ONLINE SUPPLEMENT

Bacterial load and defective monocyte-derived macrophage bacterial phagocytosis in biomass-smoke COPD

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METHODS

COPD cohort

The Chest Research Foundation (CRF) – COPD cohort was jointly established by Vadu Rural Health Program (KEM Hospital Research Centre, Pune, India) and CRF (Pune, India). This cohort includes subjects from 22 rural villages approximately 30 kilometres from Pune city. The subjects were identified through the health camps, based on post-bronchodilator (FEV_1/FVC <70%), clinical examinations conducted by the chest physicians of CRF, history of chronic exposure to noxious stimuli and high resolution computerised tomography (HRCT) imaging.

Sputum induction

Sputum induction was performed as described by Pizzichini and co-workers [1]. Briefly, sputum induction was performed by nebulizing 3% ($^{w}/_{v}$) hypertonic saline through an ultrasonic nebulizer (Omron, Kyoto, Japan) for 15 to 20 minutes. Subjects were asked to cough repeatedly and the whole expectorate was collected in a sterile sample container. Sputum plugs were selected from the whole expectorates, weighed and divided into aliquots to perform various assay (i.e. differential cell count, qPCR assay, and microbial culture assay).

Sputum plugs were diluted with Dulbecco's phosphate buffer saline (D-PBS, Sigma-Aldrich, St. Louis, Missouri) and stored at –80°C to perform qPCR assay. For qPCR assay the aliquots were not treated with any reducing/mucolytic agents (sputolysin or dithiothreitol).

Differential cell counts of sputum cells

Differential cell count was performed within 2 hours as described by Pizzichini *et al* [1]. Briefly, the sputum plugs were treated with 0.1% dithiothreitol (Sigma-Aldrich, St. Louis,

Missouri) and D-PBS (Sigma-Aldrich, St. Louis, Missouri). The resulting suspension was filtered and a cell count of total non-squamous cells was performed using a haemocytometer and expressed as millions of cells per gram of selected induced sputum. The proportion of salivary squamous cells was noted and cell viability was determined by the Trypan blue staining exclusion method. From the remainder filtrate, two cytospins were made and stained with differential staining solutions (Diff Quik stain, Dade Behring, USA). For differential cell count, a total of 400 non-squamous cells were counted and percentage of eosinophils, neutrophils, macrophages, lymphocytes and bronchial epithelial cells present in the total non-squamous cell count were reported.

Quantitative PPBs culturing

The sputum aliquots was homogenized in D-PBS by vortexing for 15 seconds (Swirlex – Vrotex Shaker, Abdos Labware, Kolkata, India). This was further serially – diluted in D-PBS (1:10, 1:100 and 1:1000). Diluted samples was inoculated in Columbia blood agar plates (general growth media), for 48 hours at 37°C and 5% (v/v) carbon dioxide (CO_2) (MSC-Advantage^{TM} Class II, Thermo Fisher Scientific, Waltham, Massachusetts). The enumerated bacterial colonies were identified using standard methods [2]. The specific potentially pathogenic bacterial species (PPBs) were sub-cultured on specific agar plates such as COBA agar for *Streptococcus pneumoniae*, chocolate agar for *Haemophilus influenzae* and *Moraxella catarrhalis*, and Cetrimide agar for *Pseudomonas aeruginosa* for 48 hours at 37°C and 5% CO_2. Results was expressed in colony forming units per mL (CFU mL^{-1}). PPBs with counts of $\geq 10^3$ CFU ml^{-1} in the sputum samples were considered significant [3].

Monocyte isolation and differentiation to macrophages

Peripheral blood mononuclear cells (PBMCs) were isolated from 40 mL venous blood using Percoll density gradient and negative selection using the Miltenyl Monocyte Isolation Kit II (Miltenyi Biotec, Bergisch Galdbach, Germany) as per manufacturer's instructions [4]. The isolated monocytes were re-suspended in MDM complete media (Macrophage-SFM media supplemented with 10% ($^{v}/_{v}$) fetal calf serum, and 1% ($^{v}/_{v}$) antibiotic-antimycotic) at 1×10^{6} cells/mL. The cells were seeded in 96-well flat clear bottom black polystyrene tissue culture plates (1×10^{5} cells/well) at 37°C, and 5% CO_2 ($^{v}/_{v}$) for 2h to allow the monocytes to adhere. After 2 hours, the non-adherent cells were aspirated and the monocytes were incubated with fresh MDM-complete media containing 2 ng mL^{-1} granulocyte-monocyte colony stimulating factor (GM-CSF) (Sigma-Aldrich, St. Louis, Missouri) for 12 days to allow complete differentiation [4]. Media was changed every 4^{th} and 7^{th} day until adequate MDMs were developed to conduct phagocytic assays [4].

Phagocytosis assay

MDMs phagocytic assay was measured as described previously [4]. MDMs were exposed for 4 hours, to fluorescently-labelled polystyrene beads of 2 µm at a concentration of 50×10^{6} beads mL^{-1} or to heat-killed non-opsonized *Streptococcus pneumoniae* (labelled with Alexa-Fluor 488 conjugate) at a concentration of 1.2×10^{9} CFU/mL and *Haemophilus influenzae* (labelled with Alexa-Fluor 488) at a concentration of 1.5×10^{8} CFU/mL. MDMs were washed with D-PBS and extracellular fluorescence was quenched with 1% ($^{w}/_{v}$) Trypan blue at room temperature. Trypan blue was aspirated and phagocytosis of beads and bacteria was measured using a fluorimeter (Fluoroskan Ascent FL, ThermoFisher Scientific, Massachusetts, USA) at

an excitation of λ485 nm and emission λ538 nm. Data is expressed as relative fluorescent units (RFU).

Viability assay

After performing the phagocytic assay, the cells were washed with D-PBS and 0.5 mg/mL of MTT dissolved in D-PBS was added and incubated for 1 hour at 37°C, and 5% CO_2. 100 μL Dimethyl sulfoxide (DMSO) was added to each well. The plates were gently shaken for 10 minutes and the absorbance was recorded at 570 nm using a microplate spectrophotometer (Multiskan Spectrum, ThermoFisher Scientific, Massachusetts, USA). The cell viability was expressed as the percentage of absorbance of exposed cells to non-exposed cells.

REFERENCES

1. Pizzichini E, Pizzichini MMM, Efthimiadis A.; Evans S, Morris MM, Squillace, D, Gleich, GJ, Dolovich, J, Hargreave F. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid phase measurements. *Am. J. Respir Crit Care Med* 1996; 154: 308–317.

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3. Tumkaya M, Atis S, Ozge C, Delialioglu N, Polat G, Kanik A. Relationship between airway colonization, inflammation and exacerbation frequency in COPD. *Respir. Med.* 2007; 101: 729–737.

4. Taylor AE, Finney-Hayward TK, Quint JK, Thomas CMR, Tudhope SJ, Wedzicha JA, Barnes PJ, Donnelly LE. Defective macrophage phagocytosis of bacteria in COPD. *Eur. Respir. J.* 2010; 35: 1039–1047.

Table S1: Primer sequences for amplification using cDNA as template

	Name of the organism	Primer Name	Sequence (5' – 3')	No. of bases	Expected length of cDNA (bp)
1.	*Streptococcus pneumoniae*	*Spn*9802 – F Spn9802 – R	AGTCGTTCCAAGGTAACAAGTCT ACCAACTCGACCACCTCTTT	23 20	157
2.	*Haemophilus influenzae*	*P4 Liporotein* – F *P4 Liporotein* –R	CCGGGTGCGGTAGAATTTAATAA CTGATTTTTCAGTGCTGTCTTTGC	23 24	91
3.	*Moraxella catarrhalis*	*Cop B* – F *Cop B* – R	GTGAGTGCCGCTTTACAACC TGTATCGCCTGCCAAGACAA	20 20	71
4.	*Pseudomonas aeruginosa*	*Pa23* – F *Pa23* – R	TCCAAGTTTAAGGTGGTAGGCTG ACCACTTCGTCATCTAAAAGACGAC	23 25	94

Table S2: qCR program of organism

	Name of the organism	Initialization temperature	Denaturation temperature	Annealing temperature	Extension temperature	Final elongation temperature
1.	*Streptococcus pneumoniae*	94ºC	94ºC	51ºC	72ºC	72ºC
2.	*Haemophilus influenzae*	94ºC	94ºC	50ºC	72ºC	72ºC
3.	*Moraxella catarrhalis*	94ºC	94ºC	52ºC	72ºC	72ºC
4.	*Pseudomonas aeruginosa*	94ºC	94ºC	52ºC	72ºC	72ºC

Table S3: Demographic characteristics of the subjects

	Healthy (N = 49)			COPD (N = 42)	
Characteristics	**H-NS**	**HS**	**H-BMS**	**S-COPD**	**BMS-COPD**
N	18	15	16	19	23
Age (years)	64.28 ± 3.72	64.60 ± 6.89	62.50 ± 9.45	63.58 ± 7.56	63.30 ± 6.70
Male : Female	18 : 0	15 : 0	0 : 16	19 : 0	0 : 23
Smoking history (pack-years)	0.00 ± 0.00[bc]	37.41 ± 19.81	0.00 ± 0.00[bc]	31.92 ± 15.95	0.00 ± 0.00[bc]
Smoking status (Current : Ex)	0 : 0	11 : 4	0 : 0	15 : 4	0 : 0
Biomass-smoke exposure history (Hours-year)	0 (0 – 0)	0 (0 – 0)	144 (130 – 269.3)[abd]	0 (0 – 0)	134 (120 – 210)[abd]
Biomass-smoke exposure status (current : ex)	0 : 0	0 : 0	16 : 0	0 : 0	23 : 0
Total CAT Score	-NA-	-NA-	-NA-	25.32 ± 7.40	18.91 ± 6.67[d]
Total SGRQ Score	-NA-	-NA-	-NA-	64.11 ± 16.74	63.66 ± 18.71
GOLD (Mild : Moderate :: Severe : Very Severe)	-NA-	-NA-	-NA-	3 : 6 :: 7 : 3	4 : 7 :: 8 : 4
β agonists	-NA-	-NA-	-NA-	42.10 % (8)	43.48 % (10)
Anti-cholinergic	-NA-	-NA-	-NA-	15.78 % (3)	17.39 % (4)
FEV_1/FVC (%)	80.1 ± 8.6	78.3 ± 4.1[a]	79.6 ± 5.2	51.7 ± 11.0[abc]	54.5 ± 11.1[abc]
Post-bronchodilator					
FEV_1 % predicted	104.6 ± 13.4	98.2 ± 20.7	95.8 ± 11.1	49.8 ± 16.9[abc]	50.4 ± 20.1[abc]
FVC % predicted	104.3 ± 17.7	99.7 ± 21.2	100.1 ± 10.1	72.9 ± 16.9[abc]	72.6 ± 17.7[abc]

Shapiro-Wilk normality test was performed. Parametric data are expressed as mean ± SD; whereas non-parametric data are expressed as median (interquartile range). Within the group comparison was performed by Kruskal-Wallis/Dunn's multiple comparisons test for non-parametric data and ANOVA/Tukey's multiple comparisons test for parametric data. [a]$P \leq 0.05$ *versus* H-NS. [b]$P \leq 0.05$ *versus* HS. [c]$P \leq 0.05$ *versus* H-BMS. [d]$P \leq 0.05$ *versus* S-COPD.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; CAT, COPD assessment test; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, global initiative for chronic obstructive pulmonary disease; SGRQ, St. George's Respiratory Questionnaire; H-NS, healthy non-smokers; HS, smokers without COPD; H-BMS, biomass-smoke exposed healthy; S-COPD, tobacco-smoke associated COPD; BMS-COPD, biomass-smoke associated COPD, SD, standard deviation; NA, not applicable]

Table S4: Multivariable linear regression analysis of demographic characteristics on load of potentially pathogenic bacterial species in induced sputum samples of the subjects

	Load of potentially pathogenic bacteria (Dependent variable)							
Independent Variables	***Streptococcus pneumoniae***		***Haemophilus influenzae***		***Moraxella catarrhalis***		***Pseudomonas aeruginosa***	
	β (*P*–value)	**95% CI**	**β (*P*–value)**	**95% CI**	**β (*P*–value)**	**95% CI**	**β (*P*–value)**	**95% CI**
Age (in years)	-0.022 (0.686, ns)	-550.39 – 364.146	0.028 (0.679, ns)	-31827.85 – 48602.85	-0.022 (0.686, ns)	-550.39 – 364.15	0.121 (0.148)	-4901.32 – 31981
Smoking history (Pack years)	0.228 (0.001)	132.03 – 497.69	0.364 (0.000)	18768.04 – 50138.99	0.228 (0.001)	132.03 – 497.69	-0.199 (0.061, ns)	-14685.23 – 333.36
Biomass smoke exposure history (hours-year)	0.265 (0.000)	38.11 – 115.05	0.586 (0.000)	8573.09 – 15256.94	0.265 (0.000)	38.11 – 115.05	0.336 (0.002)	985.58 – 4103.72

The independent variables are age, smoking history and biomass-smoke history. The linear regression analysis of load of each potentially pathogenic bacterium was performed with the independent variables. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations:* β, Beta for standardised regression coefficients; CI, confidence interval]

Table S5: Multivariable linear regression analysis of demographic characteristics on MDMs phagocytic activity of pathogenic bacteria among the subjects.

Independent variables	MDMs phagocytosis activity (RFU) (Dependent variable)			
	Streptococcus pneumoniae		***Haemophilus influenzae***	
	β (*P*–value)	**95% CI**	**β (*P*–value)**	**95% CI**
Age (in years)	0.115 (0.08, ns)	-1.344 – 23.348	0.027 (0.709, ns)	-9.728 – 14.237
Gender	-0.247 (0.073, ns)	-0.687.891–31.012	-0.223 (0.073, ns)	-595.788 – 84.011
Smoking history (Pack years)	-0.433 (0.000)	-19.577 – -9.361	-0.389 (0.000)	-16.212 – -6.181
Biomass smoke exposure history (hours-year)	-0.229 (0.006)	-2.626 – -0.442	-0.258 (0.009)	-2.591 – -0.380

The independent variables are age, smoking history and biomass-smoke history. The linear regression analysis of MDMs phagocytic activity for pathogenic bacterial was performed with the independent variables. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations:* MDMs, Monocyte-derived macrophages; β, Beta for standardised regression coefficients; CI, confidence interval]

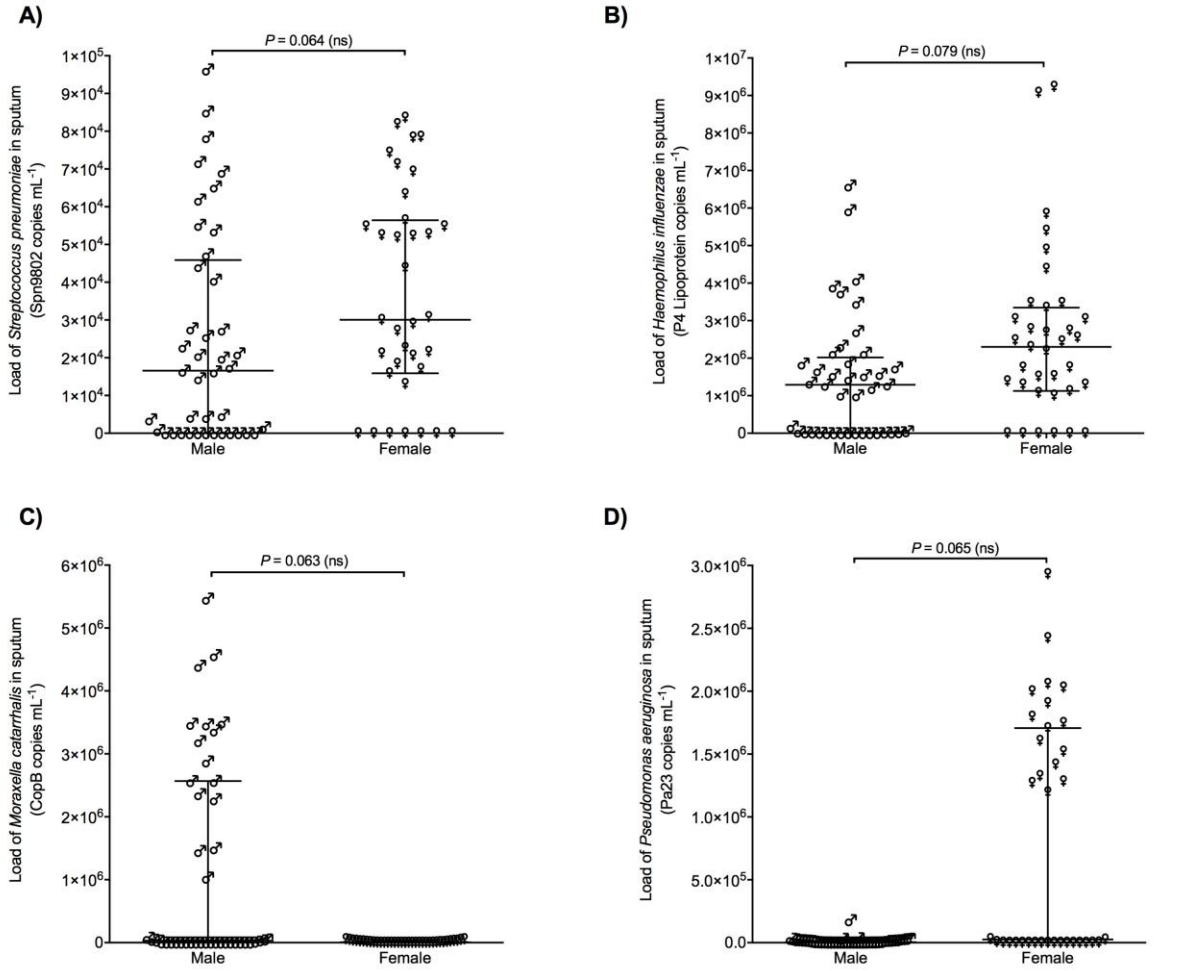


Figure S1: Gender-related comparison of each potentially pathogenic bacterial load in the induced sputum samples among study subjects using qPCR assay.

Bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C), and *Pseudomonas aeruginosa* (D) in male (♂ n = 41) and female (♀ n = 35) subjects. Shapiro-Wilk normality test was performed. Data are presented as median (interquartile range). Non-parametric Mann-Whitney test was performed. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: qPCR, quantitative polymerase chain reaction]

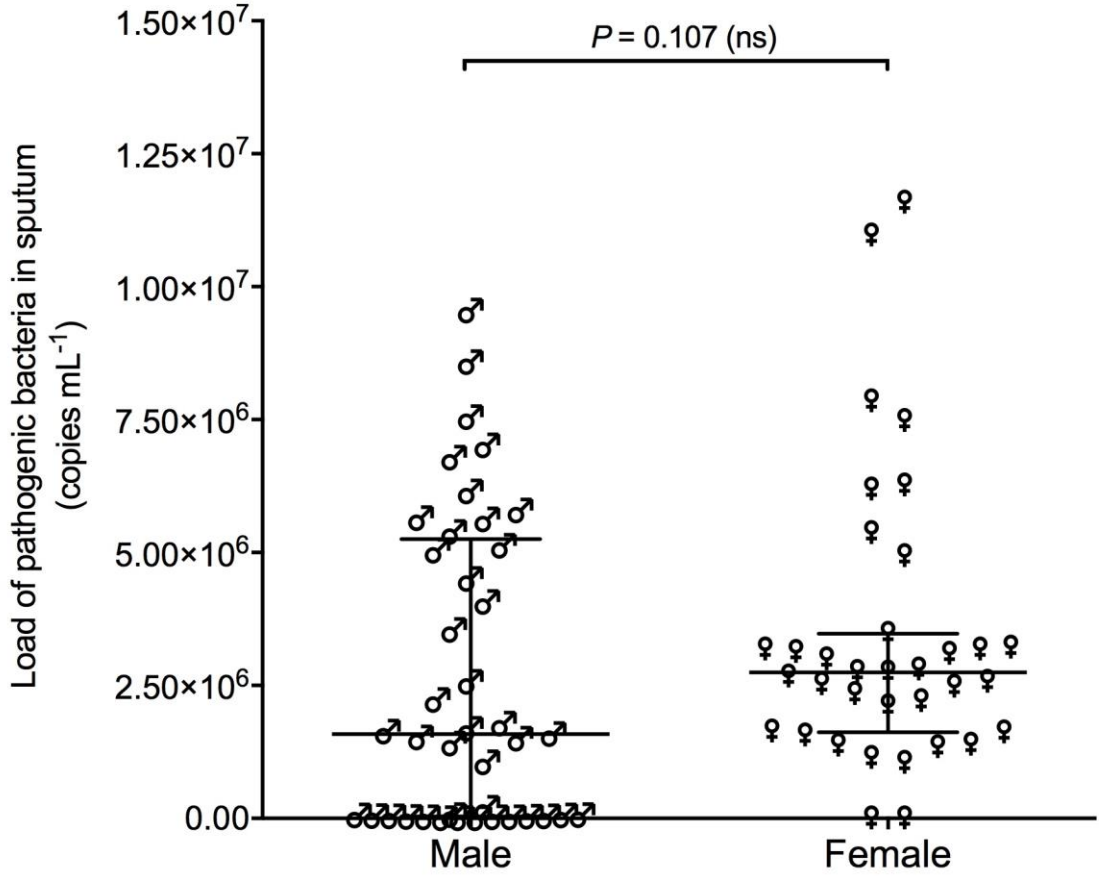


Figure S2: Gender-related comparison of overall load of potentially pathogenic bacterial load in the induced sputum samples among study subjects using qPCR assay.

Overall load of potentially pathogenic bacteria is the mean load of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa* in male (♂ n = 41) and female (♀ n = 35) subjects. Shapiro-Wilk normality test was performed. Data are presented as median (interquartile range). Non-parametric Mann-Whitney test was performed. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: qPCR, quantitative polymerase chain reaction]

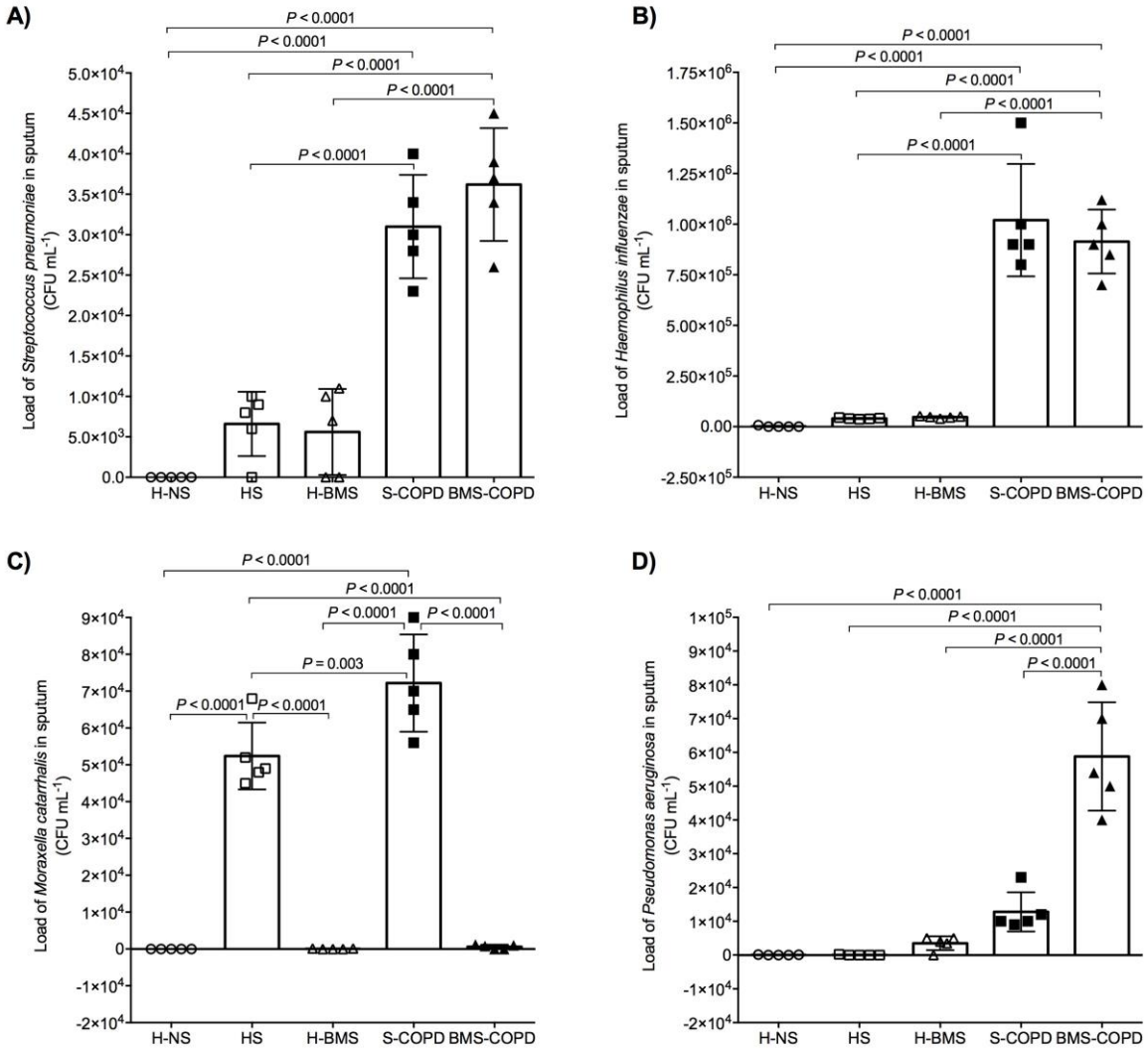


Figure S3: Load of potentially pathogenic microbes in the induced sputum samples among study subjects using microbial culture.

Bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C), and *Pseudomonas aeruginosa* (D) in H-NS (○ n = 5), HS (□ n = 5), H-BMS (△ n = 5), S-COPD (■ n = 5) and BMS-COPD (▲ n = 5). Shapiro-Wilk normality test was performed. Data are presented as mean (standard deviation). One-way ANOVA followed by Holm-Sidak multiple comparisons test was performed for within the group comparisons. $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; H-NS, healthy non-smokers; HS, smokers without COPD; H-BMS, biomass-smoke exposed healthy; S-COPD, tobacco-smoke associated COPD; BMS-COPD, biomass-smoke associated COPD]

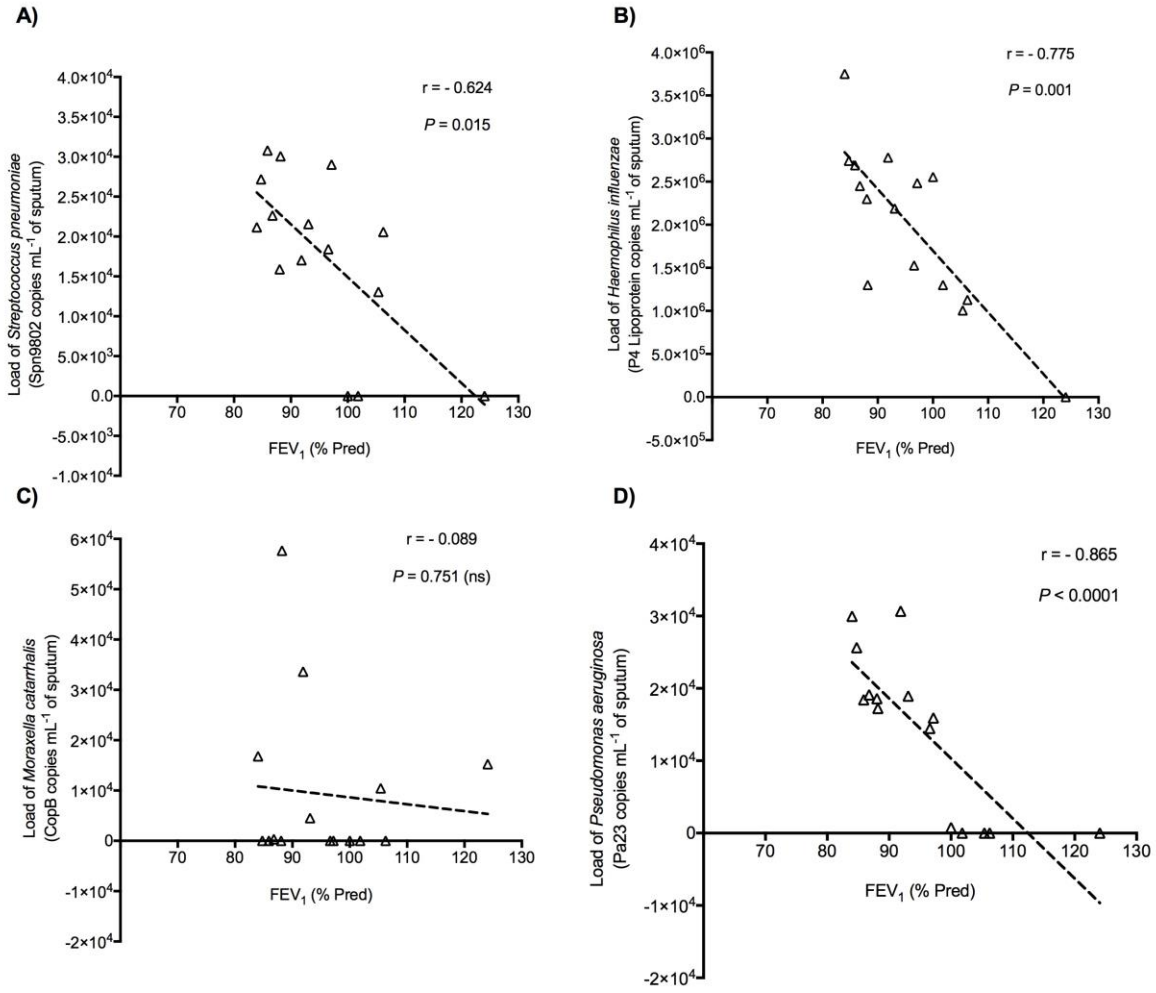


Figure S4: Relationship between bacterial load in induced sputum samples and FEV_1 % predicted in biomass-smoke exposed healthy subjects.

The correlation between FEV_1 % predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in H-BMS (△ n = 15). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; H-BMS, biomass-smoke exposed healthy]

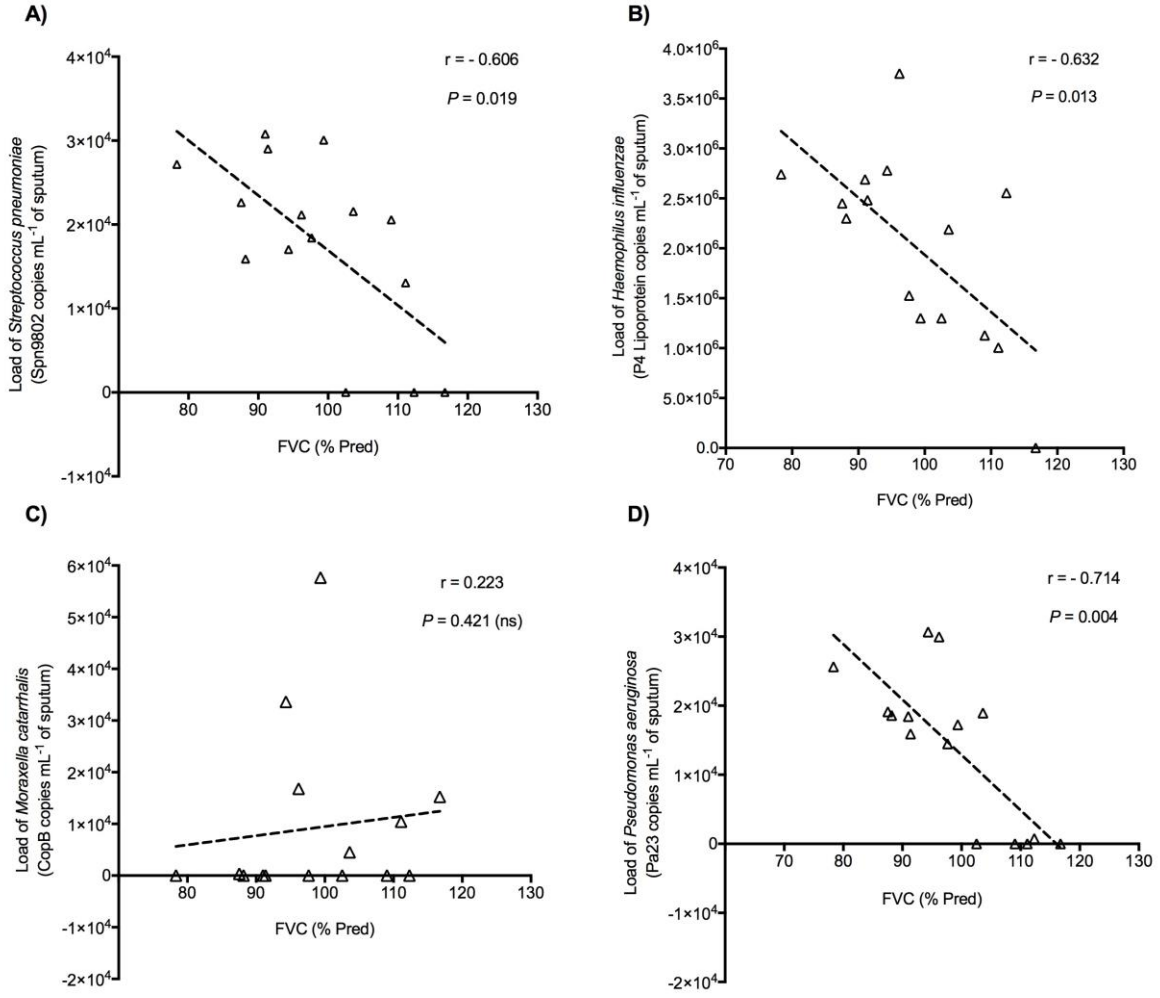


Figure S5: Relationship between bacterial load in induced sputum samples and lung FVC % predicted in biomass-smoke exposed healthy subjects.

The correlation between FVC % predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in H-BMS (△ n = 15). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity; H-BMS, biomass-smoke exposed healthy]

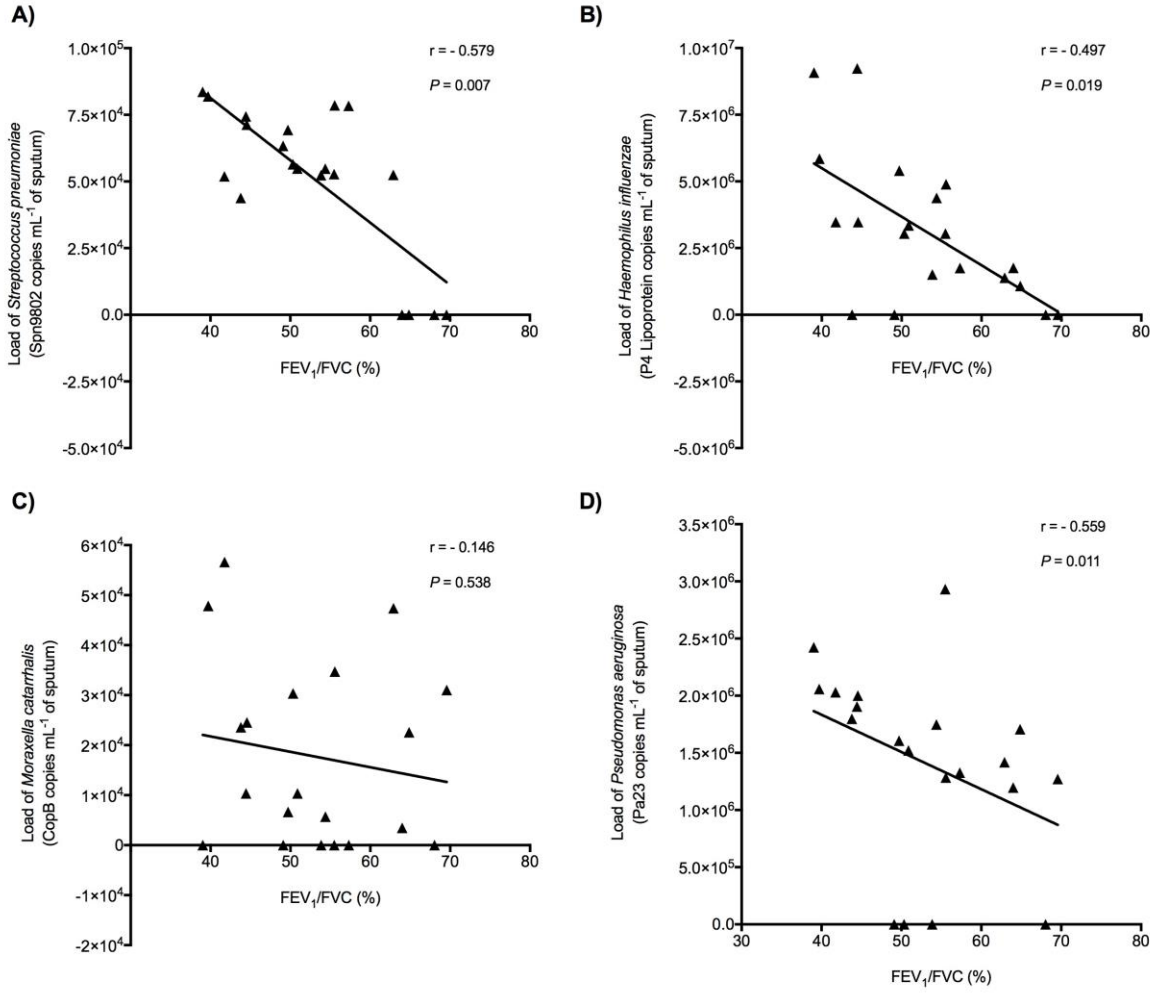


Figure S6: Relationship between bacterial load in induced sputum samples and FEV_1/FVC in biomass-smoke associated COPD subjects.

