

A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis

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Background: Commensal microbiota play a critical role in maintaining oral tolerance. The effect of food allergy on the gut microbial ecology remains unknown.

Objective: We sought to establish the composition of the gut microbiota in experimental food allergy and its role in disease pathogenesis.

Methods: Food allergy-prone mice with a gain-of-function mutation in the IL-4 receptor α chain (*Il4raF709*) and wild-type (WT) control animals were subjected to oral sensitization with chicken egg ovalbumin (OVA). Enforced tolerance was achieved by using allergen-specific regulatory T (Treg) cells. Community structure analysis of gut microbiota was performed by using a high-density 16S rDNA oligonucleotide microarrays (PhyloChip) and massively parallel pyrosequencing of 16S rDNA amplicons.

Results: OVA-sensitized *Il4raF709* mice exhibited a specific microbiota signature characterized by coordinate changes in the abundance of taxa of several bacterial families, including the Lachnospiraceae, Lactobacillaceae, Rikenellaceae, and Porphyromonadaceae. This signature was not shared by similarly sensitized WT mice, which did not exhibit an OVA-induced allergic response. Treatment of OVA-sensitized *Il4raF709* mice

with OVA-specific Treg cells led to a distinct tolerance-associated signature coincident with the suppression of the allergic response. The microbiota of allergen-sensitized *Il4raF709* mice differentially promoted OVA-specific IgE responses and anaphylaxis when reconstituted in WT germ-free mice. **Conclusion:** Mice with food allergy exhibit a specific gut microbiota signature capable of transmitting disease susceptibility and subject to reprogramming by enforced tolerance. Disease-associated microbiota may thus play a pathogenic role in food allergy. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Food allergy, microbiome, microbiota, regulatory T cells, tolerance, anaphylaxis, IgE, 16S rDNA, IL-4 receptor

It is unquestionable that food allergy has become a major health problem in developed countries, where the prevalence reaches up to 6% among children and 3% among adults.^{1,2} Like other atopic diseases, food allergies have a strong genetic component.³ However, the incidence of food allergy has increased dramatically in the last decades, particularly in affluent societies, pointing to lifestyle-associated environmental factors acting on genetically susceptible hosts to promote disease.⁴ Evidence suggests that the microbial flora are a key environmental influence in programming oral tolerance.⁵ Their lack in germ-free (GF) mice is associated with the development of T_H2 and IgE responses to dietary antigens.^{6,7} Microbial signals, such as those delivered by polysaccharide A of the commensal bacterium *Bacteroides fragilis* or by a mix of clostridial species, induce mucosal tolerance by promoting the formation of induced regulatory T (iTreg) cells from naive CD4⁺ T-cell precursors.^{8,9} Polymorphisms in or deficiency of genetic elements encoding microbial sensors, such as CD14, a high-affinity receptor for bacterial LPSs, and Toll-like receptor 4, which mediates responses to LPS, are associated with food allergy.^{10,11}

Changes in the microbial flora have been implicated in the pathogenesis of several disorders associated with the more affluent lifestyle common in developed countries. Obesity in both human subjects and experimental mouse models is associated with alterations in the intestinal microbiota that appear to be pathogenic, given that the microbiota of obese subjects promote weight gain when transferred into GF mice.¹²⁻¹⁴ More limited data have been accrued in the study of allergic diseases. In suboptimally controlled asthmatic subjects, both bacterial burden and bacterial diversity were significantly higher compared with those seen in control subjects and correlated with bronchial hyperresponsiveness.¹⁵ The development of atopy and atopic dermatitis is associated with altered early postnatal gut flora.^{16,17} In the

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Abbreviations used

Foxp3:	Forkhead box protein 3
GF:	Germ free
HC-AN:	Hierarchical clustering-average-neighbor
IL-4R:	IL-4 receptor
iTreg:	Induced regulatory T
KW:	Kruskal-Wallis
mMCP-1:	Murine mast cell protease 1
NMDS:	Nonmetric multidimensional scaling
OTU:	Operational taxonomic unit
OVA:	Ovalbumin
PAM:	Prediction Analysis for Microarrays
rDNA:	Ribosomal DNA
SEB:	Staphylococcal enterotoxin B
STAT6:	Signal transducer and activator of transcription 6
TCR:	T-cell receptor
Treg:	Regulatory T
WT:	Wild-type

case of food allergy and despite the well-known role of the commensal flora in oral tolerance induction, there is meager information on the status of the intestinal microbiota on disease onset and after tolerance establishment. One study found alterations in the microbiota of infants with milk allergy on diagnosis and again after treatment.¹⁸ Another study in which atopic dermatitis cohorts were examined for changes in the microbiota in association with allergic food sensitization found no such relationship.¹⁹ Limitations to the latter set of studies include reliance on bacterial culture methods, limited 16S RNA genotyping approaches, or both for microbiota analysis.^{18,19}

In this study we employed a phylotyping approach using high-density 16S ribosomal DNA (rDNA) oligonucleotide microarrays (PhyloChip assay; Second Genome, San Bruno, Calif) and massively parallel pyrosequencing of 16S rDNA amplicons to investigate whether oral allergic sensitization to a dietary antigen is associated with a distinct intestinal microbiota signature. These studies were enabled by the use of a novel mouse model of food allergy that well replicates many of the features of the human disease. Using the same model, we also examined whether therapy with allergen-specific regulatory T (Treg) cells imparts a tolerance-associated signature on the host microbiota.

METHODS**Animals**

BALB/cTac mice (wild-type [WT] and *Il4ra*^{F709} mice) were originally from Taconic Farms (Germantown, NY) and maintained as a separate line in the investigator's colony from which mice were used for the current studies. *C.129.II4ra^{F709/F709}* (*Il4ra*^{F709}) mice were bred onto the investigator's BALB/cTac line for 11 generations.²⁰ Foxp3^{EGFP} and DO11.10⁺Foxp3^{EGFP} mice, both on a BALB/c background, were previously described.²⁰⁻²² All mice were kept on an ovalbumin (OVA)-free diet (Harlan 1818SX, Indianapolis, Ind). They were housed together in the same colony in a specific pathogen-free environment and were 8 to 12 weeks old when used for studies. All experiments were carried out in accordance with the Institutional Animal Care and Use Committee policies and procedures of the University of California–Los Angeles and the Boston Children's Hospital.

Sensitization and challenge protocols

Studies were conducted on female mice to eliminate confounding effects of sex on the results of the microbiota analyses. In separate experiments there were

no significant differences between male and female *Il4ra*^{F709} mice in terms of sensitization and response to allergen challenge. For sensitization, female WT and *Il4ra*^{F709} mice were treated intragastrically with sterile PBS or 100 µg of OVA alone or together with 10 µg of staphylococcal enterotoxin B (SEB; Sigma-Aldrich, St Louis, Mo) in 100 µL of sterile PBS (saline) once weekly for 8 weeks. On the ninth week, mice were challenged intragastrically with 5 or 150 mg of OVA in 100 µL of PBS. Anaphylaxis was assessed in challenged mice by measuring changes in body temperature and recording symptom scores. Temperature changes were measured with a rectal temperature probe (RET3) coupled to the Physitemp Thermalert Model TH-5 (Physitemp, Clifton, NJ). After OVA challenge, temperatures were measured every 5 minutes. Symptom scores were determined according to previously detailed criteria.²²

For studies on flora-reconstituted GF mice, fecal pellets of either OVA/SEB-sensitized WT or *Il4ra*^{F709} mice were collected on the eighth week of sensitization. The pellets were dissolved at 1 pellet (20–25 mg) in 100 µL of PBS and administered to GF mice at 200 µL per mouse. The mice were then sensitized with OVA/SEB for 8 weeks and challenged with OVA, as described above.

Tolerance induction

CD4⁺DO11.10⁺Foxp3^{EGFP} T cells, representing thymus-derived (natural) Treg cells that express the DO11.10 T-cell receptor (TCR), which recognizes the OVA_{323–339} peptide in the context of I-A^d, were isolated by means of cell sorting from *DO11.10⁺Foxp3^{EGFP}* mice. On day 0 of the sensitization protocol, *Il4ra*^{F709} mice were given 5×10^5 CD4⁺DO11.10⁺Foxp3^{EGFP} T cells by means of retro-orbital transfer. The mice were then sensitized intragastrically with 100 µg of OVA in 100 µL of PBS once weekly for 8 weeks. They were challenged on week 9 with 5 mg of OVA in 100 µL of PBS administered intragastrically and monitored for their core body temperature and symptom scores, as described above.

ELISA for murine mast cell protease 1 and total and OVA-specific IgE

A murine mast cell protease 1 (mMCP-1) ELISA was performed on serum samples by using a kit (eBioscience, San Jose, Calif). Total and OVA-specific IgE concentrations were measured by using sandwich ELISAs. For total IgE, the capture and biotinylated detection antibodies (rat anti-mouse IgE clones R35-72 and R35-118, respectively) and purified mouse IgE isotype standard antibody (clone C38-2) were from BD Biosciences (San Jose, Calif). For OVA-specific IgE, the plates were coated with 100 µg/mL OVA. The detection antibody was as above, whereas the standard was a purified anti-OVA monoclonal IgE antibody (AbD Serotec, Oxford, United Kingdom).

Histologic analysis and enumeration of mast cells

Intestinal mast cells were enumerated by means of microscopic examination of sections of paraffin-embedded jejunal tissues stained with toluidine blue. Mast cells were counted and averaged across 10 high-power fields spanning the entire jejunum.

PhyloChip sample processing and hybridization

Fecal pellets were obtained from sham- and allergen-sensitized mice just before allergen challenge. DNA extraction was performed with the UltraClean Fecal extraction kit (Mo Bio, Carlsbad, Calif), according to the manufacturer's instructions. A total of 50 to 100 ng of extracted DNA per sample was used to amplify bacterial 16S rDNA gene sequences by using PCR with the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTTGTACGACTT-3'). Amplified products were hybridized to G3 PhyloChip arrays (Second Genome), which display 1,016,064 16S rDNA oligonucleotide probes that track microbial population shifts across greater than 59,000 operational taxonomic units (OTUs), each mapped to the Greengenes taxonomy.²³⁻²⁵ OTUs are defined as organisms that share sequence similarity of their 16S rDNA, which for most OTUs is greater than 99%. The hybridization procedures were as described in the aforementioned references.

Bioinformatics

Details of the PhyloChip data analyses, including data preprocessing and reduction, construction of sample-to-sample distance functions, ordination, clustering, and classification methods and phylogenetic tree construction and visualization, are detailed in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Statistical analysis

Results of allergen challenge studies were analyzed by using 2-way ANOVA. The Adonis test was used for finding significant microbiota differences associated with discrete categorical or continuous variables. Taxa were in some cases filtered to those significantly increased in their ranked HybScore in one category compared with the alternate categories by using the Kruskal-Wallis (KW) test. A *P* value of less than .05 was considered significant. Subgroup analysis for phylogenetic diversity was carried out by using 1-way ANOVA.

Other methods

Flow cytometric analyses and intracellular cytokine staining, PhyloChip and 16S rDNA pyrosequencing methods, and related statistical analyses are detailed in the [Methods](#) section in this article's Online Repository.

