

Supplemental Material:

Randomized, double-blind, placebo-controlled trial with azithromycin selects for anti-inflammatory microbial metabolites in the emphysematous lung

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Methods

Study design and participants

Subjects were enrolled from the Early Detection Research Network (EDRN, 5U01CA086137-13, IRB# 8896, PI Rom). All subjects signed informed consent to participate in this study and the research protocol was approved by the New York University and Bellevue Hospital Center (New York, NY) institutional review boards (IRB# 8896). This cohort has 1984 subjects receiving yearly follow up including chest CT; and 447 have CT-defined emphysema. We enrolled 20 EDRN subjects who fulfilled the following inclusion criteria: diagnosis of emphysema as documented in report by a radiologist; significant smoking history (>20pack/year). For all subjects, exclusion criteria included: FEV₁ < 70% predicted; >70 years old; known cardiovascular, liver, or renal disease (due to increased bronchoscopic risk); recent treatment with antibiotics or steroids in the prior 3 months. To enroll these subjects, we sent letters to all eligible subjects (n=115). Subjects were then scheduled for a screening visit at the NYU Clinical and Translational Science Institute (CTSI) where we obtained the following as part of the bronchoscopy screening: blood sample for CBC, CMP, PT/INR, and PTT; twelve-lead EKG; chest X-ray; and spirometry pre and post bronchodilators (albuterol) according with standard protocols.¹ Results of baseline samples for 14 of these subjects were previously reported utilizing a 454 sequencing approach.² For this report, all baseline samples and post-treatment samples were re-processed and sequencing performed using different (Illumina) primers on the MiSeq platform (see below). None of the subjects had received any inhaled medication for at least one month. For scoring of emphysema, a 6-point score of CT emphysema was performed.

Scoring of emphysema

For scoring of emphysema, a 6-point score of CT emphysema was performed. Briefly, the lungs were divided into three zones (upper zone above the carina, mid zone between carina and inferior pulmonary veins, lower zone below the inferior pulmonary veins), with right and left lung being considered together. The score sheet recorded the extent of emphysema in upper, mid and lower zones, according to a 6-point scale, (absent, < 5%, 5–25%, 25–50%, 50–75%, and > 75%).³⁻⁵ The emphysema score assigned to each subject was the average of all three zones. The predominant pattern of emphysema (centrilobular or panlobular) also was recorded. Automated quantitative analysis of the percentage of the entire lung comprised of voxels less than -950 HU⁵ was performed using an automated segmentation and analysis program (Pulmo3D, SyngoVia vb10, Siemens AG).

Bacterial 16S rRNA-encoding genes quantification and sequencing

DNA was then extracted from background, supraglottic and BAL samples with an ion exchange column (Qiagen). Total bacterial and human DNA levels were determined by quantitative PCR (qPCR) as previously described.⁶ The BAL samples utilized in this study were acellular BAL fluid. High-throughput sequencing of bacterial 16S rRNA-encoding gene amplicons encoding the V4 region⁷ (150bp read length, paired-end protocol) was performed with MiSeq. Reagent controls were sequenced and analyzed as quality controls. Each unique barcoded amplicon was generated in pairs of 25µl reactions with the following reaction conditions: 11µl PCR-grade H₂O, 10µl Hot MasterMix (5 Prime Cat# 2200410), 2µl of forward and reversed barcoded primer

(5 μ M), and 2 μ l template DNA. Reactions were run on a C1000 Touch Thermal Cycler (Bio-Rad) with the following cycling conditions: initial denaturing at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 58°C for 1 minute, and extension at 72 C for 90 seconds, with a final extension of 10 min at 72°C. Amplicons were quantified using Agilent 2200 TapeStation system and pooled. Purification was then performed using Ampure XT (Beckman Coulter Cat# A63882) as per the manufacturer instructions.

Analysis of 16S sequences

The obtained 16S rRNA gene sequences were analyzed using the QIIME package for analysis of community sequence data.⁸ Reads were demultiplexed and quality filtered with default parameters. Sequences were then clustered into operational taxonomic units (OTUs) using a 97% similarity threshold with UCLUST⁹ and the Greengenes 16S reference dataset and taxonomy.¹⁰ For each sample, the proportion of reads at the OTU or genus levels was used as a measure of the relative abundance of each type of bacteria in a specimen. PERMANOVA (Adonis) testing was used to compare β diversity of groups. β diversity refers to between-samples similarity based on bacterial composition. After curation and removal of sequences potentially derived from reagent controls, the absolute OTU sequence counts were normalized to obtain the relative abundances of the microbiota in each sample. These relative abundances at 97% OTU similarity and each of the 5 higher taxonomic levels (phylum, class, order, family, genus) were tested for univariate associations with clinical variables. To decrease the number of features, we only focused on major taxa and OTUs, defined as those having a relative abundance >1% in at least one sample. Since lower airway samples have low

biomass, we used SourceTracker to estimate the contribution of background microbiota to BAL samples.¹¹ Predicted metagenome was calculated using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt),¹² a software tool that predicts the functional profile of a bacterial community using only 16S rRNA gene sequence data.

We used the ade4 package in R to construct Principal Coordinate Analysis (PCoA) for β diversity based on weighted UniFrac distances.^{13 14} To avoid negative eigenvalues in the analysis, we used the Cailliez method to convert the weighted UniFrac distance matrix into a closest corresponding matrix with Euclidean properties, which was further used for PCoA.¹⁵ Nonmetric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarity was used to visualize potential clustering patterns among samples based on the estimated β diversity, and its relationship with dominant taxa.¹⁶ For comparisons of α diversity, β diversity, or taxonomy between groups at baseline, non-parametric tests were used (Mann-Whitney). To evaluate for change in α diversity, β diversity post-treatment with AZM or placebo, paired non-parametric comparisons were used (Wilcoxon signed-rank test).

