SUPPLEMENTARY TABLES

Online Data Supplement:

Methods:

qPCR Analysis:

Table S1: qPCR Standard Curve Formulas

	16S Total Bacteria Assay	***Lactobacillus* Assay**
Plate 1	y = -3.25x + 29.03	y = -3.15x + 30.65
Plate 2	y = -3.29x + 29.53	y = -3.22x + 30.75

qPCR Plate Correction:

In order to accurately compare plates against each other four samples were run on both plates and the plate that gave larger values was divided by the plate with smaller values to obtain a multiplicative factor. This was averaged for all four samples and an average multiplicative factor was obtained and used for all samples in the plate with smaller values. This was done for both the 16S total bacteria assay and the *Lactobacillus* assay.

Quantitative Histology:

Table S2: Immunostaining of Inflammatory Cells*****

Antibody Name	Antibody Type	Host Species	Against	Company	Catalog Number	Clone Name	Dilution	Pre-Treatment
CD1a+ (Langerhans Cell)	Monoclonal	Mouse	Human	DAKO	MS3571	010	1/50	Acetone 10 min. RT
CD35+ (Dendritic Reticulum Cell)	Monoclonal	Mouse	Human	DAKO	M0846	Ber-MAC-DRC	1/75	Acetone 10 min. RT
CD68+ (Macrophage)	Monoclonal	Mouse	Human	DAKO	M0718	EBM11	1/100	Acetone 10 min. RT
CD79α+ (B-lymphocyte)	Monoclonal	Mouse	Human	DAKO	M7050	JCB117	1/75	Acetone 10 min. RT
CD8+ (Cytotoxic T-lymphocyte)	Monoclonal	Mouse	Human	DAKO	M7103	C8/144b	1/50	Acetone 10 min. RT
NK1+ (Natural Killer Cell)	Monoclonal	Mouse	Human	DAKO	M1014	DAKO-NK1	1/100	Acetone 10 min. RT
Neutrophil Elastase (Neutrophil)	Monoclonal	Mouse	Human	DAKO	M752	NP57	1/50	Acetone 10 min. RT
CD4+ (Helper-inducer T-lymphocyte)	Monoclonal	Mouse	Human	DAKO	M0716	MT310	1/100	Acetone 10 min. RT

* Hansel stain used for Eosinophils

For both intraobserver and interobserver error assessment twenty slides were chosen at random spanning all the different inflammatory groups that were analyzed. Values with zero were omitted from the comparison and analysis of intra- and inter- observer error.

Results:

Table S3: Breakdown of adequate qPCR samples used for the analysis by relative location in the lung

	Top	Middle	Bottom
Control	18	21	12
GOLD 1	13	15	8
GOLD 2	18	19	12

Table S4: List of P-values for Comparisons of Total Bacteria between different Lung Locations

	Top vs. Middle	Top vs. Bottom	Middle vs. Bottom
Control	0.54	0.90	0.65
GOLD 1	0.30	0.72	0.45
GOLD 2	0.69	0.50	0.60

Table S5: Clinical characteristics separated by Lactobacillus positive or negative

	Lactobacillus Positive (mean ± SD)	**Lactobacillus Negative (mean ± SD)**
Age	65.1 ± 9.0	64.2 ± 10.0
Gender (M:F:Unknown)	32:19:1	15:7
FEV_1/FVC	67.6 ± 8.0	70.7 ± 10.5
FEV_1pp (percent predicted)	86.1 ± 16.6	88.6 ±16.3
Smoking History (cigarette-years)	1014.5 ± 585.6	818.9 ± 411.6

