

Abbreviations used

ACQ:	Asthma Control Questionnaire
BH:	Benjamini-Hochberg
BMI:	Body mass index
BOBCAT:	Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma
FKBP5:	FK506 binding protein
PB:	Protected brush
PERMANOVA:	Permutational multivariate analysis of variance
PICRUST:	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
qPCR:	Quantitative PCR
16S rRNA:	16S Ribosomal RNA

In patients with mild-to-moderate asthma, relationships between the airway microbiome and clinical features have been observed.⁸ Whether similar relationships exist in patients with severe asthma are unknown. To address this knowledge gap, we examined the bacterial microbiome in protected bronchial brushings collected from patients with severe asthma in the Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma (BOBCAT) study.¹² Bacterial composition was analyzed by using 16S ribosomal RNA (16S rRNA)-based methods, followed by *in silico* predictive metagenomic analysis of bacterial groups of interest. We identified significant relationships between airway microbiota members and features of severe asthma. Additional analyses demonstrated differences in bronchial microbiota composition between this severe asthma cohort and healthy control subjects and patients with mild-to-moderate asthma, whose bronchial microbiomes were examined in an earlier study.⁸ Our primary findings invite speculation that features of the bronchial microbiome can contribute to endotypes of severe asthma.

METHODS**Patients with severe asthma and sample processing for microbiome analysis**

As previously described,¹² the BOBCAT study was a multicenter, 3-visit study that enrolled 67 patients with severe asthma, which was defined as an FEV₁ of 40% to 80% of predicted value, an Asthma Control Questionnaire (ACQ) score of greater than 1.50, and a daily inhaled corticosteroid dose equivalent to 1000 µg of beclomethasone or greater. Subjects underwent sputum induction (visit 2), bronchoscopy (visit 3), and blood sampling. Medications were kept constant during the study; no corticosteroids or antibiotics were started or changed from 30 days before visit 1 through visit 3.

To ensure total DNA was extracted from the same number of protected brushes (PBs) for all evaluated subjects and on the basis of our prior experience⁸ that brush extractions yield variable amounts of DNA, we required at least 2 brushes to evaluate for microbiome analysis. Forty subjects fulfilled this criterion, and 2 PBs (stored in RNAlater at -80°C) were extracted. Subjects' characteristics were analyzed for differences between those whose samples were (n = 40) or were not (n = 27) evaluated for microbiome analysis by using nonparametric or parametric tests. Amplified 16S rRNA gene products (using universal primers 27F and 1492R¹³) were processed for profiling by using the 16S rRNA-based PhyloChip platform (Second Genome, South San Francisco, Calif), as previously described.^{8,14}

Data analysis

Raw array data were processed, as previously described,¹⁵⁻¹⁷ including scaling to spiked-in quantitative standards and normalization to the mean intensity of all samples. Bacterial taxa were identified based on 97% or greater

identity in 16S rRNA gene sequences (Greengenes 2011 iteration¹⁸) by using published methods.^{14,15,19} Details are available in the **Methods** section in this article's Online Repository at www.jacionline.org. Log₂-transformed fluorescence intensities, which are correlative with the relative abundance of taxa,²⁰ were used for all analyses in R software. Array data were normalized separately for exploratory analyses of microbiota differences between patients with severe asthma and previously studied⁸ healthy subjects and patients with mild-to-moderate asthma from whom PB-sampled bacterial composition had been profiled by the same platform (see data sets in **Table E1** in this article's Online Repository at www.jacionline.org). The healthy control data set included 2 subjects not in the earlier study⁸ but who had been evaluated at the University of California San Francisco by using the same criteria to exclude asthma.

α-Diversity for each sample was calculated,^{21,22} including Shannon and inverse Simpson indices (number and relative distribution of taxa) and Faith phylogenetic diversity, which additionally weights phylogenetic relationships.²³ Bray-Curtis and UniFrac distance measures^{24,25} were calculated to evaluate between-sample compositional differences by means of ordination analyses and in PERMANOVA tests with clinical and inflammatory variables.²⁶ Correlation analyses or the Significance of Analysis of Microarrays method, based on a univariate penalized regression approach,²⁷ were used to identify specific taxa-to-variable relationships. Adjustments for multiple comparisons were applied (Benjamini-Hochberg [BH] correction or *q* values²⁸). For predictive metagenomic analyses, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST)²⁹ was used to predict gene functions and KEGG pathways.

Airway epithelial gene expression analysis

RNA extracted from a separate epithelial brush was amplified for Agilent (Santa Clara, Calif) 2-color Whole Human Genome 4x44k gene expression microarray analysis. Universal Human Reference RNA (Stratagene, La Jolla, Calif) was used for the reference channel. T_H2 and T_H17 scores were calculated by means of case-wise averaging of zero-centered gene expression data after annotation-based independent filtering³⁰; that is, if multiple probes correspond to an Entrez gene, the probe with the highest interquartile range was selected. *SERPINE2*, *CLCA1*, and *POSTN* were used as IL-13-responsive T_H2 signature genes, as described previously.³¹ *CXCL1*, *CXCL2*, *CXCL3*, *IL8*, and *CSF3* were used as IL-17-responsive T_H17 signature genes based on previous evidence that expression of these genes is involved in IL-17-driven pathways.³²⁻³⁴ The data have been deposited in the National Center for Biotechnology Information's NCBI GEO database (accession no. GSE65584).

Quantitative PCR studies

Total 16S rRNA gene copies, as well as copy number determinations to validate specific species of interest, were assessed by using quantitative PCR (qPCR). Further details are provided in the **Methods** section in this article's Online Repository.

RESULTS**Characteristics of patients with severe asthma**

PBs from 40 of 67 subjects in the BOBCAT study underwent evaluation for bacterial microbiome analysis. Bacterial evaluation was not pursued in the other 27 subjects because they lacked the requisite number of 2 PBs for technical consistency in source DNA extractions. Of the evaluated subjects, 30 demonstrated sufficient amplified 16S rRNA product (prespecified threshold of ≥100 ng) for array-based profiling.

Characteristics of these subjects are summarized in **Table I**. Mean FEV₁ percent predicted (average of visit 1 and visit 2) was 59%, with a mean ACQ score of 2.4, which is indicative of poor control. qPCR experiments confirmed that this group had

TABLE I. Characteristics of the 30 patients with severe asthma for whom bronchial microbiome analysis was performed

Age (y)	46 (20 to 63)
White race (%)	24 (80)
Female sex, no. (%)	11 (37)
BMI (kg/m ²)	28.7 ± 6.9 (19.3 to 52.1)
FEV ₁ /FVC ratio	0.73 (0.55 to 1.0)
FEV ₁ (% predicted), visit 1	59.9 (34.3 to 77.7)
FEV ₁ (% predicted), visit 2	58.3 (33.6 to 82.8)
Bronchodilator reversibility (%)	13.7 (−8.6 to 38.9)
ACQ score, visit 1	2.4 ± 0.6
ACQ score, visit 2	2.4 ± 0.8
Prednisolone use, no. (%)	6 (20)
FENO (ppb)	42.8 ± 37.9 (9.7-141.5)
Sputum eosinophils (%)	5.5 ± 5.9 (0.0 to 18.8)
Sputum neutrophils (%)	52.4 ± 28.3 (0 to 88.3)
Blood absolute eosinophils (× 10 ³ /μL)	0.29 ± 0.2
Serum IgE (IU/mL)	180.8 ± 202.9 (9 to 835.2)
Serum periostin (ng/mL)	24.2 ± 7.3

Values are means ± SDs (percentages or ranges).

FENO, Fraction of exhaled nitric oxide representing the mean from visits 1 and 2; FVC, forced vital capacity.

significantly higher total 16S rRNA copy numbers compared with the other 10 evaluated subjects who had insufficient 16S rRNA amplicons (ie, low bacterial burden) for array-based profiling ($P < .05$, Wilcoxon rank sum test).

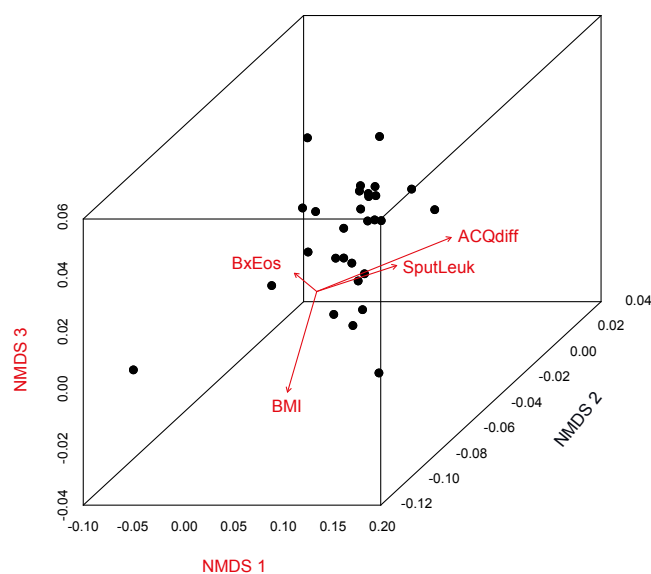
We examined whether characteristics differed among the evaluated subjects between those in whom bacterial compositional profiles were or were not successfully obtained. The subjects in whom microbiome analysis could be performed had significantly lower sputum eosinophil percentages ($5.5\% \pm 5.9\%$ vs $17.3\% \pm 21.1\%$, $P < .05$) and a trend toward lower bronchial biopsy eosinophil numbers (31.8 ± 33.7 vs 45.2 ± 19.3 eosinophils/mm², $P = .09$). We also analyzed whether characteristics differed between BOBCAT study participants whose samples were ($n = 40$) or were not ($n = 27$) evaluated for microbiome profiling. The evaluated group had lower mean body mass index (BMI; 29 vs 34 kg/m², $P < .01$) but higher numbers of biopsied IL-17A- and IL-17F-positive cells ($P < .001$ and $P < .05$, respectively). In both sets of analyses, there were no significant differences between the compared subgroups in other clinical and inflammatory parameters, including 3 examined gene expression patterns (FK506 binding protein [*FKBP5*], T_H2 , and T_H17).

Severe asthma features are associated with differences in airway bacterial composition

Distance-based PERMANOVA²⁶ analyses (Bray-Curtis or weighted UniFrac distances) were performed to identify factors that might explain variation in microbial community composition among samples (Table II). Identified factors included BMI ($P < .05$), ACQ scores at visit 2 ($P < .05$), and changes in ACQ scores during the study (visit 2 minus visit 1, $P < .01$). Associations that trended toward significance included sputum total leukocyte percentages ($P = .06$) and bronchial biopsy eosinophil numbers (per square millimeter, $P = .07$). These observations indicate that specific bacterial compositions are associated with particular disease features in this cohort. No associations with other assessed variables, including inflammatory measures from blood or bronchoalveolar lavage fluid, were found (results for all evaluated variables are available in Table E2 in this article's

TABLE II. Clinical and inflammatory features of patients with severe asthma demonstrating associations with differences in bacterial community composition based on Bray-Curtis distance measures

Variable	Distance-based PERMANOVA result (P value)
BMI	.05
ACQ score, visit 2	.05
ACQ score change (visit 2 – visit 1)	.01
Sputum total leukocyte value	.06
Biopsy eosinophil cells (mm ^{−2})	.07

**FIG 1.** Nonmetric multidimensional scaling analysis (NMDS) based on Bray-Curtis distances showing differences in bronchial bacterial composition among 30 patients with severe asthma. Each dot represents the overall bacterial community in each subject. Vectors indicate linear regression-based fits for variables found by using independent distance-based PERMANOVA testing to be associated with distinct bacterial compositions. Vector directions indicate that the specific communities associated with differences in ACQ scores (ACQdiff) and sputum total leukocyte values (SputLeuk) are similar to each other and differ from specific communities associated with BMI and biopsy eosinophil values (BxEos).

Online Repository at www.jacionline.org). To visualize these relationships, ordination analysis was used (nonmetric multidimensional scaling, Fig 1). Regression-based linear fits to this ordination indicated that change in ACQ score and sputum total leukocyte values were associated with microbiome compositions distinct from those associated with BMI and biopsy eosinophil numbers.

Distinct microbiota members are associated with different features of severe asthma

Taxon-level correlation analyses were performed between the relative abundance of each detected taxon and each variable across all subjects to identify specific bacterial taxa associated with these variables of interest. Results confirmed the ordination analyses in that taxa significantly associated with BMI were distinct from those associated with the other features.

