceae*, both microbial families upregulated by E2 in the current study, produce short chain fatty acids (SCFA; fermented products of dietary fibers) that protect against inflammation [63-65]. In particular, SCFA, including butyrate, maintain a low pH in the gastrointestinal tract and aid in nutrient absorption and pathogen inactivation [66]. In

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support of the important role of *S24-7* and *Ruminococcaceae* in inflammation, both of these families are downregulated in inflammatory conditions, including Crohn's disease, colitis and type I diabetes [67-69]. Taken together, the current findings suggest that E2 provides protection against diet-induced metabolic disorders in females by maintaining healthy levels of microbial populations, such as *S24-7* and *Ruminococcaceae.*

Firmicutes, another phylum that was altered by E2 in the current study, is generally associated with obesity in rodents and humans [70]. Studies in males have shown that a high caloric diet profoundly affects this phylum [29,71,72]. Consistent with these findings in males, in the present study, Veh female mice on a HFD gained weight and had increased levels of *Firmicutes*. However, E2-treated mice resisted this HFD-induced increase, suggesting that modulation of these microbes contributes to the regulation of energy homeostasis by estrogens. *Firmicutes* contain more OTUs that are efficient energy producers than *Bacteroidetes*, leading to increased calorie absorption and weight gain [73]. Additionally, some members of *Firmicutes* promote the formation of lipid droplets resulting in increased fatty acid absorption and weight gain [74]. In the current study, E2 altered relative abundances of *Firmicutes* in both Het and *ob/ob* mice, suggesting that E2 affects this phylum independent of body weight and leptin levels.

Obesity alters gut microbial community diversity in female mice on a HFD

The obese leptin-deficient (*ob/ob*) mice had less diverse microbiota as observed by species richness, suggesting that leptin is required for an enriched microbial ecosystem. In support, obesity is associated with a decreased microbial diversity in humans [47]. Furthermore, a Danish study on men and women found that higher levels of leptin in obese populations were associated with a lower richness of the gut microbial communities, suggesting that optimum levels of leptin are associated with metabolic health and a more diverse gut microbiota [48]. Taken together, the present results indicate that a shift in leptin levels in either direction disrupts metabolic homeostasis and alters microbial homeostasis.

Potential implications of obesity-induced alterations in gut microbiota composition in female mice on a HFD

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While we observed a direct correlation of the relative abundances of *Bacteroidetes* and its family *S24-7* with leptin (in Het mice) at the beginning of the study, by day 23, their levels dropped to even lower levels than *ob/ob* mice, suggesting a pronounced negative effect of HFD on *Bacteroidetes* in Hets. Within *Firmicutes*, *ob/ob* mice had lower relative abundances of *Clostridium* and *Lactobacillus* compared to control Het mice. In support, high leptin levels associate with *Lactobacillales*, the order containing *Lactobacillus* spp., in African-American men [75]. Most *Lactobacilli* spp. are negatively correlated with inflammation and exert their beneficial effects through modulation of the immune system via macrophage and dendritic cell functions [76,77]. These findings suggest that regulation of gut microbiota by leptin is an important mechanism for metabolic homeostasis.

While *ob/ob* mice weighed almost twice that of Het mice in the beginning, the percent weight gain was greater in Veh Hets compared to Veh *ob/ob* mice. Interestingly, the phylum *Actinobacteria* showed a greater relative abundance in Hets compared to *ob/ob* mice, primarily due to an increase in its family *Coriobacteraceae*. *Actinobacteria* is increased in obese humans and is associated with ulcerative colitis [47,62]. More than two-thirds of the obesity-related human gut microbes belong to *Actinobacteria* [47,78]. In particular, *Coriobacteriaceae* have been positively associated with increased cholesterol absorption in hamsters and obese humans [79,80]. A profound increase in the abundance of *Coriobacteriaceae* in Veh Het mice, the group that showed the highest percent weight gain, but not in E2 Hets, suggests this OTU is associated with HFD-induced weight gain.

Possible mechanisms for estradiol- and leptin-regulation of gut microbiota

Estrogens elicit many effects on physiology and behavior by binding to their intracellular and membrane receptors [81-84]. While estrogen receptor-α (ERα) and ERβ have been implicated in the effects of estrogens on metabolism [85-87], ERα appears to be the primary contributor to energy balance [85,88-90]. ERα knock-out mice exhibit increased visceral adiposity, impaired glucose tolerance and elevated insulin levels [90]. Further, systemic activation of ERα, but not ERβ, decreases food intake and body weight in female rats [89]. While the effects of ERα on the gut microbiota have not been investigated directly, a study using ERβ knock-out female mice

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suggests that ERβ influences gut microbiota in a diet-specific manner [91]. In support of this finding, ERβ is expressed in colon epithelial cells [92,93].

Estrogens also act as direct substrates for gut microbiota. For example, microbes with β-glucuronidase and β-glucosidase enzymes, including *Lactobacillus, Bifidobacterium, Clostridium* spp., convert inactive estrogens into their active forms through deconjugation [94-96]. Estrogens can also alter metabolic outcome and immune response through direct actions on gut microbiota. For example, production of lipopolysaccharides (LPS), a component of Gram-negative bacteria, is elevated after 4 weeks of HFD and is associated with obesity and insulin resistance in male mice [97,98]. E2 treatment may protect from HFD-induced metabolic disorders through blocking LPS activation, suggesting the attenuation of microbial LPS production as a mechanism of E2-mediated protection against HFD-induced obesity [99]. Additionally, E2 up-regulates production and activity of intestinal alkaline phosphatase, a protein with protective function in the gut epithelium through attenuation of the production of pro-inflammatory signals in the [42]. Taken together with the present findings, estrogens can alter gut microbiota through multiple direct and indirect mechanisms to protect from metabolic disorders.

The mechanisms by which leptin affects gut microbiota are not well understood. While intestinal epithelium expresses leptin receptors (LepR), ablation of these receptors in male mice does not alter gut microbiota or body weight [100]. In females, however, leptin could act in concert with ERß, which is expressed in the gut epithelium [92,93]. While genotype effects may be primarily dependent on leptin, it is important to note that *ob/ob* mice are obese and Het mice are lean. Obesity is an independent modulator of gut microbiota. Obese and lean twins exhibit differences in gut microbial diversity [47], and the obese phenotype can be transferred to lean recipients through the transplant of gut microbiota from obese donors [29,30]. In addition, female *ob/ob* mice have reduced estrogen levels and are sterile, and thus have altered hormone-dependent development [26,101]. These findings suggest that leptin affects host physiology, including gut microbial homeostasis through multiple mechanisms.

This study provides critical insights into the roles of estradiol and leptin in HFD-induced obesity and gut microbiota. While the identification of taxa that differ between treatments is the first step

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towards defining their functions, many microbes share redundant metabolic functions [61]. Metagenomic analysis, or correlation analysis between gut microbiota and metabolic changes, will provide further important insights on microbial community-specific functions and their effects on metabolism.

Conclusion

This study provides evidence for the function of estrogens in energy homeostasis through novel actions on gut microbiota. Estrogens altered the microbiota community in female mice that were fed a HFD. Furthermore, the use of female *ob/ob* mice that lack leptin suggests a novel function for this adipokine in the regulation of gut microbiota in females. Finally, given that many metabolic and inflammatory bowel diseases are characterized by disturbances in the gut microbiome, understanding the functions of E2 and leptin in modulating gut microbiome in females has important therapeutic applications for women with metabolic disorders.

MATERIALS AND METHODS

Animals

Seven week-old lean heterozygote (Het) and obese *ob/ob* (lacking leptin) mice (n=24/genotype) were purchased from Jackson Laboratory (Bar Harbor, Maine) and kept on a 12:12 light:dark cycle. Het mice, which have a metabolic phenotype similar to wildtype mice as evidenced by normal serum glucose and insulin levels, body temperature and energy expenditure [19], were used as controls [21] to account for genetic background. While phenotypically very similar, Het mice have lower leptin levels than wildtype mice [102]. Mice were ovariectomized (OVX) and subcutaneously implanted with a silastic capsule containing E2 (50µg in 25µl of 5% ethanol/sesame oil) or Veh (5% ethanol/sesame oil) to get the following four experimental groups: control E2, 2) control Veh, 3) *ob/ob* E2, and 4) *ob/ob* Veh. Following surgery, mice were pair-housed with a mouse of the same genotype and treatment.

On day 7, all mice underwent intracranial surgery for the implantation of an intraventricular cannula attached to a subcutaneous osmotic pump filled with bromodeoxyuridine (BrdU), to label newborn neurons for another study. The osmotic pumps were removed after 10 days. On day 35, animals received an intraperitoneal injection of leptin (5 mg/kg) 45 minutes prior to

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being euthanized. Animals that lost weight and/or sustained infection from pump implants or stereotaxic surgery were excluded from the study. All procedures were approved by the Institutional Animal Care and Use Committee of Wellesley College.

Food intake and body weight measurements and fecal sample collection

Following OVX (day 0), all mice were maintained on a standard diet for three days (13.5% calories from fat, Purina, #5001) before switching to a HFD (58.4% calories from fat Harlan Teklad, #03584) on day 4 and maintained on a HFD for the remainder of the study. Throughout the experiment, food intake and body weights were recorded every four days. For cages in which one mouse died, the amount of food eaten by the remaining mouse was doubled to match the amount with cages containing two mice. Fecal samples were collected from the cages on days 4, 7, 15, 23, 31, and 35 and immediately stored at -80°C.

DNA extraction from fecal samples, 16S rDNA sequencing and bioinformatics processing

DNA was extracted from fecal samples using a MoBio PowerSoil® DNA Isolation Kit (Valencia, CA) with minor adjustments to the manufacturer's protocol. A 5-minute incubation with the elution buffer before centrifugation was added to increase the DNA yield. The quality and quantity of the DNA samples were measured using Nanodrop (Thermo Scientific, Waltham, MA). The samples were stored at -20°C until sequencing.

The V3-V4 region of the 16S rDNA was amplified in all samples using the following universal 16S rDNA primers: forward 341F (5'-CCTACGGGAGGCAGCAG-3') and reverse 806R (5'-GGACTACHVGGGTWTCTAAT-3') with sequence adapters on both primers and sample-specific Golay barcodes on the reverse primer [103]. The PCR products were quantified by PicoGreen (Invitrogen, Carlsbad, CA) using a plate reader. After quantification, amplicons were pooled in equal concentrations, cleaned up using UltraClean PCR Clean-Up Kit (MoBio, Carlsbad, CA), and again quantified using the Qubit (Invitrogen, Carlsbad, CA). The pooled samples were then sequenced using paired-end v2 chemistry using Illumina Miseq sequencing technology (Illumina, San Diego, CA) at the Microbiome Core, Mayo Clinic (Rochester, Minnesota).

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Paired R1 and R2 sequence reads were then processed via the *hybrid-denovo* bioinformatics pipeline, which clustered a mixture of good-quality paired-end and single-end reads into operational taxonomic units (OTUs) at 97% similarity level [104]. OTUs were assigned taxonomy using the RDP classifier trained on the GreenGenes database (v13.5) [105]. A phylogenetic tree based on FastTree algorithm was constructed based on the OTU representative sequences [106]. Singleton OTUs as well as samples with less than 2,000 reads were removed from downstream analysis.

Statistical analysis

Food intake and weight data analysis

Repeated measures ANOVAs (SPSS, v.24) were performed to examine the effects of treatment and genotype on food intake and body weight over time. After a main effect was confirmed, ANOVA without corrections was performed on measures from each day to identify the days when an effect of one or both variables were present. One–way ANOVAs were then conducted to identify differences between specific groups when effects of treatment or genotype were present. Differences were considered statistically significant at $p < 0.05$.

16S rDNA sequence analysis

Analyses were first performed on the aggregated data, in which sequence reads from all samples from the same mice across days were aggregated. To study specific longitudinal trends, stratified analyses on individual days were also performed if needed.

Diversity analysis

Both α-diversity and β-diversity were analyzed on the rarefied OTU data. α-diversity (within-sample diversity) reflects species richness and evenness within the microbial populations. Two representative α-diversity measures were investigated: the observed number of OTUs, an index of the species richness, and the Pielou's evenness index [107]. A multiple linear regression model ("lm" function in R) was used to test the association between α-diversity (outcome) and treatment/genotype (covariates, both included in the model).

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β-diversity (between-sample diversity) reflects the shared diversity between bacterial populations in terms of ecological distances; pair-wise distance measure allows quantification of the overall compositional difference between samples. Different β-diversity measures provide distinctive views of the community structure. The β-diversity measures were calculated using Bray-Curtis Dissimilarity, which measures differences in bacterial composition based on taxa abundances ("vegdist" function in the R "vegan" package, v2.4-3). To test the association between β-diversity measures and treatment or genotype, we used PERMANOVA (999 permutations, "adonis" function in the R "vegan" package, v2.4-3) when adjusting the effect of the other covariate. Ordination plots were generated using principal coordinate analysis (PCoA) on the distance matrix ("cmdscale" function in R) [108].

Taxa level analysis

Differential abundance analyses were performed at the phylum, class, order, family and genus levels. Taxa with prevalence less than 10% or with a maximum proportion less than 0.2% were excluded from analysis to reduce the number of tests. An overdispersed Poisson model was fitted to the taxa counts with treatment and genotype as covariates ("glm" function in R) [109]. Wald test was used to assess significance. To account for variable sequencing depths, the GMPR size factor was estimated and used as an offset (log scale) in the regression model [110]. False discovery rate (FDR) control (B-H procedure, 'p.adjust' in R) was used to correct for multiple testing at each taxonomical level, and FDR-adjusted p-values (q-values) < 0.1 were considered significant [111]. The differential taxa were visualized on a cladogram using GraPhlAn [112]. All statistical analyses were performed in R (v. 3.3.2, R Development Core Team).

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Conflicts of Interest: None

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Figure Legends

Figure 1. Estradiol and leptin alter weight gain and food intake in adult female *ob/ob* and control mice. (A) Average weight, (B) percent weight gain and (C) food intake per cage of *ob/ob* and heterozygote control mice with arrow indicating the start of high fat diet. The effect of genotype on average weight (A) and food intake (C) were present on all days. Days with effects of E2 are denoted by * ($p<0.05$). Error bars indicate ±SEM.

Figure 2. *ob/ob* mice have decreased species richness compared to controls, while estradiol treatment decreases species evenness. (A) *ob/ob* genotype is associated with lower species richness as measured by Observed Taxonomic Units. (B) E2 treatment is associated with lower species evenness as measured by the Pielou's evenness index. "a" and "b" indicate groups with different species richness while "c" and "d" indicate groups with different species evenness ($p<0.05$).