RESULTS

Oral allergic sensitization of *Il4raF709* mice is associated with the acquisition of a specific microbiota signature

Il4raF709 mice carry a gain-of-function mutation in the IL-4 receptor (IL-4R) α chain that disrupts the binding of the Src homology domain 2-containing protein tyrosine phosphatase 1 (SHP-1) to the receptor subunit and leads to augmented signal transducer and activator of transcription 6 (STAT6) activation by IL-4 and IL-13.²⁰ *Il4raF709* mice, but not WT control mice, are particularly susceptible to oral sensitization with innocuous food allergens, such as the chicken egg protein OVA, and sensitized mice respond to oral challenge with anaphylaxis that proceeds in a mast cell- and IgE-dependent manner.²² Mutant *Il4raF709* mice were either sham sensitized with PBS or sensitized with OVA, either alone or mixed with the oral adjuvant SEB, by means of oral gavage once weekly for 8 weeks to determine whether oral allergic sensitization results in a change in the intestinal microbiota.^{22,26} As noted previously, OVA- and OVA/SEB-sensitized, but not sham-sensitized, *Il4raF709* mice had anaphylaxis on oral challenge with OVA, as evidenced by the decrease in core body temperature, and onset of diarrhea and tissue edema ([Fig 1, A](#), and data not shown).²² Anaphylaxis was associated with increased total and OVA-specific serum IgE antibody levels, mast cell expansion in small intestinal tissues, and increased serum concentrations of the mast cell protease mMCP-1 compared with unsensitized *Il4raF709* mice ([Fig 1, B-E](#)). Comparison of OVA- and OVA/SEB-sensitized mice revealed concordant temperature and biomarker responses on OVA challenge but more robust responses with the latter. In contrast, WT mice subjected to the same sensitization protocol with PBS or OVA/SEB did not have anaphylaxis and exhibited marginal or no increases in the aforementioned biomarkers.

Oral tolerance is dependent on adaptive forkhead box protein 3 (Foxp3)-positive Treg cells and is abrogated on their depletion or inhibition.^{27,28} Significantly, anaphylaxis was abrogated by the treatment of *Il4raF709* mice with CD4⁺DO11.10⁺Foxp3^{EGFP+} Treg cells, which harbor a bicistronic *Foxp3*^{EGFP} allele and express the OVA₃₂₃₋₃₃₉ peptide-specific TCR DO11.10. The

core temperature decrease and the small intestinal tissue edema pursuant to the OVA challenge were prevented ([Fig 1, A and E](#)). Treg cell treatment also suppressed total and OVA-specific IgE production, mast cell expansion and tissue infiltration, and mMCP-1 release ([Fig 1, B-E](#)).

To determine the effect of oral sensitization on the intestinal microbiota, we analyzed the relative abundance of bacterial phyla in fecal pellets of the different mouse groups by using PhyloChip 16S rDNA oligonucleotide microarrays ([Fig 2, A](#)). The microbiota of OVA-sensitized *Il4raF709* mice (including the OVA/SEB group and the combination of OVA+OVA/SEB-sensitized groups), but not that of sensitized WT mice, exhibited a significant decrease in the relative abundance of the Firmicutes family Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae compared with that seen in sham-sensitized mice ([Fig 2, B](#)). They also exhibited a significant increase in the relative abundance of the Proteobacteria family Gammaproteobacteria, Enterobacteriales, and Enterobacteriaceae ([Fig 2, C](#)).

Analysis of fecal pellets collected at the end of the sensitization period revealed that the intestinal microbiota of OVA- and OVA/SEB-sensitized *Il4raF709* mice were not significantly different when directly compared by using the presence/absence of taxa and abundance of metric criteria (Adonis *P* = .594 and .361, respectively). Also, the 97 taxa found to be differentially abundant between the 2 groups, as determined by using the KW test, could not partition the samples into distinct groups (data not shown). Accordingly, we combined the 2 groups in many of our subsequent statistical analyses.

Direct comparison of the intestinal microbiota of the combination of OVA- and OVA/SEB-sensitized *Il4raF709* mice with that of sham (PBS)-sensitized *Il4raF709* mice by using the presence/absence of taxa criterion revealed a significant difference in microbial profiles (Adonis *P* = .042 for the OVA plus OVA/SEB vs PBS groups). Of a total of 3422 OTUs found to be present in at least 1 of the samples, 251 were identified by using the KW filter to have significantly different abundances between the OVA+OVA/SEB versus PBS groups. We used 2 ordination methods, nonmetric multidimensional scaling (NMDS) and hierarchical clustering-average-neighbor (HC-AN) analyses, which examine relationships between ecologic communities, such as those of the microbial flora, to determine whether those OTUs identified by using the KW filter discriminate between sham- and allergen-sensitized *Il4raF709* mice.^{29,30} Results revealed that the identified taxa successfully partitioned the mice into 2 distinct groups, with only 1 of 9 OVA plus OVA/SEB-sensitized mice clustering with the sham-sensitized mice ([Fig 3, A and B](#)). Investigation of significant taxa the abundance of which best discriminates between sham- and allergen-sensitized *Il4raF709* mice was carried out by using Prediction Analysis for Microarrays (PAM), an algorithm that uses a nearest shrunken centroid method to determine those OTUs most characteristic of a community.³¹ Results identified 10 OTUs, mainly Bacteroidetes, that discriminated between the 2 categories, including several expanded Porphyromonadaceae, Barnesiella, and Rikenellaceae species ([Fig 3, C and D](#), and see [Table E1](#) in this article's Online Repository at www.jacionline.org).

The alterations in the microbiota affected by oral allergen sensitization were further visualized by using a circular phylogenetic tree that displayed 45 differentially abundant OTUs, representing the 41 bacterial families covered by the 251 taxa that passed the KW filter. The abundance of the represented OTUs among the different samples was revealed by means of a heat map that ranges

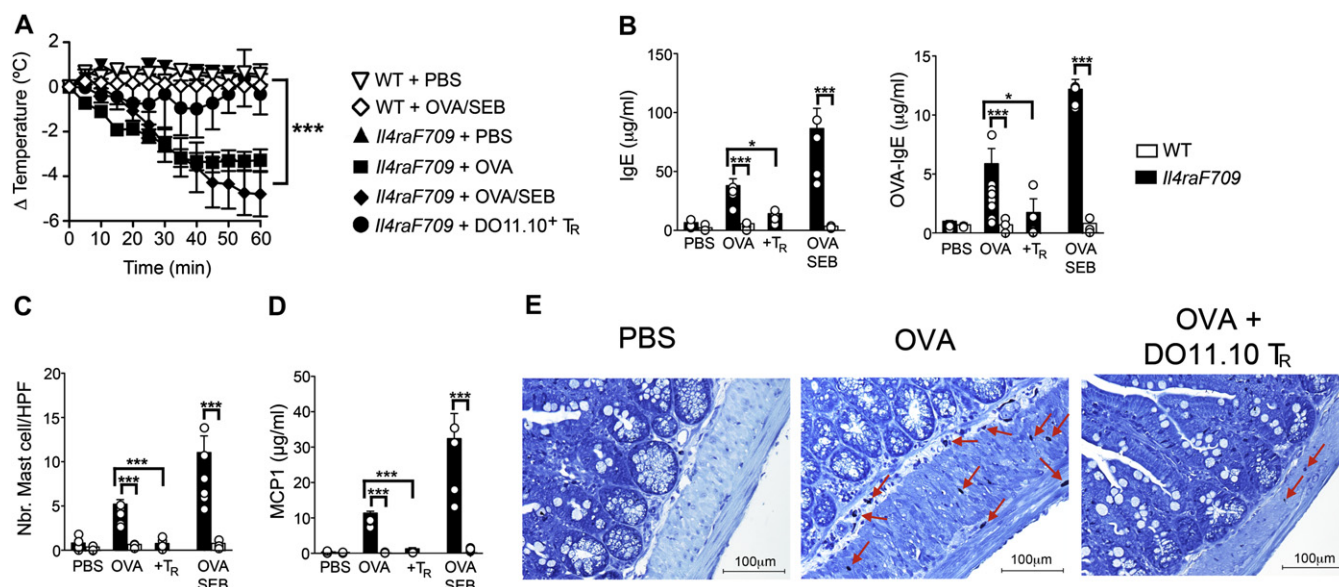


FIG 1. Oral sensitization and anaphylaxis in *Il4raF709* mice: prevention of oral sensitization by antigen-specific Treg cells. **A**, *Il4raF709* and WT BALB/c control mice were either sham sensitized with PBS or sensitized with OVA (100 μ g) or OVA/SEB (100 μ g/10 μ g) by means of gastric gavage once weekly for 8 weeks. Select groups of mice were administered intravenously at the start of the sensitization protocol (day 0), either 5×10^5 cells of DO11.10⁺ Treg (T_R) cells isolated from DO11.10⁺ Foxp3^{EGFP} mice or PBS. At the end of the sensitization period, mice were challenged with 5 mg of OVA by means of gastric gavage and monitored for rectal temperature changes. **B**, Total (left) and OVA-specific (right) serum IgE levels measured after OVA challenge. **C**, Enumeration of mast cell infiltration of small intestinal tissues of sensitized mice. **D**, Serum mMCP-1 levels in mice after OVA challenge. **E**, Small intestinal histopathology of sham (PBS)-sensitized, OVA-sensitized, and OVA-sensitized/DO11.10⁺ Treg cell-treated mice (toluidine blue staining; original magnification $\times 200$). Results represent data on 5 to 10 mice per group derived from 2 independent experiments. * $P < .05$ and *** $P < .001$, 2-way ANOVA.

from blue (decreased) to red (increased). There was a broad decrease in the abundance of a majority of the selected OTUs, especially those of the Lachnospiraceae families, in allergen-compared with sham-sensitized *Il4raF709* mice, although there were notable exceptions, such as Lactobacillaceae, Porphyromonadaceae, and some Rikenellaceae and Lachnospiraceae species (Fig 4 and see Table E2 in this article's Online Repository at www.jacionline.org). Overall, the results shown in Figs 3 and 4 confirmed that the establishment of oral allergic sensitization in the *Il4raF709* mice is associated with dysbiosis of the intestinal flora.

To confirm the results obtained with the PhyloChip analysis, we used direct, highly parallel pyrosequencing of 16S rDNA amplicons derived from fecal samples of separate independent cohorts of sham- and OVA-sensitized *Il4raF709* mice. Results revealed that the microbial communities were significantly different between the 2 groups of mice (see Fig E1 in this article's Online Repository at www.jacionline.org). With the caveat that the 16S rDNA pyrosequencing method had different depth of coverage and other technical differences compared with the PhyloChip analysis, several families and genera were also found by using the former method to differentiate between the 2 groups, including OTUs classifying to the genera *Clostridium*, *Bacteroides*, *Alistipes*, and *Streptococcus* (see Table E3 in this article's Online Repository at www.jacionline.org).

Allergen-specific Treg cell therapy induces a tolerance-associated microbiota signature

We next examined whether the induction of a food allergy-associated microbiota signature can be suppressed by enforced

tolerance with allergen-specific Treg cells. Accordingly, we compared the intestinal microbiota of *Il4raF709* mice that have been sensitized with OVA or OVA/SEB with those of similarly sensitized mice that have been intravenously administered Treg cells bearing the DO11.10 TCR specific for the immunodominant OVA peptide 323-339 (OVA₃₂₃₋₃₃₉). Therapy with Treg cells suppressed allergen sensitization and prevented the clinical, histologic, and serologic correlates of anaphylaxis (Fig 1). In contrast, therapy with CD4⁺DO11.10⁺Foxp3^{EGFP} cells or with CD4⁺DO11.10⁺ cells that express a nonfunctional Foxp3 mutant protein did not induce tolerance (Noval Rivas et al, manuscript in preparation). Direct comparison of the microbiota of the 2 categories revealed that the intestinal microbiota of DO11.10⁺ Treg cell-treated and OVA-sensitized mice were significantly different in the abundance metrics from those of sham (PBS)-treated, OVA-sensitized *Il4raF709* mice (Adonis $P = .007$). Analysis of those taxa passing the KW test for differences between the 2 categories identified 627 taxa to have different abundance levels. NMDS with these taxa shows relatively tight clustering of Treg cell-suppressed samples and a broader variation in the samples of the OVA- and OVA/SEB-sensitized but Treg cell-untreated samples. HC-AN with these KW-filtered OTUs showed complete segregation of the OVA- and OVA/SEB-sensitized samples from the Treg cell-suppressed samples (Fig 5, A and B). PAM analysis identified 10 OTUs that discriminate between the 2 groups: 8 members of the Firmicutes Lachnospiraceae family that are increased on tolerance induction and 2 in the Bacteroidetes Porphyromonadaceae family that are decreased on tolerance induction (Fig 5, C and D, and see Table E4 in this article's Online Repository at www.jacionline.org).

