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The Role of Early-Life Gut Microbiota Development and Functions in Protection Against Childhood Allergic Asthma Development

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The Role of Early-Life Gut Microbiota Development and Functions in Protection Against Childhood Allergic Asthma Development

by
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The Role of Early-Life Gut Microbiota Development and Functions in Protection Against Childhood Allergic Asthma Development

Ariane Renee Panzer

ABSTRACT

Allergic asthma is a chronic inflammatory disease characterized by Th2 inflammation and elevated circulating immunoglobulin E (IgE) concentrations.[1] Although risk alleles associated with atopy and asthma have been identified, the rapid increase in prevalence, particularly amongst school-aged children in industrialized nations, cannot be explained by host genetics alone.[2] This indicates that environmental factors must play a role in disease development.

Dog ownership represents an early-life environmental exposure associated with a decreased relative-risk of atopy[3] and lower levels IgE in childhood.[4,5] Alterations in the gut microbiome in early life have also been linked to development of childhood atopy and asthma, thus we hypothesized that the reduced risk of atopy associated with dog ownership may be due in part to changes in gut microbial composition and development. To determine the impact of dog exposure on the gut microbiome over the first year of life we performed 16S rRNA gene bacterial profiling focusing on the V4 region in longitudinally collected infant stool samples (1 week, and 1, 3, 6, and 18 months) from the children of mothers living with ≥1 dog in the home for ≥6 months pre-delivery or mothers living in a pet-free home. We found that prenatal and early-life indoor dog exposure associates with increased gut microbial richness ($p=0.046$) and diversity ($p=0.036$) during infancy, with the latter being most apparent between 3 and 6 months of age. Several organisms with immunomodulatory capacities including *Ruminococcaceae*, *Lachnospiraceae*, and *Clostridiaceae* were found to be enriched

across the sampling period in dog-exposed infants. Interestingly, statistically significant effects of dog exposure on β-diversity metrics were restricted to formula-fed children suggesting that in the absence of breast milk, dogs may provide an alternative source of environmental microbes that influence development of the infant gut microbiome. Thus, the emerging data suggests that early life dog exposure enriches microbes in the gut capable of modulating responses to inflammatory stimuli.

Differences in gut microbiota community composition[6–8] and changes in the fecal and urinary metabolic microenvironment in early life[6–8] have also been shown to precede development of childhood asthma. Additionally, cell-free fecal products from low-risk for asthma infants[6] and from high-risk for asthma infants supplemented with a *Lactobacillus rhamnosus* GG (LGG) probiotic during the first 6 months of life[8] have been shown to promote T regulatory (Treg) cell expansion *ex vivo*. While these studies highlight the importance of microbial metabolites in promoting early-life immune tolerance, the specific metabolites contributing to this effect remain unknown. We thus used shotgun metagenomic sequencing and metabolomics to determine gut microbiota functional features that distinguish healthy controls (HC) from high-risk for asthma (HRP) infants. We observed divergent amino acid biosynthesis and metabolism in the gut microbiomes of HC compared to HRP infants. Specifically, HC infant microbiomes were statistically significantly enriched for ornithine biosynthesis and the metabolite L-ornithine while HRP infants showed enrichment for ornithine degradation. We next sought to examine the impact of L-ornithine or the HC fecal metabolic milieu on immune cell phenotypes. These experiments remain ongoing.

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CHAPTER 1: INTRODUCTION

1.1 ASTHMA

1.1.1 Clinical Features & Global Impact of Asthma

Asthma is a heterogeneous disease characterized by chronic inflammation that leads to periodic swelling and narrowing of the airway ultimately causing difficulty breathing, coughing, wheezing, and chest tightness.[9] Although epidemiological and basic science studies have led to improvements in asthma management and a decrease in asthma mortality rates[10], this chronic inflammatory disease remains a significant global public health concern. In the United States, the Center for Disease Control reported a 1.5% increase per year in asthma prevalence between 2001 (7.3%) and 2010 (8.4%).[10] Worldwide, it is estimated that by the year 2025 400 million people will suffer from asthma.[11] Asthma significantly reduces quality of life, confers a substantial economic burden on health care systems[11,12], and disproportionately impacts low-income groups, people of color, and children.[13]

In the US, the majority of asthma cases develop during preschool years[14], with a common risk factor being sensitization to multiple allergens by age 2.[15] Sensitization, also known as atopy, is defined as having one or more allergen-specific serum immunoglobulin E (IgE) level(s) of 0.35 IU/mL or greater.[15] Serum IgE antibody responses against common dietary and inhalant allergens develop over the first few years of life.[16] In non-atopic children, allergen-specific IgE levels fluctuate over these years but typically remain below the sensitization threshold and eventually stabilize.[17,18] However, in atopic individuals allergen-specific IgE levels continue to rise[17,18] and can result in early-life allergic asthma development that persists into adulthood.

Indeed, IgE-mediated allergic asthma is one of the most prevalent types of asthma accounting for over 50% of adult cases and 80% of childhood cases.[19] This type

of asthma involves a heightened IgE response to allergens; an inappropriate inflammatory response commonly characterized by $CD4^+$ T helper type 2 (Th2) cell activation and accompanied by eosinophilic infiltrates; and is not managed appropriately by regulatory immune cells. In the asthmatic airway, chronic inflammation is a result of an inappropriate immune response to non-pathogenic exposures and this inflammation continues even in the absence of the allergen.[1]

1.1.2 The Basic Immunology of Allergic Asthma

Lung epithelial cells are generally the first cell type to recognize and respond to inhaled allergens via surface expression of pattern recognition receptors such as toll-like receptors (TLRs).[9] Allergen-induced activation of these receptors leads to the production of cytokines such as interleukin-33 (IL-33), thymic stromal lymphopoietin (TSLP), interleukin-1 alpha, or granulocyte-macrophage colony stimulating factor (GM-CSF) which target dendritic cells (DCs).[9] Upon activation, these DCs migrate to the lung-draining lymph nodes to promote Th2 cell development. DC priming of the Th2 response has been well established.[9] Indeed, studies have shown that following *in vitro* allergen exposure both adoptively transferred bone-marrow-derived myeloid DCs[20,21] and lung-derived DCs[21–23] induce Th2 responses in murine models. Th2 cells are defined by the expression of the transcription factors GATA3[24] and signal transducer and activator of transcription (STAT) 6[25] as well as chemokine receptors such as CCR3[26], CCR4[27], and CCR8.[27] These Th2 cells interact with local B cells and promote maturation while the Th2-associated cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) induce IgE production.[9]

This IgE circulates through the bloodstream and eventually binds with high affinity to the constitutively expressed Fc epsilon Receptor I (FcεRI) on mast cells located in the airway submucosa.[28] Upon subsequent inhalation of the allergen, antigen can crosslink adjacent, receptor-bound IgE molecules on mast cells leading to cell activation and release of granules containing bioactive molecules such as the anticoagulant histamine and the cytokine tumor necrosis factor alpha (TNFα).[29] This marks the early-phase response in which histamine increases vascular permeability and induces constriction of bronchial smooth muscle while TNFα activates and increases adhesion molecule expression on endothelial cells ultimately leading to the recruitment of inflammatory leukocytes and lymphocytes to the lung.[29] Activated mast cells also generate and release: Th2 promoting cytokines, such as IL-4 and IL-13 – which promote the cycle of inflammation; chemokines – which recruit monocytes, neutrophils, and macrophages; and leukotrienes – which recruit and activate additional inflammatory leukocytes and promote further smooth muscle contraction as well as mucus production.[29]

The late-phase asthmatic response is characterized by Th2 effector cell and inflammatory eosinophil recruitment.[29] Th2 cells secrete IL-13 and IL-5 which promote eotaxin production in epithelial cells leading to inflammatory eosinophil infiltration from the blood-stream into the lung.[1,9] In the presence of IL-5, these recruited eosinophils are activated and release cytotoxic granule proteins such as major basic protein and eosinophil peroxidase which evolved to protect against parasitic infections but in this context also cause damage to the lung tissue.[1,9] Additionally, these eosinophils synthesize proinflammatory mediators such as prostaglandins, leukotrienes, and

lipoxins as well as Th2 promoting cytokines which can perpetuate injury to airway vascular tissue, negatively impact smooth muscle reactivity, and promote further influx of inflammatory cells.[29] IgE can also bind FcεRI on DCs and cause allergen presentation and restimulation of Th2 memory cells.[9] Importantly, this type of allergy presentation rapidly stimulates Th2 cell responses even in the presence of low levels of allergen and further stimulates allergen-specific IgE production by B cells.[9] Repetitive tissue damage and inflammatory cell infiltration can lead to persistent inflammation and these pathogenic mechanisms contribute to the development of a chronic condition.[1]

1.1.3 The Role of T regulatory Cells in Managing Airway Inflammation & Asthma

Pro-inflammatory mechanisms drive the chronic inflammation that is the hallmark of allergic asthma, but lack of appropriate suppressive immune responses to resolve inflammation are another contributing factor. The ability of cells to suppress immune responses associated with antigen inhalation in the context of respiratory allergy was first described in the 1980s. Holt *et al.* (1981) demonstrated that intranasal administration of ovalbumin (OVA) led to OVA-specific IgE synthesis that diminished despite continued exposure to the allergen. Additionally, even under maximal antigenic stimulation (parenteral injection with OVA and adjuvant) these mice still exhibited tolerance. The authors then showed that this tolerance had a cellular basis as mice receiving spleen cells from OVA-primed donor animals exhibited suppressed IgE production following OVA challenge.[30] However, following adoptive transfer of splenocyte cultures depleted of T cells the suppressive effect was no longer observed.[31]

Future studies determined that these $CD4^+$ T regulatory (Treg) cells are further characterized by increased expression of CD25, the alpha chain of the IL-2 receptor[32]

(IL-2Rα), and can be of either thymic origin (tTregs) or induced in the periphery (iTregs). tTregs are derived from CD4 single positive thymocytes expressing T cell receptors (TCRs) that have an intermediate affinity to self-antigen (stronger affinity than a naive T cell, weaker affinity than cells that go on to be clonally deleted) presented by Major Histocompatibility Complex II (MHCII).[33] Alternatively, iTregs are derived from naive $CD4^+$ T cells in the presence of cytokines such as TGF-β and IL-2 and following antigen-stimulated TCR activation.[34] In addition to being $CD4^+CD25^+$ both tTregs and iTregs also express the transcription factor Forkhead box protein P3 (FoxP3).[32,35,36]

The importance of Treg function in allergic airway disease has been well established in animal models. Transfer of OVA-specific $CD4^+CD25^+$ T cells to sensitized donor mice 24 hours before airway challenge led to a decrease in hallmark features of allergic airway inflammation including eosinophil infiltration and Th2 cytokine production.[37] Importantly, $CD4^+CD25^+$ T cells administered after allergic sensitization are also able to dampen airway remodeling and inflammation, indicating the importance of this cell type in chronic inflammatory models as well.[38]

Furthermore, depletion of this regulatory T cell population in mice using anti-CD25 not only led to exacerbated inflammation and airway hyperresponsiveness but also increased the number of DCs in the airways expressing activation markers capable of promoting T effector cell proliferation.[39] Later studies using techniques that deplete regulatory cell populations that are also $FoxP3^+$ corroborated these findings. Indeed, depleting Tregs during the initial phase of allergic sensitization increased eosinophil infiltration in bronchoalveolar lavage fluid (BAL) and increased IL-4 and IL-5 cytokine secretion by mediastinal lymph node cells.[40]

Interestingly, antigen specificity is not necessary for regulation of lung allergic responses as OVA-specific TCR $CD4^+CD25^+$ T cells are capable of suppressing the inflammatory response to the non-OVA allergen ragweed and still show suppressive capacity when the TCR was blocked.[41] However, Tregs do appear rapidly following antigen stimulation with increased populations observed in the mediastinal lymph node and airway mucosa as soon as 24 hours after exposure.[42] Importantly, lack of continued exposure to allergens leads to decreases in Treg cell numbers and activity.[43] In long term allergen exposure models an increase in $CD4^+CD25^+FoxP3^+$ cells is observed in BAL and lymph nodes**,** but a decrease is observed in the lung tissue suggesting a clearing of tolerogenic cells from the site of inflammation, but a retention of these regulatory lymphocytes potentially poised to help in future inflammatory incidents.[44]

Tregs inhibit Th2 cell proliferation[45] and reduce Th2 proinflammatory cytokine production[46], and there are several mechanisms by which these cells exert their suppressive effects. Contact between T regulatory cells and T effector cells appears to be important for tolerance as when these cell populations are separated in culture by a transwell, suppression of effector cells by Tregs is mitigated.[47,48] Furthermore, neither supernatant from stimulated $CD4^+CD25^+$ cells nor supernatant from co-cultured $CD4^+CD25^+/CD4^+CD25^-$ is capable of suppressing effector cell responses in independent cultures.[47] This suppression seems to be associated with IL-2 as $CD4^+CD25^-$ cells cultured with Tregs showed undetectable IL-2 mRNA expression compared to cells cultured alone and the addition of IL-2 to co-cultures neutralized suppressive effects of Tregs.[47] Later studies, however, showed that while high concentrations of IL-2 abrogate the suppressive capacity of Tregs, low doses of IL-2

actually enhance their suppressive effects.[49] Despite the role of IL-2 in Treg proliferation and function[50], the expression of FoxP3 by Tregs leads to reduced transcription of IL-2[51] necessitating the presence of exogenous IL-2. FoxP3-promoted constitutive expression of the high affinity IL-2 receptor CD25 allows Tregs to preferentially accrue IL-2 thus reducing the availability of IL-2 molecules for T effector cells and limiting their ability to phosphorylate STAT 5 which drives upregulation of IL-2Rα.[52,53]

Adoptive transfer of $CD4^+CD25^+$ cells followed by allergen sensitization is also associated with higher levels of TGF-β[54] as well as increased pulmonary IL-10 production.[37,54] Importantly, neutralization of IL-10[37] or TGF-β[48] during the allergen challenge phase leads to worse airway outcomes, whereas treatment with cells expressing TGF-β[55] or IL-10[56] prevents airway hyperreactivity and inflammation.

Secreted soluble TGF-β has been found to be involved in the suppressive function of Tregs, but membrane-bound TGF-β is required as $CD4^+$ T cells lacking membrane-bound TGF-β – but capable of secreting normal levels of soluble TGF-β – did not express FoxP3 nor did they dampen airway inflammation.[48] TGF-β inhibits expression of the transcription factor GATA3 which is a defining feature of the Th2 cell phenotype and induces IL-4.[57] This cytokine also appears to exert its effects in part by impairing phosphorylation of IL2-inducible T-cell kinase (Itk) and thus the influx of $Ca2^+$ into the cell necessary for T cell differentiation.[34]

Conversely, IL-10 binds to receptor complexes on target cells and can inhibit monocyte production of pro-inflammatory cytokines[58] and suppress T cell proliferation and cytokine production by inhibiting the co-stimulatory molecule CD28.[59] Pulmonary

DCs are also an important source of IL-10 capable of inducing IL-10 secreting regulatory T cells.[60,61]

The importance of Tregs in managing allergic asthma has also been observed in humans. Persuasive evidence can be seen in studies of children with immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) which is caused by mutations at the FoxP3 locus and leads to reduced or non-functional $CD4^{+}CD25^{hi}$ Tregs.[62] Importantly, patients with IPEX have high rates of eczema and food allergy as well as increased IgE levels and eosinophilic inflammation.[63] There is also evidence to suggest that Treg function is impaired in asthmatics. Compared to healthy controls, $CD4^{+}$ T cells from patients with moderate asthma showed reduced TGF-β1 gene expression.[64] Additionally, while FoxP3 expression moderately correlated with TGF-β1 expression, it was significantly correlated with IL-10 expression. In an independent study, Tregs from allergic donors were unable to suppress Th2 proliferation and cytokine secretion as efficiently as Tregs from non-allergic donors.[65]

1.2 ENVIRONMENTAL MICROBIAL EXPOSURES & ASTHMA DEVELOPMENT

1.2.1 Introduction to the Hygiene Hypothesis

While there was some suggestion that allergic disorders were increasing in frequency as early as the 1930s[66], research investigating changes in disease prevalence did not appear until later in the 20th century. Following an initial examination of asthma prevalence in school-aged children in Birmingham between 1956-57, Smith and colleagues (1971) repeated their study in a 1968-69 cohort and observed an increase in asthma diagnosis from 1.8% to 2.3%. The authors also observed that while children born in the United Kingdom (UK) to immigrant parents had a similar asthma prevalence to European children (6.8% and 4.3% respectively), children born in the West Indies who immigrated to the UK had a significantly lower prevalence (1.1%). This observation led the authors to postulate that these differences may be related to factors such climate or as standard of living.[67]

Additional evidence for the rapid increase in allergic diseases was reported by Taylor *et al.* (1984) who observed a steady rise in eczema rates from 5.1% in children born in 1946 to 7.3% in children born in 1958 to 12.2% in children born in 1970 – a pattern which was consistent with similar studies conducted in other developed nations. Furthermore, the authors noted that children born into higher social classes had higher rates of eczema.[68] While family history was an accepted risk factor, both of the aforementioned studies also proposed a potential role of environmental factors.

Independent studies pointed to additional environmental factors of interest. A study in Canada found that asthma and eczema were less frequent amongst indigenous communities living traditionally in rural areas than in urban-dwelling Caucasians living in the same region.[69] Conversely, infections were more frequent in the indigenous

communities leading the authors to hypothesize a potential inverse relationship between helminth, viral, and bacterial infections and allergic disease frequencies.[69] In 1989 David Strachan built on this idea that exposure to microorganisms may be inversely correlated with allergic disease. Previous studies had observed differences in allergy and asthma prevalence in rural versus urban areas, and Strachan showed an additional relationship between allergy rates and the number of older children in the household. Based on these findings, he postulated that declining family size and increased home and personal cleanliness led to a decreased transfer of infections in early life and that this may be contributing to the pattern of increased diagnosis of atopic disease.[70] This concept later came to be referred to as the hygiene hypothesis.[71]

A study by Braback *et al.* (1995) evaluating risk factors of atopic sensitization in 10 to 12 year old children from Sweden, Poland, and Estonia also found that an increased risk of sensitization was associated with a decreasing number of persons per room in the household, or less domestic crowding. The author's noted that this finding was in agreement with the hypothesis that less frequent infections during infancy may confer protection against allergic disorders.[72] More specifically, it was thought that infections may be training the immune system to recognize foreign invaders or may be promoting T helper type 1 (Th1) immune responses that block the Th2 responses associated with an allergic response. While some epidemiological studies did observe an inverse relationship between infections and allergic disease[73], this did not fully explain the differences in allergic disease prevalence in rural compared to urban communities and pointed to the importance of other environmental factors.

Additional evidence that expanded the hygiene hypothesis came from studies examining asthma and allergy prevalence in school age children living under different conditions in Europe. A 1992 study by Von Mutius and colleagues focused on children living in eastern and western Germany. Although genetically similar, these populations were exposed to different living conditions up until the then recent unification in 1989. The authors hypothesized that increased exposure to air pollutants such as sulphur dioxide and particulate matter would correlate with increased levels of asthma and allergies, however, they found the opposite to be true. Hay fever and allergic symptoms (as reported by the parents) were more common in West Germany than in East Germany where there were higher concentrations of air pollutants.[74] These results were strengthened by findings from others studies showing an increased number of positive skin prick tests[75] and higher specific-IgE levels[76,77] in West compared to East German school children. Studies such as this changed the notion that environmental pollution was driving increased incidence of allergic disease leading researchers to shift their focus to other environmental factors.

1.2.2 The Farming Environment & Asthma Risk

In the early 1990s the specific environmental factors associated with asthma risk remained unclear, however, it appeared that rural living conditions were related to a decreased risk of disease. To tease apart the factors of rural living that contributed to this effect, Braun-Fahrländer and colleagues (1999) focused on farming and non-farming communities. They observed that Swiss school-age children (13 to 15 years of age) with farming parents reported lower allergic disease and lower rates of positive specific IgE antibodies to common allergens. Furthermore, the risk of atopic

sensitization was lowest in the children of full-time farmers compared to part-time farmers.[78] This relationship was also observed in an independent study using a younger cohort (aged 5 to 7 years) living in Bavarian farming districts.[79] Ernst and Cormier (2000) further added to these findings by examining empirical markers of atopy and asthma in secondary school students (ages 12 to 19) living rurally in Canada either on farms or with regular exposure to farming environments. Consistent with other studies, recurrent wheeze, airway hyperresponsiveness, and positive skin-prick allergy test results were all less common in farm-raised children compared to farm-exposed children.[80] Taken together, these studies indicate that constant exposure to the farm environment is necessary for the observed protective effect against asthma development.

In examining specific farming exposures and their relationship to asthma in a cohort of Austrian children aged 8 - 10 years old, Reidler *et al.* (2000) found that regular contact with livestock was a farm characteristic highly correlated with a reduced risk of atopic sensitization. Importantly, children in the study who did not live on a farm but who had regular exposure to livestock also displayed a lower prevalence of allergic sensitization.[81] Independent studies found that asthma diagnosis was decreased in farm children who had regular contact with pigs, consumed farm milk, stayed in animal sheds, and those involved in haying or use of silage.[82] Interestingly, in a different study researchers found a 59% reduction of total IgE in children who consumed unpasteurized farm milk[83] and independent studies suggest that part of this protective effect may be due to the higher levels of omega-3 polyunsaturated fatty acids found in farm milk compared to industrially processed milk.[84]

Additional studies showed that only specific types of farms were found to exert a protective effect on asthma. Using latent class analysis, Illi and colleagues (2012) identified three farm types – (1) farms that kept animals such as pigs, poultry, and horses but not cows, and which cultivated and stored grain; (2) farms that kept dairy cows and bred cattle but did not cultivate grain; and (3) farms that kept dairy cows, bred cattle, and cultivated grains and corn. A protective effect against asthma was only observed for children living on the third type of farm, with both cows and cultivation. Furthermore, the farm characteristics that accounted for most of the protective effect were contact with a cow and straw and consumption of farm milk.[85] Together, the above studies offer strong evidence that only specific farm exposures impact allergy and asthma risk.

Timing of these specific exposures has also been found to be important. Only children exposed during the first year of life to stables and farm milk had lower rates of asthma (1% compared to 11%) and atopic sensitization (12% compared to 29%).[86] Furthermore, the lowest frequencies of these disorders were observed in children who had continual exposure to these factors throughout the first five years of life. Douwes *et al*. (2008) observed that a combination of prenatal and early-life farm exposures was strongly associated with a lower risk of asthma.[87] Supporting this, Illi and colleagues (2012) found that first exposure to farm characteristics occurred most frequently when the child was *in utero*, and additional studies proposed that exposure to stables during pregnancy was a key factor related to decreased asthma risk.[82] Interestingly, maternal exposure during pregnancy was associated with enhanced expression of microbial-sensing receptors TLR2 and TLR4 in school-age children, and the number of farm

animal species the mother was exposed to was related to offspring gene expression in a dose-dependent manner.[82] Thus specific farm exposures during critical periods of fetal and neonatal development are crucial for the associated protection from childhood atopic sensitization.

1.2.3 Farming Practices as a Source of Enriched Microbial Exposures

Consistent findings correlating decreased allergic disease frequencies with increased contact with livestock/livestock sheds and/or increased consumption of unpasteurized milk suggest the protective effect associated with these factors may be mediated in part by microorganisms. Indeed, one factor highly prevalent in livestock stables is endotoxin[88], a key feature of the outer membrane of gram-negative bacteria which is capable of promoting Th1 immune responses, thus selecting against Th2.[89] von Mutius *et al.* (2000) further hypothesized that endotoxin may be more prevalent in the homes of children living on farms. They collected dust from the homes of Swiss and Bavarian farmers and non-farmers and found that endotoxin concentrations were not only higher in the dust from farmers' kitchens but were also significantly higher in farmers' children's mattresses.[90] In an independent study, increased endotoxin levels in mattress dust was correlated with a decreased risk of atopic sensitization.[91] To further investigate this relationship, the authors isolated peripheral blood leukocytes from study participants. They found that upon exposure to lipopolysaccharide, cells from participants exposed to high loads of endotoxin in their mattress produced less TNF, interferon gamma (IFNγ), IL-12, and IL-10.[91] Thus exposure to high levels of environmental endotoxin – a marker of increased microbial exposure – suppressed cytokine production and subsequent innate immune activation resulting in tolerance to

the exposure. Interestingly, Karvonen *et al.* (2014) found that while individual markers of microbial exposure were not significant predictors of asthma, that a sum of the total quantity of microbial exposures (including indicators for gram-positive bacteria, gram-negative bacteria, and fungi) was a strong predictor, with a higher score being associated with a decreased incidence of asthma.[92]

Not only were farm-raised children exposed to different concentrations of microbial components they were also found to be exposed to more microbial species. Culturing techniques and single-strand conformation polymorphism (SSCP) analysis of dust from children's mattresses showed increased microbial diversity in samples collected from farming compared to non-farming homes.[93] Birzele *et al.* (2017) carried out 16S rRNA amplicon sequencing of mattress dust and nasal samples from rural children and found that bacterial richness in the mattress dust of farm children, but not in nasal samples, was inversely associated with asthma risk, thus suggesting this microbial exposure may be impacting host-associated microbes at sites beyond the upper airways.[94] Overall, these studies highlight the importance of expanded environmental microbial exposures in early life in the prevention of asthma. Of additional interest are findings from Kirjavainen (2019) showing that dust from non-farm homes with a microbial community composition similar to that found in dust from farm homes is also capable of conferring protection against asthma[95], indicating relatively conserved environmental microbial exposures in asthma prevention.

1.2.4 Other Environmental Microbial Exposures & Asthma

Different microbial exposures and rates of asthma were also observed in children of similar genetic ancestry living in Finland and Russian Karelia. Children from Russian

Karelia had lower allergen-specific IgE levels and an increased prevalence of specific microbial antibodies compared to their Finish counterparts.[96] This increased prevalence suggests that children from Russian Karelia have different microbial exposures than children from Finland. Drinking water was found to be one source of differing microbial exposure, with drinking water from Russian Karelia having a microbial cell content that is 9-fold higher than that of Finish drinking water.[96] Interestingly, von Hertzen (2007) found a dose-dependent association between reduced microbial cell content in water and an increased risk of atopy.[97] In a follow up study they also found that amongst Finnish children increased microbial components in house dust was associated with a decreased risk of atopic sensitization.[98]

These findings highlight the importance of contact with microbes from the natural environment. Indeed, independent studies have observed that more green area around the home is associated with decreased sensitization to inhaled allergens.[99] Another study of 118 children in Finland found that the environmental biodiversity surrounding participants' homes – specifically more forest and agricultural land use – was associated with skin bacterial community composition. Importantly, compared to atopic individuals, healthy participants were exposed to increased environmental biodiversity and had a more diverse skin microbial community.[100]

Exposure to pets, especially dogs, represents another environmental exposure associated with protection from allergy and asthma. Epidemiological studies have shown that exposure to 2 or more dogs in the first year of life correlated with a significantly lower prevalence of atopy (15.4% compared to 33.6% with no pet exposure, $p=0.005$).[3] A study of pet-exposure in children from the neighboring towns of

Imatra, Finland and Svetogorsk, Russia found that asthma risk was increased among Finish children but dog exposure had a protective effect. Additionally, of the Russian children exposed to indoor dog(s) during the first year of life, none went on to develop allergic asthma.[101] Studies have also shown that prenatal dog exposure (i.e. maternal exposure during pregnancy) is significantly associated with a decrease in offspring IgE levels in cord blood as well as over time (assessed at birth, 6-, and 18-months of age).[4,5] This epidemiological association between dog exposure and a decreased risk of asthma seems to be explained in part by increased microbial exposure. This is supported by research from Fujimura and colleagues (2010) showing that the bacterial content of dust from dog-owning homes is compositionally distinct and enriched for over 300 taxa compared to dust from pet-free residences.[102]

1.2.5 Linking Microbial Exposures to Immunological Features of Allergy & Asthma

Once it was determined that there was a correlation between microbial exposures and allergic disease, researchers began focusing on the mechanisms by which microbial exposures may confer protection. In a study by Loss *et al.* (2012) they found that traditional farming practices, especially the consumption of raw milk, was associated with altered expression of innate immunity receptor genes in early life thus linking microbial exposures to changes in host immunity. Additionally, maternal farming status at the time of pregnancy was associated with increased TLR7 and TLR8 gene expression in the offspring at birth (as measured in cord blood).[103] Ege and colleagues (2007) found that children who grew up on a farm exhibited increased expression of CD14 and several TLRs (specifically 1, 2, 4, 7, 8.1 and 8.2) and that different farm exposures had different effects on innate gene expression.[104] In a separate study,

maternal exposures were also seen to relate to immune outcomes in the infant with exposure to animal sheds during pregnancy being inversely correlated with cord blood allergy-specific IgE levels.[105] Maternal contact with hay enhanced the protective effect of this exposure specifically in grass-pollen associated cord blood IgE levels.[106]

Additional studies have compared the environmental microbial exposures of Amish versus Hutterite populations. Though genetically similar, prevalence of asthma in school-aged Amish compared to Hutterite children is strikingly different, 5.2%[107] versus 21.3%[108] respectively. These populations engage in differing farming practices—traditional farming by the Amish compared to industrialized farming by the Hutterites—which are thought to alter their respective environmental microbial exposures and account for asthma disparities.[104] Indeed, house dust from Amish and Hutterite homes is microbiologically distinct, with Amish house dust being characteristically increased in LPS concentrations compared to Hutterite dust.[109] Amish subjects resident in these households exhibited lower proportions of eosinophils and less mature neutrophils compared to Hutterites, and nasal exposure of mice to the microbiologically distinct house-dust largely recapitulated the immune phenotypes of the human subjects.[109] This suggests that despite genetic similarity, distinct environmental microbial exposures can influence immune cell populations and functions, offering a potential mechanism by which increased and commensal-rich microbial exposure in early life may offer protection against atopy and asthma.

Adaptive immune responses have also been examined in Amish and Hutterite children. In Amish children, conventional $CD4^+$ T cells showed reduced expression of the costimulatory molecule CD28 or the activation marker inducible T-cell co-stimulator

(ICOS).[110] Conversely, $CD4^+ICOS^+$ T cells were increased in Hutterite children and positively correlated with total IgE levels. Furthermore, while both populations of children had similar proportions of Tregs, Tregs from Amish children expressed increased CD45RO and ICOS, whose expression on Tregs is characteristic of enhanced suppressive function.[111,112]

In regards to dog-ownership, a follow up study by Fujimura *et al.* (2014) showed that mice supplemented with house dust from dog-owning homes were protected against airway allergic sensitization – characterized by reduced mucin secretion and Th2 cytokine production – when compared to mice supplemented with house dust from pet-free homes.[113] Importantly, protection of these animals was associated with a distinct cecal microbiota[113], and, in a more recent study, with metabolic reprogramming in both the lumen and in circulation.[114]

Thus, although risk alleles associated with atopy and asthma such as ORMDL3 have been identified, the rapid increase in prevalence cannot be explained by host genetics alone.[2] The above studies offer strong evidence that environmental factors, specifically increased microbial exposures in early life, are associated with protection from allergic disease development and confer protection by influencing the development of immune functions.

1.3 THE EARLY-LIFE GUT MICROBIOTA, IMMUNE DEVELOPMENT, & ASTHMA PATHOGENESIS

1.3.1 Features of the Infant Gut Microbiota Relate to Risk of Asthma in Childhood

Following observations associating early-life microbial exposures with allergy and asthma outcomes researchers hypothesized that human-associated microbial communities may serve as the link between these factors. While microorganisms exist across a range of human body sites, the majority reside in the gastrointestinal tract. This complex community of microbes, or the gut microbiota, is established over the first three years of life[115–117] and multiple studies using independent birth cohorts have provided evidence for a relationship between early-life gut microbiota constituents and risk of allergic disease and asthma in later childhood.

In one of the earliest studies employing culture-based approaches, Bjorksten and colleagues (1999) observed that stool from 1-year old allergic children from Estonia as well as the more westernized Sweden exhibited an increased proportion of aerobic microorganisms and coliforms compared to non-allergic children.[118] Subsequent studies using stool samples from earlier timepoints (3 weeks, 1 month, and 3 months) corroborated these findings, noting an increased abundance of *Escherichia coli*[119] or *Clostridia* species[119–121] associates with a higher risk of childhood atopy. The absence of specific species in neonatal stool, including *Enterococcus*[121], *Lactobacillus*[118,122], or *Bifidobacterium*[121,122] , has also been associated with an increased risk of allergic disease. Differences in a small set of bacteria present in the first stool (meconium) and sustained over the first year of life have also been observed between high-risk for asthma infants and healthy controls.[8] Taken together, these studies indicate that

differences in early-life gut bacterial colonization patterns may influence subsequent allergic disease development.

1.3.2 Early-Life Factors Shaping Gut Microbiota Development & the Risk of Asthma

Given that both environmental exposures and differences in gut microbial community members in early life have been associated with asthma development it is of interest to understand how environmental exposures influence the gut microbiome during the critical window of assembly. Equally important is the ecological principal of pioneering colonizers, which are species that dictate the conditions of an ecosystem and initiate a chain of ecological succession that ultimately impacts the profile of the steady-state ecosystem.[116] The crucial role of initial colonizing species and early-life factors in shaping the gut microbiota suggests that inappropriate early-life gut microbiome colonization patterns and aberrant gut microbiome development could have long-lasting effects on host physiology.

1.3.2.1 *Birthing Method*

While several studies suggest that the first introduction to microbes occurs *in utero*[123–125], birth represents the first exposure to vast human-associated and environmental microbial ecosystems. Different birthing methods have a strong influence on the initial colonizing organisms, and distinct oral, skin, and gut microbial communities have been observed between vaginal and Cesarean-delivered babies.[126] Unsurprisingly, vaginally born infants are first inoculated by microbes present in the vaginal tract, mainly *Lactobacillus* and *Sneathia* species, while the microbiomes of babies delivered via Cesarean are characterized by skin-associated organisms, including *Staphylococcus* and *Streptococcus.*[126]

In addition to microbial community composition, microbial functional differences have also been observed between vaginally born and C-section babies. Studies using both predictive and functional metagenomic profiling have shown that neonates delivered via Cesarean section have a lower abundance of pathways related to metabolism of D-glutamine and D-glutamate[127] and amino and nucleotide sugars[128], but are enriched for pathways related to xenobiotic degradation[127,128] and biosynthesis of ubiquinone and other terpenoid-quinones.[127] Interestingly, the latter act as electron carriers in oxidative phosphorylation, a metabolic process favored by unactivated T cells and thus perhaps indicative of a naive immune state.

Adding to this research, Wampach *et al.* (2018) found that many of the microbial functions observed in vaginally born babies on day 3 of life were lacking in Cesarean delivered babies. One of the functional pathways enriched in vaginally born neonates was LPS biosynthesis, which is interesting given the correlation between increased exposure to endotoxin and reduced risk of allergic disease discussed in the previous section. Additionally, the authors used purified LPS extracted from 3 day old neonatal stool to treat human monocyte-derived DCs and observed that LPS from vaginally-born babies had enhanced immunostimulatory capacity.[129] Differential microbial metabolic capacities and their impact on developing immune cells may explain findings from epidemiological studies showing that, compared to vaginal birth, children born via Cesarean section have an increased risk of developing allergic disease.[130]

1.3.2.2 *Mode of Nutrition*

Some epidemiological studies have observed a protective effect of breastfeeding on allergic sensitization[131] while others examining an association with asthma did not observe the same effect.[132,133] Despite this, mode of nutrition represents one of the

most significant influences on early-life gut microbiome structure[134] and may represent a possible risk factor for childhood allergic disease.

Formula-fed infants appear to have a precocious gut microbial community structure and are enriched for bile acid biosynthesis and methanogenesis pathways, microbial functions more commonly observed in the adult microbiome.[117] This may indicate that the microbiome of formula-fed children develops too rapidly and prevents key commensals with immunomodulatory capacity from exerting their influence. Differences in individual microbial community members have also been observed. A study examining fecal samples from breast-fed compared to formula-fed babies found that the breast-fed infant gut microbiome was dominated by *Bifidobacteria* and was additionally enriched for fermentative *Lactobacillus* and *Streptococcus* species.[135] While formula-fed infants were also enriched for *Bifidobacteria* their fecal microbiome was co-dominated by *Bacteroides* species. Additionally, the formula-associated neonatal gut microbiome was distinct in its enrichment for *Staphylococcus*, *Escherichia,* and *Clostridium*.[135]

These data complement previously mentioned studies correlating increased *E. coli* and *Clostridium* and decreased *Lactobacillus* and *Bifidobacterium* with an increased risk of allergic sensitization and, notably, many of these organisms have been shown to have immunomodulatory effects.[119–122] The enrichment of *Bifidobacterium* in the gut of breast-fed infants is likely related to enrichment of human milk oligosaccharides which these bacteria use as a substrate to produce short-chain fatty acids (SCFAs) which have anti-inflammatory effects.[136,137] *Lactobacillus* species are capable of producing tryptophan-derived indoles which can act as ligands for the aryl hydrocarbon receptor

(Ahr).[138] Importantly, Ahr has been shown to not only be necessary for Treg generation and FoxP3 expression[139], but it is also important in the context of asthma as Ahr knock out mice exhibit an exacerbated inflammatory response to allergen challenge.[140] Furthermore, in animal models, *Lactobacillus* species present in maternal milk and transferred to the neonatal gut have the capacity to regulate perinatal immune development such as immunoglobulin production.[141]

1.3.2.3 *Antibiotic Treatment*

Antimicrobial exposure during early infancy is another factor associated with an increased risk of childhood atopy[142] as well as childhood asthma, rhinoconjunctivitis, and eczema.[143] Antibiotic treatment also leads to alterations in the neonatal gut microbiome. An enrichment of *Enterobacteriaceae* and *Enterococcaceae* as well as a depletion of *Bifidobacteriaceae* and *Bacteroidaceae* have been repeatedly observed in pre-term as well as full-term neonates exposed to antibiotics in the first week or first month of life.[144,145] Additionally, antibiotic-associated differences in community members[146] and overall community structure[147] are still apparent weeks to months post treatment. Indeed, antibiotic treatment led to delayed infant gut microbiota development that only converged with communities of antibiotic-naive near-term infants between 12 and 15 months of life.[147]

Independent studies have also reported differences in predicted microbial functions and decreased fecal concentrations of microbially-produced SCFAs in antibiotic-exposed babies.[148] This suggests that the aforementioned long term impacts of antibiotics on gut microbiota members and overall community structure likely extend to microbial functional capacity, and this altered functional capacity may lead to inappropriate immune cell education during a critical period of immune development.

Support for this comes from studies using murine models in which early-life pulsed antibiotic treatment led to both metagenomic and metabolomic alterations.[149] Independent studies in mice have also linked perinatal antibiotic exposure to not only changes in the gut microbiota but exacerbation of allergic disease.[150] Specifically, OVA-challenged mice treated with antibiotics during the neonatal period showed an increase in both serum and surface-bound IgE as well as a decrease in colonic Treg numbers.[151] Furthermore, *ex vivo* stimulated splenocytes from mice treated with kanamycin (a broad spectrum antibiotic targeting gram-negative bacteria) in early-life, but not during adolescence, exhibited decreased IFNγ secretion and enhanced IL-4 secretion, thus suggesting the potential of this antibiotic to promote Th2-dominant immunity.[152]

The research presented above strongly suggests that the neonatal gut microbiome serves as the link between early-life environmental exposures and childhood allergic disease development. Early-life factors such as delivery mode, mode of nutrition, and antibiotic treatment not only dictate who the initial bacterial colonizers are but also impact subsequent microbial community assembly. Aberrant gut microbiome maturation also means aberrant microbial functions capable of negatively impacting immune development and leading to long-term consequences for the host.

1.3.3 Impact of the Gut Microbiota on Immunological Features of Asthma

The first year of life marks a critical and dynamic period of microbial community assembly marked by a rapid diversification of bacterial community members.[115–117] Importantly, the establishment of the gut microbiome is concurrent with immune development, and an increasing body of research indicates that there is a critical

window in early life during which the gut microbiota exerts a strong influence on shaping asthma associated immune responses.

1.3.3.1 *Gut Microbial Communities and Immune Cell Education*

Herbst and colleagues (2011) were among the first to provide experimental evidence that commensal bacteria impact immune function in the context of allergic airway inflammation using the OVA model in germ free (GF) mice. Not only did GF mice have an exacerbated response to OVA, marked by airway hyperresponsiveness as measured by methacholine challenge and enhanced Th2 cytokine production in T cells in BAL fluid, they also had an altered number and activation status of conventional DCs in the lung. Additionally, microbial colonization of GF mice conferred protection against allergic airway inflammation.[153]

Olszak *et al.* (2012) showed that GF mice had an increased accumulation of invariant natural killer T (iNKT) cells in the colonic lamina propria and lung. This observation was seen throughout the course of the animal's life, suggesting that a lack of microbial exposure has a persistent impact on the host. Importantly, establishing the microbiota of GF mice in early life (pregnant females colonized just before delivery such that offspring are exposed to microbes on the first day of life), but not in adulthood, led to normalization of iNKT cell levels that persisted. Additionally, neonatal, but not adult, exposure to conventional microbiota reversed aberrant iNKT cell accumulation in the lung and led to decreased response to OVA as measured by a decrease in eosinophil infiltration in the lung and reduced IgE levels.[154]

The above findings associating the gut microbiome with immune cell education that leads to protection against airway inflammation have also been supported by findings from human cohorts. In a large study examining the gut bacterial community of

1-year-old children categorized as atopic wheezers (AW), Arrieta *et al.* (2015) found that at 3 months of age these communities were characteristically depleted of members of the *Faecalibacterium*, *Lachnospira*, *Veillonella*, and *Rothia* genera compared with healthy controls. Furthermore, GF mice humanized with the feces of AW subjects and concurrently supplemented with these four bacteria showed reduced expression of pro-inflammatory cytokines and protection from airway inflammation following airway allergen challenge.[7]

In an independent study conducted by Fujimura and colleagues (2016), 1-month-old infants at the highest relative-risk of developing atopy and asthma at age 2 and 4 years, respectively, exhibited the same taxonomic depletions.[6] Additionally, cell-free fecal water (CFW) from these high-risk neonates induced increased proportions of $CD4^+IL\text{-}4^+$ T cells and increased concentrations of IL-4 – quintessential immunologic hallmarks of atopy and asthma – compared with CFW from the low-risk group. Importantly, the high-risk infants in this study had significantly lower detectable dog allergen (Can f1) concentrations in their homes.[6] Therefore there is an increasing body of evidence indicating that very early life gut microbiota perturbations, including loss of bacterial taxa and encoded metabolic capacity, precede development of atopy and asthma in childhood.

1.3.3.2 *Individual Gut Symbionts Impact Treg Populations*

The role of the gut microbiota in Treg development has become apparent through GF mouse models. GF animals not only exhibit lower numbers of $CD4^+CD25^+FoxP3^+$ cells in the mesenteric lymph nodes[155] and colon lamina propria[156], but the Treg cells that are present produce less IL-10[155,157] and TGF-β.[157] That these

cells have impaired regulatory function can also be seen in their inability to induce oral tolerance to OVA-antigen in GF mice.[157]

Many gut microbial species are capable of promoting the generation of Tregs. Atarashi and colleagues screened both the mouse and human gut microbiota and found that murine *Clostridia* clusters IV and XIVa[156] and human *Clostridia* clusters IV, XIVa and XVIII[158] both induce Treg expansion in the lamina propria and impact Treg activity including promoting increased IL-10 expression. These bacteria preferentially colonize the cecum and proximal colon, forming a layer on intestinal epithelial cells.[156] This close contact with the gut epithelium can stimulate TGF-β cytokine production thus creating an environment in the gut that promotes peripheral Treg differentiation.[159] *Clostridia* can also stimulate T cells to produce IL-2[160], an important factor for Treg proliferation.

Bacteroides fragilis is another gut symbiont capable of promoting Tregs in the lamina propria and mesenteric lymph node via the capsular molecule polysaccharide A (PSA).[161] PSA is required in Treg generation as *B. fragilis* mutants that do not produce PSA cannot induce Tregs. PSA-induced Tregs not only have increased IL-10 and TGF-β2 gene expression, but also have an increased capacity to suppress T effector cell proliferation. PSA treatment of mice also increased transcription of CCR6, a chemokine receptor involved in Treg migration. These effects seem to be mediated via TLR2 as the expansion of Tregs and increased production of IL-10 seen in wildtype mice is not observed in TLR2$^{-/-}$ mice.[161]

Interestingly, in their study Atarashi *et al.* (2011) observed that while *Clostridia* species were potent inducers of Tregs, monocolonization with *B. fragilis* did not significantly impact the proportion of Tregs in the colon lamina propria.[156] It is important

to note, however, that experiments with *B. fragilis* carried out by Atarashi *et al.* were done in IQI mice, which is a mouse model of chronic autoimmunity.[156] This suggests that different bacteria may have a distinct impact on Treg phenotypes occurring via different mechanisms in a context-dependent manner.

Other human gut microbiota symbionts have also been implicated in protection from allergic airway disease. Supplementation with the human fecal isolate *Lactobacillus reuteri* led to an increased $CD4^+CD25^+FoxP3^+$ and $CD4^+IL\text{-}10^+$ T cell populations in the spleen. Additionally, $CD4^+CD25^+$ cells from *L. reuteri* supplemented animals when transferred to OVA-sensitized mice led to reduced eosinophil infiltration, decreased levels of IL-5 in BAL, and attenuation of airway hyperresponsiveness.[162] In an independent study *Bifidobacterium longum* (strain AH1206) supplementation increased Treg cell numbers – both locally in the Peyer's patches and distally in the spleen – and their suppressive function, with the strongest impact occurring in mice treated in infancy.[163] Treatment with this strain also led to increased expression of retinoic acid metabolism associated genes in Peyer's patches[163], which is of interest given that retinoic acid has been shown to induce FoxP3 expression.[164,165] Additionally, murine consumption of *B. longum* and subsequent OVA-challenge resulted in reduced eosinophil infiltration and lower levels of OVA-specific IgE.[163]

While thymic derived Tregs are important for autoimmunity – as mice who underwent a thymectomy at 3 days of age developed organ-specific autoimmunity[166] – extrathymic Tregs seems to be crucial for managing type 2 immunity. The conserved non-coding sequence 1 (CNS1) is a region essential for extrathymic Treg differentiation, and CNS1 knockout mice show pronounced Th2 pathologies in the gastrointestinal tract

and lungs as well as increased serum IgE levels.[167] Importantly, the gut microbiota of these mice is altered.[167] Thus not only do extrathymic Tregs appear to serve a distinct function of inhibiting Th2-associated inflammation, this function may be inhibited, in part, due to a lack of appropriate microbiota-derived TCR ligands or microbial impact on the local cytokine environment.

In vitro studies using human primary cells also indicate a role for certain bacteria in Treg generation. Treatment with *L. reuteri* and other *Lactobacillus* species primed human monocyte-derived DCs to skew naive $CD4^+$ T cells toward a regulatory phenotype marked by increased IL-10 production and enhanced suppression of T effector cell proliferation.[168] This effect seems to occur via binding of the DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) as treatment with DC-SIGN blocking antibodies inhibited Treg induction blocking.[168] Furthermore, a study using naive lymphocytes from cord blood observed that *Bacteroides* species promoted robust IL-10 secretion.[169]

1.3.3.3 *The Impact of Gut Symbionts on Th2 Responses*

The absence of microbial colonization in GF mice also impacts the Th1/Th2 immune cell balance, skewing naive T cells toward a Th2 phenotype marked by increased IL-4 production. Interestingly, this imbalance can be corrected by colonization of GF mice with *B. fragilis* which initiates STAT6 signaling in DCs and subsequent production of IL-12, which favors Th1 expansion.[170]

Lactic acid bacteria (LAB) also appear to play a role in mediating the Th1/Th2 balance. Using peripheral blood mononuclear cells (PBMCs) from house dust mite (HDM) allergic patients and healthy controls, Pochard *et al.* (2002) showed that incubation with heat-killed LAB and subsequent restimulation of the cells with the HDM

allergen *Dermatophagoides pteronyssinus* (dpt) inhibited secretion of Th2-associated cytokines IL-4 and IL-5 in allergic PBMCs and did so in a dose dependent manner.[171] The ability of bacteria to attenuate IL-4 and IL-5 cytokine secretion in dpt-stimulated allergic donor PBMCs was also observed in an independent study following preincubation with live *Lactobacillus* and *Bifidobacterium* species.[172] Interestingly, this effect was also observed when the PBMCs were treated with genomic DNA from these bacteria, suggesting a potential TLR-associated mechanism. Furthermore, viable *Lactobacillus* and *Bifidobacterium* species also induced IFNy secretion in unstimulated PBMCs from allergic patients, which is associated with Th1 induction.[172] Thus there is growing evidence that bacteria may play a role in restoring the Th1/Th2 imbalance characteristic of allergic disease through promoting secretion of cytokines associated with a Th1 response.

1.3.4 The Immunomodulatory Capacity of Microbial Metabolites

The genetic capacity of the gut microbiota exceeds that of humans by several orders of magnitude[173], and bestows them with the ability to modify a number of diet- or host-derived molecules to produce metabolites with bioactive potential.[174] Disease associated differences in the intestinal metabolome have been observed as early as 2000 when Bottcher *et al.* found lower levels of microbially-produced propionic, butyric, and valeric acid in early-life fecal samples of allergic compared to non-allergic children.[175] These findings have continued to be corroborated today with studies showing differences in meconium microbial community composition[8,176] and metabolic profiles[176] between healthy and high-risk for asthma infants[8] or neonates who go on to develop atopy.[176] Furthermore, Petersen and colleagues (2021) showed childhood

atopy development can be accurately predicted using the metabolic and microbial profile of meconium. Based on these findings, researchers have begun to examine the ability of microbial metabolites to modulate the phenotype of immune cell populations relevant to allergy and asthma pathogenesis.

1.3.4.1 *Short Chain Fatty Acids*

Multiple independent studies have reported increased levels SCFAs in the gastrointestinal tract of specific pathogen free (SPF) mice compared to GF or antibiotic-treated mice.[177,178] This is also seen in mice fed a high-fiber compared to a low-fiber diet[179,180], which is unsurprising given that one of the major products of gut bacterial fermentation of dietary fiber is SCFAs.[181] Indeed, Thorburn (2015) found that mice fed a high fiber diet exhibited a distinct gut microbial community and increased levels of the SCFA acetate. Furthermore, when mice were fed acetate or a high fiber diet and subsequently challenged with HDM the authors observed a reduction in allergic airway disease (AAD) phenotypes, increased FoxP3 expression in the lung, and an increased proportion of Tregs in peripheral lymph nodes. Acetate-associated suppression of AAD was dependent on Tregs as ablation of this population with anti-CD25 ameliorated the protective effect.[180]

Butyrate and propionate are other SCFAs capable of potentiating Treg differentiation, although different experimental models have led to differing results. Some studies have reported that butyrate led to enhanced $CD4^+CD25^+Foxp3^+$ T cells in the colon of treated animals[178,179], while others have only observed an impact on Treg populations in the spleen and peripheral lymph nodes.[177] These differences may be due to animal facility associated variation in the murine gut microbiota[182] and further investigation may lead to some interesting insights into the specific factors contributing

to local and distal Treg promotion. Despite these discrepancies, multiple studies have found that, independent of the site of Treg promotion, SCFA treatment leads to increased IL-10 production[178,179] and enhanced capacity of Tregs to suppress T effector cell proliferation.[177,178]

Importantly, gut-microbe-mediated fatty acid production can also act systemically to mitigate pro-allergic immune responses. Trompette *et al*. (2014) found that SCFA production by gut bacteria was associated with decreased inflammation, mucus production, and expression of T-cell-activation co-stimulatory molecules on DCs in a murine allergen challenge model. Treatment of mice with one of these SCFAs, propionate, prior to allergen challenge led to increased DC precursors, and resulted in lung-resident DCs that were less effective at reactivating pro-allergic Th2 cells.[183]

Several mechanisms appear to be involved in SCFA promotion of Treg differentiation. Smith *et al.* (2013) reported that the impact of propionate on Treg generation was mediated by the G protein-coupled receptor (GPR) 43. Not only was GPR43 significantly reduced in colonic Tregs isolated from GF mice – suggesting expression may depend on microbiota-derived signals – but propionate treatment only induced colonic Treg differentiation and enhanced suppressive activity in GPR43-sufficient and not deficient mice.[178]

SCFAs are also potent inducers of FoxP3 and are even capable of promoting its expression in naive $CD4^+$ T cells under Th17 or Th1 polarization conditions.[179] This increased induction of FoxP3 appears to be related to epigenetic modifications. Thorburn *et al.* (2015) showed that acetate treatment of mice led to reduced histone deacetylase (HDAC) activity, increased acetylation of H4 histone at the FoxP3

promoter, and higher levels of FoxP3 expression in the lung.[180] Additional studies have also shown propionate or butyrate treatment increases acetylation of histones at either the FoxP3 promoter region[177] or the CNS1 enhancer[179], which is essential for Treg generation in the periphery.[167] Importantly, in CNS1-deficient lymphocytes and mice, butyrate treatment fails to induce $FoxP3^{+}CD4^{+}$ T cells[177], highlighting the importance of this SCFA for extrathymic Treg generation. Propionate has also been shown to promote the generation of splenic Tregs in a CNS1-dependent manner, but propionate and acetate promoted colonic Tregs in a CNS1-independent manner, indicating different activities for specific SCFAs.[179] Taken together, these studies suggest that the inhibitory effect of SCFAs on HDAC activity leads to increased histone acetylation and subsequent FoxP3 transcription which promotes the differentiation and enhanced function of Tregs such that they can confer protection against AAD.