The correlation between FEV_1/FVC (%) predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in BMS-COPD (▲ n=20). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; BMS-COPD, biomass-smoke associated COPD]

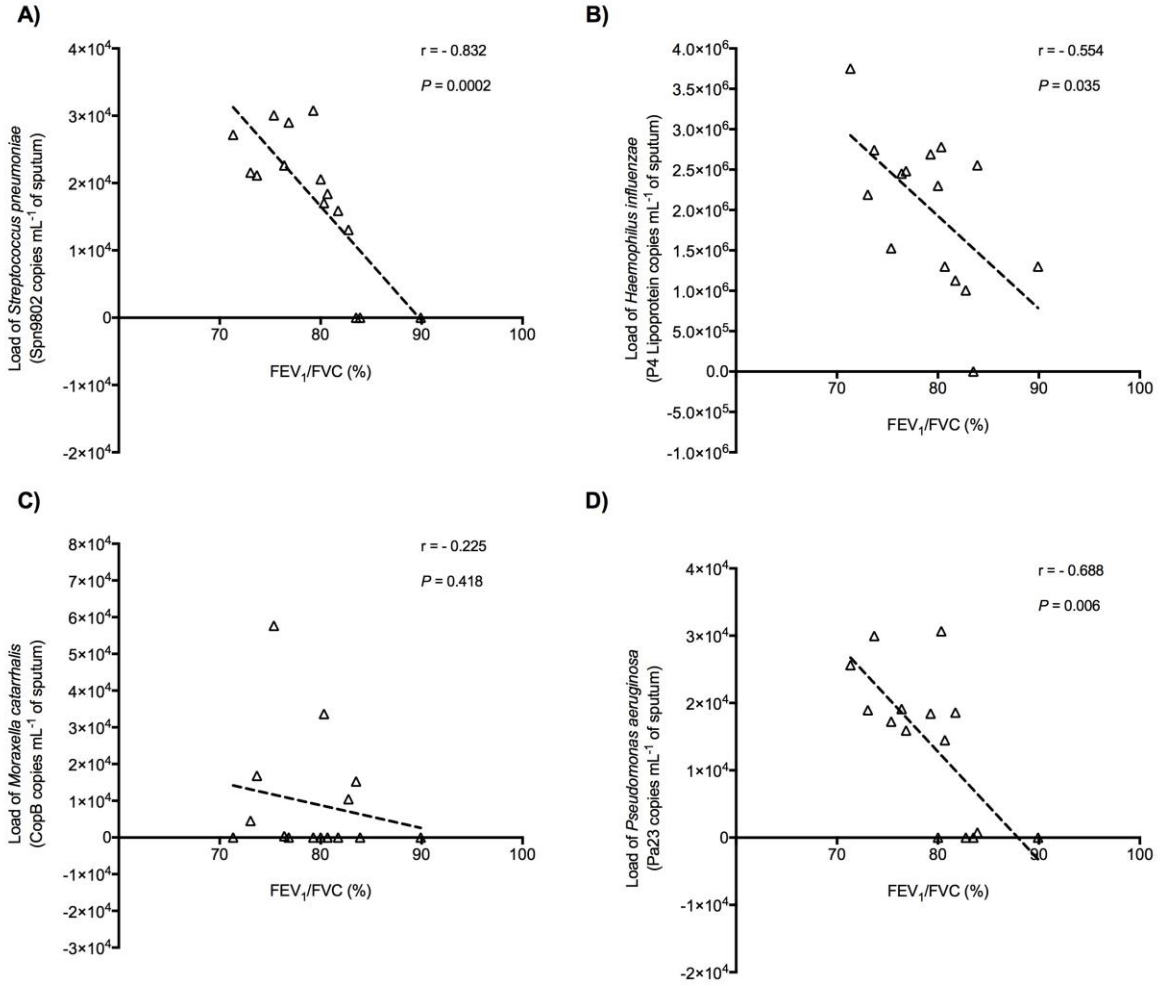


Figure S7: Relationship between bacterial load in induced sputum samples and FEV_1/FVC in biomass-smoke exposed healthy subjects.

The correlation between FEV_1/FVC (%) predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in H-BMS (△ n = 15). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; H-BMS, biomass-smoke exposed healthy]

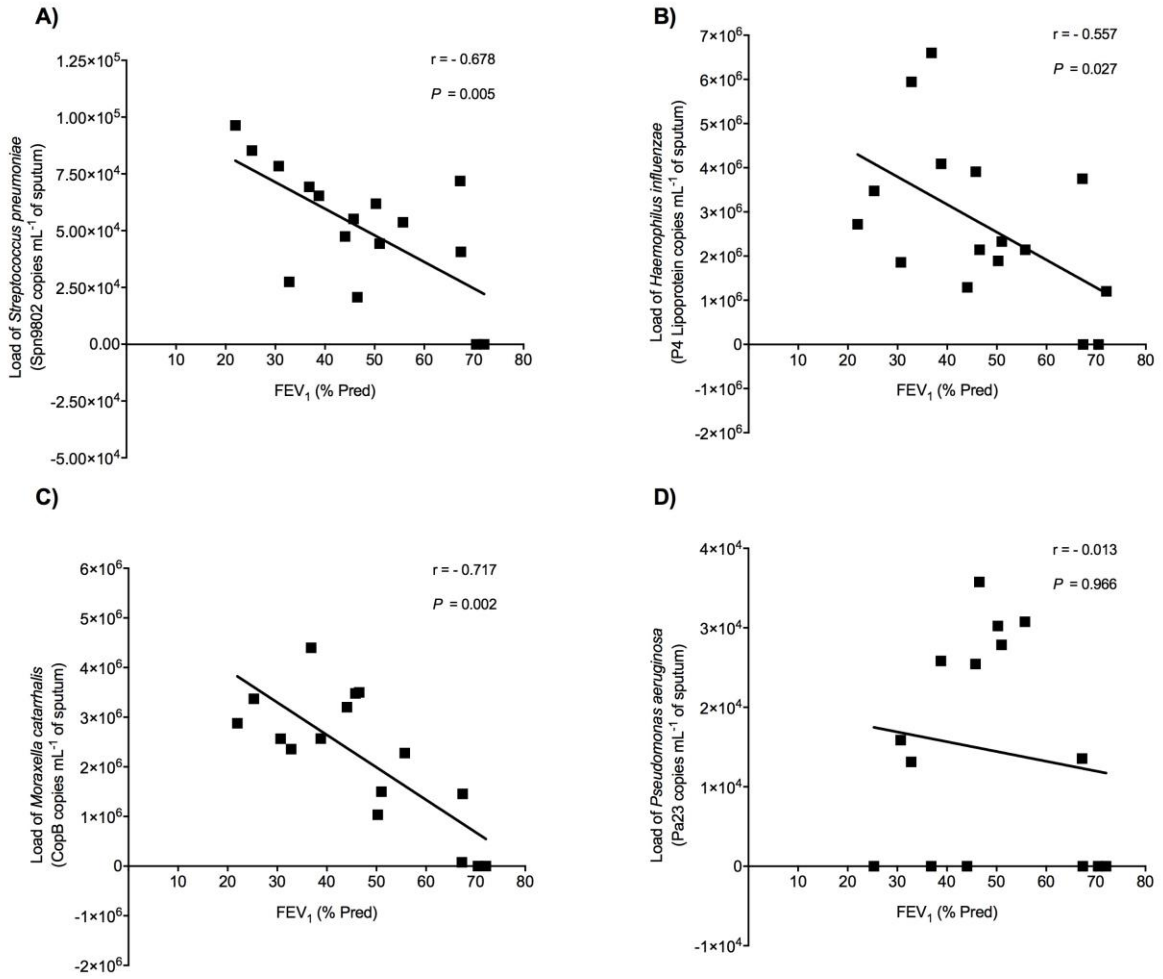


Figure S8: Relationship between bacterial load in induced sputum samples and FEV_1 % predicted in tobacco-smoke associated COPD subjects.

The correlation between FEV_1 % predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in S-COPD (■ n = 16). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; S-COPD, tobacco-smoke associated COPD]

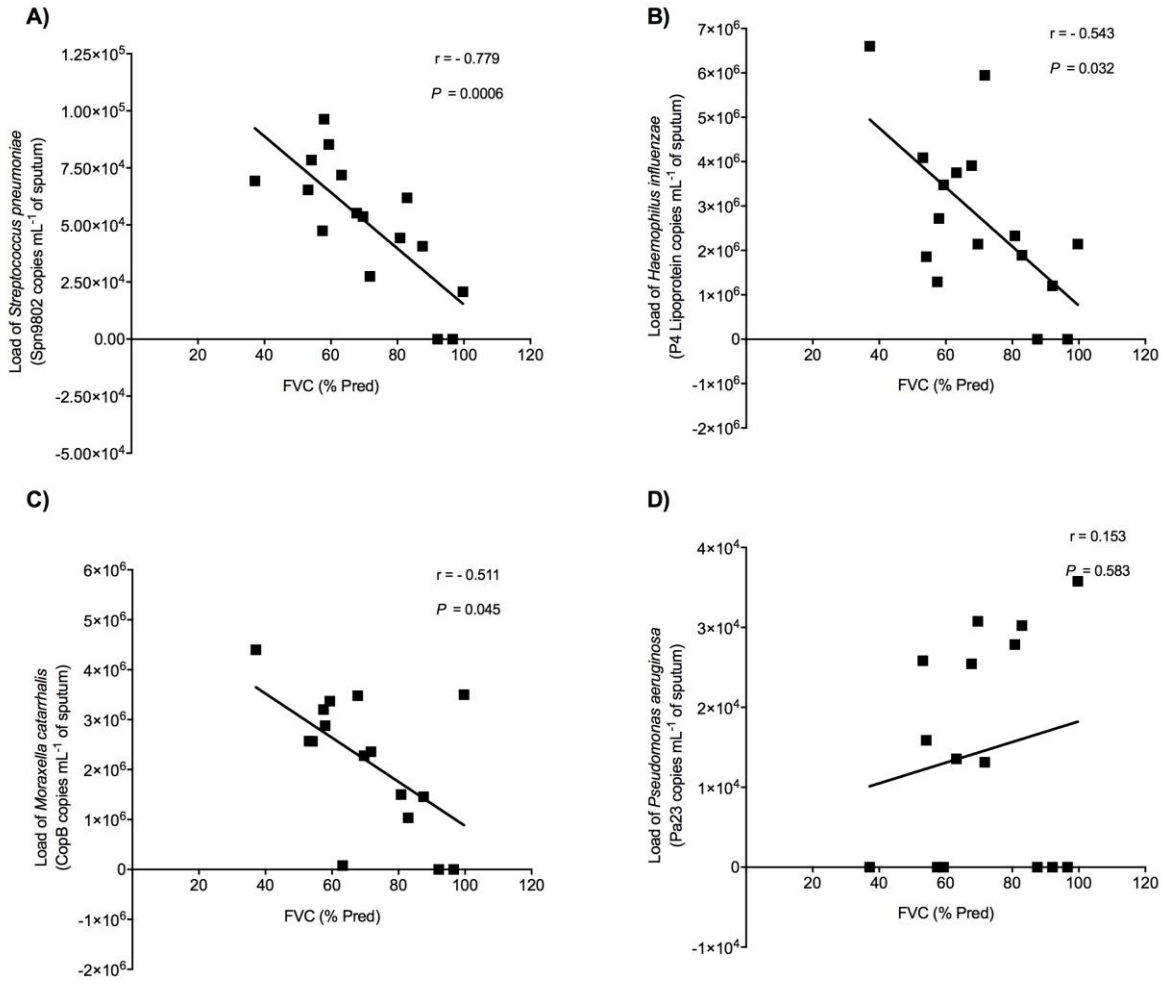


Figure S9: Relationship between bacterial load in induced sputum samples and FVC % predicted in tobacco-smoke associated COPD subjects.

The correlation between FVC % predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in S-COPD (■ n = 16). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity; S-COPD, tobacco-smoke associated COPD]

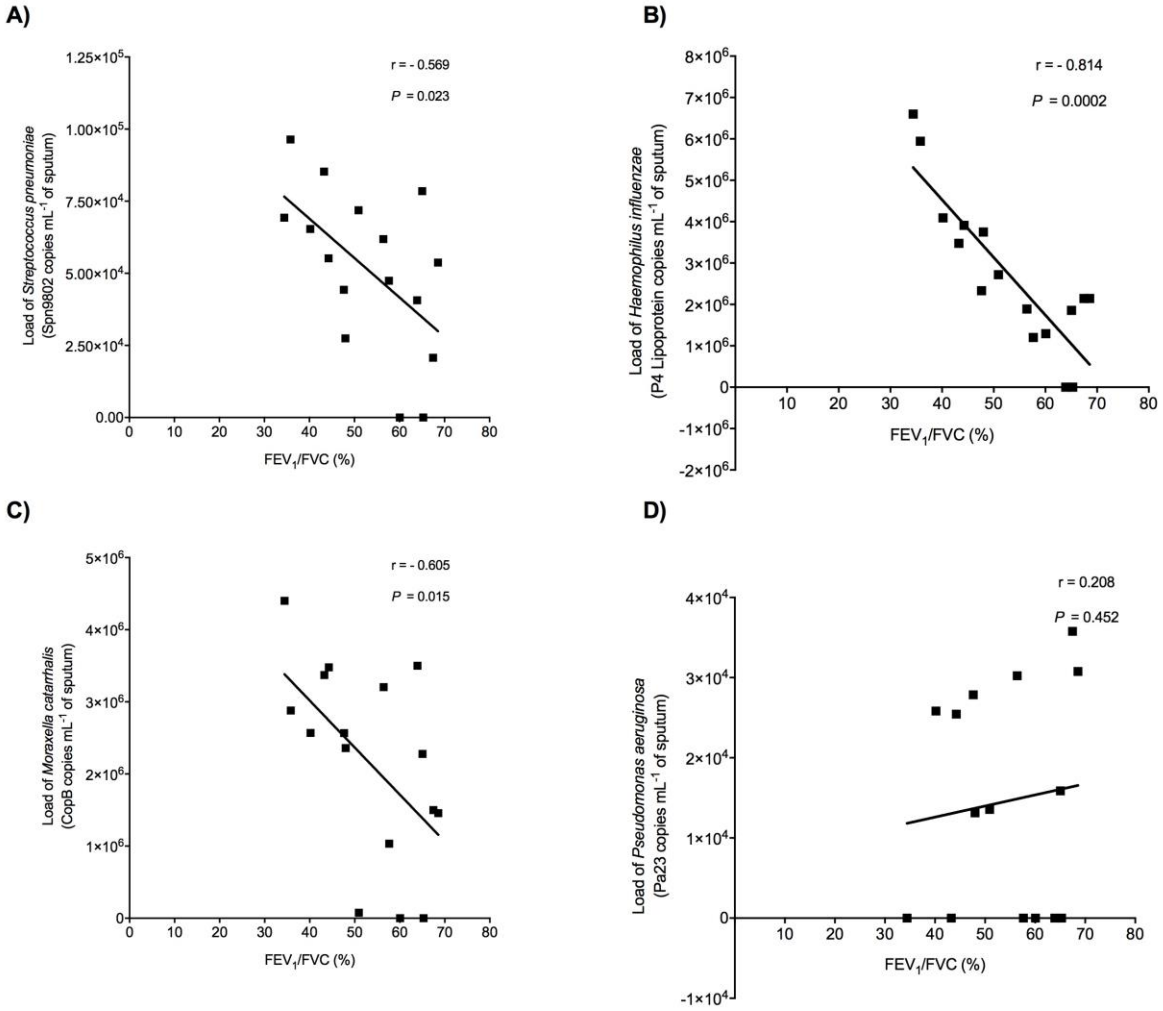


Figure S10: Relationship between bacterial load in induced sputum samples and FEV_1/FVC (%) in tobacco-smoke associated COPD subjects.