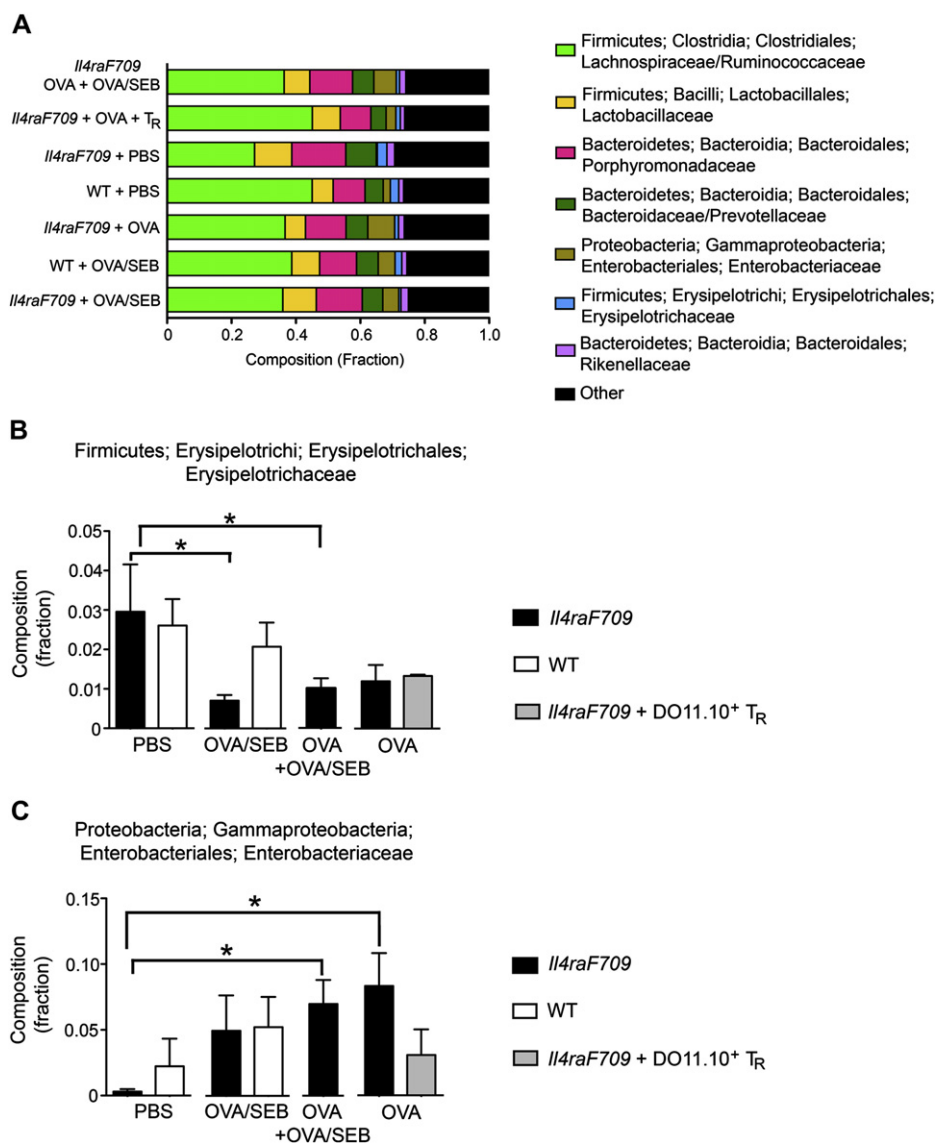


FIG 2. Effect of allergen sensitization on phylogenetic diversity. **A**, Relative abundance of the different microbial phyla in fecal samples of WT and *Il4raF709* mice that were either sham sensitized with PBS or sensitized with OVA or OVA/SEB. The combined profile of OVA- and OVA/SEB-sensitized *Il4raF709* mice is also shown, as is that of OVA-sensitized *Il4raF709* mice that have been treated with allergen-specific Treg cells. **B**, Relative abundance of the *Firmicutes Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae* family in the different groups. **C**, Relative abundance of the *Proteobacteria/Gammaproteobacteria/Enterobacteriales/Enterobacteriaceae* family in the different groups. Results represent 4 to 6 mice per group. **P* < .05, 1-way ANOVA with posttest analysis.

A global representation of the effects of Treg cell therapy on the microbiota of allergen-sensitized mice was provided by a phylogenetic tree representation of 86 differentially abundant OTUs, representing the 78 bacterial families covered by the 627 taxa that passed the KW filter (Fig 6). There was a broad resetting by means of Treg cell therapy of the changes in the microbiota induced by allergen sensitization, with reversal of the shifts in the abundance of several Firmicutes Lachnospiraceae and Bacteroidetes Porphyromonadaceae and Rikenellaceae families induced on sensitization (see Table E5 in this article's Online Repository at www.jacionline.org). However, although Treg cell therapy altered the microbiota of allergen-sensitized mice, it did not shift it back to baseline. Direct comparison of the

microbiota of tolerized and sham-treated *Il4raF709* mice revealed the 2 categories to be significantly different both in incidence and abundance (Adonis *P* = .013 and .011, respectively). KW filtering identified 786 taxa that had significantly different abundance levels in samples from sham-sensitized compared with tolerized mice and that completely segregated the 2 groups of samples into separate clusters (Fig E2, A and B, in this article's Online Repository at www.jacionline.org). PAM analysis identified 10 OTUs that best segregated the 2 groups, most from the Firmicutes Lachnospiraceae family (see Fig E2, C and D, and Table E6 in this article's Online Repository at www.jacionline.org). Overall, these results established that enforcement of oral tolerance by means of Treg cell therapy inhibited the emergence of

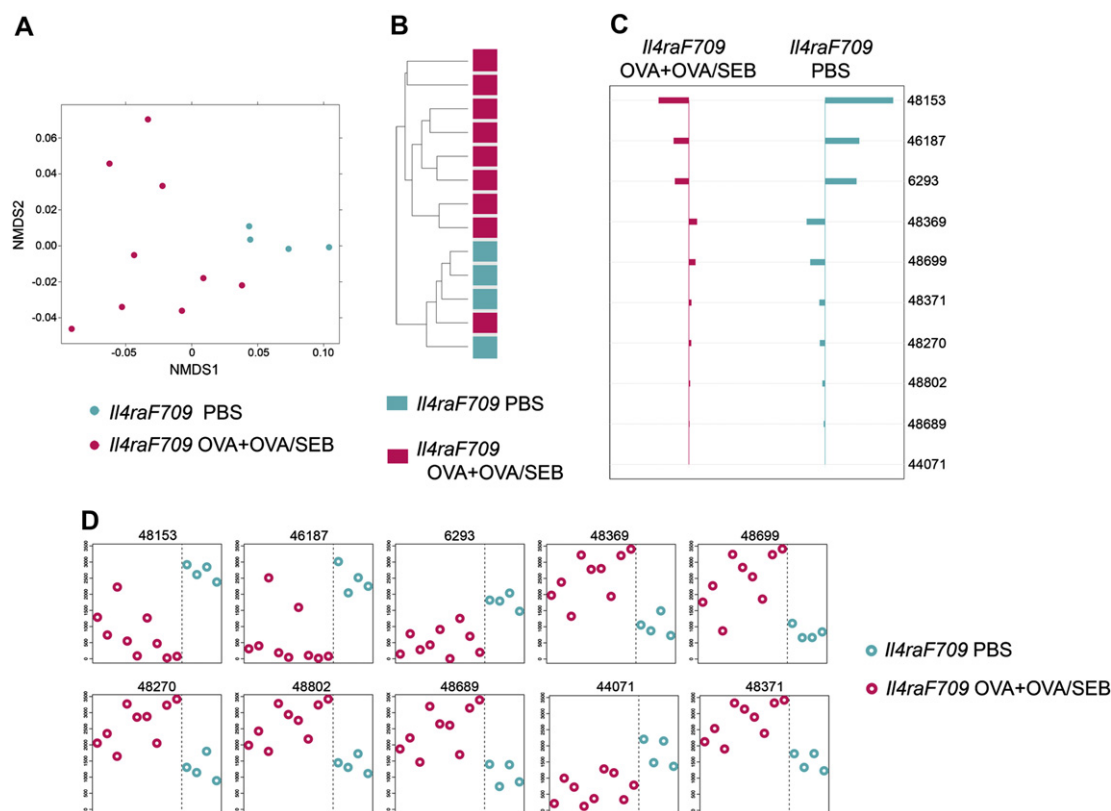


FIG 3. Allergen sensitization of *Il4raF709* mice is associated with a microbiotic signature. **A**, NMDS based on weighted UniFrac distance between samples of sham-sensitized versus OVA- and OVA/SEB-sensitized mice performed on the 251 taxa the abundance of which was significantly different between groups (sham versus allergen sensitized) by using the KW test. **B**, Hierarchical clustering (average linkage) based on weighted UniFrac distance between samples given abundance of 251 taxa with significant abundance differences across at least 1 of the categories. **C**, Nearest shrunken centroid analysis of OTUs that best characterize the difference between the sham- versus allergen-sensitized groups. The direction of the horizontal bars reflects either overrepresentation or underrepresentation of the indicated OTUs (left- and right-sided bars, respectively). The length of the bar represents the magnitude of the effect. **D**, Representation of the abundance of the OTUs identified by the nearest shrunken centroid analysis using the PAM method. Nine mice were used for the OVA+OVA/SEB group ($n = 5$ and 4 mice, respectively), and 5 mice were used for the PBS group.

the microbiota signature associated with experimental food allergy.

The microbiota changes of *Il4raF709* and WT mice are nonoverlapping

Oral sensitization of WT BALB/c mice with OVA/SEB does not induce appreciable IgE responses and systemic anaphylaxis on oral challenge with OVA.²² We set out to determine whether oral allergen sensitization of WT was associated with changes in the microbiota. Direct comparison of the microbiota of OVA/SEB-sensitized versus sham-sensitized WT mice did not reveal statistically significant differences between the 2 groups (data not shown). To determine whether the dysbiosis observed with allergen-sensitized *Il4raF709* mice was distinct from that of WT mice, we first compared the microbiota of sham-sensitized *Il4raF709* and WT mice. Direct comparison identified the microbiota of the respective category to be significantly different both in the incidence and abundance metrics (Adonis $P = .031$ and $.032$, respectively; see Fig E3 and Table E7 in this article's Online Repository at www.jacionline.org). In a separate analysis direct, highly parallel pyrosequencing was performed

on 16S rDNA amplicons from fecal samples of otherwise unmanipulated WT and *Il4raF709* littermate mice that were derived from matings of *Il4raF709* heterozygous parents and sharing the same cages. The results confirmed that the microbial communities of the respective group were significantly different (see Fig E4 in this article's Online Repository at www.jacionline.org).