1.3.4.2 *Mono- and Poly-unsaturated Fatty Acids*

Studies have also shown the metabolic capacity of microbes to convert linoleic acid to the metabolite 12,13-diHOME, a molecule that has been associated with increased risk of allergic disease.[6,184] Not only did this metabolite strongly discriminate the feces of high-risk from low-risk for asthma infants[6], but microbial genes capable of producing 12,13-diHOME were also found to be enriched in the gut of high-risk infants.[184] Furthermore, high concentrations of 12,13-diHOME in 1-month old stool samples from multiple independent cohorts was associated with an increased probability of atopy and asthma development later in childhood.[184] Interestingly, a different study showed an inverse relationship between 12,13-diHOME and asthma at 3 years of age[185], suggesting the importance of this molecule specifically in early life before the development of disease. This association between this molecule and asthma

risk was supported by experimental models showing that 12,13-diHOME reduced the frequency and IL-10 productivity of $CD4^+CD25^+FoxP3^+$ regulatory T cells *in vitro.*[6] Additionally, in mice challenged intratracheally with cockroach allergen, intraperitoneal treatment with 12,13-diHOME or supplementation with genetically engineered bacteria capable of synthesizing 12,13-diHOME led to exacerbation of airway inflammation and decreased numbers of Tregs in the lung.[184]

In addition, bacterial supplementation can promote metabolites with anti-inflammatory potential. Enrichment of *Lactobacillus johnsonii* in the gastrointestinal tract of mice was associated with subsequent protection against AAD.[113] Oral supplementation of animals with a strain of this bacteria isolated from the ceca of protected mice recapitulated the previously observed protective effect against allergic sensitization.[113] Mechanistically, this effect appears to be mediated, at least in part, through a reprogrammed circulating metabolic environment.[114] Amongst the metabolites enriched in protected animals are a range of anti-inflammatory lipids, including docosahexionic acid (DHA), that reduce activation and inflammatory cytokine productivity of bone marrow-derived DCs *in vitro*.[114] Thus, metabolic products or metabolic reprogramming associated with gut microbes act both locally and at remote mucosal sites to reprogram contemporary and precursor immune cell populations.

1.4 AIMS OF STUDY

While studies have indicated that early-life environmental exposures and infant gut microbiota composition and metabolites are associated with allergy and asthma, much work remains to be done to understand both the critical window of exposure and the mechanisms by which these factors work together to protect against childhood allergic disease development. Thus, the aim of this study is three-fold: 1) to understand how early-life microbial exposures, specifically dog ownership, impact infant gut microbial community assembly over the first year of life, 2) to elucidate gut microbial functions and associated metabolites that correlate with a decreased risk of childhood allergic asthma development, and 3) to determine how these protective metabolites modulate immune cell phenotypes. Findings from this study could be exploited for designing novel interventions not only for allergic disease, but for other diseases characterized by immune dysregulation.

CHAPTER 2: THE IMPACT OF DOG-KEEPING ON INFANT GUT MICROBIOTA DEVELOPMENT.

Materials for this chapter were modified from:

Panzer, A.R., Sitarik, A.R., Fadrosh, D., Havstad, S., Jones, K., Davidson, B., Finazzo, S., Wegienka, G., Woodcroft, K., Lukacs, N.W., Levin, A.M., Ownby, D.R., Johnson, C.C., Lynch, S.V., and Zoratti, E.M. “The impact of dog-keeping on infant gut microbiota development.” *In review.*

2.1 ABSTRACT

Background: Prenatal and early-life dog exposure has been linked to reduced childhood atopy and asthma. A potential mechanism includes altered immunity in response to changes in the gut microbiome among dog-exposed infants. We sought to determine whether infants born into homes with indoor dog(s) exhibit altered gut microbiome development. **Methods:** Pregnant women living in dog-keeping or in pet-free homes were recruited in southeast Michigan. Infant stool samples were collected at intervals between 1 week and 18 months after birth and microbiome was assessed using 16S ribosomal sequencing. Perinatal maternal vaginal/rectal swabs and stool samples were sequenced from a limited number of mothers. Mixed-effect, adjusted models were used to assess stool microbial community trajectories comparing infants from dog-keeping versus pet-free homes with adjustment for relevant covariates. **Results:** Infant gut microbial composition among vaginally born babies became less similar to the maternal vaginal/rectal microbiota and more similar to the maternal gut microbiota with advancing age. Stool samples from dog-exposed infants were microbially richer ($p=0.046$) and more diverse ($p=0.036$) through age 18 months. Enhanced diversity was most apparent between 3 and 6 months of age. Statistically significant effects of dog exposure on β-diversity metrics were restricted to formula-fed children. Across the sample collection period, dog exposure was associated with *Fusobacterium* depletion and *Citrobacter* genera enrichment, depletion of two *Lactobacillus* and one *Citrobacter* species, and enrichment of *Dorea, Collinsella, Ruminococcus, Clostridaceae* and two *Lachnospiraceae sp*. **Conclusion:** Prenatal/early-life dog exposure is associated with an altered gut microbiome during infancy and supports a potential mechanism explaining lessened atopy and asthma risk.

2.2 INTRODUCTION

Vertical transfer of maternal microbes, together with environmental exposures, shape the initial gut bacterial communities that inhabit the neonatal gastrointestinal tract.[117,186,187] These inceptive microbes shape both immune function[124,188] and subsequent trajectories of gut microbiome maturation during this critical period of development.[189] Several birth cohort studies have provided evidence that early-life gut microbiome perturbation, and subsequent metabolic dysfunction, is associated with increased risk of atopy and asthma development in later childhood.[6–8,190] We previously reported that gut microbial profiles associated with protection against atopy and asthma were more likely to be observed among babies with prenatal and early life exposure to dogs.[6,8]

Several reports indicate that household pet exposure, particularly living with indoor dogs during pregnancy and early childhood, is associated with lower total immunoglobulin E (IgE) levels, lessened allergic sensitization to common allergens, and/or lower risk of allergic disorders including asthma.[3,5,191–197] Recently we reported that dog introduction into a residence also has effects on house dust microbiota and that living with a furred pet contributes to variance in infant stool microbiota at specific time points in early life.[102,198,199]

These data support a potential gut microbiome-related mechanism linking pets to lowered risk of allergic sensitization and allergy-related disorders. In this study, we map gut microbiota taxonomic developmental trajectory over the first 18-months of life in a cohort of infants sampled longitudinally from birth. Approximately half lived in homes with an indoor dog(s), permitting us to determine whether this exposure influences early life gut microbiome development.

2.3 RESULTS

2.3.1 Participant Samples & Demographics

Among 141 maternal-child pairs enrolled, 660 samples across 134 maternal-child pairs were successfully sequenced; 131 offspring (93% of the cohort) had at least one infant stool sample successfully sequenced. Targeted sample collection times, number of samples collected, and those successfully sequenced are displayed in Table 2.1 and infant stool sample contribution by timepoint is displayed in Table S2.1.

The analyzed subset of 131 children were similar in characteristics to the full cohort and 57% of the samples were from participants residing with a dog (Table 2.2). The contribution of at least one infant stool sample (chi-square p=0.87) and the total number of samples sequenced per child did not differ by dog exposure (Wilcoxon p=0.58). Factors significantly associated with dog ownership included maternal reported race and household income. Mothers with dogs were less likely to self-identify as African American (10.7% versus 28.6%, p=0.009) and more likely to have a mid-level household income ($40,000 – $80,000; p=0.006).

2.3.2 Infant Gut Microbiota Development Over Advancing Chronological Age

Previous studies reported that the infant gut microbiota composition changes with advancing chronological age.[8,200] Thus we first examined relationships between gut microbiota development and infant age in our cohort. The gut microbiota exhibited increasing bacterial richness and diversity as well as age-related compositional changes over the first 18 months of life (Fig. 2.1A and Fig. 2.2). Using vaginal/rectal swab and maternal stool microbiota profiles as a reference, we noted that the infant gut microbiota was similar to the maternal vaginal/rectal microbiota at delivery but became increasingly similar to the maternal gut microbiome with advancing age (Fig. 2.1A [all samples] and

2.1B [vaginally born infants with paired maternal samples]), indicating compositional maturation towards an adult gut microbiome.

We next sought to identify bacterial genera related to chronological age. Of the 57 genera with a prevalence of >10%, 48 (84%) were significantly associated with infant age at the time of sample collection. Of these, 29 genera increased and 19 decreased in relative abundance with advancing age (Figure S2.1, Table S2.2, $p_{FDR}<0.05$; individual taxa associated with chronological age displayed in Table S2.3). Genera with the greatest effect size for an increase in relative abundance with advancing age included *Faecalibacterium*, *Lachnospira*, *Collinsella*, and *Dorea*, while *Staphylococcus* exhibited the greatest decrease in relative abundance with increasing age. These findings are consistent with previous observations that the infant gut microbiota develops over the first year of life and exhibits age-related accumulation of bacterial species over this developmental period.[6,7,117,147]

2.3.3 Dog-Ownership & Gut Microbial α-Diversity Metrics

Next, we examined the relationship between dog-ownership and infant gut microbiota richness, evenness, and diversity trajectories (α-diversity). For richness and evenness trajectories, linear modeling was the best fit. The overall dog effect was not statistically significant prior to covariate adjustment (Table 2.3, p=0.094 and p=0.54 respectively). After full covariate adjustment, dog-exposed children maintained a significantly higher mean trajectory of bacterial richness (p=0.046), but not evenness. Across the observation period, an average of 11 additional operational taxonomic units (OTUs) were detected in dog-exposed children compared to children living in pet-free

homes (Table 2.3 and Figure 2.2A; p=0.046). Interactions between dog exposure and the pre-specified set of effect modifiers were non-significant for richness (Table S2.4).

A quadratic trajectory fit best for the Faith's phylogenetic diversity metric. There was a significant overall dog effect in the fully adjusted model, where diversity was on average 0.72 units higher in dog-exposed children across the observation period (Table 2.3 and Figure 2.2B; p=0.036). In contrast to the other metrics, a statistically significant dog exposure by time interaction was identified (Table S2.4; p=0.012 and Figure 2.2B). While phylogenetic diversity was greater in dog-exposed children for most of the trajectory, the curves were most divergent at 3 and 6 months of age (3-months: β=1.07, p=0.003; 6-months: β=1.68, p<0.001). By 18-months the effect was greatly diminished (β=0.20, p=0.68). Around 18.5 months of age the trajectory of dog-exposed children appeared to cross the pet-free trajectory, but differences were not statistically significant at 20-months of age (p=0.27). Due to sparse data, estimates beyond 18-months should be interpreted with caution.

2.3.4 Dog-Ownership & Gut Microbiota Composition Trajectories

To understand how dog exposure impacts gut microbiota composition over time we examined β-diversity trajectories using principal coordinates. Although dog exposure was associated with overall compositional structure in some unadjusted models, the effect was non-significant after covariate adjustment (Table S2.5). Effects were not time-dependent (Table S2.4; all dog*time interaction p≥0.058). Other effect modifiers were also non-significant except for formula-feeding on the 1st principal coordinate of unweighted UniFrac (interaction p=0.036) and Canberra (interaction p=0.039)

distances. Specifically, the effect of dog ownership on β-diversity trajectory was apparent among formula fed children only (Figure 2.3).

2.3.5 Indoor Dogs & Enrichment/Depletion of Specific Taxa

We next sought to identify individual gut bacterial taxa differing between infants living with indoor dogs versus in pet-free homes. Among 120 genera, 57 were detected in >10% of samples. Following covariate adjustment, two genera reached statistical significance (Figure 2.4): *Fusobacterium* was significantly enriched in children living with dogs and *Citrobacter* was significantly depleted in these children.

We also assessed OTUs associated with dog exposure. Of the 1,286 OTUs detected, 439 were prevalent in >10% of samples. After full covariate adjustment, no OTUs had a significant dog by time interaction ($p_{FDR}<0.05$). However, nine OTUs were statistically related to living with a dog (Figure S2.2; Table S2.6). Specifically, *Lactobacillus* (OTUs 1367 and 2144) and *Citrobacter sp.* (OTU 2434) members were significantly depleted in dog-exposed children throughout early life, whereas *Collinsella stercoris* (OTU 93), *Dorea* (OTU 1015), *Ruminococcus* (OTU 1822) *Lachnospiraceae* (OTU 2277 and 575), and *Clostridiaceae* (OTU 1099) were significantly enriched.

2.3.6 Dog-Exposure in Formula-fed Infants & Taxa Trajectories

Noting the significant interaction with formula feeding observed in the β-diversity trajectory models, we examined individual OTU trajectories contributing to this interaction. A total of 75 OTUs contributed to the dog by formula feeding interaction (interaction $p_{FDR}<0.05$; Table S2.7). Most (60 of the 75 taxa) exhibited an effect of living with a dog that was stronger among formula-fed children. Of the nine OTUs with a significant main effect of dog exposure, two, *Dorea* (OTU 1015) and *Collinsella stercoris*

(OTU 93), exhibited a stronger effect of living with a dog in formula-fed versus non-formula-fed children (Table S2.7). Additionally, of 15 *Ruminococcaceae* OTUs having a significant dog exposure by formula-feeding interaction, 14 showed a stronger enrichment with dog exposure among formula-fed children. Many were identified as *Faecalibacterium prausnitzii* OTUs (Table S2.7).

2.4 DISCUSSION

We examined longitudinal development of the gut microbiota in 131 infants over the first 18 months of life. Infants in our study exhibited a similar gut microbial developmental trajectory to published studies[117,201] describing increasing richness and progression towards an adult-like gut microbiome with chronological age.

Assessment of the effect of living with a dog on gut microbiota developmental trajectory indicated that, compared to children not living with furred pets, infants living with dogs exhibit elevated gut bacterial richness and phylogenetic diversity trajectories over the first 18 months of life. Early life represents a critical period for immunological development and low-diversity or inappropriate assembly of the gut microbiota during this time has been associated with adverse health outcomes.[202–204] Alternatively, dog exposure has been associated with a decreased risk of atopy, allergy, and asthma.[3,5,191,195,205,206] Our study supports the hypothesis that this correlation may be related to a longitudinal impact of dog ownership on the developing gut microbiome. For phylogenetic diversity a statistically significant dog exposure by time interaction was observed with the most robust association at 3 and 6 months of age. This is common period of complementary food introduction known to influence the repertoire of local and circulating microbial metabolic products. Early life gut microbial metabolic products are

known to shape immune cell function and development of atopy and asthma.[6,7,183,184,207] Whether increased diversification of the gut microbiota at this key point in development results in tangible differences in the functional pathways or metabolic output of the gut microbiome or its effect on immune function clearly merits further study.

Several taxa were enriched in infants living with dogs compared to pet-free homes including *Collinsella stercoris, Dorea sp*, *Ruminococcus sp.*, several *Lachnospiraceae sp.*, and *Clostridiaceae sp.* Enrichment of *Ruminococcus* was previously reported in the gut microbiota of 3- to 4-month-old infants exposed to furry pets.[208] Separately, our group reported that *Ruminococcaceae, Lachnospiraceae,* and *Clostridiaceae* enrichment was observed in the gut microbiota of mice orally supplemented with house dust collected from homes with dogs and this microbiota restructuring was associated with protection of the recipient animals against allergic airway inflammation.[113] Further, the protective effect appeared mediated, at least in part, by alteration of the serum metabolome, specifically increased concentrations of circulating anti-inflammatory polyunsaturated fatty acids that reduced bone marrow-derived dendritic cell activation and inflammatory cytokine production *in vitro.*[114] Thus the emerging data suggests that early life dog exposure may serve to enrich for microbes in the gut that modulate response to inflammatory stimuli.

The impact of infant dog cohabitation on the gut microbiota was greatest among formula-fed infants who showed an enrichment of *Dorea sp.* (OTUs 1015) and *Collinsella stercoris* (OTU 93). Both have been identified in breast milk[209] and *Collinsella* abundance is highest in the gut of actively breast-feeding infants.[117] Whereas breast milk contains a bacterial community[210] capable of colonizing the infant gut[211–214] and

influencing community composition[117,214], formula fails to provide these microbial exposures. Thus, in the absence of the influence of breast milk, dogs may provide an alternative source of environmental microbes for the developing gut microbiome of formula-fed infants. In support of this concept, *Dorea* was recently found to be depleted in the stool of dust mite-sensitized children with allergic rhinitis at age 4-5 years compared with healthy controls and *Dorea* relative abundance negatively correlated with fecal IgE levels.[215] *Dorea* is also underrepresented in the gut microbiota of children with food allergies.[216]

Several distinct *Faecalibacterium prausnitzii* were also enriched in the gut microbiota of infants living with a dog who were formula-fed. *F. prausnitzii* is an abundant and key immunomodulatory species in the human gut microbiome, capable of exerting anti-inflammatory responses by blocking nuclear factor kB activation and by enhancing antigen-specific T-cell proliferation and IL-10 expression.[217,218] Its loss from the gut microbiota is characteristic of infants at significantly higher risk of developing allergies and asthma in later childhood and in adults with chronic inflammatory conditions such as inflammatory bowel disease.[6,8,219] Thus, the exposure of formula-fed infants to dogs in early life may serve to increase the presence of this species in early infancy.

While a small number of descriptive studies have examined the relationship between household pets and the infant gut bacterial composition, these studies were limited by small sample size, lack of pet-free control populations, and/or examination at only one time point.[208,220,221] In our study, the addition of a pet-free group of infants allowed a rigorous examination of the impact of dog-associated microbial exposures on

early life gut microbiota composition. Frequent sampling of the infant gut microbiota allowed us to evaluate trajectories to determine whether the impact of dog on the developing gut microbiome was constant or if there were critical windows of exposure. Detailed participant surveying also permitted adjustment for a wide range of potentially confounding covariates, although we note that unmeasured or residual confounding is still possible. Due to low DNA yield the vaginal/rectal swabs had a high sequencing failure rate. This does not impact analyses relating dog-keeping to the infant gut microbiome as these samples were used solely as an adult microbiota reference to compare to the infant's developing gut microbiome. To our knowledge, this study is the first to conduct longitudinal analyses examining the impact of dog exposure and the interaction of dog exposure and other early life factors on developing infant gut microbiota trajectories.

In conclusion, we observed that exposure to dogs significantly impacted gut bacterial community trajectories over the first 18 months including higher microbial diversity which was most robust at 3 and 6 months of age. The strongest compositional effects were observed among formula-fed infants and involved introduction of key immunomodulatory gut bacteria. These data support the ongoing hypothesis that associations between dog exposure and decreased risk of allergy may be due to alterations in the early-life gut microbiome. Further research is needed to confirm a causal role of dog-associated microbial exposures on infant gut microbial function and immune responses.

2.5 METHODS

2.5.1 Study Population

The Microbes, Asthma, Allergy and Pets (MAAP) birth cohort includes 141 maternal-child pairs from southeastern Michigan and was designed to evaluate potential differences in the early-life gut microbiome and immune development between children living in homes with indoor dogs versus those living in pet-free homes. Pregnant women between 18 and 49 years of age who were seeing a Henry Ford Health System (HFHS) affiliated obstetrician with planned delivery at selected HFHS hospitals and who were planning to stay in southeast Michigan for at least 2 years postpartum were recruited during their second or third trimester of pregnancy. Eligible women reported either: (i) living with a dog(s) kept indoors at least 12 hours daily for at least six months prior to pregnancy with plans to keep the dog for the study's duration OR (ii) living in furred-pet free homes for at least two years prior to pregnancy with no plans to obtain pets or have contact with pets for more than four hours weekly for the study's duration.

Recruitment spanned January 2014 to August 2016. Women were required to speak English well enough to provide written informed consent. The study was approved by the Henry Ford Hospital IRB.

2.5.2 Data Collection

Women were interviewed prenatally (recruitment), then 1-week, 6-months, and 18-months post-delivery. Questionnaires included: household demographic characteristics, tobacco smoke exposure, medication use, parental allergic history, and pet keeping. Mothers were given a dietary calendar to track weekly changes to their infant's diet (breastfeeding, formula feeding, and solid food introduction). When calendars were incomplete or missing, dietary information was collected from

questionnaires. Formula feeding was defined as any reported formula use since the previous maternal contact. Pregnancy and delivery information were abstracted from electronic health records.

2.5.3 Specimen Collection

Maternal Vaginal/Rectal Swabs (Epicentre Catch-All™) were collected by an obstetrician during routine prenatal visits within six weeks prior to delivery or on the day of delivery using the standard process for Group B Streptococcus screening. Swabs were stored in RNAlater at 4°C for a minimum of 24 hours, then transferred to -80°C.

Stool Specimens from mothers were collected during the last trimester and at approximately 1-week, 6-months, and 18-months postpartum. Infant stool specimens were collected at approximately 1-week, 1-month, 3-months, 6-months, and 18-months after birth. All samples were stored at -80°C.

2.5.4 16S ribosomal RNA Sequencing

2.5.4.1 *DNA Extraction*

A 4 mm punch biopsy (VWR International) was used to transfer ~0.3 grams of frozen stool to Lysing Matrix E tubes (LME; MP Biomedicals). Vaginal/rectal swabs in RNAlater were thawed on ice and transferred to LME tubes. RNAlater was transferred into a sterile tube and centrifuged at 16,000 x g for 5 minutes at 4°C. Pellets were re-suspended using cetyltrimethylammonium bromide (CTAB) and transferred to the LME tube containing the swab. All prepped LME tubes were stored at -80°C. Genomic DNA was extracted using a modified CTAB buffer protocol.[6]

2.5.4.2 *Sequencing Preparation*

After DNA extraction, polymerase chain reaction amplification of the 16S ribosomal RNA (rRNA) gene (variable region 4) was performed using barcoded 515F/806R primers[6] and either 2 ng/µl DNA template (swabs), 10 ng/µl DNA as template (stool), or DNA extraction buffers as no-template controls (NTCs). Amplicon triplicates were pooled, and samples with successful amplification were purified using the SequalPrep Kit (Invitrogen), quantified using the Quant-iT PicoGreen kit (Invitrogen), and pooled at 5 ng per sample. Negative controls were included in the pool at the average volume of the test samples. The pooled library underwent SPRI bead clean-up (Beckman-Coulter) and KAPA library quantification (Kapa Biosystems). The pool was then diluted to 2 nM, denatured, and a quantity of 1.5 pM was loaded onto the NextSeq cartridge (V3; Illumina) with a 60:40 ratio of library to PhiX for sequencing. 24 stool samples and 108 swab samples failed sequencing.

2.5.4.3 *Sequencing-Data Processing & Quality Control*

Paired-end sequences were merged using flasH (v1.2.11)[222], demultiplexed by barcode, and reads that contained two or more unexpected errors were discarded. Unique sequences were clustered at 97% identity into operational taxonomic units (OTU) and chimeras removed using USEARCH.[223] USEARCH was used to map all raw quality filtered reads to the unique sequence OTUs at 97% identity. Sequences were aligned using PyNast[224] and the alignment was filtered to remove gaps. A bacterial phylogenetic tree was built using FastTree.[225] The OTU table was filtered to remove unaligned sequences and taxonomy was assigned using Greengenes database (v13_5).[226] NTCs were assessed to determine potential contamination. OTUs present in over half of NTCs were removed completely from samples. For OTUs present in less

than half of NTCs the highest read count was determined and subtracted from samples. To de-noise the OTU table, taxa with less than 1/1000 of a percent of the total read count across all samples were removed. To normalize read depth across samples, data were rarefied 100 times to a read depth of 60,503 reads, using a multiple rarefaction algorithm.[6] This depth was chosen because it was a high rarefying depth that resulted in little sample loss (stool N=36, swabs N=4) and Procrustes analysis indicated the distances in this rarefied matrix were closer (lowest M_2 values) to the non-rarefied matrix compared to other rarefying depths. For number of stool samples contributed per infant refer to Table S1.

2.5.6 Statistical Analysis

Statistical significance for main and interaction effects was set at $p<0.05$. ANOVA and chi-square tests were used to compare maternal and child characteristics across groups by prenatal indoor dog keeping (yes/no). A-diversity metrics were calculated using the R packages *vegan* and *picante*. Mixed effect models were used to fit baby stool α-diversity trajectories. Exact age at stool sample collection was used rather than targeted collection time. Best-fitting shape (linear versus quadratic) was determined by Bayesian information criterion (BIC). Effect modification by age at time of stool sample collection, child sex, mode of delivery, household income, and formula feeding were selected *a priori* to be evaluated. The effect of indoor dog-keeping was assessed before and after adjusting for a set of pre-specified potentially confounding covariates: maternal reported race, household income, maternal age at birth, mode of delivery (vaginal versus caesarian-section), child sex, whether child was firstborn, age at solid food introduction, and breastfeeding duration.

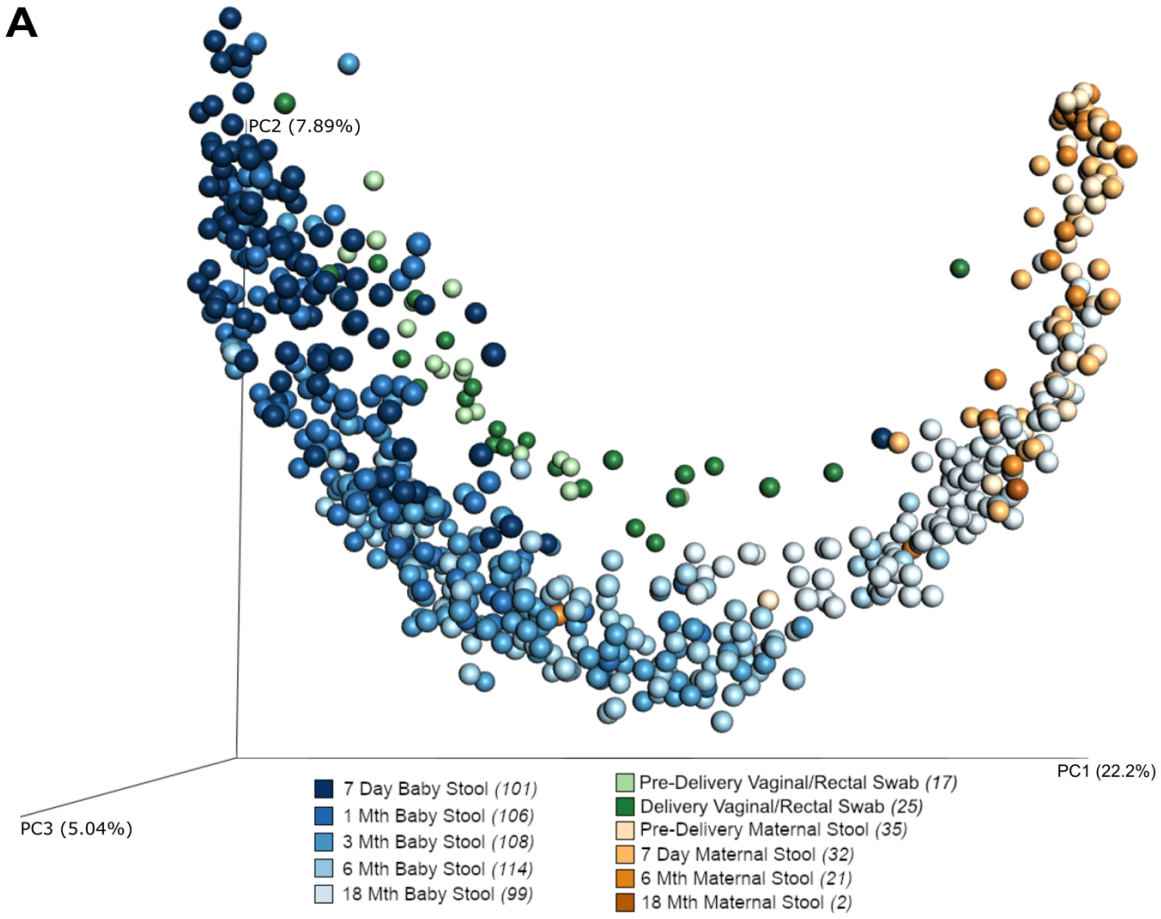
β-diversity metrics were calculated using the R packages *phyloseq* and *vegan*. Principal coordinates analysis (PCoA) was performed using the R package *labdsv* on baby stool samples with each β-diversity metric. The 1st through 4th principal coordinates were extracted and used as outcomes in mixed effect models to assess compositional differences by dog exposure—before and after covariate adjustment—as described previously for α-diversity. Compositional differences in cross-sectional datasets were also assessed using PERMANOVA, before and after covariate adjustment.

Differences in OTU and genera abundance trajectories by prenatal indoor dog exposure were fit using generalized linear mixed effect models with the R package *glmmADMB*. Briefly, each taxon was first modeled with the random subject effect and a time covariate only (continuous, actual age at sample collection); negative binomial versus zero-inflated negative binomial models were compared for best fit using BIC (unless only one of the two models were estimable; if neither model was estimable, results were not examined). Upon determining the best-fitting model, main effects of dog exposure and interaction effects with time were obtained (with subgroup effects reported for any significant interactions) with p-values for each false discovery rate-adjusted ($p_{FDR}<0.05$ considered significant). All models testing dog effects were adjusted for the pre-specified confounding covariates. Taxa were only tested if they were present in ≧10% of baby stool samples. For OTU models, interaction effects were added for potentially effect modifying covariates, as suggested by β-diversity results. All analyses were performed using R version 3.6.1 or SAS version 9.4.

2.6 ACKNOWLEDGEMENTS

We would like to acknowledge the MAAP cohort families for the substantial time and effort necessary to collect the many samples and questionnaires to complete this project. We would also like to acknowledge the continued efforts of all our supporting MAAP research team members.

2.7 FIGURES


A
PC2 (7.89%)
PC1 (22.2%)
PC3 (5.04%)
7 Day Baby Stool (101)
1 Mth Baby Stool (106)
3 Mth Baby Stool (108)
6 Mth Baby Stool (114)
18 Mth Baby Stool (99)
Pre-Delivery Vaginal/Rectal Swab (17)
Delivery Vaginal/Rectal Swab (25)
Pre-Delivery Maternal Stool (35)
7 Day Maternal Stool (32)
6 Mth Maternal Stool (21)
18 Mth Maternal Stool (2)

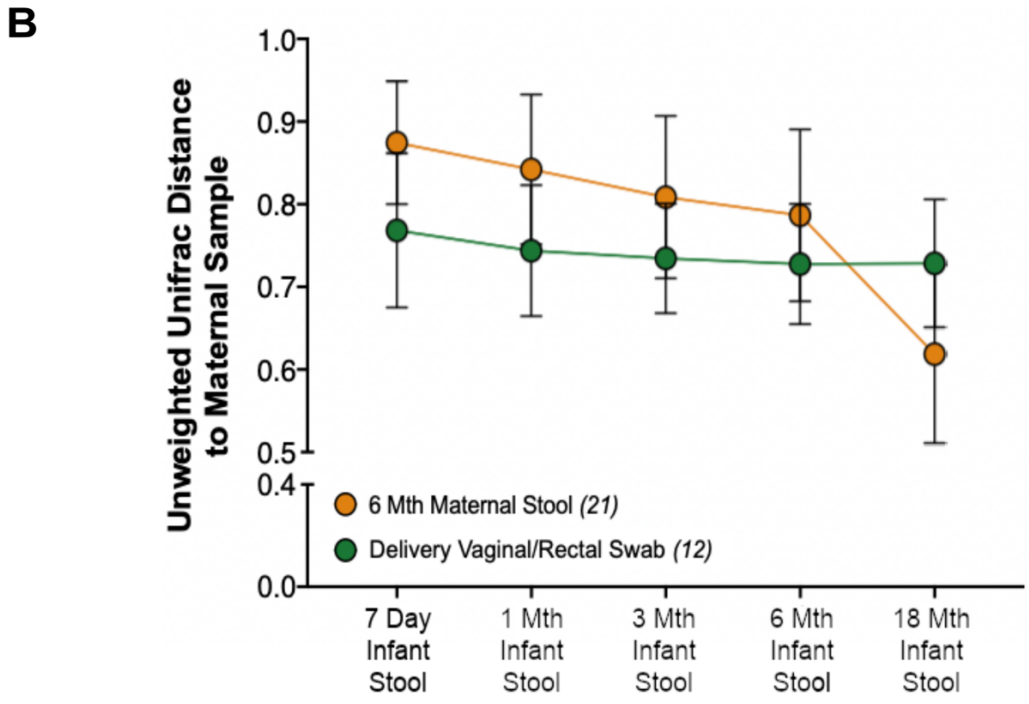

B
Unweighted Unifrac Distance to Maternal Sample
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.0
6 Mth Maternal Stool (21)
Delivery Vaginal/Rectal Swab (12)
7 Day Infant Stool
1 Mth Infant Stool
3 Mth Infant Stool
6 Mth Infant Stool
18 Mth Infant Stool

Figure 2.1 Infant Gut Microbiota Becomes More Similar to the Adult Gut Microbiota with Advancing Chronological Age. A) PCoA based on an unweighted Unifrac dissimilarity matrix showing bacterial community composition of infant stool samples (7 days and 1, 2, 6, and 18 months), maternal stool samples (pre-delivery, 7 days, 6 and 18 months), and maternal vaginal/rectal swabs (pre-delivery and delivery). B) Mean unweighted Unifrac distance of delivery maternal vaginal/rectal swabs or 6-month maternal stool to paired, vaginally born infant stool samples at each time point.

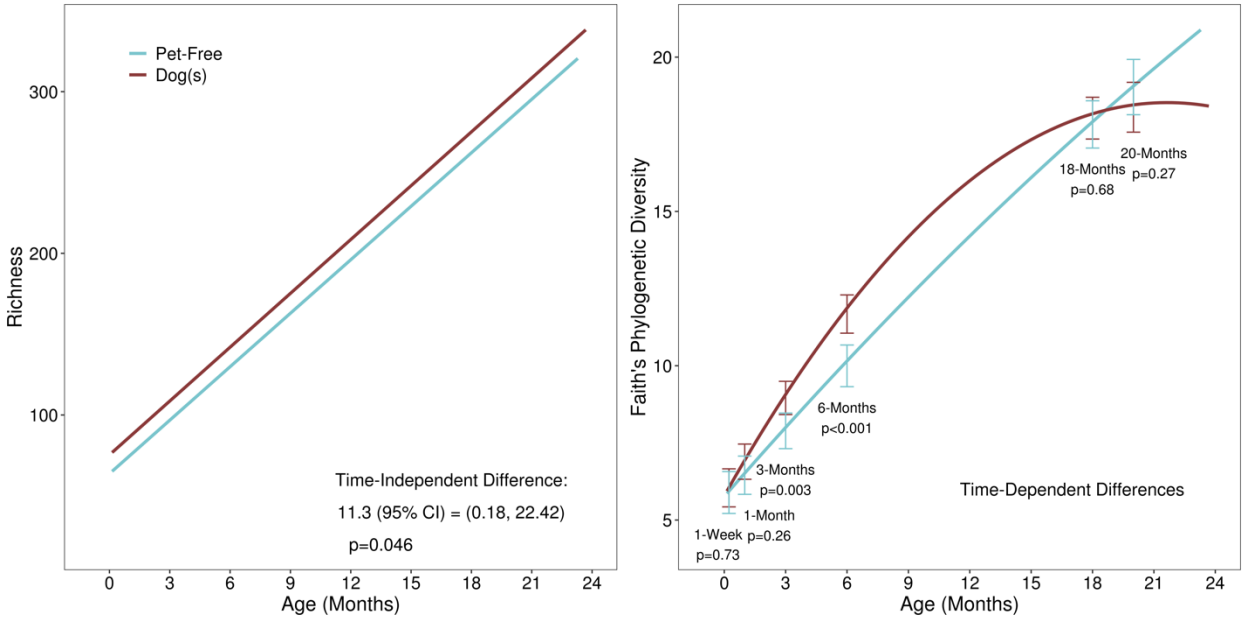


Figure 2.2 Dog-Exposed Infants Have Increased Richness and Diversity Over the First Year of Life. Fitted richness (first panel) and Faith's phylogenetic diversity (second panel) trajectories by prenatal indoor dogs. Both models use full covariate adjustment, and the phylogenetic diversity model includes terms for dog*time interaction, as this was statistically significant (p=0.012).

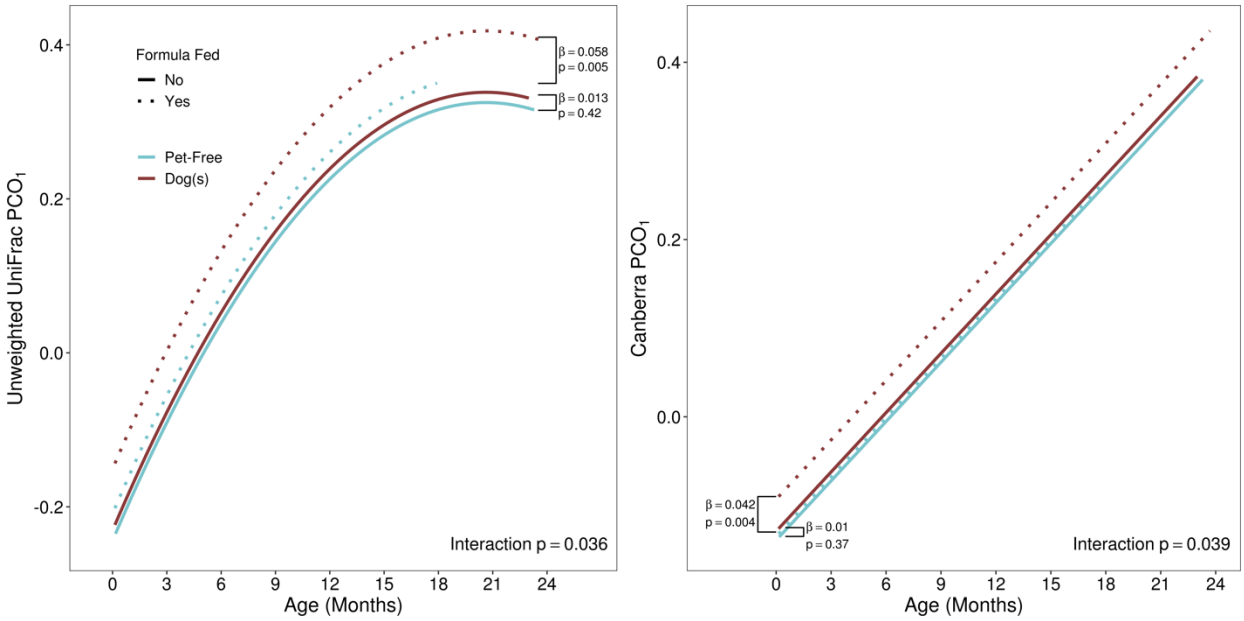


Figure 2.3 Dog Ownership Effects Gut Microbiota Composition Trajectories Only in Formula Fed Infants. Trajectories of unweighted UniFrac and Canberra metrics (1st principal coordinate), by prenatal dog exposure and type of feeding. Brackets show the difference between dog exposed and unexposed children, among children who are formula fed (dotted lines) versus not (solid lines).

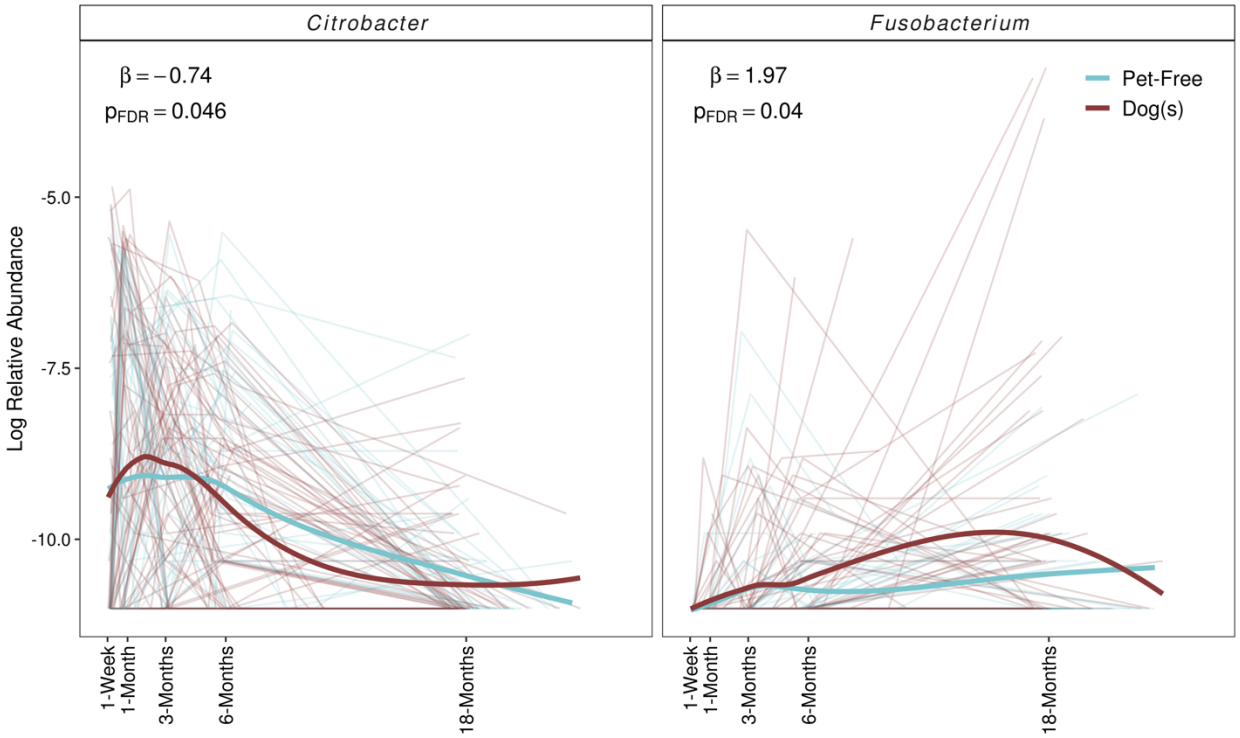


Figure 2.4 *Citrobacter* and *Fusobacterium* are Significantly Associated with Infant Dog-Exposure. Genera significantly associated with prenatal dog exposure ($p_{FDR}<0.05$) after covariate adjustment. Estimates shown represent the difference in log-transformed mean abundance, comparing infants living with dogs versus those without furred pets.

2.8 TABLES

Table 2.1: Description of 660 samples across 134 maternal-child pairs with microbiome data.

Subject	Sample	Sample Collection Time *(Number sent for sequencing)*	Observed Collection Time in days, with respect to baby date of birth (N = sequenced samples)						
			N	**Min**	**Q1**	**Median**	**Mean**	**Q3**	**Max**
Mom	Stool	Pregnancy *(36)*	35	-65	-27	-20	-21	-10	-1
		1-Week *(33)*	32	6	13	22	24	27	95
		6-Months *(21)*	21	163	175	188	188	193	258
		18-Months *(2)*	2	532	534	535	535	536	538
	Vaginal/ Rectal Swab	Pre-Delivery *(52)*	17	-45	-25	-21	-20	-10	-1
		Delivery *(102)*	25	0	0	0	0	0	0
Baby	Stool	1-Week *(119)*	101	4	7	8	8	9	14
		1-Month *(122)*	106	18	27	31	35	38	79
		3-Months *(119)*	108	81	90	95	99	99	166
		6-Months *(123)*	114	153	173	181	193	194	332
		18-Months *(103)*	99	516	532	544	560	566	720

Table 2.2: Characteristics of the overall MAAP cohort and those with infant stool microbiota measurements.

Covariate	Statistic	Level	Overall Cohort (N=141)	Has Baby Stool Micro-biota (N=131)	Indoor Dog(s) during pregnancy (N=131)		p-value[1]
					No N=56	Yes N=75	
African American Mother[2]	N (Col %)	No	112 (79.4%)	107 (81.7%)	40 (71.4%)	67 (89.3%)	**0.009**
	N (Col %)	Yes	29 (20.6%)	24 (18.3%)	16 (28.6%)	8 (10.7%)	
Maternal-Reported Marital Status	N (Col %)	No	19 (13.5%)	16 (12.2%)	6 (10.7%)	10 (13.3%)	0.651
	N (Col %)	Yes	122 (86.5%)	115 (87.8%)	50 (89.3%)	65 (86.7%)	
Household Income	N (Col %)	<$40K	21 (14.9%)	20 (15.3%)	14 (25%)	6 (8%)	**0.006**
	N (Col %)	$40K-$80K	33 (23.4%)	32 (24.4%)	7 (12.5%)	25 (33.3%)	
	N (Col %)	>$80K	64 (45.4%)	60 (45.8%)	28 (50%)	32 (42.7%)	
	N (Col %)	Refused/ Don't Know	23 (16.3%)	19 (14.5%)	7 (12.5%)	12 (16%)	
Highest level of Maternal Education	N (Col %)	HS diploma or less	22 (15.7%)	19 (14.6%)	8 (14.3%)	11 (14.9%)	0.493
	N (Col %)	Some college, Associate's degree or tech school	40 (28.6%)	37 (28.5%)	13 (23.2%)	24 (32.4%)	
	N (Col %)	Bachelor's degree	40 (28.6%)	38 (29.2%)	20 (35.7%)	18 (24.3%)	
	N (Col %)	Advanced degree	38 (27.1%)	36 (27.7%)	15 (26.8%)	21 (28.4%)	
First Born Child	N (Col %)	No	90 (63.8%)	82 (62.6%)	40 (71.4%)	42 (56%)	0.071
	N (Col %)	Yes	51 (36.2%)	49 (37.4%)	16 (28.6%)	33 (44%)	
Mode of Delivery	N (Col %)	Vaginal	96 (68.1%)	89 (67.9%)	41 (73.2%)	48 (64%)	0.264
	N (Col %)	C-section	45 (31.9%)	42 (32.1%)	15 (26.8%)	27 (36%)	

Covariate	Statistic	Level	Overall Cohort (N=141)	Has Baby Stool Micro-biota (N=131)	Indoor Dog(s) during pregnancy (N=131)		p-value[1]
					No N=56	Yes N=75	
Mother ever diagnosed with eczema	N (Col %)	No	122 (86.5%)	112 (85.5%)	47 (83.9%)	65 (86.7%)	0.66
	N (Col %)	Yes	19 (13.5%)	19 (14.5%)	9 (16.1%)	10 (13.3%)	
Mother ever diagnosed with asthma	N (Col %)	No	95 (67.4%)	90 (68.7%)	37 (66.1%)	53 (70.7%)	0.575
	N (Col %)	Yes	46 (32.6%)	41 (31.3%)	19 (33.9%)	22 (29.3%)	
Mother tobacco smoker at Pre-Delivery	N (Col %)	No	131 (93.6%)	122 (93.8%)	55 (98.2%)	67 (90.5%)	0.071
	N (Col %)	Yes	9 (6.4%)	8 (6.2%)	1 (1.8%)	7 (9.5%)	
Household tobacco smoke at Pre-Delivery	N (Col %)	No	125 (91.9%)	117 (92.1%)	53 (96.4%)	64 (88.9%)	0.121
	N (Col %)	Yes	11 (8.1%)	10 (7.9%)	2 (3.6%)	8 (11.1%)	
Child Sex	N (Col %)	Male	68 (48.6%)	63 (48.1%)	24 (42.9%)	39 (52%)	0.3
	N (Col %)	Female	72 (51.4%)	68 (51.9%)	32 (57.1%)	36 (48%)	
Antibiotic Use in First 6-Months of Life	N (Col %)	No	115 (89.8%)	114 (89.8%)	47 (88.7%)	67 (90.5%)	0.733
	N (Col %)	Yes	13 (10.2%)	13 (10.2%)	6 (11.3%)	7 (9.5%)	
Antifungal Use in First 6-Months of Life	N (Col %)	No	121 (94.5%)	120 (94.5%)	50 (94.3%)	70 (94.6%)	0.95
	N (Col %)	Yes	7 (5.5%)	7 (5.5%)	3 (5.7%)	4 (5.4%)	
Maternal Age at Birth (years)	Mean		31	31.05	31.02	31.07	0.952
	Std Dev		4.98	4.92	4.68	5.13	
Number of Previous Pregnancies	Mean		1.52	1.51	1.73	1.36	0.119
	Std Dev		1.32	1.35	1.31	1.36	
Number of Previous Live Births	Mean		0.98	0.96	1.11	0.85	0.15
	Std Dev		0.99	1	1	0.98	

Covariate	Statistic	Level	Overall Cohort (N=141)	Has Baby Stool Micro-biota (N=131)	Indoor Dog(s) during pregnancy (N=131)		p-value[1]
					No N=56	Yes N=75	
Number of Children in the Home at Pre-Delivery			141	131	56	75	0.702
	Mean		0.99	0.99	1.04	0.96	
	Std Dev		1.1	1.11	1.11	1.12	
Breastfeeding Duration (weeks)			125	124	51	73	0.239
	Mean		30.63	30.69	33.8	28.51	
	Std Dev		24.46	24.55	25.47	23.83	
First solid food introduction (weeks)			123	122	50	72	0.074
	Mean		20.72	20.75	21.92	19.94	
	Std Dev		6	6.01	4.43	6.82	

[1]*calculated by ANOVA for numerical covariates and chi-square test for categorical covariates.*
[2]*if multiracial with at least one part Black, assigned to Black category.*

Table 2.3: Association between dog exposure and infant gut α-diversity trajectories, before and after covariate adjustment.

Model	N	β[a]	SE	p-value
Richness[b]				
Unadjusted	131	10.0	6.0	0.094
Fully adjusted[c]	120	11.3	5.7	**0.046**
Evenness[b]				
Unadjusted	131	0.007	0.012	0.54
Fully adjusted[c]	120	0.001	0.012	0.96
Faith's Phylogenetic Diversity[d]				
Unadjusted	131	0.65	0.36	0.071
Fully adjusted[c]	120	0.72	0.35	**0.036**

[a]Average difference in specified α-diversity metric (comparing dog-exposed vs. pet-free), throughout early life.
[b]linear model
[c]Adjusted for maternal race, household income, maternal age at birth, mode of delivery, child sex, first born child, age at solid food introduction, and breastfeeding duration.
[d]quadratic model

2.9 SUPPLEMENTARY DATA

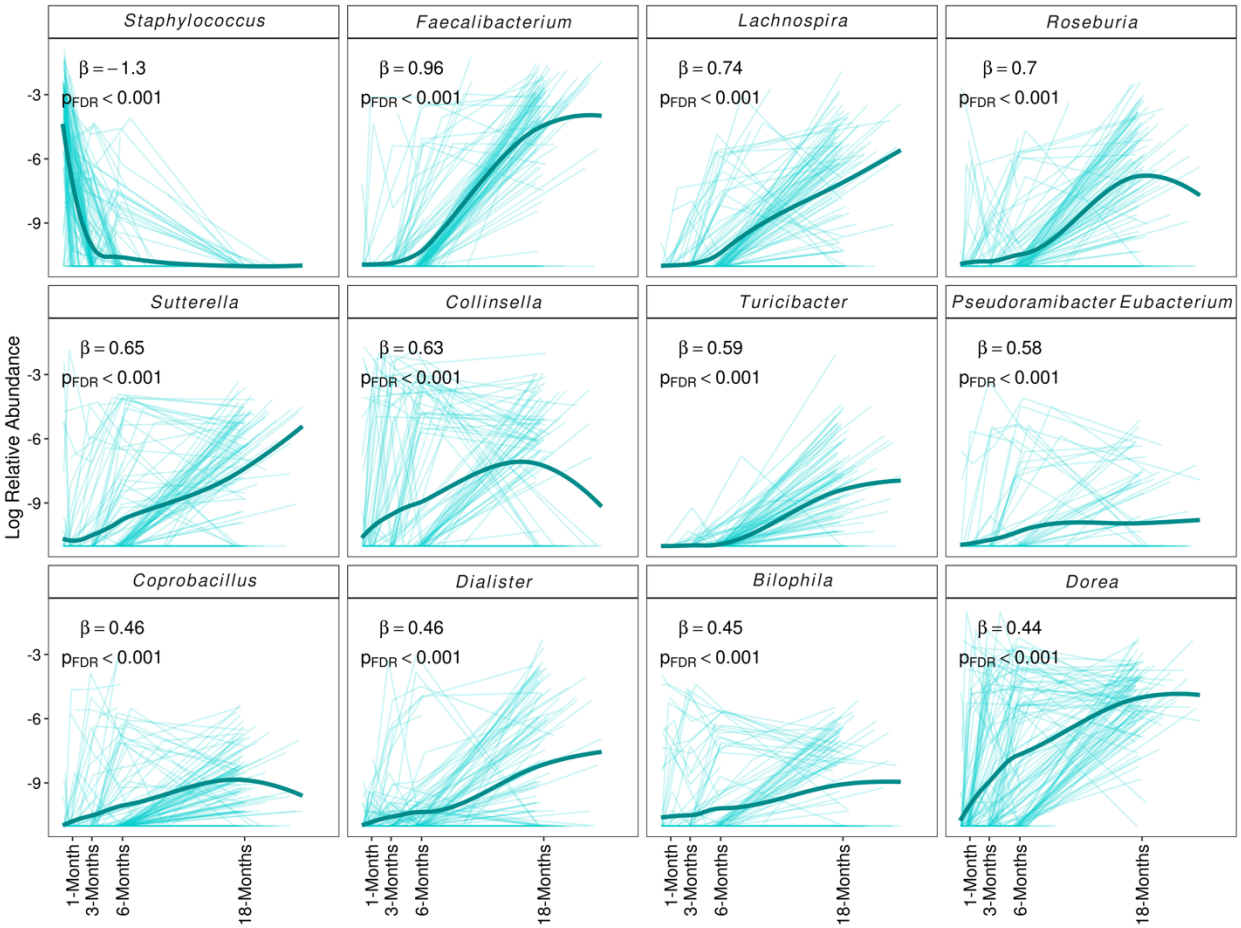


Figure S2.1 Genera Associated with Chronological Age. Top 12 genera with largest absolute effect size significantly associated with chronological age (p_{FDR}<0.05).