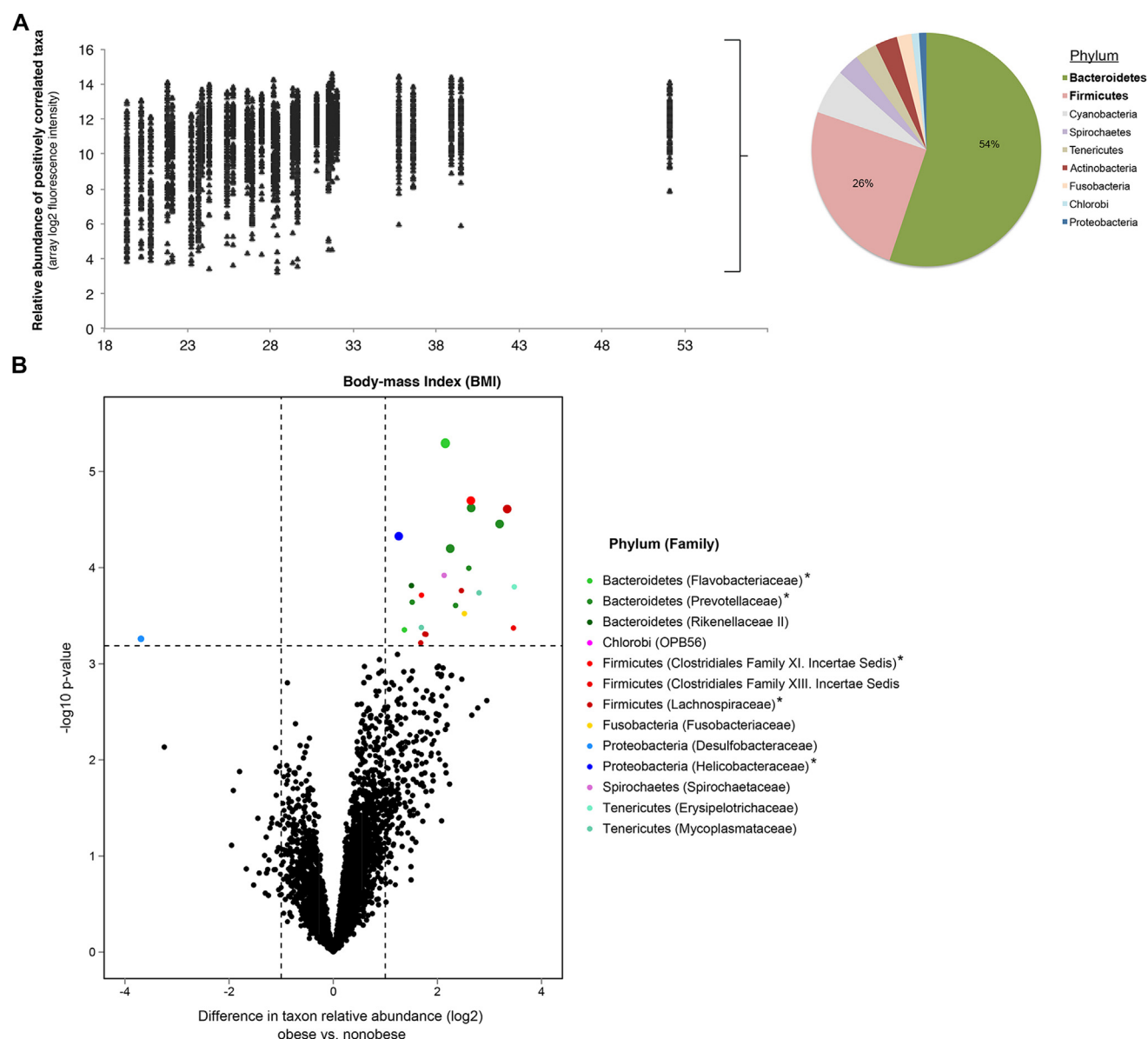


FIG 2. A, Relative abundance plotted against BMI for the 98 taxa found to be significantly correlated with BMI among the patients with severe asthma studied ($R = 0.5-0.8$, BH-adjusted $P < .05$). The distribution of bacterial phyla representing these taxa are shown, which are mainly Bacteroidetes and Firmicutes. **B**, Differences in the relative abundance of all detected taxa between obese (BMI > 30 kg/m², $n = 10$) and nonobese ($n = 19$) patients with severe asthma. Taxa significantly enriched among obese subjects (2-fold, adjusted $P < .10$) include members of the Bacteroidetes and Firmicutes phyla, such as *Prevotella* species. Dashed lines indicate a 2-fold difference ($\log_2 = 1.0$) and false discovery rate significance level of 10%, respectively. Asterisks indicate taxa most significantly greater in abundance among obese subjects (adjusted $P < .05$), which are also represented by larger circles in the plot.

Bacteroidetes and Firmicutes comprised 54% and 26%, respectively, of the 98 taxa significantly correlated with BMI (Pearson $R = 0.5-0.8$; BH-adjusted $P < .05$; Fig 2, A). This contrasted with the predominant representation of Proteobacteria among taxa uncorrelated with BMI ($P < .01$, χ^2 test). Further analysis for specific compositional differences between obese (BMI ≥ 30 kg/m²) and nonobese subjects identified several taxa more abundant among obese subjects (R package *limma* moderated t test, BH-adjusted $P < .05$; Fig 2, B). These included members of the Prevotellaceae, Mycoplasmataceae, Lachnospiraceae (*Clostridium* species), and Spirochaetaceae (*Treponema* species; see Table E3 in this article's

Online Repository at www.jacionline.org). Given this, we explored whether obese and nonobese subjects in this microbiome-profiled group differed in other characteristics. The only significant finding was that obese subjects had fewer biopsy eosinophils than nonobese subjects (mean biopsy eosinophil numbers, 11.9/mm² in subjects with high BMI vs 45.3/mm² in subjects with low BMI; $P < .01$).

In contrast to BMI, Proteobacteria represented the majority of taxa ($>90\%$) whose relative abundance correlated with worsening ACQ scores (visit 2 minus visit 1 scores; $R = 0.5-0.7$, BH-adjusted $P < .05$; Fig 3, A). These included families representing

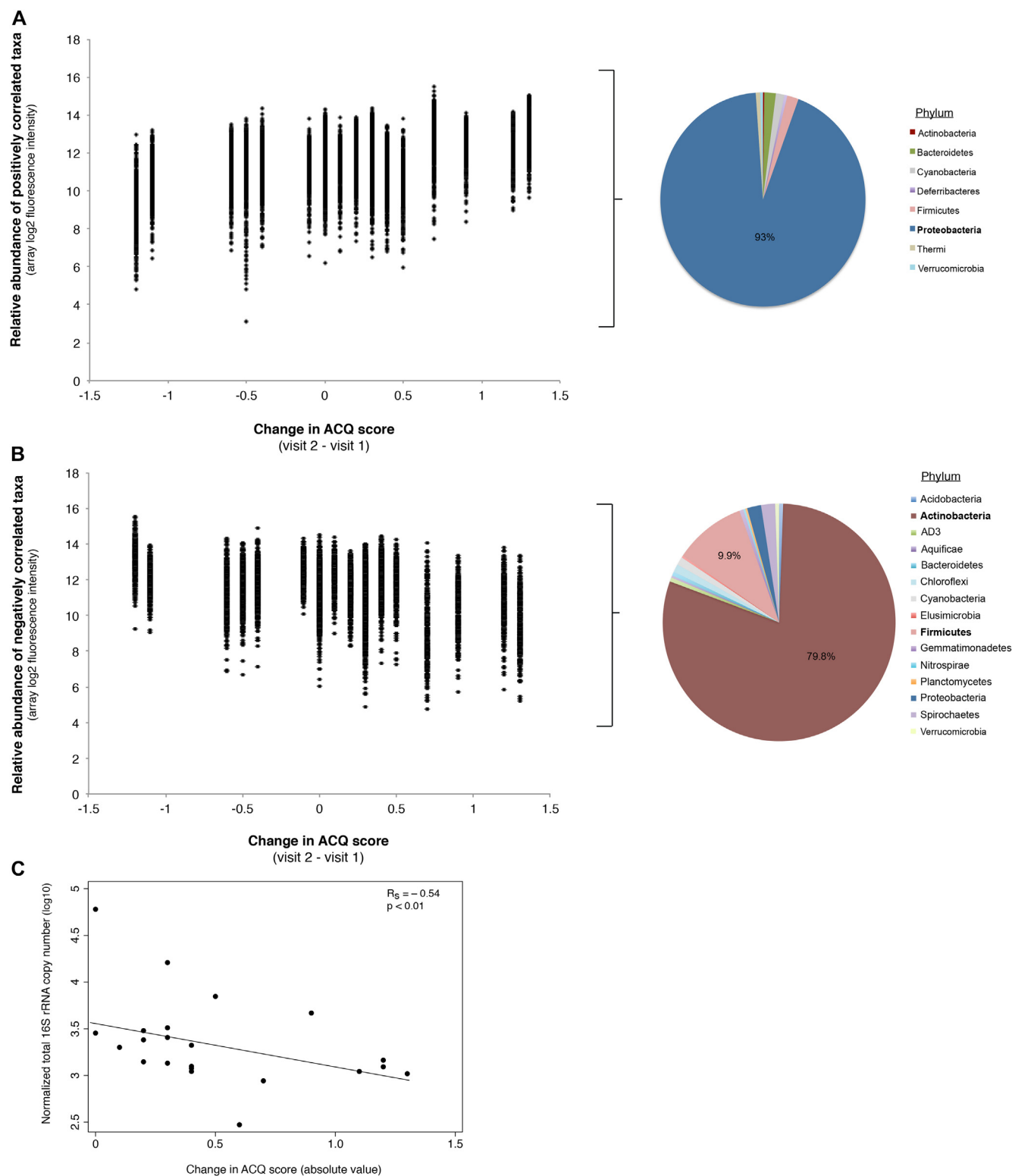


FIG 3. **A**, Relative abundance plotted against changes in ACQ scores for the bacterial taxa ($n = 448$) found to be positively correlated with this parameter ($R = 0.5$ - 0.7 , BH-adjusted $P < .05$). Increasing values of the change in ACQ score are associated with greater relative abundance predominantly of Proteobacteria members. **B**, Relative abundance plotted against change in ACQ score for the bacterial taxa ($n = 362$) found to be negatively correlated with changes in ACQ scores ($R = -0.5$ to -0.8 , BH-adjusted $P < .05$). Decreasing values of the change in ACQ score are associated with greater relative abundance of predominantly members of the Actinobacteria, followed by Firmicutes, phyla. **C**, Total 16S rRNA copy numbers, a proxy for bacterial burden, are inversely correlated with absolute values of the change in ACQ score (Spearman $\rho = -0.54$, $P < .01$).

known respiratory pathogens, such as Pasteurellaceae, Enterobacteriaceae, Neisseriaceae, Burkholderiaceae, and Pseudomonadaceae. Similar results were observed among communities significantly correlated with higher visit 2 ACQ scores (see Fig E1 in this article's Online Repository at www.jacionline.org). In contrast, taxa correlated with improving or stable ACQ scores were primarily Actinobacteria (80%; Fig 3, B), such as Streptomycetaceae, Nocardiaceae, and Mycobacteriaceae. Analyses with absolute values of the change in ACQ score, presumably a reflection of instability in asthma control, also showed Proteobacteria to most represent positively correlated communities (40/91 taxa; $R = 0.4-0.6$, $q < 0.15$). Total 16S rRNA copy numbers demonstrated an inverse relationship to absolute values of the change in ACQ score (Spearman $\rho = -0.54$, $P < .01$; Fig 3, C), with a similar trend observed with inverse Simpson diversity ($R = -0.37$, $P = .09$), suggesting that greater bacterial burden in general is associated with less variation in asthma control.

Proteobacteria also constituted the majority of taxa (71% [73/103] of taxa) significantly correlated with sputum total leukocyte numbers (mean $\pm$ SD, 812 ± 678 cells/mL). Families with members demonstrating strong correlations with this parameter ($R = 0.6-0.8$, BH-adjusted $P < .05$) included the Enterobacteriaceae and Moraxellaceae. Total 16S rRNA copy numbers significantly correlated with sputum total leukocyte numbers ($R = 0.4$, $P < .05$), which indicates that bacterial burden is associated with increased numbers of inflammatory cells.

In contrast, significant but predominantly negative associations were seen between biopsy eosinophil numbers and the relative abundance of specific bacteria (71 taxa, $R = -0.5$ to -0.74 , $q < 0.10$). These negatively correlated communities were mostly Proteobacteria (64% [45/71] of taxa), followed by Firmicutes, and included members of the Moraxellaceae and Helicobacteraceae (see Table E4 in this article's Online Repository at www.jacionline.org). Only 2 taxa demonstrated a significant positive correlation with biopsy eosinophil numbers, both Actinobacteria (*Streptomyces* and *Propionimonas* species). Total 16S rRNA copy numbers were also inversely correlated with biopsy eosinophil numbers ($R = -0.50$, $P < .01$; Fig 4, A), suggesting that higher bacterial burden is associated with less eosinophil infiltration in bronchial tissue. We did not observe relationships between bacterial composition and other indicators of eosinophilic inflammation (eg, sputum or blood eosinophil, fraction of exhaled nitric oxide, and periostin values).

Bacterial microbiota are associated with specific airway epithelial gene expression signatures

For 33 of the 40 subjects evaluated for bacterial profiling, matched airway epithelial gene expression microarray analysis was performed (23/30 microbiome-profiled and 10/10 non-microbiome-profiled subjects). Brushes analyzed for epithelial gene expression were collected concurrently with those evaluated for microbiome profiling, presenting a unique opportunity to explore contemporaneous relationships between the microbiome and local host epithelial gene expression. We specifically explored relationships to 3 gene expression patterns identified by using microarray analysis: a marker of steroid response (*FKBP5*), T_H2 -associated inflammation (*POSTN*, *CLCA1*, and *SERPINB2*),⁵ and T_H17 -associated inflammation involving genes previously shown to be involved in IL-17-driven pathways (*CXCL1*, *CXCL2*, *CXCL3*, *CXCL8*, and *CSF3*).³²⁻³⁴

Positive associations were observed between *FKBP5* expression and the relative abundance of 83 taxa ($q < 0.10$, Significance of Analysis of Microarrays), largely Actinobacteria (58%) and Proteobacteria (27%; Fig 4, B). Bacterial diversity measures were also significantly correlated with *FKBP5* expression ($R = 0.5$, $P < .01$; Fig 4, C), indicating that transcriptional evidence of steroid response is associated with increased community diversity.

No significant associations were observed between microbiota members and the T_H2 -related gene signature (at false discovery rate thresholds of up to 0.20). At a higher cutoff ($q < 0.25$), 16 taxa demonstrated negative associations with this gene signature. These included members of the Moraxellaceae and Helicobacteraceae, which were also represented among taxa negatively correlated with biopsy eosinophil numbers (see Table E4).

Positive correlations with the T_H17 -associated gene expression pattern were observed for a number of taxa. These communities were mainly Proteobacteria (72% [80/110] of taxa; $R = 0.5-0.7$, $P < .04$, $q < 0.20$), such as Pasteurellaceae, Enterobacteriaceae, and Bacillaceae (see Table E5 in this article's Online Repository at www.jacionline.org). Although Proteobacteria also represented a proportion of taxa correlated with *FKBP5* expression, the particular families largely did not overlap with those Proteobacteria families positively associated with the T_H17 expression pattern. Only 11 Proteobacteria families were represented in both gene signatures among correlated taxa, which is in contrast to the many more Proteobacteria families associated with either signature (41 families represented only among *FKBP5*-correlated taxa and 57 families represented only among T_H17 -correlated taxa). The compositional dissimilarity of taxa correlated with these 2 expression patterns was also evident by using nonmetric multidimensional scaling analysis (Fig 5). This suggests particular airway microbiota might promote T_H17 inflammation, resulting in neutrophil recruitment and contributing to neutrophilic airway inflammation independent of steroid-induced effects.

Predicted community functions of specific taxa related to severe asthma features

Shotgun metagenomic sequencing approaches can provide insight into the functional capacities of microbiota but to do so at high resolution is challenging. To explore the predicted functional capacity of identified taxa of interest in this study, we applied an alternative, informatics-based approach called PICRUSt,²⁹ which uses 16S rRNA sequence information to infer bacterial metagenomes and was shown to recapitulate results of actual metagenomic sequencing from the Human Microbiome Project.