We then compared the intestinal microbiota of OVA/SEB-sensitized *Il4raF709* mice with those of similarly sensitized WT mice. NMDS and HC-AN analysis of 352 taxa identified by the KW filter to have differential abundance in OVA/SEB-sensitized *Il4raF709* versus WT mice completely segregated the samples into 2 distinct groups (Fig 7, A and B). Of the 10 taxa found by using PAM to best discriminate between the 2 groups, 9 were increased in *Il4raF709* mice (Ruminococcaceae, Rikenellaceae, and Porphyromonadaceae species; Fig 7, C and D, and see Table E8 in this article's Online Repository at www.jacionline.org). These results were validated by using direct, highly parallel pyrosequencing of 16S rDNA amplicons derived from fecal samples of independent cohorts of OVA-sensitized WT and *Il4raF709* mice (see Fig E5 and Table E9 in this article's Online Repository at www.jacionline.org).



To explore the functional implications of the changed microbial flora in experimental food allergy, we reconstituted WT GF mice with flora derived from either OVA-sensitized WT or *Il4- α F709* mice. Flora-replete WT mice were then sensitized with OVA/SEB for 8 weeks and challenged with OVA. 16S rDNA

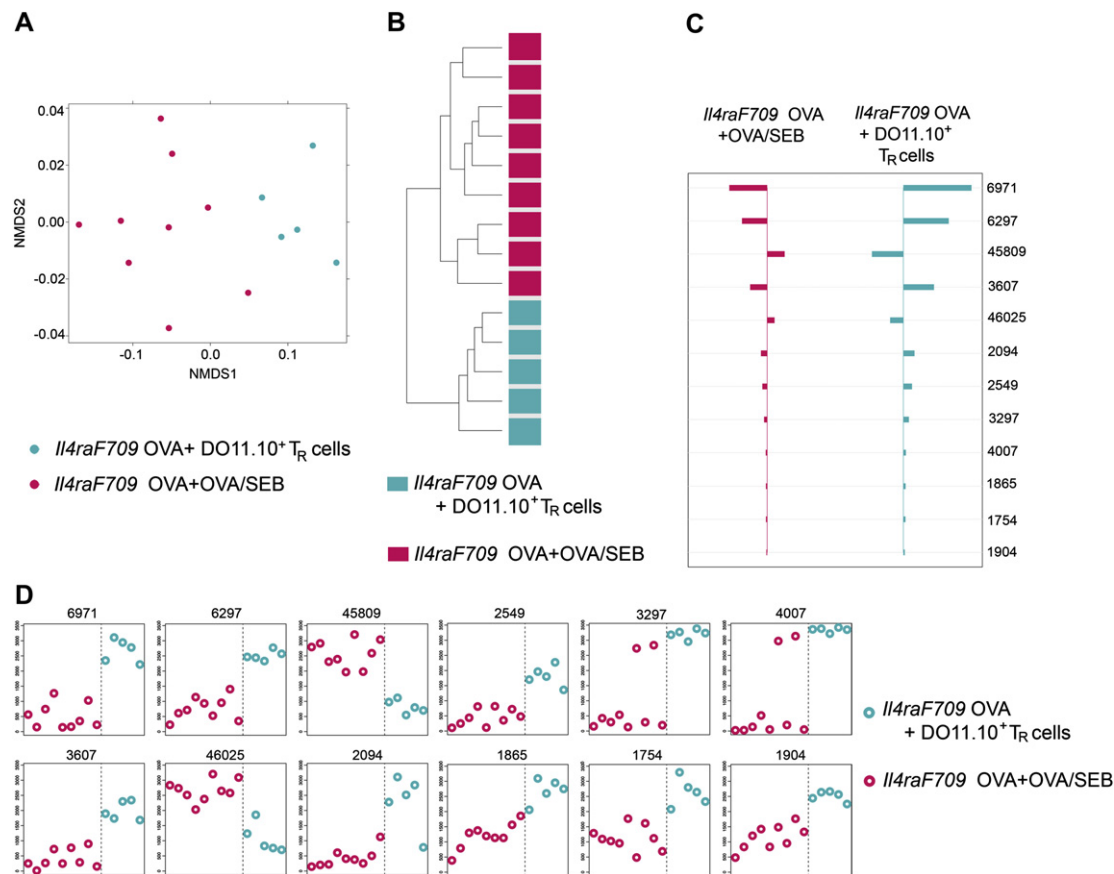


FIG 5. Enforced tolerance with allergen-specific Treg cells resets the microbiota of allergen-sensitized *Il4raF709* mice. **A**, NMDS based on weighted UniFrac distance between samples of OVA- and OVA/SEB-sensitized mice ($n = 9$) versus those of Treg cell-treated, OVA-sensitized mice ($n = 5$) based on the 627 taxa with significantly different abundance between groups (sham versus allergen sensitized) by using the KW test. **B**, Hierarchical clustering (average linkage) based on weighted UniFrac distance between samples given abundance of 627 taxa with significant abundance differences across at least 1 of the categories. **C**, Nearest shrunken centroid analysis of OTUs that best characterize the difference between allergen-sensitized versus tolerant groups. **D**, Representation of the abundance of the OTUs identified by the nearest shrunken centroid analysis using the PAM method.

pyrosequencing analysis revealed the microbiota of the flora-replete mice segregated in accordance with the source of the input microbiota (fecal samples of OVA-sensitized WT vs *Il4raF709* mice; Fig 8, A). Results revealed that the WT GF mice that were reconstituted with flora of OVA-sensitized *Il4raF709* mice but not that of OVA-sensitized WT mice underwent anaphylaxis on challenge with a heightened oral dose of OVA (150 mg), as evidenced by a decrease in core body temperature and increase in serum mMCP-1 concentrations (Fig 8, B and C). Analysis of total and OVA-specific IgE responses revealed that although the total IgE levels were not significantly different between the 2 groups, the OVA-specific IgE response was significantly higher in those mice reconstituted with flora from OVA-sensitized *Il4raF709* mice (Fig 8, D). Further analysis revealed that the microbiota of the mutant mice, but not those of the WT control animals, elicited T_H2 skewing of the OVA-responsive CD4 T cells isolated from the mesenteric lymph nodes (Fig 8, E and F). These results indicate that the dysbiosis in the OVA-sensitized *Il4raF709* mice promoted allergen-specific IgE responses and systemic anaphylaxis, which is consistent with a pathogenic function of the microbiota of allergen-sensitized *Il4raF709* mice in fostering disease.

DISCUSSION

The studies reported herein identified several novel features of the microbiota in food allergy. First, the microbial communities of food allergy-prone *Il4raF709* mice were distinct at baseline from those of congenic but food allergy-resistant WT control mice, which is consistent with the sculpting of the gut microbiota by a genetically driven T_H2 environment. Allergic sensitization of the *Il4raF709* mutant resulted in the further emergence of a food allergy-associated microbiota signature that was virtually nonoverlapping with the changes in the microbiota of similarly sensitized WT mice that otherwise did not have disease. Furthermore, tolerance enforcement by means of treatment with allergen-specific Treg cells prevented the development of the food allergy-associated microbiota signature in *Il4raF709* mice and resulted instead in the emergence of a distinct tolerance-associated signature. Finally, the functional relevance of food allergy-associated dysbiosis was revealed by the differential capacity of the flora of allergen-sensitized *Il4raF709* mice, but not those of sensitization-resistant WT mice, to upregulate OVA-specific T_H2 and IgE responses and to promote anaphylaxis when reconstituted in GF WT mice. Thus allergic dysbiosis

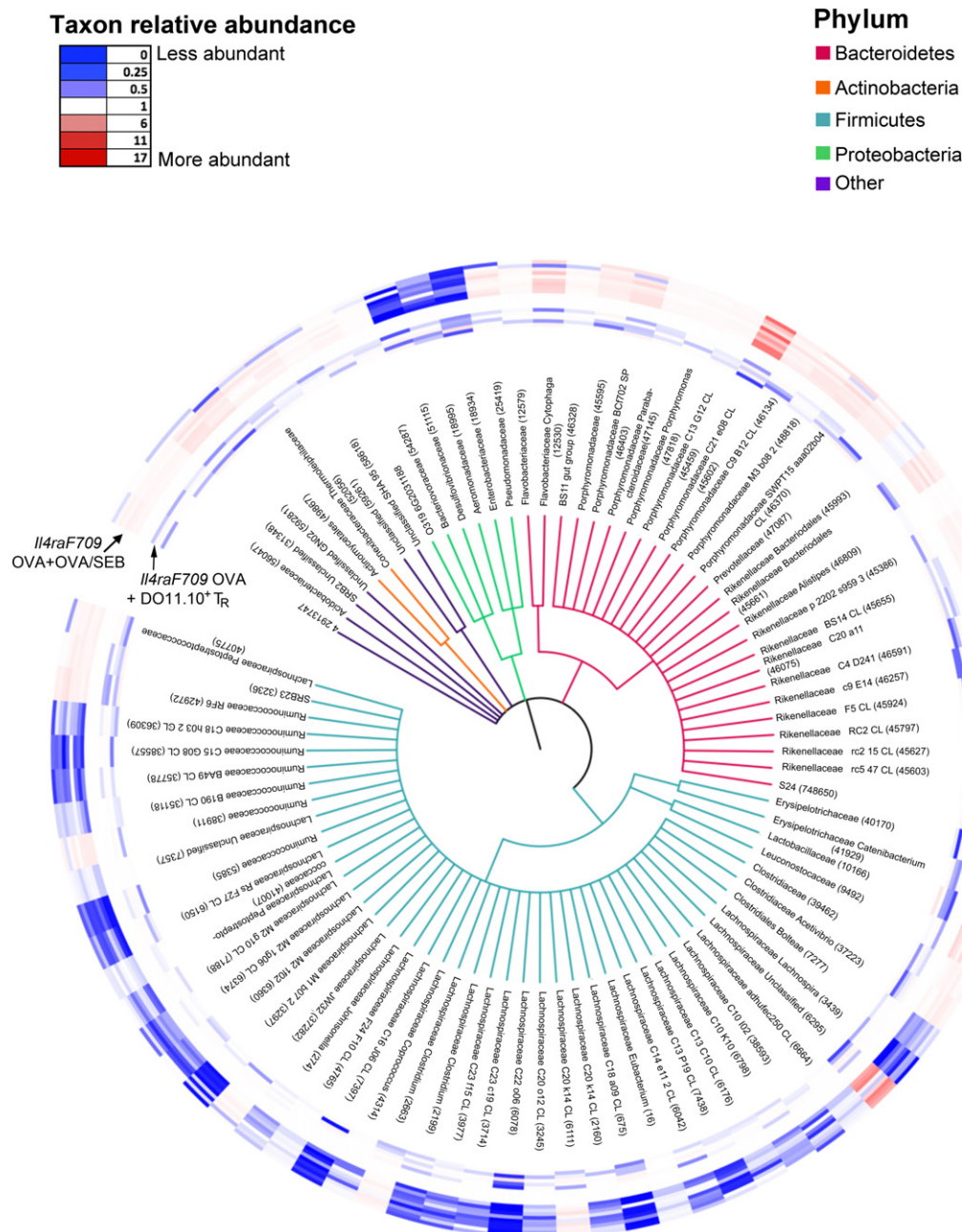


FIG 6. Phylogenetic tree based on 16S rDNA gene sequences of bacterial families with taxa showing significantly different abundances between allergen (OVA+OVA/SEB)-sensitized *Il4raF709* mice and Treg cell-treated OVA-sensitized *Il4raF709* mice shown in Fig 5. KW analysis identified 627 OTUs within 78 families that have significantly different abundance between the comparison groups. One OTU with the greatest difference between the 2 group means from each family was selected. For the 8 families that contained OTUs with both higher and lower abundance scores, both OTUs were selected. The rings around the tree comprise a heat map in which the inner rings represent the samples of tolerized mice and the outer rings represent those of allergen-sensitized mice. The color saturation indicates the degree of difference from the mean value of the sham-treated samples, where *dark blue* indicates a ratio of 0 (least abundant), *white* indicates a ratio of 1.0 (equivalent abundance), and *dark red* indicates a ratio of 17 (most abundant).

provides one mechanism contributing to the pathogenesis of food allergy.