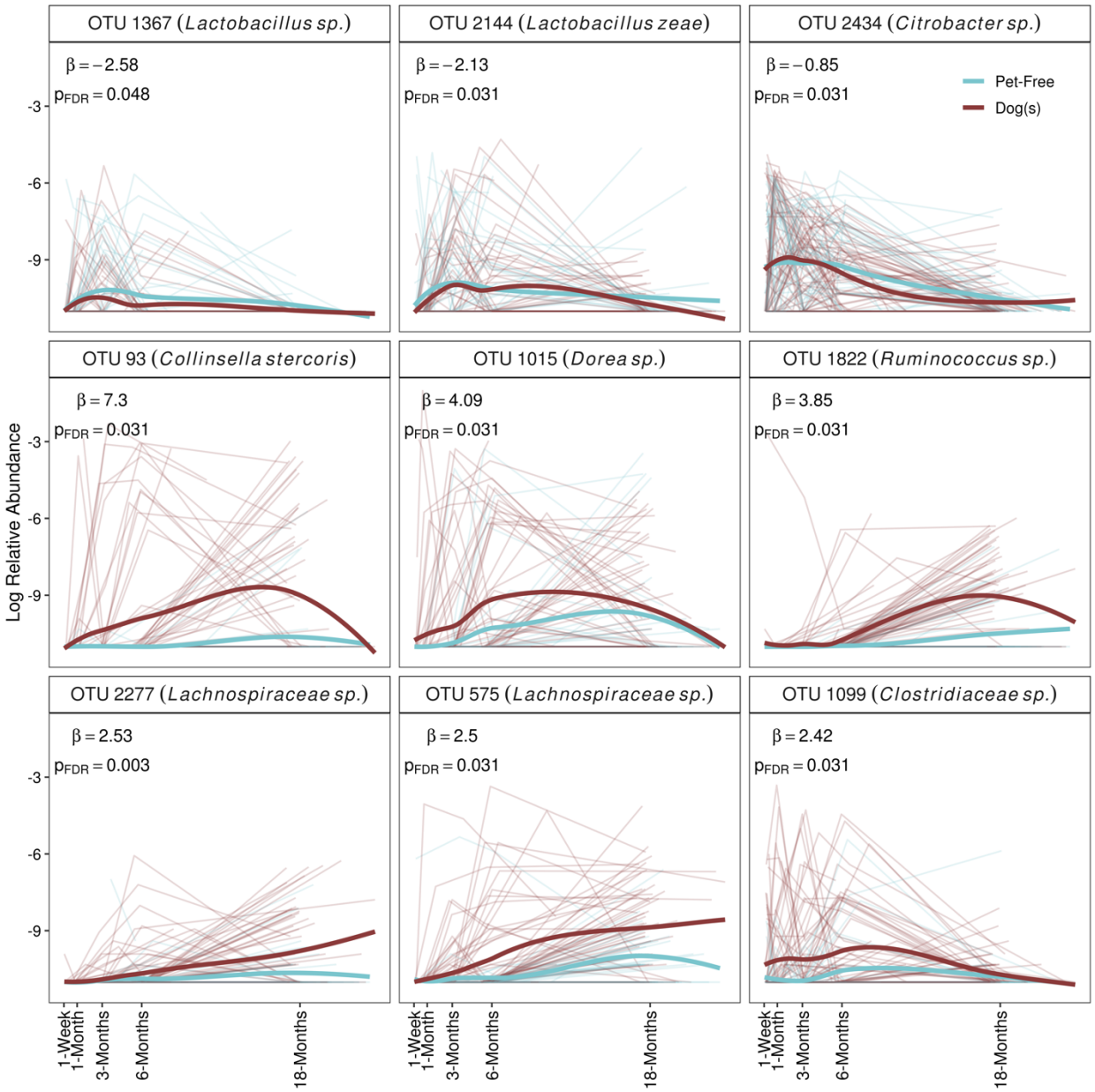


Figure S2.2 Taxa Significantly Associated with Early-Life Dog Exposure. OTUs significantly associated with prenatal dog exposure (p_{FDR}<0.05), after covariate adjustment. Estimate shown represent the difference in log-transformed mean abundance, comparing dog exposed to non-dog exposed children.

Table S2.1: Infant stool sample contribution by timepoint.

Infant	7 Day	1 Month	3 Months	6 Months	18 Months	Total
1	Y	Y	Y	Y		4
2	Y	Y	Y	Y	Y	5
3	Y	Y	Y	Y	Y	5
4	Y		Y	Y		3
5	Y	Y	Y	Y	Y	5
6	Y	Y	Y	Y	Y	5
7	Y	Y	Y	Y	Y	5
8			Y	Y	Y	3
9	Y	Y	Y	Y	Y	5
10	Y	Y	Y	Y	Y	5
11		Y	Y		Y	3
12		Y	Y		Y	3
13	Y	Y	Y	Y	Y	5
14		Y	Y	Y	Y	4
15	Y		Y	Y	Y	4
16	Y	Y	Y	Y		4
17		Y	Y	Y	Y	4
18		Y	Y	Y	Y	4
19				Y	Y	2
20	Y	Y	Y	Y	Y	5
21	Y	Y	Y	Y	Y	5
22	Y		Y	Y	Y	4
23			Y	Y		2
24		Y		Y	Y	3
25	Y	Y		Y	Y	4
26			Y	Y	Y	3
27	Y					1
28	Y	Y		Y		3
29	Y	Y	Y	Y	Y	5
30		Y	Y	Y	Y	4
31	Y	Y		Y	Y	4
32						0
33			Y			1
34	Y	Y	Y	Y	Y	5
35	Y	Y	Y			3
36		Y				1
37	Y		Y	Y	Y	4

Infant	7 Day	1 Month	3 Months	6 Months	18 Months	Total
38	Y	Y	Y	Y		4
39		Y	Y		Y	3
40	Y	Y	Y	Y		4
41	Y		Y	Y		3
42	Y	Y	Y	Y		4
43		Y	Y	Y		3
44		Y	Y	Y		3
45		Y	Y	Y	Y	4
46	Y		Y	Y	Y	4
47	Y		Y	Y		3
48	Y	Y	Y	Y	Y	5
49	Y	Y	Y	Y	Y	5
50		Y	Y	Y	Y	4
51		Y	Y	Y	Y	4
52	Y	Y	Y	Y	Y	5
53	Y	Y	Y	Y	Y	5
54	Y	Y	Y		Y	4
55	Y	Y	Y	Y	Y	5
56	Y	Y	Y	Y	Y	5
57	Y	Y	Y	Y	Y	5
58		Y	Y		Y	3
59						0
60	Y	Y			Y	3
61		Y	Y	Y	Y	4
62	Y			Y	Y	3
63	Y	Y			Y	3
64	Y	Y			Y	3
65			Y	Y	Y	3
66	Y	Y	Y	Y	Y	5
67	Y	Y	Y		Y	4
68	Y	Y	Y	Y	Y	5
69			Y	Y	Y	3
70	Y	Y		Y	Y	4
71	Y	Y	Y	Y	Y	5
72	Y	Y	Y	Y	Y	5
73	Y	Y	Y	Y	Y	5
74	Y	Y	Y	Y	Y	5
75	Y	Y	Y	Y		4

Infant	7 Day	1 Month	3 Months	6 Months	18 Months	Total
76		Y	Y	Y	Y	4
77		Y	Y	Y	Y	4
78	Y	Y	Y	Y		4
79	Y	Y	Y	Y	Y	5
80	Y	Y	Y	Y	Y	5
81						0
82	Y	Y	Y	Y	Y	5
83	Y	Y	Y	Y	Y	5
84	Y	Y	Y	Y	Y	5
85	Y	Y	Y	Y	Y	5
86	Y	Y	Y	Y	Y	5
87				Y		1
88	Y	Y	Y	Y	Y	5
89	Y	Y	Y	Y		4
90	Y	Y	Y	Y	Y	5
91		Y		Y	Y	3
92	Y					1
93	Y	Y	Y	Y		4
94	Y			Y	Y	3
95	Y	Y	Y	Y	Y	5
96		Y	Y	Y	Y	4
97	Y	Y	Y	Y	Y	5
98	Y	Y	Y	Y	Y	5
99	Y	Y	Y	Y		4
100	Y	Y		Y		3
101	Y	Y	Y	Y	Y	5
102	Y	Y	Y	Y	Y	5
103	Y	Y	Y	Y	Y	5
104	Y	Y	Y	Y	Y	5
105	Y	Y	Y	Y		4
106	Y	Y		Y	Y	4
107	Y	Y	Y	Y	Y	5
108	Y	Y	Y	Y	Y	5
109	Y	Y	Y	Y	Y	5
110	Y		Y	Y	Y	4
111	Y		Y	Y	Y	4
112	Y		Y	Y		3
113	Y	Y	Y	Y	Y	5

Infant	7 Day	1 Month	3 Months	6 Months	18 Months	Total
114	Y	Y	Y	Y	Y	5
115	Y				Y	2
116	Y	Y	Y	Y	Y	5
117	Y	Y	Y	Y		4
118	Y	Y	Y	Y	Y	5
119	Y	Y	Y	Y	Y	5
120	Y		Y	Y	Y	4
121		Y	Y	Y	Y	4
122	Y	Y		Y	Y	4
123	Y	Y			Y	3
124	Y		Y		Y	3
125	Y	Y	Y	Y		4
126	Y	Y	Y	Y		4
127	Y	Y	Y	Y	Y	5
128	Y	Y	Y	Y		4
129	Y	Y		Y		3
130	Y	Y	Y	Y	Y	5
131		Y	Y	Y	Y	4
132	Y	Y		Y		3
133	Y	Y	Y	Y	Y	5
134	Y	Y	Y	Y	Y	5

Table S2.2: Genera with significant association with chronological age.

Genus	Model	Estimate[T]	p-value	p-valueFDR
Faecalibacterium	NB	0.960736	6.30E-54	1.73E-52
Lachnospira	NB	0.738465	5.79E-41	6.37E-40
Roseburia	NB	0.695371	9.02E-42	1.24E-40
Sutterella	NB	0.647254	3.47E-37	3.18E-36
Collinsella	NB	0.627238	4.98E-24	2.11E-23
Turicibacter	NB	0.585644	2.58E-58	1.42E-56
Pseudoramibacter _Eubacterium	NB	0.584084	1.63E-13	4.07E-13
Coprobacillus	ZINB	0.461026	2.17E-25	1.08E-24
Dialister	NB	0.456429	7.26E-46	1.33E-44
Bilophila	NB	0.454404	2.97E-26	1.81E-25
Dorea	NB	0.436024	1.19E-12	2.83E-12
Anaerotruncus	NB	0.435511	1.35E-24	6.19E-24
Coprococcus	NB	0.426193	3.66E-21	1.34E-20
Parabacteroides	NB	0.424735	4.25E-21	1.46E-20
Akkermansia	NB	0.363423	1.44E-06	2.73E-06
Lactococcus	NB	0.330563	1.17E-19	3.77E-19
Phascolarcto-bacterium	NB	0.328731	1.86E-05	3.20E-05
Prevotella	NB	0.324613	9.18E-18	2.80E-17
Fusobacterium	NB	0.264104	1.19E-10	2.62E-10
Megasphaera	NB	0.216832	0.00505	0.006313
Peptostreptococcus	ZINB	0.201264	1.01E-09	2.14E-09
Blautia	NB	0.114909	0.000208	0.000336
WAL_1855D	ZINB	0.110506	0.004362	0.005712
Mogibacterium	ZINB	0.094618	9.68E-06	1.72E-05
Bulleidia	NB	0.086557	0.003214	0.004311
Oscillospira	NB	0.083277	0.000769	0.001143
Peptoniphilus	NB	0.074021	0.035671	0.040873
Ruminococcus	NB	0.071931	0.002523	0.003469
Eggerthella	NB	0.0612	0.004633	0.005926
Campylobacter	NB	0.043863	0.270353	0.28595
Bacteroides	NB	0.028467	0.137694	0.151463
Aggregatibacter	NB	0.008636	0.766156	0.795068
Eubacterium	NB	0.007529	0.873418	0.889592
Unknown	ZINB	7.68E-05	0.989563	0.989563
Anaerococcus	NB	-0.0247	0.205972	0.222127
Bifidobacterium	NB	-0.04942	0.001232	0.001783
Corynebacterium	NB	-0.05731	0.000293	0.000461

Genus	Model	Estimate[T]	p-value	p-valueFDR
Finegoldia	NB	-0.06914	0.000195	0.000325
Neisseria	NB	-0.07354	0.084078	0.094374
Atopobium	NB	-0.07781	0.002128	0.003002
Haemophilus	NB	-0.07859	0.021362	0.025541
Actinomyces	ZINB	-0.08177	6.48E-08	1.32E-07
Carnobacterium	NB	-0.08295	0.009746	0.011912
Streptococcus	NB	-0.09372	1.23E-17	3.57E-17
Veillonella	NB	-0.10029	2.15E-07	4.23E-07
Varibaculum	NB	-0.10649	0.030492	0.035682
Rothia	ZINB	-0.16142	9.13E-24	3.59E-23
Alloiococcus	NB	-0.16146	0.000598	0.000913
Enterococcus	NB	-0.19011	5.59E-16	1.54E-15
Clostridium	NB	-0.19785	7.67E-15	2.01E-14
Citrobacter	ZINB	-0.19857	6.09E-31	4.19E-30
Enterobacter	NB	-0.21442	7.08E-26	3.90E-25
Trabulsiella	NB	-0.24447	5.70E-11	1.31E-10
Acinetobacter	NB	-0.24654	1.73E-06	3.18E-06
Staphylococcus	NB	-1.30148	6.24E-36	4.90E-35
Lactobacillus	ZINB	NA*	NA*	NA*
Porphyromonas	NB	NA*	NA*	NA*

[T]Estimate represents absolute effect size, and the table is ordered based on this value.
**NA values represent genera for which association with chronological age was undetermined in this data set.*

Table S2.3: OTUs with significant association with chronological age. OTUs ordered by p-valueFDR.

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_1987	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	ZINB	-0.209895206	5.64E-86	2.42E-83
OTU_1964	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.273077475	1.35E-69	2.91E-67
OTU_1511	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.469896684	1.11E-64	1.59E-62
OTU_2375	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.660311732	1.59E-63	1.71E-61
OTU_1985	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.589414706	2.66E-63	2.28E-61
OTU_2155	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.513557693	4.10E-62	2.93E-60
OTU_2161	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.390515454	7.52E-61	4.61E-59
OTU_104	*Bacteria*	*Firmicutes*	*Bacilli*	*Turicibacterales*	*Turicibacteraceae*	*Turicibacter*	unknown	NB	0.585643559	2.58E-58	1.38E-56
OTU_1560	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.472363073	4.40E-58	2.10E-56
OTU_58	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Dialister*	unknown	ZINB	0.572640088	2.45E-57	1.05E-55
OTU_50	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	*gnavus*	NB	0.432085924	6.04E-57	2.16E-55
OTU_730	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.614852798	5.70E-57	2.16E-55
OTU_2337	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.95312939	1.50E-54	4.96E-53
OTU_10	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.91394112	2.12E-53	6.49E-52
OTU_1745	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.169318647	2.67E-52	7.65E-51
OTU_1709	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.573061436	1.42E-50	3.81E-49
OTU_1495	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.381085407	2.99E-50	7.54E-49
OTU_1192	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.544301794	3.41E-50	7.99E-49
OTU_2314	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.602319834	3.54E-50	7.99E-49
OTU_1829	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.431949816	1.07E-49	2.29E-48
OTU_2304	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.430410561	4.52E-49	9.24E-48
OTU_4	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.247869664	5.69E-47	1.11E-45
OTU_1977	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	0.470201372	3.50E-45	6.53E-44
OTU_2454	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.740729389	4.94E-44	8.84E-43
OTU_1942	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	0.543181067	8.13E-44	1.39E-42
OTU_2182	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.519372916	2.93E-43	4.83E-42
OTU_1755	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.605110416	4.21E-43	6.69E-42
OTU_1852	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.576099614	4.95E-43	7.59E-42
OTU_2168	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.48193535	8.24E-43	1.22E-41
OTU_21	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.956162248	9.67E-43	1.38E-41
OTU_7	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	NB	0.545902137	5.85E-42	8.10E-41
OTU_61	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	0.542062214	7.65E-42	1.03E-40
OTU_1013	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.507964658	1.33E-41	1.73E-40
OTU_1145	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Roseburia*	unknown	NB	0.687236364	1.89E-41	2.38E-40
OTU_1063	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.488748332	2.48E-39	3.04E-38
OTU_2011	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.26392829	4.21E-39	5.02E-38
OTU_298	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.510186378	6.12E-39	7.09E-38
OTU_1965	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.575094763	9.90E-39	1.12E-37
OTU_67	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.815919267	3.70E-38	4.07E-37
OTU_1746	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.380763589	4.39E-38	4.70E-37
OTU_1354	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.408852068	3.37E-37	3.52E-36
OTU_834	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.59104975	4.28E-37	4.37E-36
OTU_494	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.441308401	5.81E-37	5.80E-36
OTU_111	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.753146261	2.13E-36	2.08E-35
OTU_2091	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.33121743	2.86E-36	2.72E-35
OTU_14	*Bacteria*	*Firmicutes*	*Bacilli*	*Bacillales*	*Staphylococcaceae*	*Staphylococcus*	unknown	NB	-1.301213261	6.35E-36	5.92E-35
OTU_1069	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Roseburia*	unknown	NB	0.532974173	1.12E-35	1.02E-34
OTU_2248	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.547030721	2.00E-35	1.79E-34
OTU_1274	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.561346531	3.03E-35	2.65E-34
OTU_521	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	0.42423421	4.62E-35	3.96E-34
OTU_1587	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.60397556	5.01E-35	4.22E-34
OTU_147	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.453864142	2.82E-34	2.33E-33
OTU_16	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	1.014009581	3.09E-34	2.50E-33
OTU_132	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	NB	0.583814797	5.14E-34	4.08E-33
OTU_2042	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.434926371	6.68E-34	5.21E-33

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_1810	*Bacteria*	*Firmicutes*	*Bacilli*	*Bacillales*	*Planococcaceae*	Unknown	unknown	NB	-1.300710117	9.12E-34	6.99E-33
OTU_34	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*[Eubacterium]*	*dolichum*	ZINB	-0.164479883	2.71E-33	2.04E-32
OTU_568	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.552956884	5.63E-33	4.17E-32
OTU_53	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NB	0.655944555	2.77E-32	2.01E-31
OTU_27	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	ZINB	-0.234196512	7.97E-32	5.70E-31
OTU_126	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.702469284	1.02E-31	7.17E-31
OTU_114	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	0.432310788	1.62E-31	1.12E-30
OTU_2215	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.209381365	1.16E-30	7.87E-30
OTU_562	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.476920862	1.20E-30	8.05E-30
OTU_1275	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.176700108	2.78E-30	1.84E-29
OTU_2434	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Citrobacter*	unknown	ZINB	-0.194752398	3.23E-30	2.10E-29
OTU_2440	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	0.619070089	5.33E-30	3.41E-29
OTU_120	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.700307902	8.94E-30	5.64E-29
OTU_1424	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.350741523	2.37E-29	1.48E-28
OTU_278	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.453509584	2.51E-29	1.54E-28
OTU_127	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	NB	0.686423187	2.61E-29	1.58E-28
OTU_55	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.753069935	4.10E-29	2.44E-28
OTU_18	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	NB	0.502612906	6.07E-29	3.57E-28
OTU_872	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.46236105	6.92E-29	4.01E-28
OTU_1387	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.271195618	7.80E-29	4.46E-28
OTU_98	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	NB	0.795032239	1.45E-28	8.17E-28
OTU_2	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.189529374	2.12E-28	1.18E-27
OTU_155	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.496516791	2.37E-28	1.30E-27
OTU_1732	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	ZINB	0.460516321	3.70E-28	2.01E-27
OTU_44	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.733891791	4.43E-28	2.38E-27
OTU_745	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.543193023	5.09E-28	2.70E-27
OTU_2420	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.351280851	8.22E-28	4.30E-27
OTU_65	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	Unknown	unknown	NB	0.607558628	1.84E-27	9.53E-27
OTU_819	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	Unknown	unknown	NB	0.53866747	2.36E-27	1.20E-26
OTU_2187	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	0.448858862	3.92E-27	1.98E-26
OTU_23	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	unknown	NB	-0.35176277	5.32E-27	2.66E-26
OTU_1996	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	ZINB	0.393997457	6.54E-27	3.23E-26
OTU_322	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.60254643	6.63E-27	3.23E-26
OTU_19	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	NB	0.975431275	8.16E-27	3.93E-26
OTU_26	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.954215463	1.83E-26	8.72E-26
OTU_85	*Bacteria*	*Proteobacteria*	*Deltaproteobacteria*	*Desulfovibrionales*	*Desulfovibrionaceae*	*Bilophila*	unknown	NB	0.454403763	2.97E-26	1.40E-25
OTU_2057	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.283503529	3.22E-26	1.50E-25
OTU_971	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.45839094	3.37E-26	1.56E-25
OTU_575	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.428609064	3.92E-26	1.79E-25
OTU_1960	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.312971735	4.02E-26	1.82E-25
OTU_2308	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.369955879	4.30E-26	1.92E-25
OTU_1008	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.349369858	4.37E-26	1.93E-25
OTU_97	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Lachnospira*	unknown	NB	0.805212401	4.57E-26	2.00E-25
OTU_1780	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	0.312769607	5.00E-26	2.16E-25
OTU_1774	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Enterobacter*	unknown	NB	-0.214423582	7.08E-26	3.04E-25
OTU_1653	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.478305205	7.39E-26	3.14E-25
OTU_251	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.686723812	8.62E-26	3.63E-25
OTU_64	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.638922558	1.31E-25	5.45E-25
OTU_159	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.377182661	2.21E-25	9.10E-25
OTU_1788	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.332709643	6.73E-25	2.74E-24
OTU_1272	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.361439969	6.77E-25	2.74E-24
OTU_154	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	*gnavus*	NB	0.63912096	1.16E-24	4.66E-24
OTU_2106	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.316360912	1.89E-24	7.49E-24
OTU_813	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.582856804	3.38E-24	1.33E-23
OTU_1613	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.319282833	4.35E-24	1.70E-23

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_1342	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.321045691	4.39E-24	1.70E-23
OTU_51	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	0.948968206	5.10E-24	1.95E-23
OTU_2315	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.322797901	5.61E-24	2.13E-23
OTU_1839	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.307529418	5.72E-24	2.15E-23
OTU_326	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Anaerotruncus*	unknown	NB	0.424762947	6.85E-24	2.56E-23
OTU_891	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*acidifaciens*	NB	0.332048694	8.03E-24	2.97E-23
OTU_1690	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*	unknown	NB	-0.305096754	1.24E-23	4.55E-23
OTU_2044	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.348806357	1.69E-23	6.14E-23
OTU_548	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.423021772	1.92E-23	6.93E-23
OTU_2206	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.457967719	2.17E-23	7.76E-23
OTU_729	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.425185179	2.96E-23	1.05E-22
OTU_38	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.758898449	4.18E-23	1.47E-22
OTU_747	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.283565398	4.80E-23	1.67E-22
OTU_1594	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	NB	0.281935232	5.82E-23	2.01E-22
OTU_72	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.585958425	1.12E-22	3.84E-22
OTU_2383	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.488304934	1.16E-22	3.95E-22
OTU_2195	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.232654078	1.54E-22	5.22E-22
OTU_84	*Bacteria*	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	*Sutterella*	unknown	NB	0.81870245	1.82E-22	6.12E-22
OTU_2369	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.201763518	4.89E-22	1.63E-21
OTU_786	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.523662645	7.86E-22	2.59E-21
OTU_77	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.508568761	8.68E-22	2.84E-21
OTU_1795	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.221300164	9.20E-22	2.99E-21
OTU_1865	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.37960311	1.18E-21	3.79E-21
OTU_117	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	0.787258169	2.67E-21	8.56E-21
OTU_2391	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	0.84438563	2.74E-21	8.71E-21
OTU_43	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.678543273	2.78E-21	8.78E-21
OTU_1857	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.379603634	4.19E-21	1.31E-20
OTU_596	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	0.495396811	5.38E-21	1.67E-20
OTU_171	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.520668649	7.14E-21	2.20E-20
OTU_1912	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.192562266	7.94E-21	2.43E-20
OTU_131	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Lachnospira*	unknown	NB	0.671007802	9.19E-21	2.80E-20
OTU_80	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	NB	0.528295331	1.08E-20	3.26E-20
OTU_1818	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.571079834	1.19E-20	3.56E-20
OTU_1660	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	0.42071083	1.26E-20	3.74E-20
OTU_165	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	NB	0.653570927	1.26E-20	3.74E-20
OTU_162	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Coprobacillus*	unknown	NB	0.471398178	2.72E-20	8.00E-20
OTU_12	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	ZINB	-0.113745725	3.19E-20	9.31E-20
OTU_1786	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NB	0.31807514	3.37E-20	9.77E-20
OTU_226	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	NB	0.241499145	4.31E-20	1.24E-19
OTU_59	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.241156004	5.11E-20	1.46E-19
OTU_103	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*	unknown	NB	0.328970603	5.67E-20	1.61E-19
OTU_17	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.306008505	6.85E-20	1.93E-19
OTU_35	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	*aerofaciens*	NB	0.644181388	9.97E-20	2.80E-19
OTU_1614	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	0.390327674	1.01E-19	2.81E-19
OTU_66	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	NB	0.685475806	1.01E-19	2.81E-19
OTU_1158	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.322202143	1.03E-19	2.84E-19
OTU_2273	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.42156252	1.53E-19	4.18E-19
OTU_1631	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.234930252	2.23E-19	6.06E-19
OTU_2030	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.267538415	5.26E-19	1.42E-18
OTU_274	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.777401024	5.96E-19	1.60E-18
OTU_2340	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*	unknown	NB	-0.246005354	7.10E-19	1.89E-18
OTU_360	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	Unknown	unknown	NB	0.33365224	7.46E-19	1.98E-18
OTU_248	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.6521059	7.53E-19	1.98E-18
OTU_2401	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.391711248	1.01E-18	2.62E-18
OTU_109	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.752945948	1.01E-18	2.62E-18

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_1186	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.2216526	1.18E-18	3.06E-18
OTU_1591	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	Unknown	unknown	NB	-0.20530656	1.21E-18	3.11E-18
OTU_1203	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	NB	0.631720135	1.42E-18	3.62E-18
OTU_2415	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.394201049	1.45E-18	3.68E-18
OTU_2003	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.338105589	1.48E-18	3.75E-18
OTU_1471	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	*gnavus*	NB	0.329380534	1.51E-18	3.79E-18
OTU_1677	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.638083914	1.64E-18	4.09E-18
OTU_2323	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.227942469	1.67E-18	4.15E-18
OTU_967	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.341514638	3.65E-18	9.01E-18
OTU_2001	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.214187371	4.99E-18	1.22E-17
OTU_1573	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.370480722	7.62E-18	1.86E-17
OTU_2407	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.220133128	9.61E-18	2.33E-17
OTU_2052	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*acidifaciens*	NB	0.237707635	1.12E-17	2.69E-17
OTU_2277	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.388544407	1.15E-17	2.76E-17
OTU_1956	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	*Rothia*	*mucilaginosa*	ZINB	-0.171739911	1.41E-17	3.35E-17
OTU_1937	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.132772291	1.97E-17	4.66E-17
OTU_2104	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.608663513	2.70E-17	6.36E-17
OTU_99	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.411790451	2.87E-17	6.72E-17
OTU_782	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.22071215	3.07E-17	7.16E-17
OTU_771	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.399920713	4.32E-17	1.00E-16
OTU_1737	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.274716935	7.56E-17	1.74E-16
OTU_640	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.235920897	8.63E-17	1.98E-16
OTU_1773	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.241267367	1.04E-16	2.38E-16
OTU_1443	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.302362741	1.15E-16	2.60E-16
OTU_2049	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.365196625	1.24E-16	2.80E-16
OTU_1797	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.309972238	1.47E-16	3.30E-16
OTU_129	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	NB	0.690921697	1.56E-16	3.50E-16
OTU_280	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	*Rothia*	*mucilaginosa*	ZINB	-0.164618493	2.48E-16	5.52E-16
OTU_30	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	1.092101818	2.66E-16	5.88E-16
OTU_95	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	0.504798031	2.87E-16	6.31E-16
OTU_40	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.332565298	4.32E-16	9.46E-16
OTU_2051	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.611901383	4.36E-16	9.49E-16
OTU_229	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.579109817	5.96E-16	1.29E-15
OTU_1675	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.179327063	6.59E-16	1.42E-15
OTU_2378	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	ZINB	-0.07618221	6.88E-16	1.48E-15
OTU_2133	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.238596458	8.63E-16	1.84E-15
OTU_2222	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.534424013	1.01E-15	2.14E-15
OTU_1861	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.222376703	1.05E-15	2.21E-15
OTU_13	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*	unknown	NB	-0.188358385	1.51E-15	3.18E-15
OTU_631	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	ZINB	-0.192923935	2.04E-15	4.28E-15
OTU_2287	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	ZINB	0.350577541	2.37E-15	4.93E-15
OTU_300	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.289722489	3.26E-15	6.76E-15
OTU_1453	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.193197696	5.18E-15	1.07E-14
OTU_2172	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	unknown	NB	0.279417903	7.62E-15	1.56E-14
OTU_1671	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.333225477	1.22E-14	2.50E-14
OTU_2154	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	NB	0.245986935	1.24E-14	2.52E-14
OTU_381	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Corynebacteriaceae*	*Corynebacterium*	unknown	NB	-0.202980061	1.58E-14	3.21E-14
OTU_2302	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.220057991	1.59E-14	3.21E-14
OTU_1896	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.240716714	2.35E-14	4.72E-14
OTU_2008	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	ZINB	0.189285912	2.37E-14	4.73E-14
OTU_2070	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	ZINB	-0.113864419	3.08E-14	6.08E-14
OTU_344	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	NB	0.442554264	3.07E-14	6.08E-14
OTU_1073	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.384214088	5.39E-14	1.06E-13
OTU_235	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	unknown	ZINB	-0.084144128	6.25E-14	1.22E-13
OTU_37	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.392050917	7.15E-14	1.39E-13

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_1646	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.185811533	1.31E-13	2.55E-13
OTU_2380	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.478109504	2.72E-13	5.25E-13
OTU_179	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Eubacteriaceae*	*Pseudoramibacter_Eubacterium*	unknown	NB	0.584530922	3.67E-13	7.07E-13
OTU_2085	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	NB	-0.492150047	4.13E-13	7.90E-13
OTU_2207	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	NB	0.371357714	4.37E-13	8.33E-13
OTU_1643	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.42669447	4.58E-13	8.69E-13
OTU_1634	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.278250455	4.79E-13	9.05E-13
OTU_2283	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.321514864	5.52E-13	1.04E-12
OTU_2400	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	NB	0.237169752	6.90E-13	1.29E-12
OTU_597	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	NB	-0.49443996	1.09E-12	2.04E-12
OTU_25	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	NB	0.339236902	1.19E-12	2.21E-12
OTU_699	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	ZINB	0.2043158	1.27E-12	2.36E-12
OTU_1975	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.212656225	1.40E-12	2.58E-12
OTU_2043	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.116746547	1.56E-12	2.87E-12
OTU_1047	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.259687946	1.57E-12	2.87E-12
OTU_1673	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	ZINB	-0.230342615	2.84E-12	5.16E-12
OTU_2128	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	ZINB	-0.130881562	3.25E-12	5.88E-12
OTU_2325	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	NB	-0.126872823	4.72E-12	8.51E-12
OTU_2452	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	NB	0.219545051	8.97E-12	1.61E-11
OTU_2464	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.549412556	1.06E-11	1.89E-11
OTU_954	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.281962644	1.10E-11	1.95E-11
OTU_63	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.150868981	1.20E-11	2.13E-11
OTU_1601	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.286375987	2.05E-11	3.61E-11
OTU_42	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.35190033	2.40E-11	4.23E-11
OTU_2097	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Trabulsiella*	unknown	NB	-0.244469643	5.70E-11	9.99E-11
OTU_1450	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	NB	-0.142153095	6.05E-11	1.06E-10
OTU_1401	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.295517703	6.71E-11	1.17E-10
OTU_2186	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.091122647	8.84E-11	1.53E-10
OTU_2357	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.103316151	1.27E-10	2.19E-10
OTU_394	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	Unknown	unknown	NB	0.361273855	1.34E-10	2.30E-10
OTU_1946	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Citrobacter*	unknown	NB	-0.270223227	1.88E-10	3.22E-10
OTU_1951	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.347248277	2.57E-10	4.37E-10
OTU_1682	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.172988006	2.66E-10	4.51E-10
OTU_1032	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	ZINB	0.12396134	3.20E-10	5.41E-10
OTU_937	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.123974117	3.87E-10	6.51E-10
OTU_2105	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	0.370916737	4.49E-10	7.52E-10
OTU_2023	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	ZINB	0.196952603	5.97E-10	9.97E-10
OTU_1583	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*	unknown	NB	-0.177090162	6.24E-10	1.04E-09
OTU_2006	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.090687313	6.40E-10	1.06E-09
OTU_1632	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	-0.207841253	8.01E-10	1.32E-09
OTU_238	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	*Peptostreptococcus*	*anaerobius*	ZINB	0.201263598	1.01E-09	1.67E-09
OTU_2165	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	ZINB	-0.14828668	1.26E-09	2.06E-09
OTU_2061	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.303848288	1.61E-09	2.62E-09
OTU_2305	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	0.14556963	1.79E-09	2.91E-09
OTU_2090	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	*anginosus*	NB	-0.302689351	1.91E-09	3.09E-09
OTU_1990	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	NB	0.385789746	2.02E-09	3.26E-09
OTU_1599	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	NB	0.217820751	3.83E-09	6.15E-09
OTU_1916	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.213555374	4.63E-09	7.41E-09
OTU_2124	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	ZINB	0.205791011	7.35E-09	1.17E-08
OTU_2093	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	NB	-0.620314527	8.30E-09	1.32E-08
OTU_56	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.30981921	1.05E-08	1.66E-08
OTU_1490	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	NB	-0.118573697	1.38E-08	2.18E-08
OTU_1522	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.083952443	1.61E-08	2.53E-08
OTU_3	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.351778304	1.70E-08	2.65E-08
OTU_2281	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.137942131	1.81E-08	2.83E-08

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_2098	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.163684545	2.11E-08	3.27E-08
OTU_41	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	-0.227014731	2.67E-08	4.13E-08
OTU_2376	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	NB	-0.130591211	6.37E-08	9.83E-08
OTU_2309	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	NB	-0.70814364	6.96E-08	1.07E-07
OTU_49	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	0.183739521	8.06E-08	1.24E-07
OTU_1374	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	NB	0.125779719	8.26E-08	1.26E-07
OTU_1958	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.249183275	8.31E-08	1.26E-07
OTU_1434	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Dialister*	unknown	NB	0.235330402	8.54E-08	1.30E-07
OTU_1413	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.074994392	1.02E-07	1.55E-07
OTU_1549	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	NB	-0.509995872	1.48E-07	2.22E-07
OTU_76	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*eggerthii*	NB	0.336697145	1.66E-07	2.48E-07
OTU_1277	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	unknown	NB	-0.478560191	1.72E-07	2.57E-07
OTU_2078	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	NB	0.50707175	2.99E-07	4.45E-07
OTU_1966	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.127049369	3.10E-07	4.60E-07
OTU_2233	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.200713838	5.30E-07	7.84E-07
OTU_2255	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	unknown	NB	0.216085686	5.47E-07	8.06E-07
OTU_2359	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.214346979	5.51E-07	8.10E-07
OTU_669	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	ZINB	-0.05061416	5.55E-07	8.12E-07
OTU_1972	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	NB	-0.701376551	5.86E-07	8.54E-07
OTU_2258	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*perfringens*	ZINB	-0.585206643	6.76E-07	9.83E-07
OTU_1792	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.32712362	7.38E-07	1.07E-06
OTU_2303	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.217518853	8.50E-07	1.23E-06
OTU_93	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	*stercoris*	NB	0.404201061	9.58E-07	1.38E-06
OTU_1905	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	*Haemophilus*	unknown	NB	-0.164307142	1.67E-06	2.39E-06
OTU_1740	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.068815044	1.80E-06	2.57E-06
OTU_306	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	Unknown	unknown	NB	-0.267864685	1.97E-06	2.81E-06
OTU_1396	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.164830839	2.08E-06	2.95E-06
OTU_1097	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*	unknown	NB	-0.305079497	2.40E-06	3.40E-06
OTU_1716	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.111121161	3.33E-06	4.70E-06
OTU_33	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*neonatale*	NB	-0.164339955	4.20E-06	5.91E-06
OTU_2211	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	NB	-0.182112498	4.88E-06	6.82E-06
OTU_39	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	0.136346867	4.88E-06	6.82E-06
OTU_1386	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.083023655	6.04E-06	8.41E-06
OTU_1076	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.130354532	6.93E-06	9.62E-06
OTU_2465	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.062906762	1.22E-05	1.69E-05
OTU_141	*Bacteria*	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	*Sutterella*	unknown	NB	0.356154302	1.88E-05	2.60E-05
OTU_2112	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.140035769	1.94E-05	2.67E-05
OTU_2240	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*neonatale*	NB	-0.176356266	2.04E-05	2.79E-05
OTU_2157	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Eggerthella*	*lenta*	NB	0.071237924	2.14E-05	2.92E-05
OTU_2404	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	NB	-0.140233104	2.36E-05	3.22E-05
OTU_1456	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	NB	0.153917439	2.75E-05	3.73E-05
OTU_2053	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	unknown	NB	0.183806736	2.97E-05	4.02E-05
OTU_48	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	ZINB	-0.05299747	3.08E-05	4.16E-05
OTU_2166	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	-0.054726865	3.83E-05	5.15E-05
OTU_2203	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*[Eubacterium]*	*dolichum*	NB	0.130718389	4.07E-05	5.45E-05
OTU_20	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	NB	0.273545909	4.65E-05	6.20E-05
OTU_31	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	unknown	NB	0.301748164	4.65E-05	6.20E-05
OTU_1794	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	NB	-0.19178275	5.00E-05	6.64E-05
OTU_1468	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.067203842	6.18E-05	8.19E-05
OTU_1361	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	NB	0.101767412	6.62E-05	8.74E-05
OTU_1877	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	NB	0.36816595	0.000109686	0.000144341
OTU_214	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	unknown	NB	-0.320584725	0.000125437	0.000164564
OTU_1015	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	NB	0.288271448	0.000175723	0.000229833
OTU_2349	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	NB	0.170677518	0.000181948	0.000237251
OTU_32	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	unknown	NB	0.361900378	0.000183235	0.000238205

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_60	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*[Tissierellaceae]*	*Finegoldia*	unknown	NB	-0.069135762	0.000194704	0.000252351
OTU_1040	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	NB	0.320741048	0.000220327	0.000284699
OTU_2130	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	NB	-0.206109181	0.000221579	0.000285458
OTU_1358	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*parvula*	ZINB	-0.155758245	0.000321881	0.000413434
OTU_1300	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.073350702	0.000335847	0.000430085
OTU_1	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.062407864	0.000413906	0.000528469
OTU_1919	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.102877262	0.000457105	0.000581893
OTU_2002	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	-0.141536965	0.000510356	0.00064776
OTU_557	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Aerococcaceae*	*Alloiococcus*	unknown	NB	-0.161459017	0.000597671	0.000756345
OTU_583	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	unknown	NB	-0.202381152	0.000639249	0.000804217
OTU_15	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.238638609	0.000638102	0.000804217
OTU_761	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*[Mogibacteriaceae]*	*Mogibacterium*	unknown	ZINB	0.088464098	0.00067005	0.000840501
OTU_1296	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	-0.123945585	0.000727709	0.000910166
OTU_266	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*	*rhizosphaerae*	NB	-0.200642589	0.001075963	0.001341826
OTU_2457	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.064874913	0.001223267	0.001521106
OTU_995	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	NB	-0.136622619	0.001264892	0.00156832
OTU_1446	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	-0.092500235	0.001872967	0.00231557
OTU_1367	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	ZINB	-0.190503079	0.001925466	0.002373635
OTU_411	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Atopobium*	unknown	NB	-0.078638179	0.002132831	0.002621732
OTU_2050	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.104271719	0.002592527	0.003177697
OTU_36	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	*Haemophilus*	unknown	NB	-0.089679699	0.003360049	0.004106726
OTU_2450	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	-0.131299607	0.003587403	0.004372148
OTU_1999	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	ZINB	-0.089805667	0.004072452	0.004949241
OTU_62	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*[Tissierellaceae]*	*WAL_1855D*	unknown	ZINB	0.110506376	0.004361659	0.005285739
OTU_52	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Eggerthella*	*lenta*	NB	0.061163333	0.004650068	0.005619378
OTU_2005	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Varibaculum*	unknown	ZINB	-0.176329258	0.004778037	0.005757803
OTU_2432	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*[Eubacterium]*	*dolichum*	NB	-0.107134969	0.005372432	0.006455947
OTU_1058	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	Unknown	*Unknown*	*unknown*	NB	0.053451837	0.006146624	0.007365647
OTU_406	*Bacteria*	*Fusobacteria*	*Fusobacteriia*	*Fusobacteriales*	*Fusobacteriaceae*	*Fusobacterium*	unknown	NB	0.092970616	0.006475898	0.007738608
OTU_1412	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	0.105952947	0.008328164	0.009924395
OTU_2292	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.073741241	0.009009428	0.010706495
OTU_985	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Carnobacteriaceae*	*Carnobacterium*	unknown	NB	-0.082953602	0.009746164	0.011550013
OTU_847	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	NB	0.220211592	0.011916547	0.014083192
OTU_1196	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.035747642	0.012199516	0.014378001
OTU_2327	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	ZINB	-0.096305662	0.014545574	0.01709603
OTU_1871	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	NB	0.132156945	0.017167739	0.020122842
OTU_2101	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	NB	0.085460256	0.017961911	0.020996348
OTU_1822	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	unknown	NB	-0.107197341	0.019968611	0.023278625
OTU_1854	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	ZINB	-0.026720174	0.021735639	0.025269889
OTU_1992	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.046779598	0.027928492	0.032381954
OTU_86	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Varibaculum*	unknown	NB	-0.103671362	0.031529691	0.036458861
OTU_1244	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*longum*	NB	0.103441843	0.032507681	0.037488696
OTU_1462	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	NB	0.085354544	0.032875791	0.037811567
OTU_1327	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	0.117608163	0.03313627	0.038009251
OTU_87	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	*anginosus*	NB	-0.046803846	0.03763711	0.043056853
OTU_1463	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.03481506	0.041467006	0.04731209
OTU_1649	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*parvula*	NB	-0.107300482	0.043040601	0.048977236
OTU_408	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Bulleidia*	unknown	NB	0.056359918	0.0441678	0.050126948
OTU_1636	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	unknown	NB	-0.055248767	0.054022604	0.061149596
OTU_2022	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.037204071	0.058108318	0.065601233
OTU_1428	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Corynebacteriaceae*	*Corynebacterium*	unknown	NB	0.060591869	0.06728043	0.075756704
OTU_227	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Corynebacteriaceae*	*Corynebacterium*	unknown	NB	-0.054499928	0.069612091	0.078176929
OTU_2224	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	ZINB	0.045822299	0.072354285	0.081044356
OTU_511	*Bacteria*	*Proteobacteria*	*Betaproteobacteria*	*Neisseriales*	*Neisseriaceae*	*Neisseria*	*subflava*	NB	-0.07353781	0.084078484	0.093931431
OTU_257	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Megasphaera*	unknown	NB	0.115897443	0.10206217	0.113726417

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Model	Estimate	p-value	p-valueFDR
OTU_107	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*[Tissierellaceae]*	*Peptoniphilus*	unknown	NB	0.045405251	0.106227276	0.118060884
OTU_1408	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.052658982	0.107006376	0.11836543
OTU_2025	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	Unknown	unknown	NB	-0.050358688	0.107053116	0.11836543
OTU_1332	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	-0.042938039	0.130125223	0.143505709
OTU_68	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*	unknown	NB	0.05917758	0.130911191	0.14400231
OTU_1657	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*[Eubacterium]*	*dolichum*	NB	-0.060663954	0.171901093	0.188607593
OTU_1840	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	0.015447886	0.181746073	0.198900677
OTU_1835	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.022072954	0.185017265	0.201965411
OTU_1099	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	-0.066955329	0.186428922	0.202989867
OTU_1117	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*parvula*	NB	-0.032418991	0.217229726	0.235927981
OTU_1486	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	NB	0.040137441	0.236553158	0.256265921
OTU_1362	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	NB	0.032268221	0.29499467	0.318772578
OTU_57	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*zeae*	NB	-0.047202711	0.298039371	0.321253493
OTU_2009	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	0.017470454	0.319591672	0.343621121
OTU_1432	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.045972903	0.34716488	0.372334334
OTU_1289	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	NB	0.023598966	0.401310381	0.429332054
OTU_82	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*[Tissierellaceae]*	*Anaerococcus*	unknown	NB	0.01859559	0.404220472	0.431369608
OTU_2046	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	-0.017044416	0.416021433	0.442861525
OTU_2270	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.02383658	0.437969047	0.465071092
OTU_1943	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*[Eubacterium]*	*dolichum*	ZINB	-0.029269099	0.452310353	0.47911393
OTU_1724	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	NB	-0.021233433	0.465207651	0.491561779
OTU_1694	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.011557665	0.468797599	0.49413801
OTU_2204	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	unknown	NB	0.019822106	0.471948151	0.4962396
OTU_2334	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	0.016292528	0.479841006	0.503305114
OTU_2199	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	NB	-0.014330837	0.483700345	0.506115727
OTU_1301	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	ZINB	0.009632996	0.530642362	0.553882173
OTU_2451	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	-0.026634021	0.567164019	0.590566418
OTU_2077	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.012016391	0.576370568	0.598699694
OTU_1897	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	Unknown	*Unknown*	*Unknown*	NB	-0.020183328	0.593453977	0.614955932
OTU_2424	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	NB	-0.014462602	0.620404165	0.641333462
OTU_2421	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	NB	-0.012761658	0.66620976	0.687028815
OTU_898	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Aerococcaceae*	Unknown	unknown	NB	0.011211052	0.711463065	0.731936822
OTU_2245	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	NB	-0.0122641	0.717713057	0.736600243
OTU_205	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	NB	0.019699063	0.720029819	0.737214301
OTU_2256	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	ZINB	-0.012509135	0.742745034	0.758660999
OTU_541	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	*Aggregatibacter*	unknown	NB	0.008636396	0.766155962	0.780714745
OTU_9	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	*gnavus*	NB	0.009104592	0.773610278	0.786442676
OTU_2409	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	-0.011379551	0.791740453	0.802970814
OTU_2144	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*zeae*	NB	-0.010595664	0.800775335	0.810218441
OTU_1487	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	*gnavus*	NB	-0.006390926	0.853595474	0.861629314
OTU_1181	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	Unknown	unknown	NB	0.005270746	0.904351197	0.910719867
OTU_620	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	NB	0.000970746	0.957448843	0.96193338
OTU_1711	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*brevis*	NB	0.002215446	0.969096302	0.971360546
OTU_599	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	unknown	NB	-0.00135326	0.971927645	0.971927645
OTU_136	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*	unknown	NA	NA	NA	NA
OTU_1752	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	NA	NA	NA	NA
OTU_1817	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NA	NA	NA	NA
OTU_1869	*Bacteria*	*Firmicutes*	*Bacilli*	*Bacillales*	*Planococcaceae*	Unknown	unknown	ZINB	NA	NA	NA
OTU_2026	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	NA	NA	NA	NA
OTU_2148	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	ZINB	NA	NA	NA
OTU_2295	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	NB	NA	NA	NA
OTU_2342	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	NB	NA	NA	NA
OTU_5	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	NB	NA	NA	NA
OTU_8	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	ZINB	NA	NA	NA

Table S2.4: Evaluating effect modification in the association between dog exposure and infant gut α- and β-diversity trajectories.

Outcome	Covariate	Interaction p-value
α-diversity		
Richness	Time	0.41
	Child sex	0.55
	Mode of delivery	0.29
	Household income	0.4
	Formula feeding	0.59
Evenness	Time	0.53
	Child sex	0.74
	Mode of delivery	0.88
	Household income	0.14
	Formula feeding	0.3
Faith's Phylogenetic Diversity	Time	**0.012**
	Child sex	0.49
	Mode of delivery	0.26
	Household income	0.28
	Formula feeding	0.41
β-diversity		
Unweighted UniFrac, PCO_1	Time	0.058
	Child sex	0.54
	Mode of delivery	0.29
	Household income	0.4
	Formula feeding	**0.036**
Unweighted UniFrac, PCO_2	Time	0.25
	Child sex	0.35
	Mode of delivery	0.74
	Household income	0.34
	Formula feeding	0.9
Unweighted UniFrac, PCO_3	Time	0.17
	Child sex	0.93
	Mode of delivery	0.95
	Household income	0.19
	Formula feeding	0.79

Outcome	Covariate	Interaction p-value
β-diversity		
Unweighted UniFrac, PCO_4	Time	0.64
	Child sex	0.35
	Mode of delivery	0.99
	Household income	0.71
	Formula feeding	0.39
Weighted UniFrac, PCO_1	Time	0.94
	Child sex	0.15
	Mode of delivery	0.99
	Household income	0.34
	Formula feeding	0.43
Weighted UniFrac, PCO_2	Time	0.9
	Child sex	0.5
	Mode of delivery	0.95
	Household income	0.44
	Formula feeding	0.44
Weighted UniFrac, PCO_3	Time	0.39
	Child sex	0.46
	Mode of delivery	0.2
	Household income	0.73
	Formula feeding	0.49
Weighted UniFrac, PCO_4	Time	0.34
	Child sex	0.39
	Mode of delivery	0.64
	Household income	0.87
	Formula feeding	0.55
Canberra, PCO_1	Time	0.92
	Child sex	0.13
	Mode of delivery	0.25
	Household income	0.91
	Formula feeding	**0.039**
Canberra, PCO_2	Time	0.99
	Child sex	0.23
	Mode of delivery	0.85

Outcome	Covariate	Interaction p-value
β-diversity		
Canberra, PCO2	Household income	0.15
	Formula feeding	0.57
Canberra, PCO_3	Time	0.14
	Child sex	0.4
	Mode of delivery	0.99
	Household income	0.05
	Formula feeding	0.72
Canberra, PCO_4	Time	0.97
	Child sex	0.95
	Mode of delivery	0.92
	Household income	0.76
	Formula feeding	0.89
Bray-Curtis, PCO_1	Time	0.97
	Child sex	0.12
	Mode of delivery	0.96
	Household income	0.16
	Formula feeding	0.92
Bray-Curtis, PCO_2	Time	0.19
	Child sex	0.83
	Mode of delivery	0.34
	Household income	0.27
	Formula feeding	0.29
Bray-Curtis, PCO_3	Time	0.22
	Child sex	0.5
	Mode of delivery	0.78
	Household income	0.95
	Formula feeding	0.4
Bray-Curtis, PCO_4	Time	0.99
	Child sex	0.5
	Mode of delivery	0.86
	Household income	0.95
	Formula feeding	0.32

Table S2.5: Association between dog exposure and infant gut β-diversity trajectories, before and after covariate adjustment.