SUPPLEMENTARY FIGURES

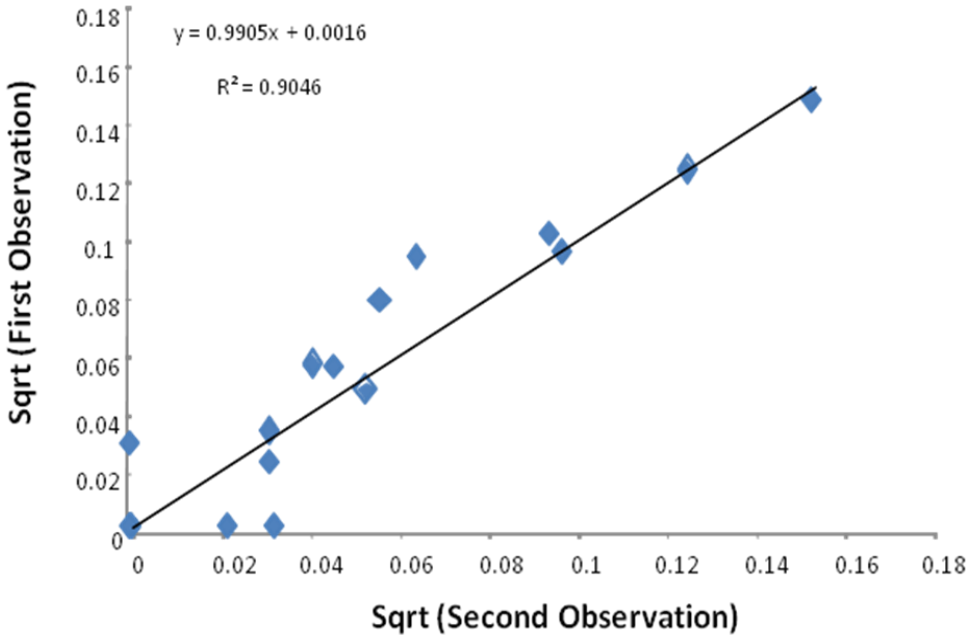

Figure S1: Intraobserver Error of Quantitative Histology Measurements of Vv in Small Airways. An R^2 of 0.9046 was observed showing good repeatability between an individual.

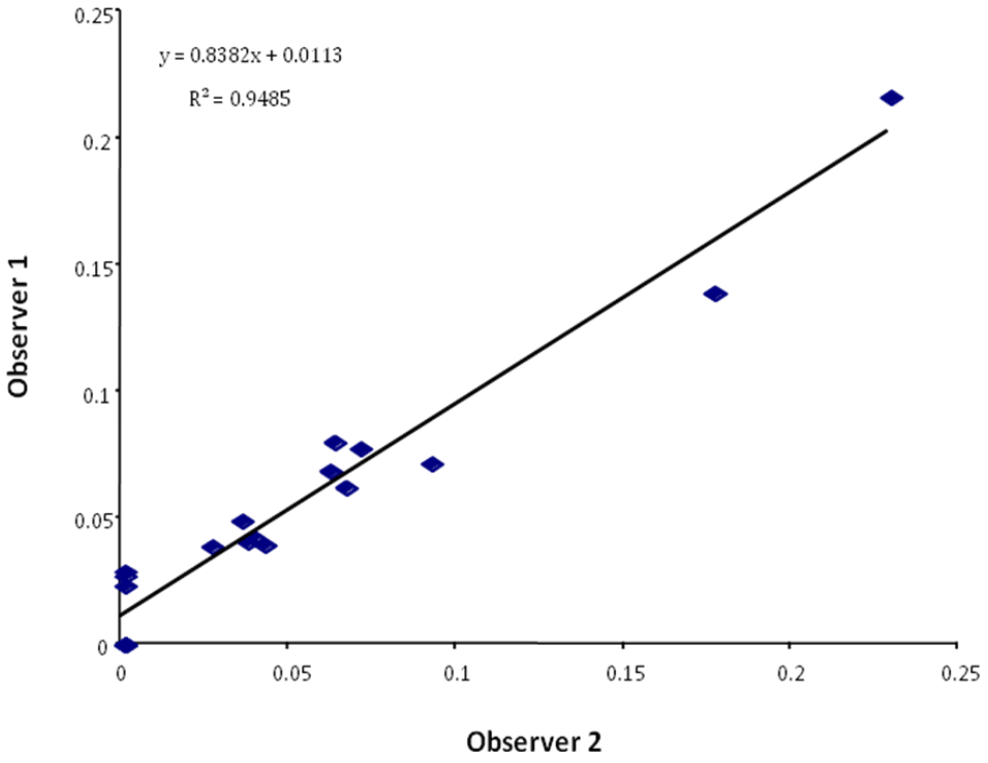

Figure S2: Interobserver Error of Quantitative Histology Measurements of Vv in Small Airways**.** An R^2 of 0.9485 was observed showing good repeatability between two different individuals.