The pathways involved in directing the changes in the microbiota of the food allergy-prone *Il4raF709* mice remain unknown. However, it can be inferred that the altered microbial ecology in *Il4raF709* mice, both at baseline and on sensitization, is related to

the augmented signaling along the IL-4R α -STAT6 axis induced by the F709 substitution.²⁰ Enhanced STAT6 signaling can act through any one of several putative mechanisms to alter the microbiota, including alterations in epithelial-commensal flora interactions in the profile of defensins expressed in the gut (which in turn reflects on the microbiota profile), alterations in other

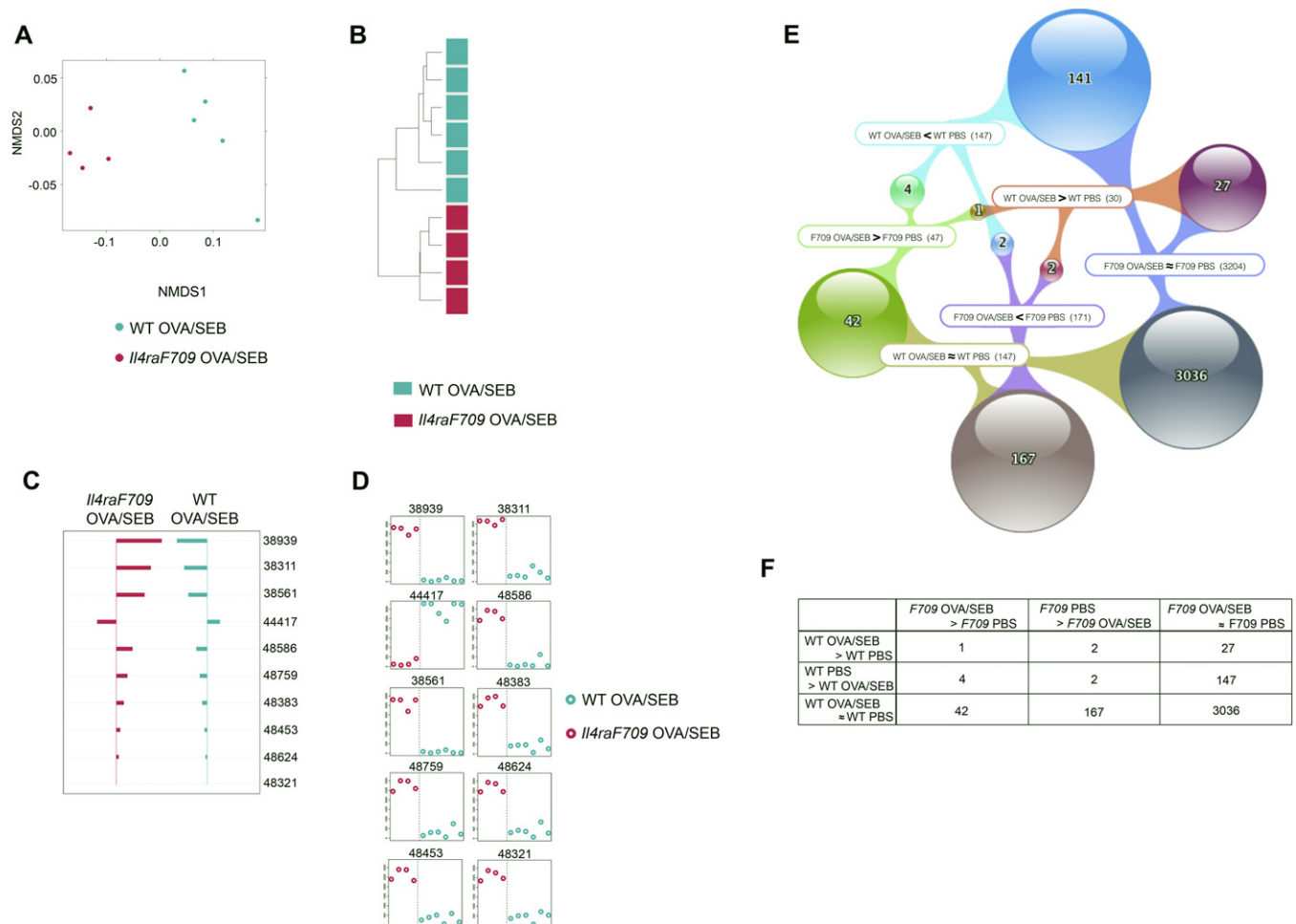


FIG 7. The microbiota signatures of allergen-sensitized *Il4raF709* and WT mice are distinct. **A**, NMDS based on weighted UniFrac distance between samples of OVA/SEB-sensitized WT versus OVA/SEB-sensitized *Il4raF709* mice based on the 352 taxa with significantly different abundance between groups by using the KW test. **B**, Hierarchical clustering based on weighted UniFrac distance between samples. **C**, Nearest shrunken centroid analysis of OTUs that best characterize the difference between the groups. **D**, Representation of the abundance of the OTUs identified by using the nearest shrunken centroid analysis with the PAM method. **E**, Venn diagram showing the abundance levels of different OTUs in relation to the sensitization state of WT and *Il4raF709* mice. The labels define the abundance states of sets of OTUs in relation to specific comparison groups, such as F709 OVA/SEB < F709 PBS, which identifies those OTUs that are less abundant in allergen-sensitized (OVA/SEB) *Il4raF709* mice compared with sham (PBS)-sensitized mice. The number of OTUs thus identified is indicated in parentheses. **Spheres** indicate intersections between 2 sets, and the **colored webs** show which intersection of sets form the spheres. **F**, Contingency table representation of the results shown in the Venn diagram. $P < .0001$ by using the χ^2 test (excluding the 3036 OTUs that did not change on sensitization in both WT and *Il4raF709* mice). Six mice for the WT OVA/SEB group versus 4 mice for the *Il4raF709* OVA/SEB group.

components of the innate and adaptive immune responses relevant to the microbial ecology, or both. Augmented *Il4raF709*-STAT6 signaling can also effect the gut microbiota by augmenting the expression of resistin-like β (*Retnlb*), which is expressed under basal conditions in gut goblet cells in a microbial flora-dependent manner and the expression of which is also induced by T_H2 signaling.³² Our previous studies have documented the exaggerated upregulation of *Retnlb* in the lungs of *Il4raF709* mice undergoing paradigms of allergic airway inflammation.²⁰

An interesting observation that emerges from these studies is the relationship of tolerance induction to the composition of the microbiota. Enforced tolerance resets the microbiota to a new signature distinct from those of both sham- and OVA-sensitized

Il4raF709 mice. Significantly, it could be demonstrated that unlike WT mice, *Il4raF709* mice exhibit defective formation and function of OVA-specific Foxp3⁺ iTreg cells on OVA sensitization (Noval Rivas et al, manuscript in preparation). Thus the loss of regulatory circuits restraining the T_H2 response in the gut might underlie the shifts in the microbial flora both at baseline and on OVA sensitization of the food allergy-prone *Il4raF709* mice.

The full mechanism or mechanisms by which the microbiota of allergen-sensitized *Il4raF709* mice contribute to disease pathogenesis remain to be determined, but previous studies provide insights into how such changes might promote disease. Components of the microbiota have been demonstrated to directly affect

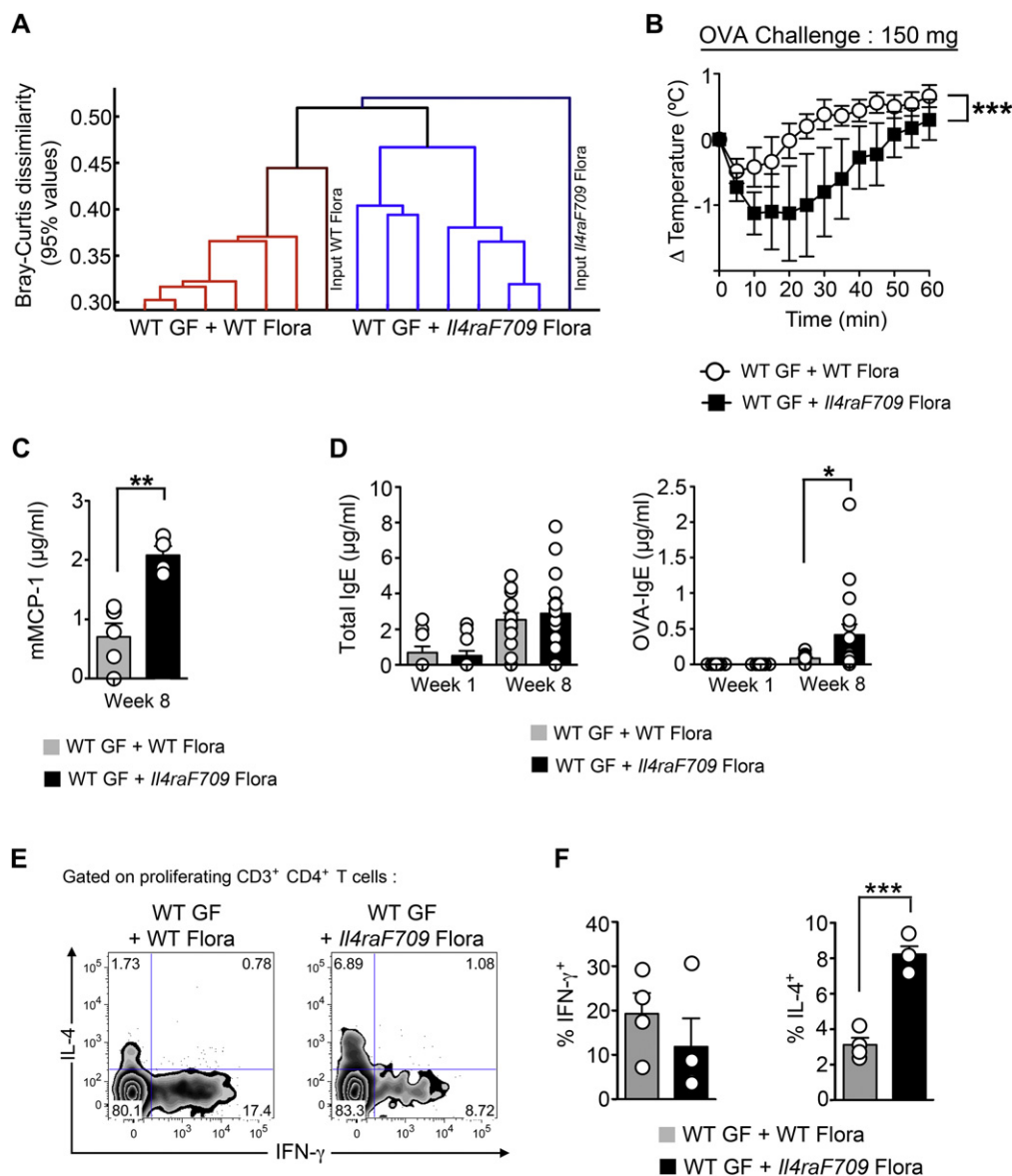


FIG 8. The gut microbiota of allergen-sensitized *Il4raF709* mice promote allergen-specific responses and anaphylaxis. **A**, Agglomerative clustering of donor and recipient stool samples based on 16S OTUs derived from 16S pyrosequencing data. The y-axis represents Bray-Curtis dissimilarity values computed on the relative abundances of OTUs in each sample. A Bray-Curtis value of 0 indicates identical microbial communities, and a Bray-Curtis value of 1 indicates communities with no overlapping OTUs. Heights of lines on the dendrogram indicate Bray-Curtis values at the 95th percentile. **B**, Core body temperature changes in flora-reconstituted, OVA/SEB-sensitized WT GF mice challenged with 150 mg of OVA. The mice were reconstituted with flora from OVA/SEB-sensitized *Il4raF709* or WT BALB/c mice and then sensitized with OVA/SEB and challenged with OVA. Conventional sham- and OVA/SEB-sensitized *Il4raF709* mice were used as control animals. **C**, Serum mMCP-1 levels in flora-reconstituted mice after OVA challenge. **D**, Total and OVA-specific IgE antibody responses in flora-reconstituted mice. **E**, Representative flow cytometric analysis of OVA-specific mesenteric $\text{CD}4^{+}$ T cells of flora-reconstituted GF mice producing IL-4 and IFN- γ . **F**, Enumeration of the percentages of cytokine-producing T cells shown in Fig 8, E. Results represent data from 6 to 12 flora-reconstituted WT GF mice per group derived from 2 independent experiments. * $P < .05$, ** $P < .005$, *** $P < .001$, 1- and 2-way ANOVA with posttest analysis.