For association with discrete factors, we used the Mann-Whitney test. Since the distributions of microbiome data are non-normal, and no distribution-specific tests are available, we used non-parametric tests of association. Paired statistics (Wilcoxon signed-rank test) were used for pre-post comparison of continuous parameters. To evaluate changes in β diversity pre vs. post treatment we performed Procrustes analysis of the principal coordinate matrices generated after β diversity determination on each

pair of BAL samples (before and after treatment) for every subject. Procrustes analysis is a statistical method based on shape analysis to test whether the conclusions obtained through two different assays performed on a given set of samples are consistent with each other. Specifically, in our study we are testing whether microbiome composition before and after treatment is similar or not. The significance of the fitness between paired samples (baseline and post treatment with either AZM or placebo) is then calculated using a Monte Carlo permutation with 1,000 iterations in order to estimate the probability that the distribution of the post treatment sample in the PCoA plot based on UniFrac distances is similar to the distribution of the baseline sample. False discovery rate (FDR) was used to control for multiple testing. To evaluate differences between groups of 16S data or inferred metagenomes, we used linear discriminant analysis (LDA) effect size (LEfSe).¹⁷ When changes between baseline and post-treatment were evaluated, we utilized the pairwise comparison feature. Features significantly discriminating among groups with LDA score > 2.0 were represented as a cladogram, as produced by LEfSe with default parameters. This ring-shaped cladogram represents the evolutionary relationship among the bacteria identified by our 16S rRNA analysis. Specifically, leaves on the outer ring indicate specific bacterial genera, while inner rings represent higher taxonomic levels, e.g. family, class, etc. Branches represent relations among bacteria, with closer branches indicating closer evolutionary history than branches further apart. This figure represents the effect size that different bacteria have in each of the groups (as measured by the LDA scores) can then be represented as well as the magnitude of the change in relative abundance among the taxa found differentially enriched. LDA scores over 2 are generally considered relevant. The LDA

effect size is utilized as a method of measuring statistical significant difference where an LDA >2 is considered significant.¹⁷ All data are publically available in Gene Expression Omnibus (GEO) under accession number GSE74396.

Measurement of metabolites in BAL fluid.

For metabolomics analysis, we utilized 4 mL of BAL samples obtained before and after treatment with AZM or placebo to perform Gas Chromatography Time of Flight (GC-TOF) Mass Spectrometry.^{18 19} Briefly, after entering the study design into the MiniX database,²⁰ samples were aliquoted and maintained at -80°C until use, at which point samples were thawed, extracted and derivatized.¹⁹ The sample was split into 15 μl aliquots which were each extracted with 1 ml of degassed acetonitrile:isopropanol:water (3:3:2) at -20°C , centrifuged and decanted with subsequent solvent evaporation to complete dryness. An acetonitrile/water (1:1) clean-up step removed lipids and the supernatant was again dried. Internal FAME standards (C8 through C30) were added and the sample was derivatized with methoxyamine hydrochloride in pyridine and subsequently by N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA, Sigma-Aldrich) for trimethylsilylation of acidic protons. An automatic liner exchange system (ALEX, Gerstel MPS2) was used to eliminate between run cross-contamination from sample matrix. One microliter of sample was injected at 50°C (ramped to 250°C) in splitless mode with a 25 sec splitless time. An Agilent 6890 gas chromatograph (Santa Clara, CA) was used with a 30 m long, 0.25 mm i.d. Rtx5Sil-MS column with $0.25\ \mu\text{m}$ 5% diphenyl film; an additional 10 m integrated guard column was used (Restek, Bellefonte

PA).²¹⁻²³ Chromatography was performed at a constant flow of 1 ml/min, ramping the oven temperature from 50°C to 330°C over 22 min. Mass spectrometry used a Leco Pegasus IV time-of-flight mass (TOF) spectrometer with 280°C transfer line temperature, electron ionization at -70 V and an ion source temperature of 250°C.

Mass spectra were acquired from m/z 85–500 at 20 spectra/sec and 1750 V detector voltage. Results²³ were exported to the netCDF format for further data evaluation with BinBase²⁰ and filtered by multiple parameters to exclude noisy or inconsistent peaks. Quantification was reported as peak height using the unique ion.²⁴ All database entries in BinBase were matched against the Fiehn mass spectral library of 1,200 authentic metabolite spectra using retention index and mass spectrum information or the NIST11 commercial library. Resulting files were exported and further processed by the metabolomics BinBase database (UC Davis). Identified metabolites were reported if present in at least 50% of the samples per study design group (as defined in the software). Data were mean-centered and divided by the standard deviation of each variable using MetaboAnalyst.²⁵

RESULTS

There were no differences in demographic and clinical characteristics or extent of emphysema between subjects in the AZM and placebo groups (**Table 1**). As per design, all subjects were current or ex-smokers (current smokers were 4/10 for the placebo and 2/10 for the AZM group, $p=ns$) with similar pack/year (median [IQR]=46.5[32.2-53.2] and 35.5[22.5-48.5] for the placebo and AZM group respectively, $p=ns$). Similarly, there

were no differences in spirometric values (FVC=98[89-110] vs. 99[85-105] percent predicted; FEV1=90[81-96] vs. 90[81-100] percent predicted; FEV1/FVC=67[61-79] vs. 72[67-74] for the placebo and AZM group respectively, p=ns). Emphysema score showed no difference between groups. Despite the presence of varying degree of emphysema in all subjects, four subjects in the placebo group and two in the AZM group met GOLD criteria for COPD.²⁶ BAL cell differential showed that both groups have predominance of alveolar macrophages (~90%) with less than 2% neutrophils, as expected for relatively healthy smokers. There were no significant differences in cell count differential between the placebo and AZM group.