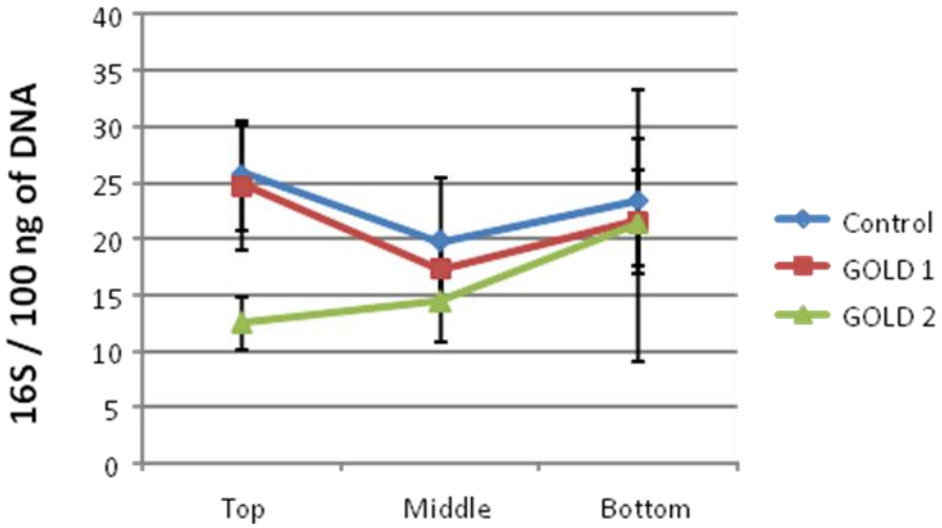

Figure S3: Total bacteria by height and GOLD stage. There is a trend for lower bacteria in the top of the lung in moderate COPD. However, no significant differences were found using ANOVA (P>0.05).

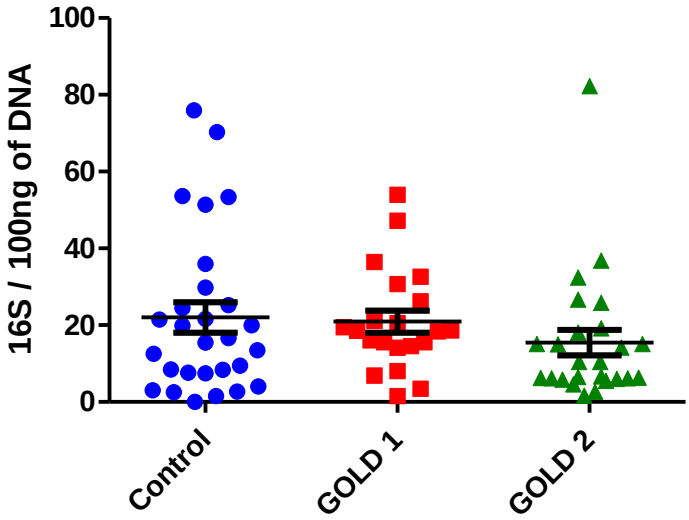

Figure S4: Average Bacterial Load per Group (error bars represent standard error). No significant difference was found in total bacteria by group (P>0.05). Controls n=28, GOLD 1 n=21, and GOLD 2 n=25.

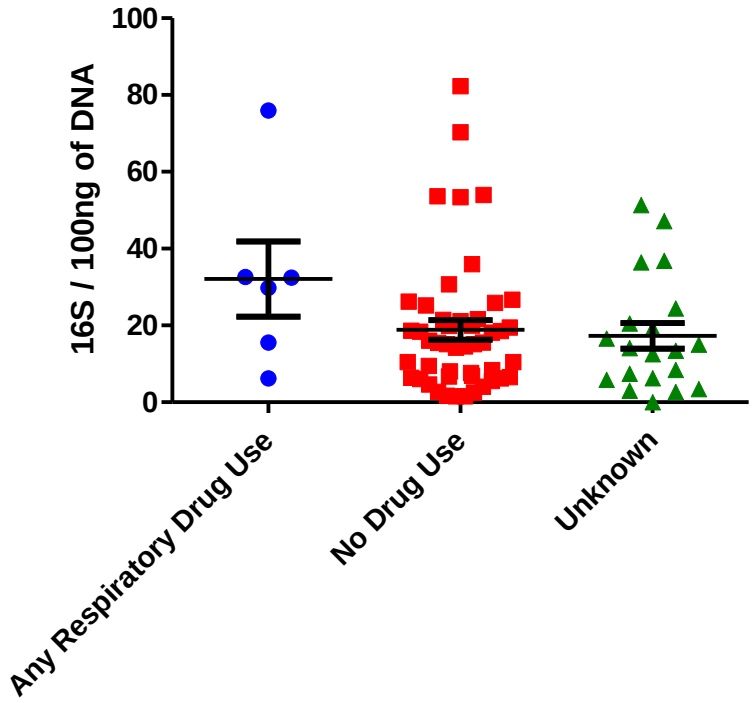

Figure S5: Total bacteria according to drug usage. No significant difference was found between the groups using ANOVA (P>0.05)