the differentiation, function, or both of Treg and effector T cells.^{8,9,33,34} Studies on mechanisms of tolerance induction by the commensal bacterium *Bacteroides fragilis* have demonstrated the capacity of its product polysaccharide A to promote Foxp3 $^{+}$ Treg cell formation by signaling through Toll-like receptor 2 receptors on T cells.³⁵ Loss of polysaccharide A abrogated tolerance induction by *B fragilis* and promoted instead T_H17

formation. These findings raise the possibility that the changes in the commensal flora in *Il4raF709* mice might adversely affect iTreg cell formation and function. Yet another mechanism could involve the direct promotion of allergic inflammation by components of the food allergy-associated microbiota in a manner similar to the promotion of T_H17 cells by segmented filamentous bacteria.³⁶⁻³⁸

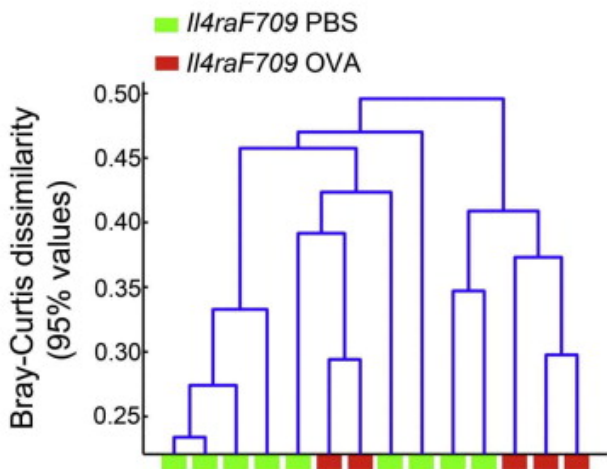
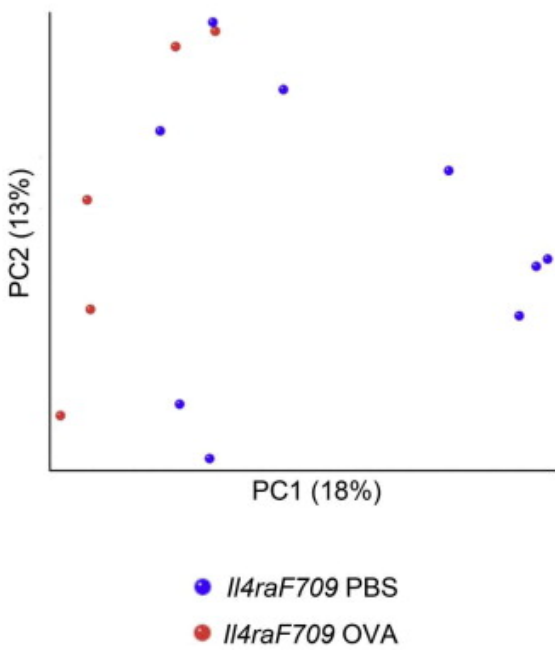
The association of oral allergic sensitization in a food allergy-prone experimental model with specific changes in the microbiota suggests that similar changes might be operative in the human disease. Some previous studies with bacterial cultures and early-generation 16S rDNA assays have intimated that such changes can indeed occur in the human host, although those observations have been conflicted, possibly because of the technical limitations of these methodologies.^{18,19} Profiling of bacterial 16S ribosomal RNA by using rDNA arrays and deep sequencing approaches promises detailed characterization of the microbiota in different subsets of patients with food allergy.³⁹ The results of such approaches might be useful in guiding the diagnosis and classification of different phenotypes of food allergy and in aiding therapeutic interventions, including those with probiotic bacteria, that aim to restore a tolerogenic microbiota.⁴⁰

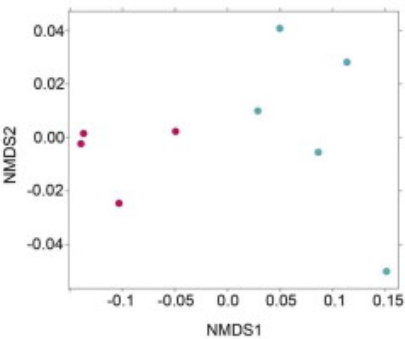
Key messages

- The microbiota of food allergic hosts are altered in a specific, reproducible manner.
- They transmit disease susceptibility to naive, germ-free recipients.
- They are subject to reprogramming upon enforced tolerance with allergen-specific regulatory T cells.

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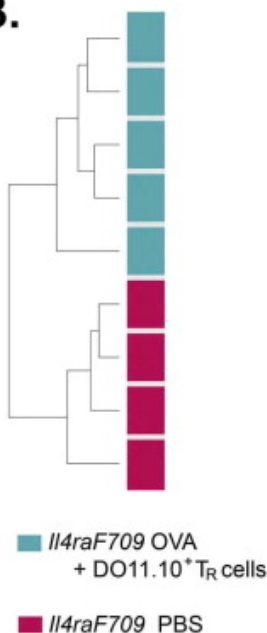
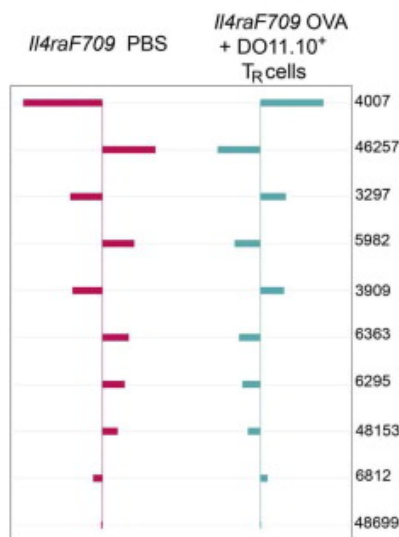
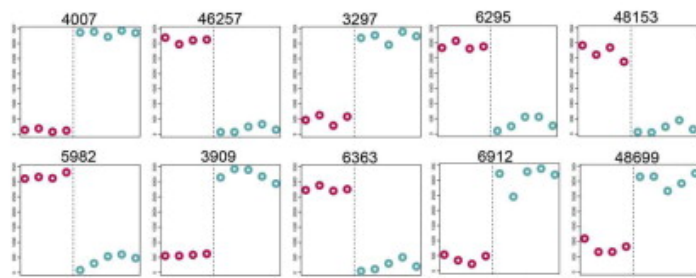
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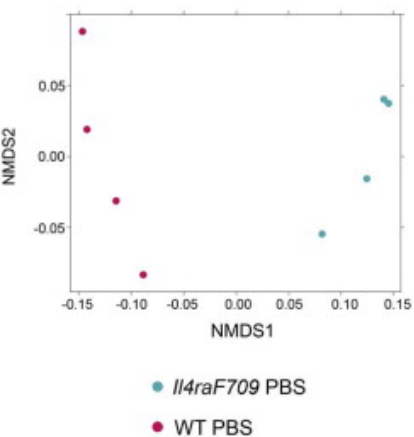
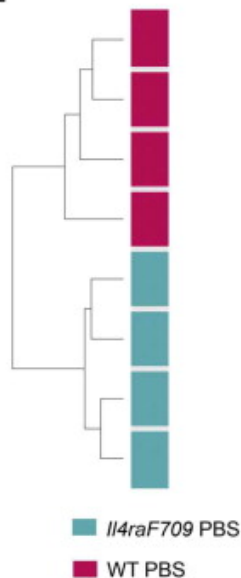
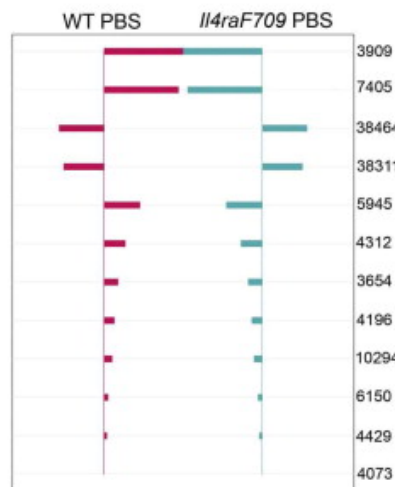
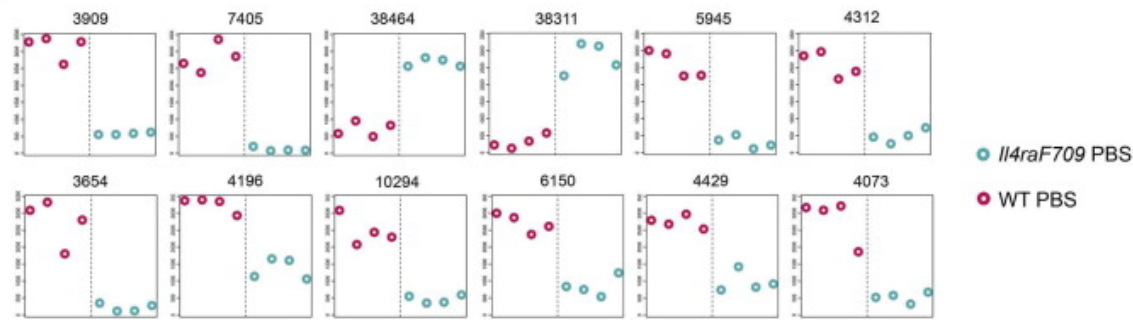
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● *Il4raF709* PBS