The correlation between FEV_1/FVC (%) *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in S-COPD (■ n = 16). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume; FVC, forced vital capacity; S-COPD, tobacco-smoke associated COPD]

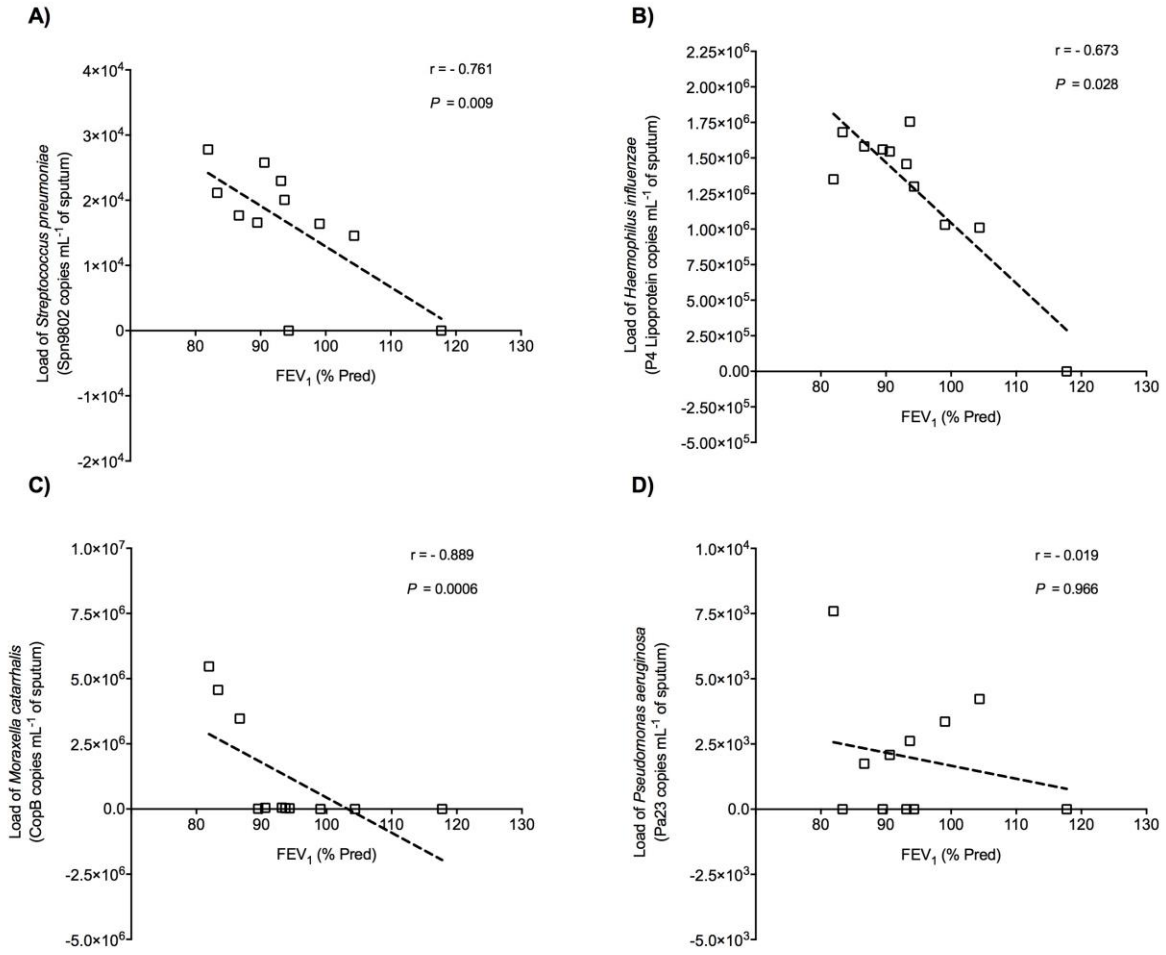


Figure S11: Relationship between bacterial load in induced sputum samples and FEV_1 % predicted in smokers without COPD subjects.

The correlation between FEV_1 % predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in HS (□ n = 11). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; HS, smokers without COPD]

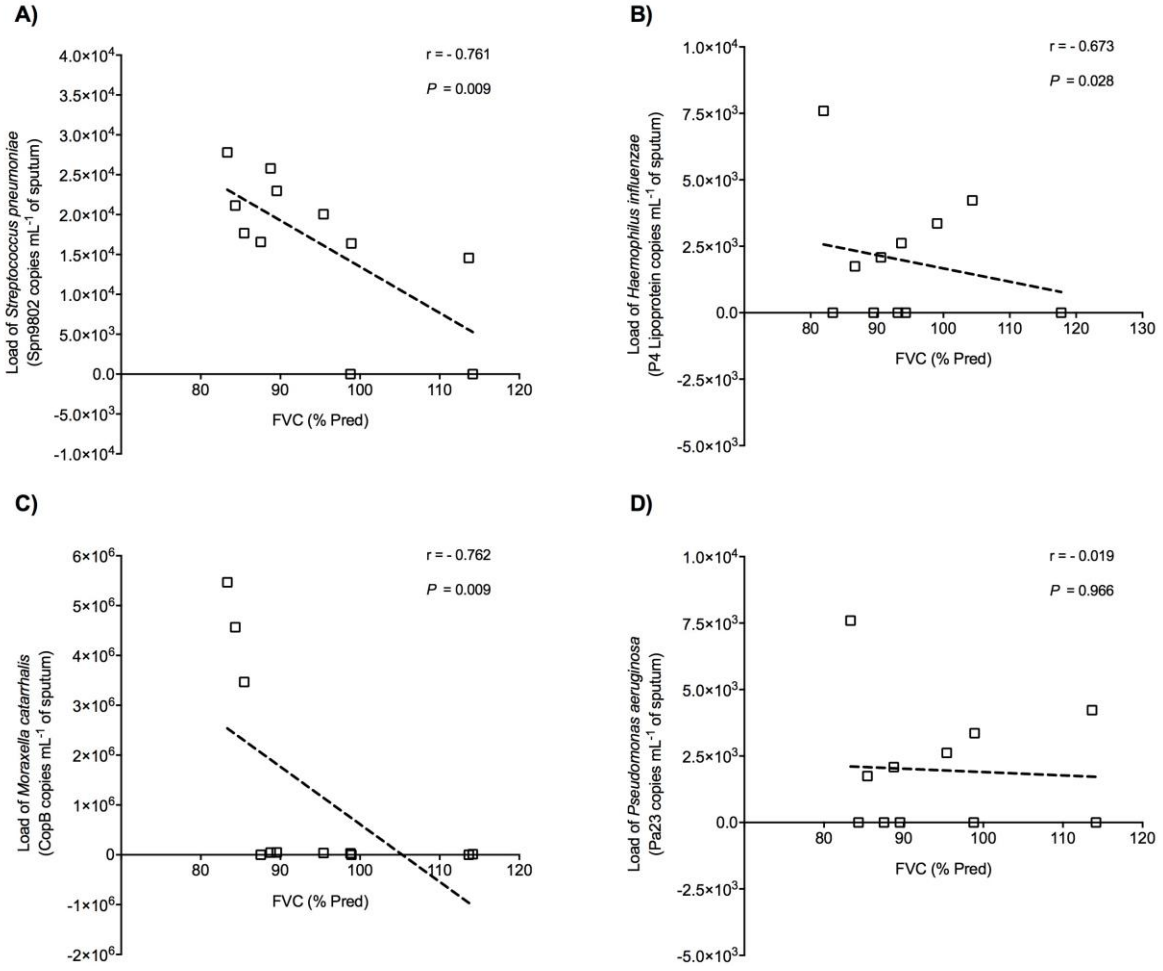


Figure S12: Relationship between bacterial load in induced sputum samples and FVC % predicted in smokers without COPD subjects.

The correlation between FVC % predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in HS (□ n = 11). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity; HS, smokers without COPD]

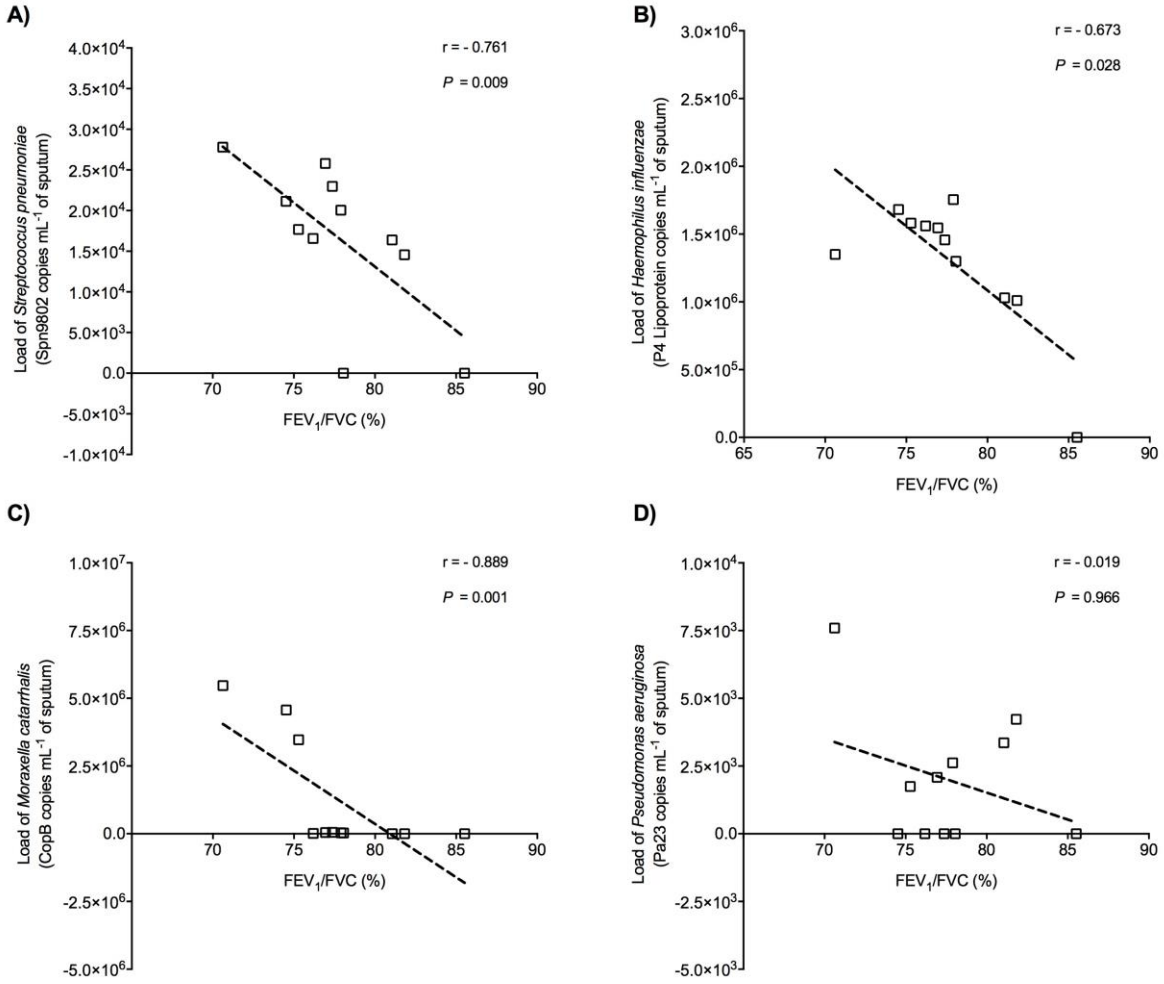


Figure S13: Relationship between bacterial load in induced sputum samples and FEV_1/FVC (%) in smokers without COPD subjects.

The correlation between FEV_1/FVC (%) predicted *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in HS (□ n = 11). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume; FVC, forced vital capacity; HS, smokers without COPD]

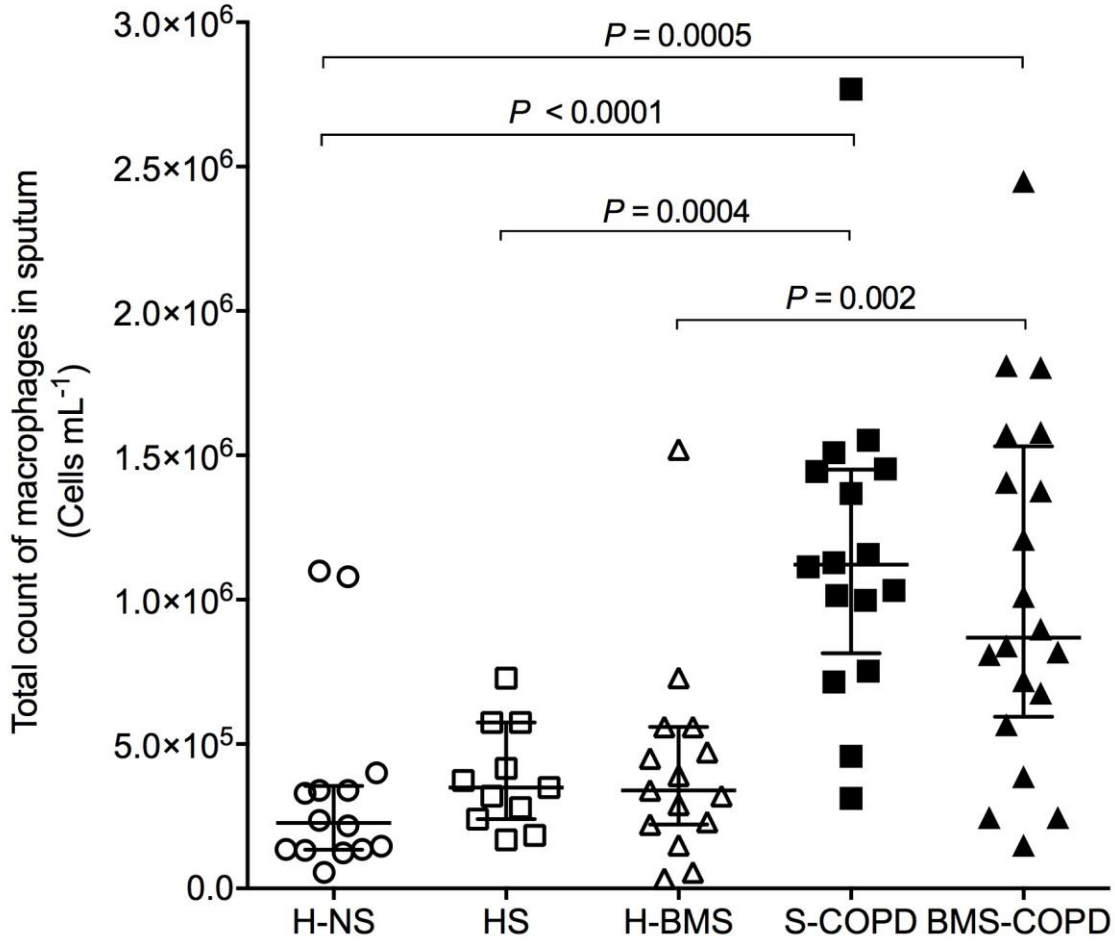


Figure S14: Comparison of absolute count of sputum macrophages count in the sputum samples of the subjects.

Total count of sputum macrophages was determined in the induce sputum samples of H-NS (○ n = 14), HS (□ n = 11), H-BMS (△ n = 15), S-COPD (■ n = 16) and BMS-COPD (▲ n = 20). Shapiro-Wilk normality test was performed. Data are presented as dot plots with median (interquartile range). Kruskal-Wallis/Dunn's multiple comparisons test was performed for within the group comparisons. $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; H-NS, healthy non-smokers; HS, smokers without COPD; H-BMS, biomass-smoke exposed healthy; S-COPD, tobacco-smoke associated COPD; BMS-COPD, biomass-smoke associated COPD]

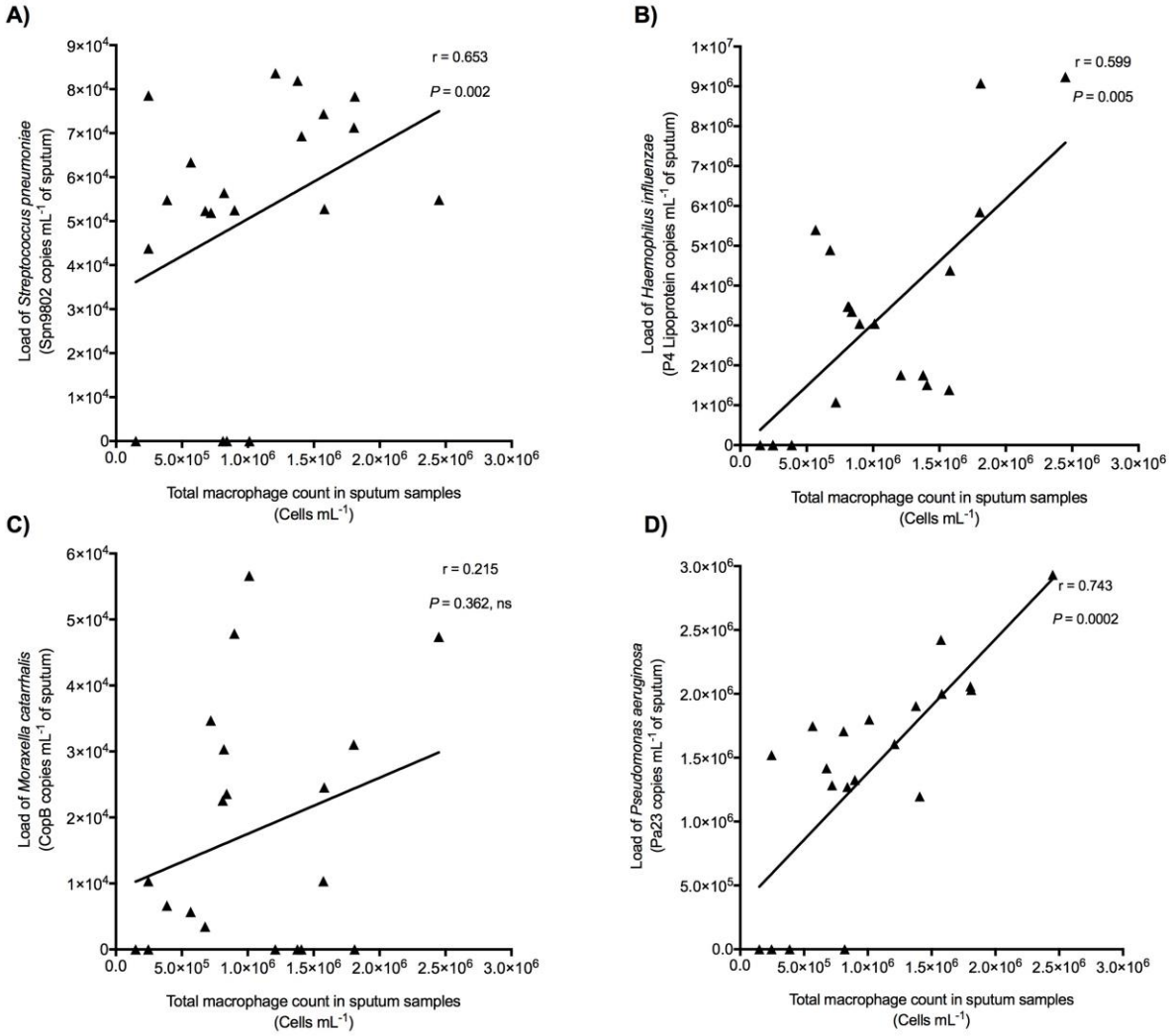


Figure S15: Relationship between total sputum macrophages and pathogenic bacterial load in the airways of biomass-smoke associated COPD subjects.