Representative 16S rRNA sequences for taxa demonstrating significant associations (positive and negative) with variables of interest in this study were inputted into PICRUSt. The outputs are predicted functions defined as KEGG gene orthologs and represented as KEGG pathways where these functions are known to be involved. For example, predicted functions present among taxa correlated with BMI (see Fig E2 in this article's Online Repository at www.jacionline.org) included pathways in carbohydrate digestion and absorption, insulin signaling, and microbially related signaling and inflammation (eg, NOD-like receptor signaling, bacterial toxins and epithelial cell signaling in *Helicobacter* species infection). This suggests that particular airway-associated microbiota might incite inflammatory responses among overweight/

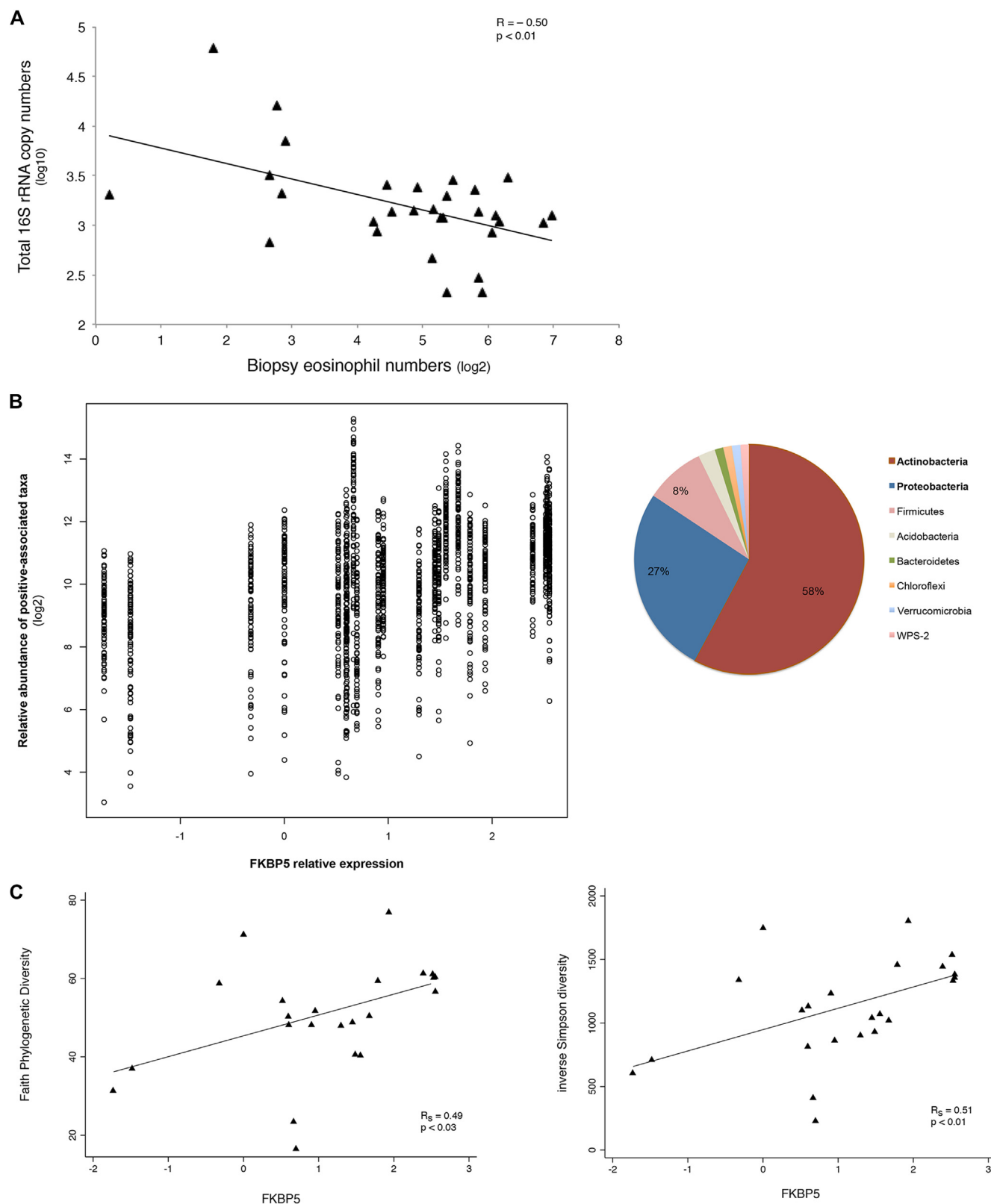


FIG 4. **A**, Total 16S rRNA copy numbers, a proxy for bacterial burden, are inversely correlated with biopsy eosinophil cell numbers ($R = -0.50$, $P < .01$). Data shown represent 29 of 40 subjects, including the subjects in whom array-based bacterial community profiling could not be performed because of low bacterial content. **B**, The relative abundance of bacterial taxa ($n = 83$, $q < .10$) associated with *FKBP5* expression plotted against the relative expression values for this gene. Phylum-level classification of these correlated communities is shown. **C**, Strongly significant correlations are seen between *FKBP5* expression and 2 different measures of bacterial community diversity: Faith phylogenetic diversity and the inverse Simpson diversity index. The former weights phylogenetic relationships among communities in the diversity determination, whereas the latter reflects primarily community richness and evenness.

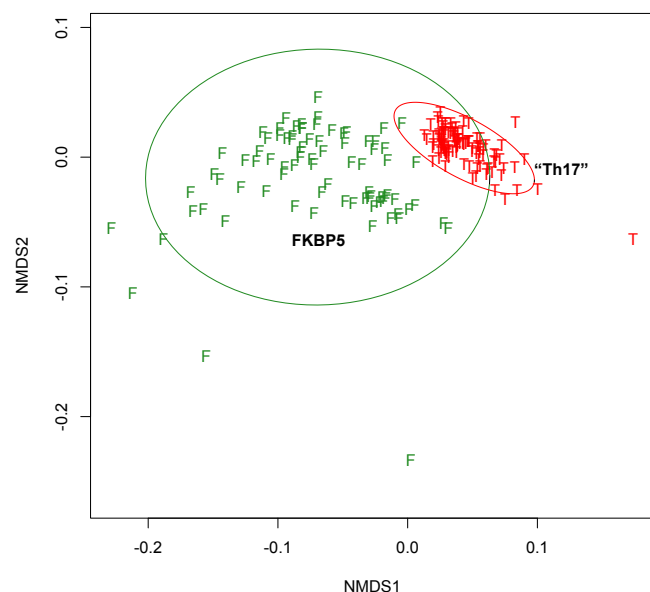


FIG 5. Compositionally distinct bacterial taxa are positively associated with *FKBP5* expression (green Fs; $n = 83$; 58% Actinobacteria) and the T_H17 -related gene expression pattern (red Ts; $n = 110$; 72% Proteobacteria). The nonmetric multidimensional scaling (NMDS) ordination is based on a Bray-Curtis distance matrix for all associated taxa in both expression patterns. Ellipses represent the 95% CIs for the SDs of the distances for each group.

obese asthmatic patients in this cohort. Analysis results for predicted functions among taxa associated with asthma control (ACQ score change) and with *FKBP5* and T_H17 -related gene expression pattern are shown in Figs E3 and E4 in this article's Online Repository at www.jacionline.org.

Bronchial microbiota differences between patients with severe asthma, healthy control subjects, and patients with mild-to-moderate asthma

We explored whether bronchial bacterial composition differs in patients with severe asthma from that profiled from previously studied healthy control subjects ($n = 7$) or patients with mild-to-moderate asthma who were receiving low-dose inhaled steroids ($n = 41$, 88 μg of fluticasone propionate twice daily).⁸ Compared with healthy control subjects (Fig 6, A, and see Table E6 in this article's Online Repository at www.jacionline.org), patients with severe asthma were enriched in several taxa, with the largest fold difference seen in a *Klebsiella* species (moderated t statistic linear models, R package *limma*, BH-adjusted $P < .001$). Compared with patients with mild-to-moderate asthma (Fig 6, B, and see Table E6), patients with severe asthma also were significantly enriched in 53 taxa, with the majority being Actinobacteria (47/53) and the remaining 5 taxa classifying to Gammaproteobacteria (all Enterobacteriaceae). The latter included a *Klebsiella* species that, as in the comparison with healthy control subjects, exhibited the highest fold difference in relative abundance (7.9-fold increase in patients with severe asthma, BH-adjusted $P < .0001$). Conversely, 42 taxa were more abundant in patients with mild-to-moderate asthma relative to the group with severe asthma. These taxa represented members from multiple phyla, with several different families of Proteobacteria (19 taxa) being most represented. This contrasted from the reverse

comparison wherein Enterobacteriaceae was the sole family of Proteobacteria with enriched members in patients with severe asthma.

qPCR studies

qPCR with primers specific for *Haemophilus influenzae*,¹⁴ a Pasteurellaceae member associated with worsening ACQ scores and expression of T_H17 -related genes, confirmed a significant correlation between β -actin-normalized log copies of *H influenzae*-represented taxon ($\rho = 0.67$, $P < .02$). qPCR also confirmed a strong correlation between *Prevotella* species abundance in samples and increased BMI ($\rho = 0.61$, $P < .05$; see Fig E5 in this article's Online Repository at www.jacionline.org) by using primers targeting specific *Prevotella* species represented in the BMI-associated taxa. *Prevotella* species copy numbers were also significantly higher in obese versus nonobese subjects ($P < .05$).

DISCUSSION

In this cohort of adults with severe asthma, we observed significant relationships between the bronchial airway microbiome and clinical and inflammatory features. The findings suggest that specific members of the airway microbiota are associated with putative phenotypes of severe asthma, such as those characterized by obesity-associated disease, corticosteroid-responsive disease, or T_H17 -associated airway inflammation. Interestingly, microbiome-associated clinical features in this study (eg, obesity and poor symptom control) are characteristics previously described among patients with some severe asthma phenotypes.⁴ A key strength of this study was the capacity to explore microbiome relationships to severe asthma-related clinical and inflammatory measures in different compartments, including blood and the lower airways. This enabled a comprehensive examination of possible microbiota relationships to underlying pathophysiologic processes reflected in these measures. Also, we explored and identified differences in bronchial bacterial composition between patients with severe asthma and healthy control subjects and patients with mild-to-moderate asthma whose bronchial microbiota had previously been characterized.⁸ Overall, our findings invite speculation that patterns of airway dysbiosis can contribute to putative endotypes of severe asthma or even asthma in general.

Among patients with severe asthma, BMI was strongly correlated with particular microbiota members, specifically Bacteroidetes and Firmicutes. This pattern is in striking contrast to the increased prevalence of Proteobacteria observed in patients with milder asthma⁷⁻⁹ and other feature correlations observed in this study. Obese patients with severe asthma also had fewer biopsy eosinophil numbers than subjects with low BMI. This invites speculation about whether members of the Bacteroidetes and Firmicutes might influence the degree of eosinophilic inflammation seen in airway biopsy specimens or, alternatively, stimulate non-eosinophil-associated immune responses. These 2 phyla also represent gastrointestinal microbiota, suggesting the possibility of aspiration. Whether the observed BMI-associated taxa contribute to asthmatic airway inflammation cannot be answered from this study, but other lines of evidence suggest they could. Species of *Prevotella* species represented among these taxa are potential pathogens previously associated with periodontitis and arthritis,^{35,36} whereas Lachnospiraceae belong to a Clostridia

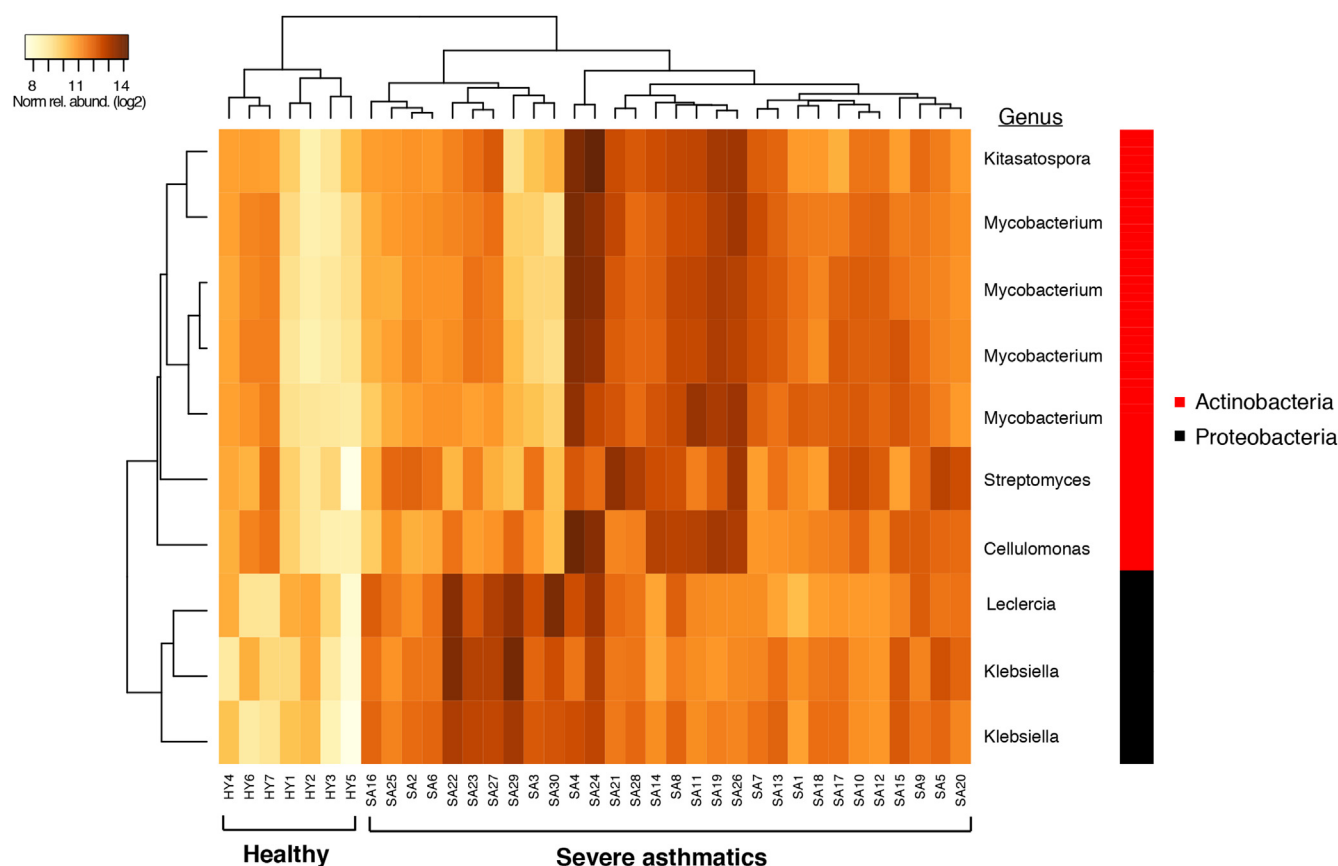


FIG 6. A, Heat map of the 10 taxa found to be significantly enriched among patients with severe asthma (n = 30) compared with healthy control subjects (n = 7; BH-adjusted $P < .15$; R package *limma*). B, Heat map of the 95 total taxa found to significantly differ in relative abundance between patients with severe asthma (n = 30) and those with mild-to-moderate asthma (n = 41; ≥ 2 -fold difference; BH-adjusted $P < .01$; R package *limma*). Relative to the other group, patients with severe asthma were enriched in 53 taxa, whereas patients with mild-to-moderate asthma were conversely enriched in 42 taxa.