Figure 3. Gut microbial communities distinctly cluster as an effect of estradiol treatment and leptin. PCo1 and PCo2 clustering of each group. Bray-Curtis Distance was used to calculate principal coordinates 1 and 2. Each data point represents the microbiota of each mouse aggregated over all days. Ob E = Estradiol-treated *ob/ob*, Ob V = Vehicle *ob/ob*, Het E= Estradiol-treated Het, and Het V= Vehicle Het mice.

Figure 4. Gut microbiota associate with estradiol treatment and genotype at multiple taxa levels. Microbiota community structure at the (A) phylum, (B) family and (C) genus level, separated by treatment and genotype. Data are shown as relative proportion of the taxa identified. Taxa with prevalence of >10% or with a maximum proportion of > 0.2% were included. Ob E =

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Estradiol-treated *ob/ob*, Ob V = Vehicle *ob/ob*, Het E= Estradiol-treated Het, and Het V= Vehicle Het mice.

Figure 5. Estradiol and obesity alter the gut microbial composition. (A) Phyla and lower level taxa associated with estradiol (E2) or vehicle (Veh) treatment. (B) Phyla and lower level taxa associated with *ob/ob* or Het genotype.

Figure 6. Gut microbiota associate with estradiol treatment and obesity at multiple taxa levels. Boxplots showing microbial taxa that are significantly altered by (A) E2 treatment and (B) genotype. Analysis was done on aggregate data from all days. Data are shown as relative proportion of the taxa identified. Taxa with prevalence of >10% or with a maximum proportion of > 0.2% were included.

Figure 7. Estradiol and obesity alter the relative abundances of *Bacteroidetes* (phylum) and *S24-7* (family) following the start of HFD. Relative abundances of the (A) the phylum *Bacteroidetes* and (B) its family *S24-7* over time. * indicates effects of E2 and # indicates effects of genotype (q<0.1).

Figure 8. Estradiol and obesity alter the relative abundances of the phylum *Firmicutes* and its lower taxa. Relative abundances over time of the (A) phylum *Firmicutes and genera* (B) *Clostridium*, (C) *Lactobacillus* and (D) *Coprococcus*. * indicates effects of E2 and # indicates effects of genotype (q<0.1).

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Figure 9. Estradiol and obesity resist increases in the relative abundances of the phylum *Actinobacteria* and its lower taxa. Relative abundances of the (A) phylum *Actinobacteria* and (B) family *Coriobacteriaceae* over time. * indicates effects of E2 and # indicates effects of genotype (q<0.1).