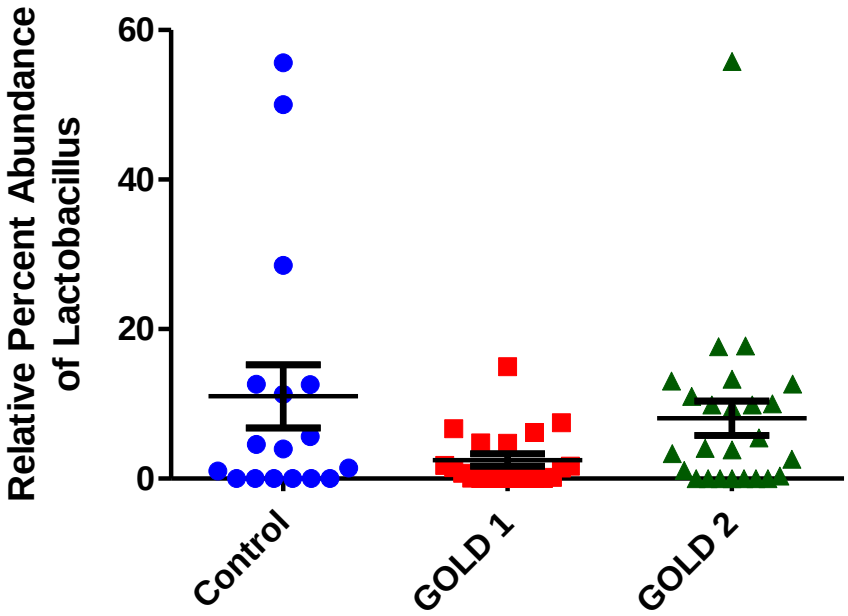

Figure S6: Average Lactobacillus abundance by sample group (error bars represent standard error). No significant difference was found between the three groups (P>0.05). Controls n=28, GOLD 1 n=21, GOLD 2 n=25.