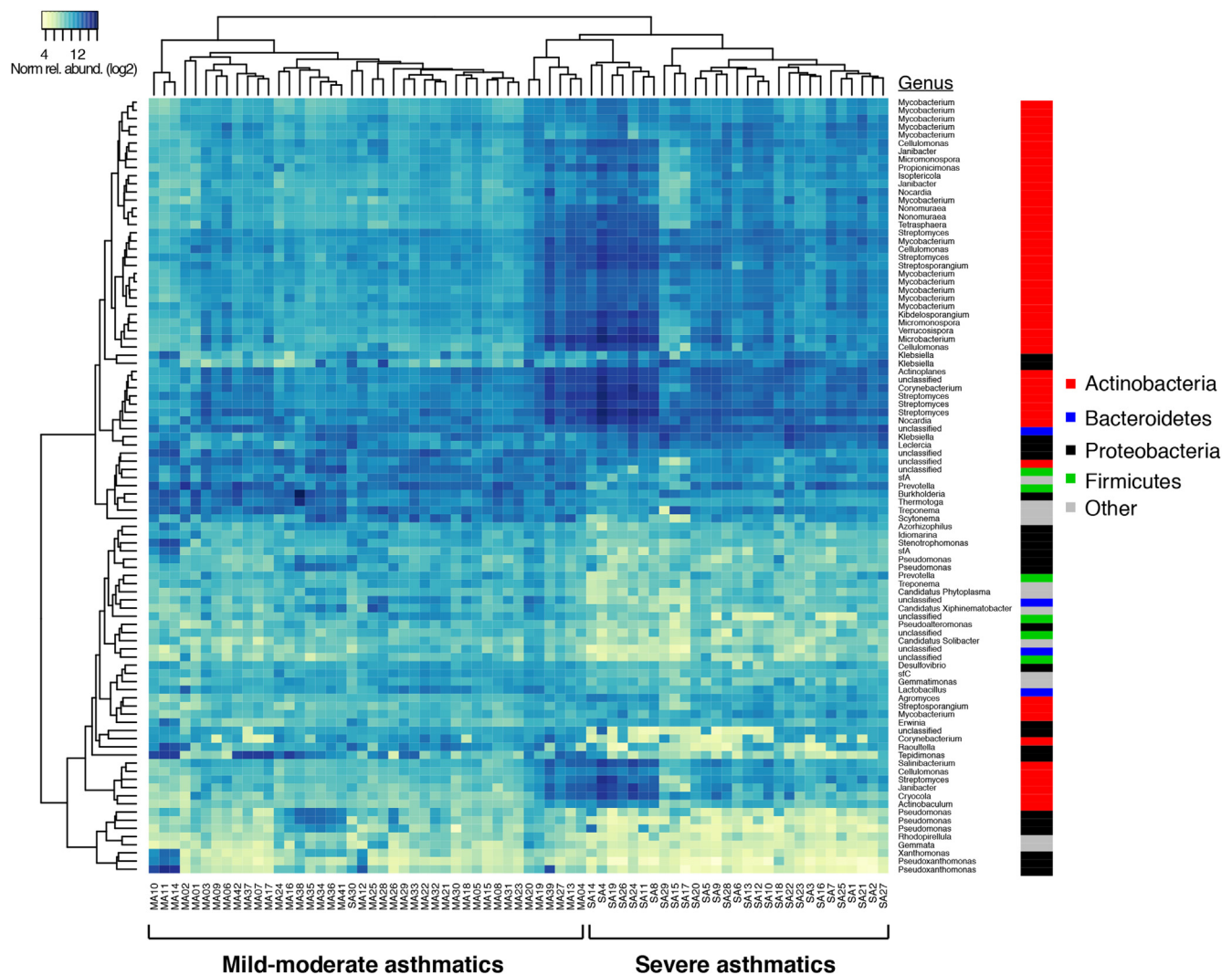
clade known to induce colonic CD4⁺ forkhead box protein 3–positive regulatory T cells.^{37,38} Thus there is precedent for the capacity of these organisms to potentially promote or regulate inflammation in the respiratory tract. This paralleled results from our predictive metagenomic analysis, suggesting the inflammation-eliciting potential of these communities.

Worsening ACQ scores among patients with severe asthma were associated with greater relative abundance of Proteobacteria (eg, Enterobacteriaceae, Neisseriaceae, and Pasteurellaceae, families representing potential respiratory pathogens), whereas improving/stable ACQ scores were associated with Actinobacteria. Actinobacteria are metabolically diverse and prolific producers of secondary metabolites, including many with antimicrobial and anti-inflammatory properties.³⁹ Interestingly, Actinobacteria also were the main group of organisms associated with molecular evidence of response to steroids (*FKBP5* expression). This suggests the possibility of certain airway microbiota being indicative of asthma responsive to steroids, adherence to prescribed therapy, or even their involvement in mechanisms of steroid response.

Study limitations include the site and timing of PB collection and the exclusion of other parent study subjects. PBs evaluated for microbiome analysis sampled at least 2 mucosal sites, with concurrently collected brushes used for gene expression studies, a

strength of this study. Although bronchoscopy followed earlier visit measurements of variables considered in our analyses (eg, ACQ score), there were no respiratory illnesses or medication changes to have influenced the bacteria identified. We cannot completely rule out possible biases related to the exclusion, for technical reasons, of other parent study subjects. However, a systematic analysis found only significant differences between evaluated and nonevaluated BOBCAT study subjects in BMI and biopsied numbers of IL-17⁺ cells. Finally, the primary aim of our study was to examine bronchial microbiome relationships to features of patients with severe asthma. No control group was enrolled and identically studied in the BOBCAT study. Therefore we performed a focused comparison of bronchial bacterial composition in patients with severe asthma with that profiled previously by using the same methods from PBs of healthy control subjects and patients with mild-to-moderate asthma studied earlier.⁸

Despite study differences and other potential biases that cannot be completely controlled, our findings suggest the following. First, airway dysbiosis is a feature of severe asthma and differs from that seen in patients with milder asthma receiving lower doses of inhaled corticosteroids. Second, different microbiota, even within the same bacterial phylum (eg, Proteobacteria), family, or even genus are implicated in different phenotypic



features of asthma, from putative disease-driven mechanisms (eg, T_H17 -related or non-type 2 airway inflammation) to potential modulation of treatment responses (eg, corticosteroids).

A surprising observation was the overall absence of positive relationships between bacterial composition and indicators of type 2-driven inflammation, from epithelial expression of type 2-responsive genes to various markers of eosinophil-related inflammation. Indeed, only biopsy eosinophil values demonstrated significant but predominantly negative correlations with bacterial microbiota members. The use of high-dose inhaled corticosteroids could suppress these measures, although the mean sputum eosinophil percentage was increased among subjects in whom microbiome profiling could be performed and these percentages correlated with relative expression of the type 2-responsive epithelial gene signature (Spearman $\rho = 0.53$, $P < .05$). Other markers (eg, fraction of exhaled nitric oxide, periostin, and blood or sputum eosinophil values) also did not demonstrate relationships to bacterial composition. Antibacterial effects of eosinophils are a consideration for these findings. However, because DNA-based detection methods were used, we expect that any DNA present from killed bacteria should still be detectable. Other possibilities include that heterogeneity within the cohort masked

bacterial associations with type 2-related inflammation or that other microbial kingdoms, such as fungi, which were previously observed to be reciprocally related to bacterial abundance,⁴⁰ play a larger role in driving these responses.

Finally, we identified a group of mainly Proteobacteria significantly associated with epithelial expression of T_H17 inflammation-related genes.⁴¹ T_H17 cells, through IL-17A and IL-17F, promote neutrophil recruitment and have been invoked as a noneosinophilic/non-type 2 pathway in asthmatic patients.⁴² Asthmatic patients without evident type 2-driven inflammation are less responsive to inhaled corticosteroids.⁵ In mice T_H17 cell-mediated airway inflammation is resistant to steroid treatment.⁴³ Our finding suggests that neutrophil-predominant airway inflammation in asthmatic patients could also represent responses to bacterial infection and not solely reflect steroidal effects. Although we did not identify specific taxa associated with sputum neutrophil values, this is not entirely surprising for in this cohort, sputum neutrophil percentages were not found to be concordant with other measures of airway inflammation.⁴⁴

In summary, results of this study suggest the airway microbiome might be highly pertinent to non-type 2 pathways in patients with severe asthma and influence manifestations of

particular disease features. Further dissection of the microbiome's role in asthma-related immune responses will require additional study of asthmatic patients with varying disease severities and phenotypes and consideration of other microbiota (eg, fungi). Our findings also suggest future mechanism-oriented investigations of specific microbial targets. Future investigations involving larger cohorts of patients with well-characterized asthma will likely be necessary to dissect the complex relationships between the airway microbiome and distinct asthma phenotypes.

We thank Ali Faruqi for his guidance in the PICRUST analyses.

Clinical implications: In patients with severe asthma, specific airway bacterial microbiota are associated with clinical and inflammatory features, including obesity and asthma control, but not markers of eosinophilic or type 2-related airway inflammation.

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Supplemental Methods:

Sample processing and PhyloChip-based bacterial community profiling

Sterilized forceps were used to transfer two brushes from each evaluated BOBCAT subject into a single lysing matrix B tube containing 0.1mm silica beads (MP Biomedical). Original collection tubes were centrifuged at 13,000 rpm for 10 minutes, RNALater removed and the remaining pellet re-suspended in 600 µl of Buffer RLT (AllPrep Kit; Qiagen) containing 1% beta-mercaptoethanol. The re-suspended pellet was transferred to the lysing matrix B tube containing the brushes. Sample bead beating was carried out using the FastPrep system (MP Biomedicals) at 5.5 m/s for 30 seconds. Afterwards tubes were centrifuged at 2,000 rpm for 3 minutes and the supernatant carefully transferred to a DNA spin column of the AllPrep Kit. DNA/RNA was extracted according to the kit instructions.

Samples were screened by PCR for presence of a 16S rRNA gene product, using 100 ng of extracted DNA and 3 µmol each of the universal 16S rRNA primers, Bact-27F and Bact-1492R in 50-µL volume reactions at an annealing temperature of 53 °C for 40 cycles in an Eppendorf MasterCycler® gradient PCR machine. Sterile water controls were used in all PCR runs and all samples were analyzed on 1% ethidium bromide-stained agarose gel to identify expected PCR product size. Those samples without visible evidence of 16S rRNA PCR product on this screen underwent repeat PCR screening with a positive control (*Pseudomonas aeruginosa* DNA) to rule out presence of sample-specific PCR inhibition. Samples with a PCR positive screen (i.e. any evidence of appropriate size 16S rRNA PCR product) proceeded to further preparation for PhyloChip

hybridization. Twelve-25 µl PCR reactions, each containing 50 ng of extracted sample DNA and 3 µmol each of Bact-27F and Bact-1492R primers, were performed across a gradient of annealing temperatures (48-58°C) for 25 cycles. Amplicons from all twelve reactions per sample were pooled, purified, and gel-quantified (E-gel® 2% and E-gel® Low Range Quantitative Ladder, Invitrogen Inc.). Samples were fragmented, biotin-labeled, and spike-in quantitative standards added comprising of known concentrations of synthetic 16S rRNA and non-16S rRNA gene fragments for within-array normalization. Twenty-four samples had 250 ng of amplicon hybridized, two samples at 175 ng, and four samples at 100 ng. To assess for potential differences in detected community composition related to different hybridization quantities, two samples with abundant 16S rRNA amplicon concentrations were hybridized at all three quantities. No significant within-sample differences in detected community composition were identified between different hybridization quantities by the following evaluations: concordance correlation coefficients for reproducibility in reported taxon abundances (Lin Biometrics 1989; CCC values 0.74 – 0.93) and kappa statistics for concordance in taxa identified as present or absent (kappa 0.62 – 0.74, $p < 0.001$). In addition, PERMANOVA analyses based on Bray-Curtis or Unifrac distance matrices demonstrated no significant differences in community composition across all samples related to different hybridization quantities ($p = 0.65$ for Bray-Curtis, $p = 0.47$ for weighted Unifrac).

Microbiome data analyses

Scaled fluorescence intensities were normalized to the mean intensity across all sample profiles in the dataset. Presence/absence determinations for taxa were determined using

algorithms described by Hazen et al. but with modified scoring thresholds selected for this dataset. For Stage 1 probe set scoring, quartile threshold values were selected based on analysis of Stage 1 scores for the spiked-in quantitative standards, applying an approach (Vilchez-Varga et al. Environ Microbiol 2013) that calibrates array hybridization signal values based on true positive and false discovery rates of internal standards. Minimum threshold Stage 1 values that for the PhyloChip quantitative standards yielded a true-positive rate of 1.0 and false-negative rate of 0 were determined (which corresponded to Stage 1 quartile ranked r scores of ≥ 0.32 , ≥ 0.53 , and ≥ 0.71). Taxa passing Stage 1 based on these r scores were included for Stage 2 scoring, which applies additional threshold criteria to exclude taxa called present due to potential cross-hybridization (here a 3rd quartile value >0.1 was used, which is less stringent). The finalized severe asthma dataset used for all downstream analyses comprised of ~3,900 OTUs or taxa. Array data were normalized independently for the severe asthma-only analysis, and for the subsequent combined analysis with microarray data previously published in an earlier study⁸ of the bronchial microbiome in healthy and non-severe asthma subjects (datasets in **Table E1**). Two healthy subjects included this analysis were not part of the earlier study⁸ but had been screened and evaluated by the same clinical procedures at the University of California, San Francisco.

For predictive metagenomic analyses, PICRUSt analyses were performed using 16S rRNA sequences retrieved for representative species of taxa of interest, i.e. those taxa demonstrating significant correlations with particular variables. The PICRUSt algorithm

utilizes these sequences to predict a microbe's gene content by inferring from the last phylogenetic common ancestor with one or more sequenced genomes, based on ancestral state reconstruction algorithms (Langille et al. Nature Biotech 2013). Predicted KEGG orthologs (Kyoto Encyclopedia of Genes and Genomes Database, release 67.1) from these inferred metagenomes were summarized at higher levels of functional classification, and associated KEGG pathways for these KOs were generated as output for concise reporting.