Supplemental Figure 1 shows that background samples are clearly distinct from supraglottic samples and was used to define upper airway taxa and background taxa. The background samples (sterile saline and bronchoscope prior to bronchoscopy) were enriched with the following genera: *Pseudomonas*, *Sphingomonas*, *Corynebacterium*, *Clostridium*, *Stenotrophomonas*, *Staphylococcus*, and *Acidocella* among others. In contrast, the supraglottic samples (obtained with a separate bronchoscope) were enriched with the following genera: *Prevotella*, *Veillonella*, *Streptococcus*, *Rothia*, *Fusobacterium*, *Haemophilus*, *Porphyromonas*, *Catonella*, and *Selenomonas* among others.

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Supplemental Figure Legend

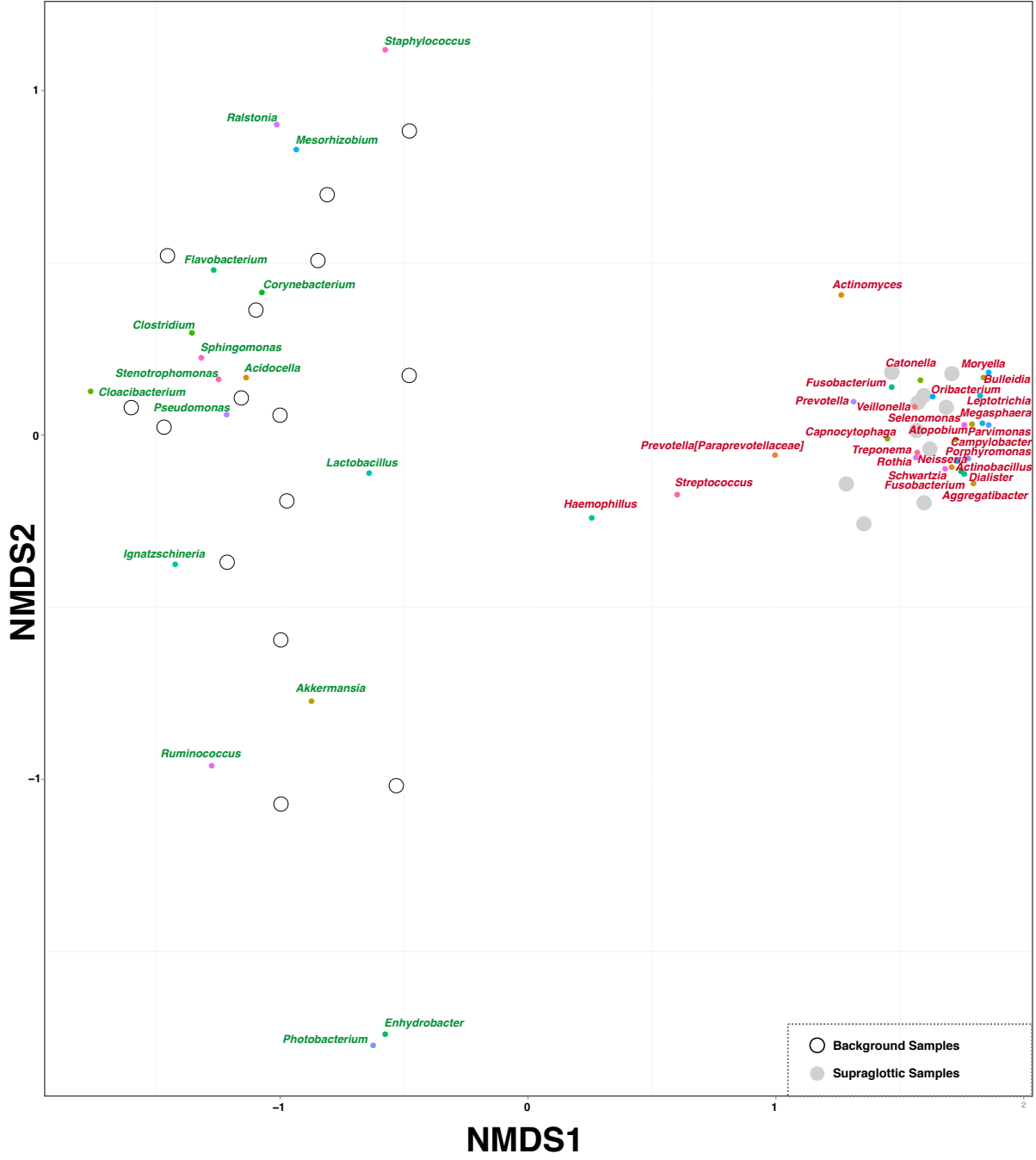
Supplemental Figure 1: Microbiome of background and supraglottic samples.

NMDS analysis was utilized to visualize sample clustering based on β diversity distances (open circles for background samples, e.g. bronchoscope, and grey circles for supraglottic samples). Small colored circles represent most abundant taxa (more than 1000 reads in at least 2 samples) with their taxonomic assignment at a genus level (green for genera most dominant in background samples and red for genera most dominant in supraglottic samples). Analysis showed that background samples and supraglottic samples clustered distinctly.