B.**C.****D.**

● *Il4raF709* OVA + DO11.10⁺ T_R cells

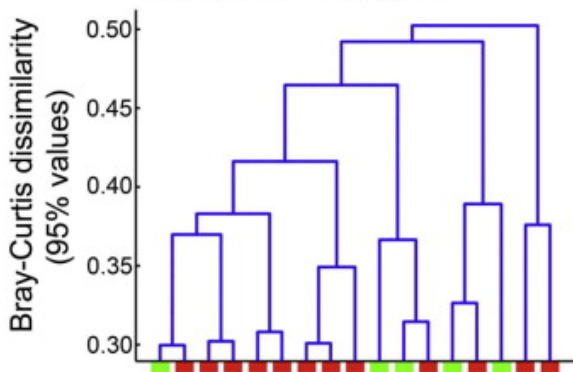
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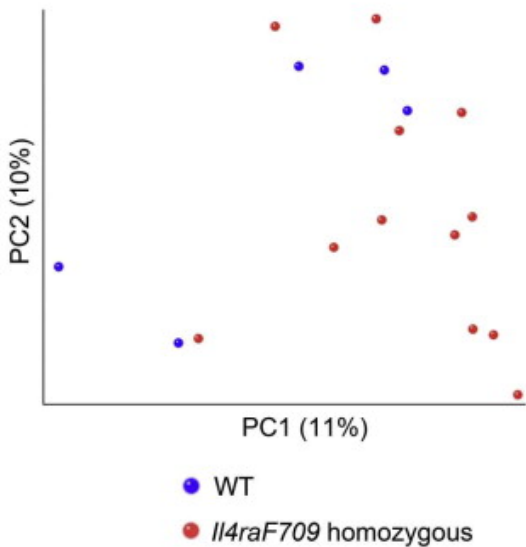
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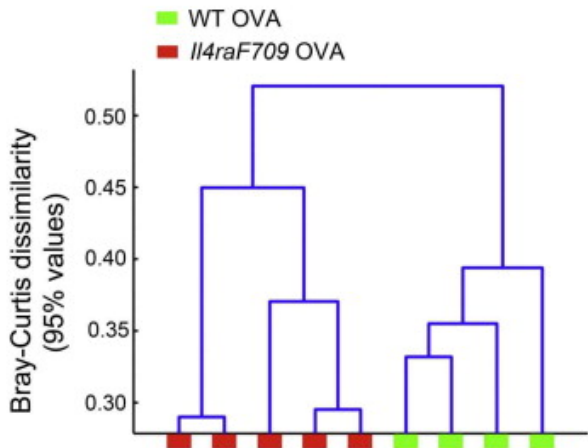
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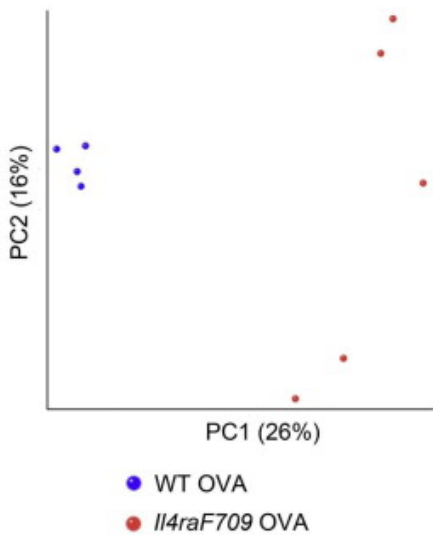
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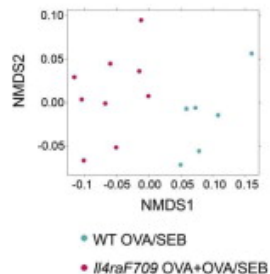
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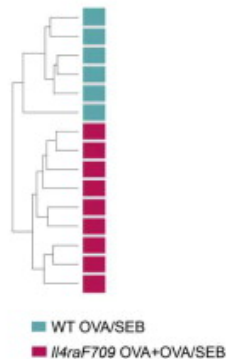
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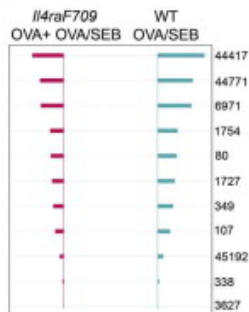
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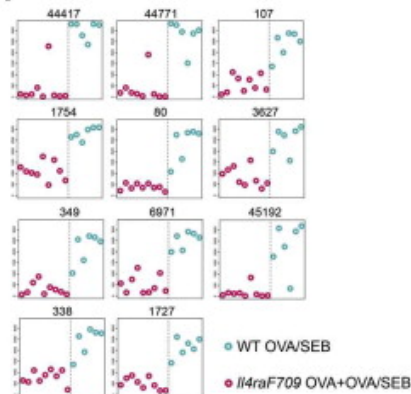
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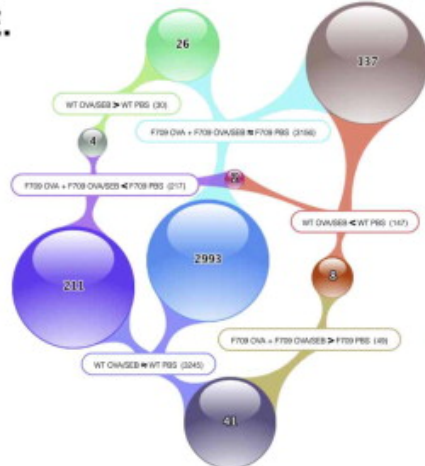
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F.

	<i>F709</i> OVA+OVA/SEB > <i>F709</i> PBS	<i>F709</i> PBS > <i>F709</i> OVA+OVA/SEB	<i>F709</i> OVA+OVA/SEB = <i>F709</i> PBS
WT OVA/SEB > WT PBS	0	4	26
WT PBS > WT OVA/SEB	8	2	137
WT OVA/SEB = WT PBS	41	211	2993

Table E1

OTU	Phylum	Class	Order	Family	Genus
48153	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes
46187	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified
6293	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia
48369	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified
48699	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified
48371	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella
48270	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified
48802	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella
48689	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella
44071	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides

OTU id	Phylum	Class	Order	Family	Genus	Species	GenBank Acc	Greengenes ID	pvals_good	meanF709P BS Group	meanF709OVA+ OVA /SEB Groups
1003	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	unclassified	EF096485	192000	0.030753561	1760	583.8888889
1727	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	AY992372	133313	0.005478553	1274.5	505.4444444
1920	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Marvinbryantia	unclassified	EF097989	194878	0.013554666	476.25	1395.666667
2199	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Dorea	unclassified	EU510853	265754	0.005478553	789.25	298.8888889
2293	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	AY991947	130302	0.005478553	1404	682.1111111
2727	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	DQ793370	180257	0.030753561	864.5	338.1111111
2933	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	unclassified	DQ808786	192305	0.008711913	2282.25	1487
3204	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU503959	260375	0.013554666	2064.5	1707.888889
3508	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU462087	295880	0.013554666	1038.25	502.2222222
3739	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF096332	181789	0.020637558	671.25	1912.444444
3887	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyri vibrio	unclassified	DQ794828	197557	0.008620018	1601.25	708.4444444
4721	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unclassified	DQ804371	184276	0.005478553	1454.25	879.3333333
5512	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU344425	277063	0.044568465	1278	938.2222222
5963	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU508687	259665	0.005478553	2311	1592.222222
6052	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium	unclassified	EU511783	272969	0.005478553	1034.5	409.8888889
6153	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU505504	267763	0.013554666	1183.75	811.2222222
6808	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	unclassified	EF098644	199674	0.030524232	78	333.1111111
7405	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU504971	264472	0.044862271	115.5	1132.444444
9680	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	unclassified	EU504798	273339	0.036982017	57.25	1728.222222
12142	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Saprospiraceae	unclassified	unclassified	EF688369	243774	0.030753561	1343	562.5555556
13243	Elusimicrobia	Elusimicrobia	Rs-M47	unclassified	unclassified	unclassified	AB089107	100887	0.005478553	2927.75	2571.555556
18934	Proteobacteria	Gammaproteobacteria	B38	unclassified	unclassified	unclassified	EF406662	214508	0.020637558	526	173.3333333
35554	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	unclassified	EF098196	197863	0.030753561	1889.25	2477.222222
36926	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	unclassified	DQ804803	193505	0.044862271	2329.25	1999.222222
38766	Firmicutes	Clostridia	Clostridiales	Catabacteriaceae	unclassified	unclassified	DQ815614	166791	0.013554666	2606.75	1090.777778

38832	Firmicutes	Clostridia	Clostridiales	Catabacteriaceae	unclassified	unclassified	EU508608	276402	0.030753561	2743.5	2247.888889
39465	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Sarcina	unclassified	EU774113	352729	0.030753561	2271.25	1751.777778
41031	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF436322	206909	0.005478553	2155	1460.666667
42301	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU465568	290338	0.030753561	2052	2566.666667
44071	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unclassified	DQ793474	196596	0.005478553	1799.25	664.3333333
45444	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EU655810	284926	0.008711913	3379.75	2986.666667
45986	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF603052	232866	0.005478553	3281.5	3152.777778
46111	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF097070	190752	0.013554666	1444.75	500.2222222
46187	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF603016	228844	0.013554666	2456	582.7777778
46257	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	AY990511	133135	0.008711913	3098.5	1568.555556
46312	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EU006270	245595	0.013554666	2978.25	3310.666667
46591	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF603044	227767	0.044862271	2253.5	1586.222222
46917	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	unclassified	AY991932	133674	0.044862271	1606	3016.777778
48153	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	unclassified	EU655823	280240	0.005478553	2688.5	747.4444444
48287	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF604721	233799	0.013554666	872.75	2167.222222
48371	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	AY992812	135659	0.005478553	1518.75	2784.888889
48689	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	EF096787	181385	0.005478553	1087	2471.666667
55191	Proteobacteria	Deltaproteobacteria	Sh765B-TzT-29	unclassified	unclassified	unclassified	EU491396	268600	0.030753561	2055	1645.111111
59023	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Helicobacter	Helicobacter hepaticus	EU508580	270331	0.008711913	1718.75	931.7777778

OTU id	Phylum	Class	Order	Family	Genus	Species	GenBank Acc	Greengenes ID	pvals_good	meanF709 PBS Group	meanF709OVA+ OVA /SEB Groups
1003	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	unclassified	EF096485	192000	0.030753561	1760	583.8888889
1727	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	AY992372	133313	0.005478553	1274.5	505.4444444
1920	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Marvinbryantia	unclassified	EF097989	194878	0.013554666	476.25	1395.666667
2199	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Dorea	unclassified	EU510853	265754	0.005478553	789.25	298.8888889
2293	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	AY991947	130302	0.005478553	1404	682.1111111
2727	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	DQ793370	180257	0.030753561	864.5	338.1111111
2933	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	unclassified	DQ808786	192305	0.008711913	2282.25	1487
3204	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU503959	260375	0.013554666	2064.5	1707.888889
3508	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU462087	295880	0.013554666	1038.25	502.2222222
3739	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF096332	181789	0.020637558	671.25	1912.444444
3887	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyri vibrio	unclassified	DQ794828	197557	0.008620018	1601.25	708.4444444
4721	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unclassified	DQ804371	184276	0.005478553	1454.25	879.3333333
5512	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU344425	277063	0.044568465	1278	938.2222222
5963	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU508687	259665	0.005478553	2311	1592.222222
6052	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium	unclassified	EU511783	272969	0.005478553	1034.5	409.8888889
6153	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU505504	267763	0.013554666	1183.75	811.2222222
6808	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	unclassified	EF098644	199674	0.030524232	78	333.1111111
7405	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU504971	264472	0.044862271	115.5	1132.444444
9680	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	unclassified	EU504798	273339	0.036982017	57.25	1728.222222
12142	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Saprospiraceae	unclassified	unclassified	EF688369	243774	0.030753561	1343	562.5555556
13243	Elusimicrobia	Elusimicrobia	Rs-M47	unclassified	unclassified	unclassified	AB089107	100887	0.005478553	2927.75	2571.555556
18934	Proteobacteria	Gammaproteobacteria	B38	unclassified	unclassified	unclassified	EF406662	214508	0.020637558	526	173.3333333
35554	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	unclassified	EF098196	197863	0.030753561	1889.25	2477.222222
36926	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	unclassified	DQ804803	193505	0.044862271	2329.25	1999.222222
38766	Firmicutes	Clostridia	Clostridiales	Catabacteriaceae	unclassified	unclassified	DQ815614	166791	0.013554666	2606.75	1090.777778