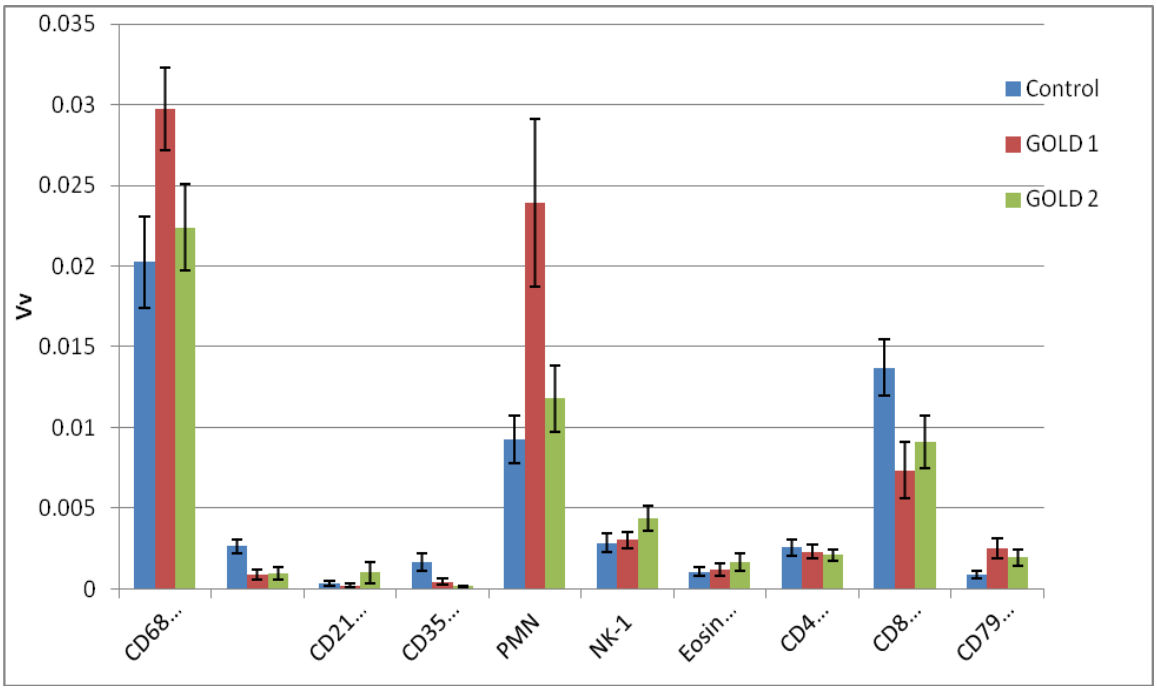

Figure S7: Volume fraction of inflammatory cells separated by GOLD grade. A significant difference was found between GOLD 1 and control for CD68+ macrophages and PMN ($P<0.05$) as well as GOLD 1 and GOLD 2 for CD68+ macrophages and PMN ($P<0.05$).

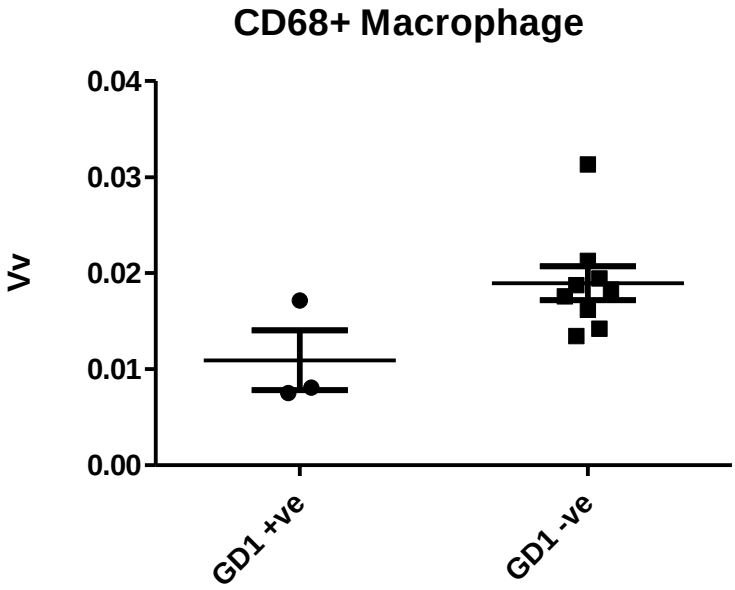

Figure S8: Volume fraction of CD68+ macrophages in control samples by GD1 positivity. There was an increased Vv in GD1 negative samples versus the positive group ($P<0.05$).

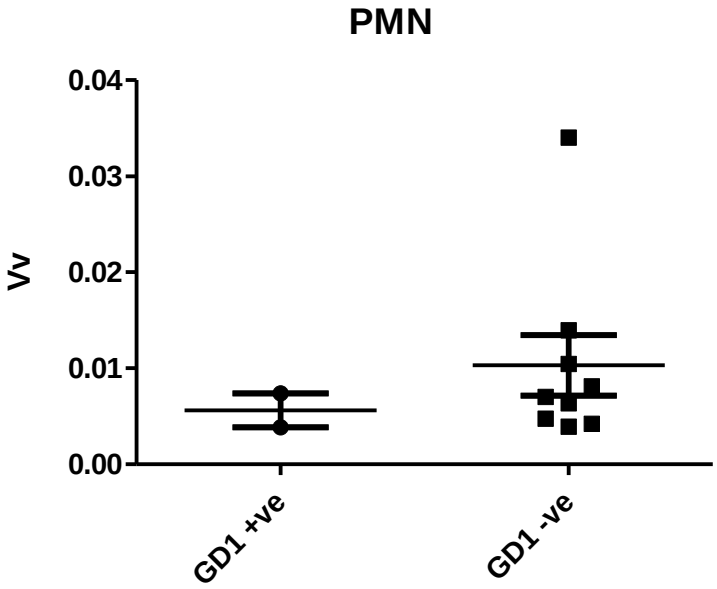

Figure S9: Volume fraction of PMN in control samples by GD1 positivity. There was a trend for increased Vv in GD1 negative samples versus the positive group ($P>0.05$).