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Figure 1

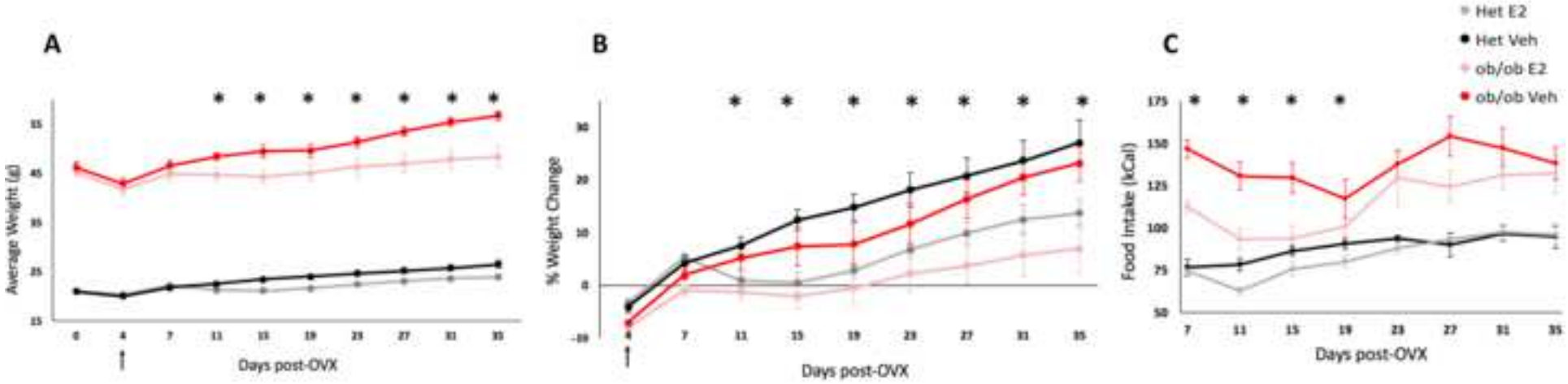

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Figure 2

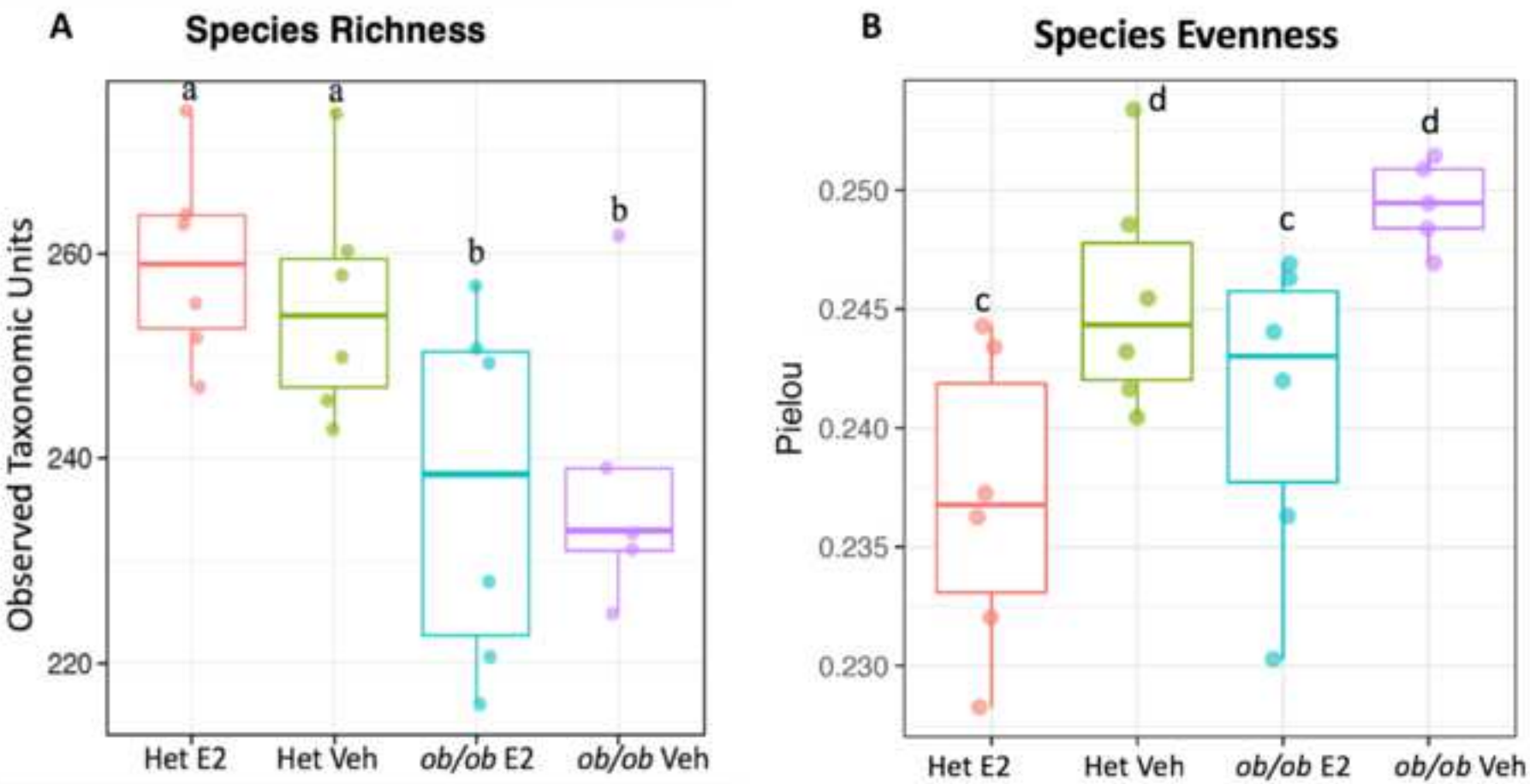

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Figure 3

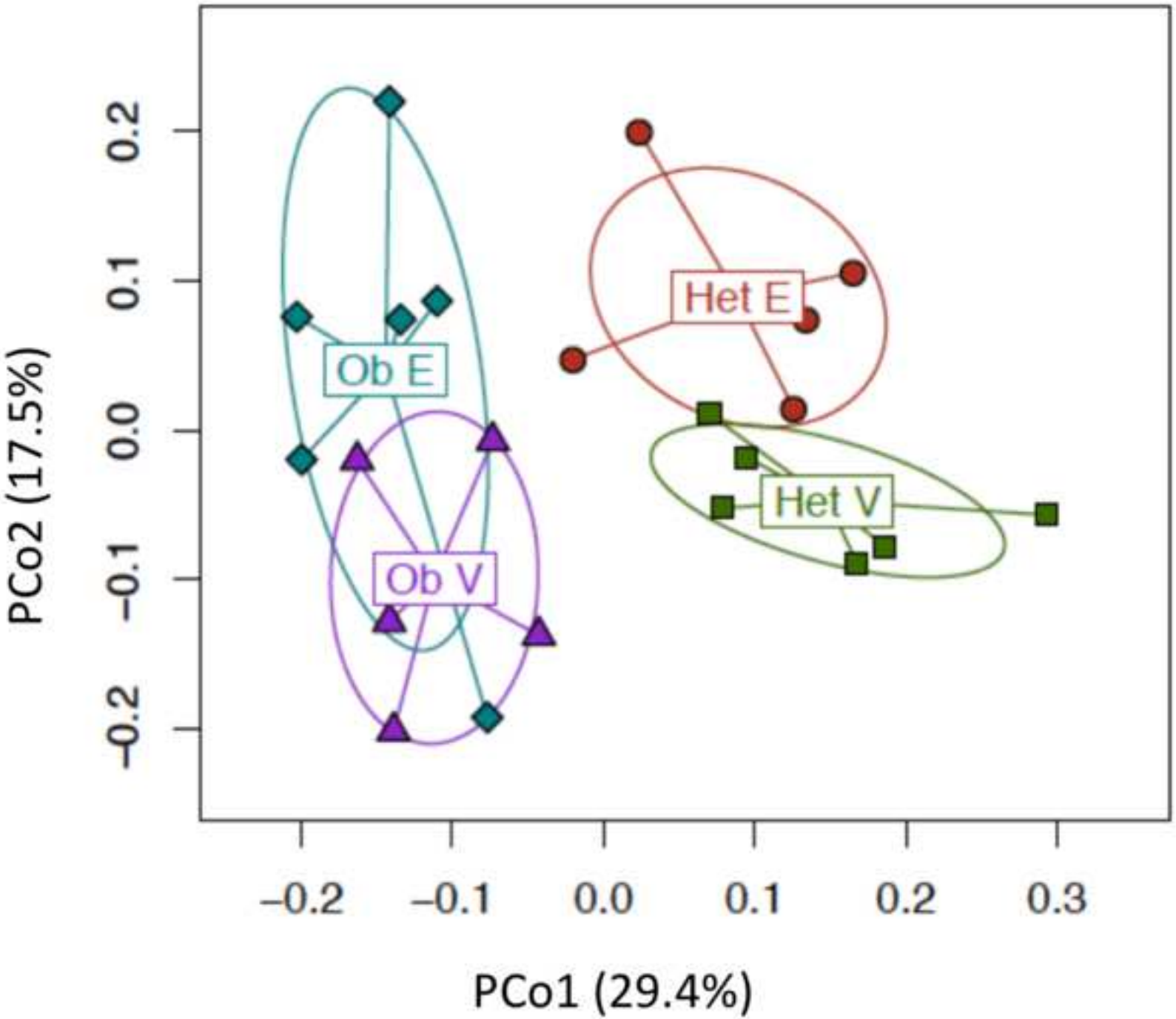

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Figure 4

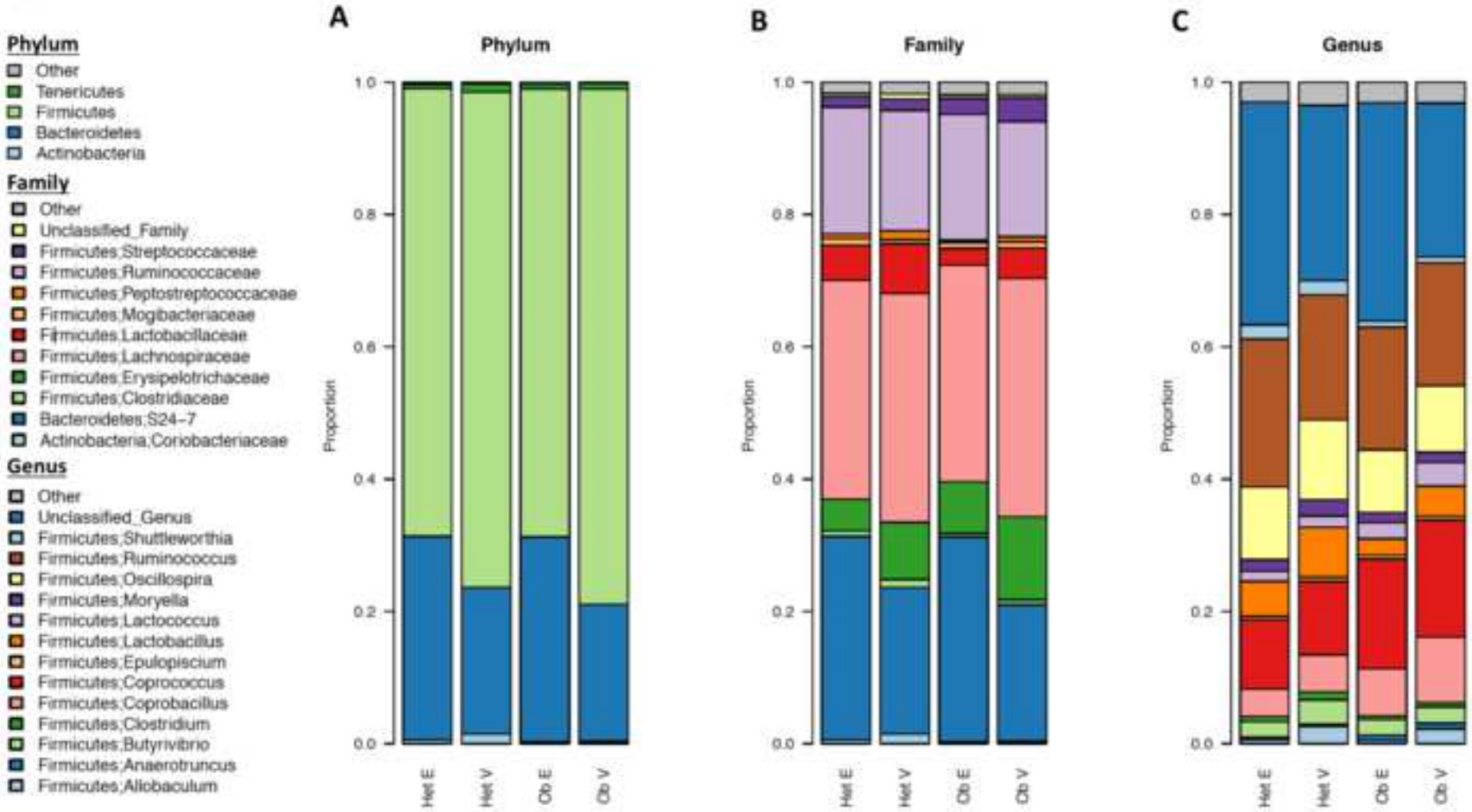

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Figure 5

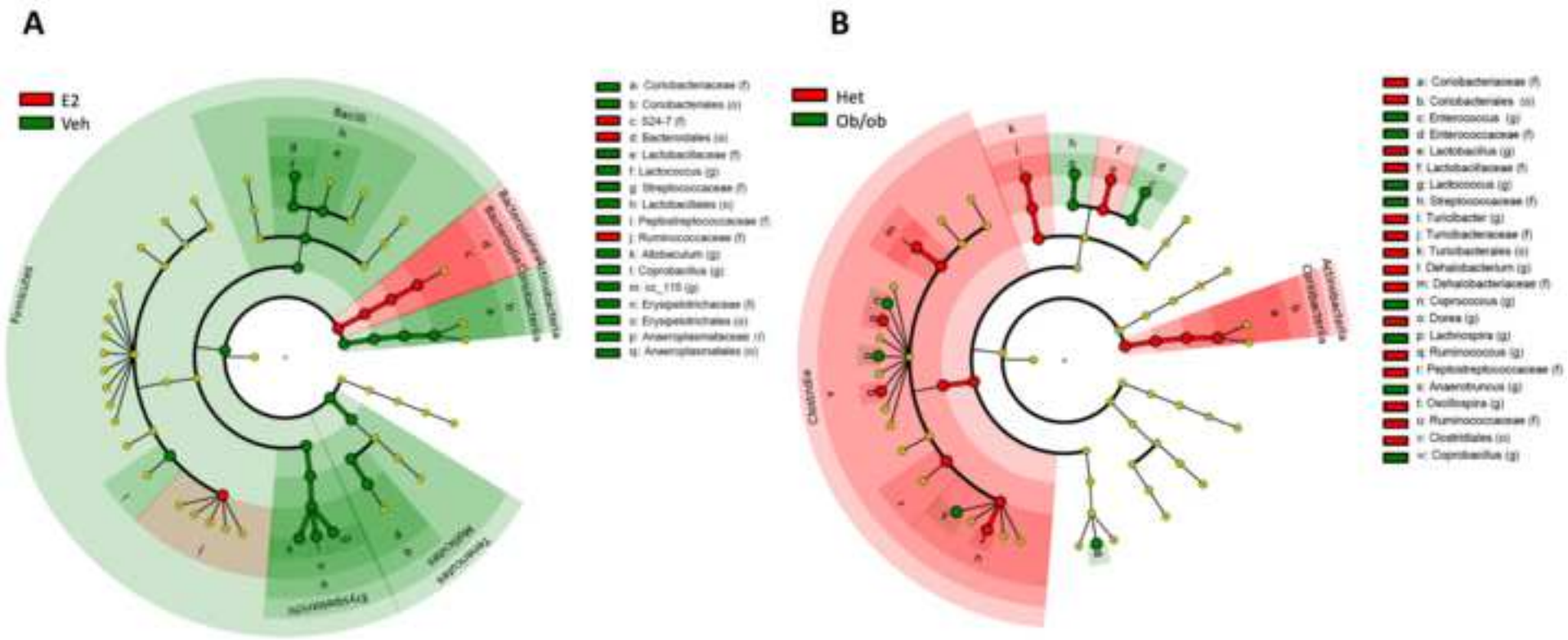

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Figure 6

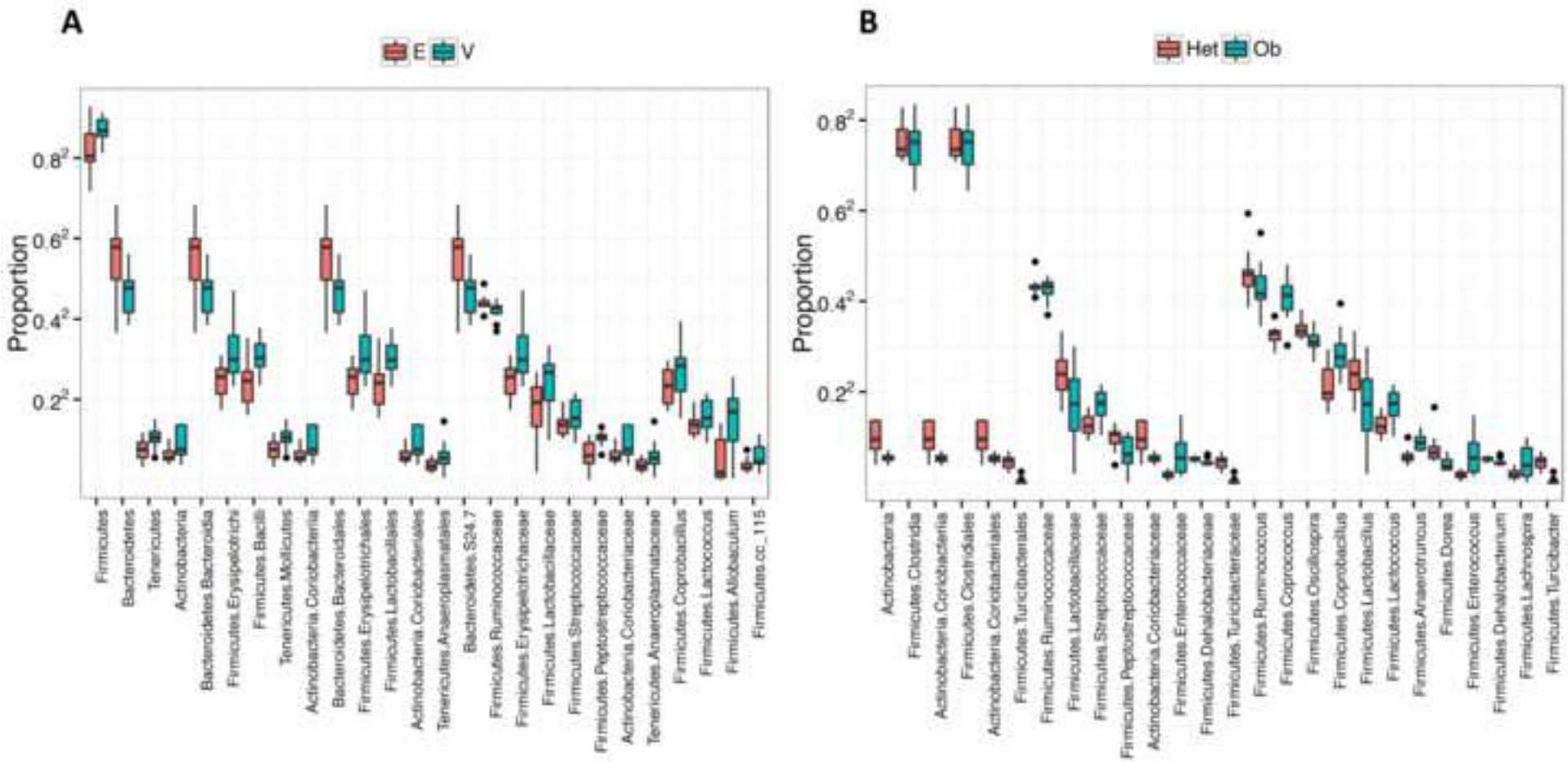

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Figure 7

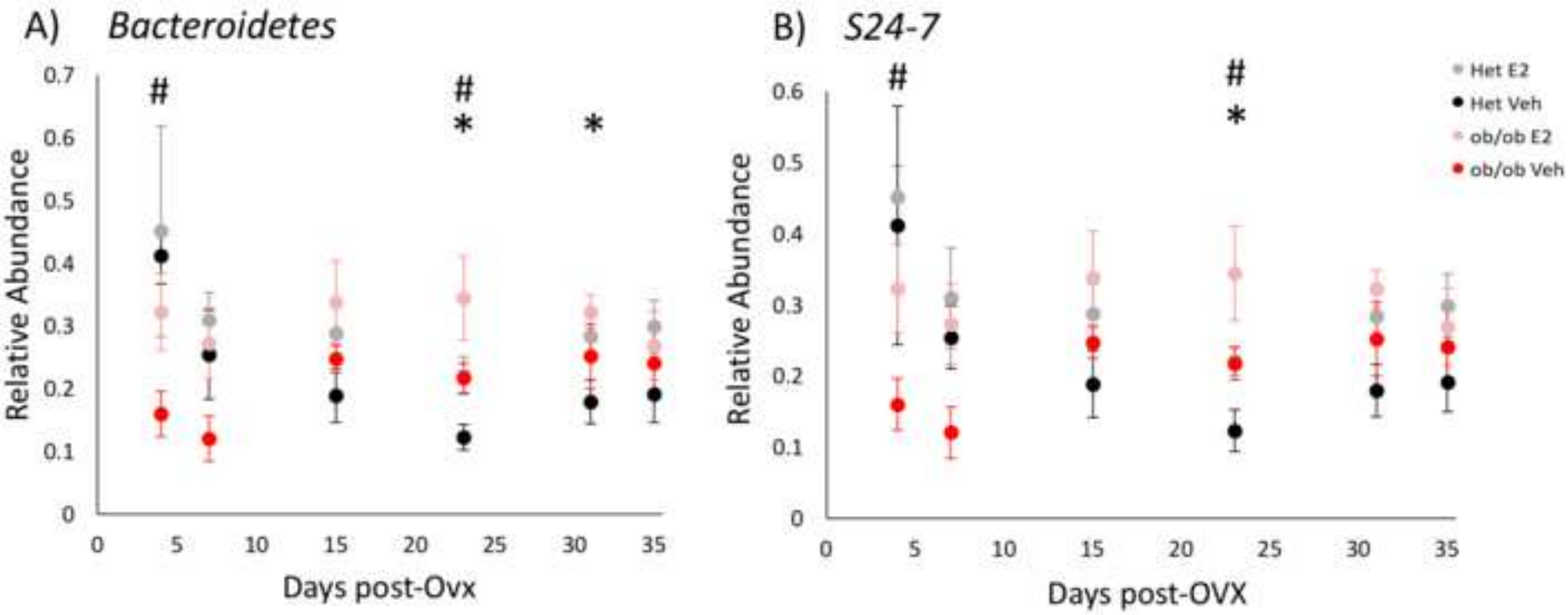

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Figure 8

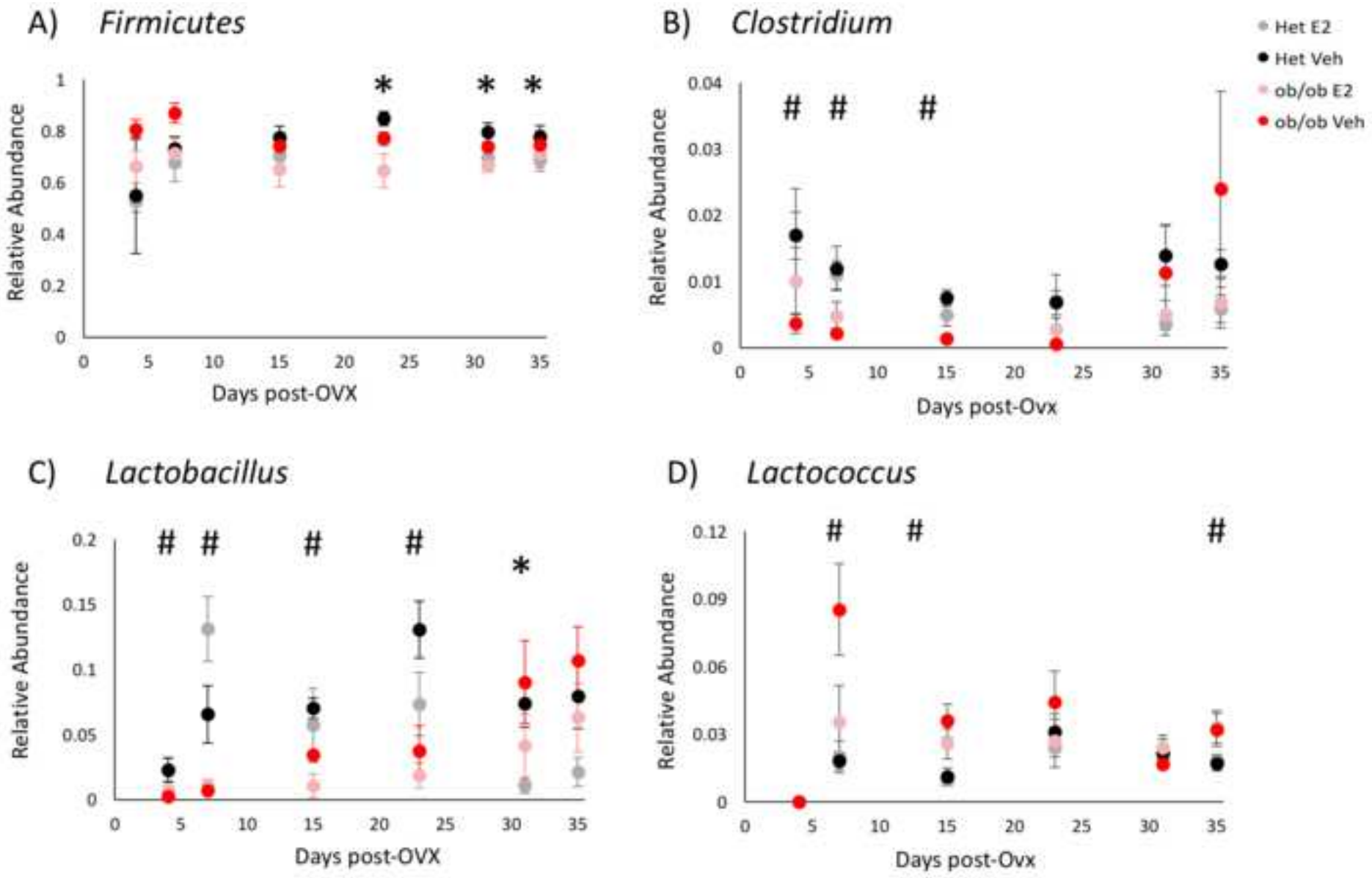

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Figure 9

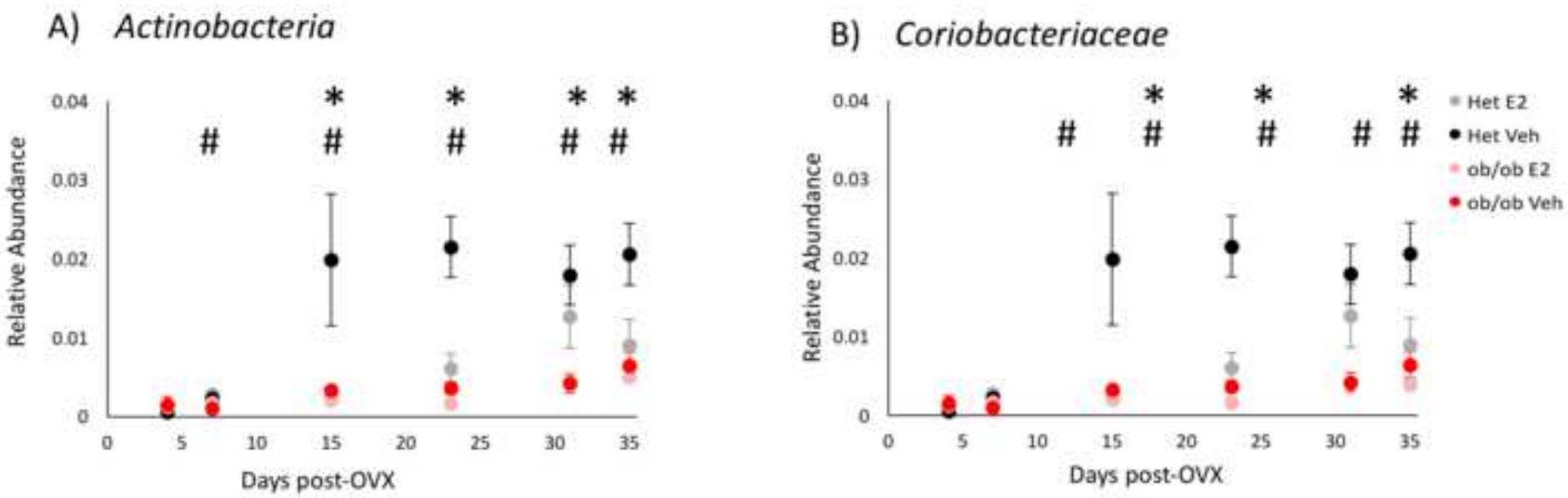

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Table 1

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Table 1. PERMANOVA P-values for individual days based on Bray-Curtis Distance

Day	Day 4	Day 7	Day 15	Day 23	Day 31	Day 35
Treatment	0.095	0.234	0.011*	0.044*	0.023*	0.022*
Genotype	0.081	0.004*	0.009*	0.001*	0.06	0.011*

* indicate a p-value of <0.05.

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Supplemental Table 1: Changes in taxa relative abundances across days as an effect of estradi

	DAY 4				DAY 7	
			Confidence Interval			
	q-value	Fold change (Veh/E2)	2.5%	97.5%	q-value	Fold change (Veh/E2)
Firmicutes	0.719	0.624	-1.066	2.313	0.629	0.049
Bacilli	0.700	-0.298	-0.953	0.358	0.891	-0.098
Bacillales	0.896	-0.208	-0.752	0.336	0.795	-0.273
Bacillaceae	0.997	0.008	-0.495	0.512	0.873	-0.221
Bacillus	0.998	0.008	-0.495	0.512	0.957	-0.219
Lysinibacillus	0.998	-1.049	-4.481	2.383	0.998	-18.618
Planococcaceae	0.997	-1.049	-4.481	2.383	0.998	-18.618
Staphylococcaceae	0.773	-2.093	-5.859	1.673	0.432	-1.926
Staphylococcus	0.811	-2.093	-5.859	1.673	0.448	-1.926
Lactobacillales	0.734	-0.505	-1.314	0.304	0.998	-0.064
Lactobacillaceae	0.773	-0.465	-1.261	0.330	0.343	-0.620
Lactobacillus	0.788	-0.465	-1.261	0.330	0.448	-0.620
Mogibacteriaceae	0.773	-0.430	-1.240	0.380	0.873	0.297
Streptococcaceae	0.773	0.624	-0.300	1.548	0.432	0.533
Lactococcus	0.593	1.661	-0.237	3.559	0.448	0.532
Streptococcus	0.828	0.510	-0.453	1.472	0.448	0.614
Enterococcaceae	0.773	-2.195	-4.817	0.427	0.407	1.621
Enterococcus	0.593	-2.195	-4.817	0.427	0.448	1.621
Clostridia	0.700	0.048	-0.048	0.145	0.757	0.052
Clostridiales	0.844	0.048	-0.048	0.145	0.795	0.052
Epulopiscium	0.659	-0.460	-1.114	0.194	0.957	-0.152
Christensenellaceae	0.997	-0.181	-0.959	0.597	0.432	-1.086
Christensenella	0.998	-0.182	-0.976	0.612	0.448	-1.075
Clostridiaceae	0.997	-0.119	-0.956	0.718	0.873	-0.179
Anaerotruncus	0.998	0.119	-0.396	0.634	0.998	0.094
Faecalibacterium	0.998	19.499	-12900.395	12939.393	0.998	-18.977
Lachnospiraceae	0.773	0.120	-0.031	0.271	0.873	0.054
Anaerostipes	0.998	-0.460	-2.095	1.175	0.448	-2.294
Blautia	0.929	-1.049	-3.276	1.178	0.998	-0.270
Butyrivibrio	0.593	0.361	-0.016	0.738	0.957	0.172
Clostridium	0.998	-0.106	-0.889	0.677	0.957	-0.211
Coprococcus	0.998	0.052	-0.168	0.272	0.885	-0.143
Dorea	0.998	-0.260	-1.011	0.491	0.957	0.176
Lachnospira	0.593	-0.781	-1.706	0.143	0.998	0.182
Moryella	0.998	0.141	-0.465	0.747	0.998	0.087

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Oribacterium	0.593	1.385	0.090	2.680	0.957	-0.276
Roseburia	0.998	-0.708	-2.542	1.126	0.464	-1.550
Shuttleworthia	0.998	-0.043	-0.733	0.647	0.998	0.108
Peptostreptococcace	0.997	-0.211	-2.209	1.786	0.936	0.204
Ruminococcaceae	0.773	-0.114	-0.330	0.101	0.873	0.075
Ruminococcus	0.998	-0.003	-0.182	0.177	0.931	0.105
Ethanoligenens	0.619	-1.290	-2.909	0.329	0.448	0.847
Oscillospira	0.998	-0.008	-0.195	0.180	0.885	0.088
Erysipelotrichi	0.873	-0.156	-0.709	0.398	0.655	0.512
Erysipelotrichales	0.996	-0.156	-0.709	0.398	0.576	0.512
Erysipelotrichaceae	0.997	-0.156	-0.709	0.398	0.454	0.512
Allobaculum	0.998	-0.646	-2.821	1.530	0.885	-0.963
Coprobacillus	0.593	-0.744	-1.590	0.101	0.621	0.433
Turicibacter	0.780	0.640	-0.410	1.690	0.448	-0.709
cc_115	0.641	0.504	-0.166	1.173	0.448	1.227
Turicibacteraceae	0.773	0.640	-0.410	1.690	0.432	-0.709
Turicibacterales	0.734	0.640	-0.410	1.690	0.576	-0.709
Dehalobacteriaceae	0.773	-0.251	-0.592	0.091	0.998	-0.052
Dehalobacterium	0.641	-0.251	-0.592	0.091	0.998	-0.052
Candidatus_Arthrom	0.998	-2.345	-7.870	3.181	0.998	0.453
SMB53	0.998	-19.367	-11221.152	11182.418		