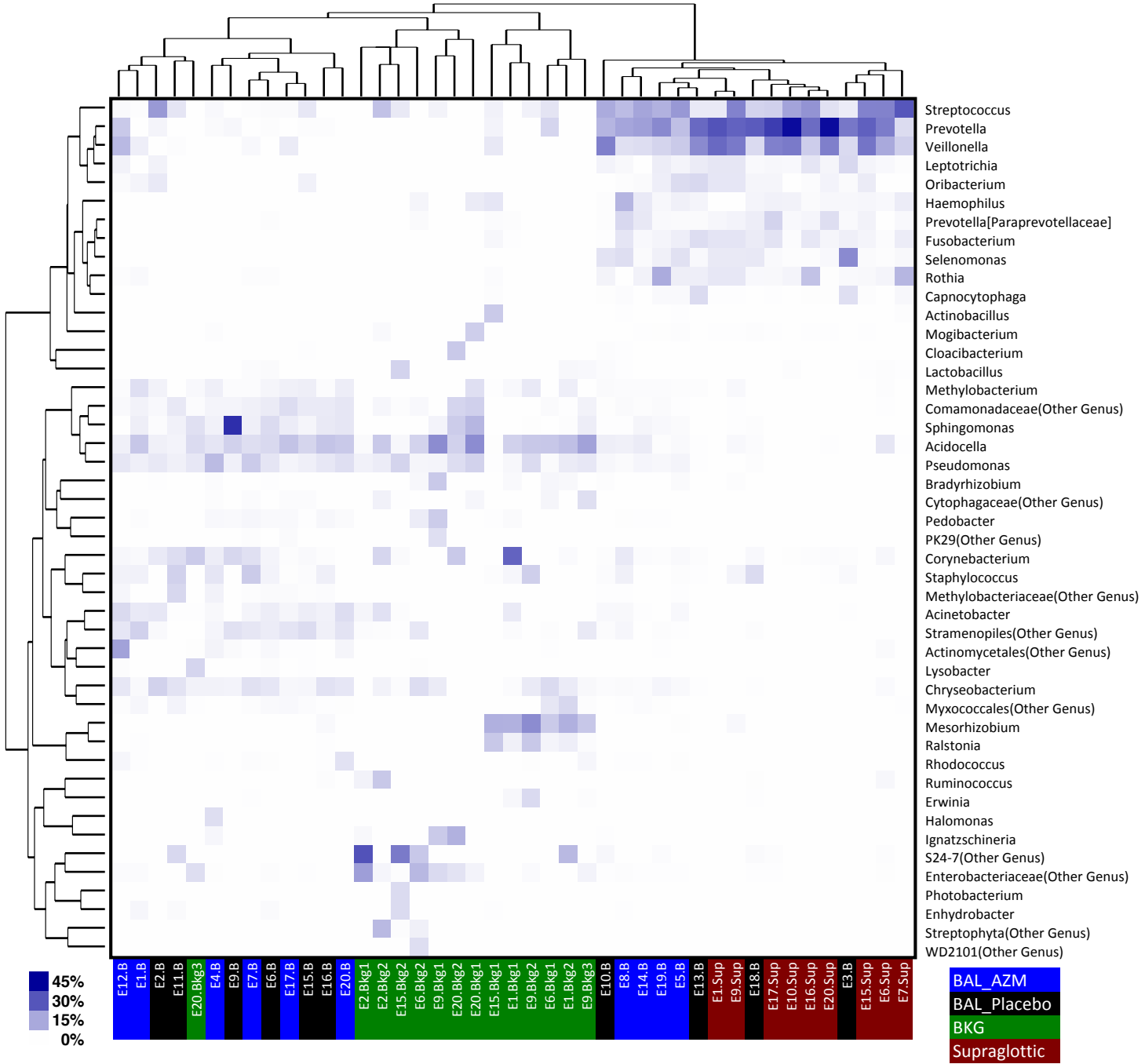
Supplemental Figure 2: Unsupervised hierarchical clustering of most abundant taxa for BAL samples, upper airway and background samples.

Upper airway samples were obtained by oral wash or by separate bronchoscope. BAL samples were obtained after passing the upper airways without suctioning and wedging in a sub segment of the lower airways. Dendrogram demonstrates two well-separated clusters. One dominated by supraglottic samples and 8/20 BAL samples (4 from the AZM group and 4 of the Placebo group). The second cluster contained background samples and the remaining BAL samples.

Supplemental Figure 1



Supplemental Figure 2



Supplementary Table 1: Demographic, pulmonary function and BAL cell differential for 8 subjects from the “Lung Microbiome and Inflammation in Early COPD” study utilized for ex-vivo experiments (Figure 6 Panels B and C)

	n=8
Age (years)	57.5 (49.7-64.5)
Male (%)	6 (75)
Caucasian (%)	5 (63)
BMI	23.0 (21.1-27.5)
Current Smoker (%)	2 (25)
Pack-years	28.8 (26.6-31.4)
PFT*	
FVC post BD †	86.0 (75.7-90.7)
FEV ₁ post BD †	83.5 (76.7-89.7)
FEV ₁ /FVC post BD	79.4 (70.2-86.5)
COPD GOLD Criteria	
GOLD 1	1
GOLD Criteria not met	7
BAL cells (%)	
Macrophages	95.3 (94.1-95.8)
Lymphocytes	3.9 (3.4-4.4)
Neutrophils	0.4 (0.2-0.6)
Eosinophils	0.0 (0.0-0.1)

Data presented as Median (IQR)