Model	N	β[a]	SE	p-value
Unweighted UniFrac, PCO_1[c]				
Unadjusted	131	0.03	0.016	0.064
Fully adjusted[d]	120	0.025	0.015	0.088
Unweighted UniFrac, PCO_2[c]				
Unadjusted	131	-0.015	0.012	0.2
Fully adjusted[d]	120	-0.018	0.013	0.18
Unweighted UniFrac, PCO_3[c]				
Unadjusted	131	0.008	0.017	0.62
Fully adjusted[d]	120	0.003	0.017	0.88
Unweighted UniFrac, PCO_4[c]				
Unadjusted	131	0.022	0.011	0.052
Fully adjusted[d]	120	0.012	0.012	0.32
Weighted UniFrac, PCO_1[c]				
Unadjusted	131	0.018	0.011	0.11
Fully adjusted[d]	120	0.014	0.012	0.23
Weighted UniFrac, PCO_2[c]				
Unadjusted	131	-0.014	0.009	0.15
Fully adjusted[d]	120	-0.013	0.01	0.2
Weighted UniFrac, PCO_3[c]				
Unadjusted	131	-0.008	0.008	0.3
Fully adjusted[d]	120	-0.008	0.008	0.34
Weighted UniFrac, PCO_4[b]				
Unadjusted	131	-0.001	0.005	0.85
Fully adjusted[d]	120	0.002	0.005	0.7
Canberra, PCO_1[b]				
Unadjusted	131	0.02	0.01	0.057
Fully adjusted[d]	120	0.016	0.01	0.11

Model	N	β[a]	SE	p-value
Canberra, PCO_2[c]				
Unadjusted	131	-0.026	0.012	**0.035**
Fully adjusted[d]	120	-0.023	0.014	0.1
Canberra, PCO_3[c]				
Unadjusted	131	0.027	0.012	**0.02**
Fully adjusted[d]	120	0.016	0.011	0.15
Canberra, PCO_4[b]				
Unadjusted	131	0.008	0.012	0.49
Fully adjusted[d]	120	0.014	0.013	0.28
Bray-Curtis, PCO_1[c]				
Unadjusted	131	0.046	0.024	0.052
Fully adjusted[d]	120	0.04	0.027	0.14
Bray-Curtis, PCO_2[c]				
Unadjusted	131	-0.009	0.017	0.57
Fully adjusted[d]	120	-0.016	0.019	0.38
Bray-Curtis, PCO_3[b]				
Unadjusted	131	-0.005	0.021	0.82
Fully adjusted[d]	120	-0.042	0.023	0.077
Bray-Curtis, PCO_4[b]				
Unadjusted	131	-0.001	0.017	0.93
Fully adjusted[d]	120	-0.006	0.019	0.76

[a]Average difference in specified principle coordinate (comparing dog-exposed vs. pet-free), throughout early life.
[b]linear model
[c]quadratic model
[d]Adjusted for maternal race, household income, maternal age at birth, mode of delivery, child sex, first born child, age at solid food introduction, and breastfeeding duration.

Table S2.6: Association between indoor prenatal dog exposure and OTU trajectories. OTUs ordered by main effect p-value.

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_2277	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	5.25E-01	7.68E-01	2.533	6.88E-06	2.81E-03
OTU_2434	*Bacteria*	*Proteobacteria*	*Gammaproteob*	*Enterobacteriales*	*Enterobacteriaceae*	*Citrobacter*	unknown	*Citrobacter sp.*	ZINB	5.22E-01	7.68E-01	-0.846	1.49E-04	3.06E-02
OTU_93	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	*stercoris*	*Collinsella stercoris*	NB	6.95E-01	8.56E-01	7.304	2.55E-04	3.06E-02
OTU_1822	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	NB	5.56E-01	7.68E-01	3.852	3.32E-04	3.06E-02
OTU_1099	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	*Clostridiaceae sp.*	NB	4.71E-02	3.40E-01	2.417	4.62E-04	3.06E-02
OTU_575	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	5.30E-01	7.68E-01	2.504	4.84E-04	3.06E-02
OTU_2144	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*zeae*	*Lactobacillus zeae*	NB	2.41E-01	6.17E-01	-2.126	5.37E-04	3.06E-02
OTU_1015	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	*Dorea sp.*	NB	6.27E-02	3.62E-01	4.09	5.99E-04	3.06E-02
OTU_1367	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	ZINB	2.69E-02	2.67E-01	-2.583	1.06E-03	4.81E-02
OTU_1358	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*parvula*	*Veillonella parvula*	ZINB	4.78E-03	1.40E-01	-2.067	1.61E-03	6.57E-02
OTU_967	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	*Dorea sp.*	NB	NA	NA	1.933	3.13E-03	1.16E-01
OTU_2165	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	ZINB	2.65E-02	2.67E-01	1.247	3.90E-03	1.20E-01
OTU_2093	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	NB	1.40E-01	4.87E-01	3.801	4.04E-03	1.20E-01
OTU_1614	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	NB	6.70E-02	3.62E-01	1.781	4.29E-03	1.20E-01
OTU_596	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Unknown*	Unknown	unknown	*Clostridiales sp.*	NB	1.74E-01	5.36E-01	2.288	4.39E-03	1.20E-01
OTU_1810	*Bacteria*	*Firmicutes*	*Bacilli*	*Bacillales*	*Planococcaceae*	Unknown	unknown	*Planococcaceae sp.*	NB	NA	NA	-1.269	5.48E-03	1.37E-01
OTU_1634	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.42E-02	3.62E-01	1.47	5.69E-03	1.37E-01
OTU_1773	*Bacteria*	*Proteobacteria*	*Gammaproteob*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	4.07E-03	1.40E-01	-1.058	8.62E-03	1.96E-01
OTU_1073	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	NB	2.43E-01	6.18E-01	2.041	1.02E-02	2.10E-01
OTU_2104	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	4.60E-01	7.45E-01	3.166	1.03E-02	2.10E-01
OTU_1996	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	ZINB	NA	NA	1.081	1.24E-02	2.27E-01
OTU_2085	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	NB	4.67E-02	3.40E-01	4.158	1.25E-02	2.27E-01
OTU_57	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*zeae*	*Lactobacillus zeae*	NB	2.97E-02	2.79E-01	-1.91	1.29E-02	2.27E-01
OTU_1549	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	NB	2.15E-02	2.57E-01	2.861	1.42E-02	2.27E-01
OTU_1972	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	NB	2.49E-02	2.67E-01	2.411	1.44E-02	2.27E-01
OTU_2380	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	2.76E-01	6.33E-01	2.891	1.44E-02	2.27E-01
OTU_597	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	NB	2.56E-02	2.67E-01	3.841	1.51E-02	2.28E-01
OTU_38	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	4.07E-01	7.27E-01	2.865	1.58E-02	2.31E-01
OTU_1332	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	*Clostridiaceae sp.*	NB	2.09E-01	5.78E-01	1.067	1.65E-02	2.32E-01
OTU_2240	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*neonatale*	*Clostridium neonatale*	NB	7.40E-02	3.67E-01	1.682	1.75E-02	2.39E-01
OTU_2440	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	*Clostridiales sp.*	NB	1.79E-01	5.43E-01	2.004	1.84E-02	2.43E-01
OTU_111	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	9.89E-01	9.96E-01	1.881	1.92E-02	2.46E-01
OTU_1631	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	2.66E-01	6.33E-01	1.519	2.11E-02	2.53E-01
OTU_2168	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	8.36E-02	3.90E-01	1.465	2.21E-02	2.53E-01
OTU_1296	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	*Clostridiaceae sp.*	NB	8.48E-01	9.23E-01	1.359	2.23E-02	2.53E-01
OTU_248	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	1.50E-01	5.07E-01	2.37	2.28E-02	2.53E-01
OTU_3	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	9.73E-02	4.22E-01	2.083	2.29E-02	2.53E-01
OTU_298	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	7.71E-01	8.86E-01	1.516	2.55E-02	2.64E-01
OTU_300	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	5.89E-01	7.91E-01	1.433	2.55E-02	2.64E-01
OTU_2258	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*perfringens*	*Clostridium perfringens*	ZINB	3.61E-01	7.08E-01	1.302	2.58E-02	2.64E-01
OTU_1774	*Bacteria*	*Proteobacteria*	*Gammaproteob*	*Enterobacteriales*	*Enterobacteriaceae*	*Enterobacter*	unknown	*Enterobacter sp.*	NB	5.01E-01	7.68E-01	-0.55	2.73E-02	2.72E-01
OTU_2009	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	*Bifidobacterium adolescentis*	NB	2.94E-01	6.48E-01	-0.68	2.91E-02	2.79E-01
OTU_2409	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	7.58E-01	8.82E-01	1.346	2.93E-02	2.79E-01
OTU_599	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	unknown	*Actinomyces sp.*	NB	4.95E-01	7.68E-01	1.115	3.10E-02	2.88E-01
OTU_1277	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	unknown	*Clostridium sp.*	NB	1.02E-01	4.26E-01	1.407	3.48E-02	3.16E-01
OTU_745	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.84E-02	3.62E-01	1.702	3.62E-02	3.22E-01
OTU_1897	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	Unknown	Unknown	unknown	*Lactobacillales sp.*	NB	1.96E-01	5.74E-01	1.152	3.83E-02	3.30E-01
OTU_2407	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	4.21E-01	7.27E-01	1.476	3.87E-02	3.30E-01
OTU_132	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	*Oscillospira sp.*	NB	4.10E-01	7.27E-01	1.696	4.03E-02	3.33E-01
OTU_2224	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	ZINB	3.86E-02	3.34E-01	-1.01	4.11E-02	3.33E-01
OTU_5	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	1.12E-02	1.75E-01	2.495	4.15E-02	3.33E-01
OTU_2133	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	4.63E-02	3.40E-01	1.096	4.38E-02	3.44E-01
OTU_1632	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	*Clostridiaceae sp.*	NB	1.40E-01	4.87E-01	0.762	4.63E-02	3.51E-01
OTU_1951	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	*Dorea sp.*	NB	2.57E-01	6.31E-01	1.255	4.69E-02	3.51E-01

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_2309	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	unknown	Lactobacillus sp.	NB	2.62E-01	6.31E-01	2.397	4.72E-02	3.51E-01
OTU_360	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Unknown	unknown	Coriobacteriaceae sp.	NB	5.51E-02	3.55E-01	1.385	4.94E-02	3.61E-01
OTU_2357	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	1.72E-01	5.36E-01	-0.704	5.25E-02	3.77E-01
OTU_2050	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	2.74E-01	6.33E-01	-1.048	5.69E-02	3.91E-01
OTU_1361	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	fragilis	Bacteroides fragilis	NB	4.43E-01	7.43E-01	-1.258	5.77E-02	3.91E-01
OTU_37	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	unknown	Dorea sp.	NB	1.22E-01	4.66E-01	1.5	5.82E-02	3.91E-01
OTU_2287	Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown	Unknown	unknown	Clostridiales sp.	ZINB	3.96E-01	7.20E-01	1.216	5.83E-02	3.91E-01
OTU_1854	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	ZINB	9.92E-01	9.96E-01	-0.299	6.01E-02	3.93E-01
OTU_2030	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	8.33E-01	9.19E-01	1.427	6.05E-02	3.93E-01
OTU_2130	Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar	Veillonella dispar	NB	5.46E-01	7.68E-01	0.975	6.32E-02	4.04E-01
OTU_2052	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	acidifaciens	Bacteroides acidifacien	NB	1.77E-01	5.40E-01	-1.135	6.55E-02	4.12E-01
OTU_1937	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	unknown	Streptococcus sp.	NB	5.62E-01	7.69E-01	-0.624	6.69E-02	4.14E-01
OTU_40	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	8.34E-01	9.19E-01	1.678	6.80E-02	4.15E-01
OTU_1671	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	unknown	Dorea sp.	NB	7.48E-01	8.73E-01	1.186	7.09E-02	4.17E-01
OTU_2148	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	ZINB	4.99E-01	7.68E-01	1.861	7.19E-02	4.17E-01
OTU_33	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	neonatale	Clostridium neonatale	NB	2.78E-01	6.33E-01	1.313	7.28E-02	4.17E-01
OTU_1795	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	1.92E-03	1.14E-01	-1.015	7.29E-02	4.17E-01
OTU_898	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Unknown	unknown	Aerococcaceae sp.	NB	8.57E-01	9.27E-01	-0.849	7.33E-02	4.17E-01
OTU_2182	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	9.21E-03	1.63E-01	1.249	7.75E-02	4.22E-01
OTU_64	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	unknown	Ruminococcus sp.	NB	5.42E-01	7.68E-01	1.664	7.79E-02	4.22E-01
OTU_98	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	unknown	Ruminococcus sp.	NB	1.82E-01	5.47E-01	1.937	7.83E-02	4.22E-01
OTU_1673	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown	unknown	Enterobacteriaceae sp.	ZINB	1.97E-03	1.14E-01	-0.452	7.88E-02	4.22E-01
OTU_1709	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	2.03E-02	2.57E-01	1.177	8.01E-02	4.22E-01
OTU_557	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Alloiococcus	unknown	Alloiococcus sp.	NB	4.72E-01	7.48E-01	0.988	8.11E-02	4.22E-01
OTU_1792	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown	unknown	Enterobacteriaceae sp.	NB	7.88E-02	3.77E-01	0.825	8.28E-02	4.22E-01
OTU_1186	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	9.61E-02	4.22E-01	0.809	8.30E-02	4.22E-01
OTU_2206	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	unknown	Ruminococcus sp.	NB	NA	NA	1.121	8.42E-02	4.22E-01
OTU_1711	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	brevis	Lactobacillus brevis	NB	4.87E-03	1.40E-01	-1.451	8.48E-02	4.22E-01
OTU_2105	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	unknown	Ruminococcus sp.	NB	5.72E-01	7.76E-01	1.315	8.63E-02	4.22E-01
OTU_2464	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	5.34E-01	7.68E-01	2.183	8.66E-02	4.22E-01
OTU_2424	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Unknown	unknown	Erysipelotrichaceae sp	NB	6.35E-03	1.51E-01	0.727	9.23E-02	4.44E-01
OTU_155	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	5.80E-02	3.59E-01	1.301	9.51E-02	4.52E-01
OTU_2172	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	unknown	Actinomyces sp.	NB	6.20E-01	8.14E-01	1.039	9.82E-02	4.55E-01
OTU_406	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	unknown	Fusobacterium sp.	NB	1.26E-01	4.66E-01	0.795	9.89E-02	4.55E-01
OTU_1869	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Unknown	unknown	Planococcaceae sp.	ZINB	1.18E-01	4.58E-01	-0.604	9.98E-02	4.55E-01
OTU_165	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	unknown	Oscillospira sp.	NB	4.31E-01	7.33E-01	1.838	1.00E-01	4.55E-01
OTU_274	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	unknown	Ruminococcus sp.	NB	2.08E-01	5.78E-01	1.761	1.01E-01	4.55E-01
OTU_1987	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	ZINB	NA	NA	-0.28	1.05E-01	4.68E-01
OTU_1275	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	unknown	Streptococcus sp.	NB	1.28E-01	4.66E-01	0.489	1.08E-01	4.77E-01
OTU_2465	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	9.41E-01	9.85E-01	-0.473	1.11E-01	4.81E-01
OTU_1788	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	unknown	Dorea sp.	NB	1.30E-01	4.66E-01	0.858	1.13E-01	4.85E-01
OTU_2002	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	adolescentis	Bifidobacterium adoles	NB	4.59E-01	7.45E-01	-0.62	1.20E-01	5.06E-01
OTU_1327	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	5.95E-01	7.97E-01	0.822	1.22E-01	5.06E-01
OTU_14	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	unknown	Staphylococcus sp.	NB	1.04E-02	1.73E-01	-0.844	1.23E-01	5.06E-01
OTU_971	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	6.90E-01	8.54E-01	1.073	1.23E-01	5.06E-01
OTU_30	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	2.00E-01	5.77E-01	-2.34	1.24E-01	5.06E-01
OTU_1413	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	unknown	Streptococcus sp.	NB	6.67E-01	8.37E-01	-0.476	1.28E-01	5.15E-01
OTU_1076	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	8.01E-01	9.11E-01	-0.803	1.29E-01	5.15E-01
OTU_1560	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	2.08E-02	2.57E-01	0.823	1.30E-01	5.17E-01
OTU_1587	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	prausnitzii	Faecalibacterium prau	NB	7.62E-01	8.82E-01	-0.907	1.34E-01	5.28E-01
OTU_2155	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	7.20E-02	3.67E-01	0.846	1.36E-01	5.28E-01
OTU_1058	Bacteria	Firmicutes	Bacilli	Lactobacillales	Unknown	Unknown	unknown	Lactobacillales sp.	NB	7.31E-01	8.71E-01	0.468	1.38E-01	5.28E-01
OTU_985	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	unknown	Carnobacterium sp.	NB	9.66E-01	9.95E-01	0.663	1.38E-01	5.28E-01
OTU_2106	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	4.23E-01	7.27E-01	0.697	1.40E-01	5.30E-01

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_1244	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*longum*	*Bifidobacterium longum*	NB	3.94E-01	7.20E-01	1.171	1.41E-01	5.30E-01
OTU_2452	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	*Bacteroides fragilis*	NB	5.69E-01	7.75E-01	-1.148	1.44E-01	5.37E-01
OTU_2022	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	7.36E-01	8.71E-01	-0.606	1.53E-01	5.58E-01
OTU_1453	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	3.55E-01	7.08E-01	0.518	1.53E-01	5.58E-01
OTU_63	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	2.35E-01	6.12E-01	-0.487	1.54E-01	5.58E-01
OTU_1857	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	9.90E-01	9.96E-01	1.277	1.56E-01	5.61E-01
OTU_21	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.70E-03	1.51E-01	1.366	1.60E-01	5.62E-01
OTU_43	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	8.61E-02	3.97E-01	1.468	1.61E-01	5.62E-01
OTU_1468	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	3.83E-01	7.09E-01	-0.508	1.63E-01	5.62E-01
OTU_2077	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	5.06E-01	7.68E-01	-0.544	1.63E-01	5.62E-01
OTU_120	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	3.43E-01	6.94E-01	1.397	1.63E-01	5.62E-01
OTU_2420	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	3.13E-01	6.64E-01	1.163	1.66E-01	5.62E-01
OTU_68	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*	unknown	*Prevotella sp.*	NB	3.65E-01	7.08E-01	0.792	1.66E-01	5.62E-01
OTU_2369	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	ZINB	1.01E-01	4.26E-01	0.383	1.70E-01	5.69E-01
OTU_2011	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	7.30E-01	8.71E-01	-0.631	1.71E-01	5.70E-01
OTU_381	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Corynebacteriaceae*	*Corynebacterium*	unknown	*Corynebacterium sp.*	NB	8.19E-01	9.17E-01	0.443	1.74E-01	5.71E-01
OTU_2349	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	*Blautia producta*	NB	5.55E-01	7.68E-01	0.849	1.75E-01	5.71E-01
OTU_2161	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.87E-02	3.62E-01	0.599	1.78E-01	5.77E-01
OTU_1732	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	ZINB	9.55E-01	9.89E-01	-0.712	1.82E-01	5.77E-01
OTU_562	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	4.72E-03	1.40E-01	0.982	1.83E-01	5.77E-01
OTU_95	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	NB	5.50E-02	3.55E-01	1.176	1.83E-01	5.77E-01
OTU_1852	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	2.35E-01	6.12E-01	-0.954	1.83E-01	5.77E-01
OTU_344	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	*Blautia producta*	NB	3.05E-01	6.64E-01	-1.18	1.85E-01	5.77E-01
OTU_77	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.28E-01	6.01E-01	1.315	1.86E-01	5.77E-01
OTU_229	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	6.12E-01	8.13E-01	0.825	1.89E-01	5.82E-01
OTU_2023	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	ZINB	3.67E-01	7.09E-01	0.819	1.91E-01	5.82E-01
OTU_1362	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	*Erysipelotrichaceae sp.*	NB	1.37E-02	1.91E-01	0.567	1.93E-01	5.84E-01
OTU_159	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	3.94E-04	1.14E-01	-1.257	1.96E-01	5.87E-01
OTU_2128	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	*Veillonella dispar*	ZINB	NA	NA	-0.408	1.97E-01	5.87E-01
OTU_1835	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	1.16E-01	4.58E-01	-0.499	1.99E-01	5.89E-01
OTU_1443	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	NB	9.41E-01	9.85E-01	0.646	2.08E-01	6.11E-01
OTU_761	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Mogibacteriaceae*	*Mogibacterium*	unknown	*Mogibacterium sp.*	ZINB	4.64E-01	7.45E-01	-0.565	2.10E-01	6.12E-01
OTU_1374	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	*Erysipelotrichaceae sp.*	NB	1.83E-03	1.14E-01	0.538	2.12E-01	6.15E-01
OTU_937	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	2.16E-01	5.87E-01	0.632	2.18E-01	6.26E-01
OTU_2211	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	*Veillonella dispar*	NB	9.66E-01	9.95E-01	0.545	2.19E-01	6.26E-01
OTU_1716	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	3.72E-01	7.09E-01	0.719	2.23E-01	6.32E-01
OTU_179	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Eubacteriaceae*	*Pseudoramibacter_Eubacterium*	unknown	*Pseudoramibacter_Eubacterium sp.*	NB	3.81E-01	7.09E-01	2.167	2.25E-01	6.32E-01
OTU_813	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	5.01E-01	7.68E-01	1.227	2.26E-01	6.32E-01
OTU_1522	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	8.97E-01	9.54E-01	-0.224	2.28E-01	6.35E-01
OTU_2359	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	7.89E-01	9.00E-01	-0.801	2.33E-01	6.44E-01
OTU_2049	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	4.14E-01	7.27E-01	0.899	2.35E-01	6.46E-01
OTU_2195	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	6.42E-01	8.24E-01	-0.447	2.38E-01	6.46E-01
OTU_1912	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	ZINB	9.84E-01	9.96E-01	-0.302	2.42E-01	6.46E-01
OTU_1646	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	1.12E-01	4.55E-01	0.832	2.42E-01	6.46E-01
OTU_2005	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Varibaculum*	unknown	*Varibaculum sp.*	ZINB	2.67E-01	6.33E-01	-0.682	2.43E-01	6.46E-01
OTU_1424	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	3.64E-01	7.08E-01	0.549	2.44E-01	6.46E-01
OTU_322	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	5.52E-01	7.68E-01	-1.043	2.45E-01	6.46E-01
OTU_954	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	5.83E-02	3.59E-01	0.795	2.48E-01	6.51E-01
OTU_1486	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadacea*	*Parabacteroides*	*distasonis*	*Parabacteroides distasonis*	NB	5.60E-01	7.68E-01	1.46	2.51E-01	6.54E-01
OTU_2315	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	3.02E-01	6.63E-01	-0.723	2.58E-01	6.67E-01
OTU_2248	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	9.88E-01	9.96E-01	-0.648	2.59E-01	6.67E-01
OTU_280	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	*Rothia*	*mucilaginosa*	*Rothia mucilaginosa*	ZINB	2.55E-01	6.31E-01	0.368	2.64E-01	6.74E-01
OTU_2305	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	5.49E-02	3.55E-01	0.464	2.80E-01	7.03E-01
OTU_2334	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	*Bifidobacterium adolescentis*	NB	6.88E-01	8.54E-01	-0.268	2.80E-01	7.03E-01

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_141	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	unknown	Sutterella sp.	NB	4.34E-01	7.35E-01	1.512	2.81E-01	7.03E-01
OTU_1063	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown	unknown	Ruminococcaceae sp.	NB	7.06E-01	8.60E-01	-0.534	2.82E-01	7.03E-01
OTU_2166	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	adolescentis	Bifidobacterium adolescentis	NB	4.66E-01	7.45E-01	-0.182	2.86E-01	7.05E-01
OTU_129	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	unknown	Oscillospira sp.	NB	3.72E-01	7.09E-01	1.354	2.87E-01	7.05E-01
OTU_1097	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	unknown	Acinetobacter sp.	NB	5.24E-01	7.68E-01	1.022	2.88E-01	7.05E-01
OTU_631	Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar	Veillonella dispar	ZINB	9.26E-01	9.79E-01	-0.432	2.90E-01	7.05E-01
OTU_2101	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	distasonis	Parabacteroides distasonis	NB	4.23E-01	7.27E-01	1.275	2.92E-01	7.07E-01
OTU_2051	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	unknown	Ruminococcus sp.	NB	3.86E-01	7.10E-01	-1.066	2.99E-01	7.14E-01
OTU_1694	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	8.06E-01	9.13E-01	-0.431	3.01E-01	7.14E-01
OTU_2303	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	NA	NA	0.67	3.01E-01	7.14E-01
OTU_50	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	gnavus	Ruminococcus gnavus	NB	2.63E-02	2.67E-01	0.536	3.02E-01	7.14E-01
OTU_511	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	subflava	Neisseria subflava	NB	2.73E-01	6.33E-01	-0.619	3.07E-01	7.17E-01
OTU_1289	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Unknown	unknown	Peptostreptococcaceae sp.	NB	5.07E-02	3.47E-01	0.377	3.10E-01	7.17E-01
OTU_1158	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	9.78E-02	4.22E-01	0.791	3.12E-01	7.17E-01
OTU_1975	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	unknown	Streptococcus sp.	NB	5.27E-01	7.68E-01	0.482	3.13E-01	7.17E-01
OTU_620	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	4.54E-01	7.45E-01	-0.307	3.14E-01	7.17E-01
OTU_2154	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	uniformis	Bacteroides uniformis	NB	4.79E-01	7.53E-01	0.702	3.14E-01	7.17E-01
OTU_2207	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Unknown	unknown	Peptostreptococcaceae sp.	NB	3.37E-03	1.40E-01	0.898	3.15E-01	7.17E-01
OTU_326	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerotruncus	unknown	Anaerotruncus sp.	NB	8.68E-01	9.32E-01	0.599	3.17E-01	7.17E-01
OTU_1354	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	5.76E-04	1.14E-01	-0.85	3.22E-01	7.21E-01
OTU_1755	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	1.56E-01	5.19E-01	0.694	3.23E-01	7.21E-01
OTU_1946	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	unknown	Citrobacter sp.	NB	8.51E-01	9.23E-01	0.491	3.28E-01	7.24E-01
OTU_104	Bacteria	Firmicutes	Bacilli	Turicibacterales	Turicibacteraceae	Turicibacter	unknown	Turicibacter sp.	NB	6.39E-01	8.24E-01	0.593	3.31E-01	7.24E-01
OTU_1737	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	2.66E-02	2.67E-01	0.616	3.32E-01	7.24E-01
OTU_1599	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	fragilis	Bacteroides fragilis	NB	5.11E-01	7.68E-01	-1.056	3.33E-01	7.24E-01
OTU_1992	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	7.48E-01	8.73E-01	-0.361	3.33E-01	7.24E-01
OTU_62	Bacteria	Firmicutes	Clostridia	Clostridiales	Tissierellaceae	WAL_1855D	unknown	WAL_1855D sp.	ZINB	1.37E-01	4.84E-01	0.541	3.34E-01	7.24E-01
OTU_86	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Varibaculum	unknown	Varibaculum sp.	NB	8.10E-01	9.13E-01	-0.593	3.37E-01	7.25E-01
OTU_171	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	4.24E-01	7.27E-01	0.824	3.39E-01	7.25E-01
OTU_39	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Unknown	unknown	Clostridiaceae sp.	NB	6.41E-01	8.24E-01	-0.345	3.41E-01	7.27E-01
OTU_25	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	distasonis	Parabacteroides distasonis	NB	6.76E-01	8.44E-01	1.361	3.47E-01	7.34E-01
OTU_1203	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	producta	Blautia producta	NB	2.63E-01	6.31E-01	-1.245	3.48E-01	7.34E-01
OTU_2325	Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar	Veillonella dispar	NB	4.93E-01	7.68E-01	-0.254	3.52E-01	7.36E-01
OTU_55	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	6.97E-01	8.56E-01	-1.046	3.54E-01	7.36E-01
OTU_1573	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	3.30E-01	6.84E-01	1.055	3.54E-01	7.36E-01
OTU_1196	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	unknown	Streptococcus sp.	NB	5.55E-01	7.68E-01	-0.268	3.59E-01	7.37E-01
OTU_1660	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	unknown	Coprococcus sp.	NB	5.99E-02	3.62E-01	0.566	3.59E-01	7.37E-01
OTU_1965	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	9.11E-01	9.65E-01	-0.514	3.60E-01	7.37E-01
OTU_1877	Bacteria	Verrucomicrobi	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	muciniphila	Akkermansia muciniphila	NB	4.26E-01	7.27E-01	1.416	3.64E-01	7.37E-01
OTU_2001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	6.81E-02	3.62E-01	-0.481	3.65E-01	7.37E-01
OTU_2457	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	unknown	Streptococcus sp.	NB	6.44E-01	8.24E-01	-0.362	3.66E-01	7.37E-01
OTU_42	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	3.99E-01	7.23E-01	0.566	3.80E-01	7.62E-01
OTU_2097	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Trabulsiella	unknown	Trabulsiella sp.	NB	3.08E-01	6.64E-01	0.311	3.83E-01	7.64E-01
OTU_1342	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	unknown	Blautia sp.	NB	NA	NA	0.471	3.85E-01	7.64E-01
OTU_103	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	unknown	Lactococcus sp.	NB	1.01E-01	4.26E-01	0.554	3.87E-01	7.65E-01
OTU_2421	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Unknown	unknown	Clostridiaceae sp.	NB	6.40E-02	3.62E-01	0.459	3.94E-01	7.67E-01
OTU_2090	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus	Streptococcus anginosus	NB	3.16E-01	6.64E-01	0.639	3.95E-01	7.67E-01
OTU_1450	Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar	Veillonella dispar	NB	4.67E-01	7.45E-01	-0.274	3.96E-01	7.67E-01
OTU_521	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Unknown	unknown	Clostridiaceae sp.	NB	7.40E-01	8.71E-01	-0.621	3.98E-01	7.67E-01
OTU_1690	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	unknown	Enterococcus sp.	NB	5.55E-01	7.68E-01	-0.383	3.99E-01	7.67E-01
OTU_1591	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Unknown	unknown	Enterococcaceae sp.	NB	1.19E-01	4.58E-01	0.36	4.00E-01	7.67E-01
OTU_2292	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	6.63E-01	8.36E-01	0.403	4.08E-01	7.73E-01
OTU_2199	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	adolescentis	Bifidobacterium adolescentis	NB	6.16E-01	8.14E-01	-0.254	4.08E-01	7.73E-01
OTU_1274	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown	unknown	Ruminococcaceae sp.	NB	2.05E-01	5.78E-01	0.426	4.08E-01	7.73E-01

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_891	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*acidifaciens*	*Bacteroides acidifaciens*	NB	3.11E-01	6.64E-01	0.656	4.20E-01	7.87E-01
OTU_205	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	4.46E-02	3.40E-01	0.775	4.24E-01	7.87E-01
OTU_61	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	*Clostridiales sp.*	NB	2.77E-01	6.33E-01	0.495	4.24E-01	7.87E-01
OTU_2124	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	ZINB	8.15E-01	9.14E-01	0.509	4.25E-01	7.87E-01
OTU_2046	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	*Bifidobacterium adolescentis*	NB	4.14E-01	7.27E-01	-0.317	4.27E-01	7.87E-01
OTU_782	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	2.09E-02	2.57E-01	-0.418	4.27E-01	7.87E-01
OTU_107	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Tissierellaceae*	*Peptoniphilus*	unknown	*Peptoniphilus sp.*	NB	3.02E-02	2.79E-01	0.371	4.34E-01	7.96E-01
OTU_729	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	*Dorea sp.*	NB	4.76E-01	7.51E-01	0.514	4.47E-01	8.14E-01
OTU_1985	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	5.13E-02	3.47E-01	0.417	4.48E-01	8.14E-01
OTU_1181	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	Unknown	unknown	*Lactobacillaceae sp.*	NB	7.65E-01	8.82E-01	-0.458	4.56E-01	8.18E-01
OTU_1456	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	*Bacteroides uniformis*	NB	7.14E-01	8.65E-01	0.789	4.57E-01	8.18E-01
OTU_1942	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	NB	2.11E-02	2.57E-01	0.461	4.57E-01	8.18E-01
OTU_2308	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	1.59E-01	5.22E-01	0.404	4.60E-01	8.18E-01
OTU_1462	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	*Parabacteroides distasonis*	NB	7.42E-02	3.67E-01	1.226	4.61E-01	8.18E-01
OTU_238	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	*Peptostreptococcus*	*anaerobius*	*Peptostreptococcus anaerobius*	ZINB	3.60E-01	7.08E-01	-0.379	4.62E-01	8.18E-01
OTU_1990	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	*distasonis*	*Parabacteroides distasonis*	NB	1.59E-01	5.22E-01	1.386	4.66E-01	8.21E-01
OTU_1	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	8.15E-01	9.14E-01	-0.163	4.68E-01	8.21E-01
OTU_411	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Atopobium*	unknown	*Atopobium sp.*	NB	3.37E-01	6.88E-01	0.273	4.70E-01	8.22E-01
OTU_85	*Bacteria*	*Proteobacteria*	*Deltaproteobacteria*	*Desulfovibrionales*	*Desulfovibrionaceae*	*Bilophila*	unknown	*Bilophila sp.*	NB	8.41E-01	9.20E-01	0.748	4.76E-01	8.29E-01
OTU_126	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	NB	7.75E-02	3.75E-01	0.734	4.81E-01	8.30E-01
OTU_747	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	1.90E-01	5.68E-01	0.512	4.82E-01	8.30E-01
OTU_1905	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	*Haemophilus*	unknown	*Haemophilus sp.*	NB	4.04E-01	7.26E-01	-0.369	4.84E-01	8.30E-01
OTU_226	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	NB	7.40E-01	8.71E-01	-0.237	4.87E-01	8.30E-01
OTU_114	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	*Clostridiales sp.*	NB	8.28E-01	9.19E-01	-0.475	4.87E-01	8.30E-01
OTU_2044	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	9.70E-01	9.95E-01	0.677	4.97E-01	8.40E-01
OTU_2186	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	8.09E-01	9.13E-01	0.205	4.97E-01	8.40E-01
OTU_59	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	5.06E-02	3.47E-01	0.283	5.04E-01	8.48E-01
OTU_834	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.60E-01	6.31E-01	0.5	5.09E-01	8.54E-01
OTU_2053	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	unknown	*Parabacteroides sp.*	NB	6.45E-01	8.24E-01	1.088	5.14E-01	8.58E-01
OTU_847	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	*Akkermansia muciniphila*	NB	2.77E-01	6.33E-01	0.739	5.17E-01	8.60E-01
OTU_2222	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	NB	2.52E-01	6.29E-01	-0.599	5.21E-01	8.61E-01
OTU_80	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	NB	3.11E-01	6.64E-01	0.63	5.22E-01	8.61E-01
OTU_2340	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*	unknown	*Enterococcus sp.*	NB	9.94E-01	9.96E-01	0.286	5.24E-01	8.61E-01
OTU_1490	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	*Veillonella dispar*	NB	3.11E-01	6.64E-01	-0.208	5.31E-01	8.69E-01
OTU_84	*Bacteria*	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	*Sutterella*	unknown	*Sutterella sp.*	NB	4.17E-01	7.27E-01	0.765	5.38E-01	8.77E-01
OTU_1047	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	4.64E-02	3.40E-01	0.385	5.42E-01	8.79E-01
OTU_1040	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	*Akkermansia muciniphila*	NB	3.61E-01	7.08E-01	0.859	5.50E-01	8.88E-01
OTU_1916	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.57E-01	8.34E-01	0.299	5.53E-01	8.88E-01
OTU_1797	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	7.27E-03	1.55E-01	-0.269	5.54E-01	8.88E-01
OTU_1446	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescentis*	*Bifidobacterium adolescentis*	NB	5.12E-01	7.68E-01	-0.183	5.57E-01	8.90E-01
OTU_2098	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	4.36E-01	7.35E-01	0.23	5.64E-01	8.95E-01
OTU_1401	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	2.37E-01	6.14E-01	-0.549	5.64E-01	8.95E-01
OTU_2376	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*dispar*	*Veillonella dispar*	NB	2.88E-01	6.42E-01	-0.179	5.68E-01	8.96E-01
OTU_995	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	*Peptostreptococcaceae sp.*	NB	3.39E-02	2.99E-01	0.228	5.70E-01	8.96E-01
OTU_1740	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	7.02E-01	8.58E-01	0.106	5.72E-01	8.96E-01
OTU_67	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.18E-01	8.14E-01	0.571	5.79E-01	8.97E-01
OTU_1396	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	8.60E-01	9.28E-01	0.275	5.80E-01	8.97E-01
OTU_35	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	*aerofaciens*	*Collinsella aerofaciens*	NB	2.84E-01	6.41E-01	0.785	5.82E-01	8.97E-01
OTU_1300	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	7.13E-01	8.65E-01	-0.236	5.82E-01	8.97E-01
OTU_1511	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	4.15E-01	7.27E-01	-0.218	5.84E-01	8.97E-01
OTU_1463	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	8.63E-01	9.30E-01	-0.178	5.87E-01	8.99E-01
OTU_1956	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	*Rothia*	*mucilaginosa*	*Rothia mucilaginosa*	ZINB	3.32E-01	6.84E-01	0.206	5.89E-01	8.99E-01
OTU_1013	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	4.51E-01	7.45E-01	-0.296	5.97E-01	9.05E-01
OTU_1434	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Dialister*	unknown	*Dialister sp.*	NB	3.84E-01	7.09E-01	0.477	5.97E-01	9.05E-01

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_2057	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown	unknown	Enterobacteriaceae sp.	NB	4.49E-02	3.40E-01	0.208	6.03E-01	9.10E-01
OTU_1428	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	unknown	Corynebacterium sp.	NB	1.22E-02	1.83E-01	0.434	6.10E-01	9.14E-01
OTU_235	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	unknown	Actinomyces sp.	ZINB	3.37E-01	6.88E-01	-0.1	6.10E-01	9.14E-01
OTU_72	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	1.69E-01	5.36E-01	-0.577	6.13E-01	9.14E-01
OTU_583	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	unknown	Clostridium sp.	NB	1.29E-02	1.87E-01	0.352	6.25E-01	9.24E-01
OTU_1896	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown	unknown	Enterobacteriaceae sp.	NB	3.50E-03	1.40E-01	0.225	6.27E-01	9.24E-01
OTU_27	Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar	Veillonella dispar	ZINB	NA	NA	-0.161	6.32E-01	9.24E-01
OTU_2112	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	2.80E-01	6.35E-01	0.522	6.36E-01	9.24E-01
OTU_2270	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	1.25E-01	4.66E-01	-0.273	6.37E-01	9.24E-01
OTU_36	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	unknown	Haemophilus sp.	NB	2.02E-01	5.78E-01	-0.209	6.39E-01	9.24E-01
OTU_214	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	unknown	Actinomyces sp.	NB	6.98E-01	8.56E-01	-0.471	6.39E-01	9.24E-01
OTU_1960	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	1.28E-01	4.66E-01	0.37	6.42E-01	9.24E-01
OTU_15	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	5.37E-01	7.68E-01	0.872	6.42E-01	9.24E-01
OTU_1008	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	1.97E-01	5.74E-01	-0.361	6.44E-01	9.24E-01
OTU_2327	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	ZINB	9.53E-01	9.89E-01	-0.17	6.45E-01	9.24E-01
OTU_19	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	unknown	Ruminococcus sp.	NB	NA	NA	-0.524	6.47E-01	9.24E-01
OTU_1412	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	4.03E-01	7.26E-01	0.656	6.50E-01	9.24E-01
OTU_32	Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	unknown	Veillonella sp.	NB	6.40E-01	8.24E-01	0.737	6.50E-01	9.24E-01
OTU_2042	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown	unknown	Ruminococcaceae sp.	NB	NA	NA	0.23	6.58E-01	9.28E-01
OTU_1471	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	gnavus	Ruminococcus gnavus	NB	1.51E-01	5.08E-01	0.259	6.58E-01	9.28E-01
OTU_2337	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown	unknown	Ruminococcaceae sp.	NB	5.22E-01	7.68E-01	0.4	6.61E-01	9.29E-01
OTU_1487	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	gnavus	Ruminococcus gnavus	NB	2.09E-01	5.78E-01	0.208	6.65E-01	9.31E-01
OTU_66	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	producta	Blautia producta	NB	4.55E-01	7.45E-01	0.418	6.70E-01	9.31E-01
OTU_1919	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	NB	1.14E-01	4.58E-01	-0.237	6.71E-01	9.31E-01
OTU_1682	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	unknown	Streptococcus sp.	NB	1.63E-01	5.24E-01	0.167	6.76E-01	9.31E-01
OTU_2203	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Eubacterium	dolichum	Eubacterium dolichum	NB	1.29E-01	4.66E-01	-0.217	6.78E-01	9.31E-01
OTU_2256	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	distasonis	Parabacteroides distasonis	ZINB	3.75E-01	7.09E-01	-0.394	6.79E-01	9.31E-01
OTU_87	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus	Streptococcus anginosus	NB	9.53E-01	9.89E-01	0.217	6.81E-01	9.31E-01
OTU_60	Bacteria	Firmicutes	Clostridia	Clostridiales	Tissierellaceae	Finegoldia	unknown	Finegoldia sp.	NB	3.15E-01	6.64E-01	0.15	6.82E-01	9.31E-01
OTU_548	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	unknown	Dorea sp.	NB	6.02E-03	1.51E-01	0.311	6.85E-01	9.31E-01
OTU_2432	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Eubacterium	dolichum	Eubacterium dolichum	NB	7.44E-01	8.73E-01	-0.218	6.85E-01	9.31E-01
OTU_2415	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	7.74E-01	8.88E-01	-0.267	6.93E-01	9.39E-01
OTU_117	Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown	Unknown	unknown	Clostridiales sp.	NB	2.42E-01	6.17E-01	0.48	6.99E-01	9.40E-01
OTU_41	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Unknown	unknown	Clostridiaceae sp.	NB	3.20E-01	6.66E-01	-0.223	7.02E-01	9.40E-01
OTU_1653	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	7.22E-02	3.67E-01	-0.253	7.05E-01	9.40E-01
OTU_2454	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown	unknown	Ruminococcaceae sp.	NB	6.36E-01	8.24E-01	0.291	7.06E-01	9.40E-01
OTU_2283	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown	unknown	Enterobacteriaceae sp.	NB	1.18E-01	4.58E-01	-0.164	7.08E-01	9.40E-01
OTU_44	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	9.29E-02	4.19E-01	-0.424	7.10E-01	9.40E-01
OTU_1387	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown	unknown	Enterobacteriaceae sp.	NB	1.05E-01	4.31E-01	0.12	7.11E-01	9.40E-01
OTU_640	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	1.95E-01	5.74E-01	-0.266	7.13E-01	9.40E-01
OTU_1272	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	unknown	Dorea sp.	NB	8.81E-01	9.44E-01	0.239	7.15E-01	9.40E-01
OTU_669	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	unknown	Bifidobacterium sp.	ZINB	5.16E-01	7.68E-01	0.061	7.26E-01	9.52E-01
OTU_1069	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	unknown	Roseburia sp.	NB	5.36E-01	7.68E-01	-0.27	7.30E-01	9.54E-01
OTU_16	Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown	Unknown	unknown	Clostridiales sp.	NB	6.63E-02	3.62E-01	0.391	7.36E-01	9.58E-01
OTU_1794	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Unknown	unknown	Peptostreptococcaceae sp.	NB	4.52E-02	3.40E-01	0.174	7.41E-01	9.62E-01
OTU_2450	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Unknown	unknown	Clostridiaceae sp.	NB	8.80E-03	1.62E-01	0.2	7.45E-01	9.63E-01
OTU_12	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Unknown	unknown	Erysipelotrichaceae sp.	ZINB	NA	NA	0.078	7.49E-01	9.63E-01
OTU_1840	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	adolescentis	Bifidobacterium adolescentis	NB	8.37E-01	9.19E-01	-0.052	7.57E-01	9.63E-01
OTU_9	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Ruminococcus	gnavus	Ruminococcus gnavus	NB	4.67E-01	7.45E-01	0.142	7.58E-01	9.63E-01
OTU_2323	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	unknown	Bacteroides sp.	NB	6.22E-01	8.14E-01	0.189	7.61E-01	9.63E-01
OTU_1677	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	unknown	Lachnospiraceae sp.	NB	1.98E-01	5.74E-01	-0.319	7.62E-01	9.63E-01
OTU_1583	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	unknown	Enterococcus sp.	NB	3.72E-01	7.09E-01	0.138	7.63E-01	9.63E-01
OTU_1636	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	unknown	Actinomyces sp.	NB	4.63E-01	7.45E-01	-0.109	7.70E-01	9.63E-01
OTU_1824	Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	parvula	Veillonella parvula	NB	7.78E-01	8.90E-01	-0.199	7.73E-01	9.63E-01

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_8	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	ZINB	3.53E-01	7.08E-01	-0.059	7.83E-01	9.63E-01
OTU_408	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Bulleidia*	unknown	*Bulleidia sp.*	NB	2.72E-01	6.33E-01	-0.096	7.84E-01	9.63E-01
OTU_1839	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	8.17E-03	1.58E-01	-0.132	7.86E-01	9.63E-01
OTU_786	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	1.25E-01	4.66E-01	0.199	7.89E-01	9.63E-01
OTU_730	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	5.35E-01	7.68E-01	0.151	7.96E-01	9.63E-01
OTU_1432	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	8.51E-04	1.14E-01	0.229	7.97E-01	9.63E-01
OTU_2233	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.87E-01	6.42E-01	0.136	7.98E-01	9.63E-01
OTU_2400	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	*Bacteroides uniformis*	NB	9.37E-01	9.85E-01	-0.146	7.99E-01	9.63E-01
OTU_1966	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.59E-01	8.34E-01	-0.109	8.00E-01	9.63E-01
OTU_541	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	*Aggregatibacter*	unknown	*Aggregatibacter sp.*	NB	5.83E-01	7.86E-01	-0.123	8.03E-01	9.63E-01
OTU_53	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	5.08E-01	7.68E-01	-0.213	8.07E-01	9.63E-01
OTU_2245	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	4.78E-02	3.40E-01	-0.299	8.08E-01	9.63E-01
OTU_17	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	3.17E-01	6.64E-01	-0.205	8.12E-01	9.63E-01
OTU_2078	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	*Clostridiales sp.*	NB	2.07E-01	5.78E-01	-0.401	8.13E-01	9.63E-01
OTU_2375	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	6.45E-01	8.24E-01	-0.14	8.18E-01	9.63E-01
OTU_1594	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	*Bacteroides uniformis*	NB	5.44E-01	7.68E-01	-0.15	8.20E-01	9.63E-01
OTU_2302	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	ZINB	5.50E-01	7.68E-01	0.052	8.20E-01	9.63E-01
OTU_1386	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	3.76E-01	7.09E-01	-0.069	8.22E-01	9.63E-01
OTU_1032	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	*Oscillospira sp.*	ZINB	5.21E-01	7.68E-01	0.072	8.24E-01	9.63E-01
OTU_18	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	*Bacteroides uniformis*	NB	2.49E-01	6.24E-01	0.271	8.26E-01	9.63E-01
OTU_1780	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	*Clostridiaceae sp.*	NB	3.10E-02	2.79E-01	-0.137	8.29E-01	9.63E-01
OTU_1613	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	6.02E-01	8.04E-01	0.081	8.29E-01	9.63E-01
OTU_699	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	*Peptostreptococcaceae sp.*	ZINB	NA	NA	0.118	8.30E-01	9.63E-01
OTU_872	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	1.96E-03	1.14E-01	0.142	8.31E-01	9.63E-01
OTU_2006	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	6.57E-01	8.34E-01	-0.069	8.32E-01	9.63E-01
OTU_56	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	7.32E-01	8.71E-01	0.163	8.32E-01	9.63E-01
OTU_2003	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	3.58E-01	7.08E-01	-0.178	8.32E-01	9.63E-01
OTU_51	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	NB	4.26E-01	7.27E-01	0.29	8.33E-01	9.63E-01
OTU_227	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Corynebacteriaceae*	*Corynebacterium*	unknown	*Corynebacterium sp.*	NB	8.93E-01	9.53E-01	0.08	8.35E-01	9.63E-01
OTU_1408	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	7.58E-02	3.71E-01	0.107	8.36E-01	9.63E-01
OTU_2281	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	4.60E-02	3.40E-01	-0.074	8.36E-01	9.63E-01
OTU_1657	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Eubacterium*	*dolichum*	*Eubacterium dolichum*	NB	6.28E-02	3.62E-01	-0.118	8.38E-01	9.63E-01
OTU_2378	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*adolescenti*	*Bifidobacterium adolescentis*	ZINB	1.33E-01	4.75E-01	-0.029	8.43E-01	9.63E-01
OTU_2314	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	3.78E-01	7.09E-01	0.122	8.45E-01	9.63E-01
OTU_251	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	9.36E-01	9.85E-01	0.181	8.45E-01	9.63E-01
OTU_2255	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	unknown	*Collinsella sp.*	NB	6.05E-01	8.06E-01	0.161	8.56E-01	9.71E-01
OTU_1601	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	2.46E-01	6.20E-01	-0.111	8.57E-01	9.71E-01
OTU_2061	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.26E-02	2.63E-01	-0.132	8.60E-01	9.72E-01
OTU_31	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*	unknown	*Parabacteroides sp.*	NB	5.82E-01	7.86E-01	0.32	8.66E-01	9.75E-01
OTU_2	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	8.08E-02	3.82E-01	-0.05	8.67E-01	9.75E-01
OTU_1986	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	4.46E-01	7.45E-01	-0.085	8.70E-01	9.75E-01
OTU_1649	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*parvula*	*Veillonella parvula*	NB	9.96E-01	9.96E-01	-0.109	8.80E-01	9.83E-01
OTU_109	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.62E-01	6.31E-01	0.17	8.84E-01	9.83E-01
OTU_65	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	Unknown	unknown	*Rikenellaceae sp.*	NB	8.88E-02	4.05E-01	-0.147	8.86E-01	9.83E-01
OTU_13	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*	unknown	*Enterococcus sp.*	NB	1.70E-01	5.36E-01	0.049	8.89E-01	9.83E-01
OTU_1145	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Roseburia*	unknown	*Roseburia sp.*	NB	5.23E-01	7.68E-01	0.119	8.90E-01	9.83E-01
OTU_2404	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	6.85E-02	3.62E-01	0.046	8.91E-01	9.83E-01
OTU_76	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*eggerthii*	*Bacteroides eggerthii*	NB	9.81E-01	9.96E-01	0.145	8.94E-01	9.83E-01
OTU_1192	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	6.84E-01	8.52E-01	0.068	9.04E-01	9.90E-01
OTU_52	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Eggerthella*	*lenta*	*Eggerthella lenta*	NB	6.68E-01	8.37E-01	-0.029	9.11E-01	9.90E-01
OTU_394	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	Unknown	unknown	*Coriobacteriaceae sp.*	NB	5.45E-01	7.68E-01	0.106	9.11E-01	9.90E-01
OTU_7	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	*Bacteroides fragilis*	NB	5.19E-01	7.68E-01	-0.145	9.11E-01	9.90E-01
OTU_2451	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	2.27E-01	6.01E-01	0.088	9.16E-01	9.90E-01
OTU_2043	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	NB	7.31E-01	8.71E-01	-0.029	9.20E-01	9.90E-01

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_1495	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	NB	2.20E-01	5.88E-01	0.042	9.21E-01	9.90E-01
OTU_20	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	*Akkermansia muciniphila*	NB	9.56E-02	4.22E-01	0.112	9.22E-01	9.90E-01
OTU_154	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Ruminococcus*	*gnavus*	*Ruminococcus gnavus*	NB	7.63E-01	8.82E-01	0.108	9.22E-01	9.90E-01
OTU_1943	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Eubacterium*	*dolichum*	*Eubacterium dolichum*	ZINB	1.07E-02	1.73E-01	-0.046	9.24E-01	9.90E-01
OTU_97	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Lachnospira*	unknown	*Lachnospira sp.*	NB	5.48E-01	7.68E-01	0.097	9.32E-01	9.92E-01
OTU_2008	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	*Dorea sp.*	ZINB	4.60E-01	7.45E-01	0.034	9.37E-01	9.92E-01
OTU_162	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Coprobacillus*	unknown	*Coprobacillus sp.*	NB	9.73E-01	9.95E-01	-0.059	9.42E-01	9.92E-01
OTU_99	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	1.42E-01	4.89E-01	-0.061	9.48E-01	9.92E-01
OTU_1675	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	NB	4.37E-02	3.40E-01	-0.021	9.48E-01	9.92E-01
OTU_49	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	Unknown	unknown	*Clostridiaceae sp.*	NB	4.68E-01	7.45E-01	0.035	9.55E-01	9.92E-01
OTU_147	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	7.37E-01	8.71E-01	0.042	9.55E-01	9.92E-01
OTU_771	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	9.80E-01	9.96E-01	0.045	9.56E-01	9.92E-01
OTU_257	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Megasphaera*	unknown	*Megasphaera sp.*	NB	7.17E-01	8.66E-01	-0.042	9.62E-01	9.92E-01
OTU_1958	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.14E-01	5.87E-01	0.027	9.65E-01	9.92E-01
OTU_1724	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptostreptococcaceae*	Unknown	unknown	*Peptostreptococcaceae sp.*	NB	1.50E-01	5.07E-01	-0.016	9.66E-01	9.92E-01
OTU_131	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Lachnospira*	unknown	*Lachnospira sp.*	NB	9.53E-01	9.89E-01	0.052	9.66E-01	9.92E-01
OTU_2025	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pasteurellales*	*Pasteurellaceae*	Unknown	unknown	*Pasteurellaceae sp.*	NB	9.70E-01	9.95E-01	0.018	9.66E-01	9.92E-01
OTU_2383	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	1.61E-01	5.23E-01	0.049	9.66E-01	9.92E-01
OTU_306	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	Unknown	unknown	*Veillonellaceae sp.*	NB	8.43E-01	9.20E-01	0.033	9.66E-01	9.92E-01
OTU_2204	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	unknown	*Veillonella sp.*	NB	8.36E-01	9.19E-01	-0.019	9.69E-01	9.92E-01
OTU_2215	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	ZINB	3.29E-03	1.40E-01	-0.009	9.71E-01	9.92E-01
OTU_26	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	1.74E-01	5.36E-01	0.047	9.72E-01	9.92E-01
OTU_127	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	Unknown	unknown	*Erysipelotrichaceae sp.*	NB	8.36E-01	9.19E-01	-0.031	9.73E-01	9.92E-01
OTU_494	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	5.17E-03	1.40E-01	0.019	9.75E-01	9.92E-01
OTU_568	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	3.80E-01	7.09E-01	0.016	9.82E-01	9.92E-01
OTU_10	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	5.50E-01	7.68E-01	-0.017	9.84E-01	9.92E-01
OTU_2391	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	NB	3.44E-01	6.94E-01	0.024	9.85E-01	9.92E-01
OTU_819	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	Unknown	unknown	*Rikenellaceae sp.*	NB	5.84E-02	3.59E-01	-0.015	9.85E-01	9.92E-01
OTU_266	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*	*rhizosphaerae*	*Acinetobacter rhizosphaerae*	NB	8.94E-01	9.53E-01	-0.009	9.92E-01	9.95E-01
OTU_82	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Tissierellaceae*	*Anaerococcus*	unknown	*Anaerococcus sp.*	NB	5.58E-01	7.68E-01	-0.003	9.95E-01	9.95E-01
OTU_1117	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Veillonella*	*parvula*	*Veillonella parvula*	NB	7.23E-01	8.71E-01	0.003	9.95E-01	9.95E-01
OTU_1301	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	ZINB	7.05E-02	3.67E-01	NA	NA	NA
OTU_136	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*	unknown	*Prevotella sp.*	NA	NA	NA	NA	NA	NA
OTU_1643	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	NA	NA	NA	NA	NA
OTU_1745	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	ZINB	NA	NA	NA	NA	NA
OTU_1746	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	NA	NA	NA	NA	NA
OTU_1752	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NA	NA	NA	NA	NA	NA
OTU_1786	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NB	2.20E-01	5.88E-01	NA	NA	NA
OTU_1817	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NA	NA	NA	NA	NA	NA
OTU_1829	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	NA	NA	NA	NA	NA
OTU_1861	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	NB	7.85E-03	1.58E-01	NA	NA	NA
OTU_1865	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	NB	NA	NA	NA	NA	NA
OTU_1871	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	*Blautia producta*	NB	NA	NA	NA	NA	NA
OTU_1964	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	ZINB	NA	NA	NA	NA	NA
OTU_1999	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*fragilis*	*Bacteroides fragilis*	ZINB	NA	NA	NA	NA	NA
OTU_2026	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	NA	NA	NA	NA	NA	NA
OTU_2070	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	ZINB	NA	NA	NA	NA	NA
OTU_2091	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	1.03E-01	4.26E-01	NA	NA	NA
OTU_2157	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Eggerthella*	*lenta*	*Eggerthella lenta*	NB	NA	NA	NA	NA	NA
OTU_2187	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	*Clostridiales sp.*	NB	NA	NA	NA	NA	NA
OTU_2273	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	NA	NA	NA	NA	NA
OTU_2295	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	NB	NA	NA	NA	NA	NA
OTU_23	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	unknown	*Clostridium sp.*	NB	8.29E-01	9.19E-01	NA	NA	NA
OTU_2304	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	NB	NA	NA	NA	NA	NA

										Dog*Time Interaction[b]		Dog Main Effect[b]		
OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Model[a]	p-value	p-valueFDR	Estimate[c]	p-value	p-valueFDR
OTU_2342	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	NA	NA	NA	NA	NA
OTU_2401	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.84E-02	2.75E-01	NA	NA	NA
OTU_278	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	NB	2.17E-01	5.87E-01	NA	NA	NA
OTU_34	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Eubacterium*	*dolichum*	*Eubacterium dolichum*	ZINB	2.89E-01	6.42E-01	NA	NA	NA
OTU_4	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	ZINB	NA	NA	NA	NA	NA
OTU_48	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	*Oscillospira sp.*	ZINB	1.74E-01	5.36E-01	NA	NA	NA
OTU_58	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Dialister*	unknown	*Dialister sp.*	ZINB	5.32E-01	7.68E-01	NA	NA	NA

[a]*model selected based on minimum BIC, unless only one of the two models were estimable. If neither model was estimable, NA values are provided.*

[b]*after adjusting for maternal race, household income, maternal age at birth, mode of delivery, child sex, first born child, age at solid food introduction, and breastfeeding duration.*

[c]*difference in log-transformed mean OTU abundance, comparing children exposed to dogs versus not.*

Table S2.7: OTUs with a significant dog*formula feeding interaction. OTUs ordered by interaction p-value.