Paenibacillaceae	0.997	-18.935	-7296.780	7258.910		
Paenibacillus	0.998	-18.935	-7296.780	7258.910		
Geobacillus						
Bacteriodetes	0.397	-0.366	-0.897	0.165	0.503	-0.438
Bacteriodia	0.530	-0.366	-0.897	0.165	0.655	-0.439
Bacteroidales	0.734	-0.366	-0.897	0.165	0.576	-0.439
S24-7	0.773	-0.366	-0.897	0.165	0.432	-0.439
Cytophagia	0.996	0.033	-0.955	1.022	0.957	0.156
Cytophagales	0.996	0.033	-0.955	1.022	0.998	0.156
Flammeovirgaceae	0.997	0.033	-0.955	1.022	0.998	0.156
Persicobacter	0.998	0.033	-0.955	1.022	0.998	0.156
Tenericutes	0.371	0.711	-0.091	1.514	0.957	-0.021
Mollicutes	0.530	0.711	-0.091	1.514	0.957	-0.021
Acholeplasmatales	0.996	-18.733	-5100.110	5062.644	0.998	-19.309
Acholeplasmataceae	0.997	-18.733	-5100.110	5062.644	0.998	-19.309
Candidatus_Phytopla	0.998	-18.733	-5100.110	5062.644	0.998	-19.309
Anaeroplasmatales	0.734	0.971	-0.001	1.943	0.652	0.560
Anaeroplasmataceae	0.773	0.971	-0.001	1.943	0.699	0.560
Anaeroplasma	0.593	0.971	-0.001	1.943	0.756	0.560
RF39	0.844	0.421	-0.483	1.324	0.947	-0.238
Unclassified_Family	0.848	0.418	-0.469	1.306	0.873	-0.251
Unclassified_Genus	0.664	-0.345	-0.853	0.163	0.448	-0.407

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Anaeroplasmatales						
Verrucomicrobia	0.371	-1.916	-3.995	0.163	0.503	-5.468
Verrucomicrobiae	0.530	-1.916	-3.995	0.163	0.655	-5.468
Verrucomicrobiales	0.734	-1.916	-3.995	0.163	0.652	-5.468
Verrucomicrobiaceae	0.773	-1.916	-3.995	0.163	0.699	-5.468
Akkermansia	0.593	-1.916	-3.995	0.163	0.756	-5.468
Fusobacteria	0.719	0.624	-1.066	2.313	0.503	1.423
Fusobacteriia	0.783	0.624	-1.066	2.313	0.655	1.423
Fusobacteriales	0.896	0.624	-1.066	2.313	0.652	1.423
Leptotrichiaceae	0.997	0.624	-1.066	2.313	0.699	1.423
Streptobacillus	0.998	0.624	-1.066	2.313	0.756	1.423
Actinobateria	0.836	-0.079	-0.632	0.473	0.629	-0.187
Actinobacteria	0.612	1.653	-1.131	4.437	0.927	-0.357
Actinomycetales	0.734	1.653	-1.131	4.437	0.998	-0.357
Micrococcaceae	0.997	19.577	-7133.763	7172.917	0.998	-18.878
Nesterenkonia	0.998	19.577	-7133.763	7172.917		
Coriobacteriia	0.991	-0.100	-0.663	0.462	0.757	-0.183
Coriobacteriales	0.996	-0.100	-0.663	0.462	0.795	-0.183
Coriobacteriaceae	0.997	-0.100	-0.663	0.462	0.873	-0.183
Adlercreutzia	0.998	-0.106	-0.677	0.466	0.957	-0.169
Nocardiaceae	0.997	19.517	-11426.279	11465.313	0.873	0.841
Rhodococcus	0.998	19.517	-11426.279	11465.313	0.957	0.841
Bifidobacteriales						
Bifidobacteriaceae						
Bifidobacterium						
Atopobium						
Arthrobacter					0.998	-18.878
Microbacteriaceae						
Microbacterium						
Propionibacteriaceae						
Propionibacterium						
Coriobacterium						
Proteobacteria	0.836	-0.317	-1.989	1.355	0.937	0.176
Alphaproteobacteria	0.996	0.053	-1.260	1.366	0.655	-1.088
Rhizobiales	0.996	-0.170	-1.667	1.326	0.576	-1.778
Rhizobiaceae	0.997	-0.170	-1.667	1.326	0.454	-1.778
Agrobacterium	0.998	-0.170	-1.667	1.326	0.472	-1.778
Comamonadaceae	0.997	19.436	-7771.184	7810.056	0.998	0.154
Delftia	0.998	19.201	-7641.235	7679.638	0.998	19.611
Betaproteobacteria	0.996	19.436	-7771.184	7810.056	0.957	0.154
Gammaproteobacter	0.530	-1.739	-4.234	0.757	0.891	0.416
Enterobacteriales	0.996	-18.821	-7714.520	7676.878	0.998	0.135
Enterobacteriaceae	0.997	-18.821	-7714.520	7676.878	0.998	0.135

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Klebsiella	0.998	-18.821	-7714.520	7676.878	0.998	0.135
Pseudomonadales	0.996	0.055	-1.856	1.967	0.998	20.243
Pseudomonadaceae	0.997	0.055	-1.856	1.967		
Pseudomonas	0.998	0.055	-1.856	1.967		
Xanthomonadales	0.996	0.055	-1.440	1.550	0.998	-0.233
Xanthomonadaceae	0.997	0.055	-1.440	1.550	0.998	-0.233
Stenotrophomonas	0.998	0.055	-1.440	1.550	0.998	-0.233
Burkholderiales					0.998	0.154
Leptothrix					0.998	-19.494
Moraxellaceae					0.998	21.162
Acinetobacter					0.998	21.162
Caulobacterales						
Caulobacteraceae						
Phenylobacterium						
Bradyrhizobiaceae						
Afipia						
Sphingomonadales						
Sphingomonadaceae						
Sphingomonas						
Rhodobacterales						
Rhodobacteraceae						
Paracoccus						
Deltaproteobacteria						
Desulfovibrionales						
Desulfovibrionaceae						
Desulfovibrio						
Chlorobi	0.836	-0.098	-1.026	0.830	0.503	-1.397
BSV26	0.996	-0.098	-1.026	0.830	0.655	-1.397
BSN164	0.996	-0.098	-1.026	0.830	0.576	-1.397
Cyanobacteria	0.397	0.831	-0.325	1.987	0.503	-1.729
Chloroplast	0.530	0.831	-0.325	1.987	0.655	-1.729
Streptophyta	0.734	0.831	-0.325	1.987	0.652	-1.729
Acidobacteria						
DA052						
Ellin6513						

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ol treatment. Highlighted cells are considered statistically significant at q-values <0.1.