Supplemental Table 2: Baseline and Post Treatment BAL Metabolome

Identified Metabolites	Placebo			Azithromycin		
	Baseline	Post	p-value	Baseline	Post	p-value
benzoic acid	679925[331277-1210183]	1355826[894921-2349333]	0.33	519990[343424-975669]	1202138[843110-1592804]	0.01*
indole-3-acetate	26473[16902-67875]	75186[32444-91336]	0.07	51265[28460-88768]	114436[54926-149237]	0.01*
glycolic acid	59034[33058-84747]	119052[51072-277146]	0.09	67431[40507-91334]	153725[94168-200436]	0.01*
linoleic acid	47794[32706-117408]	139611[60235-178711]	0.20	79747[58834-106961]	133049[84809-281600]	0.01*
myristic acid	312792[145061-781892]	830770[575898-1101248]	0.14	456919[322885-651979]	650671[419004-1338945]	0.01*
dihydroabiatic acid	195975[96846-418924]	232604[147591-480440]	0.51	203772[160778-426965]	393816[256817-821487]	0.02*
glycerol	840280[465942-3199227]	2256936[1238100-5079733]	0.39	1135594[545730-1799274]	3784072[2004333-7693163]	0.02*
1-hexadecanol	36235[21577-55660]	76709[45072-99984]	0.09	37593[21128-77509]	75699[40502-106484]	0.02*
pelargonic acid	266076[125452-1342536]	871869[432469-2273577]	0.58	362378[170469-663044]	644735[405906-2180462]	0.02*
pentadecanoic acid	422981[241154-953080]	1016767[580300-1441876]	0.14	479790[367548-850743]	1011859[610962-1735166]	0.02*
capric acid	128685[82200-315752]	361127[180959-816932]	0.24	177135[54818-264712]	283436[182414-882691]	0.03*
3,6-anhydro-D-hexose	80794[55297-290946]	182501[120171-1099303]	0.33	82102[45197-165765]	236065[140200-432409]	0.03*
butyrolactam NIST	352959[171521-2461418]	256781[202829-1138936]	0.72	341688[93616-1950917]	2198378[420854-6154763]	0.04*
222049 M+H 570.3	161903[107642-284973]	399540[216442-804926]	0.07	176821[111545-315314]	398001[181144-846971]	0.04*
gluconic acid	11549[10453-15756]	17067[12570-34565]	0.17	16099[6605-23825]	31755[14626-60137]	0.05
methionine	19825[14032-52918]	57787[27151-138533]	0.03*	22083[17082-35281]	60037[16909-119238]	0.05
methylhexadecanoic acid	375255[238652-915188]	879665[508512-1170058]	0.14	584393[374206-846164]	1014113[476553-1881106]	0.05
palmitic acid	4100008[2074089-7106503]	7044057[4111397-10852973]	0.09	5467752[3319768-7763547]	6675427[4047929-13475967]	0.05
palmitoleic acid	161405[111108-332945]	258730[163470-546780]	0.39	239221[99200-318557]	310881[237504-928806]	0.05
tocopherol alpha	89512[71007-112855]	146425[53625-355144]	0.33	141644[73471-210806]	207279[130893-517808]	0.05
cholesterol	12441169[7940379-17825050]	18807835[8243892-41771866]	0.20	13370046[9727895-24807039]	24872877[10633299-46231338]	0.07
gamma-tocopherol	29439[14613-42305]	50549[30493-73534]	0.09	47286[15066-58434]	75435[33844-160593]	0.07
heptadecanoic acid NIST	41078[33861-85626]	141451[79637-213605]	0.06	87028[29950-108712]	130532[58704-221262]	0.07
leucine	211870[154950-785057]	625528[404527-1345927]	0.04*	158961[122326-302364]	623686[166592-1241871]	0.07
1,5-anhydroglucitol	611537[388980-1083021]	1361975[806891-2288241]	0.24	591223[208574-1122287]	1309966[558922-4010445]	0.07
oleic acid	144372[88729-479394]	280867[159351-535625]	0.51	207990[172596-332046]	361802[232018-827698]	0.07
threonine	131246[91008-303799]	222519[185232-433494]	0.28	169071[86520-236589]	419860[183706-713357]	0.07
citric acid	27740030[19274247-54077039]	45210000[25820743-82168731]	0.07	26926590[12642262-51972991]	57565023[33780791-69227307]	0.09
creatinine	196984[115963-420089]	728193[398990-1047084]	0.09	135048[62471-342433]	460511[314361-652940]	0.09
ethanolamine	2529719[980554-4377351]	2006473[1615126-3571008]	0.88	525316[344679-1222085]	4830077[676272-11189025]	0.09
hydroxycarbamate NIST	41036[17343-77766]	64989[38723-120484]	0.17	36817[23290-49005]	90698[48279-113005]	0.09
indole-3-lactate	39533[10960-69191]	52412[28458-121387]	0.09	36203[21887-102126]	41802[14437-155302]	0.09