Quantitative PCR studies

Q-PCR for total 16S rRNA gene copies was performed on 37 of 40 samples with sufficient remaining DNA after array experiments, using the QuantiTect SYBR Green PCR master mix kit (Qiagen), 10 ng of extracted DNA, and the 16S rRNA primers 338-F (5'-ACTCCTACGGGAGGCAGCAG-3') and 518-R (5'-ATTACCGCGGCTGCTGG-3') at a final concentration of 0.3 μ M in 25- μ l triplicate reactions. PCR conditions were 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 10 min. Standard curves were generated from parallel PCR reactions of serial log-concentrations ($1 \times 10^2 - 1 \times 10^8$) of 16S rRNA gene product generated using the universal bacterial primers, Bact-27F and Bact-1492R. Regression coefficients (r^2) for all standard curves were ≥ 0.98 .

Total 16S rRNA copy numbers for each sample were normalized to mammalian β -actin copy numbers in each sample, which were determined by Q-PCR using, the QuantiTect

SYBR Green PCR master mix kit (Qiagen), 10 ng of extracted DNA, and β -actin primers actinB_F (5'-ACCAACTGGGACGACATGGAGAAGA-3') and actinB_R (5'-TACGGCCAGAGGCGTACAGGGATAG-3') at a final concentration of 0.3 μ M under the following conditions: 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 10 min. Actin gene copy numbers were determined using standard curves at serial log-concentrations of $1 \times 10^2 - 1 \times 10^8$. Actin gene standards were generated from PCR product using actin primers Actin1127_F and Actin1127_R. Regression coefficients (r^2) for all standard curves were ≥ 0.99 .

QPCR studies were also carried out for *H. influenzae* and specific *Prevotella* species (e.g. *P. nigrescens*, *P. intermedia*, *P. oris*) represented by taxa significantly associated with the variables of interest. Primers targeting these species (Huang et al. JCM 2014, Larsen et al. PLoS One 2010) were used in triplicate 25- μ l reactions under the following conditions: 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s (*H. influenzae*) or 15 sec (*Prevotella*), annealing at 58 °C for 30 sec (*H. influenzae*) or 1 min (*Prevotella*), extension at 72 °C for 30 s, and final extension at 72 °C for 10 min.

Standard curves for *Prevotella* QPCR were generated from *P. intermedia* DNA (ATCC 25611D-5). Linear regression of cycle threshold values and log-transformed fluorescence intensities was performed to validate reported relative abundances of these taxa.

Supplemental Figures Legends.

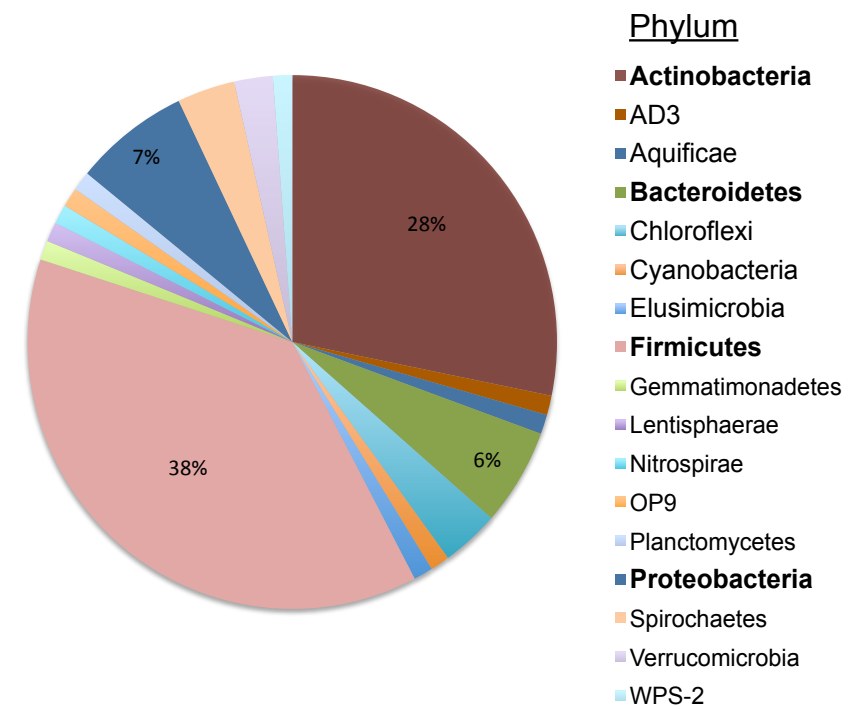
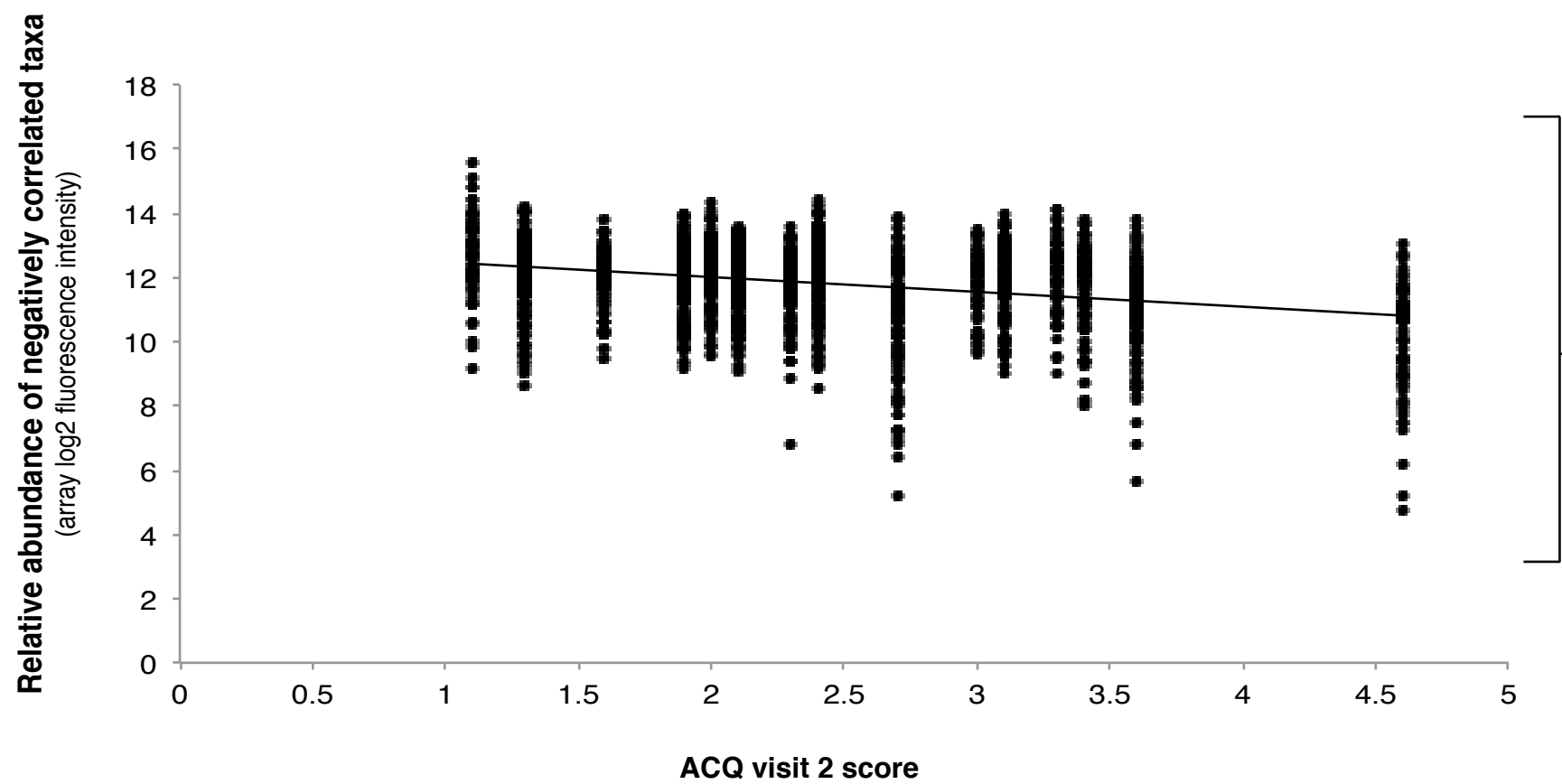
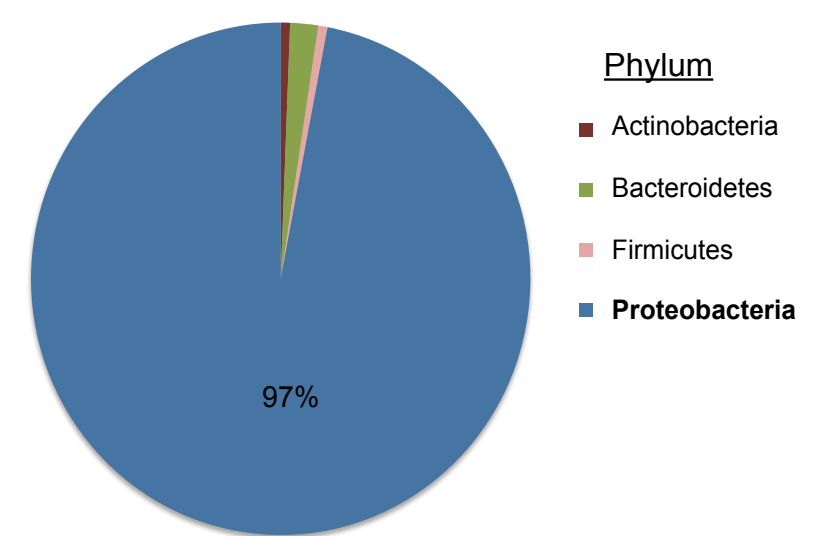
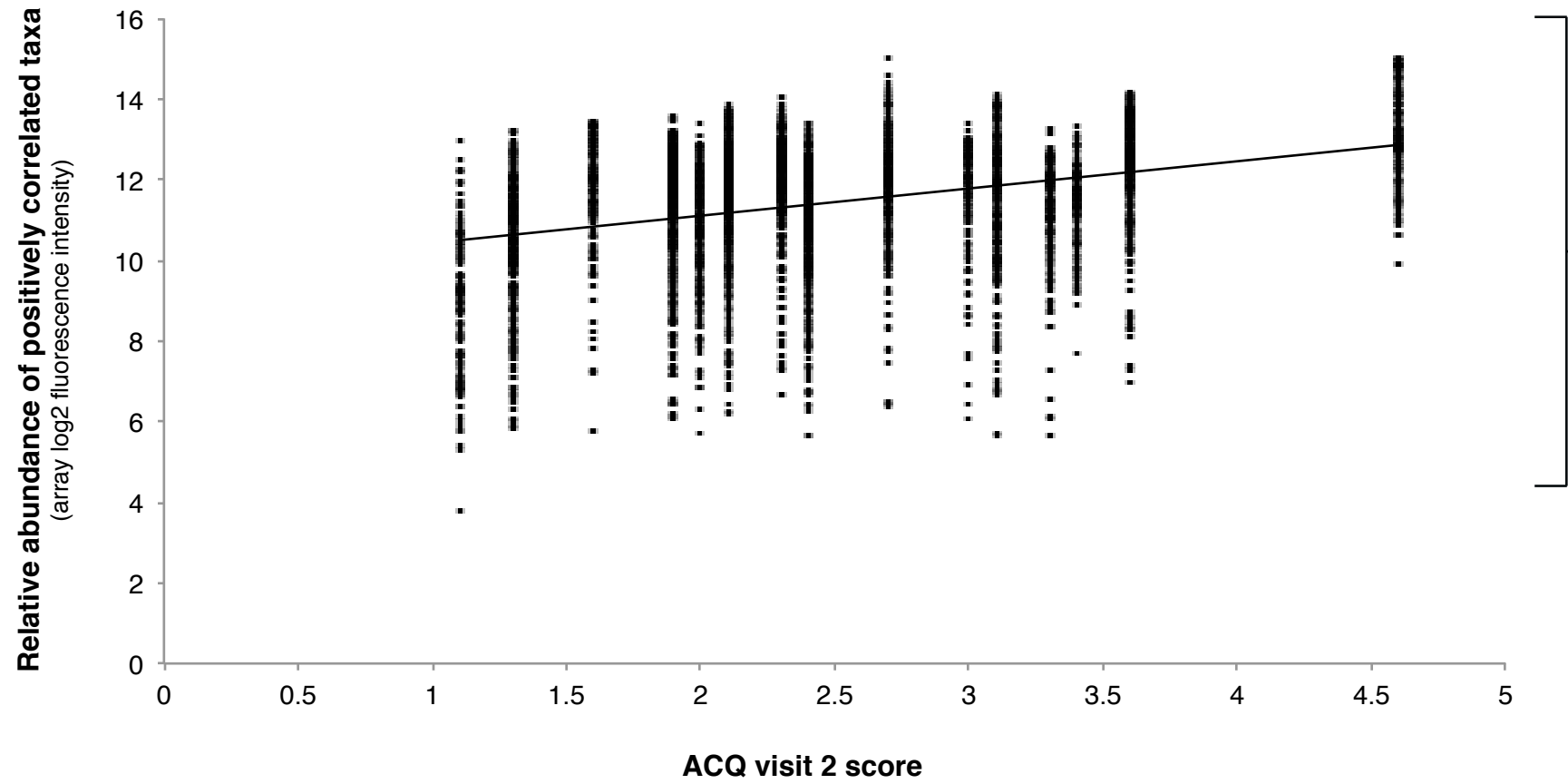
Figure E1. Bacterial phyla representing taxa positively (n=166) and negatively (n=85) correlated with ACQ visit 2 scores (Pearson R = 0.5 – 0.7, q-value < 0.05).

Figure E2. Predicted bacterial community functions represented among taxa positively and negatively correlated with BMI. Representative 16S rRNA gene sequences for taxa significantly correlated with BMI were used as inputs for PICRUSt (Langille et al. 2013). KEGG pathways shown in the figure are biologic pathways in which the predicted functions are known to participate.

Figure E3. Predicted bacterial community functions (KEGG pathways) represented among taxa significantly associated with change in ACQ.