38832	Firmicutes	Clostridia	Clostridiales	Catabacteriaceae	unclassified	unclassified	EU508608	276402	0.030753561	2743.5	2247.888889
39465	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Sarcina	unclassified	EU774113	352729	0.030753561	2271.25	1751.777778
41031	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF436322	206909	0.005478553	2155	1460.666667
42301	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU465568	290338	0.030753561	2052	2566.666667
44071	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unclassified	DQ793474	196596	0.005478553	1799.25	664.3333333
45444	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EU655810	284926	0.008711913	3379.75	2986.666667
45986	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF603052	232866	0.005478553	3281.5	3152.777778
46111	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF097070	190752	0.013554666	1444.75	500.2222222
46187	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF603016	228844	0.013554666	2456	582.7777778
46257	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	AY990511	133135	0.008711913	3098.5	1568.555556
46312	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EU006270	245595	0.013554666	2978.25	3310.666667
46591	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF603044	227767	0.044862271	2253.5	1586.222222
46917	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	unclassified	AY991932	133674	0.044862271	1606	3016.777778
48153	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	unclassified	EU655823	280240	0.005478553	2688.5	747.4444444
48287	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF604721	233799	0.013554666	872.75	2167.222222
48371	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	AY992812	135659	0.005478553	1518.75	2784.888889
48689	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	EF096787	181385	0.005478553	1087	2471.666667
55191	Proteobacteria	Deltaproteobacteria	Sh765B-TzT-29	unclassified	unclassified	unclassified	EU491396	268600	0.030753561	2055	1645.111111
59023	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Helicobacter	Helicobacter hepaticus	EU508580	270331	0.008711913	1718.75	931.7777778

Table E3

OTUs	Change
Clostridium	Up in OVA-sensitized mice
Bacteroides	Up in OVA-sensitized mice
Alistipes	Up in OVA-sensitized mice
Bacteroides	Up in OVA-sensitized mice
Streptococcus	Up in PBS-sensitized mice

OTU ID	Phylum	Class	Order	Family	Genus	Species	GenBank Acc	Greengenes ID	pvals_good	meanF709OVA+ D011.10+ TR Cell Group	meanF709OVA+O VA /SEB Groups
16	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF096140	191785	0.002641739	3389.6	3064.111111
274	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	AY984484	126076	0.009322376	3043.4	2372.777778
675	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF096987	194051	0.013637725	1856	435.5555556
2160	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU506487	260705	0.009322376	2054.2	1291.555556
2199	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	unclassified	EU510853	265754	0.002699796	752.2	298.8888889
2663	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium	unclassified	AY992468	129526	0.038765574	1554.6	2167.555556
3245	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	AY992440	135500	0.013637725	2957.8	2028.444444
3297	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	DQ014740	135329	0.002699796	3205	844
3439	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	unclassified	EF099065	197605	0.004148197	2093.4	692.5555556
3714	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium	unclassified	EU504190	258283	0.006269685	3028.6	1783.555556
3977	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU655820	282552	0.002699796	2718.6	1440.111111
4314	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU472497	297677	0.002670671	3383.8	2559.777778
4765	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU845293	335032	0.006269685	3346.2	3142
5385	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unclassified	EU467221	290412	0.004148197	1279.4	346.2222222
6042	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU510566	277090	0.038765574	1327	817.7777778
6078	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	DQ014905	131066	0.009167784	1209	128
6111	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium	unclassified	EU510550	270765	0.027806895	801	1326
6150	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU655796	283186	0.006269685	2653.6	1200.777778
6176	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU506249	268844	0.038765574	3093.4	2437.888889
6295	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF098339	180805	0.006212643	344.8	1944.888889
6360	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	DQ015311	131283	0.019630657	2851.4	1697.888889
6374	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU508022	269317	0.002699796	2653.6	1479.222222
6664	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium	unclassified	DQ824714	179311	0.002699796	3398.6	3062.444444
6798	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU504406	264803	0.019630657	1895	1374.444444
7188	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	AY990633	129785	0.038765574	3072.4	2189
7277	Firmicutes	Clostridia	Clostridiales	Clostridiales	Clostridium	bolteae	AY990174	134932	0.004148197	3047.6	2225.222222
7357	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU510660	263382	0.002584451	3411.2	3107
7397	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	DQ015661	137692	0.038765574	1313.8	286.4444444

7438	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU511837	260533	0.00924492	2692.4	1110
9492	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus	unclassified	AY169967	101369	0.019630657	592.2	1139.333333
10166	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	unclassified	DQ815579	167922	0.038765574	681.4	1657.666667
12530	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Sediminicola	unclassified	EF157264	238644	0.027806895	530.6	1566.333333
12579	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	unclassified	unclassified	EU003223	253574	0.019630657	2083	2592.888889
13747	Nitrospirae	Nitrospira	Nitrospirales	29-Apr	unclassified	unclassified	AM943596	261009	0.038765574	1561.6	2184.444444
18934	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	unclassified_Enterobacteriaceae	unclassified	EF406662	214508	0.013637725	712.6	173.3333333
18995	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	unclassified	DQ303266	143929	0.038551579	1787.8	1035.333333
25419	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	unclassified	EU537183	287223	0.019630657	653	1115.222222
31188	Proteobacteria	Deltaproteobacteria	Myxococcales	0319-6G20	unclassified	unclassified	EU373907	264962	0.027806895	1381.4	1875.111111
31348	Fibrobacteres	Fibrobacteria	P.palm C70	unclassified	unclassified	unclassified	EU283593	278037	0.027806895	2141.8	2681.777778
33236	Firmicutes	Clostridia	Thermoanaerobacterales	SRB2	unclassified	unclassified	AM778006	238092	0.013637725	1314	2051.777778
35118	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	unclassified	EU474940	289731	0.006269685	2636.4	2080.333333
35778	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillibacter	unclassified	EU508276	264785	0.019630657	2480.8	1229.666667
36309	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	unclassified	unclassified	EU622715	279949	0.027806895	2414.2	1521.666667
37223	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	unclassified	AB298753	213289	0.027806895	1317.6	2073.333333
37282	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU459375	303222	0.038765574	1353.6	1735.888889
38557	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Eubacterium	unclassified	EU006202	236392	0.019496367	2399.2	1037.555556
38593	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EF406702	214116	0.006269685	3064.8	1826.111111
38911	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	unclassified	unclassified	EU465630	300458	0.019630657	1037.4	1780.111111
39462	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae 1	Clostridium	AF332717	46741	0.019630657	1375	1970.111111
40170	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum	unclassified	EF099557	194080	0.009322376	2584.4	658.4444444
40775	Firmicutes	Clostridia	Clostridiales	unclassified XIII	Anaerovorax	unclassified	EU344440	277051	0.013637725	831	1199.666667
41007	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	EU748342	308321	0.038765574	3049.6	2802.555556
41929	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Catenibacterium	unclassified	EU469379	296667	0.013637725	1880.6	934.2222222
42972	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	unclassified	unclassified	EU470107	299345	0.004148197	1161	2261

45386	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	RC9 gut group	unclassified	EU469558	298032	0.013637725	1795.2	2552.333333
45459	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	EF406544	208409	0.006269685	2244.4	2911.444444
45595	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	EF098500	194770	0.002699796	1223.4	2546.555556
45602	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	unclassified_Porphyromonadaceae	unclassified	EF406873	208486	0.002699796	272.4	1913.666667
45603	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF099607	182382	0.002699796	1572.2	2710.333333
45627	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	AY239412	97151	0.004148197	814.2	1924.888889
45655	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EU470252	295171	0.038765574	1584	2208
45661	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	RC9 gut group	unclassified	EU468670	295780	0.004148197	884.4	1909.111111
45797	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF686575	249825	0.019630657	1294	2209.444444
45924	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF099074	180126	0.002699796	531.8	1681
45993	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	RC9 gut group	unclassified	EU468654	298356	0.019496367	3316.6	3189.444444
46075	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	AY991717	130984	0.019630657	1124.8	2207.666667
46134	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	EF098889	180729	0.002699796	1183.4	2106.111111
46257	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	AY990511	133135	0.006212643	177.8	1568.555556
46328	Bacteroidetes	Bacteroidia	Bacteroidales	BS11 gut group	unclassified	unclassified	EU469555	302918	0.038765574	2039.4	2529.444444
46370	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Barnesiella	unclassified	EF099501	193408	0.013637725	908.4	2039.888889
46403	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Candidatus Symbiothrix	unclassified	AB192209	144106	0.013637725	808.8	1915.777778
46591	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	unclassified	unclassified	EF603044	227767	0.002670671	468.4	1586.222222
46809	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	unclassified	EU510354	270248	0.013534674	3098	3265.888889
47087	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	unclassified	EU475110	301251	0.006269685	1108	2044.222222
47145	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	unclassified	EU463133	294104	0.019630657	2030	2509.777778
47818	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	unclassified	EU289079	246308	0.013637725	2318	2888.111111
48650	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	unclassified	unclassified	EF097666	178662	0.006269685	2051.4	2689.222222
48818	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	unclassified_Porphyromonadaceae	unclassified	EF614751	227615	0.004148197	933.2	1931.555556
49867	Actinobacteria	Actinobacteria	Actinobacteridae	Actinomycetales	Frankineae	unclassified	EF016820	254924	0.038765574	868.2	1624.777778
51115	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	LE30	unclassified	DQ815907	170836	0.027806895	1947	753.555556

52056	Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	unclassified	unclassified	EU132646	243412	0.032705381	1048.4	1530.777778
54287	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoraceae	Peredibacter	unclassified	AM991200	278094	0.023258499	2300.4	2612.333333
56047	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	unclassified	unclassified	EU050885	256652	0.027806895	1954	2515
58618	Candidate division OD1	unclassified	unclassified	unclassified	unclassified	unclassified	EU385818	266622	0.038765574	2161.8	2581
59261	Candidate division OD1	unclassified	unclassified	unclassified	unclassified	unclassified	EU385705	265484	0.019630657	1247.8	1986.444444
59281	GN02	GN07	unclassified	unclassified	unclassified	unclassified	DQ234160	144617	0.027806895	833.8	1679.555556

Table E6

OTU	Phylum	Class	Order	Family	Genus
4007	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
46257	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
3297	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
5982	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
3909	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
6363	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
6295	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
48153	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes
6912	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
48699	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified

Table E6

OTU	Phylum	Class	Order	Family	Genus
4007	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
46257	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
3297	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
5982	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
3909	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
6363	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
6295	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
48153	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes
6912	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
48699	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified

Table E8

OTU	Phylum	Class	Order	Family	Genus
38939	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified
38311	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified
38561	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified
44417	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
48586	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48759	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48383	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48453	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48624	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48321	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unclassified

Table E8

OTU	Phylum	Class	Order	Family	Genus
38939	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified
38311	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified
38561	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified
44417	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
48586	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48759	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48383	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48453	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48624	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
48321	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unclassified

Table E9

OTUs	Change
Alistipes	Up in WT OVA-sensitized mice
Alistipes	Up in WT OVA-sensitized mice
Clostridium	Up in WT OVA-sensitized mice
Anaeroplasma	Up in WT OVA-sensitized mice
Lachnobacterium	Up in WT OVA-sensitized mice
Clostridium	Up in Il4raF709 OVA-sensitized mice
Clostridium	Up in Il4raF709 OVA-sensitized mice
Helicobacter	Up in Il4raF709 OVA-sensitized mice
Bacteroides	Up in Il4raF709 OVA-sensitized mice
Bacteroides	Up in Il4raF709 OVA-sensitized mice
Bacteroides	Up in Il4raF709 OVA-sensitized mice
Clostridium	Up in Il4raF709 OVA-sensitized mice