isoleucine	116105[70927-382503]	288086[185381-573124]	0.04*	96343[64000-172516]	281059[108602-514551]	0.09
isothreonic acid	20715[10707-26745]	24263[14998-48594]	0.51	17459[9821-38546]	45678[16772-88306]	0.09
lauric acid	414658[342817-1146370]	876271[572705-2334716]	0.44	829400[385000-1062360]	914078[575475-1599731]	0.09
1-deoxyerythritol	26399[15662-106264]	53342[42133-193023]	0.33	25400[19915-82453]	91017[45594-251284]	0.09
2-deoxyerythritol NIST	76973[42867-131276]	178606[103203-354633]	0.11	78608[42272-149179]	135522[61413-508331]	0.09
2-deoxytetronic acid	137017[72742-313583]	379842[200460-931784]	0.06	93956[79187-222074]	231927[120570-1517361]	0.09
serine	60552[34107-84950]	99355[48920-313295]	0.20	56640[45289-83887]	161168[37906-336906]	0.09
sorbitol	33798[26084-46758]	63153[48837-91787]	0.14	32746[20810-50014]	89836[28189-212238]	0.09
threitol	14540[10904-44224]	63794[40702-84127]	0.07	18408[14564-36050]	28432[16707-105229]	0.09
arabinose	166568[105787-351294]	541444[210029-782386]	0.28	126985[104645-176118]	244206[193686-763580]	0.11
beta-alanine	10223[7260-17851]	20479[13364-30608]	0.07	10756[5775-26704]	42031[15421-55300]	0.11
fumaric acid	48843[20738-100903]	127407[38781-251701]	0.04*	57358[36552-82102]	85438[36055-336853]	0.11
glyceric acid	197727[140445-355486]	365826[215601-1088639]	0.24	192620[96246-393736]	548470[215488-1306638]	0.11
guanosine	72099[11771-140654]	102781[45501-243242]	0.20	34893[23430-60192]	127540[33477-414908]	0.11
mannose	111589[66581-172590]	163776[96696-325662]	0.24	45727[30841-165338]	232345[60069-447615]	0.11
1-monopalmitin	64854[38534-100823]	158048[74683-192424]	0.07	93956[45555-117296]	122543[75516-197387]	0.11
nonadecanoic acid	41428[31537-77023]	92507[53728-256395]	0.20	73892[24484-79853]	53800[33546-114359]	0.11
pseudo uridine	55275[26946-96926]	86681[50441-129611]	0.17	33621[22509-76114]	79188[39136-168488]	0.11
thymine	18625[14004-37449]	70084[50452-113046]	0.06	29960[14207-55617]	68473[26054-112422]	0.11
azelaic acid	16022[8099-89159]	124268[23403-284521]	0.03*	47231[33296-97454]	53543[23741-505291]	0.14
conduritol beta expoxide	27229[18965-54279]	30687[16576-145127]	0.58	39259[22180-64704]	88573[19510-219611]	0.14
cytidine-5'-diphosphate	278005[140361-599237]	510454[144004-944608]	0.39	126040[69549-484267]	589751[102188-1516353]	0.14
inosine	1015753[377137-1911421]	2226462[659084-3087468]	0.24	506018[325835-967271]	1400808[392793-4604289]	0.14
malic acid	15269[9992-28290]	32759[13276-49183]	0.06	21267[13155-36449]	54414[15385-87439]	0.14
1-monostearin	45425[22956-62876]	102182[44134-150705]	0.09	42228[26303-75033]	69006[53242-143924]	0.14
2-hydroxybutanoic acid	135005[69281-1113756]	532543[192370-757837]	0.65	138708[63928-234978]	307410[157735-1069430]	0.14
methanolphosphate	183863[79542-435400]	311201[140035-559997]	0.24	151533[94320-324101]	403917[169064-630362]	0.14
phenylethylamine	80527[12065-340215]	44488[29469-619347]	0.33	13723[9576-26756]	28784[20446-114907]	0.14
tagatose	49678[40394-97281]	123934[82926-230453]	0.07	54341[38469-91392]	159287[55335-274924]	0.14
alanine	211952[81123-396375]	401878[241109-1084937]	0.14	211603[170821-412552]	562856[166617-900923]	0.17
arachidonic acid	219161[149301-860541]	409292[270733-872363]	0.58	263035[154267-450189]	406713[202567-1193051]	0.17
aspartic acid	74206[41369-159346]	88708[46835-195609]	0.44	55430[34195-97592]	96187[47549-359957]	0.17
behenic acid	24984[20509-39623]	54594[25882-96074]	0.14	32957[17518-58884]	62485[16258-81850]	0.17
cellobiose	73647[51256-115552]	69228[36618-198672]	0.80	35960[30457-152057]	187715[50512-380661]	0.17
erythritol	43393[30836-147898]	209138[99262-317654]	0.07	44258[36025-97338]	60390[38504-445089]	0.17
inositol myo- delta	528620[421421-923272]	520271[334480-2175482]	0.88	326853[180533-1091986]	1717177[208134-2646930]	0.17
2-picolinic acid	34082[21211-179455]	91910[25373-270095]	0.06	52056[25265-69586]	72045[41546-506061]	0.17