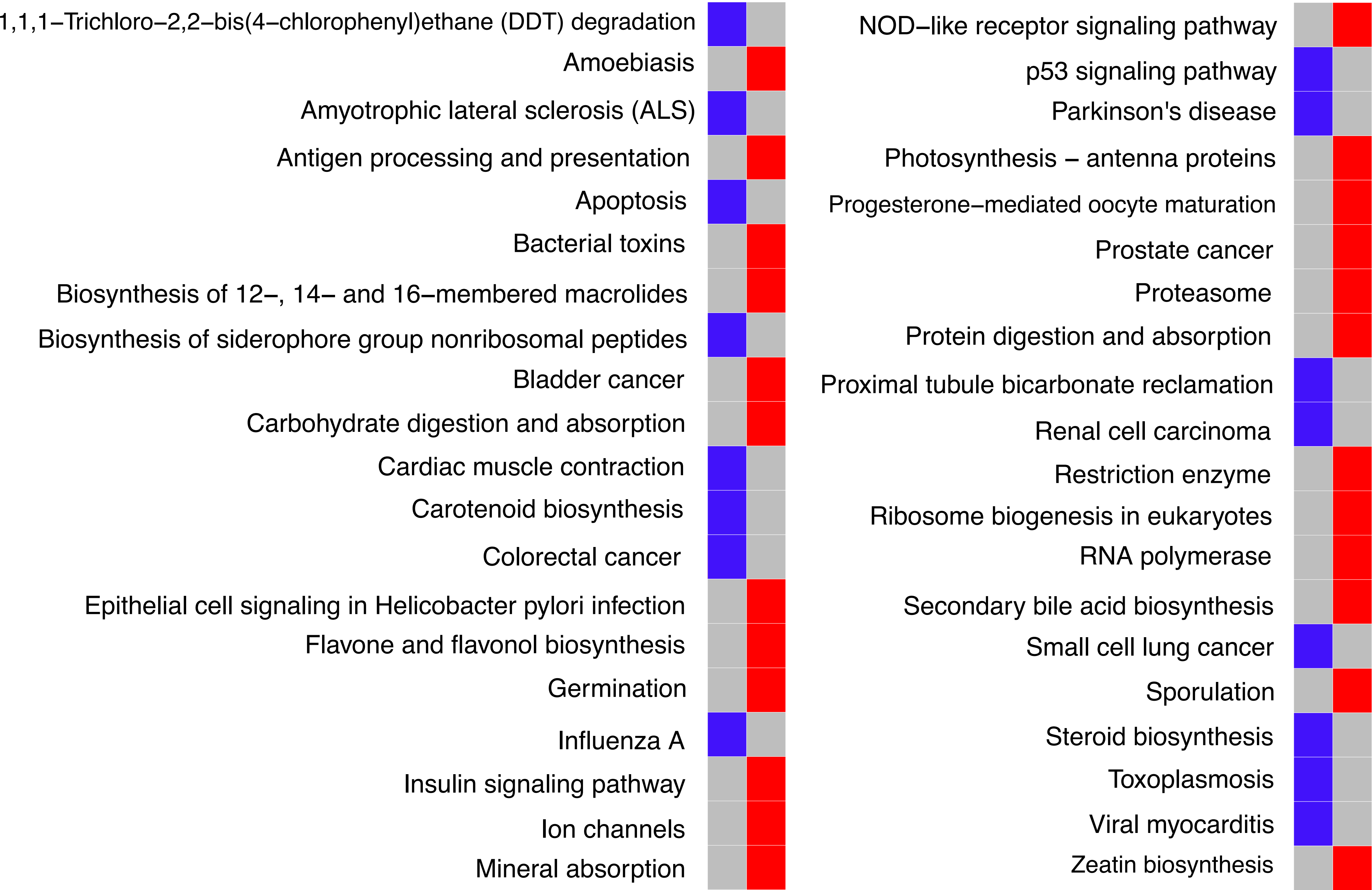
Figure E4. Predicted bacterial community functions represented among taxa positively associated with FKBP5 expression and the Th17 inflammation gene signature.

Figure E5. Significant correlation is observed between body mass index (BMI) and *Prevotella* spp. copy numbers (normalized to b-actin) determined by quantitative PCR.



KEGG pathways representing predicted functions among:

Taxa POSITIVELY correlated with BMI
Taxa NEGATIVELY correlated with BMI



Aldosterone–regulated sodium reabsorption

Basal transcription factors

Caffeine metabolism

Circadian rhythm – plant

Fatty acid elongation in mitochondria

Cytochrome P450

Flavone and flavonol biosynthesis

Isoflavonoid biosynthesis

Leishmaniasis

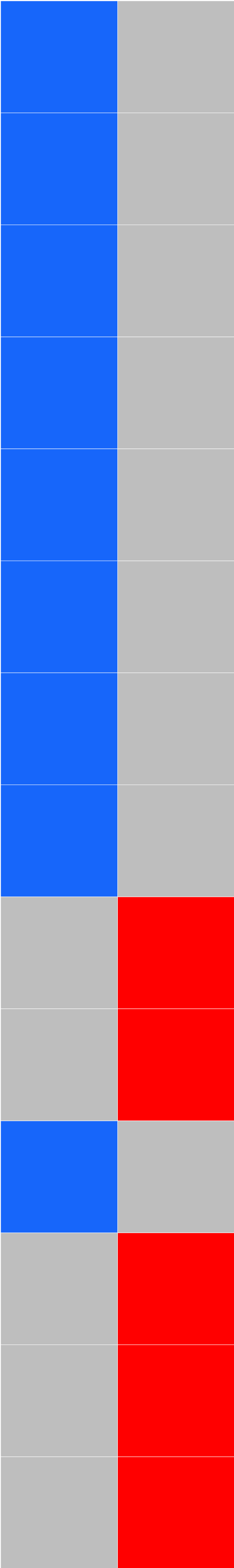
Melanogenesis

mRNA surveillance pathway

Phagosome

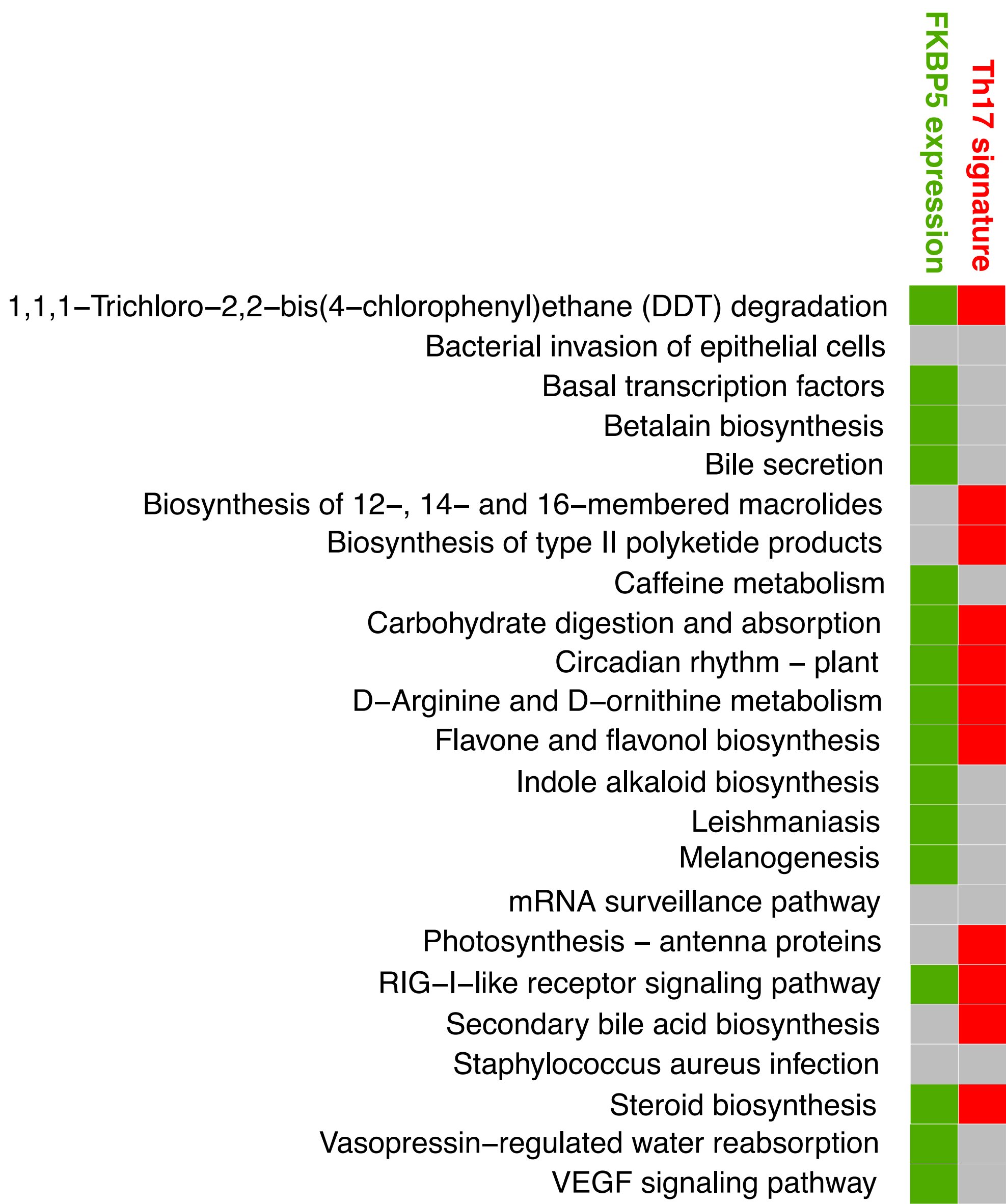
Rheumatoid arthritis

VEGF signaling pathway



KEGG pathways represented predicted functions among taxa:

- Taxa NEGATIVELY correlated with ACQ change
- Taxa POSITIVELY correlated with ACQ change



KEGG pathways representing predicted functions among:

- Taxa positively correlated with steroid response (FKBP5)
- Taxa positively correlated with Th17 gene signature

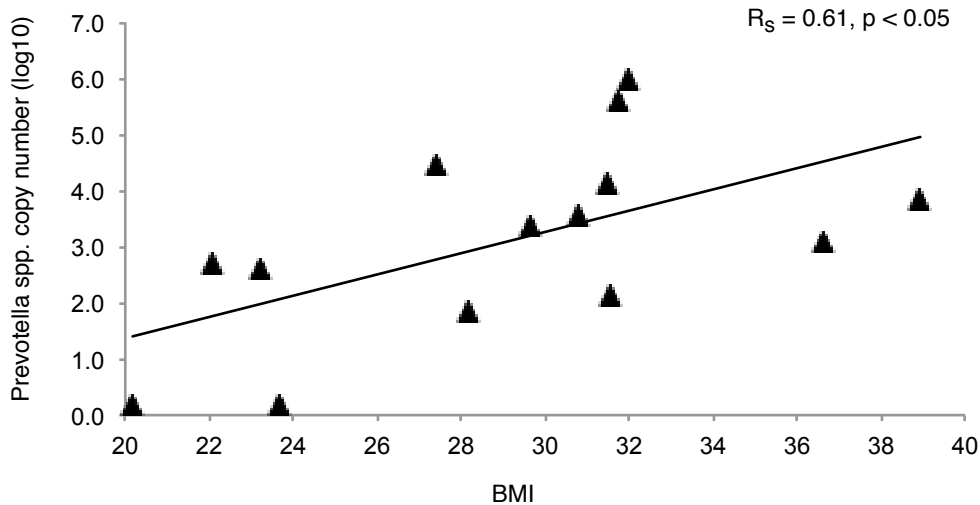


Table E3. Airway bacterial communities demonstrating the largest fold-differences in relative abundance in obese vs. non-obese severe asthma subjects (Benjamini-Hochberg adjusted $p < 0.10$).

Fold-difference (higher in obese)	adjusted p-value	Phylum	Class	Family	Genu	NCBI accession # for taxon representative species
11.1	0.05	Tenericutes	Erysipelotrichi	Erysipelotrichaceae	Bulleidia	AY044916.1
10.1	0.02	Firmicutes	Clostridia	Lachnospiraceae	unclassified	U13038.1
9.2	0.03	Bacteroidetes	Bacteroidia	Prevotellaceae	Prevotella	AY689229.1
7.0	0.05	Tenericutes	Mollicutes	Mycoplasmataceae	Mycoplasma	AF125583.1
6.3	0.02	Bacteroidetes	Bacteroidia	Prevotellaceae	Prevotella	U43698.1
6.2	0.02	Firmicutes	Clostridia	Clostridiales Family XI. Incertae Sedis	Parvimonas	AY323523.1
6.1	0.05	Bacteroidetes	Bacteroidia	Prevotellaceae	Prevotella	AF183402.1
5.5	0.05	Firmicutes	Clostridia	Lachnospiraceae	Clostridium	AJ576354.1
4.7	0.04	Bacteroidetes	Bacteroidia	Prevotellaceae	Prevotella	X81876.1
4.4	0.02	Bacteroidetes	Flavobacteria	Flavobacteriaceae	Zobellia	AB121975.1
4.4	0.05	Spirochaetes	Spirochaetes	Spirochaetaceae	Treponema	AY369247.1
3.2	0.05	Firmicutes	Clostridia	Clostridiales Family XI. Incertae Sedis	Peptoniphilus	AY167967.1
2.8	0.05	Bacteroidetes	Bacteroidia	Rikenellaceae	unclassified	AY160852.1
2.4	0.03	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Sulfurimonas	AY532570.1
1.9	0.05	Firmicutes	Clostridia	Clostridiales Family XI. Incertae Sedis	Finegoldia	AF542227.1

Table E4. Bacterial taxa significantly correlated with bronchial biopsy eosinophil numbers. The majority of correlations were negative (Pearson R = -0.5 to -0.7, q-value <0.10). Only two taxa exhibited positive correlations at the specified level of significance.