The correlation between total sputum macrophages and airway load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C), and *Pseudomonas aeruginosa* (D) was determined in the sputum samples of BMS-COPD (▲ n = 20) subjects. Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; BMS-COPD, biomass-smoke associated COPD]

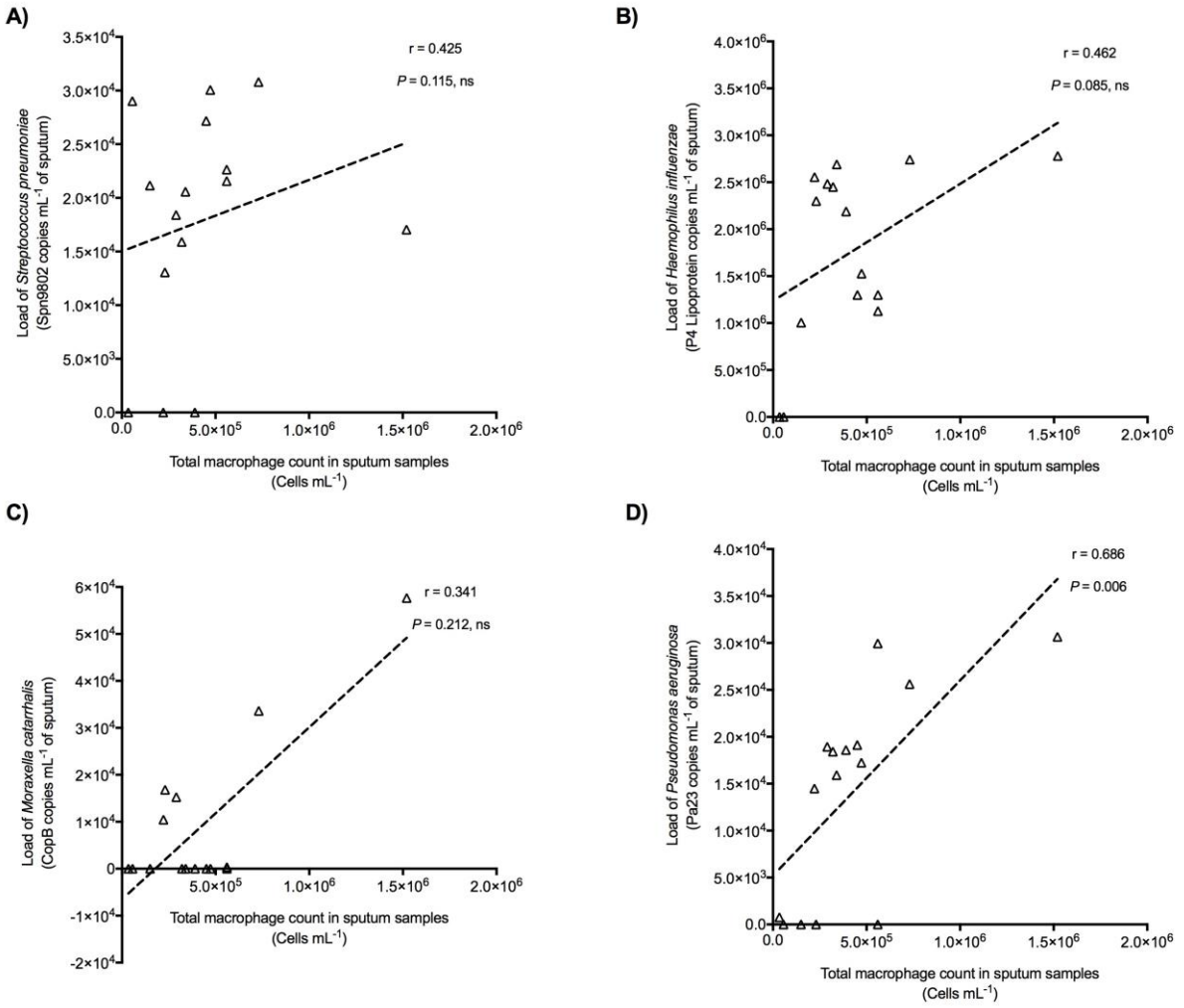


Figure S16: Relationship between total sputum macrophages and pathogenic bacterial load in the airways of biomass-smoke exposed healthy subjects.

The correlation between total sputum macrophages and airway load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C), and *Pseudomonas aeruginosa* (D) was determined in the sputum samples of H-BMS (△ n = 15) subjects. Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; H-BMS, biomass-smoke exposed healthy]

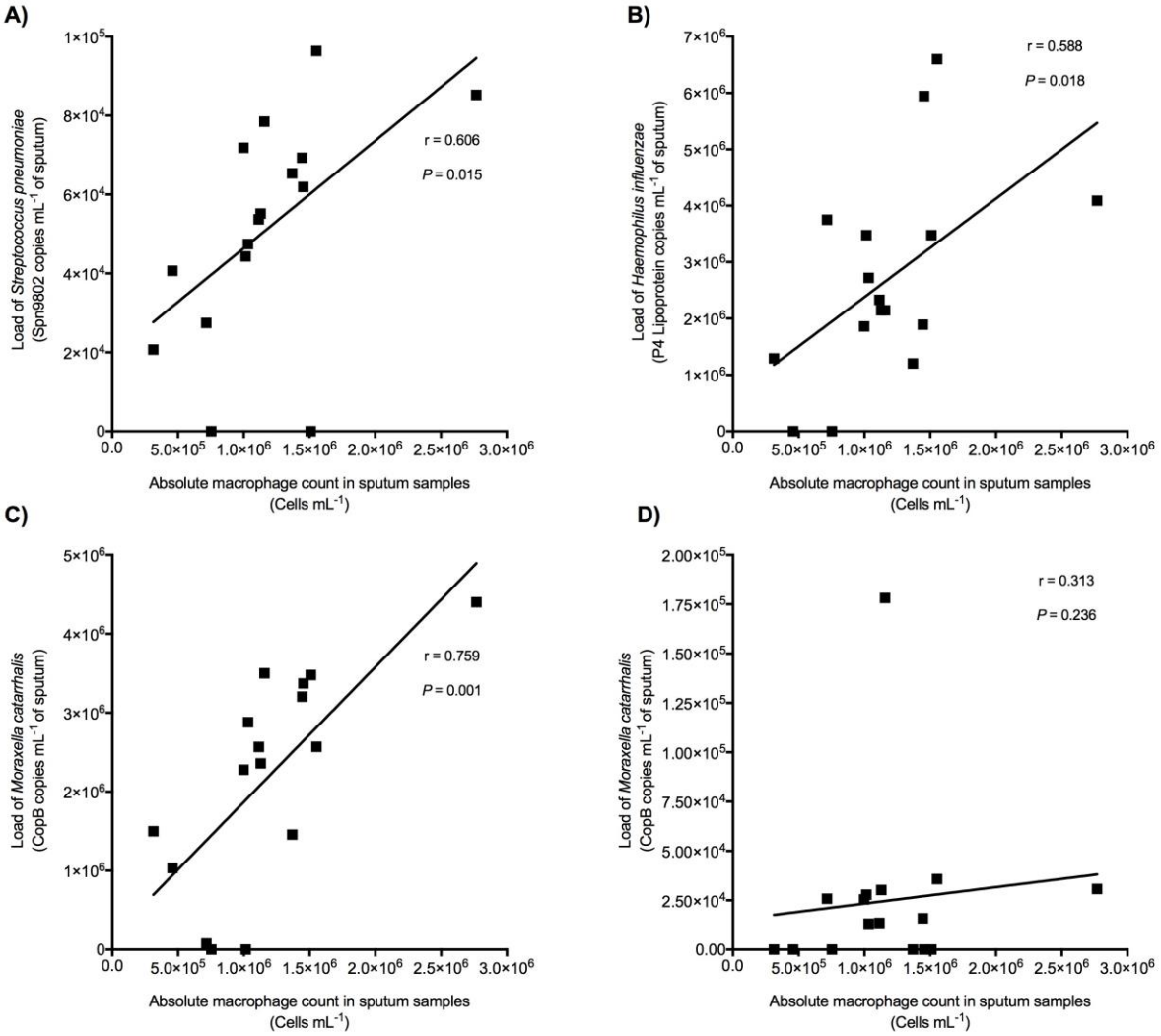


Figure S17: Relationship between total sputum macrophages and pathogenic bacterial load in the airways of tobacco-smoke associated COPD subjects.

The correlation between total sputum macrophages and airway load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C), and *Pseudomonas aeruginosa* (D) was determined in the sputum samples of S-COPD (■ n = 16) subjects. Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; S-COPD, tobacco-smoke associated COPD]

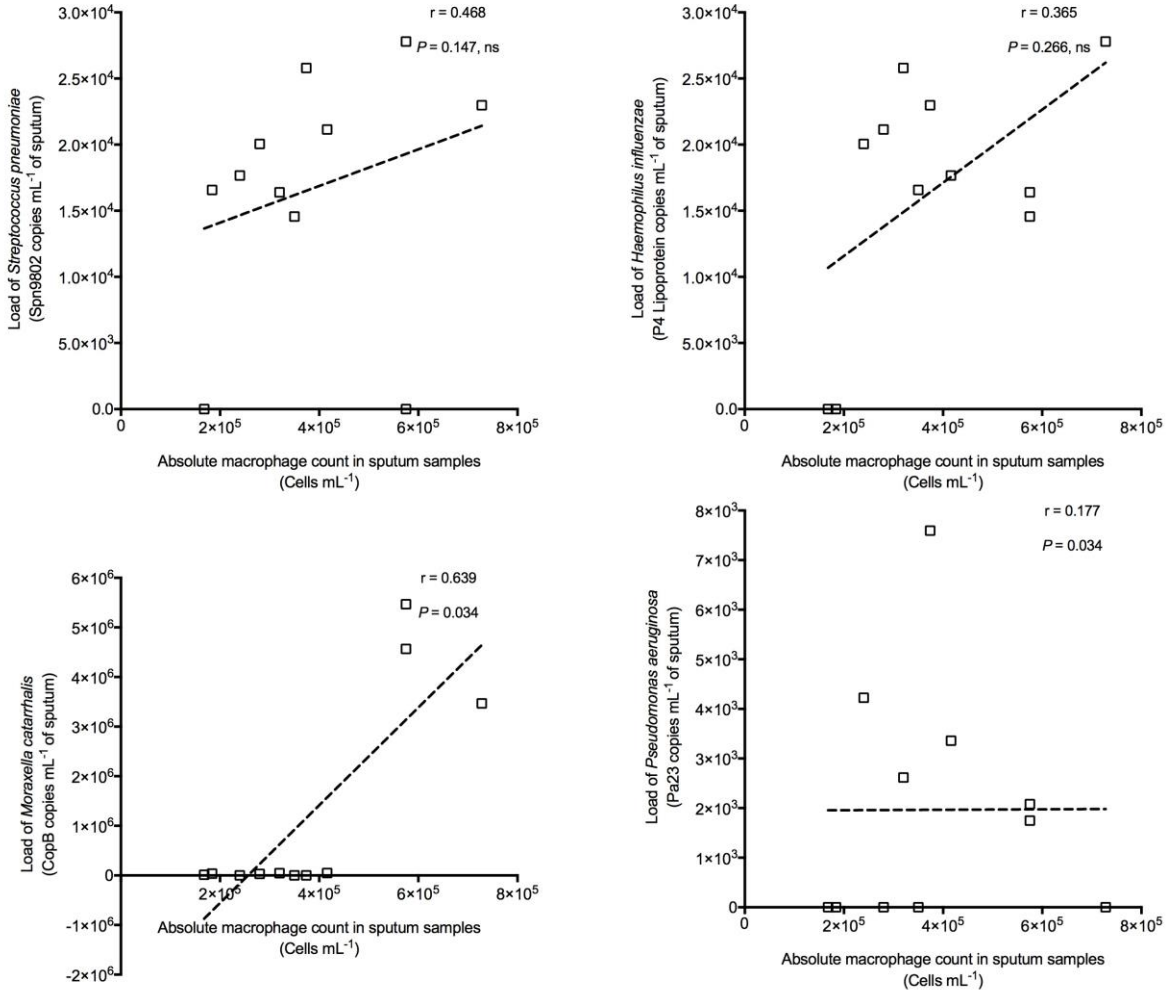


Figure S18: Relationship between total sputum macrophages and pathogenic bacterial load in the airways of smokers without COPD subjects.

The correlation between total sputum macrophages and airway load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C), and *Pseudomonas aeruginosa* (D) was determined in the sputum samples of HS (□ n = 11) subjects. Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; HS, smokers without COPD]

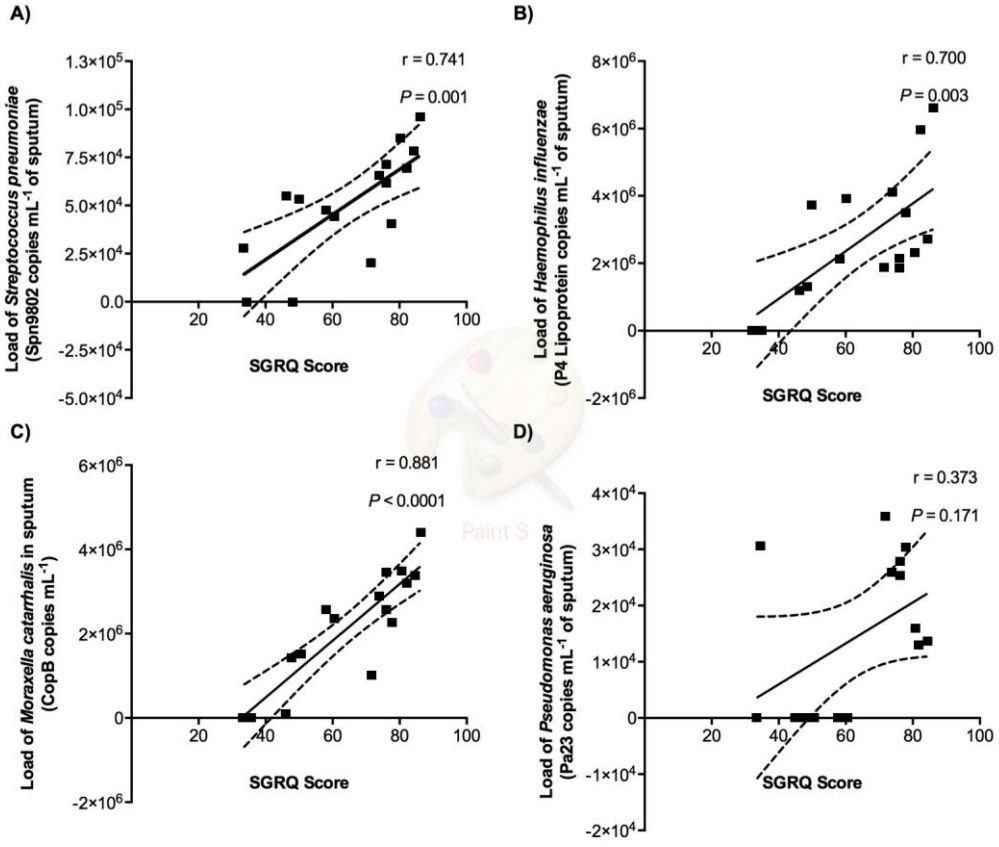


Figure S19: Relationship between bacterial load in induced sputum samples and total SGRQ score in tobacco-smoke associated COPD subjects.

The correlation between total SGRQ score *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in S-COPD (■ n = 16). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; S-COPD, tobacco-smoke associated COPD; SGRQ, St. George's Respiratory Questionnaire]

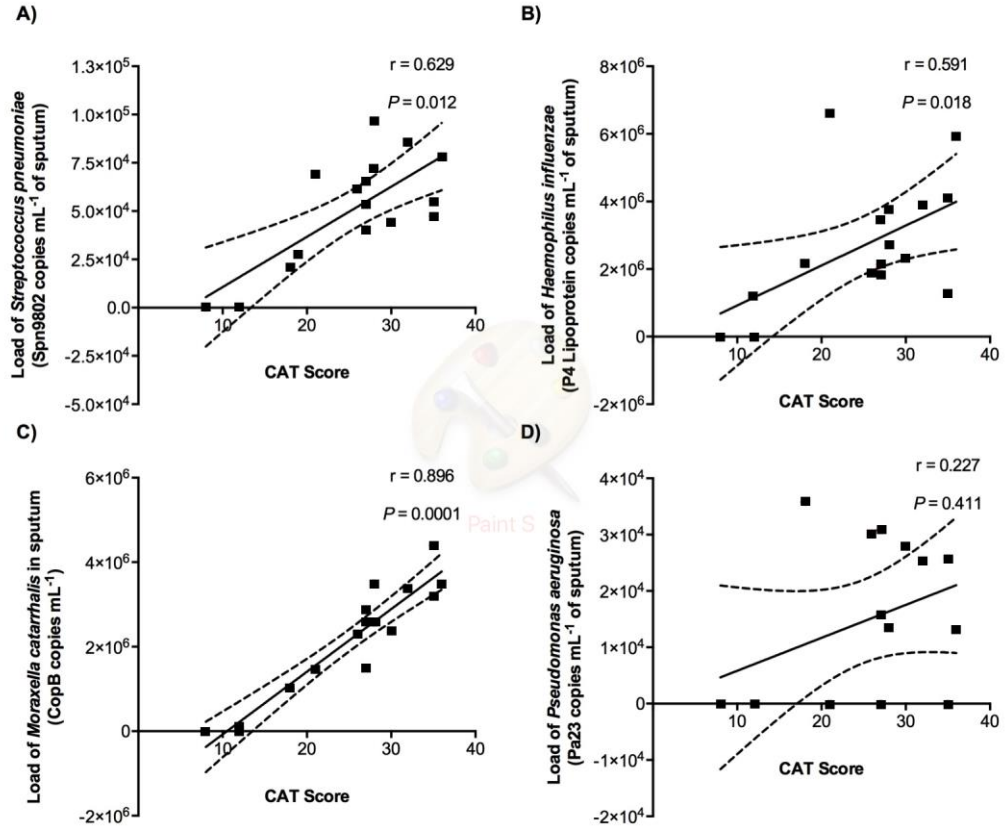


Figure 20: Relationship between bacterial load in induced sputum samples and total CAT score in tobacco-smoke associated COPD subjects.