		DAY 15				DAY 23	
Confidence Interval				Confidence Interval			
2.50%	97.5%%	q-value	Fold change (Veh/E2)	2.50%	97.50%	q-value	Fold change (Veh/E2)
-0.090	0.188	0.997	0.001	-0.096	0.099	0.012	0.132
-0.528	0.331	0.129	0.542	0.022	1.062	0.026	0.574
-0.972	0.426	0.834	-0.175	-0.683	0.334	0.536	-0.237
-0.936	0.493	0.976	-0.115	-0.692	0.463	0.997	0.039
-0.934	0.496	0.997	-0.107	-0.687	0.472	0.997	0.033
-6076.192	6038.956	0.997	-0.401	-2.406	1.604	0.997	0.174
-6076.192	6038.956	0.976	-0.401	-2.406	1.604	0.997	0.174
-4.010	0.159	0.675	-0.591	-1.859	0.678	0.187	-1.073
-4.010	0.159	0.803	-0.591	-1.859	0.678	0.254	-1.073
-0.506	0.378	0.185	0.553	0.017	1.089	0.031	0.587
-1.101	-0.140	0.478	0.638	-0.165	1.440	0.187	0.602
-1.101	-0.140	0.532	0.638	-0.165	1.440	0.254	0.602
-0.562	1.155	0.397	-0.420	-0.875	0.036	0.640	-0.259
-0.017	1.084	0.728	0.169	-0.262	0.599	0.316	0.543
-0.019	1.083	0.849	0.169	-0.263	0.600	0.356	0.554
-0.074	1.302	0.997	0.073	-0.535	0.681	0.997	-0.028
0.183	3.059	0.217	1.951	0.266	3.636	0.316	1.034
0.183	3.059	0.357	1.951	0.266	3.636	0.356	1.034
-0.078	0.182	0.241	-0.084	-0.185	0.017	0.912	-0.029
-0.078	0.182	0.345	-0.084	-0.185	0.017	0.996	-0.029
-0.717	0.413	0.997	0.048	-0.179	0.276	0.946	-0.075
-2.273	0.101	0.997	-0.042	-0.743	0.659	0.316	0.640
-2.307	0.157	0.997	-0.101	-0.834	0.632	0.467	0.688
-0.803	0.445	0.675	0.270	-0.278	0.818	0.997	-0.030
-0.442	0.630	0.997	0.017	-0.506	0.541	0.997	-0.015
-6055.877	6017.923	0.894	-4.778	-18.216	8.660	0.997	-18.923
-0.139	0.247	0.714	-0.062	-0.208	0.085	0.997	-0.010
-4.762	0.173	0.997	0.307	-1.508	2.121	0.997	0.042
-1.848	1.308	0.668	0.997	-0.538	2.531	0.798	1.700
-0.309	0.653	0.532	0.359	-0.116	0.834	0.488	0.329
-0.833	0.412	0.803	0.246	-0.288	0.779	0.997	-0.043
-0.463	0.178	0.532	0.234	-0.062	0.530	0.379	-0.232
-0.411	0.762	0.532	0.592	-0.087	1.272	0.356	0.544
-1.168	1.532	0.532	3.175	-0.845	7.195	0.826	-1.072
-0.387	0.560	0.532	0.452	-0.114	1.019	0.997	-0.100

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-1.317	0.765	0.997	-0.084	-0.912	0.743	0.826	-0.505
-3.510	0.410	0.997	-20.043	-9914.922	9874.837	0.997	0.183
-0.756	0.972	0.997	-0.104	-1.710	1.502	0.798	0.547
-0.692	1.101	0.570	0.518	-0.281	1.317	0.369	0.554
-0.102	0.251	0.406	-0.131	-0.282	0.019	0.471	-0.073
-0.154	0.365	0.035	-0.384	-0.608	-0.161	0.997	-0.052
-0.172	1.866	0.849	-0.312	-1.058	0.433	0.997	0.113
-0.111	0.288	0.803	0.078	-0.082	0.237	0.997	0.046
-0.161	1.186	0.262	0.243	-0.103	0.589	0.004	0.565
-0.161	1.186	0.421	0.243	-0.103	0.589	0.005	0.565
-0.161	1.186	0.524	0.243	-0.103	0.589	0.007	0.565
-3.020	1.094	0.997	19.182	-9289.834	9328.199	0.254	2.487
-0.213	1.078	0.803	0.194	-0.177	0.565	0.356	0.296
-1.543	0.125	0.997	22.127	-7527.969	7572.223	0.997	20.695
-0.070	2.524	0.408	0.944	0.064	1.824	0.997	0.514
-1.543	0.125	0.997	22.127	-7527.969	7572.223	0.997	20.695
-1.543	0.125	0.997	22.127	-7527.969	7572.223	0.997	20.695
-0.397	0.292	0.670	-0.192	-0.545	0.161	0.337	-0.205
-0.397	0.292	0.803	-0.192	-0.545	0.161	0.405	-0.205
-2.107	3.013						
		0.997	19.311	-7453.486	7492.108	0.997	19.792
		0.997	-19.491	-8329.763	8290.780	0.997	18.642
-0.962	0.085	0.103	-0.402	-0.775	-0.030	0.007	-0.635
-0.962	0.085	0.129	-0.402	-0.775	-0.030	0.008	-0.635
-0.962	0.085	0.185	-0.402	-0.775	-0.030	0.010	-0.635
-0.962	0.085	0.239	-0.402	-0.775	-0.030	0.015	-0.635
-1.453	1.765					0.997	19.670
-1.453	1.765					0.997	19.670
-1.453	1.765					0.997	19.670
-1.453	1.765					0.997	19.670
-0.791	0.748	0.058	1.274	0.271	2.278	0.256	-0.674
-0.791	0.748	0.089	1.274	0.271	2.278	0.359	-0.674
-13323.065	13284.447						
-13323.065	13284.447						
-13323.065	13284.447						
-0.434	1.554	0.490	2.279	-1.564	6.121		
-0.434	1.554	0.624	2.279	-1.564	6.121		
-0.434	1.554	0.752	2.279	-1.564	6.121		
-1.094	0.618	0.132	1.272	0.266	2.277	0.436	-0.674
-1.107	0.604	0.189	1.249	0.258	2.241	0.337	-0.677
-0.902	0.088	0.453	-0.326	-0.650	-0.001	0.182	-0.506

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-15.375	4.440	0.276	-0.800	-1.898	0.298	0.933	0.102
-15.375	4.440	0.262	-0.800	-1.898	0.298	0.997	0.102
-15.375	4.440	0.421	-0.800	-1.898	0.298	0.997	0.102
-15.375	4.440	0.524	-0.800	-1.898	0.298	0.997	0.102
-15.375	4.440	0.543	-0.800	-1.898	0.298	0.997	0.102
-1.043	3.890	0.540	1.303	-1.487	4.093	0.997	20.189
-1.043	3.890	0.458	1.303	-1.487	4.093	0.997	20.189
-1.043	3.890	0.655	1.303	-1.487	4.093	0.997	20.189
-1.043	3.890	0.675	1.303	-1.487	4.093	0.997	20.189
-1.043	3.890	0.803	1.303	-1.487	4.093	0.997	20.189
-0.669	0.295	0.000	1.440	0.778	2.101	0.000	1.275
-2.480	1.766	0.798	0.248	-0.944	1.439	0.912	0.468
-2.480	1.766	0.997	-0.163	-1.454	1.127	0.996	0.468
-7695.698	7657.942	0.867	-0.444	-1.926	1.039	0.978	-0.503
		0.849	-1.032	-3.648	1.584		
-0.661	0.296	0.000	1.444	0.780	2.108	0.000	1.277
-0.661	0.296	0.000	1.444	0.780	2.108	0.000	1.277
-0.661	0.296	0.001	1.444	0.780	2.108	0.000	1.277
-0.667	0.330	0.338	0.541	0.106	0.976	0.254	0.559
-2.152	3.834					0.997	19.657
-2.152	3.834					0.997	19.657
		0.997	20.472	-7326.518	7367.462		
		0.997	20.472	-7326.518	7367.462		
		0.997	20.472	-7326.518	7367.462		
		0.532	1.477	-0.470	3.423	0.356	0.883
-7695.698	7657.942	0.997	0.067	-2.206	2.341	0.997	-20.059
						0.254	1.981
-1.460	1.812	0.734	-0.420	-1.874	1.033	0.423	-0.843
-3.172	0.995	0.262	1.144	-0.477	2.765	0.997	-0.293
-4.099	0.544	0.997	18.883	-5515.701	5553.467	0.997	-0.381
-4.099	0.544	0.997	18.883	-5515.701	5553.467	0.997	-0.381
-4.099	0.544	0.997	18.883	-5515.701	5553.467	0.997	-0.381
-2.671	2.979	0.997	20.821	-9561.355	9602.996	0.997	0.169
-8535.288	8574.510	0.997	19.607	-6748.532	6787.746		
-2.671	2.979	0.997	20.821	-9561.355	9602.996	0.997	0.169
-1.391	2.223	0.280	-1.800	-4.553	0.954	0.641	-1.329
-1.789	2.059	0.997	-18.964	-7645.230	7607.303	0.536	-2.427
-1.789	2.059	0.997	-18.964	-7645.230	7607.303	0.471	-2.427

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-1.789	2.059	0.997	-18.964	-7645.230	7607.303	0.577	-2.427
-8057.879	8098.366	0.456	1.189	-0.650	3.028	0.997	20.530
		0.997	20.380	-11082.623	11123.382	0.997	19.657
		0.997	20.380	-11082.623	11123.382	0.997	19.657
-1.949	1.483						
-1.949	1.483						
-1.949	1.483						
-2.671	2.979	0.997	20.821	-9561.355	9602.996	0.997	0.169
-11990.017	11951.029						
-11990.404	12032.727						
-11990.404	12032.727						
		0.997	0.045	-2.327	2.416		
		0.997	0.045	-2.327	2.416		
		0.997	0.045	-2.327	2.416		
-3.239	0.445	0.104	-1.338	-2.653	-0.023	0.430	-0.739
-3.239	0.445	0.129	-1.338	-2.653	-0.023	0.646	-0.739
-3.239	0.445	0.185	-1.338	-2.653	-0.023	0.686	-0.739
-4.834	1.376						
-4.834	1.376						
-4.834	1.376						
		0.997	-19.520	-10683.960	10644.920		
		0.997	-19.520	-10683.960	10644.920		
		0.997	-19.520	-10683.960	10644.920		

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		DAY 31				DAY 35	
Confidence Interval				Confidence Interval			
2.50%	97.50%	q-value	Fold change (Veh/E2)	2.50%	97.50%	q value	Fold change (Veh/E2)
0.041	0.223	0.060	0.215	0.057	0.372	0.042	0.183
0.154	0.994	0.005	0.987	0.415	1.559	0.029	0.761
-0.616	0.142	0.005	0.626	0.260	0.992	0.915	-0.287
-0.474	0.552	0.066	0.502	0.126	0.878	0.998	-0.039
-0.476	0.542	0.126	0.502	0.126	0.878	0.998	-0.044
-1.741	2.089	0.997	-0.091	-1.488	1.306	0.998	-0.173
-1.741	2.089	0.997	-0.091	-1.488	1.306	0.998	-0.173
-2.033	-0.114	0.243	1.267	-0.194	2.727	0.152	-1.596
-2.033	-0.114	0.389	1.267	-0.194	2.727	0.300	-1.596
0.158	1.016	0.005	0.993	0.410	1.576	0.038	0.780
0.044	1.161	0.021	1.392	0.507	2.278	0.038	1.069
0.044	1.161	0.050	1.392	0.507	2.278	0.173	1.069
-0.784	0.265	0.997	-0.107	-0.540	0.326	0.722	-0.254
-0.051	1.138	0.982	0.161	-0.302	0.624	0.995	0.117
-0.050	1.158	0.961	0.164	-0.303	0.630	0.998	0.121
-0.492	0.437	0.997	-0.054	-0.482	0.375	0.998	-0.131
-0.153	2.221	0.368	0.911	-0.321	2.143	0.998	-0.084
-0.153	2.221	0.551	0.911	-0.321	2.143	0.998	-0.084
-0.133	0.075	0.938	-0.005	-0.140	0.129	0.791	-0.042
-0.133	0.075	0.997	-0.005	-0.140	0.129	0.915	-0.042
-0.269	0.120	0.389	0.196	-0.024	0.416	0.629	0.170
-0.109	1.389	0.982	0.314	-0.651	1.278	0.998	0.229
-0.246	1.621	0.994	0.296	-0.860	1.452	0.998	0.178
-1.078	1.017	0.080	1.078	0.201	1.955	0.081	1.085
-0.492	0.463	0.826	-0.303	-0.887	0.281	0.537	-0.356
-6494.104	6456.259	0.997	-18.353	-5614.412	5577.706	0.998	-4.213
-0.171	0.151	0.997	0.047	-0.150	0.243	0.998	-0.032
-1.377	1.462	0.994	-0.736	-3.106	1.635	0.998	0.067
-1.573	4.974	0.997	0.239	-1.273	1.750	0.537	1.135
-0.137	0.794	0.126	0.468	0.098	0.838	0.998	0.229
-1.102	1.016	0.131	1.075	0.197	1.953	0.173	1.085
-0.512	0.047	0.961	0.132	-0.223	0.487	0.998	-0.006
-0.083	1.172	0.997	0.022	-0.857	0.901	0.998	0.312
-3.380	1.236	0.796	0.928	-0.705	2.560	0.998	-0.383
-0.675	0.475	0.994	0.168	-0.437	0.772	0.335	0.539