octadecane-1,12-diol NIST	23300[14767-26572]	50075[26578-70176]	0.09	31987[15879-50472]	50949[22529-73682]	0.17
oxoproline	669421[387718-935305]	895224[711563-1093093]	0.14	433476[181425-683614]	1266951[264877-1786470]	0.17
urea	12328286[9051874-18245632]	24852080[23153893-37275978]	0.07	18634224[7461860-26728407]	21731399[17282025-43816779]	0.17
valine	264563[194489-647093]	678485[482716-1223600]	0.07	217256[137136-440210]	507513[122045-1479465]	0.17
fructose	33974[15909-72076]	70988[20909-112230]	0.24	33762[17206-50572]	69907[26041-174362]	0.21
glucose	7292124[5787357-13790523]	13910100[5426338-26899768]	0.28	4065947[3033592-17284625]	15201713[5287551-38775473]	0.21
glutamate	55544[23499-591582]	152110[46058-702684]	0.24	29826[16892-67991]	74959[42417-138795]	0.21
glutamic acid	150845[44704-229646]	74060[50304-245313]	0.96	74320[25434-131068]	216419[42388-395460]	0.21
glycine	1825752[1197647-3018261]	2288399[764250-3671555]	0.80	1117430[234342-1998697]	5012636[401870-7465132]	0.21
lactic acid	196489[139807-1675833]	785127[515703-1660697]	0.28	488183[268303-664979]	555973[216155-2719915]	0.21
levoglucosan	28610[19293-45781]	68869[45554-157920]	0.06	31987[27204-73005]	55677[26040-96112]	0.21
maltose	35687[26171-70622]	45915[26581-108323]	0.28	31929[25294-89018]	70713[40100-148100]	0.21
pimaric acid NIST	23557[10745-44589]	35658[26955-63041]	0.28	22834[8632-60032]	37744[21849-82765]	0.21
salicylaldehyde	77189[44539-160950]	213699[50943-477884]	0.44	73186[24379-243444]	168980[65742-326826]	0.21
succinic acid	97820[49763-234285]	156500[57683-552914]	0.04*	69722[45041-155215]	108365[33763-696300]	0.21
xylitol	22485[13905-57231]	67895[25440-119730]	0.24	16741[13296-46686]	21301[14934-69574]	0.21
beta-sitosterol	21658[13788-54624]	45713[27274-94299]	0.17	23643[12523-39420]	45349[15752-88214]	0.26
threonic acid	16712[8122-60753]	31359[16444-47865]	0.88	9717[5975-58175]	28784[10411-97687]	0.26
fucose + rhamnose	159015[115739-410336]	402565[253875-710902]	0.07	132166[78153-224427]	181053[104332-805653]	0.31
glutamine	296667[86068-527042]	270733[146491-1275971]	0.44	475555[142515-1260969]	986215[546126-1301202]	0.31
proline	172443[92233-581918]	935404[292356-2017627]	0.03*	120499[86502-555697]	766041[238500-1327758]	0.31
uridine	71888[15335-283007]	113715[66430-363704]	0.33	27741[19816-39630]	55827[15064-205671]	0.31
elaidic acid	10919[7470-46406]	43090[22717-235985]	0.09	22965[12790-37570]	48801[15775-60237]	0.37
glutaric acid	38869[15175-90699]	73350[19151-218254]	0.03*	37803[20476-76818]	30509[8612-304951]	0.37
phosphoric acid	1012123[299744-1739223]	2789897[346509-5584042]	0.07	1147404[571176-2390413]	1784305[429660-3997462]	0.37
ribitol	90155[37396-207727]	423417[81291-662931]	0.11	64225[33441-98430]	93523[46664-362132]	0.37
stearic acid	16091841[8523785-23005572]	23636012[13158520-36491870]	0.11	20120483[14254399-31967002]	22839473[12551131-49893609]	0.37
taurine	236556[62706-891649]	215502[73274-410902]	0.58	121090[29931-276589]	576610[77307-1314867]	0.37
adenosine	23824[12024-133858]	160364[42961-1043801]	0.14	30097[10464-78604]	43855[18545-152031]	0.51
hydroxylamine	503198[304634-734579]	916695[781610-1363139]	0.09	727800[330160-1158320]	1002431[675352-1189346]	0.51
200509 carbohydrate	54880[25817-101467]	38501[25461-113867]	0.80	30700[19689-89806]	52549[26399-108806]	0.51
phthalic acid	81926[43852-154329]	192809[106024-299398]	0.14	83216[47943-267281]	89454[65818-375035]	0.51
dodecanol	90491[49587-305369]	145983[99165-424348]	0.28	183922[95102-359231]	260595[100222-457883]	0.59
2-monoolein	45593[24155-111856]	84527[34212-105624]	0.51	33194[20234-90000]	45623[25844-119459]	0.59
monoolein	74398[28471-115430]	196369[95731-433387]	0.07	97932[41284-678938]	178902[59628-496419]	0.59
propane-1,3-diol NIST	69933[47074-141045]	284016[149474-464125]	0.11	172503[89485-425916]	185450[97171-265015]	0.59
sucrose	8921[6336-16763]	27276[13681-65368]	0.24	21179[7454-43083]	21891[14750-38147]	0.59

aconitic acid delta	221412[120656-539689]	283586[62352-790536]	0.58	294464[124521-463953]	204256[20334-1470789]	0.68
arabitol	12567[7293-43230]	27224[13591-57218]	0.72	12340[7195-37657]	9365[3216-42374]	0.68
2-ketoisocaproic acid	14767[9335-31626]	63441[47632-120451]	0.07	41949[21350-71671]	42631[25027-93408]	0.68
4-hydroxybenzoate	4853042[697785-10187236]	17337545[616666-23710953]	0.17	10754058[5910029-23139592]	14465555[3049833-33553337]	0.68
erythronic acid lactone	146592[89921-266195]	318374[159189-1335963]	0.11	180240[75067-1222573]	399939[184649-835516]	0.77
glycerol-alpha-phosphate	140368[105925-182087]	329534[95516-373342]	0.24	148484[85253-195416]	128337[82644-313741]	0.77
lignoceric acid	26618[14927-51559]	70479[41806-227298]	0.09	32134[18908-248229]	48557[21641-126609]	0.77
3-hydroxybutanoic acid	99705[62008-343776]	250772[106813-549333]	0.33	239089[69714-641744]	255127[91014-740552]	0.77
ribose	17980[9860-96789]	92551[51542-159972]	0.06	17986[9852-60106]	20130[11965-140829]	0.77
cysteine	76992[15826-160800]	116821[28187-286450]	0.33	48635[19837-129589]	48758[36315-126731]	0.86
octadecanol	37450[19291-57486]	72523[51409-180429]	0.06	48504[32643-104105]	78399[34599-104519]	0.86
nicotinamide	104807[75138-175623]	172285[94422-433177]	0.11	131364[68272-246056]	103641[51244-342543]	0.95
oxalic acid	715420[431665-3371264]	1157069[727347-7432795]	0.28	639795[288001-3336724]	1215190[662690-1837775]	0.95