The correlation between the total CAT score *versus* bacterial load of *Streptococcus pneumoniae* (A), *Haemophilus influenzae* (B), *Moraxella catarrhalis* (C) and *Pseudomonas aeruginosa* (D) was determined in S-COPD (■ n = 16). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation analysis. $P<0.05$ was considered statistically significant. ns: not significant as $P>0.05$.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; CAT, COPD assessment test; S-COPD, tobacco-smoke associated COPD]

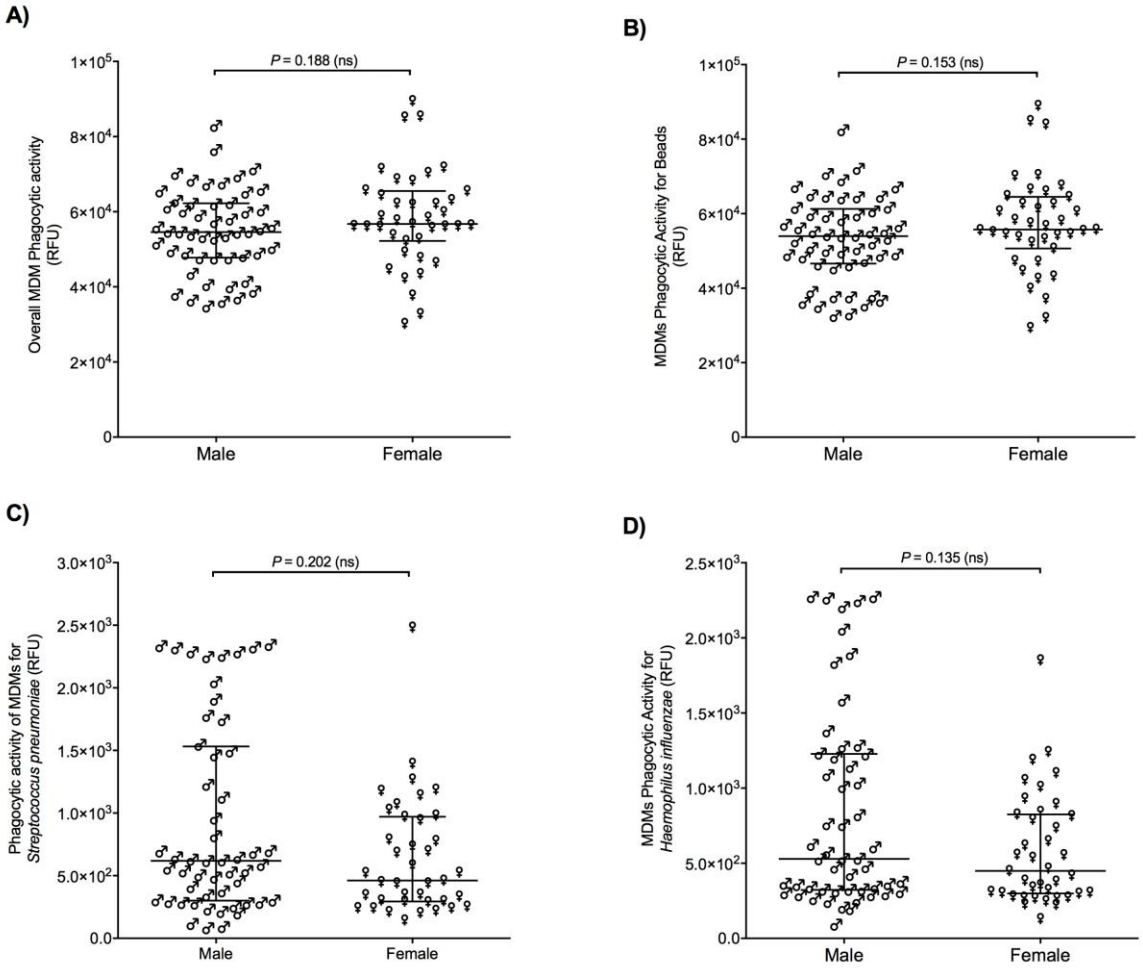


Figure S21: Gender-related comparison of MDMs phagocytic activity for fluorescently labelled beads and PPBs among study subjects.

(A) Overall comparison of MDMs phagocytic activity (mean MDMs phagocytic activity of fluorescently labelled inert beads and heat-killed *Streptococcus pneumoniae* and *Haemophilus influenzae*), (B) MDMs phagocytic activity for fluorescently-labelled inert beads, (C) MDMs phagocytic activity for fluorescently labelled heat-killed *Streptococcus pneumoniae*, and (D) MDMs phagocytic activity for fluorescently labelled heat-killed *Haemophilus influenzae* in male (♂ n = 52) and female (♀ n = 39). Shapiro-Wilk normality test was performed. Data are presented as median (interquartile range). Non-parametric Mann-Whitney test was performed. $P<0.05$ was considered statistically significant.

[*Abbreviations*: MDMs, monocyte-derived macrophages; PPBs, potentially pathogenic bacterial species]

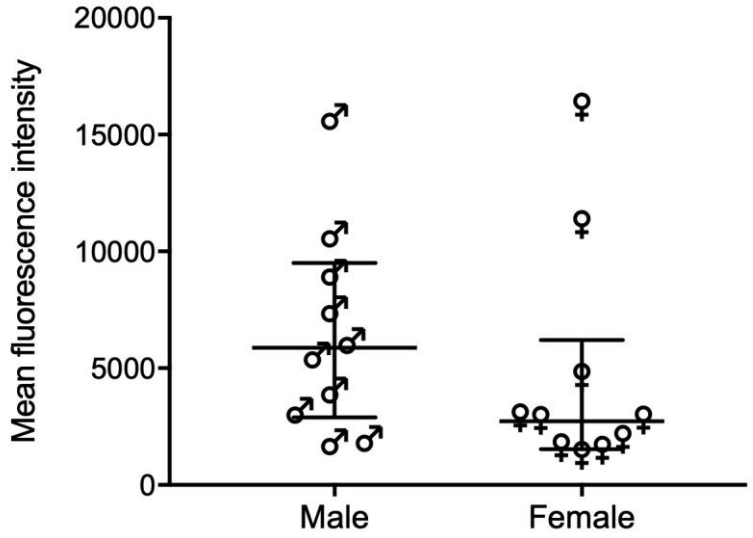


Figure S22: Gender-related comparison of MDMs phagocytic activity for fluorescently labelled bacteria among western COPD subjects.

MDMs phagocytic activity for fluorescently labelled heat-killed *Haemophilus influenzae* in male (♂ n = 10) and female (♀ n = 10). Shapiro-Wilk normality test was performed. Data are presented as median (interquartile range). Non-parametric Mann-Whitney test was performed. $P<0.05$ was considered statistically significant.

[*Abbreviations*: MDMs, monocyte-derived macrophages]

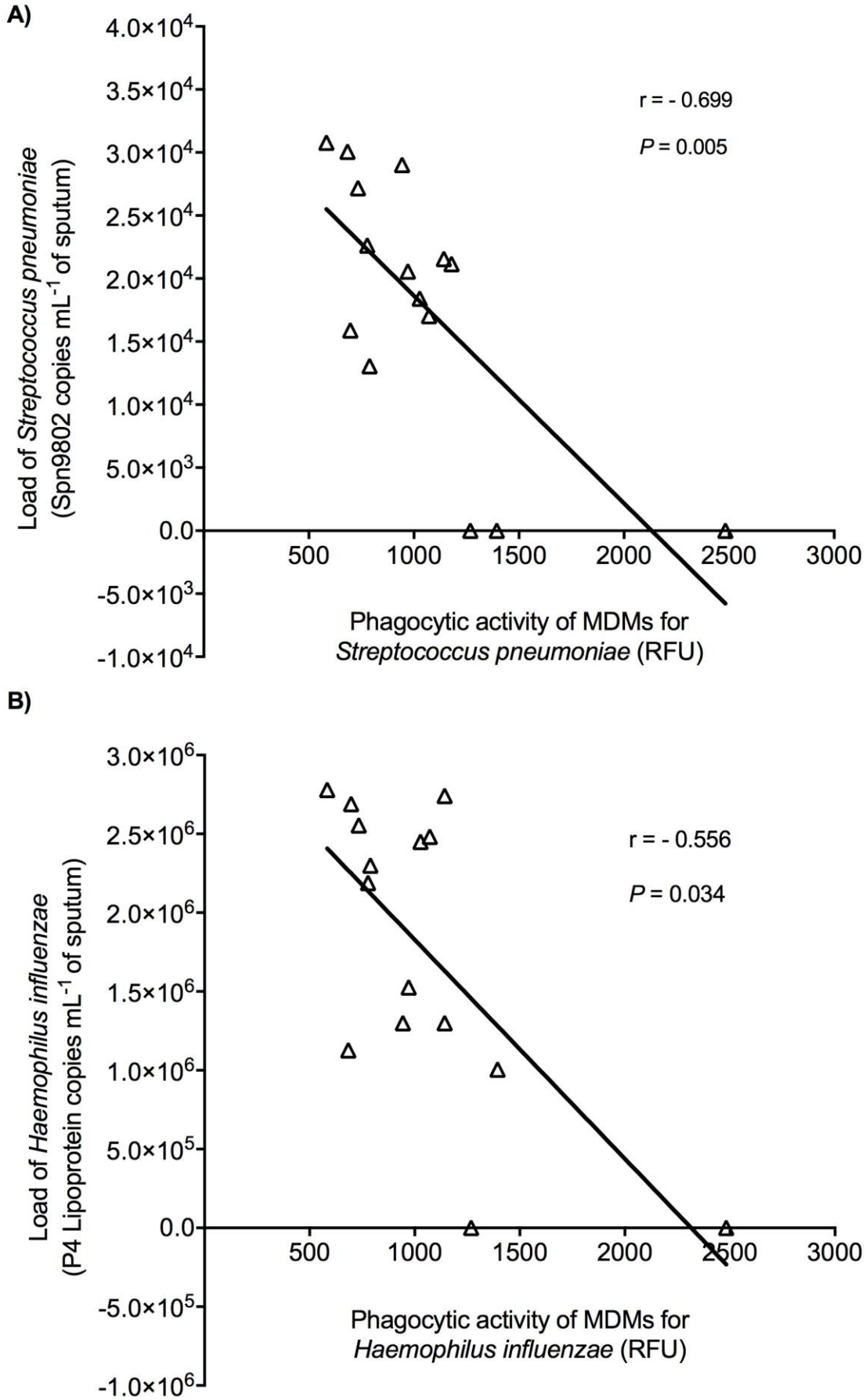
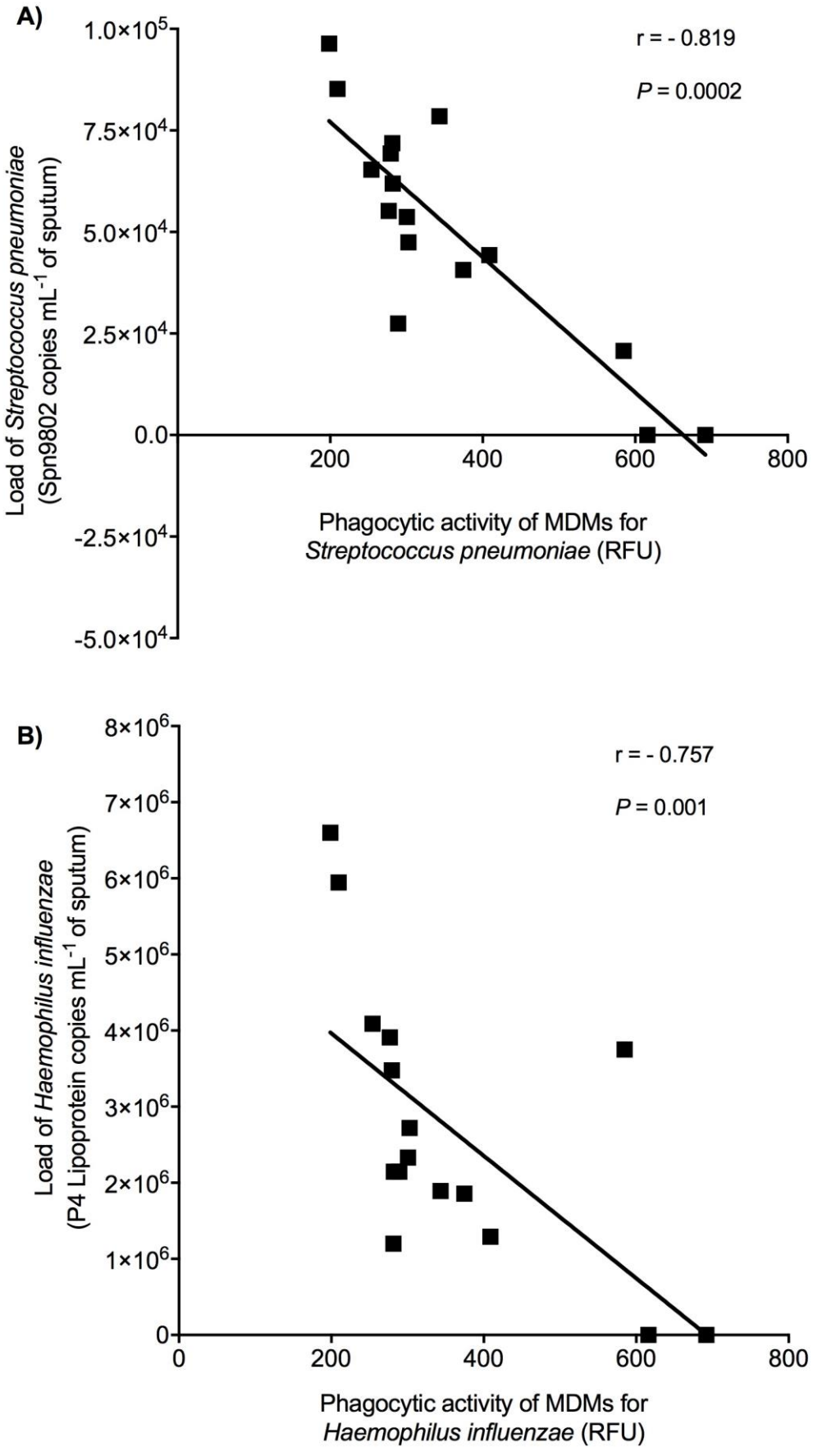

A)
4.0×10^4
3.5×10^4
3.0×10^4
2.5×10^4
2.0×10^4
1.5×10^4
1.0×10^4
5.0×10^3
0.0
-5.0×10^3
-1.0×10^4
Load of Streptococcus pneumoniae (Spn9802 copies mL^-1 of sputum)
r = - 0.699
P = 0.005
500
1000
1500
2000
2500
3000
Phagocytic activity of MDMs for Streptococcus pneumoniae (RFU)
B)
3.0×10^6
2.5×10^6
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0.0
-5.0×10^5
-1.0×10^6
Load of Haemophilus influenzae (P4 Lipoprotein copies mL^-1 of sputum)
r = - 0.556
P = 0.034
500
1000
1500
2000
2500
3000
Phagocytic activity of MDMs for Haemophilus influenzae (RFU)

Figure S23: Relationship between bacterial load in the induced sputum samples and phagocytic activity of MDMs in biomass-smoke exposed healthy subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and load of *Streptococcus pneumoniae* (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and load of *Haemophilus influenzae* (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Spearman correlation analysis in H-BMS (△ n = 15). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; H-BMS, biomass-smoke exposed healthy]


A)
r = - 0.819
P = 0.0002
Load of Streptococcus pneumoniae (Spn9802 copies mL-1 of sputum)
1.0×10^5
7.5×10^4
5.0×10^4
2.5×10^4
0.0
-2.5×10^4
-5.0×10^4
200
400
600
800
Phagocytic activity of MDMs for Streptococcus pneumoniae (RFU)
B)
r = - 0.757
P = 0.001
Load of Haemophilus influenzae (P4 Lipoprotein copies mL-1 of sputum)
8×10^6
7×10^6
6×10^6
5×10^6
4×10^6
3×10^6
2×10^6
1×10^6
0
0
200
400
600
800
Phagocytic activity of MDMs for Haemophilus influenzae (RFU)

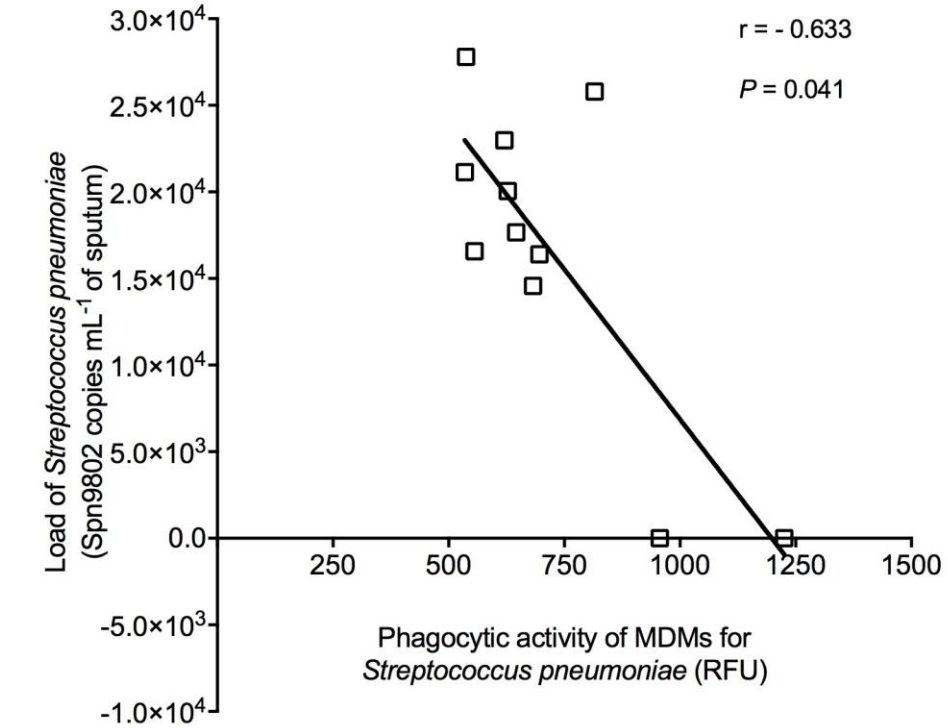
Figure

S24: Relationship between bacterial load in the induced sputum samples and phagocytic activity of MDMs in tobacco-smoke associated COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and load of *Streptococcus pneumoniae* (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and load of *Haemophilus influenzae* (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Spearman correlation analysis in S-COPD (■ n = 16). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; S-COPD, tobacco-smoke associated COPD]