R	raw P-value	q-value	Phylum	Class	Family	Genus	NCBI accession for taxon representative organism
-0.7	0.000	0.01	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AY842259.1
-0.7	0.000	0.03	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	unclassified	D14507.1
-0.7	0.000	0.03	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF363138.1
-0.7	0.000	0.05	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AJ430827.1
-0.7	0.000	0.05	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF417542.1
-0.7	0.000	0.07	Bacteroidetes	Flavobacteria	Flavobacteriaceae	Psychroserpens	U62913.1
-0.6	0.000	0.07	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Acinetobacter	X81666.1
-0.6	0.000	0.07	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Helicobacter	Y18028.1
-0.6	0.000	0.07	Proteobacteria	Gammaproteobacteria	Halomonadaceae	Halomonas	AJ564880.1
-0.6	0.000	0.07	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AJ430829.1
-0.6	0.000	0.07	Proteobacteria	Alphaproteobacteria	Rickettsiaceae	Rickettsia	L36215.1
-0.6	0.000	0.07	Firmicutes	Clostridia	Lachnospiraceae	unclassified	AJ576351.1
-0.6	0.000	0.07	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AY771717.1
-0.6	0.000	0.07	Proteobacteria	Alphaproteobacteria	Sphingomonadaceae	Sphingopyxis	AF367204.1
-0.6	0.000	0.07	Proteobacteria	Gammaproteobacteria	Halomonadaceae	Chromohalobacter	AB021386.1
-0.6	0.000	0.08	Proteobacteria	Alphaproteobacteria	Bartonellaceae	Bartonella	Z31349.1
-0.6	0.001	0.08	Proteobacteria	Alphaproteobacteria	Bartonellaceae	Bartonella	X97822.1
-0.6	0.001	0.08	Proteobacteria	Alphaproteobacteria	Hyphomicrobiaceae	Hyphomicrobium	AY305006.1
-0.6	0.001	0.08	Proteobacteria	Alphaproteobacteria	Rickettsiaceae	Rickettsia	U11012.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Halomonadaceae	Chromohalobacter	AY373448.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AJ430828.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Acinetobacter	Z93445.1

-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Candidatus Blochmannia	AY334371.1
-0.6	0.001	0.08	Proteobacteria	Alphaproteobacteria	unclassified	sfK	AJ536878.1
-0.6	0.001	0.08	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF417548.1
-0.6	0.001	0.08	Bacteroidetes	Sphingobacteria	Flexibacteraceae	unclassified	AB078048.1
-0.6	0.001	0.08	Bacteroidetes	Sphingobacteria	Balneolaceae	Balneola	AF513957.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Escherichia	AF527825.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AY513645.1
-0.6	0.001	0.08	Proteobacteria	Deltaproteobacteria	Syntrophobacteraceae	unclassified	AJ296569.1
-0.6	0.001	0.08	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Helicobacter	AF103883.1
-0.6	0.001	0.08	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF239844.2
-0.6	0.001	0.08	Bacteroidetes	Sphingobacteria	Flexibacteraceae	unclassified	AB078054.1
-0.6	0.001	0.08	Spirochaetes	Spirochaetes	Spirochaetaceae	Treponema	X87140.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Psychromonadaceae	Psychromonas	Y14697.1
-0.6	0.001	0.08	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF417556.1
-0.6	0.001	0.08	Cyanobacteria	Chloroplast	unclassified	sfB	AF289247.1
-0.6	0.001	0.08	Firmicutes	Clostridia	Clostridiales Family XI. Incertae Sedis	Finegoldia	AB109769.1
-0.6	0.001	0.08	unclassified	unclassified	unclassified	sfA	AB077817.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AY722804.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AJ309942.1
-0.6	0.001	0.08	Tenericutes	Mollicutes	Acholeplasmataceae	Candidatus Phytoplasma	AY192577.1
-0.6	0.001	0.08	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Acinetobacter	AF390087.1
-0.6	0.001	0.08	Bacteroidetes	Bacteroidia	Rikenellaceae	unclassified	AF371912.1
-0.6	0.001	0.08	Spirochaetes	Spirochaetes	Spirochaetaceae	Treponema	AJ458946.1
-0.6	0.002	0.08	Proteobacteria	Alphaproteobacteria	Rickettsiaceae	Rickettsia	L36223.1
-0.6	0.002	0.08	Proteobacteria	Epsilonproteobacteria	Campylobacteraceae	Sulfurospirillum	AY280410.1
-0.6	0.002	0.08	Firmicutes	Bacilli	Streptococcaceae	Streptococcus	AY691525.1

-0.6	0.002	0.08	Firmicutes	Clostridia	Eubacteriaceae	unclassified	AY554414.1
-0.6	0.002	0.09	Bacteroidetes	Bacteroidia	Rikenellaceae	unclassified	AY216441.1
-0.6	0.002	0.09	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AJ539102.1
-0.6	0.002	0.09	Firmicutes	Clostridia	Peptostreptococcaceae	Peptostreptococcus	AY167954.1
-0.6	0.002	0.09	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	U69636.1
-0.6	0.002	0.09	Firmicutes	Clostridia	Ruminococcaceae	unclassified	AY791247.1
-0.6	0.002	0.09	Firmicutes	Bacilli	Bacillaceae	Bacillus	AY030326.1
-0.6	0.002	0.09	Firmicutes	Clostridia	Lachnospiraceae	unclassified	AB088950.1
-0.6	0.002	0.09	Cyanobacteria	Oscillatoriothricaceae	Microcystaceae	Microcystis	U40333.2
-0.6	0.002	0.09	Firmicutes	Bacilli	Bacillaceae	Bacillus	AY310301.1
-0.6	0.002	0.09	Proteobacteria	Gammaproteobacteria	Psychromonadaceae	Psychromonas	U85849.1
-0.6	0.002	0.09	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF417545.1
-0.6	0.002	0.09	Tenericutes	Mollicutes	Acholeplasmataceae	Candidatus Phytoplasma	AY739024.1
-0.6	0.002	0.09	Proteobacteria	Gammaproteobacteria	Xanthomonadaceae	Stenotrophomonas	AB167174.1
-0.5	0.002	0.09	Tenericutes	Mollicutes	Acholeplasmataceae	Candidatus Phytoplasma	AY685054.1
-0.5	0.002	0.09	Firmicutes	Bacilli	Bacillaceae	Bacillus	AB115959.1
-0.5	0.003	0.09	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	AJ313425.1
-0.5	0.003	0.09	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Helicobacter	U96300.1
-0.5	0.003	0.09	Bacteroidetes	Bacteroidia	Rikenellaceae	unclassified	AB186809.1
-0.5	0.003	0.09	Proteobacteria	Gammaproteobacteria	Xanthomonadaceae	unclassified	AY676483.2
-0.5	0.003	0.09	Proteobacteria	Alphaproteobacteria	Caulobacteraceae	Brevundimonas	AY328801.1
-0.5	0.003	0.09	Proteobacteria	Alphaproteobacteria	Bartonellaceae	Bartonella	AY515118.1
-0.5	0.003	0.09	Tenericutes	Mollicutes	Acholeplasmataceae	Acholeplasma	AY538167.1
0.6	0.001	0.08	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	AY207594.1
0.6	0.002	0.09	Actinobacteria	Actinobacteria	Nocardioideae	Propionimonas	AB078859.2

Table E5. Bacterial taxa demonstrating the strongest correlations with the Th17/neutrophilic inflammation gene signature (Pearson R 0.5 – 0.7, q-value $\leq$ 0.20). Taxa are sorted by the magnitude of correlation from positive (110 taxa) to negative (186 taxa).

R	Unadjusted p-value	q-value	Phylum	Class	Family	Genus	NCBI accession for taxon representative organism
0.7	0.00	0.2	Proteobacteria	Alphaproteobacteria	Pelagibacteraceae	Candidatus Pelagibacter	AF382131.1
0.6	0.00	0.2	Proteobacteria	Betaproteobacteria	unclassified	sfB	AY212696.1
0.6	0.00	0.2	Firmicutes	Clostridia	Lachnospiraceae	Coproccoccus	AJ408972.1
0.6	0.00	0.2	Deferribacteres	Deferribacteres	Deferribacteraceae	Deferribacter	AJ515882.1
0.6	0.00	0.2	Planctomycetes	Phycisphaerae	unclassified	sfA	AY162125.1
0.6	0.00	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	unclassified	X80721.1
0.6	0.00	0.2	Synergistetes	Synergistia	Dethiosulfovibrionaceae	Jonquetella	AF481216.1
0.6	0.00	0.2	Firmicutes	Bacilli	Bacillaceae	unclassified	AB034710.1
0.6	0.00	0.2	Proteobacteria	Gammaproteobacteria	Sinobacteraceae	unclassified	AY627375.1
0.6	0.01	0.2	Proteobacteria	Gammaproteobacteria	Piscirickettsiaceae	Methylophaga	AY128533.1
0.6	0.01	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Avibacterium	AY498871.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Gallibacterium	AY172724.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Haemophilus	AY613497.1
0.5	0.01	0.2	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AY649433.1
0.5	0.01	0.2	Proteobacteria	Alphaproteobacteria	Acetobacteraceae	Acidomonas	AB056321.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Pseudomonadaceae	Pseudomonas	AJ297767.1
0.5	0.01	0.2	Proteobacteria	Alphaproteobacteria	Rhodospirillaceae	unclassified	AF406524.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Aeromonadaceae	Aeromonas	AB027005.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Avibacterium	AY498870.1
0.5	0.01	0.2	Bacteroidetes	Sphingobacteria	Flexibacteraceae	unclassified	AB078063.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Aeromonadaceae	Aeromonas	AF418214.1
0.5	0.01	0.2	Proteobacteria	Betaproteobacteria	Alcaligenaceae	Achromobacter	AF205370.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Aggregatibacter	AY362906.1
0.5	0.01	0.2	Actinobacteria	Actinobacteria	Nocardiopsaceae	Nocardiopsis	AY619715.2
0.5	0.01	0.2	Bacteroidetes	Sphingobacteria	Rhodothermaceae	unclassified	AY493957.1
0.5	0.01	0.2	Cyanobacteria	Synechococcophycideae	unclassified	sfA	AF448078.1
0.5	0.01	0.2	AD3	ABS-6	unclassified	sfA	AY395409.1

0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Histophilus	AF549394.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Shewanellaceae	Shewanella	AB057660.1
0.5	0.01	0.2	Proteobacteria	Epsilonproteobacteria	Campylobacteraceae	Campylobacter	AF372092.1
0.5	0.01	0.2	Proteobacteria	Deltaproteobacteria	Bdellovibrionaceae	Bdellovibrio	AY294216.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Sedimenticolaceae	Sedimenticola	X95229.1
0.5	0.01	0.2	Proteobacteria	Deltaproteobacteria	Nitrospinaceae	unclassified	AF523884.1
0.5	0.01	0.2	TM6	SBRH58	unclassified	sfA	AF268992.1
0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Halomonadaceae	Candidatus Portiera	AY266097.1
0.5	0.01	0.2	Proteobacteria	Alphaproteobacteria	Rhodobacteraceae	unclassified	AF368182.1
0.5	0.01	0.2	Proteobacteria	Deltaproteobacteria	Nitrospinaceae	unclassified	AY177804.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Serratia	AJ233433.1
0.5	0.02	0.2	Cyanobacteria	Chloroplast	unclassified	sfA	AY706947.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Chromatiaceae	unclassified	AY328773.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Xanthomonadaceae	Xanthomonas	AY576647.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Pelagibacteraceae	Candidatus Pelagibacter	AB193898.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Caulobacteraceae	unclassified	AJ459874.1
0.5	0.02	0.2	Cyanobacteria	Oscillatoriophyceae	Phormidiaceae	Hydrocoleum	AB039015.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Avibacterium	AF487726.1
0.5	0.02	0.2	Chlamydiae	Chlamydiae	Waddliaceae	Waddlia	AY184804.1
0.5	0.02	0.2	Deferribacteres	Deferribacteres	Calithriaceae	Caldithrix	AY280423.1
0.5	0.02	0.2	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Sulfurovum	AY225614.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Shewanellaceae	Shewanella	X81623.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Xanthomonadaceae	Xanthomonas	AY576648.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Brucellaceae	unclassified	AJ519939.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF208516.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Shewanellaceae	Shewanella	AF387347.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Mannheimia	AF053893.1
0.5	0.02	0.2	Proteobacteria	Deltaproteobacteria	Myxococcaceae	Myxococcus	AY724798.1
0.5	0.02	0.2	Proteobacteria	Deltaproteobacteria	Nitrospinaceae	unclassified	AF523883.1
0.5	0.02	0.2	Armatimonadetes	Armatimonadia	Armatimonadaceae	unclassified	AF316757.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Aeromonadaceae	Aeromonas	AY532688.1
0.5	0.02	0.2	Cyanobacteria	Oscillatoriophyceae	Microcystaceae	Microcystis	U40340.2
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Actinobacillus	AY362900.1

0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	unclassified	AF476103.1
0.5	0.02	0.2	Proteobacteria	Deltaproteobacteria	Syntrophobacteraceae	Syntrophobacter	AF482435.1
0.5	0.02	0.2	Cyanobacteria	Chloroplast	unclassified	sfA	V00165.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Rhodobacteraceae	Loktanela	AY654838.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Photorhabdus	Z76740.1
0.5	0.02	0.2	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Helicobacter	AY366424.1
0.5	0.02	0.2	Proteobacteria	Deltaproteobacteria	Desulfobulbaceae	unclassified	AJ535252.1
0.5	0.02	0.2	SAR406	AB16	A714017	ZA3312c	AF382116.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Salmonella	X80676.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Hyphomicrobiaceae	Hyphomicrobium	AY328652.1
0.5	0.02	0.2	Proteobacteria	Betaproteobacteria	Hydrogenophilaceae	Thiobacillus	AB181501.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AY649437.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Halomonadaceae	Candidatus Portiera	AY266096.1
0.5	0.02	0.2	Proteobacteria	Alphaproteobacteria	Sphingomonadaceae	Sphingobium	AY026948.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Basfia	AY029193.1
0.5	0.02	0.2	Bacteroidetes	Flavobacteria	Cryomorphaceae	unclassified	AF521195.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Shewanellaceae	Shewanella	U91552.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	unclassified	AF476110.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Aggregatibacter	AY362884.1
0.5	0.02	0.2	Firmicutes	Clostridia	Syntrophomonadaceae	Syntrophomonas	AY536889.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Pseudomonadaceae	Pseudomonas	AY189751.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Alteromonadaceae	ND137	AB052965.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Haemophilus	AY362911.1
0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Acinetobacter	AF509830.1
0.5	0.03	0.2	Cyanobacteria	Chloroplast	unclassified	sfG	AF289245.1
0.5	0.03	0.2	Proteobacteria	Betaproteobacteria	Oxalobacteraceae	Herminiimonas	AY728038.1
0.5	0.03	0.2	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobium	AF417553.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Aeromonadaceae	Aeromonas	AY532690.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Acinetobacter	X86591.1
0.5	0.03	0.2	Thermi	Deinococci	Thermaceae	Thermus	AB071811.1
0.5	0.03	0.2	Tenericutes	Mollicutes	Acholeplasmataceae	Candidatus Phytoplasma	AY389826.2
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Candidatus Regiella	AF293623.1