Data presented as Median (IQR) intensity

* p-values < 0.05

None of the tests yielded significance after FDR adjustment

Supplemental Table 3: Baseline and Post Treatment BAL Cytokine Levels

	Placebo			Azithromycin		
	Baseline	Post	p-value	Baseline	Post	p-value
TNF α	0.099[0.069-0.293]	0.086[0.061-0.149]	0.29	0.093[0.061-0.158]	0.059[0.045-0.082]	<0.01*
IL-13	0.025[0.021-0.042]	0.027[0.018-0.03]	0.58	0.03[0.022-0.047]	0.019[0.016-0.025]	<0.01*
CXCL1	44.0 [33.5-146.0]	54.8 [33.7-100.4]	0.52	81.3 [69.4-574.2]	53.3[37.3-217.0]	0.01*
IL-12 p40	0.076[0.032-0.105]	0.053[0.044-0.079]	0.20	0.062[0.042-0.091]	0.049[0.038-0.067]	0.02*
IL-9	0.041[0.034-0.059]	0.038[0.033-0.066]	0.44	0.039[0.034-0.095]	0.034[0.032-0.035]	0.06
sIL-2Ra	0.084[0.047-0.149]	0.068[0.042-0.107]	0.45	0.085[0.058-0.169]	0.066[0.035-0.095]	0.07
IL-5	0.026[0.019-0.095]	0.023[0.016-0.07]	0.80	0.077[0.025-0.122]	0.025[0.016-0.039]	0.09
IL-1ra	0.919[0.132-2.037]	0.352[0.093-1.663]	0.51	0.471[0.246-1.138]	0.273[0.1-0.703]	0.11
EGF	0.072[0.039-0.207]	0.072[0.039-0.344]	0.33	0.102[0.041-0.449]	0.09[0.038-0.217]	0.17
FGF-2	0.098[0.083-0.14]	0.087[0.083-0.147]	0.51	0.1[0.088-0.116]	0.087[0.085-0.098]	0.17
sCD40L	0.617[0.05-4.86]	0.121[0.036-0.942]	0.09	0.712[0.275-1.571]	0.149[0.055-1.069]	0.17
GM-CSF	0.19[0.165-0.247]	0.175[0.13-0.235]	0.33	0.197[0.172-0.216]	0.158[0.144-0.189]	0.21
VEGF	4.5[1.3-25.4]	4.6[1.5-20.3]	0.72	17.9[6.7-24.4]	6.2[3.4-19.4]	0.21
IL-1 α	0.524[0.428-1.038]	0.415[0.27-1.066]	0.51	0.413[0.22-0.644]	0.418[0.156-0.57]	0.26
IP-10	100.5 [22.2-196.5]	49.7 [19.8-106.0]	0.24	41.5 [30.0-135.4]	35.9 [35.0-64.2]	0.26
IL-12 p70	0.061[0.047-0.078]	0.051[0.047-0.066]	0.33	0.057[0.048-0.072]	0.054[0.048-0.068]	0.29
IL-15	0.665[0.309-0.778]	0.532[0.27-0.806]	0.88	0.839[0.44-1.201]	0.504[0.347-1.036]	0.31
MCP-1	52.1 [30.3-68.1]	39.8[11.4-58.0]	0.45	29.9 [18.4-54.9]	24.1 [17.4-41.2]	0.37
TGF α	0.176[0.12-0.347]	0.339[0.194-0.416]	0.45	0.297[0.173-0.421]	0.205[0.095-0.377]	0.37
MCP-3	0.354[0.192-0.62]	0.29[0.091-0.581]	0.45	0.223[0.172-0.725]	0.196[0.114-0.556]	0.40
Fractalkine	0.569[0.383-0.964]	0.677[0.322-0.919]	0.80	0.56[0.324-0.988]	0.41[0.193-0.83]	0.44
IL-7	0.111[0.04-0.178]	0.119[0.02-0.166]	0.95	0.148[0.091-0.189]	0.118[0.059-0.196]	0.44
Eotaxin	0.237[0.208-0.312]	0.239[0.169-0.326]	0.51	0.189[0.17-0.332]	0.208[0.153-0.27]	0.52
IL-8	6.7 [5.3-18.7]	8.4 [4.7-17.6]	0.96	8.6 [3.7-14.9]	6.0[2.8-11.4]	0.52
MIP-1 β	0.466[0.331-2.939]	0.446[0.245-1.401]	0.45	0.428[0.293-0.688]	0.343[0.151-0.926]	0.59
MDC	0.73[0.359-2.388]	0.888[0.415-2.427]	0.58	0.464[0.253-1.178]	0.918[0.412-1.1]	0.77
IL-6	0.368[0.228-0.762]	0.276[0.148-0.714]	0.45	0.345[0.306-0.559]	0.484[0.212-0.658]	0.86
MIP-1 α	0.448[0.216-3.587]	0.421[0.178-1.316]	0.14	0.266[0.196-0.598]	0.243[0.134-0.476]	0.86
Fit-3Ligand	0.297[0.186-0.977]	0.339[0.187-0.604]	0.72	0.359[0.227-0.751]	0.342[0.282-1.273]	0.95
G-CSF	11.2[9.4-33.1]	12.3[5.4-25.0]	0.80	10.2 [7.2-19.1]	14.2[3.4-18.6]	0.95

Data presented as Median (IQR) pg/mL

* p-values < 0.05,

None of the tests yielded significance after FDR adjustment