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Interaction p-value	Interaction p-valueFDR	Estimate: Not Formula Fed[a]	Estimate: Formula Fed[b]
OTU_1694	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	3.22E-11	1.40E-08	1.47	-2.18
OTU_10	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	1.51E-08	3.06E-06	-2.85	3.99
OTU_65	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	Unknown	unknown	*Rikenellaceae sp.*	2.11E-08	3.06E-06	-2.49	4.79
OTU_1560	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	2.90E-08	3.16E-06	0.25	4.31
OTU_2359	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	4.99E-07	4.03E-05	0.91	-3.53
OTU_5	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	6.46E-07	4.03E-05	-0.43	8.2
OTU_38	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	6.48E-07	4.03E-05	-2.54	7.03
OTU_2161	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	9.93E-07	5.14E-05	-0.18	2.87
OTU_147	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	1.06E-06	5.14E-05	-0.68	4.38
OTU_2155	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	1.36E-06	5.93E-05	1.12	5.83
OTU_2133	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	3.65E-06	1.35E-04	0.48	4.22
OTU_2375	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	3.85E-06	1.35E-04	-1.81	2.6
OTU_300	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	4.04E-06	1.35E-04	0.75	5
OTU_2391	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	2.35E-05	7.30E-04	-2.24	9.16
OTU_1013	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	4.18E-05	1.21E-03	-0.76	3.94
OTU_21	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	5.52E-05	1.50E-03	2.33	7.74
OTU_2337	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	7.39E-05	1.87E-03	-0.44	5.3
OTU_1861	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	7.73E-05	1.87E-03	0.14	2.93
OTU_26	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	1.32E-04	3.01E-03	-0.61	5.35
OTU_568	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	1.53E-04	3.33E-03	-0.89	3.7
OTU_819	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	Unknown	unknown	*Rikenellaceae sp.*	1.96E-04	4.02E-03	-0.88	3.76
OTU_1413	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	2.03E-04	4.02E-03	-0.03	-1.78
OTU_51	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	unknown	*Coprococcus sp.*	2.55E-04	4.64E-03	-1.9	7.68
OTU_103	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*	unknown	*Lactococcus sp.*	2.56E-04	4.64E-03	1.73	-1.1
OTU_1746	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	2.80E-04	4.87E-03	-0.65	4.32
OTU_40	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	unknown	*Bacteroides sp.*	3.63E-04	6.08E-03	-1.68	2.72
OTU_1186	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	4.01E-04	6.46E-03	0.41	2.86
OTU_1709	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	5.58E-04	8.67E-03	0.9	4.21
OTU_60	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Tissierellaceae*	*Finegoldia*	unknown	*Finegoldia sp.*	6.06E-04	9.09E-03	-1.01	1.15
OTU_84	*Bacteria*	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	*Sutterella*	unknown	*Sutterella sp.*	8.30E-04	1.18E-02	1.16	8.43
OTU_2303	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	8.62E-04	1.18E-02	-0.46	3.05
OTU_43	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	8.79E-04	1.18E-02	1.6	7.45
OTU_494	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	9.12E-04	1.18E-02	0.21	3.25
OTU_1244	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	*longum*	*Bifidobacterium longum*	9.25E-04	1.18E-02	-1.24	2.57
OTU_1986	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	1.05E-03	1.30E-02	-1.13	1.58
OTU_2314	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	1.20E-03	1.45E-02	0.13	3.39
OTU_61	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	*Clostridiales sp.*	1.25E-03	1.45E-02	0.22	2.93
OTU_745	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	1.30E-03	1.45E-02	1.54	4.86
OTU_19	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	1.33E-03	1.45E-02	0.21	8.45
OTU_2454	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	1.34E-03	1.45E-02	-0.07	4.03
OTU_2203	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Eubacterium*	*dolichum*	*Eubacterium dolichum*	1.39E-03	1.48E-02	0.96	-1.66
OTU_98	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	unknown	*Ruminococcus sp.*	1.54E-03	1.59E-02	0.48	6.95
OTU_326	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Anaerotruncus*	unknown	*Anaerotruncus sp.*	1.58E-03	1.60E-02	2.38	-0.07
OTU_562	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	1.86E-03	1.80E-02	1.25	4.44
OTU_120	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	1.90E-03	1.80E-02	0	5.3
OTU_1069	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Roseburia*	unknown	*Roseburia sp.*	1.95E-03	1.80E-02	-1.4	2.48
OTU_1985	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	1.95E-03	1.80E-02	0.95	4.5
OTU_1495	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	unknown	*Bifidobacterium sp.*	2.46E-03	2.23E-02	-0.25	1.83
OTU_1636	*Bacteria*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	unknown	*Actinomyces sp.*	2.62E-03	2.33E-02	0.59	-1.23
OTU_1015	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Dorea*	unknown	*Dorea sp.*	2.67E-03	2.33E-02	0.42	5.82
OTU_411	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Atopobium*	unknown	*Atopobium sp.*	2.76E-03	2.35E-02	1.08	-0.68
OTU_1058	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	Unknown	Unknown	unknown	*Lactobacillales sp.*	2.89E-03	2.42E-02	1.05	-0.41
OTU_63	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	2.95E-03	2.42E-02	-0.08	-1.72
OTU_2154	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*uniformis*	*Bacteroides uniformis*	3.17E-03	2.53E-02	-0.72	2.24
OTU_2182	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	3.20E-03	2.53E-02	1.35	4.39

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	Label	Interaction p-value	Interaction p-valueFDR	Estimate: Not Formula Fed[a]	Estimate: Formula Fed[b]
OTU_1342	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	3.29E-03	2.56E-02	-0.4	1.92
OTU_2168	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	3.50E-03	2.67E-02	1.05	3.74
OTU_129	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	unknown	*Oscillospira sp.*	3.92E-03	2.94E-02	-0.96	4.39
OTU_2091	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	4.10E-03	3.02E-02	0.11	3.44
OTU_111	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	Unknown	unknown	*Ruminococcaceae sp.*	4.36E-03	3.14E-02	1.78	6.39
OTU_2085	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	4.43E-03	3.14E-02	0.47	5.37
OTU_1192	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Faecalibacterium*	*prausnitzii*	*Faecalibacterium prausnitzii*	4.47E-03	3.14E-02	0.15	2.7
OTU_59	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	Unknown	unknown	*Enterobacteriaceae sp.*	4.81E-03	3.32E-02	1.3	-0.57
OTU_2006	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	unknown	*Streptococcus sp.*	4.98E-03	3.33E-02	0.09	-1.24
OTU_971	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	4.98E-03	3.33E-02	2.15	-0.91
OTU_93	*Bacteria*	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	*stercoris*	*Collinsella stercoris*	5.12E-03	3.37E-02	6.03	15.79
OTU_2187	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	Unknown	Unknown	unknown	*Clostridiales sp.*	5.86E-03	3.80E-02	0.96	4.46
OTU_1657	*Bacteria*	*Firmicutes*	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Eubacterium*	*dolichum*	*Eubacterium dolichum*	6.21E-03	3.93E-02	0.83	-1.65
OTU_20	*Bacteria*	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	*Akkermansia muciniphila*	6.24E-03	3.93E-02	-2.37	2.78
OTU_66	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	*Blautia producta*	6.35E-03	3.95E-02	-0.83	2.68
OTU_597	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	unknown	*Lactobacillus sp.*	6.74E-03	4.13E-02	0.3	4.99
OTU_1737	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	unknown	*Blautia sp.*	7.24E-03	4.38E-02	0.61	3.16
OTU_1434	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Dialister*	unknown	*Dialister sp.*	7.48E-03	4.46E-02	0.61	-5.07
OTU_155	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	Unknown	unknown	*Lachnospiraceae sp.*	8.06E-03	4.74E-02	-0.12	2.34
OTU_344	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*producta*	*Blautia producta*	8.16E-03	4.74E-02	-2.09	0.6

[a] *difference in log-transformed mean OTU abundance (after covariate adjustment) comparing children exposed to dogs versus not, among children not formula fed.*

[b] *difference in log-transformed mean OTU abundance (after covariate adjustment) comparing children exposed to dogs versus not, among formula fed children.*

2.10 EXETENDED DATA

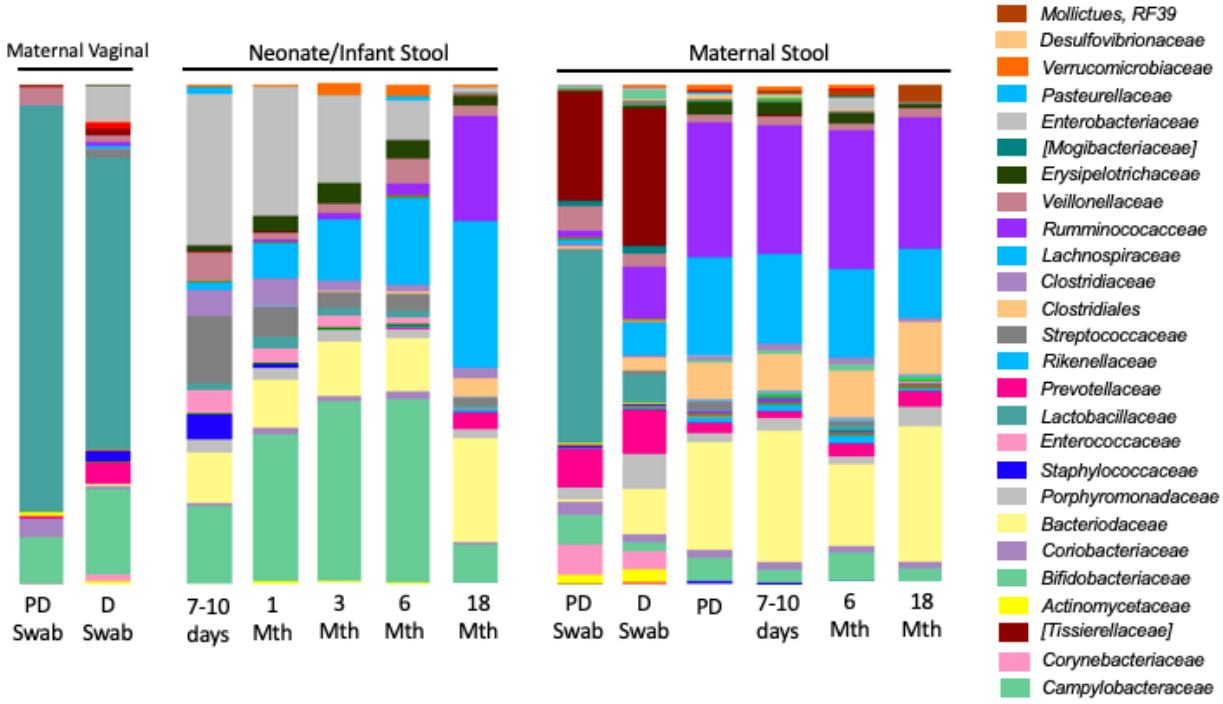


Figure E2.1 Family Relative Abundance in Neonatal and Maternal Samples. Bacterial family relative abundance summary plots by sample type and timepoint. Full bar height is 100%. PD = Pre-Delivery, D = Delivery, Mth = Month.

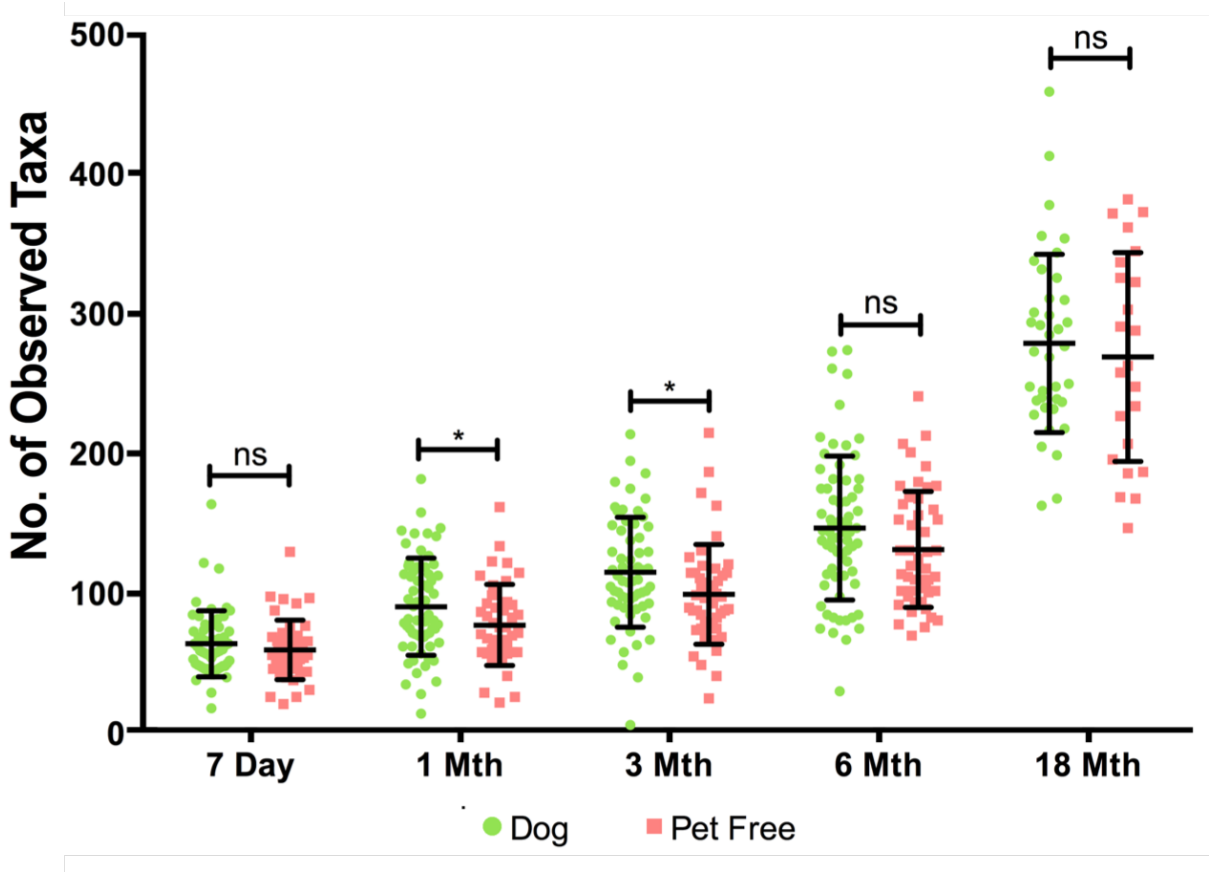


Figure E2.2 Number of Observed Taxa is Enriched in Dog-Exposed Neonates at 1 and 3 Months of Age. Taxa Cross-sectional analysis showing increased richness in dog-keeping compared to pet-free neonates. At all timepoints a trend toward increased richness in dog exposed infants is observed, with those trends being significant at 1- and 3-months of age (Mann-Whitney, *$p<0.05$). In the mixed effects model, however, there a significant dog*time interaction was not observed. Note, not all 18-month samples were included as they were still being actively collected at the time of this analysis.

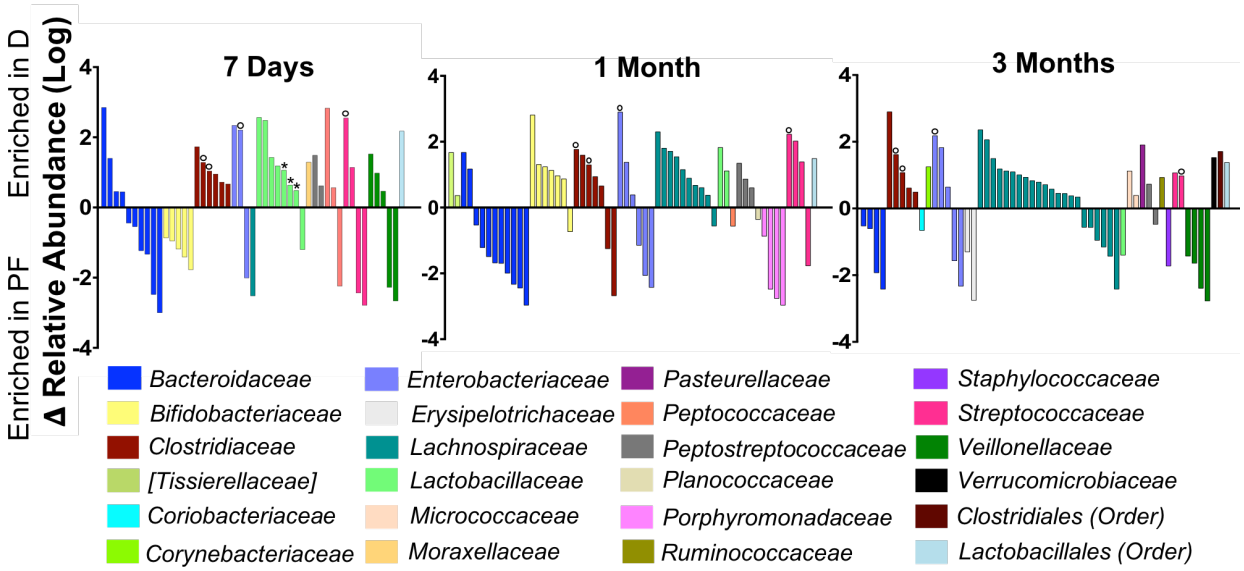


Figure E2.3 Taxa Enriched in Dog-Owning or Pet-Free Infants Over the First 3 Months of Life and Association with Total IgE. Cross-sectional differences in taxon relative abundance between dog exposed compared to pet-free infants at 7 days, 1 month, and 3 months of age determined via simultaneous application of three different regression models: Poisson regression, negative binomial, and zero inflated negative binomial. Taxa that exhibit a q-value <0.2 and p-value less than 0.05 were considered signifcant. Each bar represents a specific taxon within a bacterial family. Open circles represent taxa that persist over the first three months of life. Asterisks represent taxa that were independently found to be enriched in infants with the lowest 6-month total Immunoglobulin E (IgE) levels (Poisson Regression Model, $p<0.05$, $q<0.2$).

2.10.1 1-month Old Infant Cell Free Fecal Water Did Not Impact Gut Epithelial Cell Line Gene Expression

In addition to examining the impact of dog ownership on gut microbial community dynamics, we also wanted to understand the impact of microbial metabolites present in dog-exposed or pet-free infant stool on the gut environment. As dog exposure is associated with lower levels of IgE in childhood[4,5] we also stratified infants by high or low total IgE trajectory (Figure E2.4, Table E.1). We hypothesized that gut-associated microbial products from 1-month old neonates not exposed to dogs and with a high total IgE trajectory would promote an inflammatory environment in the gut while products from dog-exposed low total IgE neonatal stool would promote an anti-inflammatory environment.

To test this the Caco-2 gut epithelial cell line was treated with cell-free fecal water (CFW) from 1-month old infants and then assessed for changes in gene expression of 15 genes including inflammatory (IL-4, IL-6, IL-12, IL-13, IL-17, TNFα) and anti-inflammatory (IL-10, TGF-β) cytokines, microbially relevant receptors (TLR4, CYP1A1), tight junction proteins (Occludin and Claudin) and mucin related genes (Muc2, Muc3, Muc5AC). A list of primer sequences can be found in Table E2.2. For most genes tested there was not a statistically significant difference in expression when comparing treatment with stool from high IgE and low IgE infants; dog-keeping and pet free infants; or when considering both IgE and pet-keeping status (Extended Data Figure 2.5, 2.6, and 2.7).

These results may be due to several factors including: 1) Samples being chosen based on total IgE instead of specific IgE which is a stronger biomarker of atopy and allergic asthma development in later life. Alternatively, stratifying patients

microbiologically and choosing samples based on bacterial community composition may be more biologically relevant; 2) The Caco-2 cell line is derived from heterogeneous adult human epithelial colorectal adenocarcinoma cells. While this cell line is commonly used, it is possible that it is not biologically relevant for early-life studies. However, it was the best option as we do not have access to infant gut epithelial cells. Organoids may be an alternative for future studies, although these are still likely to be adult derived; 3) The experiment may have been underpowered for capturing the effects of 1-month old CFW on gut epithelial cell gene expression.

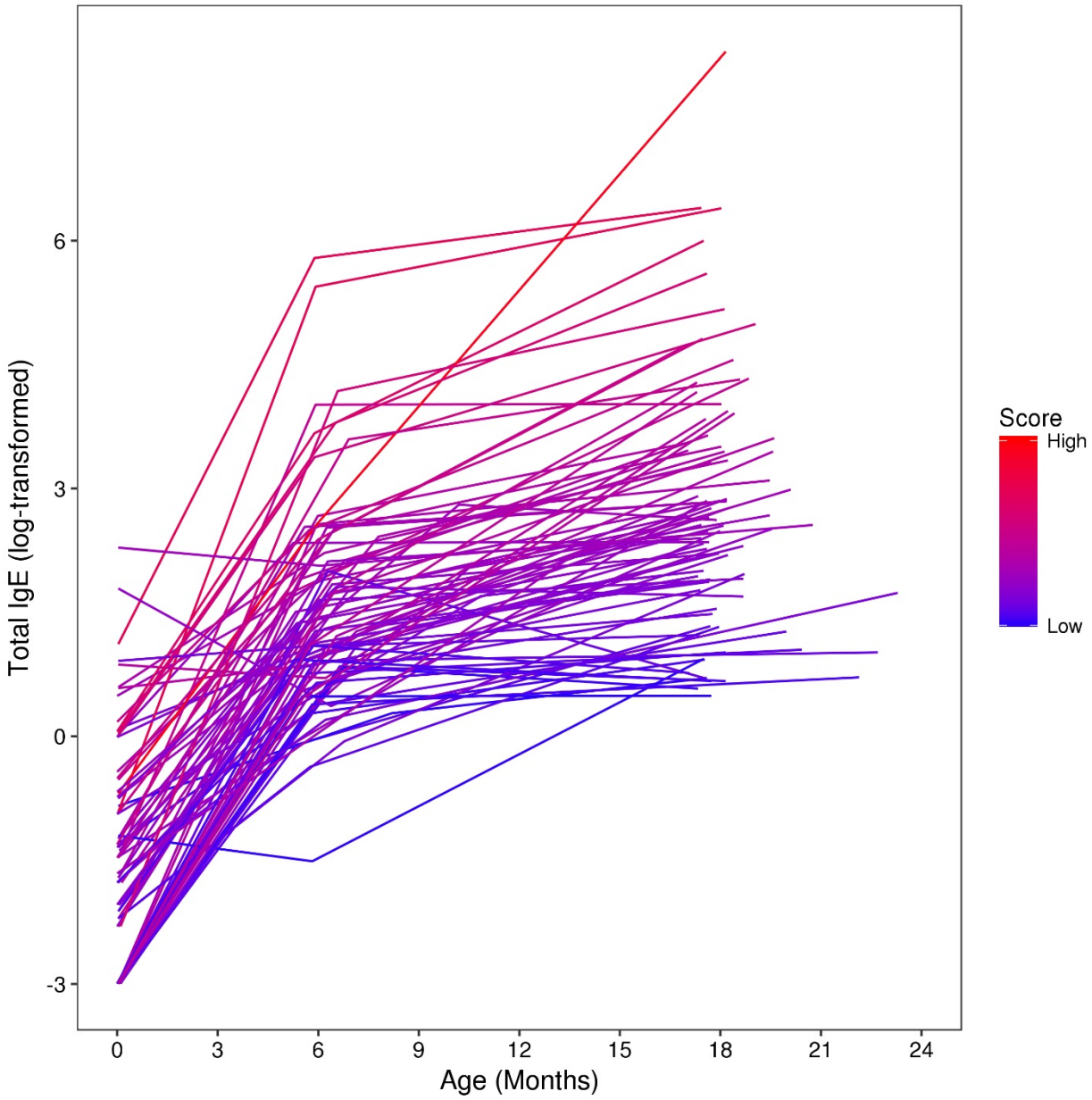


Figure E2.4 Total IgE trajectories for 83 infants from the MAAP cohort. Each line represents one infant and lines are colored by a score combining area under the curve and 18-month total IgE data.

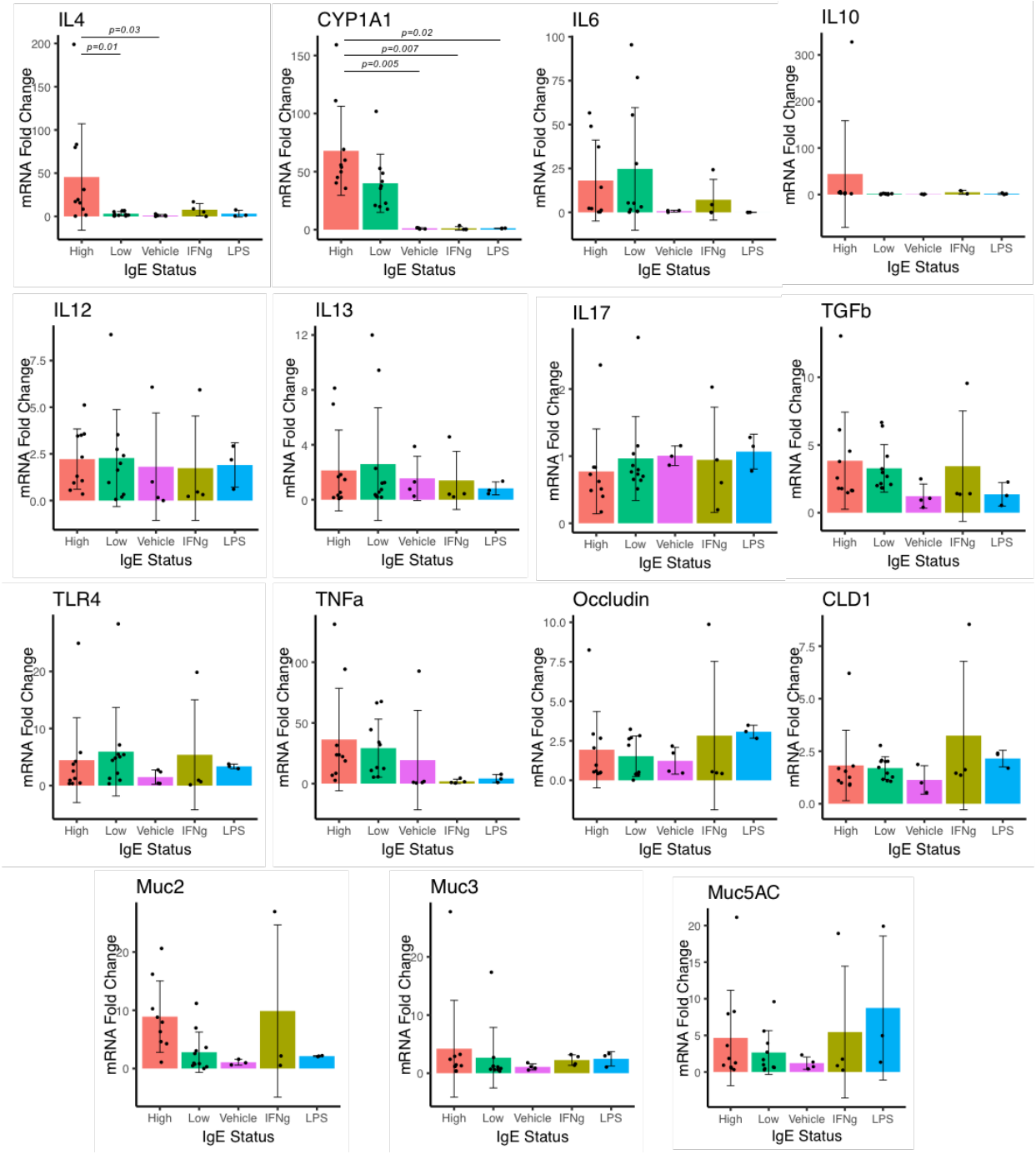


Figure E2.5 Impact of 1-Month CFW Stratified by Total IgE Levels on Gut Epithelial Cell Gene Expression. Gene expression of Caco-2 gut epithelial cells treated with 1-month old filtered fecal water stratified by total IgE trajectory. While differences were observed between high compared to low IgE trajectory infants for IL-4 and CYP1A1, it is possible the significance is being driven by outliers.

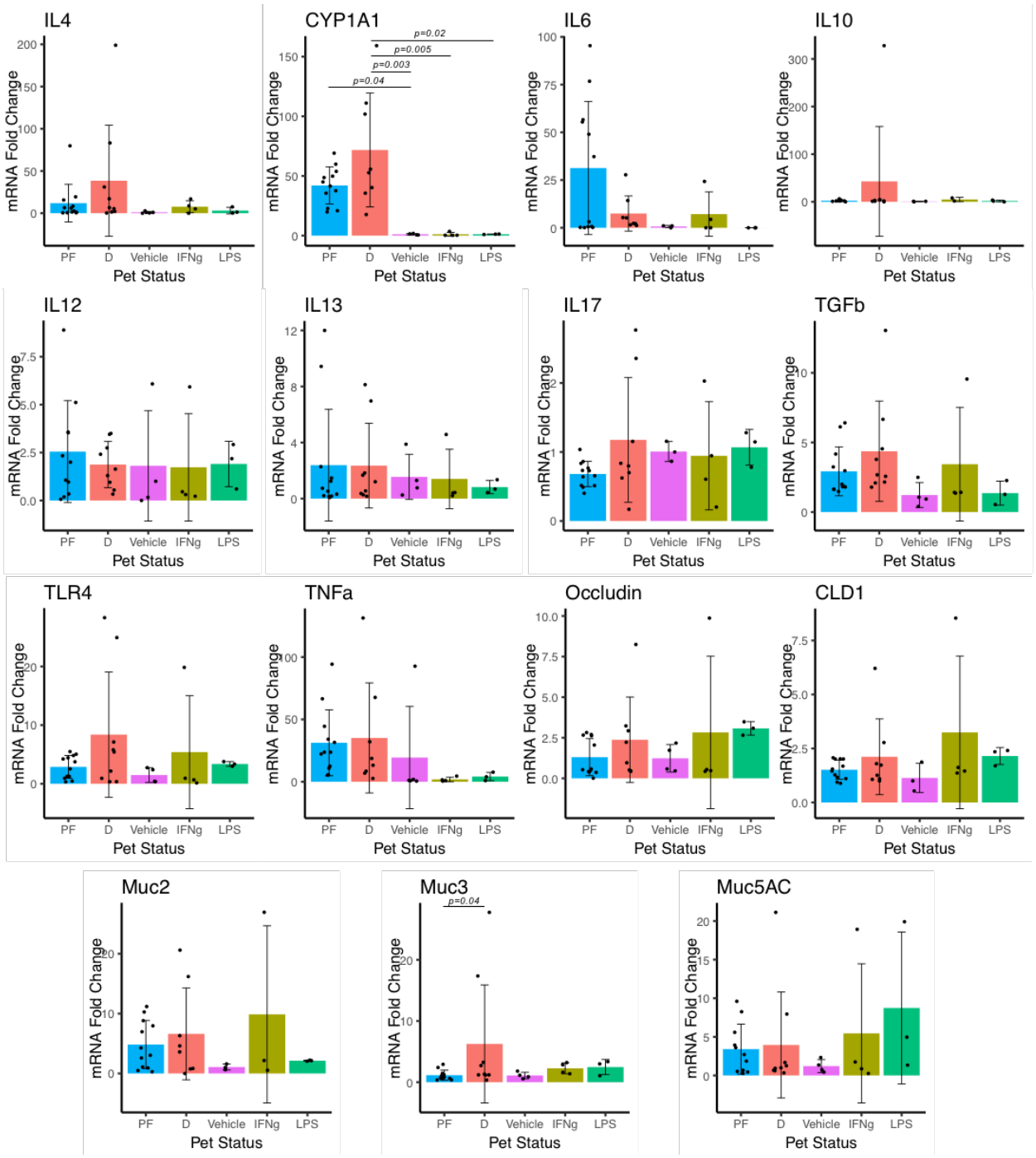


Figure E2.6 Impact of 1-Month CFW Stratified by Pet Status on Gut Epithelial Cell Gene Expression. Gene expression of Caco-2 gut epithelial cells treated with 1-month old filtered fecal water stratified by dog ownership. While differences were observed between dog-owning (D) compared to pet-free (PF) infants for CYP1A1 and Muc3, it is likely, especially for Muc3, that the significance is being driven by outliers.

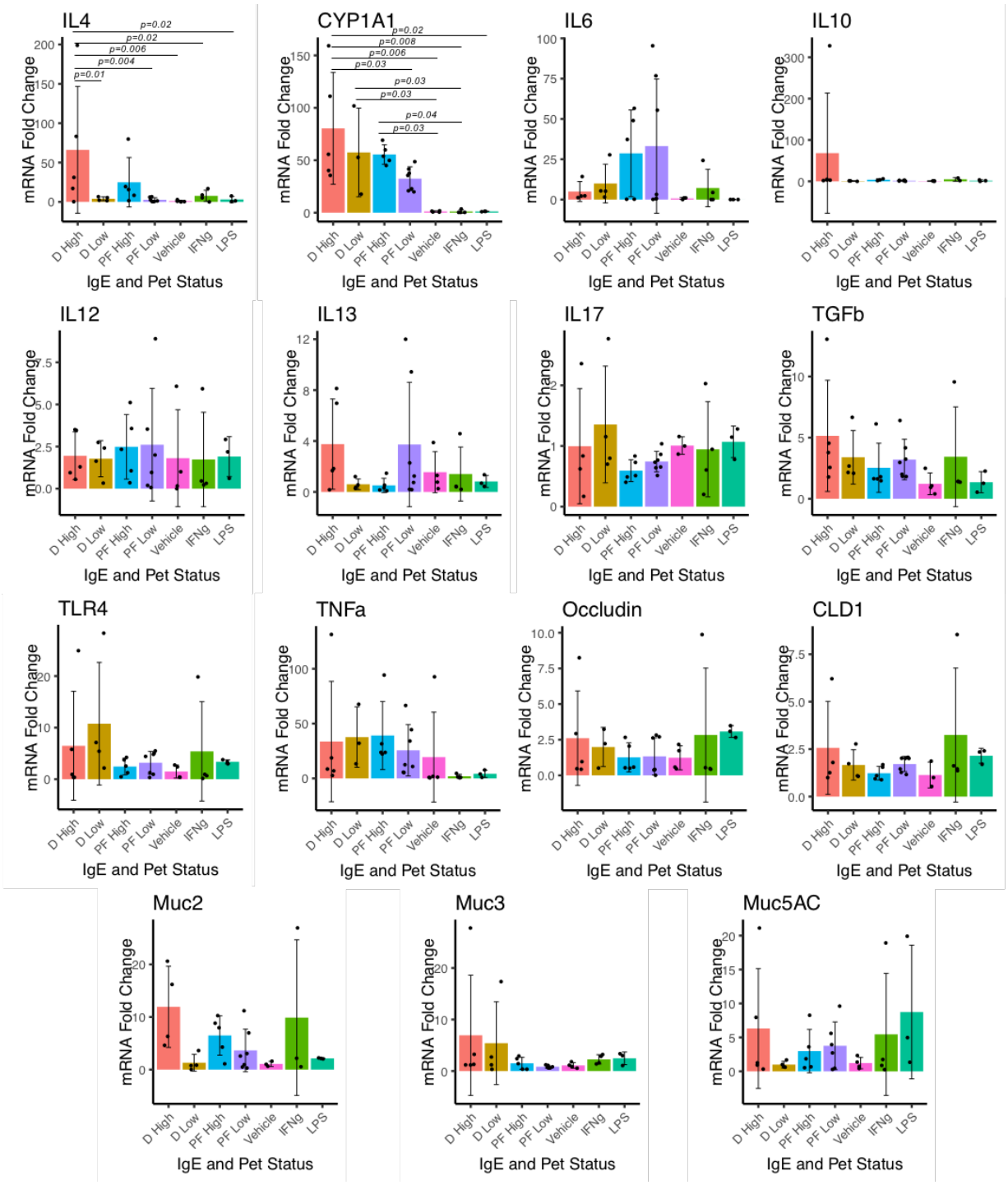


Figure E2.7 Impact of 1-Month CFW Stratified by Total IgE Levels and Pet Status on Gut Epithelial Cell Gene Expression. Gene expression of Caco-2 gut epithelial cells treated with 1-month old filtered fecal water stratified by dog ownership and IgE trajectory. While differences were observed for IL-4 and CYP1A1, the large amount of variation is likely driving the significance.

Table E2.1: N=10 highest and lowest total IgE trajectories, as determined by the combined score.

famID	LynchLabID at:					Have Baby Stool at:			
	1-Week	1-Month	3-Months	6-Months	18-Months	1-Week	1-Month	3-Months	6-Months
10 Lowest									
9658	E167	E182	E253	E346	E697	1	1	1	1
9223	E57	E70	E106	E162	E548	1	1	1	1
7172		E511	E618			0	1	1	0
9309	E86	E95	E152	E220	E699	1	1	1	1
9014	E2	E4	E14	E42	E304	1	1	1	1
7361	E521		E633	E682		1	0	1	1
9695	E190	E225		E389		1	1	0	1
9977	E347	E380	E444	E513	E780	1	1	1	1
9767	E248	E280		E442	E751	1	1	0	1
9818	E284				E756	1	0	0	0
10 Highest									
9554	E172	E195	E271	E377	E711	1	1	1	1
9900	E440		E555			1	0	1	0
7474	E507	E574	E643			1	1	1	0
9313		E92	E131	E200	E642	0	1	1	1
9769	E246	E259	E357	E457	E746	1	1	1	1
9753	E234	E263	E329	E418	E728	1	1	1	1
9388		E130	E166	E255	E652	0	1	1	1
9130		E53	E94		E573	0	1	1	0
7068	E408	E434	E510	E613		1	1	1	1
9505	E158	E169	E244	E345	E707	1	1	1	1

Table E2.2: PCR primers used for quantification of genes in Caco2 cells

Name	Forward Primer	Reverse Primer	Notes
Beta Actin	AAGATGACCCAGATCATGTTTGAGACC	AGCCAGTCCAGACGCAGGAT	Housekeeping gene
CLD1	CCACAGCATGGTATGGCAATAG	CAGCCCAGCCAGTGAAGAG	Claudin-1, Component of tight junctions
CYP1A1	GACCACAACCACCAAGAAC	AGCGAAGAATAGGGATGAAG	Aryl hydrocarbon hydroxylase, Aryl hydrocarbon receptor responsive gene
IL-10	TCA GGG TGG CGA CTC TAT	TGG GCT TCT TC TAA ATC GTT C	Anti-inflammatory cytokine
IL-12	CGTAGAATTGGATTGGTATCCGG	GCTCTTGCCCTGGACCTGAACGC	Cytokine that induces Th1 cell differentiation
IL-13	TGAGGAGCTGGTCAACATCA	CAGGTTGATGCTCCATACCAT	Cytokine that regulates IgE synthesis
IL-17	TCAACCCGATTGTCCACCAT	GAGTTTAGTCCGAAATGAGGCTG	Pro-inflmmatory cytokine
IL-4	ACTTTGAACAGCCTCACAGAG	TTGGAGGCAGCAAAGATGTC	Cytokine that induces Th2 cell differentiation
IL-6	GATGGCTGAAAAAGATGGATGC	CTGCAGGAACTGGATCAGGACT	Pro-inflmmatory cytokine
Muc2	CTGCACCAAGACCGTCCTCATG	GCAAGGACTGAACAAAGACTCAGAC	Gel-forming mucin, major component of mucus in the intestine
Muc3	CTCCAAGCCACACTGCCC	TGCTCCCCAAACTATCTG	Membrane-associated mucin
Muc5AC	TACTCCACAGACTGCACCAACTG	CGTGTATTGCTTCCCGTCAA	Gel-forming mucin, major component of mucus in the intestine
Occuludin	GATGAGCAGCCCCCCAAT	GGTGAAGGCACGTCCTGTGT	Component of tight junctions
TGF-β	GCGTGCTAATGGTGGAAAC	CGGTGACATCAAAAGATAACCAC	Cytokine that promotes Treg generation
TLR4	AAGCCGAAAGGTGATTGTTG	CTGAGCAGGGTCTTCTCCAC	Toll like receptor, ligand is lipopolysaccharide and lipoteichoic acids
TNFα	AGGCGGTGCTTGTTCCTCAG	GGCTACAGGCTTGTCACTCG	Pro-inflmmatory cytokine

2.11 EXTENDED METHODS

2.11.1 Statistical Analyses

α-Diversity (within sample) indices and bacterial family relative abundance were calculated using QIIME (Quantitative Insights into Microbial Ecology).[227] To test for significant differences in taxon abundance between dog-exposed and pet-free infants, we simultaneously applied Poisson, negative binomial, and zero-inflated negative binomial regressions (three-model approach) to taxon count data and tested for best fit on a taxon-by-taxon basis using our multiply rarefied OTU table. After correction for false discovery (Benjamini-Hochberg), taxa that exhibited a *q* value less than 0.2 and a *P* value less than 0.05 were considered significant.

Of the children with baby stool microbiome sequenced at one or more timepoints, 83 had total IgE measured at all three collection timepoints (birth, 6 months, and 18 months) and were included in IgE trajectory analysis. To account for early rapid increases in total IgE as well as high total IgE at the latest time point, extreme low and high total IgE trajectories were determined using the following two measures: 1) the area under the curve (AUC) of the total IgE trajectory, and 2) cross-sectional total IgE at the 18-month time point. Both measures were log-transformed and converted to standard normal distributions; these two transformed values were then averaged together to determine a single combined score. High values of this score represent high total IgE trajectories, and low values represent low total IgE trajectories.

2.11.2 Epithelial Cell Assay & Gene Expression Analysis

1 month old fecal samples from five high and five low IgE trajectory infants were used (biological replicates). 0.5 grams of stool was added to 500 µl pre-warmed extraction buffer containing phosphate-buffered saline (PBS) and 20% Fetal Bovine

Serum (FBS). Samples were vortexed, incubated at 37°C for 10 min, and centrifuged at 14,000 x g at room temperature to pellet solids. Supernatant was filtered through Mini-UniPrep 0.45 µm filter followed by a Mini-UniPrep 0.22 µm filter before being used in the epithelial cell assay. Extraction buffer was used as a negative control. Caco-2 cells were cultured in complete Minimum Essential Media (Gibco MEM with 1X non-essential amino acids, 1X penicillin-streptomycin, and 10X FBS). Cells were seeded at 20,000 cells/cm^2 and maintained for 14 days with media changes three times a week. After 14 days, differentiated Caco-2 cells were treated with 10% filtered cell-free fecal water (CFW), with three wells of cells being treated with the same CFW sample. After 24 hours, cells were washed with PBS and RNA was extracted and DNase treatment was performed using the RNAqueous-Micro Kit (Thermo Fisher) following the manufacturer's protocol. RNA concentration was normalized to 40 ng/µl and immediately converted to cDNA using the High-Capacity RNA to cDNA Kit (Applied Biosystem) and following the manufacturer's instructions. After reverse transcription, 220 µl of sterile water was added to cDNA. Quantitative polymerase chain reaction amplification of 15 genes was performed in 10 µl reactions using forward and reverse primers at a final concentration of 0.5 uM, Power SYBR Green PCR Master Mix (Life Technologies) at a final concentration of 1X, and 2 µl of cDNA. Reactions were run on a QuantStudio 6 qPCR machine using the following program: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 sec and 60°C for 1 min, followed by 95°C 15 sec, 60°C for 1 min, and 95°C for 15 sec. Human beta actin was used as a reference gene and gene expression was calculated using the delta delta ct method in which extraction buffer delta ct value is subtracted from the experimental ct value. Samples with a beta actin ct over 20 were

removed from analysis. Significant differences in gene expression were calculated for the following groups: high and low IgE trajectory infants; dog-exposed and pet-free infants; and dog-exposed high and low IgE trajectory and pet-free high and low IgE trajectory infants. Analysis was done in R version 3.4.0 using linear mixed effects analysis (lme4 package) and plots were made using ggplot2.

CHAPTER 3: EXAMINING THE RELATIONSHIP BETWEEN EARLY-LIFE GUT MICROBIAL FUNCTIONS AND PROTECTION AGAINST ALLERGIC ASTHMA.

Material for Section 3.3.2 was modified from:

Panzer, A.R., McKean, M., Cabana, M.D., and Lynch, S.V. “Microbial Amino Acid Synthesis and Metabolism Pathways Differentiate Healthy and High Risk for Asthma Infants at 6-Months of Age.” In preperation, September 2021.

3.1 ABSTRACT

Compared with high-risk for asthma (HR) infants, those at low risk possess distinct gut microbiomes and fecal metabolite profiles.[6,7] In a double-blind placebo-controlled trial examining the effect of daily *Lactobacillus rhamnosus* GG (LGG) supplementation over the first 6 months of life on a HR infant population, the fecal metabolome of 6-month-old probiotic-supplemented infants (HRLGG) was more similar to that of healthy control (HC) infants compared with HR infants who received a placebo (HRP).[8] Additionally, LGG-associated cell-free fecal products from 6-month stool promoted Treg expansion *ex vivo*.[8] We thus sought to determine the specific early-life gut microbial products capable of promoting the observed immune tolerance.

Both the HC and HRLGG infant fecal metabolomes were characterized by enrichment of a variety of C19 and C21 lipid steroids.[8] One of these steroids, dehydroepiandrosterone sulfate (DHEA-S), had previously been implicated in protection against asthma in murine and human models.[228,229] We therefore hypothesized that HC and HRLGG gut microbial communities have the functional capacity to produce this steroid which in turn impacts immune tolerance in the host. To address this hypothesis, we used data and stool samples from 6-month old HC, HRLGG, or HRP infants.[8] We carried out several approaches including evaluating the impact of DHEA-S treatment on dendritic cell (DC) phenotype; shotgun metagenomic sequencing of stool samples (n=25) to identify microbial steroid-associated genes; bacterial culturing to isolate bacteria capable of producing the unsulfated form of DHEA; and animal models in which mice were treated with the precursor to DHEA 17α-hydroxypregnenolone (17-OH-Preg) and challenged to induce allergic airway inflammation to determine if gut microbes were

capable of metabolizing 17-OH-Preg to DHEA and whether this led to protection against allergic airway inflammation.