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-1.621	0.611	0.961	-0.387	-1.331	0.558	0.537	-2.365
-1.666	2.031	0.997	-18.973	-7057.403	7019.458	0.998	-0.883
-0.546	1.641	0.997	0.096	-0.810	1.002	0.998	-0.232
-0.201	1.309	0.000	1.103	0.643	1.563	0.152	0.866
-0.190	0.045	0.080	-0.204	-0.367	-0.041	0.031	-0.208
-0.301	0.196	0.551	-0.186	-0.448	0.077	0.537	-0.229
-0.766	0.992	0.961	-0.196	-0.767	0.375	0.998	-0.122
-0.116	0.208	0.994	-0.052	-0.229	0.124	0.998	-0.048
0.245	0.885	0.004	0.721	0.332	1.110	0.022	0.762
0.245	0.885	0.005	0.721	0.332	1.110	0.029	0.762
0.245	0.885	0.004	0.721	0.332	1.110	0.031	0.762
0.237	4.737	0.015	1.886	0.863	2.909	0.173	1.426
-0.028	0.620	0.126	0.463	0.107	0.819	0.173	0.522
-6610.962	6652.352	0.997	20.237	-7582.521	7622.995	0.998	19.121
-1.012	2.041	0.551	-0.527	-1.283	0.229	0.998	0.381
-6610.962	6652.352	0.997	20.237	-7582.521	7622.995	0.998	19.121
-6610.962	6652.352	0.997	20.237	-7582.521	7622.995	0.997	19.121
-0.463	0.053	0.982	-0.110	-0.426	0.206	0.998	-0.101
-0.463	0.053	0.961	-0.110	-0.426	0.206	0.998	-0.101
-8767.607	8807.191	0.389	1.580	-0.176	3.336	0.998	19.910
-6678.268	6715.552					0.998	19.666
-1.032	-0.239	0.089	-0.299	-0.619	0.021	0.664	-0.244
-1.032	-0.239	0.125	-0.299	-0.619	0.021	0.791	-0.244
-1.032	-0.239	0.143	-0.299	-0.619	0.021	0.915	-0.244
-1.032	-0.239	0.210	-0.299	-0.619	0.021	0.767	-0.244
-9463.821	9503.160						
-9463.821	9503.160						
-9463.821	9503.160						
-9463.821	9503.160						
-1.543	0.194	0.089	0.868	-0.054	1.789	0.664	0.520
-1.543	0.194	0.125	0.868	-0.054	1.789	0.791	0.520
-1.543	0.194	0.143	0.868	-0.054	1.789	0.915	0.520
-1.541	0.188	0.210	0.838	-0.068	1.744	0.808	0.516
-0.852	-0.160	0.551	-0.186	-0.447	0.074	0.998	-0.145

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-0.762	0.966	0.406	-0.412	-1.287	0.462	0.664	-0.391
-0.762	0.966	0.513	-0.412	-1.287	0.462	0.791	-0.391
-0.762	0.966	0.604	-0.412	-1.287	0.462	0.915	-0.391
-0.762	0.966	0.820	-0.412	-1.287	0.462	0.995	-0.391
-0.762	0.966	0.853	-0.412	-1.287	0.462	0.998	-0.391
-10543.363	10583.741	0.722	-0.475	-3.089	2.139	0.997	20.406
-10543.363	10583.741	0.848	-0.475	-3.089	2.139	0.997	20.406
-10543.363	10583.741	0.997	-0.475	-3.089	2.139	0.997	20.406
-10543.363	10583.741	0.997	-0.475	-3.089	2.139	0.998	20.406
-10543.363	10583.741	0.997	-0.475	-3.089	2.139	0.998	20.406
0.777	1.773	0.089	0.518	-0.001	1.038	0.032	0.802
-1.186	2.123	0.362	-1.482	-3.864	0.899	0.791	-1.630
-1.186	2.123	0.420	-1.482	-3.864	0.899	0.915	-1.630
-2.126	1.119	0.997	-18.740	-5415.071	5377.592	0.767	1.317
						0.998	0.293
0.776	1.777	0.125	0.525	0.002	1.048	0.022	0.851
0.776	1.777	0.143	0.525	0.002	1.048	0.029	0.851
0.776	1.777	0.192	0.525	0.002	1.048	0.031	0.851
0.098	1.021	0.796	0.219	-0.180	0.619	0.537	0.468
-8194.362	8233.675	0.997	19.449	-11011.398	11050.296		
-8194.362	8233.675	0.997	19.449	-11011.398	11050.296		
-0.126	1.892	0.994	0.358	-1.062	1.777	0.998	0.146
-8744.604	8704.486	0.997	-18.740	-5415.071	5377.592	0.173	1.827
		0.997	-19.936	-11921.968	11882.097		
		0.997	-19.936	-11921.968	11882.097		
		0.997	-19.167	-8120.994	8082.661	0.998	-19.335
		0.997	-19.167	-8120.994	8082.661	0.998	-19.335
0.208	3.755	0.389	0.917	-0.117	1.952	0.537	1.245
-2.324	0.638	0.089	-1.425	-2.713	-0.138	0.771	-0.306
-2.119	1.532	0.125	-1.510	-3.114	0.094	0.888	-0.275
-2.561	1.798	0.997	-0.353	-2.039	1.333	0.997	0.244
-2.561	1.798	0.997	0.044	-1.847	1.935	0.722	1.235
-2.561	1.798	0.997	0.044	-1.847	1.935	0.664	1.235
-1.849	2.186	0.997	-0.257	-2.079	1.565	0.998	-0.143
		0.853	1.364	-1.494	4.221	0.998	0.441
-1.849	2.186	0.848	-0.257	-2.079	1.565	0.997	-0.143
-3.715	1.057	0.637	-0.854	-3.279	1.571	0.888	-0.455
-6.221	1.367	0.997	-19.702	-9799.957	9760.553	0.997	-0.496
-6.221	1.367	0.997	-19.702	-9799.957	9760.553	0.998	-0.496

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-6.221	1.367	0.997	-19.702	-9799.957	9760.553	0.998	-0.496
-9122.354	9163.414	0.997	18.954	-6406.095	6444.003		
-8194.362	8233.675	0.997	18.954	-6406.095	6444.003		
-8194.362	8233.675	0.997	18.954	-6406.095	6444.003		
						0.997	0.441
						0.998	0.441
						0.998	0.441
-1.849	2.186	0.997	-0.257	-2.079	1.565	0.997	-0.143
		0.997	-19.295	-10015.386	9976.796	0.998	-0.260
		0.997	-19.481	-9593.444	9554.482	0.998	-18.928
		0.997	-19.481	-9593.444	9554.482	0.998	-18.928
		0.143	-2.395	-4.802	0.012		
		0.192	-2.395	-4.802	0.012		
		0.997	18.954	-6406.095	6444.003		
						0.997	-19.420
						0.998	-19.420
						0.998	-19.420
						0.997	19.486
						0.997	19.486
						0.998	19.486
						0.998	19.486
-2.203	0.726	0.089	-1.410	-2.876	0.055	0.664	-0.529
-2.203	0.726	0.125	-1.410	-2.876	0.055	0.791	-0.529
-2.203	0.726	0.143	-1.410	-2.876	0.055	0.915	-0.529

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Confidence Interval	
2.50%	**97.50%**
0.043	0.324
0.217	1.306
-0.830	0.256
-0.635	0.557
-0.641	0.553
-2.959	2.613
-2.959	2.613
-3.094	-0.099
-3.094	-0.099
0.219	1.340
0.319	1.818
0.319	1.818
-0.664	0.156
-0.208	0.443
-0.208	0.450
-0.624	0.362
-1.817	1.649
-1.817	1.649
-0.165	0.082
-0.165	0.082
-0.078	0.419
-1.018	1.477
-1.281	1.637
0.220	1.950
-0.832	0.120
-13.077	4.651
-0.219	0.154
-2.083	2.217
-0.318	2.588
-0.247	0.705
0.220	1.950
-0.290	0.278
-0.518	1.141
-2.200	1.434
0.005	1.074

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-5.322	0.592
-3.708	1.942
-1.167	0.703
0.058	1.674
-0.343	-0.072
-0.533	0.075
-0.989	0.745
-0.161	0.065
0.273	1.250
0.273	1.250
0.273	1.250
0.373	2.479
0.093	0.950
-6308.088	6346.330
-0.453	1.216
-6308.088	6346.330
-6308.088	6346.330
-0.406	0.205
-0.406	0.205
-8583.759	8623.578
-10430.797	10470.129
-0.697	0.209
-0.697	0.209
-0.697	0.209
-0.697	0.209
-0.532	1.572
-0.532	1.572
-0.532	1.572
-0.531	1.563
-0.520	0.230

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-1.435	0.654
-1.435	0.654
-1.435	0.654
-1.435	0.654
-1.435	0.654
-11351.938	11392.750
-11351.938	11392.750
-11351.938	11392.750
-11351.938	11392.750
-11351.938	11392.750
0.255	1.349
-5.155	1.896
-5.155	1.896
-1.062	3.697
-2.908	3.494
0.286	1.417
0.286	1.417
0.286	1.417
-0.131	1.067
-1.842	2.134
0.318	3.336
-13994.878	13956.208
-13994.878	13956.208
-0.327	2.816
-1.737	1.125
-1.664	1.113
-1.438	1.926
-0.667	3.138
-0.667	3.138
-1.713	1.427
-1.258	2.140
-1.713	1.427
-2.637	1.726
-2.797	1.804
-2.797	1.804

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-2.797	1.804
-1.664	2.546
-1.664	2.546
-1.664	2.546
-1.713	1.427
-1.782	1.263
-8370.841	8332.985
-8370.841	8332.985
-11357.471	11318.632
-11357.471	11318.632
-11357.471	11318.632
-9410.643	9449.615
-9410.643	9449.615
-9410.643	9449.615
-9410.643	9449.615
-2.057	1.000
-2.057	1.000
-2.057	1.000

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Supplemental Table 2: Changes in taxa relative abundances across days as an effect of genotype.

	DAY 4				DAY 7	
			Confidence Interval			
	q-value	Fold change (*obob* /Het)	2.5%	97.5%	q-value	Fold change (*obob* /Het)
Firmicutes	0.239	0.089	-0.015	0.192	0.412	0.065
Bacilli	0.001	-1.637	-2.412	-0.862	0.041	-0.659
Bacillales	0.082	-0.629	-1.172	-0.085	0.831	0.279
Bacillaceae	0.137	-0.529	-1.034	-0.024	0.861	0.291
Bacillus	0.134	-0.529	-1.034	-0.024	0.829	0.289
Lysinibacillus	0.876	0.699	-2.733	4.132	0.998	-0.165
Planococcaceae	0.893	0.699	-2.733	4.132	0.998	-0.165
Staphylococcaceae	0.581	-1.236	-3.760	1.288	0.998	-0.169
Staphylococcus	0.610	-1.236	-3.760	1.288	0.998	-0.169
Lactobacillales	0.012	-1.639	-2.574	-0.704	0.099	-0.594
Lactobacillaceae	0.008	-1.797	-2.765	-0.829	0.000	-2.599
Lactobacillus	0.013	-1.797	-2.765	-0.829	0.000	-2.599
Mogibacteriaceae	0.871	0.245	-0.583	1.074	0.936	0.199
Streptococcaceae	0.997	-0.011	-0.914	0.892	0.017	1.032
Lactococcus	0.427	1.224	-0.674	3.122	0.014	1.038
Streptococcus	0.998	-0.072	-1.022	0.877	0.441	0.506
Enterococcaceae	0.603	0.850	-1.006	2.706	0.162	1.884
Enterococcus	0.643	0.850	-1.006	2.706	0.158	1.884
Clostridia	0.017	0.145	0.045	0.245	0.329	0.100
Clostridiales	0.019	0.145	0.045	0.245	0.472	0.100
Epulopiscium	0.017	1.639	0.648	2.630	0.014	1.137
Christensenellaceae	0.893	-0.182	-0.960	0.595	0.936	-0.227
Christensenella	0.876	-0.171	-0.965	0.623	0.998	-0.220
Clostridiaceae	0.070	-1.089	-1.974	-0.205	0.009	-1.223
Anaerotruncus	0.017	1.071	0.412	1.730	0.014	1.038
Faecalibacterium	0.998	-19.826	-12794.555	12754.902	0.896	-0.501
Lachnospiraceae	0.067	0.202	0.045	0.360	0.092	0.243
Anaerostipes	0.855	-0.424	-2.008	1.161	0.218	-3.451
Blautia	0.816	0.699	-1.528	2.926	0.998	19.297
Butyrivibrio	0.020	-0.570	-0.944	-0.196	0.705	-0.243
Clostridium	0.038	-1.128	-1.962	-0.294	0.013	-1.227
Coprococcus	0.021	0.344	0.109	0.580	0.243	0.287
Dorea	0.141	-0.773	-1.530	-0.017	0.014	-1.011

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Lachnospira	0.042	2.215	0.531	3.898	0.731	0.709
Moryella	0.807	0.210	-0.423	0.844	0.602	0.286
Oribacterium	0.374	0.949	-0.346	2.243	0.998	-0.240
Roseburia	0.795	-0.606	-2.327	1.115	0.998	-0.236
Shuttleworthia	0.486	0.442	-0.308	1.192	0.243	-0.842
Peptostreptococcacea	0.893	-0.402	-2.383	1.580	0.527	-6.278
Ruminococcaceae	0.574	0.118	-0.104	0.340	0.861	-0.071
Ethanoligenens	0.998	-0.064	-1.397	1.270	0.998	-0.190
Oscillospira	0.427	0.129	-0.065	0.323	0.243	-0.176
Ruminococcus	0.017	0.328	0.136	0.520	0.069	0.345
Erysipelotrichi	0.334	0.371	-0.221	0.963	0.240	0.687
Erysipelotrichales	0.355	0.371	-0.221	0.963	0.311	0.687
Erysipelotrichaceae	0.454	0.371	-0.221	0.963	0.240	0.687
Allobaculum	0.952	0.296	-1.880	2.472	0.998	18.991
Coprobacillus	0.855	0.222	-0.600	1.043	0.130	0.804
Turicibacter	0.021	-3.161	-5.293	-1.030	0.998	-20.844
cc_115	0.427	0.456	-0.256	1.168	0.998	0.109
Turicibacteraceae	0.033	-3.161	-5.293	-1.030	0.998	-20.844
Turicibacterales	0.019	-3.161	-5.293	-1.030	0.998	-20.844
Dehalobacteriaceae	0.067	0.469	0.099	0.838	0.936	0.097
Dehalobacterium	0.051	0.469	0.099	0.838	0.998	0.097
SMB53	0.998	19.176	-12109.558	12147.911		
Candidatus_Arthromi	0.795	1.995	-3.531	7.520	0.998	-0.482
Paenibacillaceae	0.317	-1.792	-4.069	0.486		
Paenibacillus	0.340	-1.792	-4.069	0.486		
Geobacillus						
Bacteriodetes	0.039	-0.771	-1.300	-0.242	0.148	-0.556
Bacteriodia	0.017	-0.771	-1.300	-0.242	0.164	-0.556
Bacteroidales	0.019	-0.771	-1.300	-0.242	0.230	-0.556
S24-7	0.033	-0.771	-1.300	-0.242	0.162	-0.556
Cytophagia	0.017	-2.878	-4.733	-1.024	0.740	-0.384
Cytophagales	0.019	-2.878	-4.733	-1.024	0.963	-0.384
Flammeovirgaceae	0.033	-2.878	-4.733	-1.024	0.936	-0.384
Persicobacter	0.020	-2.878	-4.733	-1.024	0.998	-0.384
Tenericutes	0.396	-0.401	-1.161	0.359	0.412	-0.366
Mollicutes	0.410	-0.401	-1.161	0.359	0.499	-0.366
Anaeroplasmatales	0.905	-0.154	-1.037	0.729	0.748	-0.500
Anaeroplasmataceae	0.908	-0.154	-1.037	0.729	0.802	-0.500
Anaeroplasma	0.906	-0.154	-1.037	0.729	0.705	-0.500
RF39	0.165	-0.866	-1.779	0.047	0.832	-0.307
Unclassified_Family	0.198	-0.846	-1.740	0.049	0.903	-0.301