0.5	0.03	0.2	Bacteroidetes	Sphingobacteria	Balneolaceae	Balneola	AY162093.1
0.5	0.03	0.2	Cyanobacteria	Chloroplast	unclassified	sfG	U67742.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Piscirickettsiaceae	Thioalkalimicrobiu m	AF126548.1
0.5	0.03	0.2	Spirochaetes	Spirochaetes	Spirochaetaceae	Spirochaeta	AY605165.1
0.5	0.03	0.2	Cyanobacteria	Nostocophycideae	Nostocaceae	unclassified	AF160256.1
0.5	0.03	0.2	Bacteroidetes	Bacteroidia	RikenellaceaeII	unclassified	AY244952.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Serratia	AY040208.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	HTCC2188	unclassified	AF406527.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Pseudoalteromonadaceae	Pseudoalteromonas	AF045560.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Pasteurella	AY362897.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Klebsiella	AF390083.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Klebsiella	AF543283.1
0.5	0.03	0.2	Firmicutes	Clostridia	Ruminococcaceae	Ruminococcus	AB185810.1
0.5	0.03	0.2	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Helicobacter	AF072471.1
0.5	0.03	0.2	Proteobacteria	Deltaproteobacteria	Desulfobulbaceae	unclassified	AJ535230.1
0.5	0.03	0.2	Proteobacteria	Alphaproteobacteria	Holosporaceae	unclassified	AF422687.1
0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Leclercia	AY297724.1
0.5	0.03	0.2	Proteobacteria	Deltaproteobacteria	unclassified	sfA	AB193909.1
-0.6	0.001	0.2	Actinobacteria	Actinobacteria	Dietziaceae	Dietzia	X79290.1
-0.6	0.001	0.2	Firmicutes	Bacilli	Bacillaceae	Bacillus	AB062696.1
-0.6	0.001	0.2	Firmicutes	Bacilli	Thermoactinomycetaceae	Planifilum	AB088364.1
-0.6	0.002	0.2	Firmicutes	Bacilli	Bacillaceae	Bacillus	AF221061.1
-0.6	0.002	0.2	Acidobacteria	Solibacteres	Solibacteraceae	Candidatus Solibacter	AF529350.1
-0.6	0.003	0.2	Firmicutes	Bacilli	Bacillaceae	Bacillus	AJ581125.1
-0.6	0.003	0.2	Actinobacteria	Actinobacteria	Nocardiodaceae	Aeromicrobium	AF005021.1
-0.6	0.003	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	AJ399467.1
-0.6	0.003	0.2	Firmicutes	Bacilli	Bacillaceae	Geobacillus	AY491497.1
-0.6	0.004	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Micromonospora	X92621.1
-0.6	0.004	0.2	Spirochaetes	Spirochaetes	Spirochaetaceae	Treponema	X87139.1
-0.6	0.01	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Micromonospora	AY291589.1
-0.6	0.01	0.2	Spirochaetes	Spirochaetes	Spirochaetaceae	TM3	AY605130.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	AJ399473.1

-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Micromonospora	X92598.1
-0.5	0.01	0.2	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiaceae	unclassified	AJ401121.1
-0.5	0.01	0.2	Firmicutes	Clostridia	Sulfobacillaceae	Sulfobacillus	AF460985.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Solirubrobacteraceae	unclassified	AY234499.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Sanguibacteraceae	unclassified	AB126692.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Acidimicrobiaceae	unclassified	AY395429.1
-0.5	0.01	0.2	Firmicutes	Clostridia	Thermoanaerobacteraceae	Caldanaerobacter	NC_003869.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Nocardiodaceae	Propionificimonas	AB078820.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Micromonospora	AB159778.2
-0.5	0.01	0.2	Proteobacteria	Deltaproteobacteria	Desulfovibrionaceae	Desulfovibrio	AB089104.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	X92614.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Nocardia	AB086862.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Agromyces	AY507128.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Solirubrobacteraceae	unclassified	AY234610.1
-0.5	0.01	0.2	Proteobacteria	Alphaproteobacteria	Methylobacteriaceae	Methylobacterium	D32229.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Intrasporangiaceae	Janibacter	AY533561.1
-0.5	0.01	0.2	Proteobacteria	Alphaproteobacteria	Rhodospirillaceae	Telmatospirillum	AB111107.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Acidimicrobiaceae	unclassified	AY805539.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Actinosynnemataceae	Lentzea	AB020029.1
-0.5	0.01	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Escherichia	AE014073.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Verrucosipora	Y15523.1
-0.5	0.01	0.2	Nitrospirae	Nitrospira	Thermodesulfovibrionaceae	Candidatus Magnetobacterium	AJ863136.1
-0.5	0.01	0.2	Firmicutes	Clostridia	Clostridiaceae	Clostridium	AY330128.1
-0.5	0.01	0.2	Firmicutes	Bacilli	Bacillaceae	Bacillus	AY296812.1
-0.5	0.01	0.2	Firmicutes	Bacilli	Enterococcaceae	Enterococcus	AY365054.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Nocardia	AF430042.1
-0.5	0.01	0.2	Firmicutes	Bacilli	Paenibacillaceae	Brevibacillus	AY493971.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Intrasporangiaceae	Janibacter	AY522569.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Micromonospora	AB107231.2
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Sanguibacteraceae	Sanguibacter	X79452.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Micrococcaceae	Arthrobacter	X83406.1

-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Actinoplanes	AJ277576.1
-0.5	0.01	0.2	Nitrospirae	Nitrospira	FW	GOUTA7	AF523973.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	Y14699.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	X77443.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Micrococcaceae	Arthrobacter	AY436791.1
-0.5	0.01	0.2	Tenericutes	Erysipelotrichi	Erysipelotrichaceae	Allobaculum	AJ417075.1
-0.5	0.01	0.2	Firmicutes	Clostridia	Lachnospiraceae	Pseudobutyrvibrio Candidatus Koribacter	X95893.1 AY234578.1
-0.5	0.01	0.2	Acidobacteria	Acidobacteria	Acidobacteriaceae	Algoriphagus	AY264838.1
-0.5	0.01	0.2	Bacteroidetes	Sphingobacteria	Cyclobacteriaceae	Syntrophobacter	AF482439.1
-0.5	0.01	0.2	Proteobacteria	Deltaproteobacteria	Syntrophobacteraceae	unclassified	AB047516.1
-0.5	0.01	0.2	Actinobacteria	Actinobacteria	Pseudonocardiaceae	Amycolatopsis	AF222022.1
-0.5	0.01	0.2	Aquificae	Aquificae	Hydrogenothermaceae	unclassified	AF188332.1
-0.5	0.01	0.2	Firmicutes	Bacilli	Leuconostocaceae	Leuconostoc	AY117684.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Lactobacillaceae	Lactobacillus	X94230.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Streptomyetaceae	Streptomyces	AB026219.1
-0.5	0.02	0.2	Verrucomicrobia	Opitutae	Opitutaceae	unclassified	AY587231.1
-0.5	0.02	0.2	Chloroflexi	Chloroflexi-4	unclassified	sfH	AY592108.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Coriobacteriaceae	unclassified	AJ534681.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Micromonospora	AB196710.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Corynebacteriaceae	Corynebacterium	AY101388.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Rhodococcus	X81929.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Staphylococcaceae	Staphylococcus	X68417.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	unclassified	sfF	AY592144.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Actinomycetaceae	Actinomyces	AJ540309.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Staphylococcaceae	Staphylococcus	L37597.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Cellulomonadaceae	Cellulomonas	X79462.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Rhodococcus	AY566576.1
-0.5	0.02	0.2	Firmicutes	Clostridia	Sulfobacillaceae	Thermaerobacter	AB061441.1
-0.5	0.02	0.2	Firmicutes	Clostridia	Clostridiaceae	Clostridium	X74770.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Streptomyetaceae	Streptomyces	AJ271887.1

-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Euzebiaceae	unclassified	AF448192.1
-0.5	0.02	0.2	Spirochaetes	Spirochaetes	Spirochaetaceae	TM3	AY605143.1
-0.5	0.02	0.2	Firmicutes	Clostridia	Clostridiaceae	Clostridium	Y18175.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	AB007417.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	AB007416.1
-0.5	0.02	0.2	Cyanobacteria	Nostocophycideae	Nostocaceae	unclassified	AJ630455.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Paenibacillaceae	Paenibacillus	AJ011321.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	Y17240.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	unclassified	X93201.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	AJ399463.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Actinosynnemataceae	unclassified	AY114175.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	unclassified	AJ575552.1
-0.5	0.02	0.2	Bacteroidetes	Sphingobacteria	Sphingobacteriaceae	Pedobacter	AJ438171.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Promicromonosporaceae	Isoptericola	AJ298873.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Propionibacteriaceae	Propionibacterium	AJ704571.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Corynebacteriaceae	Corynebacterium	Y10999.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Intrasporangiaceae	Janibacter	AF176948.1
-0.5	0.02	0.2	Proteobacteria	Deltaproteobacteria	unclassified	sfl	AJ519630.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Corynebacteriaceae	Corynebacterium	AY271720.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Bacillaceae	Bacillus	D55731.1
-0.5	0.02	0.2	Firmicutes	Clostridia	Sulfobacillaceae	YNPFFP6	AF391987.1
-0.5	0.02	0.2	OP9	OPB46	unclassified	sfA	AB195913.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Cellulomonadaceae	Cellulomonas	AF064701.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Pseudonocardiaceae	Saccharomonospora	Z38023.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Sporolactobacillaceae	Sporolactobacillus	AJ634661.1
-0.5	0.02	0.2	Tenericutes	Erysipelotrichi	Erysipelotrichaceae	Pei061	AJ576412.1
-0.5	0.02	0.2	Firmicutes	Clostridia	Lachnospiraceae	unclassified	AB185642.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	unclassified	sfA	AY093463.1
-0.5	0.02	0.2	Chloroflexi	Caldilineae	Caldilineaceae	unclassified	AY216458.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Actinosynnemataceae	Actinosynnema	X84447.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Actinoplanes	U58526.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Mycobacteriaceae	Mycobacterium	AJ536033.1

-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Actinosynnemataceae	unclassified	AF328679.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Rhodococcus	AY833095.1
-0.5	0.02	0.2	Firmicutes	Clostridia	Peptococcaceae	Desulfosporosinus	AF076247.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Actinomycetaceae	Arcanobacterium	AJ300767.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Thermomonosporaceae	Actinoallomurus	AB193569.1
-0.5	0.02	0.2	Spirochaetes	Spirochaetes	Spirochaetaceae	KSA2	AY605171.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Salinibacterium	AF468436.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Mycobacteriaceae	Mycobacterium	X88908.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Micrococcaceae	unclassified	AY512631.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Agromyces	AF389342.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	AF452714.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Bacillaceae	Bacillus	AY373359.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	AJ853910.1
-0.5	0.02	0.2	Proteobacteria	Deltaproteobacteria	Nitrospinaceae	unclassified	AY211659.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Frigoribacterium	AY439262.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	BA000030.3
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Micrococcaceae	Arthrobacter	AY452082.1
-0.5	0.02	0.2	Proteobacteria	Gammaproteobacteria	Xanthomonadaceae	unclassified	AB161360.1
-0.5	0.02	0.2	Planctomycetes	Phycisphaerae	unclassified	sfB	AY162122.1
-0.5	0.02	0.2	Firmicutes	Bacilli	Staphylococcaceae	Staphylococcus	NC_004461.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	NC_003888.3
-0.5	0.02	0.2	Firmicutes	Bacilli	Lactobacillaceae	Lactobacillus	AF469172.1
-0.5	0.02	0.2	Firmicutes	Clostridia	Clostridiales Family XIII. Incertae Sedis	unclassified	AY854283.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	AB004725.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Actinoplanes	AB047505.1
-0.5	0.02	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Rhodococcus	AJ250927.1
-0.5	0.03	0.2	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Helicobacter	AF506794.1
-0.5	0.03	0.2	Firmicutes	Bacilli	Carnobacteriaceae	unclassified	X70907.1
-0.5	0.03	0.2	Firmicutes	Clostridia	Clostridiaceae	Clostridium	NC_004557.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Intrasporangiaceae	Phycococcus	AY682381.1
-0.5	0.03	0.2	Cyanobacteria	Nostocophycideae	Nostocaceae	Dolichospermum	AY701546.1

-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Bifidobacteriaceae	Bifidobacterium	AJ420965.1
-0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Piscirickettsiaceae	Piscirickettsia	U36940.1
-0.5	0.03	0.2	Bacteroidetes	Bacteroidia	Rikenellaceae	unclassified	AB100010.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Rhodococcus	AJ250926.1
-0.5	0.03	0.2	Firmicutes	Bacilli	Bacillaceae	Geobacillus	AF411065.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Candidatus Aquiluna	AJ575517.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Candidatus Rhodoluna	AJ575498.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Salinibacterium	AJ438586.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	unclassified	AJ575544.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Kineosporiaceae	Kineococcus	X77958.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Bifidobacteriaceae	Bifidobacterium	AY513712.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Micromonospora	L11306.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Nocardiodaceae	Propionimonas	AB166787.1
-0.5	0.03	0.2	Chloroflexi	Anaerolineae	Anaerolinaceae	GN14	AJ306741.2
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	AJ717353.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Dermabacteraceae	Brachybacterium	AB098579.1
-0.5	0.03	0.2	Firmicutes	Bacilli	Bacillaceae	Geobacillus	AY608935.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Cellulomonadaceae	unclassified	AY234441.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Cryocola	AF505513.1
-0.5	0.03	0.2	Firmicutes	Bacilli	Lactobacillaceae	Lactobacillus	AJ306297.1
-0.5	0.03	0.2	Proteobacteria	Epsilonproteobacteria	Helicobacteraceae	Helicobacter	AF333341.1
-0.5	0.03	0.2	Proteobacteria	Deltaproteobacteria	Myxococcaceae	Myxococcus	AJ233927.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Thermomonosporaceae	Actinomadura	AY273787.1
-0.5	0.03	0.2	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadaceae	unclassified	AY289480.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Thermomonosporaceae	Actinomadura	AF163122.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Streptomycetaceae	Streptomyces	AB026214.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Mycobacteriaceae	Mycobacterium	X55022.1
-0.5	0.03	0.2	Proteobacteria	Deltaproteobacteria	Myxococcaceae	Myxococcus	AJ233919.1
-0.5	0.03	0.2	Firmicutes	Bacilli	Alicyclobacillaceae	Alicyclobacillus	AB059672.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Nocardiaceae	Rhodococcus	AJ306401.1

-0.5	0.03	0.2	Chloroflexi	RA13C7	unclassified	sfA	AJ536886.1
-0.5	0.03	0.2	VHS-B3-43	unclassified	unclassified	sfA	AY592662.1
-0.5	0.03	0.2	Firmicutes	Bacilli	Bacillaceae	Geobacillus	AF276305.1
-0.5	0.03	0.2	Firmicutes	Bacilli	Planococcaceae	Lysinibacillus	L14015.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Brevibacteriaceae	Brevibacterium	AJ488509.2
-0.5	0.03	0.2	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Escherichia	NC_004337.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Actinomycetaceae	Actinomyces	AY221601.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Actinomycetaceae	Actinomyces	AJ427451.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Mycobacteriaceae	Mycobacterium	AJ416914.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	AB004723.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Cellulomonadaceae	unclassified	AY250883.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	D21343.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Actinoplanes	AB048216.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Micromonosporaceae	Actinoplanes	AJ277584.1
-0.5	0.03	0.2	Actinobacteria	Actinobacteria	Microbacteriaceae	Microcella	AF445364.1
-0.5	0.03	0.2	Proteobacteria	Alphaproteobacteria	Bradyrhizobiaceae	Afipia	AF041446.1
-0.5	0.03	0.2	Proteobacteria	Alphaproteobacteria	Acetobacteraceae	Swaminathania	AF459455.1