As results from the above experiments proved inconclusive, we broadened our approach. We used the shotgun metagenomic sequencing data and previously generated metabolomic data to identify microbial functional pathways and associated metabolites that differentiate the gut microbiomes of HC compared to HRP infants. We observed that HC infant microbiomes were statistically significantly enriched for ornithine biosynthesis and the metabolite L-ornithine while HRP infants showed enrichment for ornithine degradation. We next sought to examine the impact of L-ornithine or the HC fecal metabolic milieu on immune cell phenotypes. These experiments remain ongoing.

3.2 INTRODUCTION

Having shown that early life environmental microbial exposures, such as dog-keeping, impact the development of the infant gut microbiota and alter diversification trajectories in a way that may promote protection from asthma development we next sought to understand the mechanisms by which the gut microbiota may be exerting this effect. Specifically, we were interested in microbial functions enriched in the gut of low-risk for asthma infants and how products from these microbes may educate immune cells to promote hallmarks of tolerance and protection against asthma.

Several studies have shown differences in microbial community composition at 1-month or 3-months of age between healthy infants and those at increased risk for atopy and asthma.[6,7] In addition, low-risk infants were enriched for specific bacterial genera and had distinct fecal metabolic profiles compared to high-risk children.[6,7]

Independently, a longitudinal study of healthy controls (HC) and high-risk for atopy infants demonstrated divergent gut microbiota development throughout the first year of life and metabolic abnormalities at 6 months of age in the high-risk group.[8] This study was based on a trial in which half of the high-risk group received a daily oral supplement of *Lactobacillus rhamnosus* GG (HRLGG) for the first six months of life with the other half receiving a placebo (HRP). At the end of the supplementation period fecal metabolic profiles of the HRLGG group were significantly more similar to HC infants, and cell-free fecal products of the former promoted T regulatory cell expansion and IL-10 production *ex vivo*.[8] This suggests that the early-life gut microbiota may be manipulated to promote regulatory immune responses.

Evidence already exists to support the idea that microbial products from the infant gut can impact immune cells in a way that promotes Th2 responses and allergic

inflammation.[184] Yet, comparatively little is known about the specific early-life microbial metabolites that may promote a tolerogenic immune profile in healthy infants. We thus sought to address this by further interrogating metabolites enriched at 6-months of age in either healthy control infant stool samples from the Development of Infant Microbial Evolution (DIMES) cohort[8] or LGG supplemented high-risk for asthma infants from the Trial of Infant Probiotic Supplementation (TIPS) cohort.[230]

3.3 RESULTS

3.3.1 Examining the Capacity of the Early-Life Gut Microbiota to Metabolize Steroids

In their cohorts, both Fujimura *et al.* (2016) and Durack *et al.* (2018) observed a number of lipid steroids enriched in the feces of low risk for asthma infants. These lipid steroids were also enriched in the LGG supplemented infants compared to HRP infants[8], thus offering evidence that these steroids may be of microbial origin.

The ability of gut resident microorganisms to produce steroids has been recognized for over half a century. In a 1968 study comparing the fecal metabolic profiles of wild-type rats and germ-free (GF) rats, Gustafsson and colleagues observed that some steroids detectable in wild-type rats were undetectable in GF rats.[231] The microbial capacity to synthesize steroids has also been observed in human studies. Fresh fecal samples from healthy adults were collected and fecal dilutions were cultured in Brain Heart infusion (BHI) broth plus cysteine in the presence of either 16α - hydroxyprogesterone[232] or deoxycorticosterone.[233] In both instances the authors observed microbe-driven dihydroxylation of these steroids. Additionally, microbes present in human fecal samples have the ability to convert cortisol into C-21 and C-19

compounds.[234] Individual fecal isolates capable of metabolizing steroids have also been identified including a *Clostridium* isolate that synthesizes androgen from cortisol.[235]

One steroid, dehydroepiandrosterone (DHEA), became of specific interest to us as the sulfated version of this steroid was enriched in the HRLGG group compared to the HRP group and had previously been implicated in protection from allergic airway inflammation.[8] In a murine study using an ovalbumin (OVA) sensitization and challenge model, OVA treated mice supplemented with 50 or 100 mg/kg DHEA showed decreased Th2-associated cytokine levels in bronchoalveolar lavage fluid compared to OVA treated non-supplemented mice.[228] Additionally, the authors observed a decrease in eosinophil infiltration and goblet cell hyperplasia in the lungs and a decrease in OVA-specific Immunoglobulin E (IgE) levels in the DHEA supplemented mice. Another study using human peripheral blood mononuclear cells (PBMCs) isolated from subjects with airway hyperresponsiveness (AHR) showed similar results.[229] After culturing with DHEA, PBMCs from AHR patients showed suppressed IL-4 and IFNγ cytokine production compared to cells from healthy controls. Based on these studies and the enrichment of DHEA sulfate (DHEA-S) in infants who received *Lactobacillus* supplementation we hypothesized that microbes in the infant gut are capable of biosynthesizing DHEA and that this microbially-derived DHEA may confer protection against allergic asthma.

3.3.1.1 *Assessing the Impact of DHEA Treatment on Primary Human DCs*

We began by testing whether DHEA-S could promote a tolerogenic immune cell phenotype. Previous research in our lab examining the impact of microbial metabolites on immune cell phenotypes used a DC/T-cell co-culture assay. This assay takes nine

days to run, so we decided to shorten the experiment by focusing on whether DHEA-S is capable of promoting a tolerogenic DC program characterized by IL-10 secretion. DCs were isolated from PBMCs using the Pan DC Enrichment Kit and treated with various concentrations of DHEA-S in the presence of growth factors IL-4 (20 ng/mL final concentration) and GM-CSF (10 ng/mL final concentration). Supernatant was collected at 24 hours and 10 ng/mL TNFα, IL-1β, and IL-6 and 1 μM prostaglandin E2 were added to induce DC activation and maturation. Supernatant was collected again at 48 hours and cytokine secretion was assessed using cytometric bead array (CBA) cytokine detection kits.

Results from Experiment 1 (2.8.18) suggested that 48-hour supernatant from DCs treated with 0.94 μM or 2.5 μM of DHEA-S had increased levels of anti-inflammatory IL-10 compared to vehicle treated and no-treatment controls, with 2.5 μM treatment inducing more IL-10. However, these data should be interpreted with caution as there were only two biological replicates and thus statistical tests could not be performed. Additionally, cell viability was not assessed and thus we cannot be sure if cytokine levels were induced by the metabolite or if this signal came from dying cells.

For Experiment 2 (3.17.18/4.5.18) we decided a challenge was necessary to prove that DHEA-S had a protective effect. Thus 24 hours after DCs were plated with DHEA-S we added heat killed cells or cell-free supernatant (CFS) from a species of *Candida* enriched in and isolated from the gut of high risk for asthma neonates.[6] IL-10, TNF, IL-6 and IL-13 concentrations were assessed in 24- and 48-hour supernatant, however, results were only obtained for TNF due to issues with the standards for IL-10 and IL-6. No IL-13 was detected in any samples and no TNF was detected in 24-hour

supernatant. After 48 hours DHEA-S treatment in the presence of *Candida* CFS did not lower the amount of TNF being secreted by DCs whereas in the presence of heat-killed Candida DHEA-S led to a slight decrease in secreted TNF. Again, these data should be interpreted with caution due to insufficient replicates for statistical analysis and due to cell viability not being assessed.

For the next experiment we again challenged the cells after a 24-hour incubation with DHEA-S, but challenged the cells with a mix of agonists for toll-like receptors (TLR) 1 through 9 to induce a more targeted inflammatory response through a known mechanism. In Experiment 3 (5.15.18), treatment with the TLR agonist mix after a 24-hour incubation led to massive cell death. In Experiment 4 (6.12.18), TLR agonist mix at concentrations of 1000 ng, 500 ng, 250 ng, 100 ng, and 10 ng were tested for impact on cell viability. Massive cell death was again observed with the 1000 ng TLR agonist mix, but all other concentrations did not impact live $CD45^+$ cell numbers as drastically (Figure 3.1). DHEA-S concentrations of 0.5, 1, 3, and 6 μM were also tested for impact on cell viability. Treatment with 6 μM of DHEA-S had the least number of live $CD45^+$ cells whereas 3 μM DHEA-S treatment showed the best cell viability (Figure 3.1).

In Experiment 4 we also assessed IL-10, TNF, IL-6, and IL-12p70 cytokine levels in 48-hour supernatant from TLR agonist mix treated DCs. IL-12p70 was not detected in any samples. 500 ng TLR agonist mix led to the least cell death, the highest TNF and IL-6 cytokine concentrations, and low IL-10 levels, second only to cells treated with 10 ng TLR agonist mix although these cells were not hugely viable. This suggested that 500 ng of TLR agonist mix may be the optimal concentration for future experiments. Although not statistically significant, we also observed that the concentrations of

inflammatory cytokines IL-6 and TNF were higher in untreated DCs compared to TLR agonist treated DCs (Figure 3.1). From this we hypothesized that two factors may be contributing to increased levels of these inflammatory cytokines in the media of untreated DCs: 1) the DCs may be stressed following resuscitation and isolation procedures and 2) we may be detecting TNF and IL-6 only because these cytokines are included in the maturation/activation cocktail.

Thus, in Experiment 5 (7.16.18) we waited 24 hours before treating isolated DCs with steroid to allow these potentially stressed cells to return to homeostasis. Additionally, we did not treat cells with the activation/maturation cocktail. No secretion of IL-10, TNF, or IL-6 were observed 6 or 24 hours after TLR agonist stimulation. This suggests that the TNF and IL-6 levels measured previously were due to the presence of these cytokines in the activation/maturation cocktail.

The culture conditions used above had worked for other lab members in the context of DC/T-cell co-culture experiments but results from my experiments suggested a different procedure would be required to assess the impact of DHEA-S on DCs alone. A paper published by Butts and colleagues in 2007 showed that lipopolysaccharide (LPS) activated, murine bone marrow-derived DCs treated with progesterone had suppressed production of pro-inflammatory cytokines including TNFα.[236] We thus attempted to use this experimental procedure to help determine appropriate conditions for our DHEA-S treatment experiments. Unfortunately, we were unable to replicate Butts *et al.* experiment using DCs isolated from human PBMCs and observed pronounced cell death in our experiments which appeared to be due to both the vehicle the progesterone was resuspended in (DMSO) and the LPS concentration used (5 ug/mL).

3.3.1.2 *Evaluating the Genetic Potential of Early-Life Gut Microbes to Produce Steroids*

In addition to determining the impact of DHEA-S on immune cell phenotypes, we also wanted to determine whether bacteria in the gut of low-risk for asthma infants had the genetic capacity to produce steroids. To address this, we carried out shotgun metagenomic sequencing and used the tool ShortBRED.[237] For ShortBRED, the user assembles a database of protein sequences of interest and then uses this database to probe the metagenomic data for sequence reads with homology. While gut bacteria have been shown to be capable of synthesizing steroids, as mentioned previously, little information exists on steroid-associated bacterial genes. Thus, instead of focusing on just DHEA we decided to widen our search to as many steroid-associated microbial genes as possible.

To create our ShortBRED database we: 1) looked up enzymes associated with steroid synthesis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Enzyme Commission (EC) number; 2) searched each enzyme name and all synonyms on the NCBI protein database; and 3) filtered results by taxon to separate human, fungal, and bacterial genes and then manually assessed the results to create a FASTA file of relevant, non-redundant microbial enzymes.

Probing of the TIPS/DIMES metagenomic dataset with this database yielded few homologous sequences. Two hypothetical proteins found in *E. coli* with >80% identity to CYP17A, a cytochrome P450 enzyme with 17,20-lyase activity that can convert 17α-hydroxypregnenlone (17-OH-Preg) into DHEA, were found in the dataset but were not statistically significantly different between high- and low-risk for asthma infants. Additionally, one *Salmonella* enzyme 20α-hydroxysteroid dehydrogenase, an

oxidoreductase involved in metabolism of 21 carbon progestagens, was also found in the dataset, but again was not statistically significant.

We hypothesized that there may be three reasons we were unable to get significant hits: 1) the metagenomic sequencing depth was, on average, about 10 million reads and these microbial steroid-associated genes may be rare enough that this depth was too shallow for us to observe them in our dataset, 2) we did not sequence enough samples to give us the power to achieve significance with the few genes we were able to pick up, and 3) the output of ShortBRED is only as good as the input database you supply and our input database mostly included microbial steroid-associated genes which are not well characterized.

3.3.1.3 *Evaluating the Metabolic Potential of Early-Life Gut Microbes to Synthesize DHEA from the Precursor 17α-Hydroxypregnenolone*

As we were unsuccessful using genetic approaches to identify bacteria and bacterial enzymes capable of synthesizing DHEA, we next turned to culturing techniques to try to isolate DHEA-synthesizing bacteria. 6-month stool samples from four healthy controls with high DHEA levels and four high-risk infants with low DHEA levels were used. For the first bacterial isolation, stool punches from these samples were used to inoculate filter sterilized single donor human breast milk purchased from Innovative Research. We purported that breast milk was a reasonable rich media that would emulate the nutrients these microbes would come in contact with in the 6-month-old gut. Stool punches were first cultured in nutrient-rich breast milk to help resuscitate bacteria and then transferred to minimal media containing the precursor to DHEA, 17-OH-Preg. In an alternative culturing technique, breast milk was supplemented with 17-OH-Preg in an attempt to enrich only for bacteria capable of utilizing or persisting in the

presence of this metabolite. We also cultured 6-month-old stool sample punches in a variety of rich medias including BHI, Nutrient Broth (NB), de Man, Rogosa and Sharpe (MRS) broth, Luria Broth (LB), and Tryptic Soy Broth (TSB) and then streaked these liquid cultures on agar plates made with the minimal media M9 salts and supplemented with 17-OH-Preg. These culturing approaches are summarized in Figure 3.2 and were carried out in both aerobic and anaerobic conditions.

From these isolation experiments we discovered that 17-OH-Preg was non-selective at the concentration used as over 100 bacterial isolates persisted. Some isolates underwent full length 16S rRNA sequencing and the majority of these were classified as *Klebsiella*, *Escherichia-Shigella*, and *Enterococcus*. Additionally, four *Bacillus*, two *Staphylococcus*, four unclassified bacteria, one *Enterobacter*, and one *Acinetobacter* were identified. It is important to note that bacterial survival does not necessarily mean that the steroid is utilized.

We next sought to test whether the bacteria we had isolated were capable of utilizing 17-OH-Preg. To do this glycerol stocks of isolates were plated on M9 minimal media supplemented with either 17-OH-Preg or vehicle (DMSO). After 24 hours, single colonies for each isolate were picked and cultured in liquid M9 media also supplemented with 17-OH-Preg. The liquid culture was incubated for 48 hours following which supernatant was collected. Isolates were cultured both aerobically and anaerobically. Supernatant from these cultures was then either used directly for a DHEA enzyme-linked immunosorbent assay (ELISA) or underwent an ethyl acetate lipid extraction. Unfortunately, when we included a standard curve of 17-OH-Preg we observed that the antibodies used in the DHEA ELISA showed cross-reactivity with this

precursor metabolite (Figure 3.3). Based on these results we determined this method could not be reliably used to measure 17-OH-Preg utilization.

An alternative approach for measuring metabolites is thin layer chromatography (TLC) which allows for the separation of a mixture of compounds based on their differences in solubility.[238] Two phases are required for this method, a stationary phase and a mobile phase. The stationary phase is a thin, uniform layer of silica gel coated onto aluminum or glass. The procedure for TLC is pictured in Figure 3.4A and the method for calculating the metabolite retention factor is pictured in Figure 3.4B. Additional information on this procedure can be found in the methods section.

To achieve the appropriate conditions for detecting DHEA and 17-OH-Preg we tested different mobile phases, different ratios of mobile phases, different silica plates, and multiple visualization reagents[239] (Figure 3.4C and D). The final conditions for DHEA and 17-OH-Preg detection were a silica gel 60 plate for the stationary phase, a mix of hexanol and ethyl acetate (80/20, vol/vol) for the mobile phase, and Lieberman-Burchard reagent[240] was sprayed onto the plate following heating of the plate to 110°C for visualization.

For TLC experiments we began by using whole stool samples, hypothesizing that we would be more likely to get a positive result with an intact community of microbes instead of individual isolates of which we had hundreds. 6-month-old infant stool samples with high DHEA levels were serially diluted in M9 minimal media and incubated for 24 hours under aerobic or anaerobic conditions in the presence of vehicle (DMSO) or 17-OH-Preg at a concentration of 300 μM – the concentration used when optimizing TLC conditions. Following this, supernatants were collected, extracted using an ethyl

acetate extraction procedure, and run on the TLC plate using the conditions mentioned previously. Stool samples in which 17-OH-Preg or DHEA was spiked in before lipid extraction were used as controls. The TLC showed that 17-OH-Preg and DHEA spiked into stool were visible amongst the fecal metabolic milieu (Figure 3.5). After a 24-hour incubation there was some visible 17-OH-Preg but no visible DHEA (Figure 3.5). Additionally, we observed a band of unknown lipids in all samples (Figure 3.5).

These results led us to hypothesize that the bacteria may be preferentially metabolizing other lipids present in the stool sample. Thus, we sought to determine the time point at which these unknown lipids were depleted as this may be the optimal time to treat samples with 17-OH-Preg. For this experiment, 10^{-1} dilutions of stool were incubated under anaerobic conditions and supernatant was collected after 24, 48, and 72 hours and on day 6 and day 7. Following lipid extraction, supernatants were run on a TLC gel using the methods previously described (Figure 3.6). Unknown lipid bands did not disappear even after 7 days of anaerobic incubation at 37°C thus suggesting microbes in the stool community were not using these metabolites as a nutrient source.

Next steps for these experiments would entail incubating stool samples with 17-OH-Preg for longer periods of time. For example. Bokkehnheuser *et al.* (1975) incubated their fresh, adult human stool samples with their steroids of interest for up to 25 days.[233] Freshness of stool samples and the kinds of microbes that can persist in frozen stool samples may also be impacting 17-OH-Preg utilization.

3.3.1.4 *17-OH-Preg Treatment Protects Against Allergic Airway Inflammation in Mice*

As the infant stool samples being used in this study were collected and frozen seven to 11 years ago (collection occurred 2008 – 2012) and had undergone several

freeze/thaw cycles we decided it would be useful to examine whether active gut microbes in a biological system were capable of synthesizing DHEA. We hypothesized that the gut microbiota would be able to synthesize DHEA from 17-OH-Preg and that this would confer protection against allergic airway inflammation in a murine model.

To test this hypothesis, we supplemented adult mice daily with 1 mg/mL (3000 uM) 17-OH-Preg administered via oral gavage. Mice were also sensitized intratracheally with house dust mite (HDM) allergen and subsequently challenged with HDM on day 7, 8, 9, 10, and 11 consistent with the well-established HDM model (Figure 3.7A). On day 14 mice were sacrificed and blood, spleen, mediastinal and mesenteric lymph nodes, lung, small intestine, cecum, and large intestine were collected. Three groups of mice were used: 1) control mice treated intratracheally with PBS (n = 5); 2) vehicle supplemented mice (0.5% DMSO in PBS) challenged with HDM (n = 5); and 3) 17-OH-Preg supplemented mice challenged with HDM (n = 5).

Flow cytometry was performed on single-cell suspensions from the lung, spleen, and lymph nodes and total IgE was measured in plasma. Our analysis showed that 17-OH-Preg supplementation suppressed hallmarks of allergic airway inflammation. Specifically, mice challenged with HDM and supplemented with 17-OH-Preg had fewer numbers of eosinophils in the lung and lower total IgE levels compared to non-supplemented HDM-challenged mice (Figure 3.7C). To assess whether 17-OH-Preg was converted to DHEA in the gut we used an ethyl acetate extraction method to extract steroids from Day 13 mouse stool and ran the samples under our optimized TLC conditions. Despite the high dose of 17-OH-Preg (3000 uM) used to treat the mice,

DHEA was not visible in the mouse stool extracts run on the TLC plate suggesting a need for a more sensitive method for measuring steroids (Figure 3.7D).

3.3.2 Early-Life Gut Microbiota Production of Amino Acids & Polyamines

As we were unable to identify a strong allergy-protective microbial metabolite candidate based on the statistically significantly enriched metabolites in healthy and *Lactobacillus* supplemented infants we decided to broaden our approach. To this end, we further interrogated the metagenomic data generated from high-risk for asthma stool samples from the TIPS cohort and healthy control infant stool samples from the DIMES cohort to determine gut microbiota functional features and associated metabolites that may relate to tolerogenic immune cell programming and protection against chronic inflammation.[8] We focused our efforts on 6-month old samples which exhibited the most significant metabolic and immunological differences between the healthy control and high risk groups.

3.3.2.1 *Shotgun Metagenomic Sequencing Corroborates 16S ribosomal RNA Gut Microbiota Structure*

Stool samples (n=25) collected in parallel under an identical protocol in the TIPS and DIMES cohorts underwent shotgun metagenomic sequencing. Initially the shotgun metagenomic data was compared to previously generated 16S rRNA sequencing data[8] from these fecal samples to confirm that bacterial distributions in the newly generated data were consistent with those previously observed using biomarker sequencing. Family level distributions across both datasets were highly comparable as determined by Procrustes analysis which indicated a significant ($p=0.001$) positive correlation ($M^2=0.7496$) between the two data matrices (Figure 3.8A). *Bifidobacteriaceae* represented the most dominant family in the infant feces examined, followed by

Enterobacteriaceae and *Bacteroidaceae* (Figure 3.8B). Differences in the relative abundance of *Verrucomicrobiaceae*, *Streptococcaceae*, *Prevotellaceae,* and *Ruminococcaceae* were observed across specific paired sample datasets. This is likely due to 16S V4 primer bias[241], lower community coverage in shotgun data, and the application of distinct methods of DNA extraction – column-based QIAamp DNA Stool Mini kit extraction for 16S rRNA samples and modified CTAB protocol[6] for metagenomic samples (though differences are minimized due to the use of mechanical disruption via bead-beating in both methods).

3.3.2.2 *Divergent Amino Acid Biosynthesis & Metabolism Differentiate HC & HRP Gut Microbiomes*

Differences in microbial functional pathways between HC and HRP infants were examined using comparative analysis of shotgun metagenomic data. Reads-based analysis indicated a number of microbial pathways enriched in HC compared with HRP infant gut microbiota including those involved in biosynthesis of tryptophan, ornithine and glutamine, several long chain fatty-acids, lipopolysaccharide, vitamin B6 (a critical co-enzyme for amino acid, glucose, and lipid metabolism), thiamine, and Enterobactin, a bacterial siderophore recently shown to promote host mitochondrial iron uptake[242] (Figure 3.9A). Conversely, the HRP infant gut microbiota exhibited distinct biosynthetic capacity, enriched for pathways involved in biosynthesis of glutamate, histidine and threonine, menaquinol (nitric oxide reductase), the immunostimulatory bacterial cell wall lipids peptidoglycan and mycolate, and secondary metabolites including aerobactin (a virulence factor[243]). Of note, HC and HRP infant gut microbiota exhibited opposing amino acid degradation pathways, with the HC microbiota encoding pathways for histidine I and glutamate metabolism (gamma-aminobutanoate [GABA]), while HRP

microbiota were primarily enriched for arginine and ornithine metabolism pathways, suggesting that these amino acids or their metabolic products may play key biological roles in influencing asthma development in childhood (Figure 3.9A). Amino acid biosynthesis is intimately linked to cellular energy production[244], while glycolysis, pentose phosphate and the tricarboxylic acid (TCA) cycle produce substrates necessary for amino acids production.[245] Notably, HC and HRP microbiomes exhibited distinct pathways for energy biogenesis, with HRP microbiomes being enriched for the TCA cycle, indicating enhanced capacity to produce threonine and glutamate. Conversely, HC infant gut microbiomes were enriched for the acetyl-CoA to butanoate pathway, which produces GABA, an inhibitory neurotransmitter recently shown to ameliorate the effect of pulmonary neuroendocrine and group 2 innate lymphoid cells in murine models of airway allergic asthma.[246]

To determine whether these differentially enriched microbial pathways related to metabolic differentials between HC and HRP infants, we compared previously generated untargeted metabolomic data from these samples and paired it with enzyme count data from the metagenomic analysis. Several metabolic products of microbial pathways found to differentiate HC and HRP infants were respectively enriched in these groups (Figure 3.9B). Specifically, HC infant microbiomes were enriched for ornithine, the tryptophan metabolite indole, and urocanate, a product of histidine metabolism. Ornithine has been shown to induce the immunosuppressive enzyme indoleamine 2,3-dioxygenase 1 (IDO1) in both gut epithelial cells, where it promotes increased production of the aryl hydrocarbon receptor (Ahr) ligand kynurenine[247], and in DCs, where it increases expression of *tgf-β1* and the capacity to induce regulatory T cells.[248]

L-tryptophan metabolites such as indole also function as Ahr ligands.[249] Not only is Ahr necessary for Treg generation and FoxP3 expression[139], but it is also important in the context of asthma as Ahr knock out mice exhibit increased airway inflammatory responses following ovalbumin challenge.[140] Previous studies have demonstrated decreased urocanate concentration in the urine of children with atopic asthma compared with healthy controls.[250] In a mouse model of atopic dermatitis, topical application of cis-urocanate led to skin lesion improvement and a reduction in mast cell infiltration and serum IgE levels[251], while in the presence of cis-urocanate, TCR stimulated $CD4^+$ T cells had increased IL-10 expression and secretion.[252]

The HRP group also exhibited enrichment of metabolites that corroborated their microbial gene pathway enrichments, including 5-aminolevulinate, a product of heme biosynthesis from glycine and agmatine, a metabolic product of L-arginine degradation (Figure 3.9B). Both 5-aminolevulinate and agmatine are upstream in their associated metabolic pathways, suggesting that the gut microbiota of high-risk for asthma infants has reduced capacity to produce downstream metabolites such as immunoregulatory polyamines or protoheme. This may substantially alter the early life intestinal microbial colonization landscape resulting in depletion of key early life species such as *Enterococcus faecalis* or other lactic acid producing bacteria which are sensitive to inflammatory conditions[253,254] and require exogenously supplied heme.[255,256]

To validate these findings, we re-examined the pathway data in the context of 12-month eczema outcomes, which can serve as a predictor of asthma in later childhood.[257,258] Infants who did not develop eczema were enriched for microbial L-tryptophan and putrescine biosynthesis pathways, the latter representing a precursor for

GABA and polyamines such as spermidine (Figure 3.10), which have been shown to promote Treg polarization.[259] We also present divergent pathway data for HRLGG compared to HRP infants in Figure 3.11. Taken together, these results suggest that fundamental differences in early life gut microbial innate immune stimulation, energy biogenesis, and immunomodulatory amino acid biosynthesis and degradation relate to risk of asthma and to eczema development in later childhood.

3.3.3 Impact of Polyamines & Amino Acids on Asthma-Associated Immune Cell Phenotypes

Previously we observed that, compared to HRP infants, fecal metabolites in 6-month-old HRLGG infant stool stimulated higher Treg numbers and enhanced IL-10 production. Importantly, L-ornithine – and its immediate downstream metabolite putrescine – were statistically significantly enriched in HRLGG infants suggesting these molecules may have played a role in the observed effect. Based on these previous findings and our observation in this study that both the biosynthetic pathway and the metabolite L-ornithine were significantly enriched in healthy controls we decided to investigate the impact of L-ornithine and putrescine on Treg induction and Th2 inhibition using multiple techniques.

3.3.3.1 *Treg Polarization Experiments*

Prior studies have shown polyamines can promote naive $CD4^+$ T cells to preferentially differentiate into Tregs *in vitro*[259] and are capable of inducing a regulatory environment in the gut marked by increased TGF-β[260] and FoxP3 expression.[259] Thus we decided to test whether the HRLGG-enriched polyamine putrescine is also capable of promoting enhanced Treg differentiation.

For our first experiment plate-bound anti-CD3 (1 μg/mL) and soluble anti-CD28 (2 μg/mL) were used to stimulate the T cell receptor (TCR)[261]; TGF-β (50 ng/mL) and IL-2 (10 ng/mL) were used to induce Treg polarization[261]; and 10, 30, and 50 μM concentrations of putrescine were tested to determine this polyamines impact on Treg polarization. For a comparison we also treated cells with putrescine under non-polarizing Th0 conditions (anti-CD3, anti-CD28, and IL-2). Under these conditions cell viability was extremely low and under Treg conditions (Gating Strategy Figure 3.12) the viability was somewhat higher but still variable (Figure 3.13A). Untreated naive $CD4^+$ T cells cultured under Treg conditions induced the strongest Treg polarization, however, none of the putrescine concentrations tested enhanced Treg polarization (Figure 3.13B).

Based on these results we purported that the high concentration of TGF-β used in our experiment may be overwhelming the cells and inducing such strong Treg polarization that it masks any potential effects of putrescine. Alternatively, if used at lower concentrations TGF-β may work in concert with putrescine to facilitate naive T cell differentiation into Tregs. Thus, we did a titration of TGF-β based on a previously published polyamine and TGF-β titration experiment.[259] Overall, naive T cells cultured with 0.2 ng/mL or 0.5 ng/mL TGF-β had extremely low viability (Figure 3.14A) and did not lead to Treg induction amongst viable cells (data not shown). Except for cells treated with 50 ng/mL TGF-β, viability of cells treated with 1 ng/mL TGF-β was higher than for all other groups (Figure 3.14A). However, treatment with 1 ng/mL TGF-β and 10 μM of putrescine did not enhance Treg differentiation *in vitro* (Figure 3.14B).

We next wanted to address why we were seeing such low cell viability. While low TGF-β concentrations may be playing a role, it was also possible that the media we were using was not providing cells with enough nutrients to survive the robust anti-CD3/anti-CD28 TCR stimulation. Initially we used TexMacs media for our experiments as it does not include serum which may contain circulating putrescine or other polyamines that could impact our results. On the other hand, serum can provide factors critical for cell survival. We thus decided to carry out a titration using higher TGF-β concentrations under both serum-free (TexMacs media) and serum-supplemented (R10 media) conditions. Generally, cells cultured with R10 media had increased cell viability across all TGF-β and putrescine or vehicle treatment conditions (Figure 3.15A). Furthermore, more live $CD4^+$ Tregs were observed for T cells cultured with R10 compared to TexMacs for all TGF-β concentrations (Figure 3.15B). However, the 50 ng/mL TGF-β control did not yield high Treg numbers necessitating repetition of the TGF-β titration experiment using the viability promoting R10 media only (Figure 3.14B).

In addition to repeating the TGF-β titration we also decided to include more than one putrescine treatment group and use a broader range of concentrations based on the lowest and highest concentrations found in the relevant literature.[262,263] Culturing cells using R10 media again resulted in better cell viability than in previous experiments with most treatment groups having 75% viability or higher (Figure 3.16A). The percentage of live $CD4^+CD3^+$ Tregs, however, varied greatly between donors. For Donor 34, the 50 ng/mL TGF-β treatment yielded a low percentage of Tregs (Figure 3.16B). Interestingly, cells cultured in 5 ng/mL TGF-β showed increased Treg differentiation for all putrescine concentrations tested compared to no treatment and

vehicle controls (Figure 3.16B). Additionally, cells cultured in 9 ng/mL TGF-β and 25 μM putrescine showed increased Treg differentiation compared to vehicle and no treatment controls (Figure 3.16B). Compared to Donor 34, cells from Donor 35 treated with 50 ng/mL TGF-β showed a more robust Treg induction. Donor 35's cells cultured in 1 ng/mL TGF-β also had an increased percentage of Tregs for all putrescine concentrations tested compared to vehicle-treated, untreated, and 50 ng/mL TGF-β treated controls (Figure 3.16B). Additionally, cells cultured in 5 ng/mL TGF-β and 25 or 250 μM putrescine showed increased Treg differentiation compared to vehicle-treated, untreated, and 50 ng/mL TGF-β treated controls (Figure 3.16B). Due to this variability between donors, it was difficult to discern the impact of putrescine treatment on Treg induction.

Based on these findings, we decided to compare Treg induction variability between all donors tested thus far. Figure 3.17 shows the percent of live $CD4^+CD25^+FoxP3^+$ cells from Donor 34 and Donor 35 in two independent experiments. In both experiments naive T cells were cultured in R10 media supplemented with anti-CD28 (2 μg/mL), IL-2 (10 ng/mL), and TGF-β (50 ng/mL) and in the presence of plate-bound anti-CD3 (1 μg/mL). In panel A it is clear that these conditions did not yield a high percentage of Tregs, however, in the independent experiment in panel B the same conditions led to a slightly lower percentage of Tregs in Donor 34 and a higher percentage of Tregs in Donor 35.

Variability in Treg induction was also observed in experiments using cells isolated from Donor 36 and Donor 37 (Figure 3.18). Naive T cells isolated from these donors were cultured under the same Treg polarization conditions mentioned above, but

in panel A these conditions induced higher numbers of Tregs than in the independent experiment shown in panel B. Additionally, for Donor 37, naive T cells treated under Treg polarizing conditions did not consistently induce more Tregs than cells cultured under the same conditions but without the addition of TGF-β (Figure 3.18B).

In some experiments cells were treated with phorbol 12-myristate 13 acetate (PMA), ionomycin, and golgi plug (Gplug) to stimulate overproduction of additional cytokines we planned to assess via flow cytometry and to block secretion of these cytokines from the cell. We considered that this treatment may be impacting the Treg phenotype, however, a comparison of PMA/Ionomycin/Gplug treated and untreated cells showed only minor variation in Treg percentages (Figure 3.18A). In Donor 36, PMA/Ionomycin/Gplug treatment led to a slight reduction in the percent of Tregs whereas in Donor 37 this treatment led to a slight increase in the percent of Tregs (Figure 3.18A). Taken together these data suggest that Treg polarization can be quite variable, with primary cells from some donors responding more strongly to these conditions than others. It will thus be important to account for this variability in future experiments.

3.3.3.2 *Th2 Polarization Experiments*

We next examined whether polyamines are capable of suppressing Th2 polarization (Gating strategy Figure 3.19). The initial Th2 polarizing conditions we tested were from a paper by Simpson *et al.* in which 12.5 ng/mL IL-4 was used to stimulate Th2 polarization and 10ng/mL IL-2 was used to support cell viability. Isolated naive $CD4^+$ T cells were cultured in the presence of plate bound anti-CD3 (1 µg/mL, clone HIT3a) and anti-CD28 (2 µg/mL, clone CD28.2) for three days to stimulate the TCR. After three

days the cells were transferred to a new culture plate with no anti-CD28 or anti-CD3 and allowed to rest for two days before antibody staining and flow cytometry was performed. Cells were also treated with several concentrations of putrescine and agmatine, a metabolite in the arginine degradation pathway found to be enriched in HRP infants. Cell viability for this experiment was low, around 50%, and, following PMA/Iono/Gplug treatment no IL-4 cytokine production was observed (Figure 3.20A).

In our second experiment we tested multiple concentrations of IL-4 and IL-2 based on Th2 polarization conditions found in the literature.[262–265] For this experiment we also used plate-bound anti-CD3 and soluble anti-CD28 and did not transfer cells to a new plate to allow a rest from TCR stimulation as we hypothesized this may have led to the lower cell viability we observed in our first experiment. Indeed, the cell viability for this experiment was better (~75% viability), however, very little IL-4 cytokine production was observed and was highly variable (Figure 3.20B). We also assessed IFNγ production and found that 5% to 10% of live $CD4^+CD3^+$ cells were producing this cytokine which is capable of interfering with Th2 polarization by promoting Th1 (Figure 3.20B).

Based on these results, for our next experiment we cultured cells in the presence of anti-IFNγ (5 μg/mL). As in our first experiment, we treated the cells with various concentrations of putrescine and followed the protocol from Simpson *et al.*, but used the recommended clones of anti-CD3 (clone 15E8) and anti-CD28 (clone OKT3) for the three-day TCR stimulation. Although we achieved decent cell viability (~75% or higher) and observed fewer $IFN\gamma^+$ cells (minimum 0.56% of live $CD3^+CD4^+$ cells, maximum of 5.02% of live $CD3^+CD4^+$ cells, average 2.2% of live $CD3^+CD4^+$ cells) we did not see induction of $CD3^+CD4^+IL\text{-}4^+$ Th2 cells (Figure 3.21A).

We next followed an alternative Th2 polarization protocol from the Ansel lab at UCSF. This protocol differed from the above procedure in two ways: 1) IL-2 was only added to media during the two-day rest period and 2) media was supplemented with HEPES Buffer, non-essential amino acids, and 2-mercaptoethanol to support cell differentiation. These conditions improved overall cell viability (>80%), but again there were few to no IL-4$^+$CD4$^+$ cells (Figure 3.21B).

3.3.3.3 *L-Ornithine & Cell Free Fecal Water Treatment of DCs & Co-Culture with Autologous T Cells*

As we did not achieve successful polarization conditions in our previous experiments, we decided to use a less targeted approach to test the impact of L-ornithine on naive CD4$^+$ T cell differentiation (Gating strategy Figure 3.22A). Previous research from the Lynch lab showed that adult blood derived DCs treated with cell-free fecal water (CFW) from high-risk for asthma neonates and co-cultured with autologous naive CD4$^+$ T cells led to Th2 differentiation.[6] We thus decided to use this established protocol to test whether L-ornithine treatment was capable of reducing the ability of CFW derived from high-risk for asthma infants to induce a Th2 phenotype.
Although we achieved decent cell viability (~75%) (Figure 3.22B), neither CFW derived from stool of two high-risk infants nor IL-4 treatment of DCs promoted CD4$^+$IL-4$^+$ T cells (Figure 3.22C, other data not shown). To ensure that our PMA/Iono/Gplug cytokine stimulation was working we also examined flow plots of the CD4$^+$ cell population. Compared to non-treated cells, cells treated with PMA/Iono/Gplug showed a shift in the CD4$^+$ population, a known characteristic of this treatment which suggests it is working as expected and is not the cause for the lack of IL-4$^+$ cells (Figure 3.22D).

3.4 FIGURES

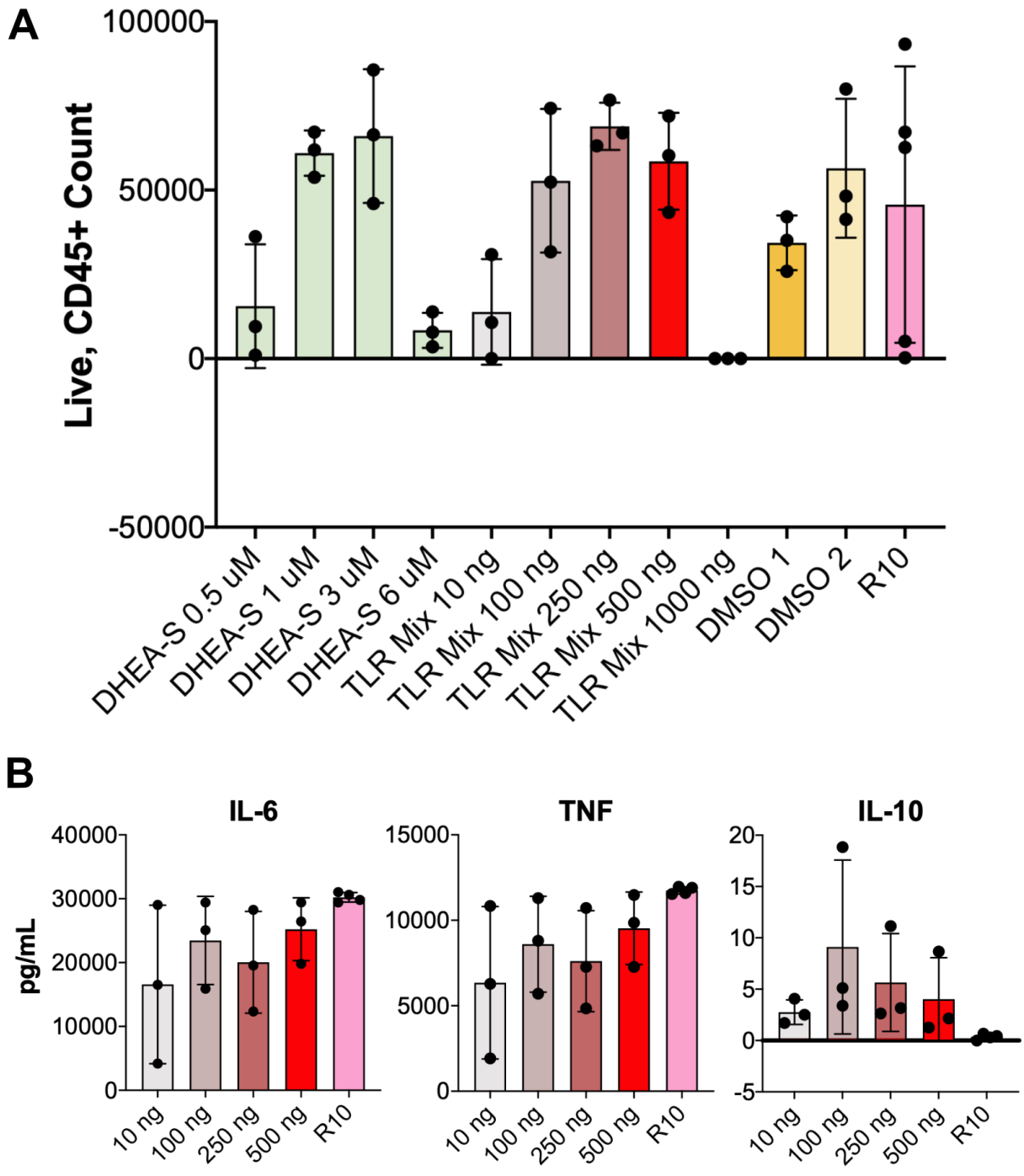


Figure 3.1 DCs treated with titrations of DHEA-S and TLR agonist mix. A) Viability of CD45^{+} cells under each treatment condition. B) Concentration of secreted cytokines following treatment with different concentrations of TLR agonist mix. In both A) and B) each dot represents a treated well.

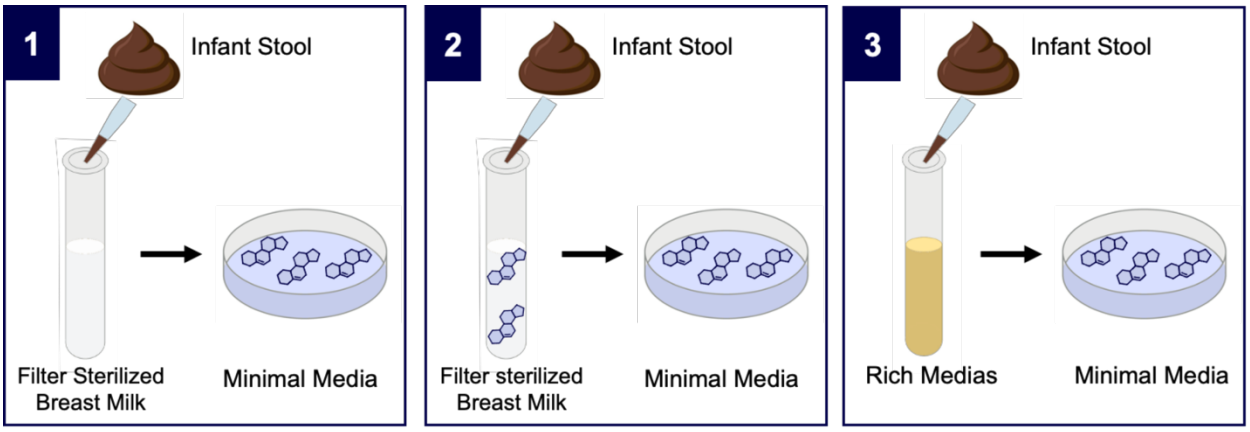


Figure 3.2 Culturing techniques used to isolate DHEA-producing bacteria from 6-month-old infant stool samples. Chemical structure represents addition of steroid to media.

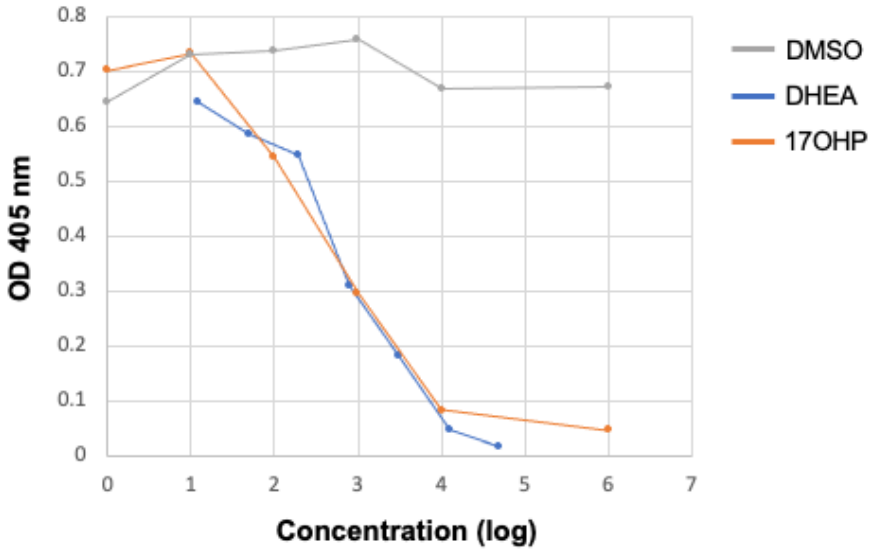


Figure 3.3 DHEA ELISA antibodies show cross reactivity with 17-OH-Preg. The optical density (OD) of six 17-OH-Preg (17-OH-P) concentrations (max 1,000,000 pg/mL, min 1 pg/mL) and seven DHEA concentrations (max 50,000 pg/mL, min 12.21 pg/mL) were measured. For the majority of concentrations tested, the OD value was highly similar between the two steroids indicating that this DHEA ELISA kit is also capable of measuring 17-OH-P.

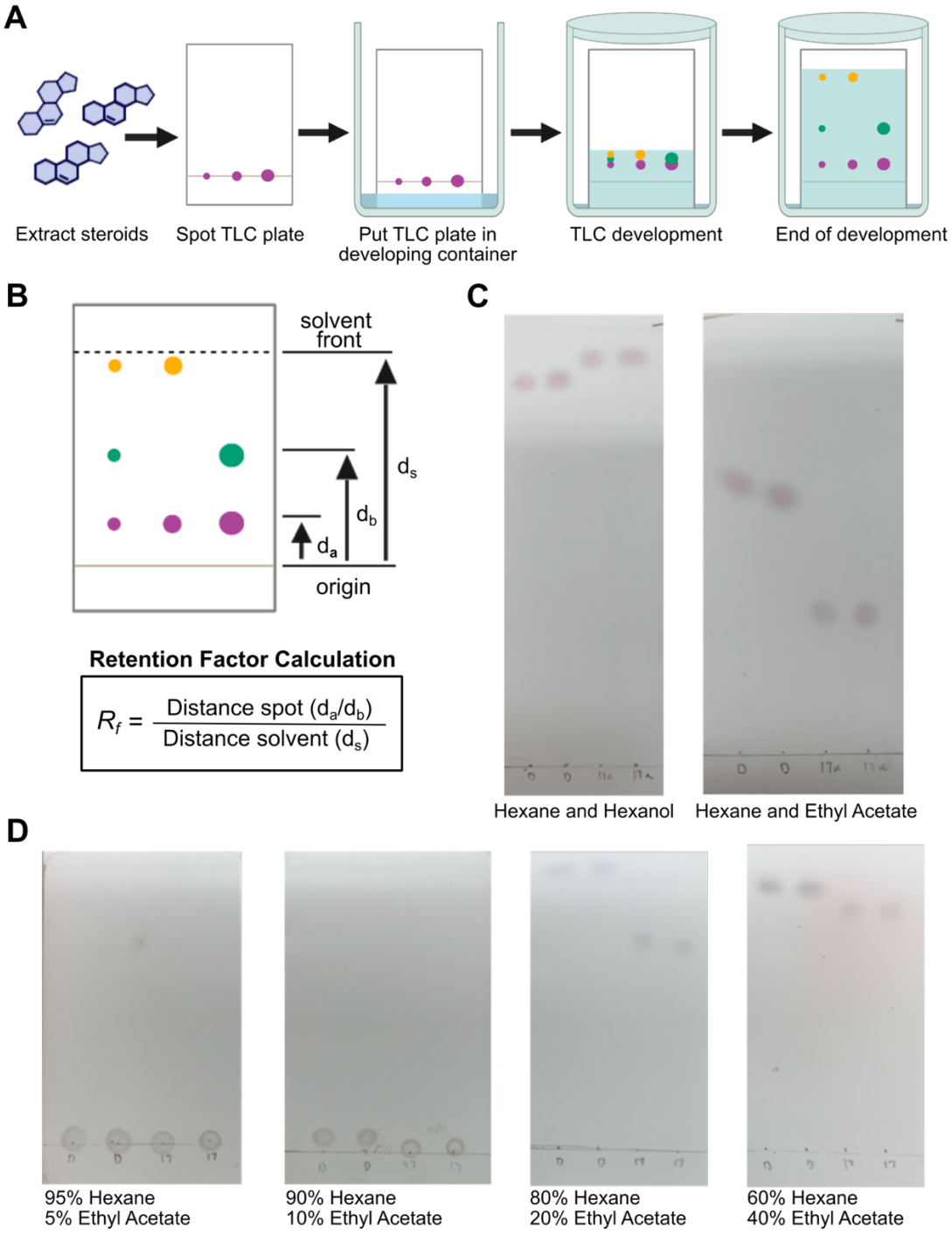


Figure 3.4 TLC procedure and condition testing. A) The TLC procedure. B) Retention factor calculation. C) Results of running a pure 17-OH-Preg (17α) standard and a pure DHEA (D) standard on silica 60 gel plates using different mobile phases: hexane and hexanol for the gel on the left and hexane and ethyl acetate for the gel on the right. Hexane and Ethyl acetate led to the clearest separation. D) Results of running a pure 17-OH-Preg (17) standard and a pure DHEA (D) standard on silica 60 gel plates using different concentrations of hexane and ethyl acetate. A solution of 80% hexane and 20% ethyl acetate led to the clearest separation between the two steroids.

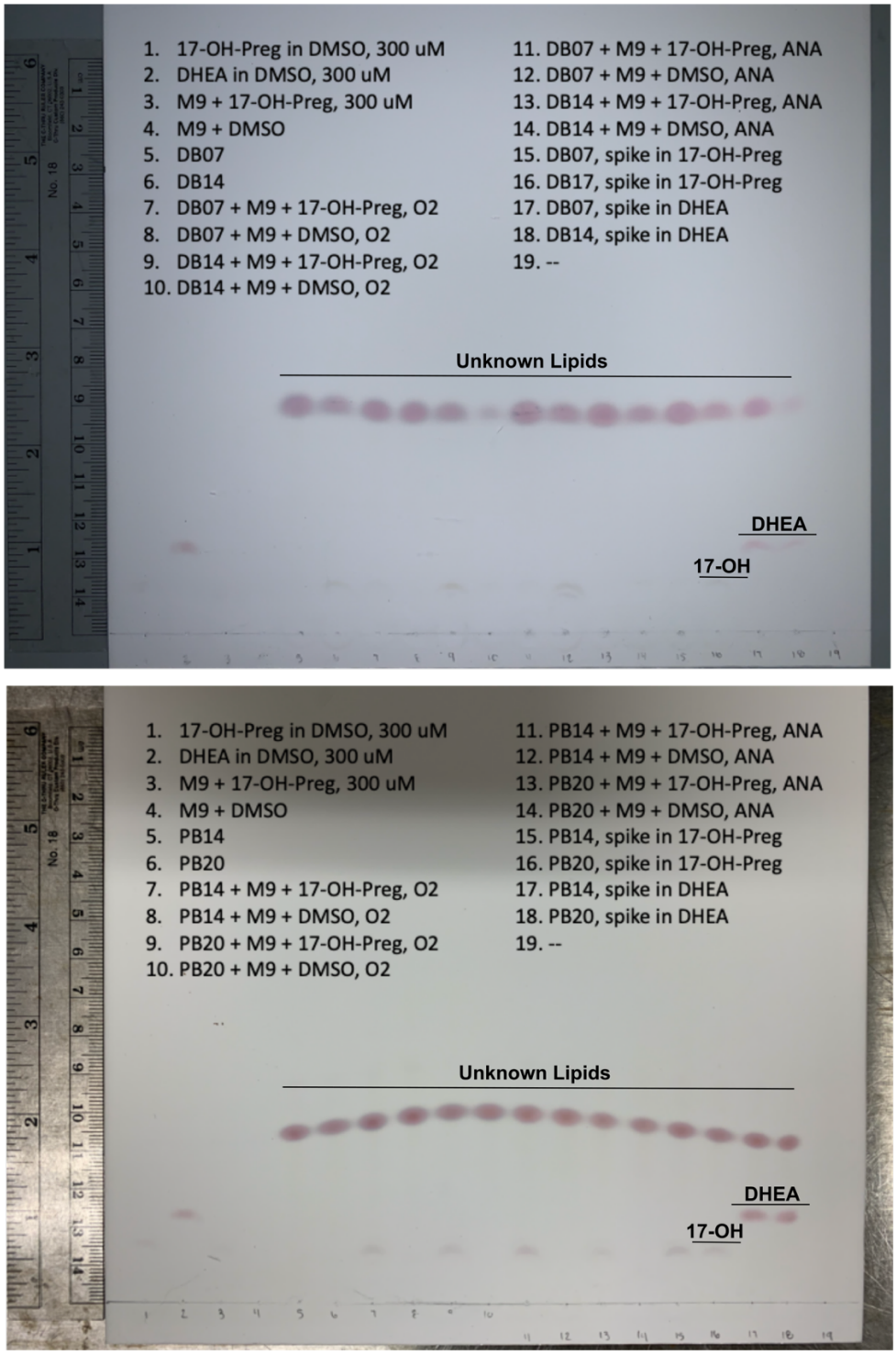


Figure 3.5 TLC results following 6-month-old stool sample culture under various conditions. "DB##" are healthy 6-month-old stool samples from the DIMES cohort and "PB##" are high-risk for asthma 6-month-old stool samples from the TIPS cohorts. All samples were cultured in M9 minimal media and cultured in the presence of either 17-OH-Preg or DMSO (vehicle). O2 denotes aerobic conditions and ANA denotes anaerobic conditions.