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Unclassified_Genus	0.020	-0.768	-1.275	-0.260	0.130	-0.545
Acholeplasmatales	0.089	-2.485	-4.726	-0.243	0.998	-19.668
Acholeplasmataceae	0.116	-2.485	-4.726	-0.243	0.998	-19.668
Candidatus_Phytoplas	0.108	-2.485	-4.726	-0.243	0.998	-19.668
Fusobacteria	0.239	-4.140	-9.540	1.261	0.412	-3.027
Fusobacteriia	0.249	-4.140	-9.540	1.261	0.329	-3.027
Fusobacteriales	0.254	-4.140	-9.540	1.261	0.518	-3.027
Leptotrichiaceae	0.317	-4.140	-9.540	1.261	0.527	-3.027
Streptobacillus	0.347	-4.140	-9.540	1.261	0.473	-3.027
Actinobateria	0.754	0.124	-0.446	0.694	0.052	-0.690
Actinobacteria	0.826	-0.385	-2.461	1.690	0.899	-0.179
Actinomycetales	0.905	-0.385	-2.461	1.690	0.998	-0.179
Micrococcaceae	0.997	19.137	-7122.826	7161.099	0.861	-0.858
Coriobacteriia	0.811	0.135	-0.446	0.716	0.041	-0.691
Coriobacteriales	0.905	0.135	-0.446	0.716	0.099	-0.691
Coriobacteriaceae	0.893	0.135	-0.446	0.716	0.041	-0.691
Adlercreutzia	0.876	0.136	-0.454	0.726	0.069	-0.647
Nocardiaceae	0.997	-19.850	-11373.377	11333.676	0.998	0.504
Rhodococcus	0.998	-19.850	-11373.377	11333.676	0.998	0.504
Nesterenkonia	0.998	19.137	-7122.826	7161.099		
Coriobacterium						
Arthrobacter					0.788	-0.858
Microbacteriaceae						
Microbacterium						
Propionibacteriaceae						
Propionibacterium						
Bifidobacteriales						
Bifidobacteriaceae						
Bifidobacterium						
Atopobium						
Proteobacteria	0.396	1.109	-1.023	3.240	0.412	0.980
Alphaproteobacteria	0.240	1.804	-0.384	3.992	0.329	-1.426
Rhizobiales	0.285	1.685	-0.682	4.051	0.311	-2.115
Rhizobiaceae	0.361	1.685	-0.682	4.051	0.247	-2.115
Betaproteobacteria	0.977	0.024	-1.591	1.639	0.899	-0.183
Burkholderiales	0.996	0.024	-1.591	1.639	0.998	-0.183
Comamonadaceae	0.997	0.024	-1.591	1.639	0.998	-0.183
Delftia	0.876	-0.382	-2.129	1.366	0.998	19.345
Agrobacterium	0.383	1.685	-0.682	4.051	0.243	-2.115
Gammaproteobacteri	0.334	1.849	-1.125	4.824	0.329	1.829
Enterobacteriales	0.501	1.467	-1.510	4.443	0.998	18.933

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Enterobacteriaceae	0.581	1.467	-1.510	4.443	0.998	18.933
Klebsiella	0.610	1.467	-1.510	4.443	0.998	18.933
Pseudomonadales	0.996	18.062	-5881.765	5917.890	0.998	19.875
Xanthomonadales	0.996	19.086	-7678.454	7716.627	0.998	-19.648
Xanthomonadaceae	0.997	19.086	-7678.454	7716.627	0.998	-19.648
Stenotrophomonas	0.998	19.086	-7678.454	7716.627	0.998	-19.648
Pseudomonadaceae	0.997	18.062	-5881.765	5917.890		
Pseudomonas	0.998	18.062	-5881.765	5917.890		
Moraxellaceae					0.998	20.805
Acinetobacter					0.998	20.805
Leptothrix					0.998	-19.748
Caulobacterales						
Caulobacteraceae						
Phenylobacterium						
Bradyrhizobiaceae						
Afipia						
Sphingomonadales						
Sphingomonadaceae						
Sphingomonas						
Rhodobacterales						
Rhodobacteraceae						
Paracoccus						
Deltaproteobacteria						
Desulfovibrionales						
Desulfovibrionaceae						
Desulfovibrio						
Verrucomicrobia	0.239	2.018	-0.471	4.507	0.589	0.338
Verrucomicrobiae	0.240	2.018	-0.471	4.507	0.737	0.338
Verrucomicrobiales	0.235	2.018	-0.471	4.507	0.952	0.338
Verrucomicrobiaceae	0.316	2.018	-0.471	4.507	0.936	0.338
Akkermansia	0.329	2.018	-0.471	4.507	0.998	0.338
Cyanobacteria	0.239	1.187	-0.237	2.611	0.412	1.217
Chloroplast	0.240	1.187	-0.237	2.611	0.499	1.217
Streptophyta	0.235	1.187	-0.237	2.611	0.769	1.217
Chlorobi	0.823	-0.107	-1.042	0.829	0.412	-0.998
BSV26	0.882	-0.107	-1.042	0.829	0.329	-0.998
BSN164	0.961	-0.107	-1.042	0.829	0.518	-0.998
Acidobacteria						
DA052						
Ellin6513						

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Highlighted cells are considered statistically significant at q-values <0.1.

		DAY 15				DAY 23	
Confidence Interval				Confidence Interval			
2.50%	97.5%%	q-value	Fold change (*obob* /Het)	2.50%	97.50%	q-value	Fold change (*obob* /Het)
-0.075	0.205	0.196	-0.101	-0.199	-0.003	0.000	-0.210
-1.097	-0.221	0.997	-0.105	-0.613	0.402	0.005	-0.752
-0.422	0.981	0.163	-0.585	-1.122	-0.049	0.009	0.607
-0.431	1.014	0.104	-0.691	-1.314	-0.068	0.387	0.374
-0.433	1.012	0.168	-0.697	-1.324	-0.071	0.400	0.375
-1.841	1.511	0.756	-1.078	-3.393	1.237	0.623	1.109
-1.841	1.511	0.675	-1.078	-3.393	1.237	0.617	1.109
-1.479	1.141	0.847	0.320	-0.893	1.534	0.054	1.212
-1.479	1.141	0.870	0.320	-0.893	1.534	0.059	1.212
-1.043	-0.146	0.997	-0.090	-0.612	0.432	0.006	-0.777
-3.414	-1.785	0.190	-0.815	-1.669	0.038	0.001	-1.449
-3.414	-1.785	0.233	-0.815	-1.669	0.038	0.001	-1.449
-0.673	1.071	0.822	-0.134	-0.580	0.313	0.997	0.109
0.388	1.676	0.001	0.909	0.445	1.373	0.997	0.049
0.393	1.683	0.002	0.913	0.448	1.378	1.000	0.051
-0.204	1.216	0.252	0.542	-0.080	1.164	1.000	0.004
0.107	3.662	0.064	2.379	0.443	4.314	0.064	2.553
0.107	3.662	0.120	2.379	0.443	4.314	0.067	2.553
-0.031	0.232	0.000	-0.217	-0.319	-0.114	0.005	-0.175
-0.031	0.232	0.000	-0.217	-0.319	-0.114	0.006	-0.175
0.452	1.821	0.843	0.070	-0.158	0.297	0.011	-0.309
-1.218	0.763	0.225	-0.674	-1.430	0.082	0.387	0.525
-1.251	0.811	0.233	-0.779	-1.585	0.027	0.614	0.484
-1.924	-0.523	0.000	-1.666	-2.441	-0.890	0.190	-1.118
0.399	1.676	0.223	0.552	0.016	1.088	0.004	0.956
-1.914	0.912	0.789	-4.706	-18.144	8.731	1.000	0.269
0.046	0.440	0.000	-0.311	-0.460	-0.161	0.131	-0.170
-7.002	0.100	0.528	-1.630	-4.160	0.899	1.000	-19.392
-7929.271	7967.864	0.236	1.600	-0.172	3.372	0.670	-1.511
-0.725	0.238	0.789	-0.197	-0.672	0.278	1.000	-0.049
-1.924	-0.529	0.000	-1.772	-2.557	-0.987	0.217	-1.142
-0.039	0.613	0.870	0.078	-0.217	0.373	0.000	0.742
-1.648	-0.373	0.233	-0.662	-1.366	0.043	0.083	-0.775

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-0.777	2.196	0.340	-2.539	-5.788	0.711	1.000	-0.207
-0.200	0.773	0.236	-0.530	-1.114	0.054	0.011	-1.020
-1.258	0.778	0.032	-2.279	-3.771	-0.787	0.067	-2.820
-1.651	1.179	0.997	20.159	-9658.541	9698.859	0.871	-0.683
-1.753	0.069	0.789	-0.596	-2.300	1.108	0.257	-1.079
-15.735	3.179	0.030	-1.407	-2.420	-0.394	0.026	-1.359
-0.248	0.106	0.813	-0.049	-0.200	0.101	0.154	-0.116
-1.150	0.771	0.997	0.124	-0.610	0.858	0.614	-0.482
-0.375	0.024	0.233	-0.161	-0.322	0.001	0.004	-0.297
0.076	0.613	0.105	-0.288	-0.511	-0.065	0.010	-0.420
-0.040	1.414	0.000	0.929	0.556	1.302	0.943	0.053
-0.040	1.414	0.000	0.929	0.556	1.302	0.997	0.053
-0.040	1.414	0.000	0.929	0.556	1.302	0.997	0.053
-7817.881	7855.863	0.997	0.111	-2.034	2.256	0.400	-1.087
0.085	1.524	0.000	0.928	0.527	1.329	0.459	0.214
-7102.513	7060.826	0.997	-21.804	-6982.617	6939.010	1.000	-20.300
-1.034	1.251	0.120	1.072	0.182	1.963	1.000	0.323
-7102.513	7060.826	0.997	-21.804	-6982.617	6939.010	0.997	-20.300
-7102.513	7060.826	0.997	-21.804	-6982.617	6939.010	0.997	-20.300
-0.249	0.443	0.621	-0.184	-0.538	0.171	0.013	-0.423
-0.249	0.443	0.680	-0.184	-0.538	0.171	0.010	-0.423
		0.997	19.369	-7403.685	7442.423	1.000	19.683
-3.042	2.078						
		0.997	0.162	-1.408	1.732	1.000	0.000
-1.066	-0.046	0.997	-0.043	-0.407	0.322	0.027	0.495
-1.066	-0.045	0.997	-0.043	-0.407	0.322	0.035	0.495
-1.066	-0.045	0.997	-0.043	-0.407	0.322	0.034	0.495
-1.066	-0.045	0.997	-0.043	-0.407	0.322	0.054	0.495
-2.001	1.233					0.997	-19.506
-2.001	1.233					0.997	-19.506
-2.001	1.233					0.997	-19.506
-2.001	1.233					1.000	-19.506
-1.136	0.404	0.997	-0.338	-1.199	0.523	0.460	0.390
-1.136	0.404	0.997	-0.338	-1.199	0.523	0.805	0.390
-1.485	0.486	0.933	-2.118	-5.960	1.724		
-1.485	0.486	0.603	-2.118	-5.960	1.724		
-1.485	0.486	0.644	-2.118	-5.960	1.724		
-1.148	0.535	0.997	-0.334	-1.197	0.530	0.838	0.390
-1.141	0.538	0.739	-0.331	-1.187	0.525	0.617	0.390

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-1.029	-0.061	0.789	-0.111	-0.432	0.210	0.125	0.353
-13467.087	13427.752						
-13467.087	13427.752						
-13467.087	13427.752						
-7.503	1.449	0.997	-0.133	-2.467	2.202	0.997	20.073
-7.503	1.449	0.997	-0.133	-2.467	2.202	0.997	20.073
-7.503	1.449	0.997	-0.133	-2.467	2.202	0.997	20.073
-7.503	1.449	0.997	-0.133	-2.467	2.202	0.997	20.073
-7.503	1.449	0.997	-0.133	-2.467	2.202	1.000	20.073
-1.180	-0.200	0.002	-1.244	-1.899	-0.590	0.000	-1.694
-2.234	1.877	0.703	-0.869	-2.202	0.463	0.943	-0.279
-2.234	1.877	0.997	-0.572	-1.933	0.788	0.997	-0.279
-2.830	1.114	0.603	-0.972	-2.629	0.686	0.997	-18.927
-1.178	-0.203	0.001	-1.245	-1.902	-0.588	0.000	-1.697
-1.178	-0.203	0.001	-1.245	-1.902	-0.588	0.000	-1.697
-1.178	-0.203	0.001	-1.245	-1.902	-0.588	0.000	-1.697
-1.153	-0.142	0.921	0.094	-0.327	0.516	0.577	-0.262
-2.489	3.496					0.997	19.533
-2.489	3.496					1.000	19.533
		0.789	-0.960	-3.576	1.656	0.789	-0.960
						1.000	-19.571
-2.830	1.114	0.789	-0.983	-3.609	1.643	1.000	-20.175
		0.997	-20.351	-7515.845	7475.142		
		0.997	-20.351	-7515.845	7475.142		
		0.997	-20.351	-7515.845	7475.142		
		0.997	-19.291	-7643.708	7605.126	1.000	-19.602
-0.941	2.900	0.997	0.217	-1.200	1.635	0.400	0.833
-3.509	0.658	0.997	0.093	-1.311	1.497	0.943	0.482
-4.437	0.206	0.997	-0.623	-2.206	0.961	0.997	0.195
-4.437	0.206	0.739	-0.623	-2.206	0.961	0.997	0.195
-3.008	2.642	0.997	-20.663	-9614.218	9572.893	0.997	18.501
-3.008	2.642	0.997	-20.663	-9614.218	9572.893	0.997	18.501
-3.008	2.642	0.997	-20.663	-9614.218	9572.893	0.997	18.501
-8845.648	8884.338	0.997	-19.451	-6801.632	6762.730	0.997	-19.451
-4.437	0.206	0.789	-0.623	-2.206	0.961	1.000	0.195
-0.948	4.607	0.997	0.642	-1.315	2.599	0.811	0.850
-8242.987	8280.854	0.933	1.260	-0.930	3.451	0.997	0.661