A)


r = - 0.633
P = 0.041
Load of Streptococcus pneumoniae (Spn9802 copies mL-1 of sputum)
3.0×10^4
2.5×10^4
2.0×10^4
1.5×10^4
1.0×10^4
5.0×10^3
0.0
-5.0×10^3
-1.0×10^4
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for Streptococcus pneumoniae (RFU)

B)

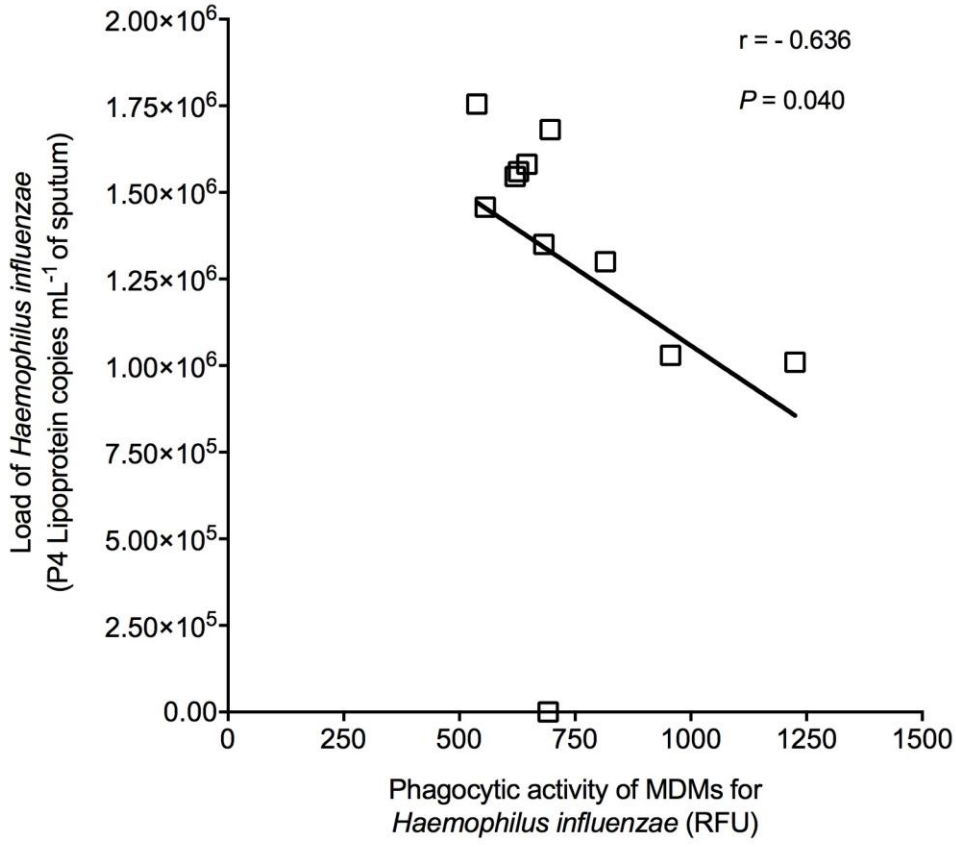
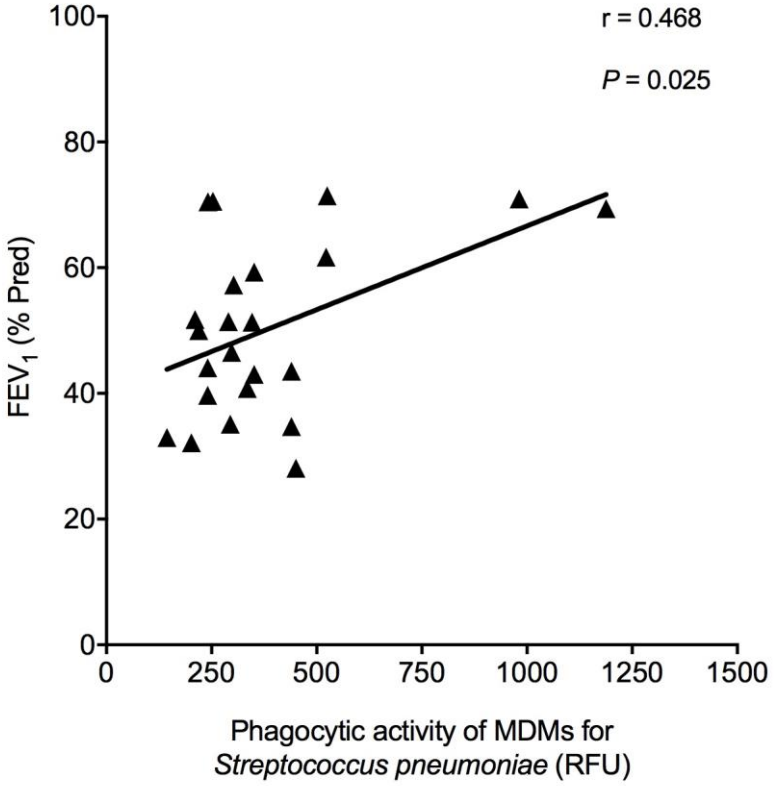

r = - 0.636
P = 0.040
Load of Haemophilus influenzae (P4 Lipoprotein copies mL-1 of sputum)
2.00×10^6
1.75×10^6
1.50×10^6
1.25×10^6
1.00×10^6
7.50×10^5
5.00×10^5
2.50×10^5
0.00
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for Haemophilus influenzae (RFU)

Figure S25: Relationship between bacterial load in the induced sputum samples and phagocytic activity of MDMs in smokers without COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and load of *Streptococcus pneumoniae* (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and load of *Haemophilus influenzae* (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Spearman correlation analysis in HS (□ n = 11). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; HS, smokers without COPD]

A)


r = 0.468
P = 0.025
FEV1 (% Pred)
100
80
60
40
20
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

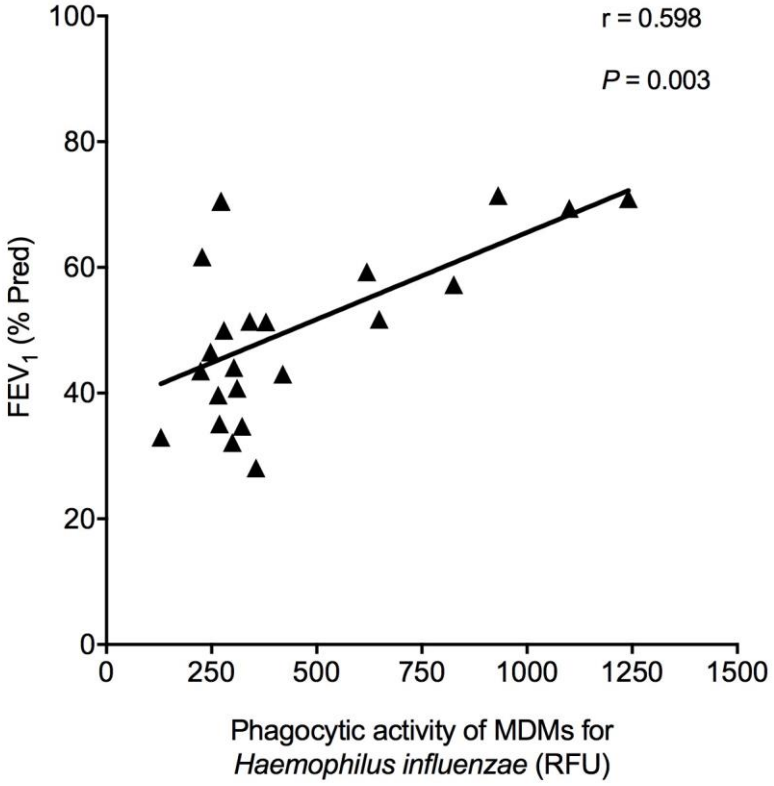
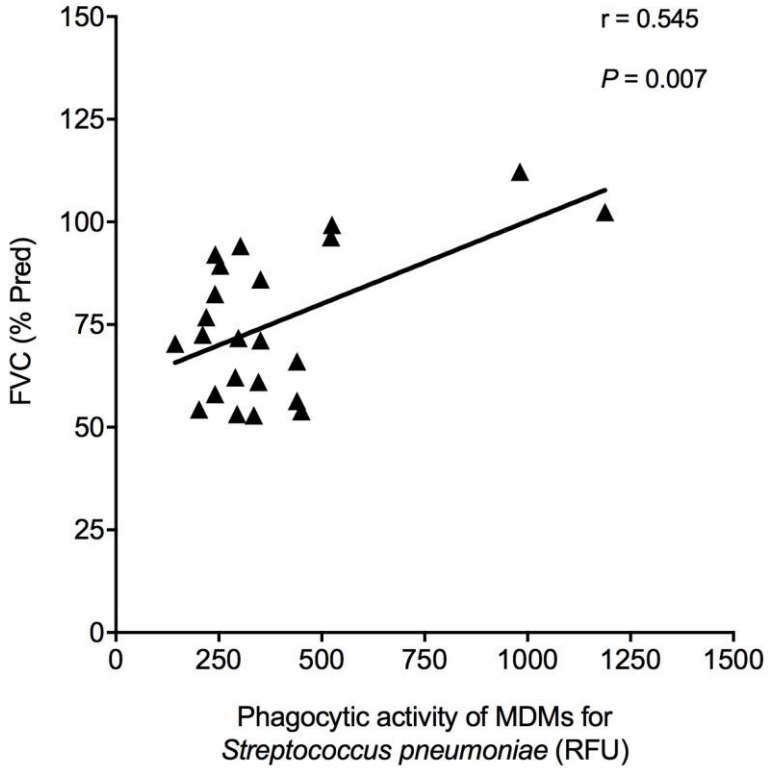

r = 0.598
P = 0.003
FEV1 (% Pred)
100
80
60
40
20
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S26: Relationship between phagocytic activity of MDMs and FEV_1 (% Pred) in biomass-smoke associated COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1 (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1 (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in BMS-COPD (▲ n = 23). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; BMS-COPD, biomass-smoke associated COPD]

A)


r = 0.545
P = 0.007
FVC (% Pred)
150
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

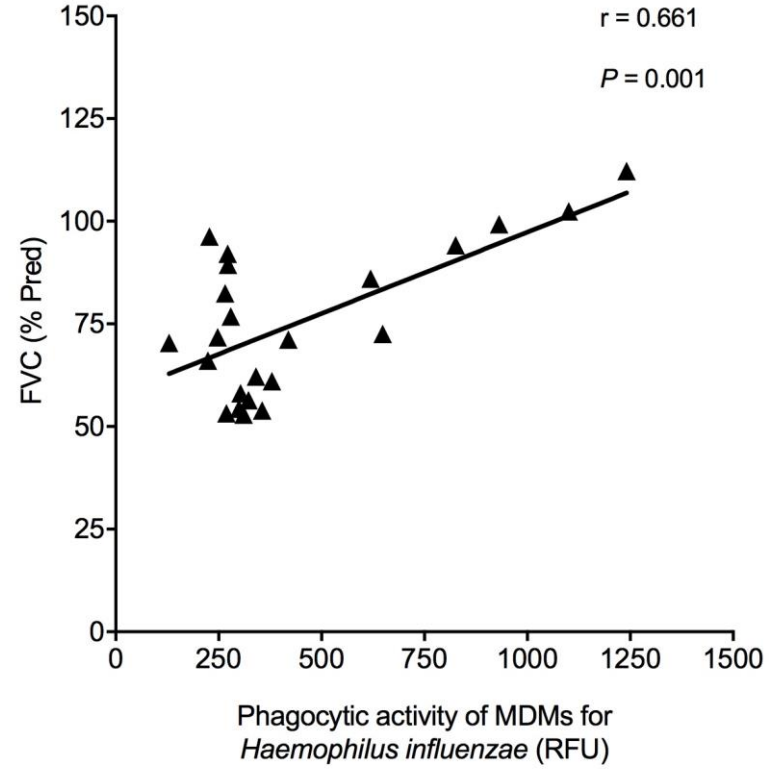
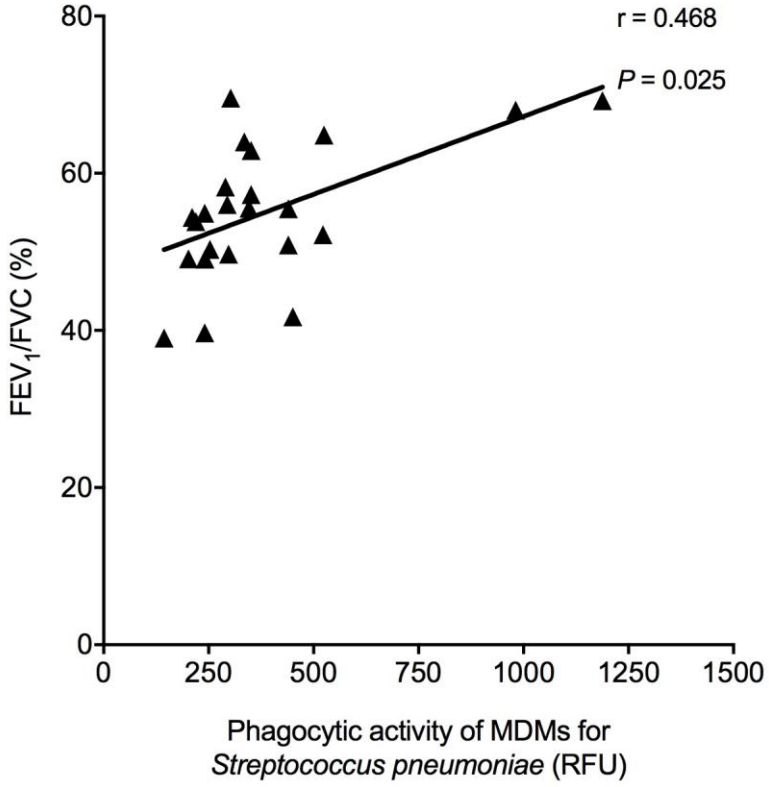

r = 0.661
P = 0.001
FVC (% Pred)
150
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S27: Relationship between phagocytic activity of MDMs and FVC (% Pred) in biomass-smoke associated COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FVC (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FVC (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in BMS-COPD (▲ n = 23). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FVC, forced vital capacity; BMS-COPD, biomass-smoke associated COPD]

A)


r = 0.468
P = 0.025
FEV1/FVC (%)
80
60
40
20
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

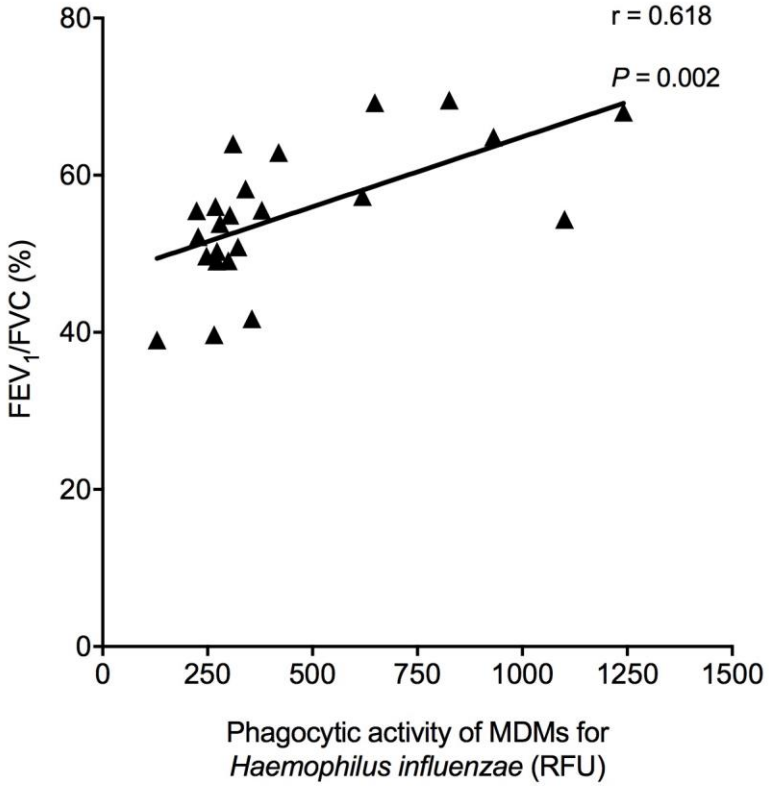
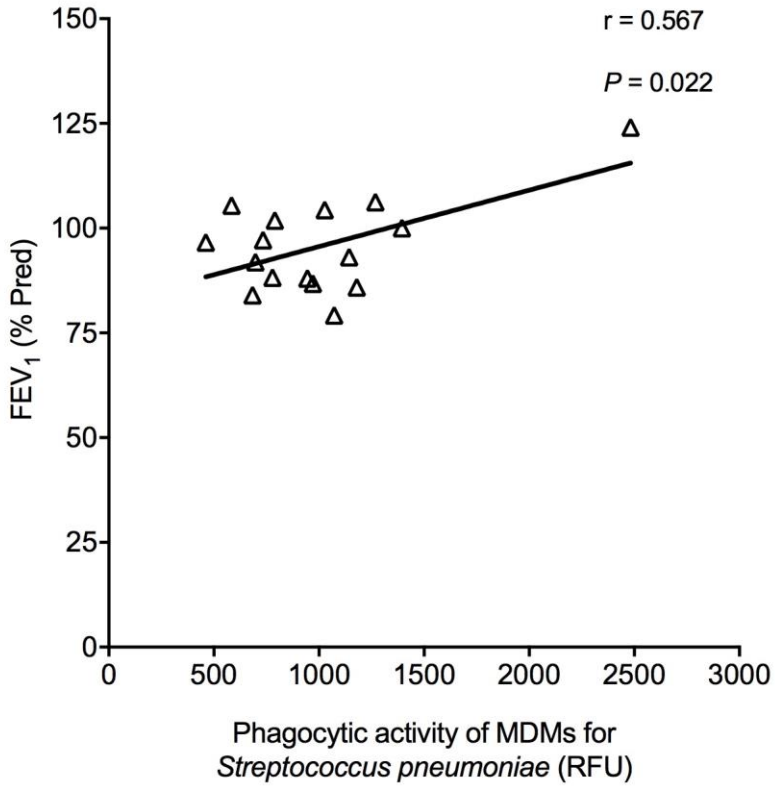

r = 0.618
P = 0.002
FEV1/FVC (%)
80
60
40
20
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S28: Relationship between phagocytic activity of MDMs and FEV_1/FVC (%) in biomass-smoke associated COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1/FVC (%) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1/FVC (%) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in BMS-COPD (▲ n = 23). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; FVC, forced vital capacity; BMS-COPD, biomass-smoke associated COPD]