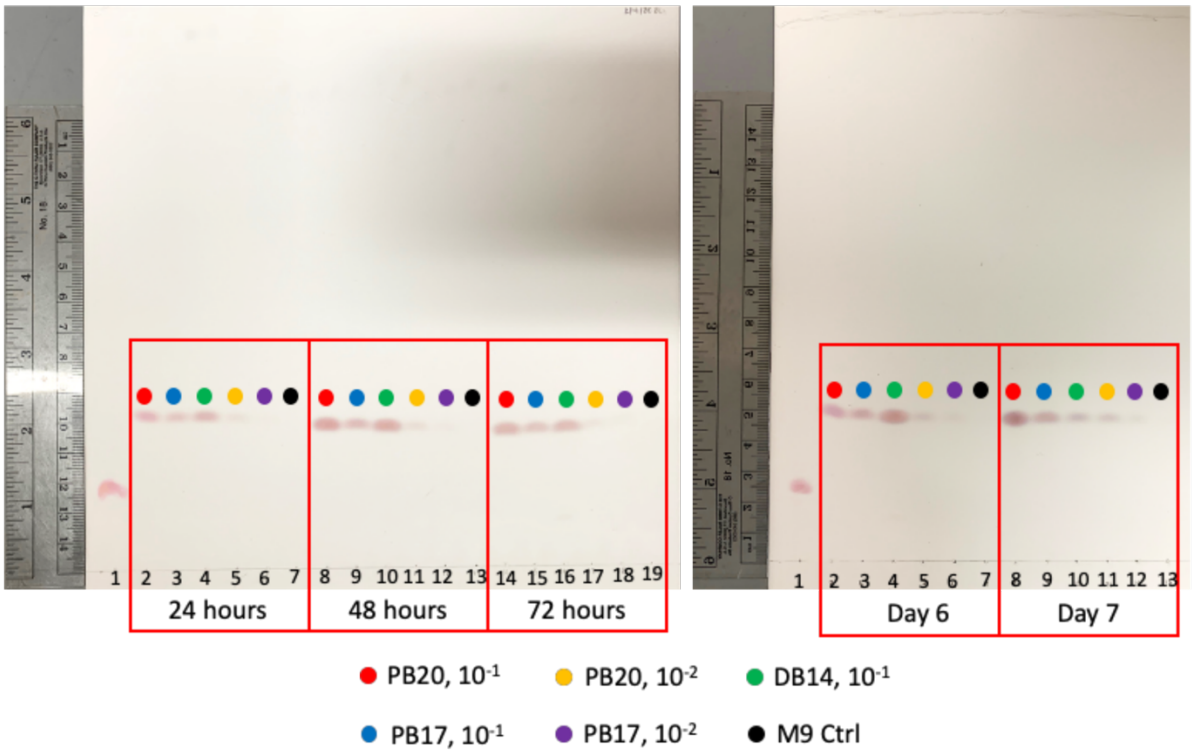


Figure 3.6 TLC results of supernatant collected from stool incubations across multiple timepoints. A DHEA standard was run in lane one. All other bands represent unknown lipids in the sample which did not disappear after 24, 48, or 72 hours or 6 or 7 days. “DB##” are healthy 6-month-old stool samples from the DIMES cohort and “PB##” are high-risk for asthma 6-month-old stool samples from the TIPS cohorts.

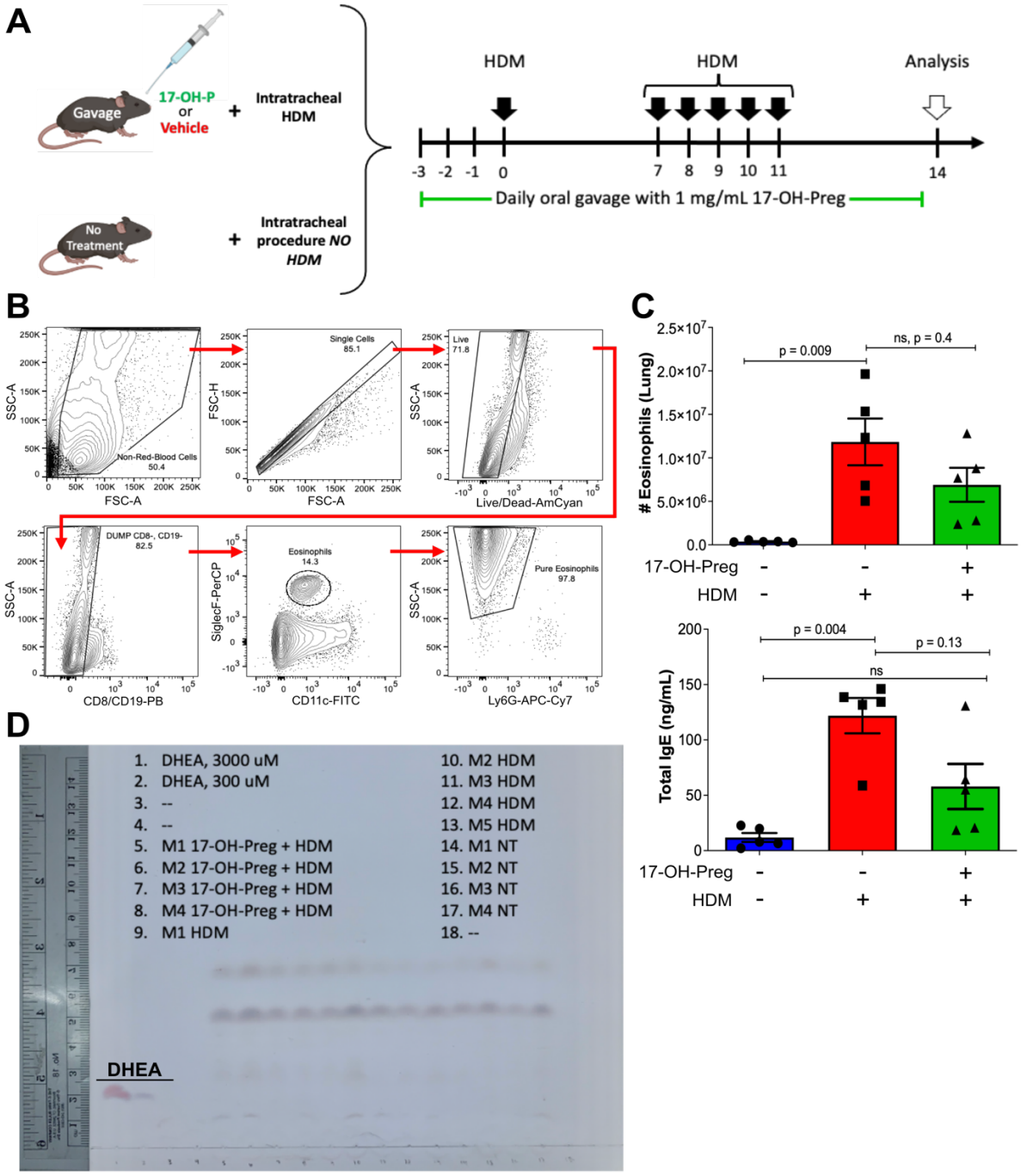


Figure 3.7 Oral supplementation with 17-OH-Preg reduces hallmarks of allergic airway inflammation in a murine model. A) Treatment groups and house dust mite (HDM)/17-OH-Preg treatment timeline. B) Gating strategy for eosinophils was defined as follows: live, $CD8^-/CD19^-$, $SiglecF^+/CD11c^-$, $SSC\text{-}A^{hi}/Ly6G^-$. C) Comparison of eosinophil numbers in the mouse lung and of total IgE levels in mouse plasma. Group differences for both were tested by Kruskal-Wallis. Benjamini-Hochberg adjusted p-values obtained from Dunn's multiple comparison test *post hoc*. (n = 5 for each group). D) TLC of mouse stool samples following ethyl acetate steroid extraction.

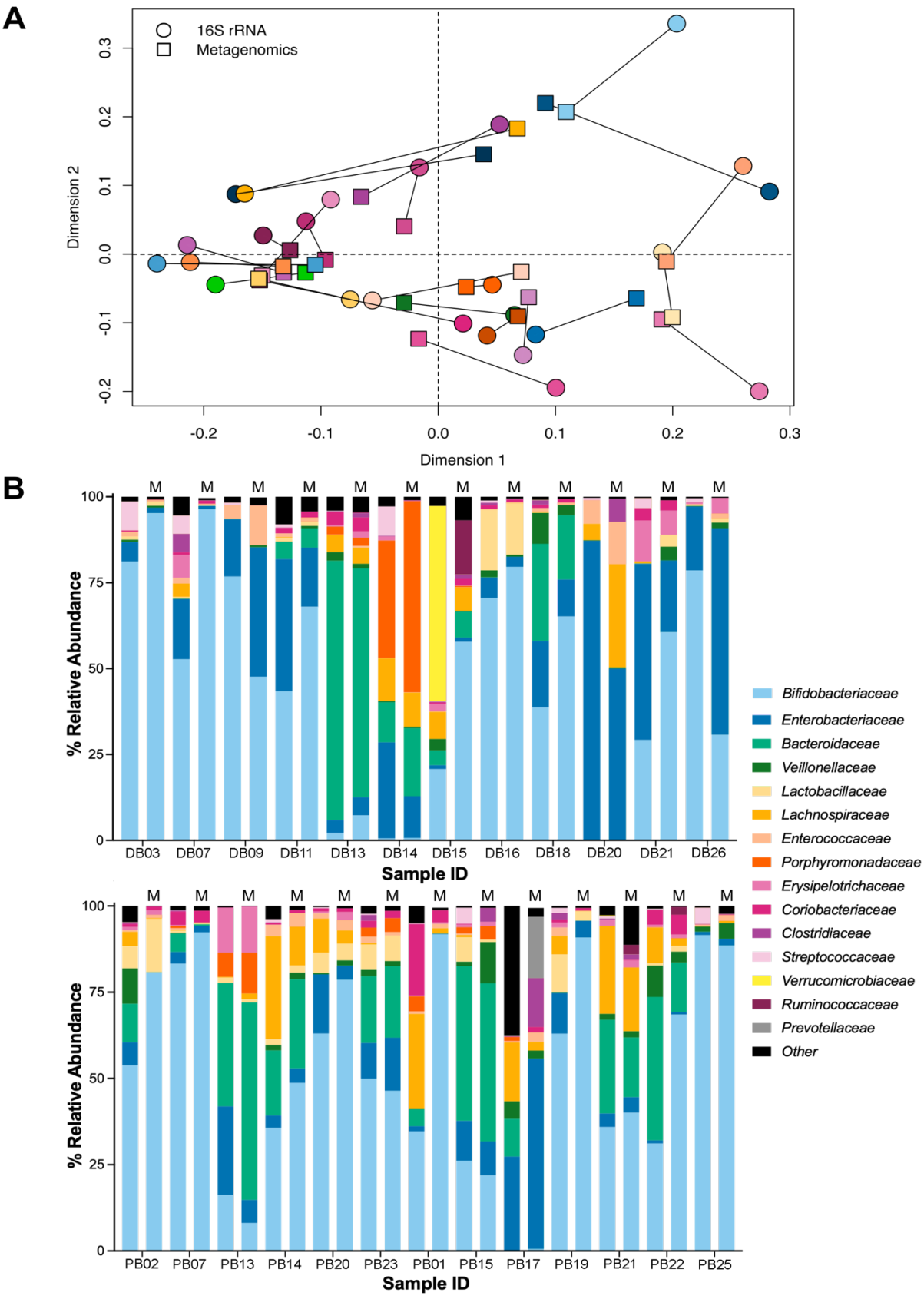

A
16S rRNA
Metagenomics
Dimension 2
Dimension 1
B
% Relative Abundance
Sample ID
DB03 DB07 DB09 DB11 DB13 DB14 DB15 DB16 DB18 DB20 DB21 DB26
PB02 PB07 PB13 PB14 PB20 PB23 PB01 PB15 PB17 PB19 PB21 PB22 PB25
Bifidobacteriaceae
Enterobacteriaceae
Bacteroidaceae
Veillonellaceae
Lactobacillaceae
Lachnospiraceae
Enterococcaceae
Porphyromonadaceae
Erysipelotrichaceae
Coriobacteriaceae
Clostridiaceae
Streptococcaceae
Verrucomicrobiaceae
Ruminococcaceae
Prevotellaceae
Other

Figure 3.8 Comparison of family level infant gut microbiota structure from shotgun metagenomic and 16S rRNA sequencing. A) Procrustes plot combining coordinate matrices generated from bacterial family profiles. Each color represents one patient's gut bacterial family profile and 16S profiles (circles) and metagenomic profiles (squares) for the same individual are connected with a line. The closer the square and circle are to each other the more similar the 16S and metagenomic profiles are for that individual. Protest permutational test of non-randomness $p = 0.001$, $M^2 = 0.7496$. ($n = 25$) B) Stacked bar plot showing family level relative abundance for each infant. 16S relative abundance is shown on the left side of the individual Sample ID and metagenomic (M) relative abundance is shown on the right. Colors represent different bacterial families.

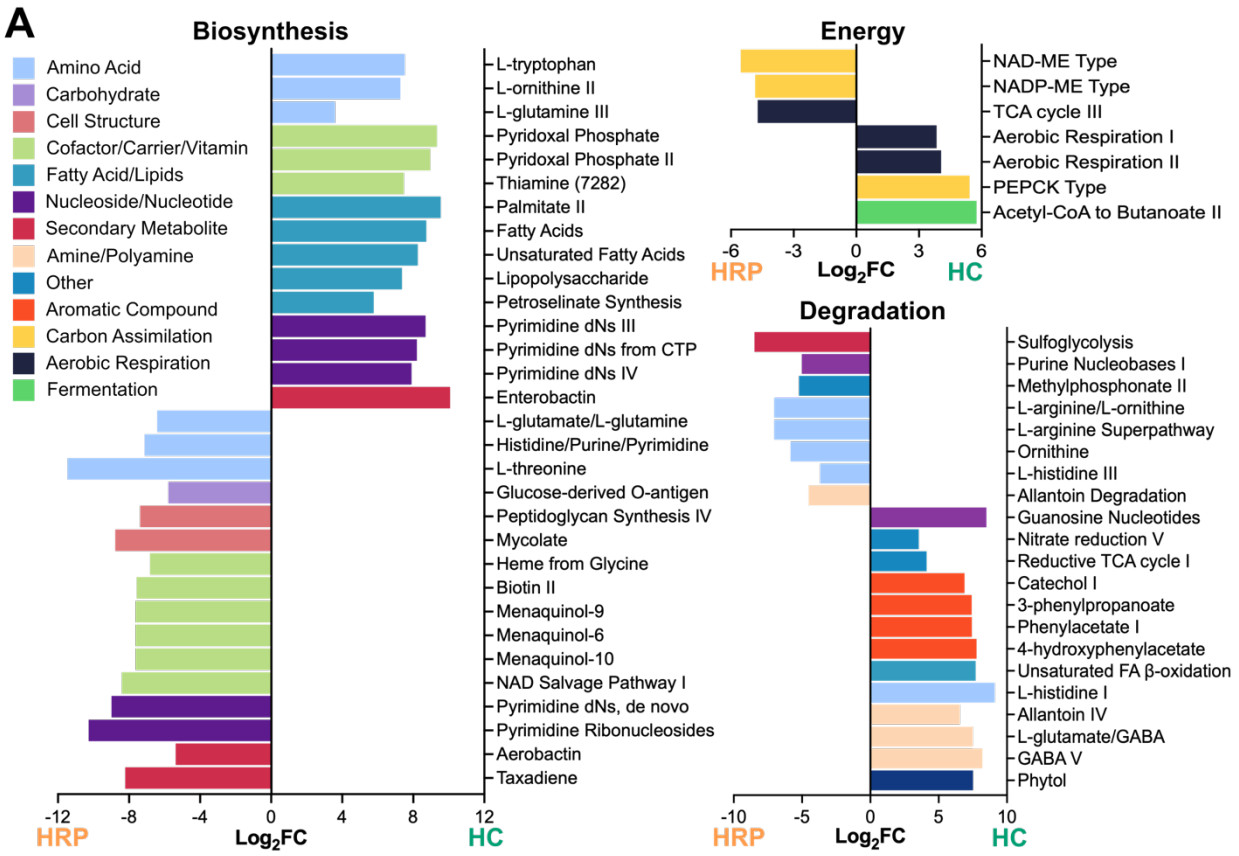

A
Biosynthesis
Amino Acid
Carbohydrate
Cell Structure
Cofactor/Carrier/Vitamin
Fatty Acid/Lipids
Nucleoside/Nucleotide
Secondary Metabolite
Amine/Polyamine
Other
Aromatic Compound
Carbon Assimilation
Aerobic Respiration
Fermentation
L-tryptophan
L-ornithine II
L-glutamine III
Pyridoxal Phosphate
Pyridoxal Phosphate II
Thiamine (7282)
Palmitate II
Fatty Acids
Unsaturated Fatty Acids
Lipopolysaccharide
Petroselinate Synthesis
Pyrimidine dNs III
Pyrimidine dNs from CTP
Pyrimidine dNs IV
Enterobactin
L-glutamate/L-glutamine
Histidine/Purine/Pyrimidine
L-threonine
Glucose-derived O-antigen
Peptidoglycan Synthesis IV
Mycolate
Heme from Glycine
Biotin II
Menaquinol-9
Menaquinol-6
Menaquinol-10
NAD Salvage Pathway I
Pyrimidine dNs, de novo
Pyrimidine Ribonucleosides
Aerobactin
Taxadiene
-12 -8 -4 0 4 8 12
HRP
Log2FC
HC
Energy
NAD-ME Type
NADP-ME Type
TCA cycle III
Aerobic Respiration I
Aerobic Respiration II
PEPCK Type
Acetyl-CoA to Butanoate II
-6 -3 0 3 6
HRP
Log2FC
HC
Degradation
Sulfoglycolysis
Purine Nucleobases I
Methylphosphonate II
L-arginine/L-ornithine
L-arginine Superpathway
Ornithine
L-histidine III
Allantoin Degradation
Guanosine Nucleotides
Nitrate reduction V
Reductive TCA cycle I
Catechol I
3-phenylpropanoate
Phenylacetate I
4-hydroxyphenylacetate
Unsaturated FA β-oxidation
L-histidine I
Allantoin IV
L-glutamate/GABA
GABA V
Phytol
-10 -5 0 5 10
HRP
Log2FC
HC
B
Key
Metabolite
Enzyme
Not enriched/detected
Enriched in HC
Enriched in HRP
L-ornithine synthesis II
2-oxoglutarate
glu
γ-L-glutamyl 5-phosphate
L-glutamate-5-semialdehyde
L-ornithine
L-arginine/L-ornithine degradation
arg
agmatine
putrescine
spermidine
γ-glutamyl-L-putrescine
4-(γ-L-glutamylamino) butanal
4-(γ-L-glutamylamino) butanoate
4-aminobutanal
1-pyrroline
4-aminobutanoate
succinate semialdehyde
succinate
Heme b synthesis
glycine
5-amino-levulinate
porpho-bilinogen
preuropor-phyrinogen
uropor-phyrinogen III
copropor-phyrinogen III
protopor-phyrinogen IX
proto-porphyrin IX
protoheme
L-histidine degradation III
histidine
urocanate
4-imidazolone-5-propanoate
N-formimino-L-glutamate
L-histidine degradation I
glutamate
a 5-formimino-tetrahydrofolate
a 5,10-methenyltetrahydrofolate
an N10-formyltetrahydrofolate
L-tryptophan synthesis
D-erythrose 4-phosphate
phosphoenolpyruvate
shikimate
chorismate
glutamine
anthranilate
indole
serine
tryptophan

Figure 3.9 Distinct microbial amino acid and metabolic pathways differentiate HC and HRP infants. A) Bar plots of biosynthesis, energy, and degradation-related functional pathways distinguishing HC (n = 12) from HRP (n = 7) microbiomes (log2FC > |1|; $p < 0.05$; pathways present in >1/3 of samples in each group). Negative binomial regression (MaAsLin2 and a three-model analysis) was used to test differences in normalized abundance (nCopies Per Million) between groups. B) Significant microbial pathways overlayed with log2FC data for enzymes and statistically significant metabolites (Welch's Test, $p<0.1$) that differentiate HC and HRP microbiomes.

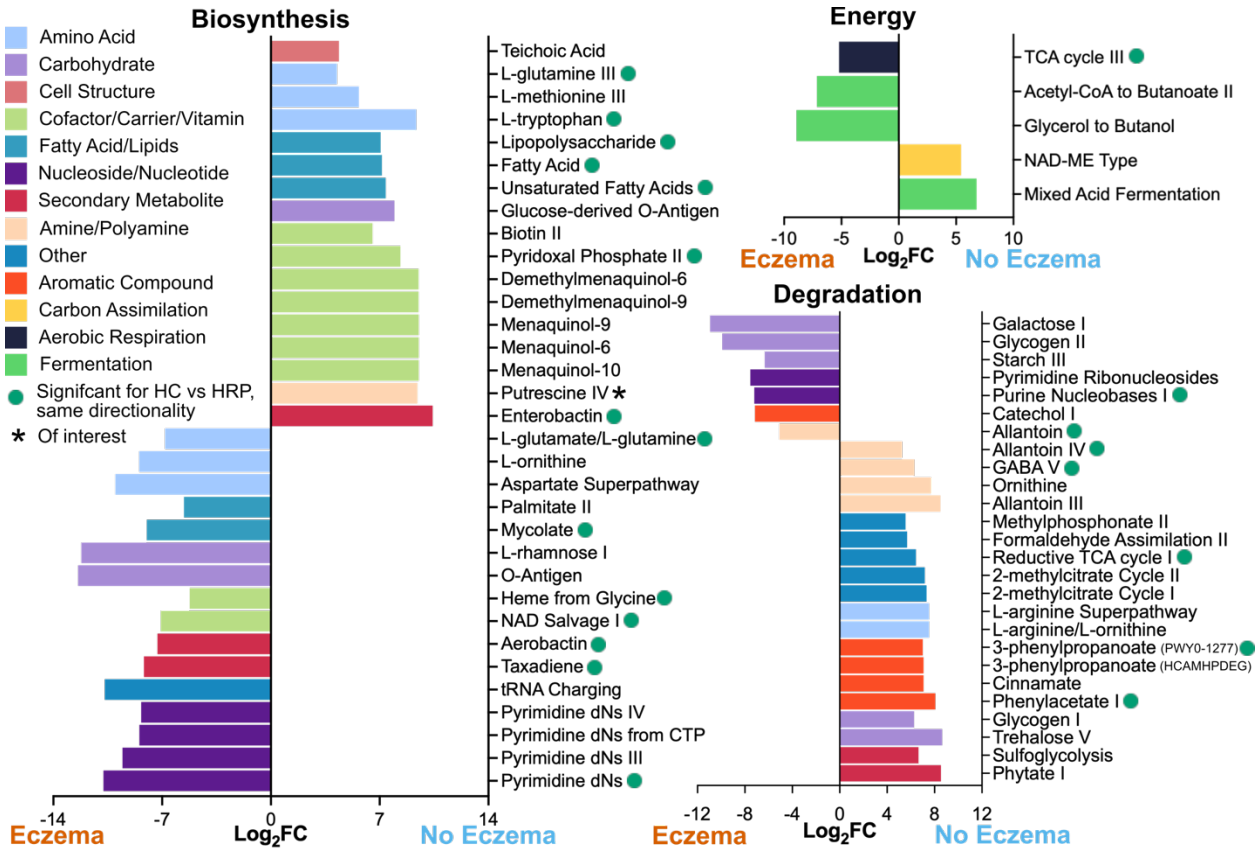


Figure 3.10 Distinct microbial amino acid and metabolic pathways also differentiate healthy infants from infants who developed eczema at 12 months. Bar plots of biosynthesis, energy, and degradation-related functional pathways distinguishing no eczema (n = 19) from eczema (n = 5) microbiomes (log2FC > |1|; $p < 0.05$; pathways present in >1/3 of samples in each group). Negative binomial regression (MaAsLin2 and a three-model analysis) was used to test differences in normalized abundance (nCopies Per Million) between groups.

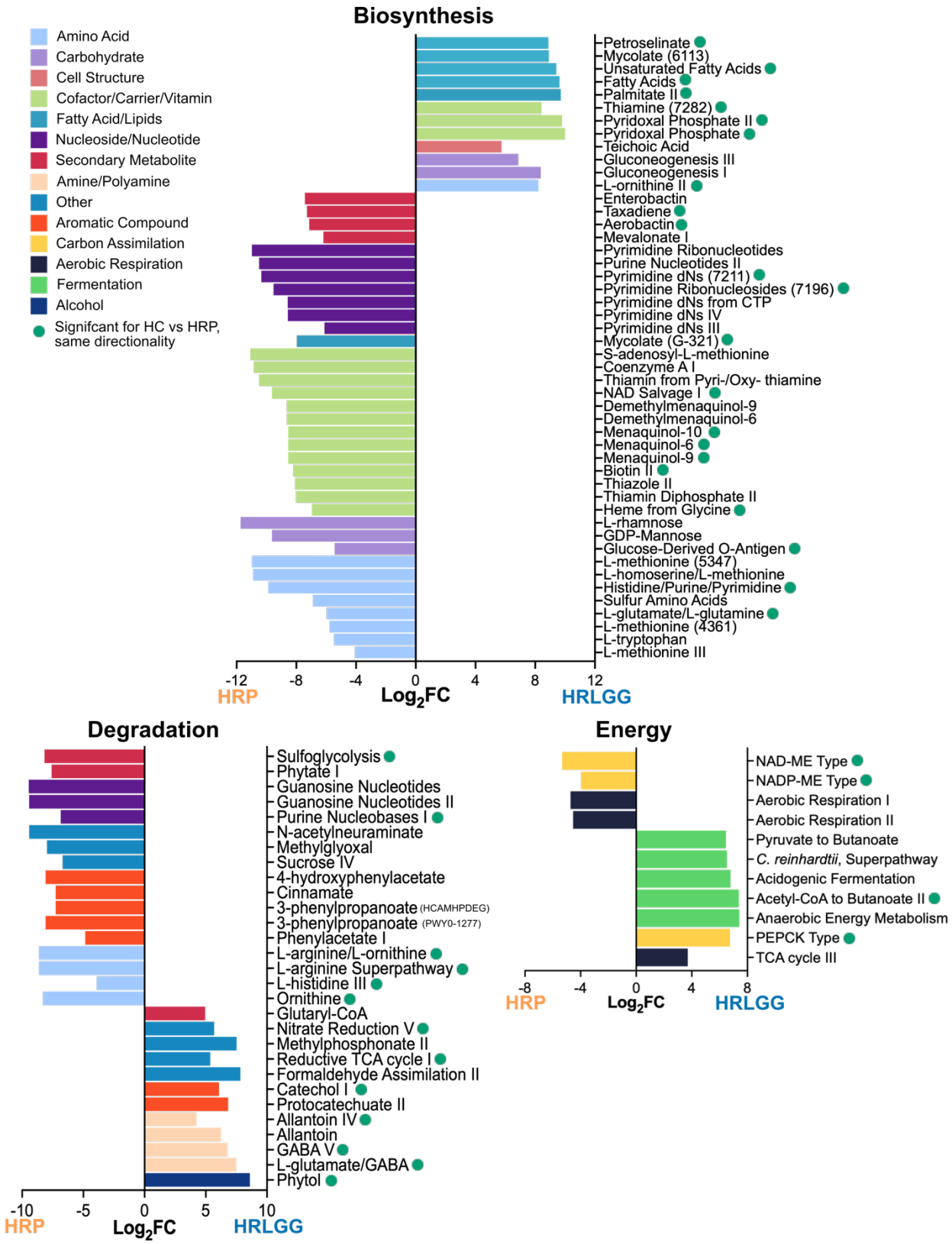

Biosynthesis
Amino Acid
Carbohydrate
Cell Structure
Cofactor/Carrier/Vitamin
Fatty Acid/Lipids
Nucleoside/Nucleotide
Secondary Metabolite
Amine/Polyamine
Other
Aromatic Compound
Carbon Assimilation
Aerobic Respiration
Fermentation
Alcohol
Signifcant for HC vs HRP, same directionality
Petroselinate
Mycolate (6113)
Unsaturated Fatty Acids
Fatty Acids
Palmitate II
Thiamine (7282)
Pyridoxal Phosphate II
Pyridoxal Phosphate
Teichoic Acid
Gluconeogenesis III
Gluconeogenesis I
L-ornithine II
Enterobactin
Taxadiene
Aerobactin
Mevalonate I
Pyrimidine Ribonucleotides
Purine Nucleotides II
Pyrimidine dNs (7211)
Pyrimidine Ribonucleosides (7196)
Pyrimidine dNs from CTP
Pyrimidine dNs IV
Pyrimidine dNs III
Mycolate (G-321)
S-adenosyl-L-methionine
Coenzyme A I
Thiamin from Pyri-/Oxy- thiamine
NAD Salvage I
Demethylmenaquinol-9
Demethylmenaquinol-6
Menaquinol-10
Menaquinol-6
Menaquinol-9
Biotin II
Thiazole II
Thiamin Diphosphate II
Heme from Glycine
L-rhamnose
GDP-Mannose
Glucose-Derived O-Antigen
L-methionine (5347)
L-homoserine/L-methionine
Histidine/Purine/Pyrimidine
Sulfur Amino Acids
L-glutamate/L-glutamine
L-methionine (4361)
L-tryptophan
L-methionine III
-12 -8 -4 0 4 8 12
HRP
Log2FC
HRLGG
Degradation
Sulfoglycolysis
Phytate I
Guanosine Nucleotides
Guanosine Nucleotides II
Purine Nucleobases I
N-acetylneuraminate
Methylglyoxal
Sucrose IV
4-hydroxyphenylacetate
Cinnamate
3-phenylpropanoate (HCAMHPDEG)
3-phenylpropanoate (PWY0-1277)
Phenylacetate I
L-arginine/L-ornithine
L-arginine Superpathway
L-histidine III
Ornithine
Glutaryl-CoA
Nitrate Reduction V
Methylphosphonate II
Reductive TCA cycle I
Formaldehyde Assimilation II
Catechol I
Protocatechuate II
Allantoin IV
Allantoin
GABA V
L-glutamate/GABA
Phytol
-10 -5 0 5 10
HRP
Log2FC
HRLGG
Energy
NAD-ME Type
NADP-ME Type
Aerobic Respiration I
Aerobic Respiration II
Pyruvate to Butanoate
C. reinhardtii, Superpathway
Acidogenic Fermentation
Acetyl-CoA to Butanoate II
Anaerobic Energy Metabolism
PEPCK Type
TCA cycle III
-8 -4 0 4 8
HRP
Log2FC
HRLGG

Figure 3.11 Distinct microbial metabolic pathways also differentiate HRLGG and HRP infants. Bar plots of biosynthesis, energy, and degradation-related functional pathways distinguishing no HRLGG (n = 6) from HRP (n = 7) microbiomes (log2FC > |1|; $p < 0.05$; pathways present in >1/3 of samples in each group). Negative binomial regression (MaAsLin2 and a three-model analysis) was used to test differences in normalized abundance (nCopies Per Million) between groups.

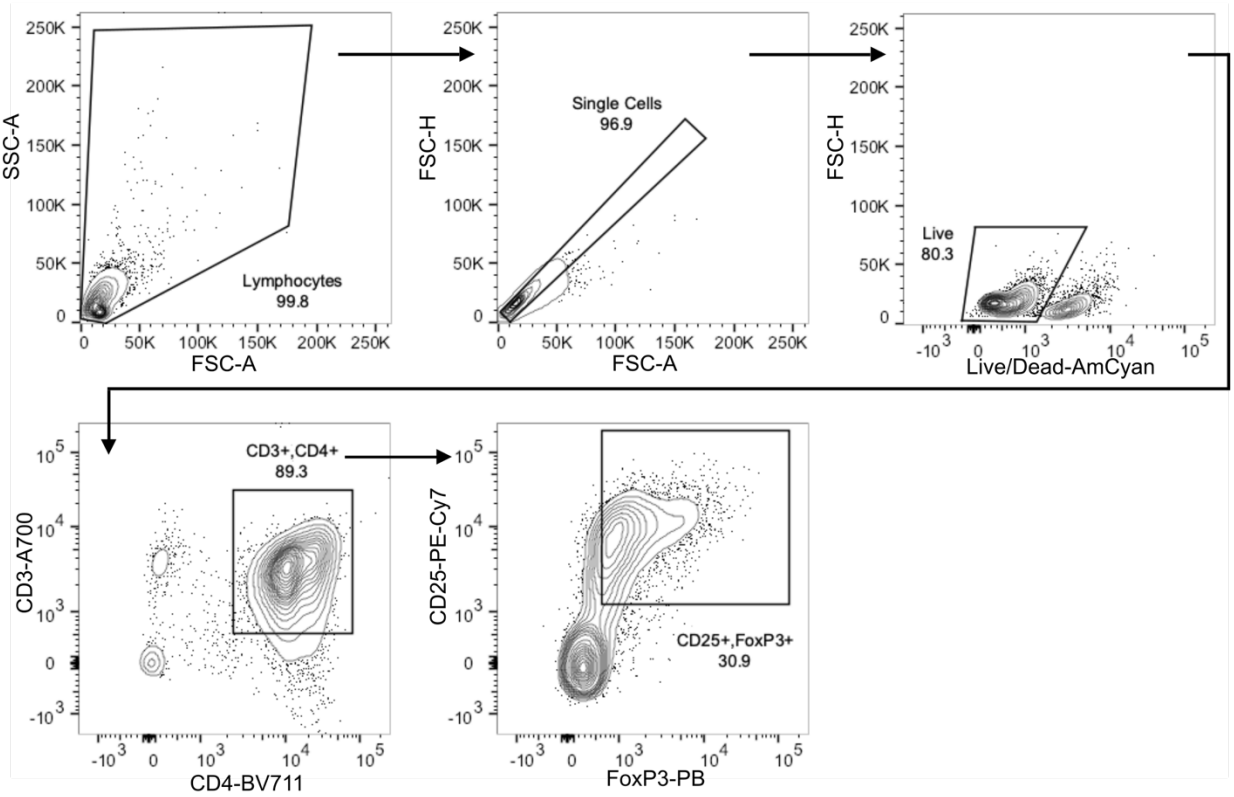


Figure 3.12 Treg Cell Gating Strategy. After 5 days of culture T cell phenotype was assessed via flow cytometry. A large lymphocyte gate was drawn following which single cells and live cells were gated. T regulatory cells were defined as $CD3^{+}CD4^{+}CD25^{+}FoxP3^{+}$ cells.

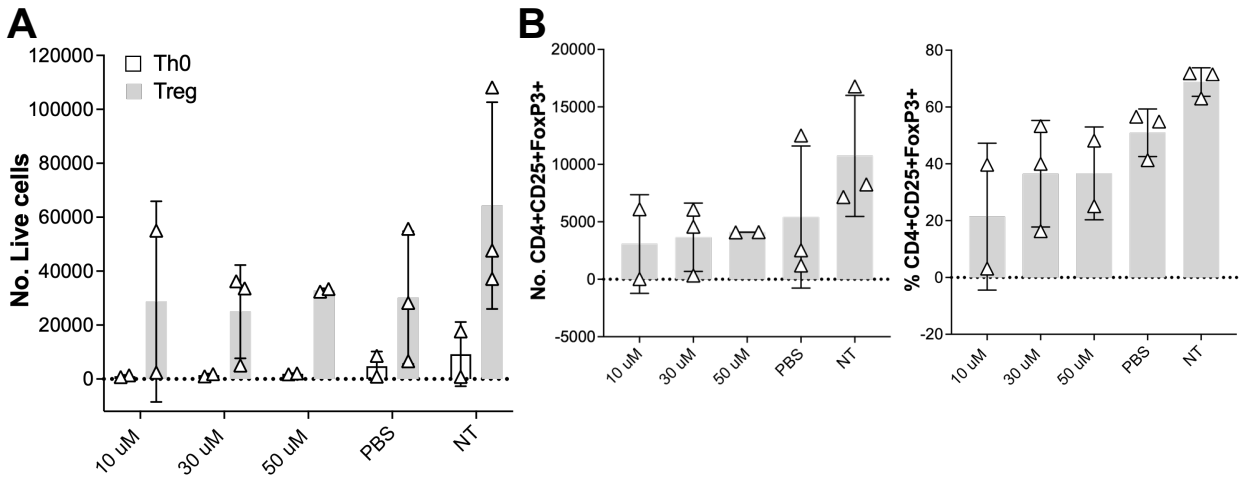


Figure 3.13 Testing the effect of putrescine treatment under strong Treg polarization conditions. A) Bar graph showing the number of live cells under Th0 conditions (1 µg/mL plate-bound anti-CD3, 2 µg/mL soluble anti-CD28, and 10 ng/mL IL-2) and Treg conditions (1 µg/mL plate-bound anti-CD3, 2 µg/mL soluble anti-CD28, 10 ng/mL IL-2, and 50 ng/mL TGF-β). B) Bar graphs showing percent of live, $CD4^+$ cells and number of live, $CD4^+$ cells that are $CD25^+FoxP3^+$. For all plots each triangle represents a single donor and standard deviation is shown. Bars are colored based on polarization conditions used. No statistics were performed due to insufficient numbers for statistical analysis.

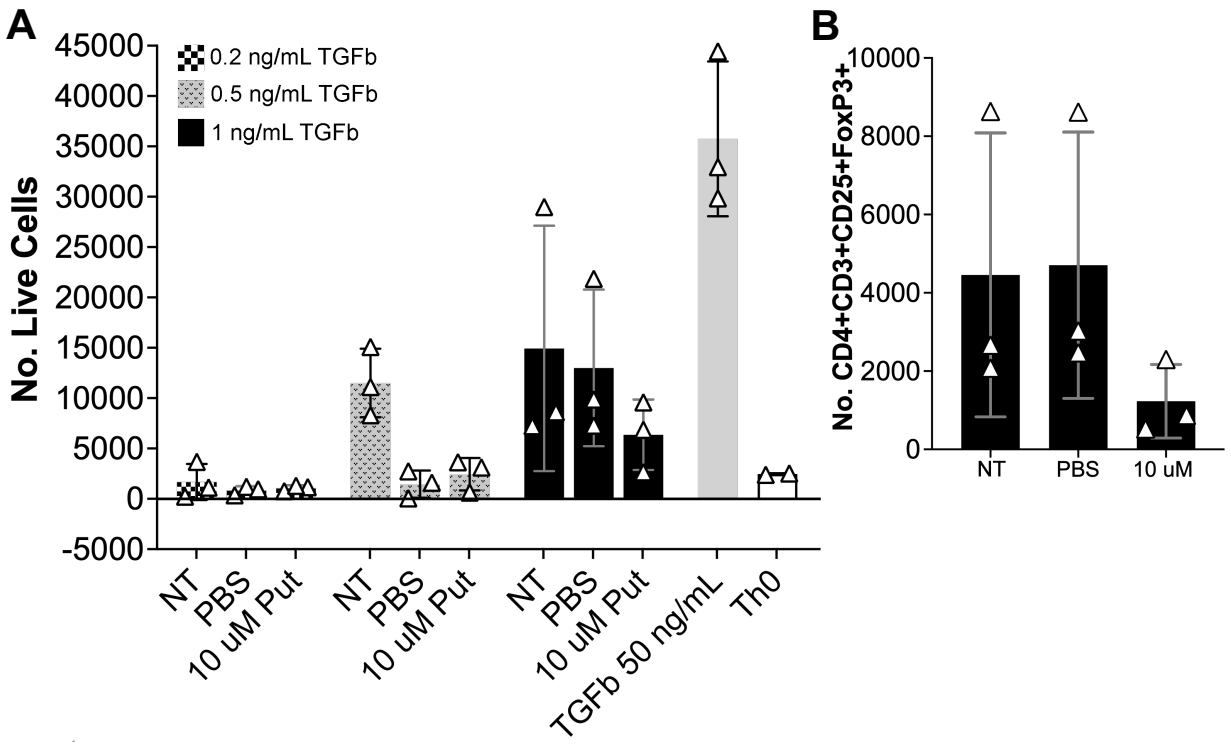


Figure 3.14 Testing the effect of putrescine treatment under different concentrations of TGF-β. A) Bar graph showing the number of live cells under various treatment conditions. Th0 conditions (1 μg/mL plate-bound anti-CD3, 2 μg/mL soluble anti-CD28, and 10 ng/mL IL-2) B) Bar graph showing the number of live $CD4^+CD3^+$ Tregs ($CD25^+FoxP3^+$) induced by treatment with 1 ng/mL TGF-β under various treatment conditions. For all plots each triangle represents a single donor and standard deviation is shown. Bars are colored according to TGF-β concentration used and cells were additionally treated with either 10 μM of putrescine (Put) or the vehicle PBS. NT = No Treatment. No statistics were performed due to insufficient numbers for statistical analysis.

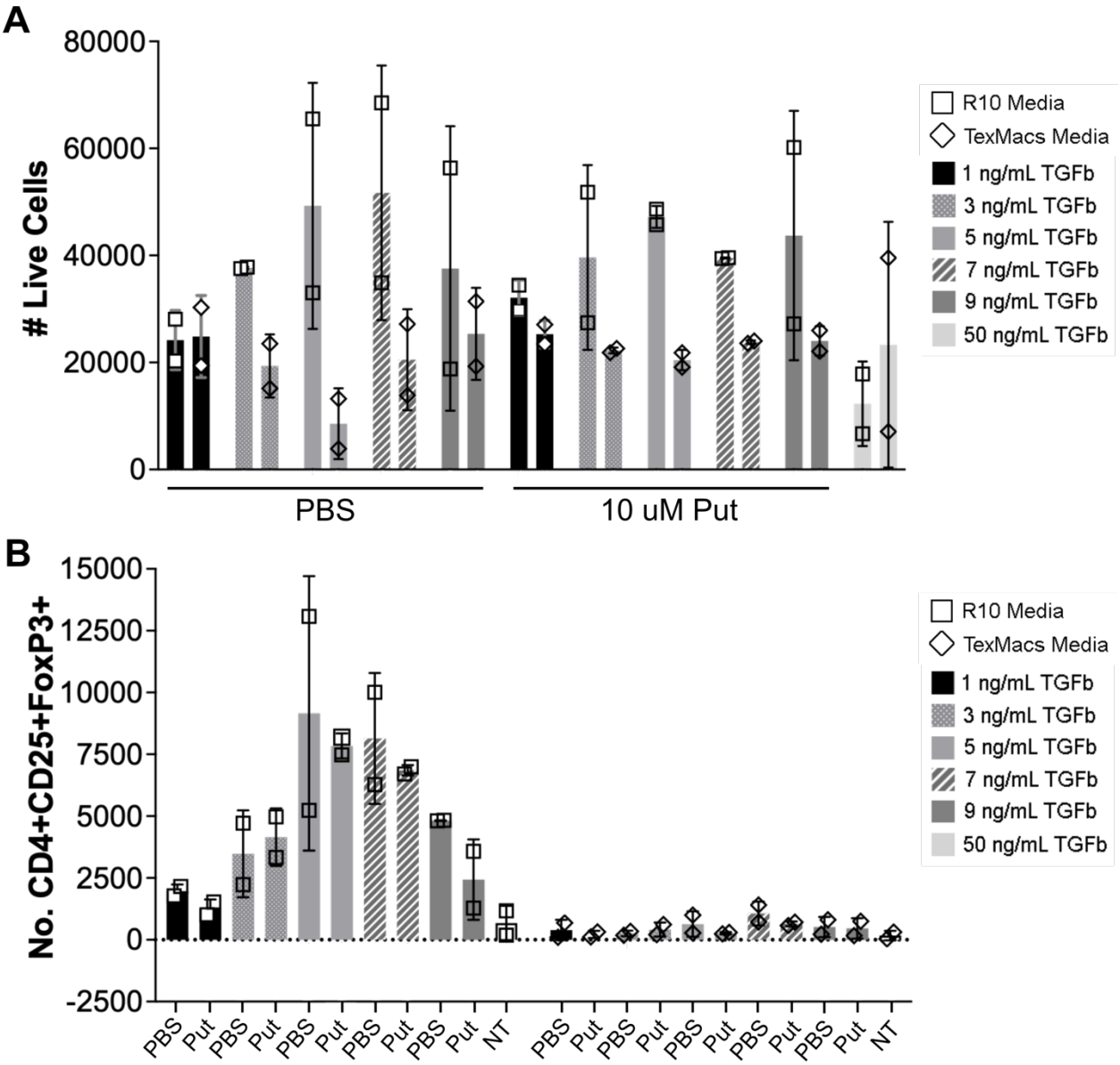


Figure 3.15 TGF-β titration under serum-free (TexMacs) or serum-supplemented (R10) conditions. A) Bar graph comparing the number of live cells in TexMacs or R10 cultured naive T cells under various treatment conditions. B) Bar graph comparing the number of live $CD4^+$ Tregs ($CD25^+FoxP3^+$) under various treatment conditions. Each square represents a well of cells from a single donor cultured using R10 media. Each diamond represents a well of cells from a single donor cultured using TexMacs media. Bars are colored according to TGF-β concentration used and cells were additionally treated with either 10 μM of putrescine (Put) or the vehicle PBS. Standard deviation is shown. NT = No Treatment. No statistics were performed due to insufficient numbers for statistical analysis.

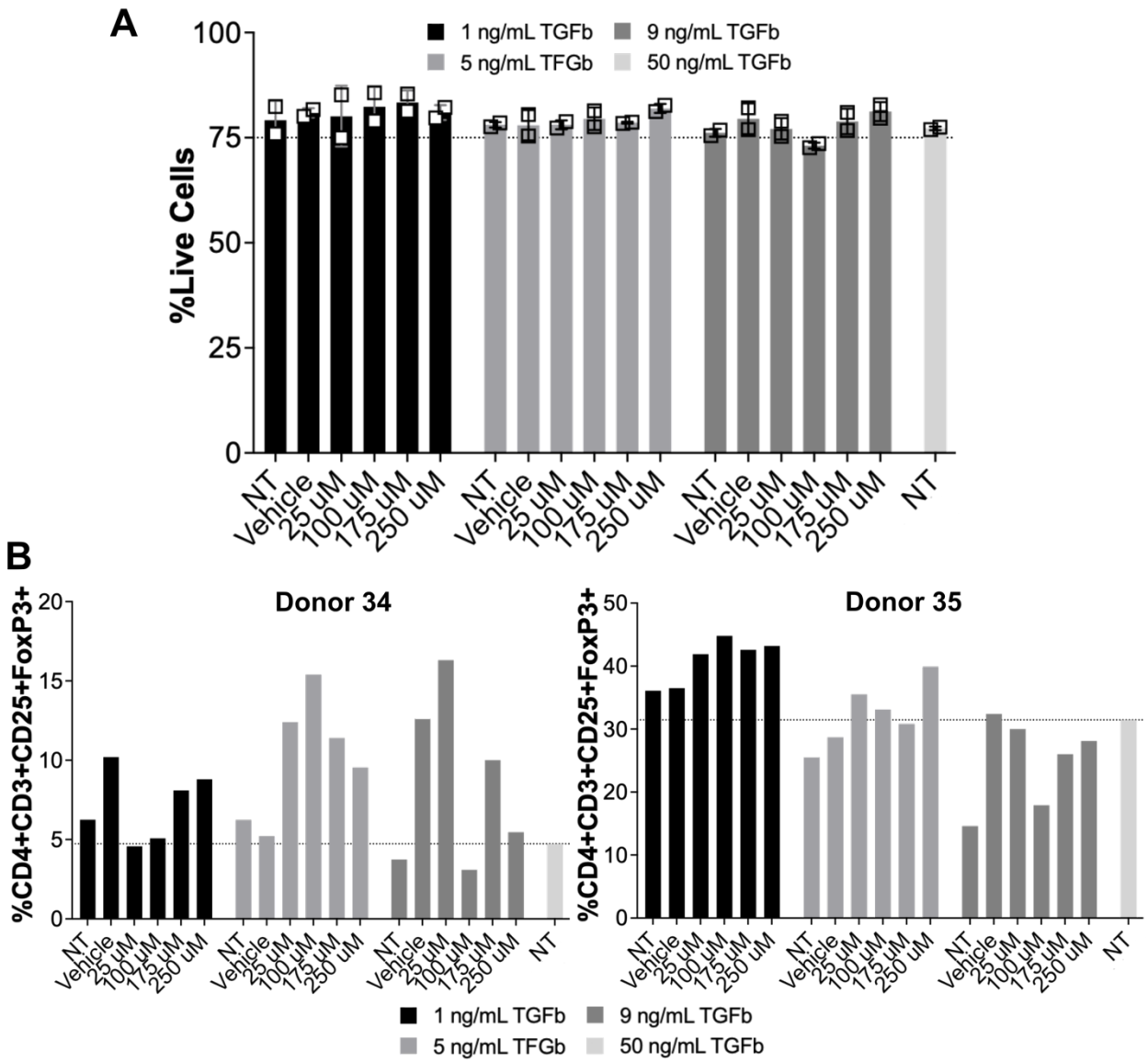


Figure 3.16 TGF-β titration under different putrescine treatment conditions. A) Bar graph comparing the number of live cells. Each square represents a well of cells from a single donor and standard deviation is shown. B) Bar graphs comparing the percent of live $CD4^+$ Tregs ($CD25^+FoxP3^+$) under various treatment conditions from the two donors used in this experiment. For all plots bars are colored according to TGF-β concentration used and cells were additionally treated with either 25, 100, 175, or 250 μM of putrescine or the vehicle PBS. NT = No Treatment. No statistics were performed due to insufficient numbers for statistical analysis.