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-8242.987	8280.854	0.603	1.260	-0.930	3.451	0.881	0.661
-8242.987	8280.854	0.628	1.260	-0.930	3.451	0.883	0.661
-7933.308	7973.058	0.997	-19.001	-6780.326	6742.325	0.997	20.416
-8628.202	8588.907						
-8628.202	8588.907						
-8628.202	8588.907						
		0.997	-20.237	-11222.247	11181.773	0.997	19.533
		0.997	-20.237	-11222.247	11181.773	1.000	19.533
-11876.963	11918.574						
-11876.963	11918.574						
-11504.790	11465.294						
		0.997	0.116	-2.255	2.487		
		0.997	0.116	-2.255	2.487		
		0.997	0.116	-2.255	2.487		
-0.890	1.567	0.997	-0.047	-1.057	0.964	0.389	-0.598
-0.890	1.567	0.997	-0.047	-1.057	0.964	0.544	-0.598
-0.890	1.567	0.997	-0.047	-1.057	0.964	0.551	-0.598
-0.890	1.567	0.997	-0.047	-1.057	0.964	0.404	-0.598
-0.890	1.567	0.997	-0.047	-1.057	0.964	0.459	-0.598
-1.422	3.855						
-1.422	3.855						
-1.422	3.855						
-2.515	0.520	0.997	-0.057	-1.114	1.000	0.752	0.302
-2.515	0.520	0.997	-0.057	-1.114	1.000	0.943	0.302
-2.515	0.520	0.997	-0.057	-1.114	1.000	0.997	0.302
		0.997	-19.459	-10738.367	10699.449		
		0.997	-19.459	-10738.367	10699.449		
		0.997161	-19.458627	-10738.367	10699.449		

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Confidence Interval		DAY 31		Confidence Interval		DAY 35
2.50%	97.50%	q-value	Fold change (*obob* /Het)	2.50%	97.50%	q value
-0.302	-0.119	0.719	-0.040	-0.198	0.119	0.997
-1.194	-0.310	0.210	0.473	-0.058	1.004	0.150
0.219	0.994	0.523	0.241	-0.115	0.597	0.197
-0.147	0.894	0.850	0.133	-0.238	0.504	0.482
-0.141	0.891	0.910	0.133	-0.238	0.504	0.654
-1.103	3.320	0.931	0.455	-0.943	1.852	0.998
-1.103	3.320	0.873	0.455	-0.943	1.852	0.949
0.266	2.158	0.850	0.531	-0.748	1.811	0.482
0.266	2.158	0.910	0.531	-0.748	1.811	0.654
-1.230	-0.324	0.356	0.478	-0.064	1.019	0.187
-2.147	-0.750	0.691	0.481	-0.267	1.229	0.455
-2.147	-0.750	0.827	0.481	-0.267	1.229	0.512
-0.403	0.622	0.879	0.129	-0.301	0.558	0.949
-0.536	0.634	0.850	0.176	-0.287	0.639	0.010
-0.542	0.644	0.910	0.177	-0.289	0.644	0.007
-0.458	0.466	0.997	0.059	-0.368	0.486	0.076
0.463	4.643	0.084	2.582	0.528	4.637	0.434
0.463	4.643	0.086	2.582	0.528	4.637	0.482
-0.278	-0.071	0.004	-0.239	-0.377	-0.102	0.002
-0.278	-0.071	0.006	-0.239	-0.377	-0.102	0.003
-0.505	-0.114	0.833	-0.128	-0.351	0.095	0.998
-0.229	1.279	0.551	0.736	-0.259	1.731	0.949
-0.445	1.414	0.827	0.741	-0.453	1.935	0.998
-2.331	0.094	0.919	-0.191	-0.999	0.617	0.926
0.426	1.485	0.086	0.722	0.134	1.311	0.076
-1.263	1.800	0.982	-0.481	-2.360	1.397	0.998
-0.331	-0.009	0.084	-0.253	-0.454	-0.051	0.012
-7034.885	6996.101	0.997	-0.216	-2.418	1.986	0.998
-4.784	1.763	0.997	-0.073	-1.598	1.451	0.998
-0.514	0.416	0.843	0.201	-0.165	0.567	0.998
-2.373	0.090	0.982	-0.196	-1.006	0.613	0.998
0.450	1.035	0.162	0.386	0.030	0.742	0.998
-1.440	-0.109	0.086	-1.516	-2.737	-0.296	0.076

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-2.138	1.724	0.982	0.384	-1.141	1.910	0.998
-1.667	-0.373	0.018	-1.228	-1.994	-0.463	0.005
-5.117	-0.523	0.828	-0.588	-1.571	0.395	0.998
-2.644	1.278	0.910	-0.691	-2.494	1.111	0.998
-2.318	0.159	0.910	-0.450	-1.404	0.504	0.798
-2.282	-0.437	0.528	-0.337	-0.766	0.091	0.949
-0.233	0.001	0.053	-0.234	-0.398	-0.069	0.028
-1.387	0.423	0.881	0.290	-0.272	0.853	0.998
-0.461	-0.134	0.000	-0.435	-0.620	-0.250	0.000
-0.672	-0.167	0.013	-0.467	-0.740	-0.194	0.076
-0.261	0.367	0.092	0.421	0.044	0.797	0.177
-0.261	0.367	0.161	0.421	0.044	0.797	0.197
-0.261	0.367	0.142	0.421	0.044	0.797	0.293
-2.577	0.403	0.997	0.094	-0.641	0.829	0.998
-0.112	0.539	0.018	0.568	0.211	0.926	0.076
-6003.957	5963.356	0.997	-19.780	-7276.919	7237.359	0.998
-1.200	1.847	0.077	1.022	0.248	1.796	0.482
-6003.957	5963.356	0.997	-19.780	-7276.919	7237.359	0.949
-6003.957	5963.356	0.997	-19.780	-7276.919	7237.359	0.993
-0.683	-0.164	0.003	-0.680	-1.024	-0.336	0.296
-0.683	-0.164	0.003	-0.680	-1.024	-0.336	0.333
-9036.523	9075.889	0.741	1.065	-0.456	2.586	0.998
-2.163	2.162					0.998
0.118	0.871	0.496	0.162	-0.150	0.474	0.997
0.118	0.871	0.403	0.162	-0.150	0.474	0.997
0.118	0.871	0.585	0.162	-0.150	0.474	0.997
0.118	0.871	0.845	0.162	-0.150	0.474	0.998
-9625.098	9586.085					
-9625.098	9586.085					
-9625.098	9586.085					
-9625.098	9586.085					
-0.420	1.200	0.496	0.511	-0.366	1.387	0.997
-0.420	1.200	0.403	0.511	-0.366	1.387	0.874
-0.420	1.200	0.585	0.511	-0.366	1.387	0.993
-0.415	1.195	0.738	0.513	-0.354	1.380	0.949

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0.022	0.684	0.910	0.095	-0.162	0.352	0.998
-10830.352	10870.499	0.750	-0.425	-3.039	2.189	0.997
-10830.352	10870.499	0.750	-0.425	-3.039	2.189	0.997
-10830.352	10870.499	0.911	-0.425	-3.039	2.189	0.997
-10830.352	10870.499	0.978	-0.425	-3.039	2.189	0.998
-10830.352	10870.499	0.997	-0.425	-3.039	2.189	0.998
-2.291	-1.096	0.005	-1.073	-1.691	-0.454	0.044
-1.934	1.375	0.279	1.846	-0.536	4.228	0.637
-1.934	1.375	0.438	1.846	-0.536	4.228	0.702
-6035.147	5997.293	0.954	-0.286	-1.738	1.166	0.482
-2.298	-1.096	0.004	-1.085	-1.710	-0.460	0.027
-2.298	-1.096	0.006	-1.085	-1.710	-0.460	0.035
-2.298	-1.096	0.010	-1.085	-1.710	-0.460	0.028
-0.719	0.196	0.997	-0.026	-0.428	0.376	0.998
-8384.177	8423.243	0.997	19.517	-11113.043	11152.078	
-8384.177	8423.243	0.997	19.517	-11113.043	11152.078	
-3.576	1.656					0.998
-6716.070	6676.928	0.997	-19.940	-6916.171	6876.292	0.998
-8518.400	8478.049	0.997	-0.286	-1.738	1.166	0.998
		0.997	20.324	-12026.885	12067.532	
		0.997	20.324	-12026.885	12067.532	
		0.997	19.589	-8324.096	8363.274	0.998
		0.997	19.589	-8324.096	8363.274	0.998
-5245.100	5205.896	0.997	-18.719	-5504.945	5467.507	0.998
-0.585	2.252	0.037	1.620	0.399	2.841	0.989
-1.343	2.308	0.092	1.693	0.181	3.206	0.653
-1.910	2.301	0.911	-0.303	-1.989	1.383	0.987
-1.910	2.301	0.850	-1.035	-3.295	1.225	0.949
-6318.984	6355.986	0.488	-0.851	-2.888	1.186	0.447
-6318.984	6355.986	0.697	-0.851	-2.888	1.186	0.479
-6318.984	6355.986	0.850	-0.851	-2.888	1.186	0.482
-6801.632	6762.730	0.910	-1.000	-3.858	1.858	0.998
-1.910	2.301	0.910	-1.035	-3.295	1.225	0.998
-1.153	2.852	0.346	2.497	-1.206	6.201	0.997
-1.362	2.683	0.997	20.183	-10355.462	10395.829	0.997

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-1.362	2.683	0.997	20.183	-10355.462	10395.829	0.998
-1.362	2.683	0.997	20.183	-10355.462	10395.829	0.998
-9378.762	9419.595	0.697	0.699	-1.118	2.516	
						0.997
						0.998
						0.998
-8384.177	8423.243	0.850	0.699	-1.118	2.516	
-8384.177	8423.243	0.910	0.699	-1.118	2.516	
		0.997	-19.228	-9925.817	9887.362	0.998
		0.997	19.820	-9437.511	9477.151	0.998
		0.997	19.820	-9437.511	9477.151	0.998
		0.997	19.762	-6637.773	6677.297	
		0.997	19.762	-6637.773	6677.297	
		0.997	19.762	-6637.773	6677.297	
						0.997
						0.998
						0.998
						0.997
						0.997
						0.998
-1.502	0.305	0.719	-0.212	-1.070	0.647	0.989
-1.502	0.305	0.682	-0.212	-1.070	0.647	0.653
-1.502	0.305	0.891	-0.212	-1.070	0.647	0.840
-1.502	0.305	0.919	-0.212	-1.070	0.647	0.833
-1.502	0.305	0.982	-0.212	-1.070	0.647	0.998
-1.035	1.639	0.496	0.626	-0.508	1.759	0.989
-1.035	1.639	0.403	0.626	-0.508	1.759	0.769
-1.035	1.639	0.585	0.626	-0.508	1.759	0.987

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	Confidence Interval	
Fold change (obob/Het)	**2.50%**	**97.50%**
-0.011	-0.152	0.130
0.594	0.051	1.137
-0.541	-1.093	0.011
-0.415	-1.027	0.196
-0.412	-1.024	0.200
-0.731	-3.709	2.248
-0.731	-3.709	2.248
-0.774	-1.897	0.348
-0.774	-1.897	0.348
0.614	0.055	1.173
0.556	-0.155	1.266
0.556	-0.155	1.266
0.089	-0.307	0.484
0.606	0.273	0.939
0.605	0.269	0.941
0.633	0.137	1.129
2.602	-0.542	5.746
2.602	-0.542	5.746
-0.238	-0.362	-0.114
-0.238	-0.362	-0.114
-0.115	-0.365	0.135
0.256	-0.992	1.505
0.291	-1.169	1.751
0.312	-0.492	1.116
0.621	0.156	1.085
-0.557	-2.521	1.406
-0.324	-0.513	-0.135
0.109	-2.028	2.246
0.076	-1.259	1.410
0.144	-0.332	0.620
0.312	-0.492	1.116
0.124	-0.157	0.405
-1.419	-2.515	-0.322

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-0.526	-2.343	1.291
-1.268	-1.936	-0.599
-0.111	-1.590	1.367
-1.026	-3.851	1.799
-0.572	-1.536	0.391
0.197	-0.579	0.973
-0.200	-0.333	-0.066
0.020	-0.830	0.871
-0.396	-0.512	-0.280
-0.405	-0.710	-0.099
0.481	-0.001	0.963
0.481	-0.001	0.963
0.481	-0.001	0.963
0.282	-0.625	1.188
0.591	0.155	1.027
-0.395	-2.166	1.376
0.710	-0.156	1.576
-0.395	-2.166	1.376
-0.395	-2.166	1.376
-0.293	-0.600	0.014
-0.293	-0.600	0.014
19.753	-8525.420	8564.926
-19.286	-10811.767	10773.194
-0.018	-0.457	0.420
-0.018	-0.457	0.420
-0.018	-0.457	0.420
-0.018	-0.457	0.420
0.262	-0.785	1.309
0.262	-0.785	1.309
0.262	-0.785	1.309
0.263	-0.780	1.305

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-0.045	-0.413	0.322
20.247	-11262.797	11303.292
20.247	-11262.797	11303.292
20.247	-11262.797	11303.292
20.247	-11262.797	11303.292
20.247	-11262.797	11303.292
-0.844	-1.440	-0.248
3.279	-2.583	9.141
3.279	-2.583	9.141
2.012	-1.060	5.083
-0.919	-1.543	-0.295
-0.919	-1.543	-0.295
-0.919	-1.543	-0.295
0.252	-0.345	0.849
0.150	-3.051	3.352
-19.001	-6161.770	6123.769
19.430	-5344.678	5383.537
20.008	-15650.315	15690.331
20.008	-15650.315	15690.331
-18.445	-6562.226	6525.336
-0.549	-2.003	0.904
-0.679	-2.129	0.771
-0.546	-2.330	1.238
0.503	-1.234	2.240
-1.491	-3.567	0.586
-1.491	-3.567	0.586
-1.491	-3.567	0.586
19.627	-8671.290	8710.544
0.503	-1.234	2.240
-0.190	-2.257	1.877
-0.293	-2.478	1.893

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-0.293	-2.478	1.893
-0.293	-2.478	1.893
18.632	-6525.978	6563.242
18.632	-6525.978	6563.242
18.632	-6525.978	6563.242
-19.042	-6542.468	6504.384
-19.087	-8439.227	8401.052
-19.087	-8439.227	8401.052
-19.577	-11440.632	11401.478
-19.577	-11440.632	11401.478
-19.577	-11440.632	11401.478
-19.040	-9440.250	9402.169
-19.040	-9440.250	9402.169
-19.040	-9440.250	9402.169
0.453	-0.545	1.451
0.453	-0.545	1.451
0.453	-0.545	1.451
0.453	-0.545	1.451
0.453	-0.545	1.451
0.499	-0.931	1.929
0.499	-0.931	1.929
0.499	-0.931	1.929