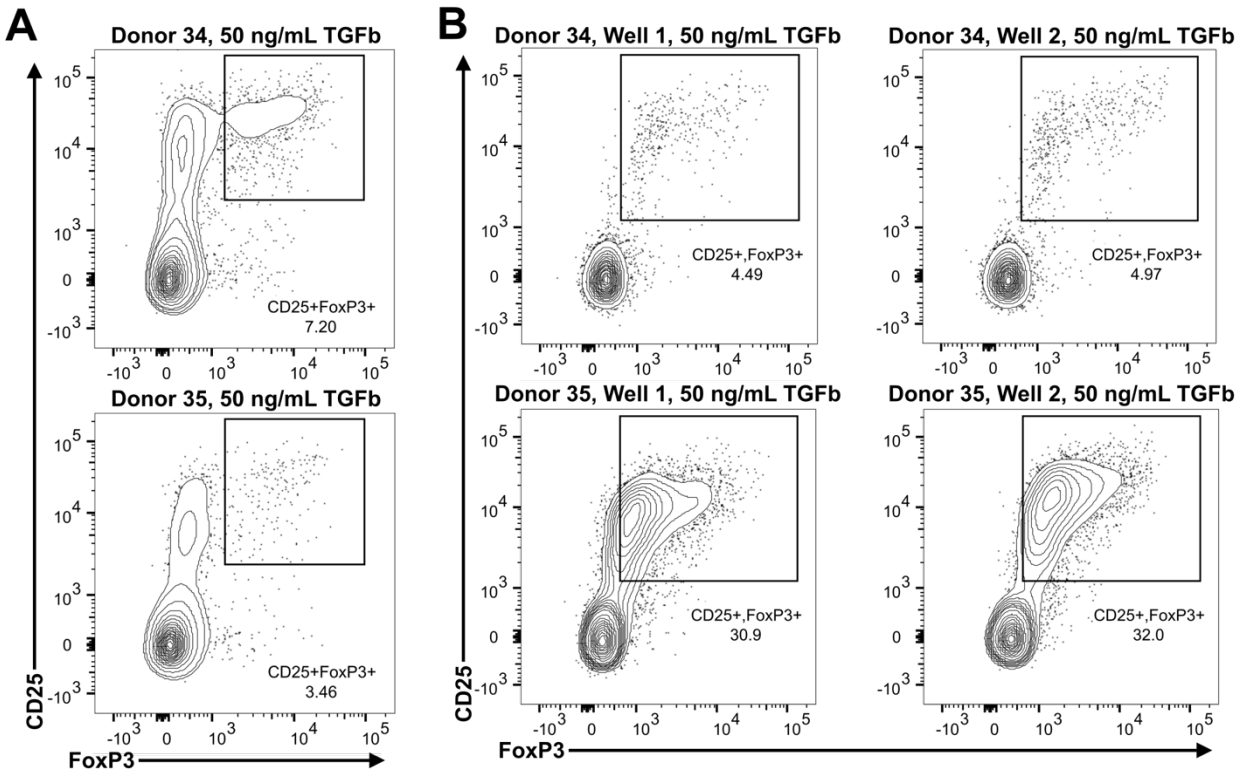


Figure 3.17 Comparing Treg induction across experiments in blood Donor 34 and 35. A) Flow plots of live $CD4^+$ T cells from two donors cultured under Treg polarization conditions and gated on CD25 (Y axis) and FoxP3 (X axis) expression. Number on graphs represents the percent of live $CD4^+CD25^+FoxP3^+$ cells. B) Flow plots from an independent experiment showing the percent of live $CD4^+$ T cells cultured under Treg polarizing conditions that express CD25 and FoxP3.

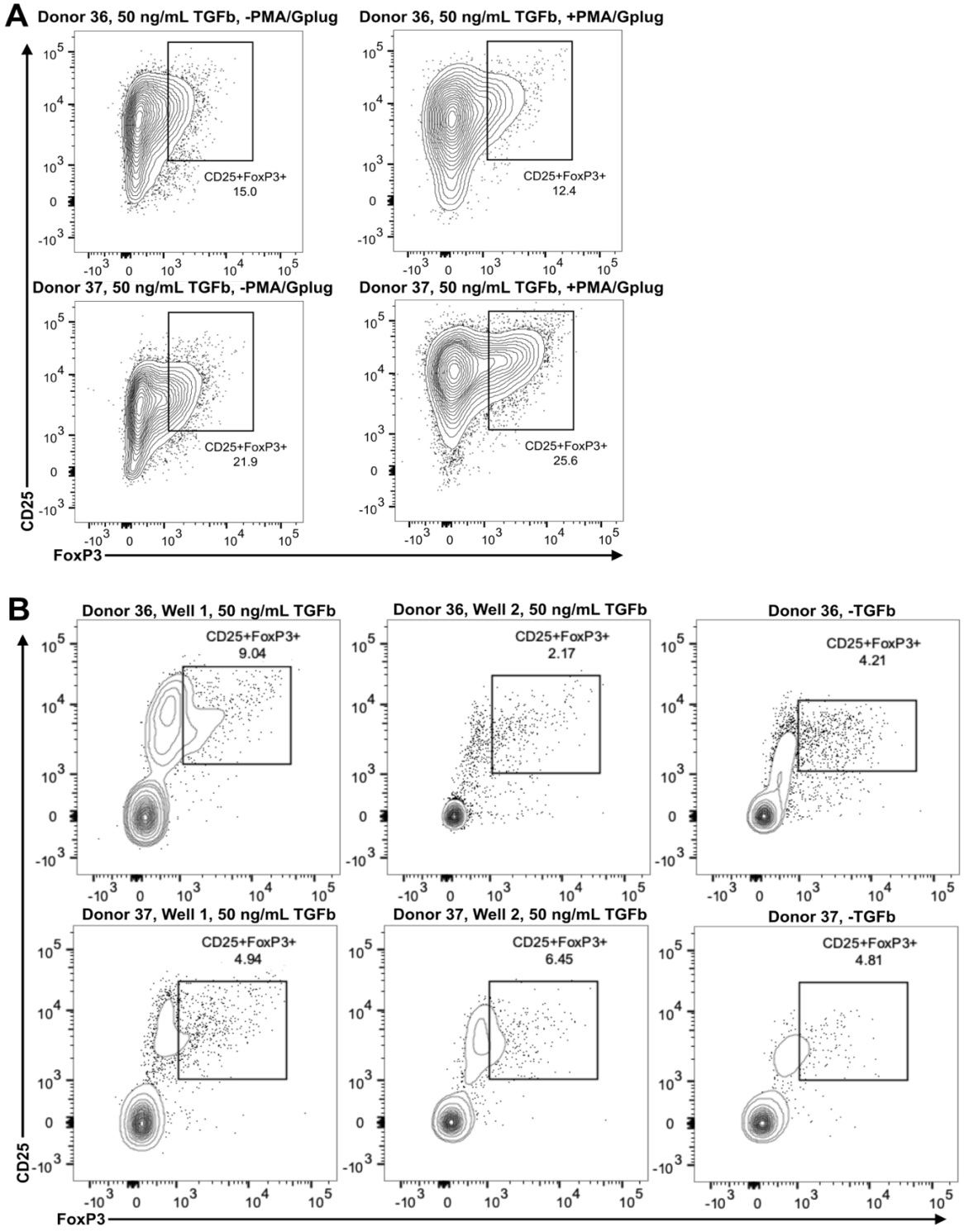


Figure 3.18 Comparing Treg induction in blood Donor 36 and 37. A) Flow plots show the percent of $CD4^+$ Tregs following cell culture under Treg polarizing conditions and either treatment with or without PMA/Ionomycin/Gplug 4 hours prior to cell staining. B) Flow plots from an independent experiment showing the percent of live $CD4^+$ Tregs following culture under Treg polarizing conditions or non-Treg polarizing conditions (-TGF-β).

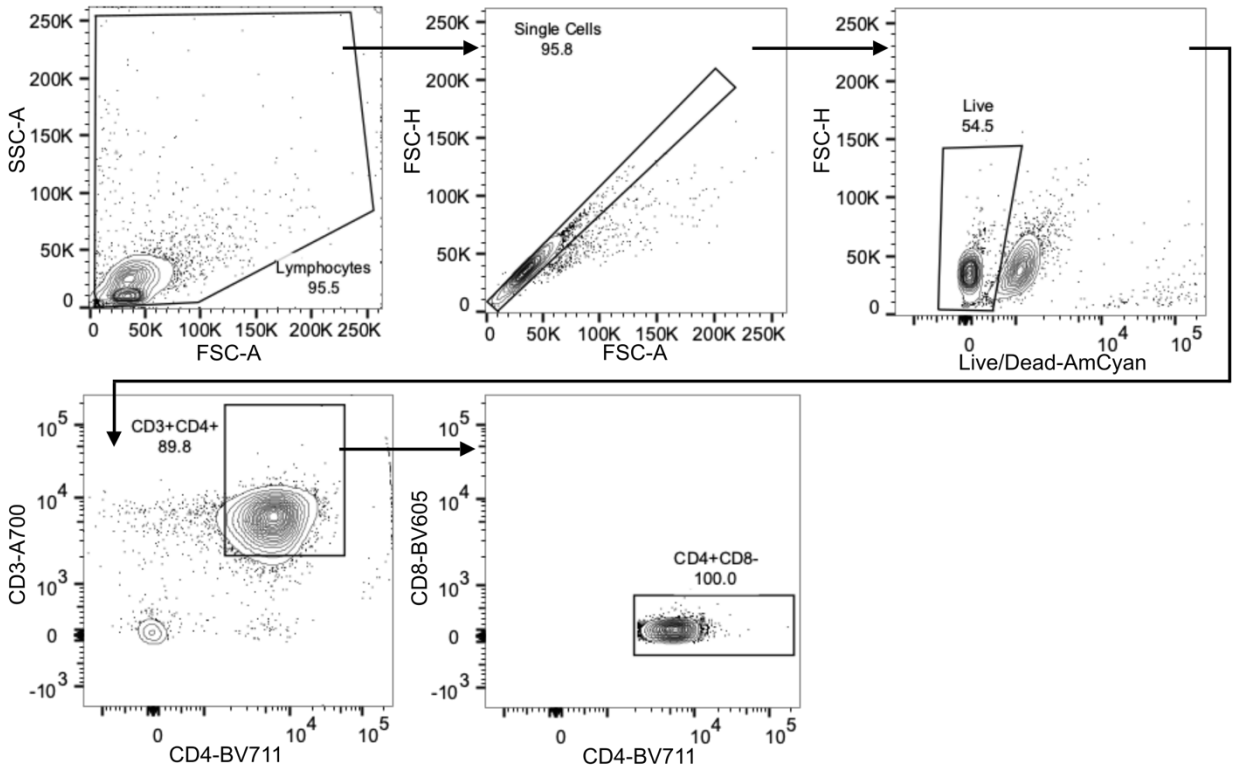
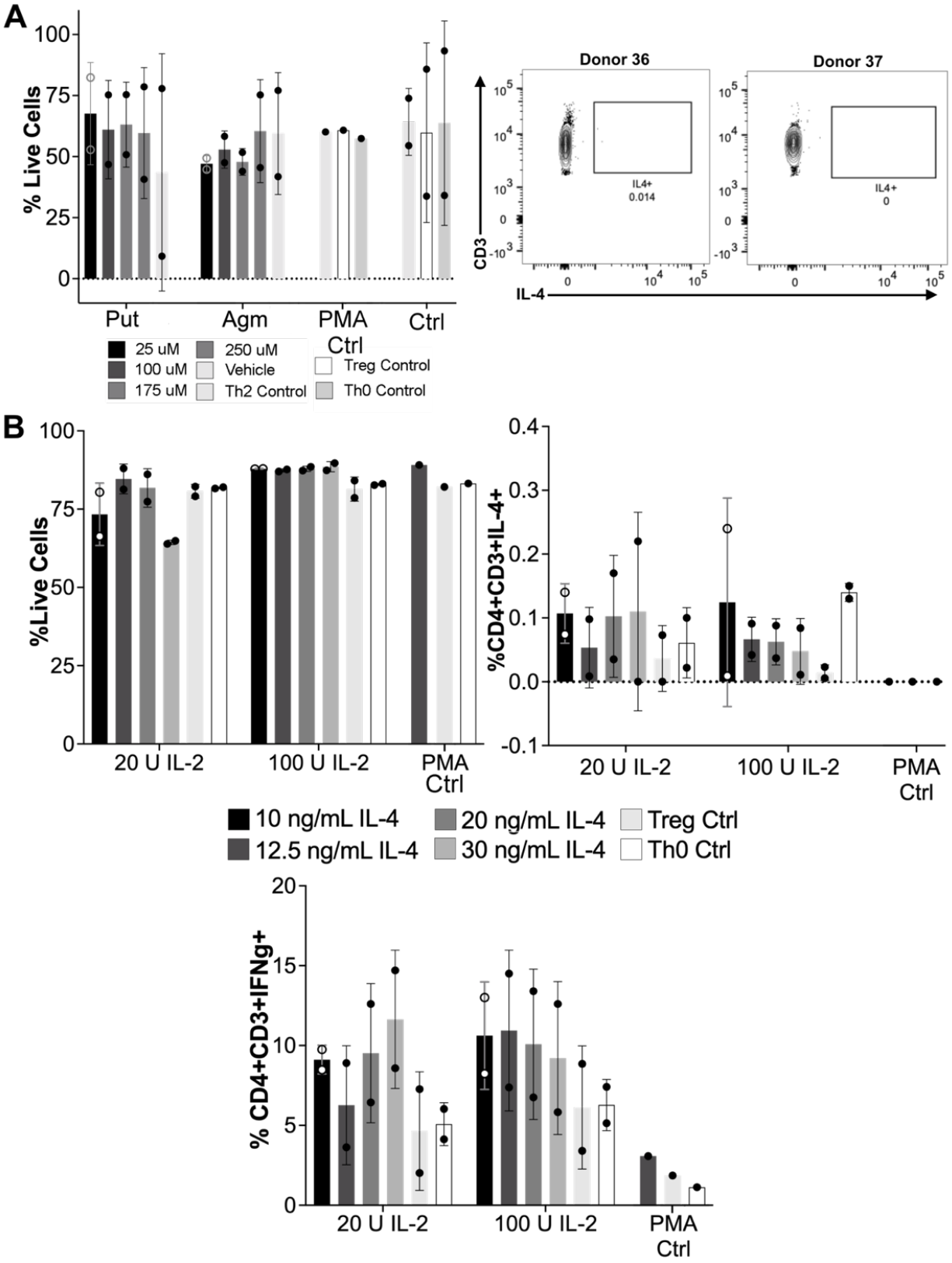


Figure 3.19 Th2 Cell Gating Strategy. T cell phenotype was assessed via flow cytometry. A large lymphocyte gate was drawn following which single cells and live cells were gated. Th2 cells were defined as IL-4$^+$ cells (not shown) that are CD3$^+$CD4$^+$CD8$^-$.


A
% Live Cells
100
75
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PMA
Ctrl
Ctrl
25 uM
100 uM
175 uM
250 uM
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Th2 Control
Treg Control
Th0 Control
Donor 36
Donor 37
CD3
IL-4
IL4+
0.014
IL4+
0
B
%Live Cells
%CD4+CD3+IL-4+
0.4
0.3
0.2
0.1
0.0
-0.1
20 U IL-2
100 U IL-2
PMA
Ctrl
10 ng/mL IL-4
12.5 ng/mL IL-4
20 ng/mL IL-4
30 ng/mL IL-4
Treg Ctrl
Th0 Ctrl
% CD4+CD3+IFNg+
20
15
10
5
0

Figure 3.20 Assessing IL-4 and IL-2 concentrations to induce Th2 polarization. A) Bar graph showing viability and flow plots showing percent live $CD4^+CD3^+IL\text{-}4^+$ cells following treatment with 12.5 ng/mL IL-4 and 10 ng/mL IL-2 (Th2 polarization conditions) and TCR stimulation with plate-bound anti-CD3 (clone HIT3a)/anti-CD28 (clone CD28.2) for 3 days followed by a 2-day resting period. Bars are colored by polyamine treatment concentration or alternative polarization condition treatment. B) Bar graphs from an independent experiment showing viability, percent live $CD4^+CD3^+IL\text{-}4^+$ cells, and percent live $CD4^+CD3^+IFN\gamma^+$ cells following treatment with various concentrations of IL-4 and IL-2 and constant TCR stim with anti-CD3/anti-CD28. Bars are colored by IL-4 concentration used. For all experiments circles represent a single donor and standard deviation is shown. No statistics were performed due to insufficient numbers for statistical analysis. PMA Ctrl = no treatment with PMA/Ionomycin/Gplug.

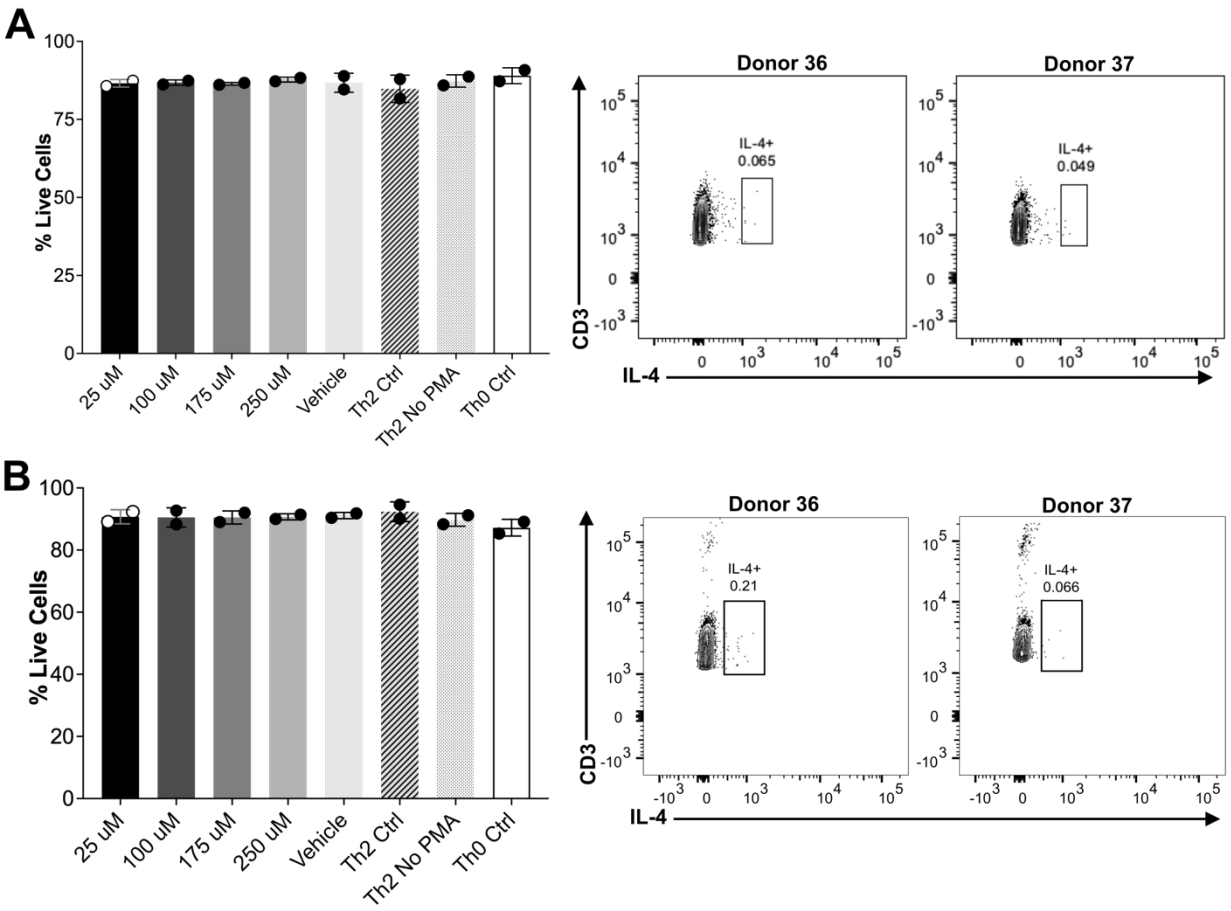

Figure 3.21 Assessing the impact of anti-IFNγ, anti-CD28/anti-CD3 clones, and different media supplements on Th2 polarization. A) Bar graph showing viability and flow plots showing percent live $CD4^+CD3^+IL\text{-}4^+$ cells following treatment with 12.5 ng/mL IL-4, 10 ng/mL IL-2, and 5 µg/mL anti-IFNγ (Th2 polarization conditions) and TCR stimulation with plate-bound anti-CD3 (clone 15E8)/anti-CD28 (clone OKT3) for 3 days followed by a 2-day resting period. B) Bar graph showing viability and flow plots showing percent live $CD4^+CD3^+IL\text{-}4^+$ cells in an independent experiment. Cells were cultured under similar conditions as those used in panel A except that IL-2 was added only during the 2-day resting period and media was supplemented with HEPES, non-essential amino acids, and 2-mercaptoethanol. For all graphs, bars are colored by polyamine treatment concentration or alternative polarization condition treatment and standard deviation is shown. No statistics were performed due to insufficient numbers for statistical analysis. No PMA = no treatment with PMA/Ionomycin/Gplug.

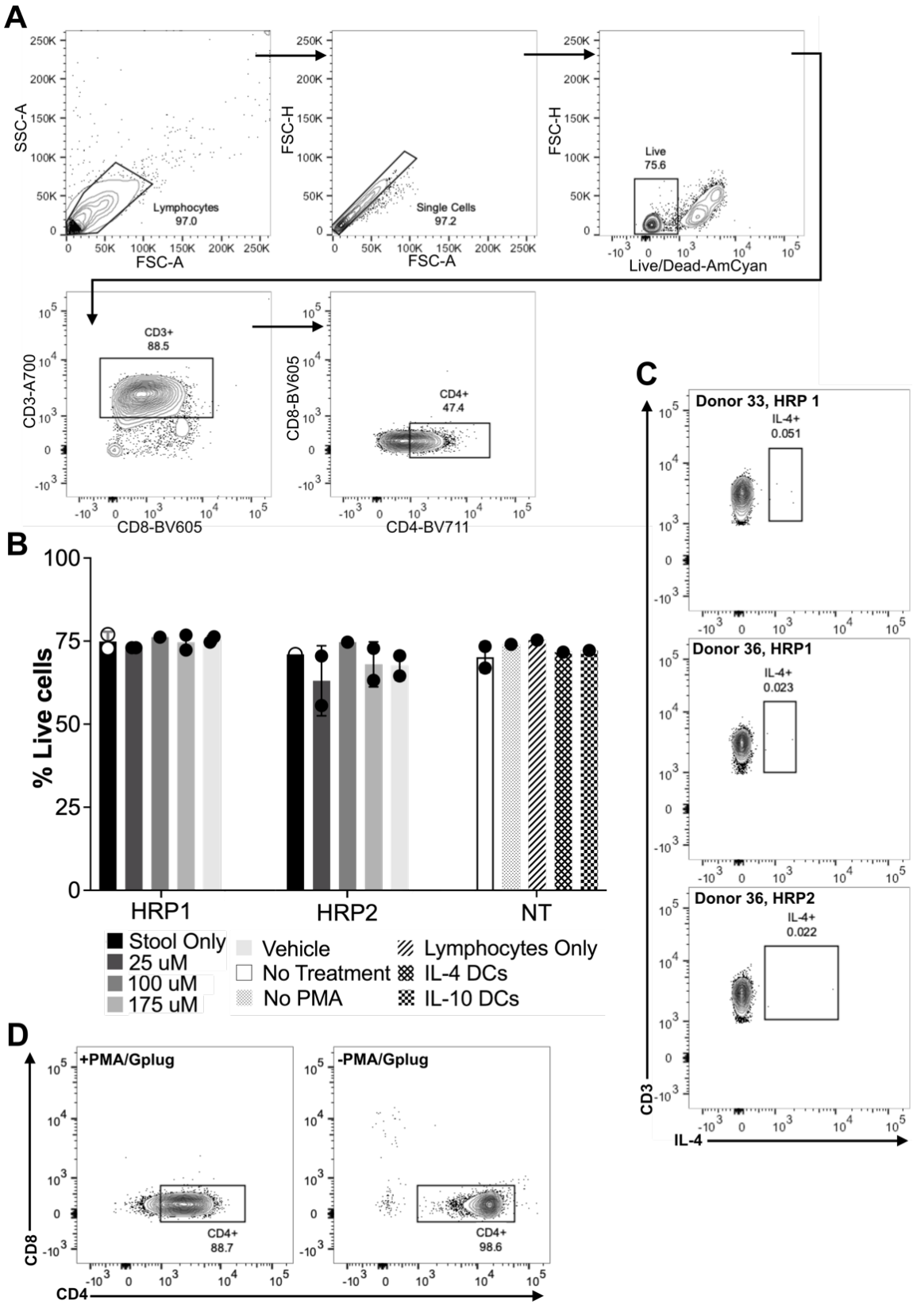
A
SSC-A
FSC-A
Lymphocytes
97.0
FSC-H
FSC-A
Single Cells
97.2
FSC-H
Live/Dead-AmCyan
Live
75.6
CD3-A700
CD8-BV605
CD3+
88.5
CD8-BV605
CD4-BV711
CD4+
47.4
B
% Live cells
HRP1
HRP2
NT
Stool Only
25 uM
100 uM
175 uM
Vehicle
No Treatment
No PMA
Lymphocytes Only
IL-4 DCs
IL-10 DCs
C
Donor 33, HRP 1
IL-4+
0.051
Donor 36, HRP1
IL-4+
0.023
Donor 36, HRP2
IL-4+
0.022
CD3
IL-4
D
+PMA/Gplug
CD4+
88.7
-PMA/Gplug
CD4+
98.6
CD8
CD4

Figure 3.22 L-ornithine treatment of DCs co-cultured with autologous naive T cells. A) T cell phenotype was assessed via flow cytometry. Lymphocytes, single cells, and live cells were gated. Th2 cells were defined as $CD8^{-}CD3^{+}CD4^{+}IL\text{-}4^{+}$. B) Bar graph showing viability of cells treated with CFW from two high-risk infants (HRP1, HRP2). Bars are colored by DC L-ornithine treatment concentration or alternative treatment conditions and standard deviation is shown. No statistics were performed due to insufficient numbers for statistical analysis. No PMA = no treatment with PMA/Ionomycin/Gplug. NT = No treatment. C) Flow plots showing percent live $CD4^{+}CD3^{+}IL\text{-}4^{+}$ cells. D) Flow plots showing a shift in the $CD4^{+}$ population following PMA/Ionomycin/Gplug treatment suggesting the stimulation is working.

3.5 METHODS

3.5.1 Steroid-Related Experiments

3.5.1.1 Human Immune Cell Assays

Peripheral blood samples were obtained from healthy, de–identified human donors from the Blood Centers of the Pacific, San Francisco, CA. Donors signed an agreement acknowledging that their blood may be used for research. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll–Paque (GE Healthcare) gradient centrifugation and washed twice with R10 media (Advanced RPMI 1640 Medium [Thermo Fisher Scientific] with 10% heat–inactivated fetal bovine serum [FBS; USA Scientific] and a solution of 2 mM L–glutamine and 100 U ml^{-1} penicillin–streptomycin [Corning/Fisher Scientific]). PBMCs (20 million cells per mL) were stored in FBS plus 10% DMSO first at -80°C for two days and then at -114°C for long term storage.

Prior to cell isolation, PBMC vials were thawed at 37°C for 90 seconds, washed once with R10 media supplemented with DNase (1:100 dilution of 1 mg/mL, Roche), washed once in R10 media, and incubated at 37°C in 5% CO_2 in R10 media for a maximum of 16 hours. DCs were isolated from PBMCs using the EasySep™ Human Pan–DC Pre–Enrichment Kit (STEMCELL Technologies) and naive T cells were isolated using the EasySep™ Human naive $CD4^+$ T cell Isolation Kit (STEMCELL Technologies).

3.5.1.2 DC Experiments with DHEA-S

DHEA-S was purchased from Cayman Chemical, solubilized in dimethylsulfoxide (DMSO), and stored at -20°C until use.

DCs were cultured in R10 media supplemented with 10 ng ml^{-1} GM–CSF (R&D Systems) and 20 ng ml^{-1} IL–4 (R&D Systems) (DC treatment media) at 37°C in 5% CO_2 with various concentrations of DHEA-S (refer to text). After 24 hours, supernatant was collected and fresh R10 media supplemented with 10 ng ml^{-1} TNF, IL-1β and IL-6 (Peprotech) and 1 mM prostaglandin E2 (STEMCELL Technologies) was added to stimulate DCs unless otherwise stated in the text. After an additional 24-hour incubation, supernatant was collected and DCs were stained with LIVE/DEAD fixable Aqua cell stain (1:500 dilution, Invitrogen) and anti-human CD45 (APC, BD Biosciences) and run on a BD LSR II flow cytometer. Cell-free supernatant (achieved via centrifugation) was stored at -80°C until use. Secreted cytokine levels were evaluated by cytometric bead array following the manufacturer's protocol (BD Biosciences).

Additionally, in some experiments cells were treated with 5 μg/mL lipopolysaccharide (Sigma-Aldrich), *Candida* cell-free supernatant or heat-killed cells (lab isolate), or a human toll-like receptor agonist mix (TLR 1-9, Invivogen). Specifics can be found in the text.

3.5.1.3 *Ethyl Acetate Extraction*

Ethyl Acetate was added to samples at a 2X volume and tubes were vortexed vigorously for 30 seconds to mix. Samples sat for 10 minutes at room temperature to allow the mix to separate into two distinct layers and the top layer was transferred into a new tube. An additional 2X volume of ethyl acetate was added following which samples were vortexed for 30 seconds and incubated for 10 minutes at room temperature. The top layer was then transferred to the corresponding sample tube and dried under nitrogen flow or using SpeedVac. Dry samples were stored at -20°C and resuspend in

DMSO using the same initial sample volume (i.e. 200 µL) immediately before running them on the TLC plate.

3.5.1.4 *Thin Layer Chromatography*

The TLC chamber was set up by adding 80 mL of hexane (Sigma-Aldrich) and 20 mL of ethyl acetate (mobile phase) and a piece of filter paper was placed directly into the mobile phase. The chamber was covered with a glass lid and left for at least 30 minutes to allow the chamber to become saturated. Once the chamber was saturated, 5 µL of extracted samples and standards were spotted 1 cm up from the bottom of an aluminum backed silica gel TLC plate (EMD Millipore) and allowed to dry. Following this the filter paper was removed, the plate was placed standing in the chamber, and the lid is replaced on top. The mobile phase rises up the TLC plate through capillary action and as it does so interactions occur between the solid phase, solvent, and sample that cause each analyte to be carried to a definite height on the plate. When the mobile phase was approximately an inch away from the top of the TLC plate (approximately an hour later) the separation is complete. The plate was then removed from the chamber and allowed to dry. While the plate dried, fresh Liebermann-Buchard Reagent (McNelis, 2009, Dissertaion) was made which includes: 8% sulfuric acid, 2% acetic acid, 24% ethanol, and water. Once the plate was dried a TLC sprayer (Thomas Scientific) was used to spray the plate with Liebermann-Buchard Reagent in a fume hood, allowed to dry again, and then placed on a hot plate set to 110°C for 10 minutes until bands appeared. Compound identification was then be completed by comparing the retention factor (Rf) value – which is equal to the distance migrated over the total distance covered by the solvent – of samples to those of the reference standards.

3.5.1.5 *Animal Experiments*

Six-week-old female C57BL/6 mice were obtained from Jackson Laboratories and randomized to treatment groups. The experiment was performed with 5 mice per cage and treatment groups were housed in independent cages. Mice were supplemented with 1 mg/mL (3000 μM) 17-OH-Preg solubilized in 10% dimethylsulfoxide (DMSO) or vehicle (10% DMSO) via oral gavage for three days. Following initial supplementation, mice were anaesthetized with isoflurane and sensitized intratracheally with 50 μL of the house dust mite (HDM) allergen *Dermatophagoides Pteronyssinus* (Greer Laboratories). Mice continued to be supplemented daily with 17-OH-Preg for the remainder of the experiment and were challenged with HDM on day 7, 8, 9, 10, and 11 consistent with the well-established HDM model. Mice were sacrificed on day 14 and blood (collected via cardiac puncture), spleen, mediastinal and mesenteric lymph nodes, lung, small intestine, cecum, and large intestine were collected.

The small intestine, cecum, and large intestine were stored in RNAlater (Ambion) at 4°C for one day and then transferred to -80°C for long-term prior to anticipated 16S rRNA sequencing. Blood samples were centrifuged at a low speed (1000 – 2000 x g) at 4°C for 10 minutes following which serum was collected. Serum concentrations of HDM-specific IgE were determined using the mouse Anti-HDM IgE Assay Kit (Chondex Inc) and following manufacturer instructions.

Following euthanasia, the right and left lungs were separated. The inferior lobes of the left lung were processed for histological analysis or saved in RNAlater (Ambion) for future gene expression analyses. The right lobe as well as the spleen and mediastinal and mesenteric lymph nodes were processed for flow cytometry. Tissue

was manually dissected, digested with 5 mg per sample collagenase (Sigma-Aldrich), and passed through a 40 μM filter to generate single-cell suspensions. Cells were counted using a BD Accuri C6 Cytometer (BD Biosciences) and half the cells were transferred to a plate for immune cell phenotype staining and the other half was transferred to a separate plate for Th2 phenotype staining.

The immune cell phenotyping panel consisted of anti-mouse CD11c (FITC, Clone: N418, Thermo Fisher); FoxP3 (PE, Clone: MF23, BD Pharmigen); SiglecF (PerCP, Clone: E50-2440, BD Pharmigen); Ly6C (Pe-Cy7, Clone: AL-21, BD Pharmigen); Ly6G (APC-Cy7, Clone: 1A8, BD Pharmigen); CD25 (APC, Clone: PC61, BD Pharmigen); CD19 (Biotin, Clone: ebio1D3, eBioscience); CD8 (Biotin, Clone: 53-6.7, BD Pharmigen); CD11b (BV605, Clone: M1/70, BioLegned); F4/80 (BV650, Clone: 6F12, BD Pharmigen); CD4 (BV711, Clone RM4-5, BD Pharmigen); LIVE/DEAD fixable Aqua cell stain (AmCyan, 1:250 dilution, Invitrogen); and CD3 (BUV395, Clone: 17A2, BD Pharmigen). All dilutions were 1:100 unless stated otherwise. Cells were classified as eosinophils ($CD8^-/CD19^-$, Siglec $F^+/CD11c^-$, SSC-$A^{hi}/Ly6G^-$); macrophages ($CD11b^+Ly6G^-Ly6C^-F4/80^+$); monocytes ($CD11b^+Ly6C^+F4/80^+$); alveolar macrophages ($CD11b^-SiglecF^+CD11c^+F4/80^+$); dendritic cells ($CD11b^+Ly6G^-CD11c^+$); neutrophils ($CD11b^+Ly6G^+$); and Tregs ($CD3^+CD8^-/CD19^-CD4^+CD25^+FoxP3^+$).

Cytokine production was assessed using 50 ng/mL PMA (Santa Cruz Biotechnology), 5 μg/mL ionomycin (Millipore Sigma), and 5 μg/mL Brefeldin A (Sigma-Aldrich). Intracellular cytokine staining was carried out using the FoxP3/Transcription Factor Staining Buffer Kit (Tonbo Biosciences) following manufacturer's instructions but with slightly shorter incubation periods (which may explain why this panel was not

successful). The Th2 cytokine phenotyping panel consisted of anti-mouse IL-5 (FITC, 1:200 dilution, Clone: TRFK5, Leinco); IL-13 (PE, Clone: ebio 13A, eBioscience); IFNγ (PeCy7, Clone: XMG1.2, BD Biosciences); IL-4 (APC, Clone: 11B11, Biolegned); and IL-17a (PB, Clone: TC11-18H10.1, Biolegned) used at 1:100 dilution unless stated otherwise. Additionally, the same CD3, CD8, and CD4 antibodies from the previous panel were also used at a 1:100 dilution and a LIVE/DEAD fixable Aqua cell stain was used at 1:250.

3.5.2 Sequencing & Metabolite Analysis of 6-Month-Old Infant Stool Samples

3.5.2.1 *Study Population*

Recruitment for the Trial of Infant Probiotic Supplementation was carried out in the San Francisco Bay Area and consisted of infants determined to be at high-risk of developing asthma based on medical diagnosis of one or both parents. Computer-generated randomization assigned infants to one of two trial arms, with one arm receiving daily supplementation with a *Lactobacillus rhamnosus* GG probiotic (LGG; strain ATCC 53103; at 1×10^{10} CFU) over the first six months of life and the other receiving a daily placebo in the form of prebiotic inulin (325 mg).[230] Stool samples were collected at birth, 1, 3, 6, and 12-months of age from this cohort as well as the Development of Infant Microbial Evolution (DIMES) cohort which enrolled infants born to non-atopic parents. Stool was collected from diapers, mailed overnight to the study team, and stored at -80°C until processing.

3.5.2.2 *DNA Extraction*

DNA was extracted from sixth-month stool samples from 12 HC, 6 HRLGG, and 7 HRP infants that had previously undergone untargeted LC-MS and for which enough

material remained. Stool samples from infants were prepared for extraction on dry ice. A 4MM punch biopsy with plunger (VWR International) was used to aliquot 0.3 grams of sample into a Lysing Matrix E tube (LME; MP Biomedicals) with 500 µl hexadecyltrimethylammonium bromide (CTAB, Sigma-Aldrich) and 500 µl phenol:chloroform:isoamyl-alcohol (25:24:1, Sigma-Aldrich). Samples were then incubated at 65C for 15 minutes. Following incubation, samples were homogenized in a Prep-24 homogenizer at 5.5 m/s for 30 seconds and centrifuged at 16,000 x g for 5 minutes at 4C. The aqueous phase was transferred to phase lock gel matrix tubes (Fisher). An additional 500 µl of CTAB buffer was added to the LME tubes and incubation, homogenization, and centrifugation were repeated. The aqueous phases from paired extractions were combined in the gel matrix tube. An equal volume of chloroform was mixed with each sample, followed by centrifugation at 16,000 x g for 10 minutes at 4°C. The aqueous phase (600 µl) was transferred to a clean microfuge tube, combined with 2 volume-equivalents (1200 µl) of polyethylene glycol (PEG, Sigma-Aldrich) and stored overnight at 4°C to precipitate DNA. Microfuge tubes were centrifuged for 10 min at 16,000 x g, then DNA pellets were washed with 300 µl of ice-cold 70% ethanol, air-dried for 10 minutes, and re-suspended in 100 µl of sterile water. DNA from stool samples was quantified using the Qubit dsDNA Broad Range Assay Kit (Thermo Fisher), diluted to 10 ng/µL.

3.5.2.3 *Metagenomic Data Analysis*

Extracted DNA was sent to the Vincent J. Coates Genomic Sequencing Laboratory at the California institute for Quantitative Biosciences for 150-bp paired-end sequencing on an Illumina HiSeq 4000 (http://www.qb3.berkeley.edu/gsl). The average

coverage of sequenced samples was around 10 million reads as determined by FastQC and aggregation of FastQC files using MultiQC. Deeper sequencing would have been more ideal, but at 6-months of age microbial communities have already gone rapid development and become more complex which may explain why we were only able to achieve this depth. It should also be noted that stool samples from the TIPS/DIMES cohort were collected in the early 2010s and thus may have lost some biological information due to freeze/thaw cycles.

Bbduk from the Joint Genome Institute (JGI) was used to remove TruSeq adapters, filter out human and PhiX reads, and quality trim raw sequences before further analysis. For each sample read 1 and read 2 were concatenated into one file and then run through HUMAnN2.[266] This produced a pathway abundance table with MetaCyc[267] pathways and a gene families table for each sample. Individual sample tables for each type of table were combined using “humann2_join_tables” and normalized to counts per million (CPM) using the command “humann2_renorm_table”. The joined, normalized pathway abundance table was then used as input for MaAsLin2[268] which determined pathways that were statistically significantly different between groups using a negative binomial model. An in-house three model script that simultaneously applies Poisson, negative binomial, and zero-inflated negative binomial regressions and tests for best fit on a pathway-by-pathway basis was used to validate these findings.

The combined, normalized gene family table was used as input for the command “humann2_regroup_table” with the flag “--groups uniref90_level4ec” to determine enzyme commission (EC) numbers associated with the uniref90 proteins called by

HUMAnN2. Average EC CPMs were calculated for all groups and Log2 Fold changes were determined between HC vs HRP, HRLGG vs HRP, and HC vs HRLGG. The same was done for statistically significant metabolomics data (Welch's test, $p<0.1$) published in Durack *et al.* (2018) Log2 fold change for both metagenomics and metabolomics data was compiled into one file that was uploaded to the MetaCyc website and overlayed onto pathways deemed statistically significant using MaAsLin2 ($p <0.05$) and where were present in 1/3 of samples in each group.

For Procrustes analysis, family level count data from 16S rRNA and shotgun metagenomic sequencing were used to generate two Bray-Curtis distance matrices. These distance matrices were then used to generate two coordinate matrices and permutational testing for non-randomness between the 16S rRNA and metagenomic matrices was carried out using the protest command. These analyses were done in R (v4.0.0) using the Vegan package. Family relative abundance was calculated for 16S rRNA sequencing data using the summarize_taxa.py command in QIIME (Quantitative Insights into Microbial Ecology).[227] For metagenomic sequencing, family relative abundance was calculated using MetaPhlAn.[269]

3.5.3 Polyamine and Amino Acid-Related Experiments

3.5.3.1 *Preparation of Polyamines and Amino Acids*

L-ornithine hydrochloride (Cayman Chemical), putrescine (1,4-Diaminobutane, Sigma-Aldrich), and agmatine sulfate salt (Sigma-Aldrich) were solubilized in 1X phosphate buffered saline (PBS), pH 7.4 (Thermo Fisher Scientific). All were stored at high concentrations at -20°C until use.

3.5.3.2 *Treg Polarization Experiments*

Peripheral blood mononuclear cells (PBMCs) and naive $CD4^+$ T cells were isolated as described in section 3.5.1.1 of this text. Depending on the experiment, isolated T cells were cultured in TexMACS (Miltenyi Biotec) or R10 media supplemented with 2 µg/mL anti-CD28 (Clone: CD28.2, BD Biosciences), 0.5 - 50 ng/mL recombinant human TGF-β (PeproTech), and 10 ng/mL recombinant human IL-2 (PeproTech) in the presence of plate-bound anti-CD3 (Clone: HIT3a, BioLegend). Cells were also treated with 10, 25, 100, 175, or 250 µM putrescine depending on the experiment (these details can be found in the results section). Cells were cultured for 5 days and media was refreshed every 48 hours.

Cells were stained using anti-human CD4 (BV711, Clone: L200, BD Biosciences); CD25 (PE-Cy7, Dilution 1:25 Clone: M-A251, BD Biosciences); Live/Dead Fixable Aqua Dead Cell Stain (1:500 Invitrogen); CD3 (Alexa 700, Clone: SP34-2, BD Biosciences); and FoxP3 (eFluor 450, Dilution: 1:20 Clone: PCH101, eBioseince). All antibodies were used at a dilution of 1:100 unless stated otherwise. Flow cytometry data were collected on a BD LSR II flow cytometer and Treg cells were defined as $CD3^+CD4^+CD25^+FoxP3^+$.

3.5.3.3 *Th2 Polarization Experiments*

Isolated T cells were cultured in R10 media supplemented with 10 ng/mL, 12.5 ng/mL, 20 ng/mL, 30 ng/mL recombinant human IL-4 (PeproTech), 10 ng/mL or 10 U or 100 U recombinant human IL-2 (PeproTech), and in, some cases, 5 µg/mL anti-IFNγ (Clone: B-B1, Thermo Scientific) to induce Th2 polarization. Plate-bound anti-CD3 (Clone: HIT3a or Clone OKT3, BioLegend) and either plate-bound or soluble anti-CD28 (Clone: CD28.2, BD Biosciences OR Clone: 15E8, Miltenyi Biotec) were used to

stimulate the T cell receptor for 3 days. After 3 days, cells were washed in fresh R10, transferred to a new plate, and allowed to rest for 2 days. Cells were also treated with 25, 100, 175, or 250 μM of putrescine depending on the experiment (these details can be found in the results section). Cells were cultured for a total of 5 days and media was refreshed every 48 hours.

In one experiment R10 media was also supplemented with 10 mM HEPES buffer (Thermo Fisher Scientific), 1X MEM Non-Essential Amino Acids Solution (100X, Thermo Fisher Scientific), and 100 μM 2-mercaptoethanol (Sigma Aldrich).

To assess cytokine production co-cultures were treated with Phorbol Myristate Acetate–Ionomycin (SIGSa, St. Louis, MO) and GolgiPlug (BD Biosciences, San Jose, CA) for 4 to 6 hours prior to cell staining. Following extracellular antibody staining, intracellular cytokine staining was carried out using the FoxP3/Transcription Factor Staining Buffer Kit (Tonbo Biosciences) following manufacturer's instructions. Cells were stained using Aqua and anti-human CD4, CD25, CD3, and FoxP3 as described in the Treg section. Cells were also stained with anti-human CD8a (BV605, Dilution: 1:100, Clone: RPA-T8, BioLegend); IFNy (FITC, Dilution 1:200, Clone: B27, BD Biosciences); IL-4 (PE, Dilution: 1:20, Clone: 7A3-3, Miltenyi Biotec); IL-17a (eFluor660, Dilution: 1:20, Clone: eBio64DEC17, eBioscience).

Flow cytometry data were collected on a BD LSR II flow cytometer (BD Biosciences) and T cell subsets were defined as follows: Th1, $CD3^+CD4^+IFN\gamma^+$; Th2, $CD3^+CD4^+IL\text{-}4^+$; Th17, $CD3^+CD4^+IL\text{-}17^+$; Treg cells, $CD3^+CD4^+CD25^+FoxP3^+$.

3.5.3.4 *DC/T cell experiments with L-Ornithine*

DCs were plated at 100,000 cells/well and cultured in R10 media supplemented with 10 ng ml^{-1} GM–CSF (R&D Systems) and 20 ng ml^{-1} IL-4 (R&D Systems) (DC treatment media) at 37 °C for 3 days with cell free fecal water (CFW) or CFW plus 25, 100, or 175 μM of L-ornithine. Some DCs were also treated with 12.5 ng/mL recombinant human IL-4 (PeproTech) or 40 ng/mL recombinant human IL-10 (PeproTech). The DC treatment media was freshly prepared and replaced every 48 hours. After three days, DCs were stimulated with 10 ng ml^{-1} TNF, IL-1β and IL-6 (Peprotech) and 1 mM prostaglandin E2 (STEMCELL Technologies) for 24 hours. DCs were subsequently washed in fresh R10 media, counted via flow cytometry, and plated in TexMACs Medium (Miltenyi Biotec) at 25,000 cells per well.

Following purification, autologous naive $CD4^+$ T cells were suspended in TexMACS Medium (Miltenyi Biotec) and added to the treated DCs at a ratio of 5:1 in the presence of 10 ng ml^{-1} anti-CD28 (Clone: CD28.2, BD Biosciences) and anti-CD49d (Clone: 9F10, BD Biosciences). T and DC cells were co-cultured for 5 days at 37 °C in 5% CO_2 and fresh TexMACS media was added every 48 hours.

Cytokine production and intracellular cytokine staining were carried out using the same methods described for Th2 experiments. Cells were stained using anti-human CD4, CD8a, CD25, CD3, IFNy, IL-4, and IL-17a as well as FoxP3 and Aqua as previously described in the text. Flow cytometry data collection and T cell subsets definitions can be found in the Th2 experimental method section.

3.5.3.5 *Cell-Free Fecal Water Preparation*

Biopsy punches (VWR International) were used to transfer 0.5 – 1.2 grams of frozen stool to a sterile tube and an equal volume of pre-warmed extraction buffer (PBS-

EDTA [Teknova], warmed to 37°C before use) was added. Samples were fully suspended in extraction buffer and then shaken for 10 min at 37°C, 1000 rpm. Solids were pelleted by centrifuging tubes at 3000k x g at 4°C for 30 minutes. The liquid layer was then transferred to a new, sterile tube and spun at 16000 x g for 10 min at 4°C. Up to 400 µL of the liquid layer was transferred to a Whatman Mini-UniPrep 0.45 µm syringeless filter (Sigma-Aldrich) and liquid was pressed through the membrane and recovered in the sterile container. Up to 400 µL of this filtrate was then transferred to a Whatman Mini-UniPrep 0.2 µm syringeless filter (Simga-Aldrich) and liquid was pressed through the membrane and recovered in the sterile container. Most samples had more than 400 µL of liquid so multiple Mini UniPrep filter units were used per sample. 0.2 µm filtered liquid was then aliquoted out in volumes of 100 µL and stored at -80°C until use in the DC/T cell assay described above.

CHAPTER 4: FUTURE DIRECTIONS

4.1 THE IMPACT OF DOG-KEEPING ON INFANT GUT MICROBIOTA DEVELOPMENT

While dog-exposure, especially between 3 and 6 months of age, seems to impact infant gut microbiota trajectories and gut community constituents over the first year of life future studies will be necessary to determine if there is a biological impact associated with the observed differences between dog-exposed and pet-free neonates. Metagenomic sequencing and untargeted mass-spectrometry could be carried out on samples from timepoints where dog-exposure imparted the greatest impact to determine microbial pathways and metabolites differentiating dog-exposed from pet-free neonates. If differences are not observed at one timepoint, metabolomic and metagenomic profiling could be done on all timepoints and the impact of dog-exposure on gut microbial community functional trajectory could be determined and pathways/metabolites that remain impacted over time could be examined.

To understand how functional differences in the gut microbiome of dog-exposed neonates impact host biology, cell-free fecal water could be generated from stool of dog-exposed or pet-free neonates and used in the established DC/T-cell co-culture assay to determine if the dog-associated microbial metabolite milieu differentially impacts T cell, or other immune cell, phenotypes. This co-culture assay could also be done using microbial metabolites identified by metagenomic and metabolomic profiling as being statistically significantly enriched in dog-exposed infants. Further experiments could also be done to determine the impact of key microbial metabolites on immune cell function.

Additionally, germ-free mice could also be treated with stool from dog-exposed or pet-free infants and the impact of these microbial communities on allergen-mediated airway pathology could be examined. Alternatively, we could use random forest analysis to determine taxa predictive of non-asthma-associated phenotypes, such as low concentrations of specific immunoglobulin E to one or more allergens at one year of age. Following identification of bacteria with potential protective capacity, we could isolate these bacteria from neonatal stool samples and either do experiments treating immune cells with cell-free supernatant from bacterial cultures or carry out supplementation studies in mice subsequently exposed to allergic airway challenge.

4.2 THE CAPACITY OF THE EARLY-LIFE GUT MICROBIOTA TO METABOLIZE STEROIDS

Future directions for the DC/T cell experiments would entail trying different vehicles for progesterone, such as ethanol, and different concentrations of lipopolysaccharide to determine a concertation that stimulates cells but does not lead to cell death. Additionally, experiments may not have been successful because the DCs used were directly isolated from adult blood. Human monocyte derived DCs may have greater plasticity than DCs directly isolated from blood and thus may be a more appropriate choice for these experiments. Additionally, it may be more relevant to use DCs isolated from cord blood instead of adult blood as the effects of our steroid of interest may only be observed in early life.

The next crucial step for this study will be to optimize and run a mass spectrometry protocol using blood and stool samples collected from mice to determine if supplementation with 17-OH-Preg leads to higher levels of DHEA. If higher concentrations of DHEA are observed in 17-OH-Preg supplemented mice we would

next use sequencing techniques to determine differences in the relative abundance of bacterial taxa in the intestine. Bacteria with a statistically significantly higher relative abundance in supplemented verses unsupplemented mice would then be isolated from mouse stool or intestinal samples, cultured in the presence of 17-OH-Preg, and supernatants would be analyzed via mass spectrometry to identify strains with the capacity to synthesize DHEA.

If we do not observe an increase in DHEA but do observe a decrease in 17-OH-Preg concertation in our murine samples this may suggest a different downstream metabolite is leading to the protection against airway inflammation observed in the supplemented mice. If this is the case, we could perform untargeted mass spectrometry to determine metabolites enriched in supplemented verses unsupplemented mice and screen murine gut isolates for their capacity to produce this metabolite. Future studies would involve supplementing mice with bacteria capable of synthesizing DHEA or the alternative steroid of interest and investigating whether this bacterial supplementation also promoted protection from allergen-induced airway inflammation.

4.3 IMPACT OF POLYAMINES & AMINO ACIDS ON ASTHMA ASSOCIATED IMMUNE CELL PHENOTYPES

Although this study had a small number of samples, the integration of shotgun metagenomic and metabolomic data offered multiple lines of evidence suggesting a divergent role of microbial amino acid metabolism in healthy compared to high-risk for asthma infants at 6 months of age. While there is support in the literature suggesting polyamines and amino acids can be produced by gut microbes and can also play a role in influencing immune cells[259,262,263] additional research will be necessary to validate our findings. This would entail analyses of microbial pathways in a larger cohort of healthy

and high-risk for asthma infants at multiple timepoints as well as complementary biological experiments.

4.3.1 T Cell Polarization Experiments

One reason we may not be able to achieve Th2 differentiation is because we are using adult lymphocytes whereas most of the published literature uses lymphocytes isolated from cord blood. Future experiments would involve testing the multiple previously tested conditions on naive $CD4^+$ T cells from cord blood.

4.3.2 DC/T Cell Co-Culture Experiments

Instead of using high-risk for asthma cell-free fecal water (CFW) to promote Th2 differentiation, future experiments should test alternative antigenic stimuli. Once we have determined the appropriate stimulus for DCs to promote a Th2 phenotype we can then test various concentrations of L-ornithine to see if it reduces Th2 induction and/or promotes Treg differentiation. Alternatively, we could use transcriptomics to examine the broad impact of the fecal metabolite milieu from healthy control (HC) infants on DCs and T cells. This experiment would entail treating DCs with CFW from high-risk placebo (HRP) or HC infants in the presence and absence of an antigenic stimulus, such as cockroach allergen (CRA). DCs would then either be harvested 12 hours after exposure or co-cultured with autologous T cells and harvested, with the T cell population, after 5 days. RNAseq would then be performed to determine the impact of fecal metabolites associated with high-risk for asthma infants and healthy infants on immune cell gene expression in the context of antigenic stimulation.

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