A)


r = 0.567
P = 0.022
FEV1 (% Pred)
150
125
100
75
50
25
0
0
500
1000
1500
2000
2500
3000
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

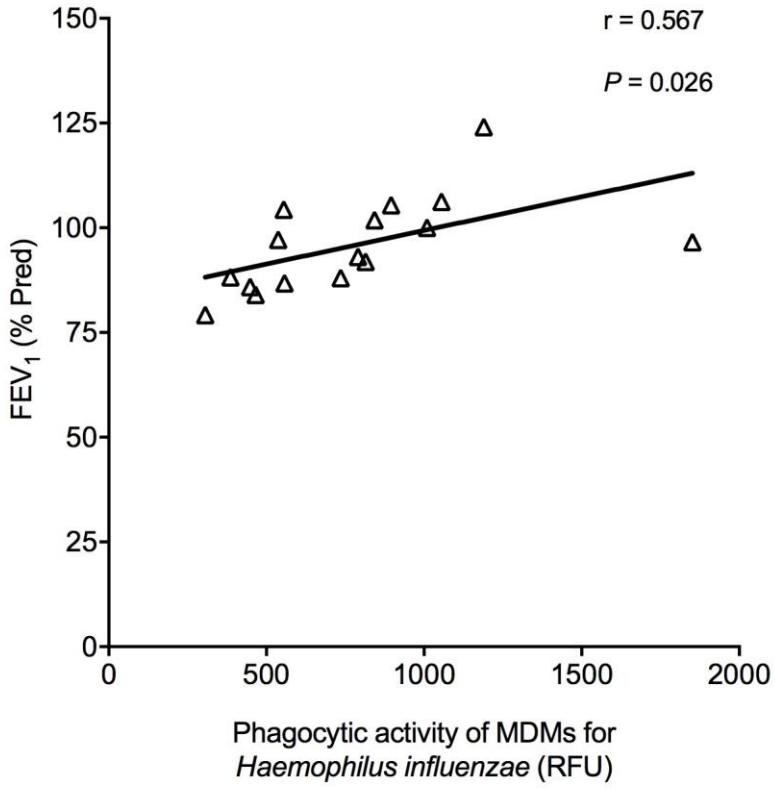
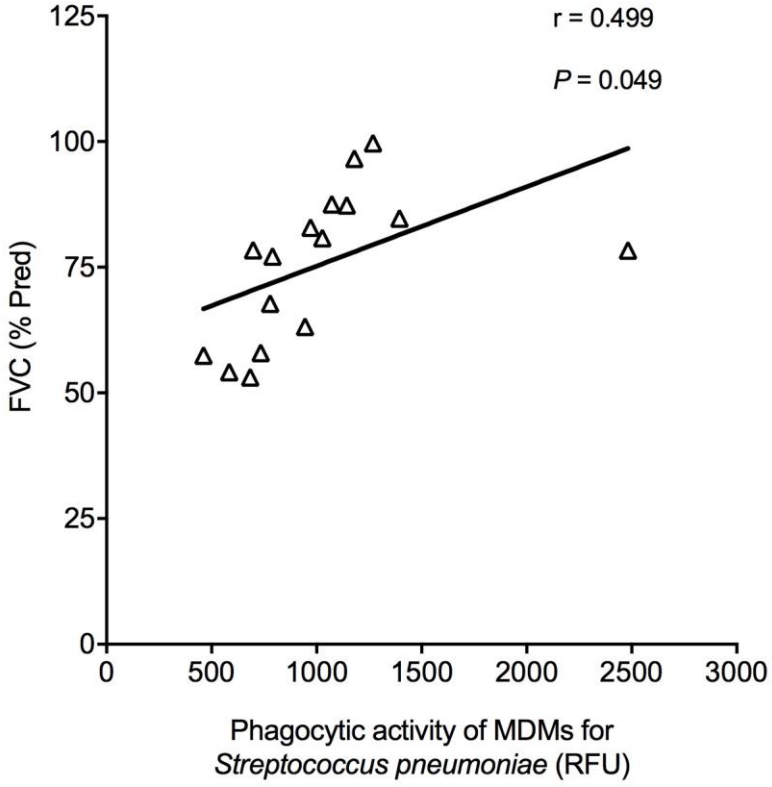

r = 0.567
P = 0.026
FEV1 (% Pred)
150
125
100
75
50
25
0
0
500
1000
1500
2000
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S29: Relationship between phagocytic activity of MDMs and FEV_1 (% Pred) in biomass-smoke exposed healthy subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1 (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1 (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in H-BMS (△ n = 16). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; H-BMS, biomass-smoke exposed healthy subjects]

A)


r = 0.499
P = 0.049
FVC (% Pred)
125
100
75
50
25
0
0
500
1000
1500
2000
2500
3000
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

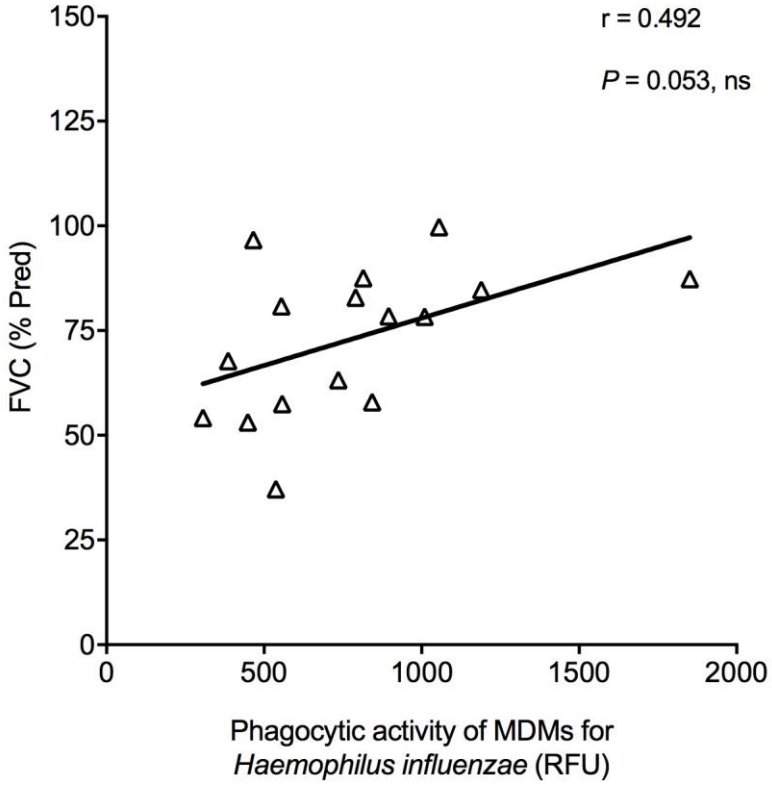
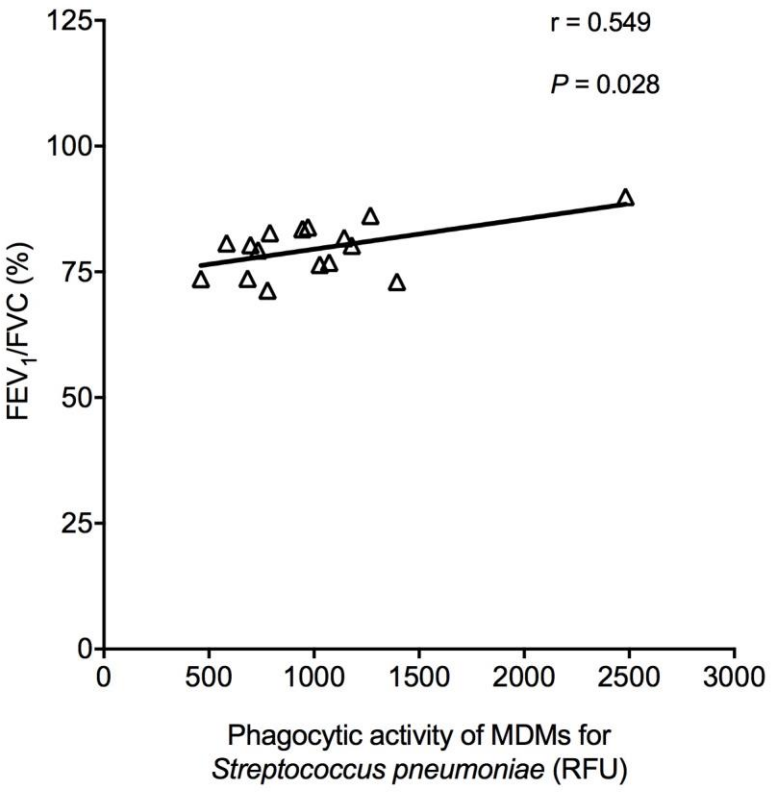

r = 0.492
P = 0.053, ns
FVC (% Pred)
150
125
100
75
50
25
0
0
500
1000
1500
2000
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S30: Relationship between phagocytic activity of MDMs and FVC (% Pred) in biomass-smoke exposed healthy subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FVC (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FVC (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in H-BMS (△ n = 16). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FVC, forced vital capacity; H-BMS, biomass-smoke exposed healthy]

A)


r = 0.549
P = 0.028
FEV1/FVC (%)
125
100
75
50
25
0
0
500
1000
1500
2000
2500
3000
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

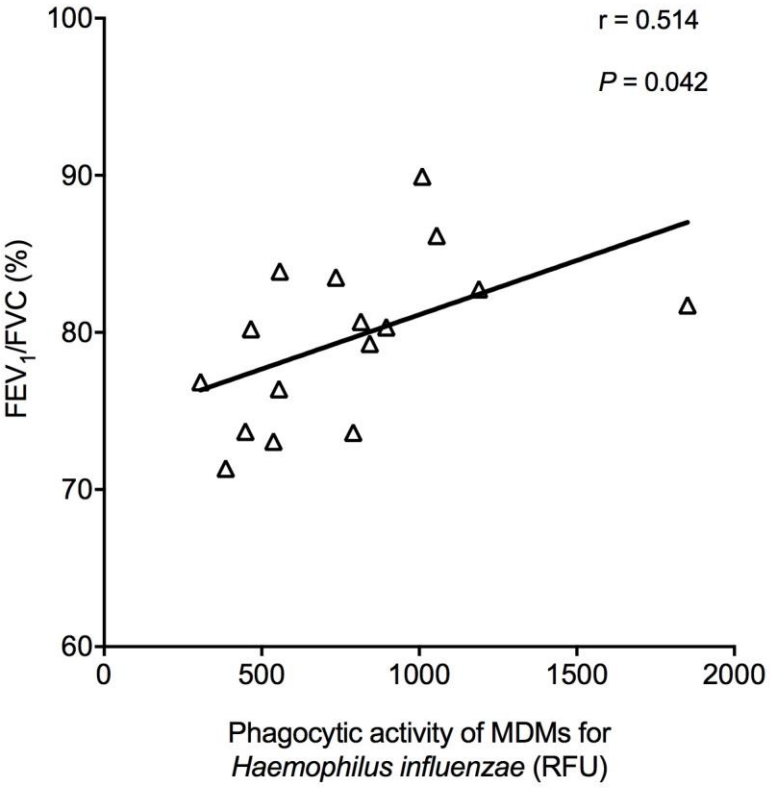
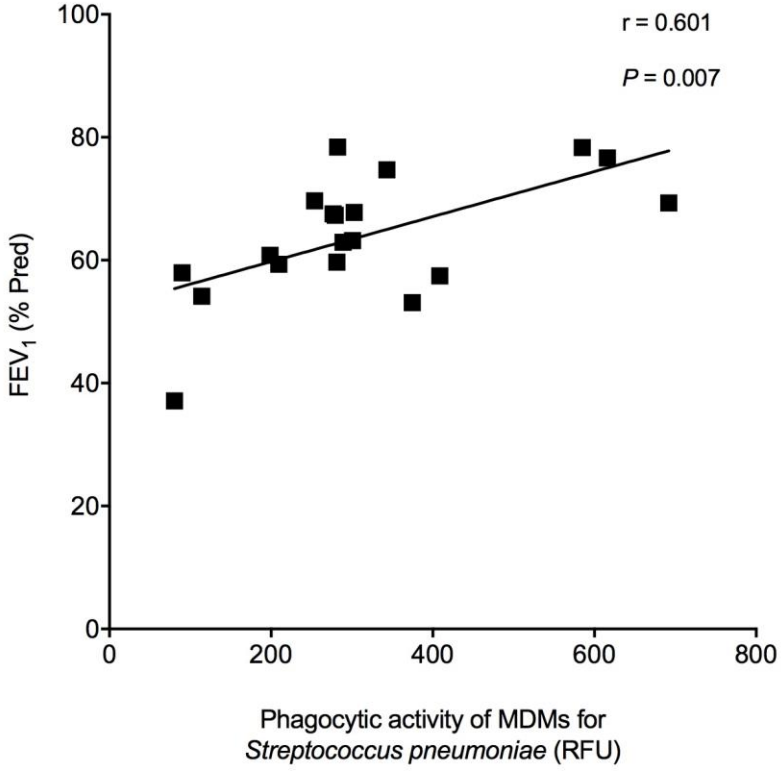

r = 0.514
P = 0.042
FEV1/FVC (%)
100
90
80
70
60
0
500
1000
1500
2000
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S31: Relationship between phagocytic activity of MDMs and FEV_1/FVC (%) in biomass-smoke exposed healthy subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1/FVC (%) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1/FVC (%) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in H-BMS (△ n = 16). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; FVC, forced vital capacity; H-BMS, biomass-smoke exposed healthy]

A)


r = 0.601
P = 0.007
FEV1 (% Pred)
100
80
60
40
20
0
0
200
400
600
800
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

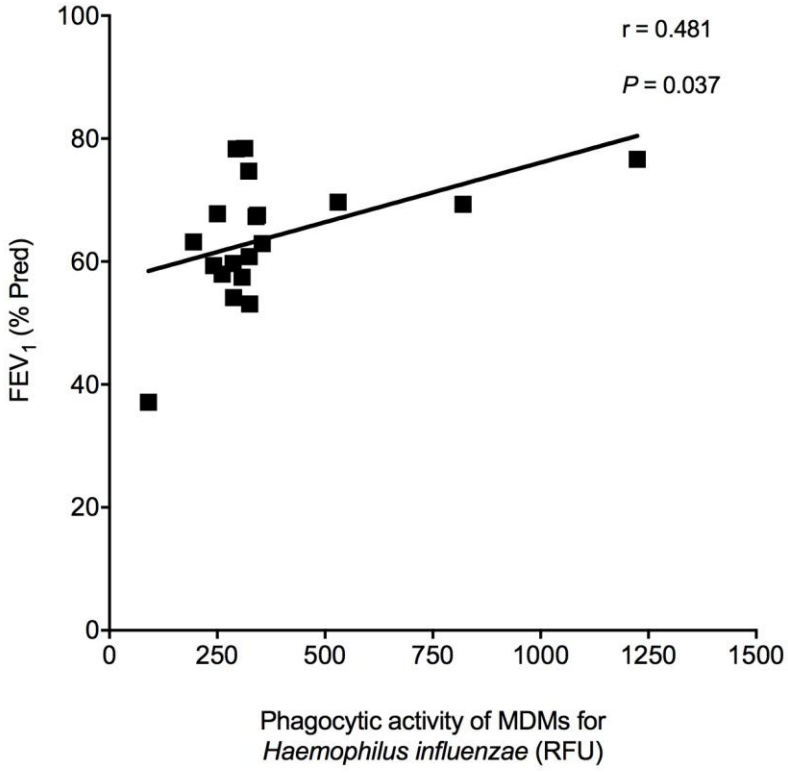
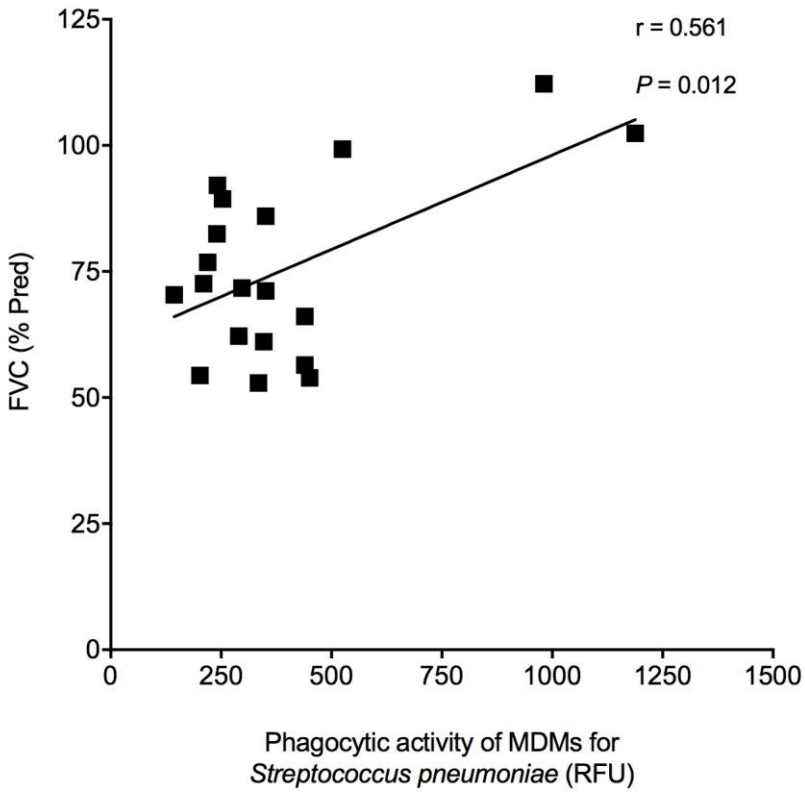

r = 0.481
P = 0.037
FEV1 (% Pred)
100
80
60
40
20
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S32: Relationship between phagocytic activity of MDMs and FEV_1 (% Pred) in tobacco-smoke associated COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1 (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1 (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in S-COPD (■ n = 19). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; S-COPD, tobacco-smoke associated COPD]

A)


r = 0.561
P = 0.012
FVC (% Pred)
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

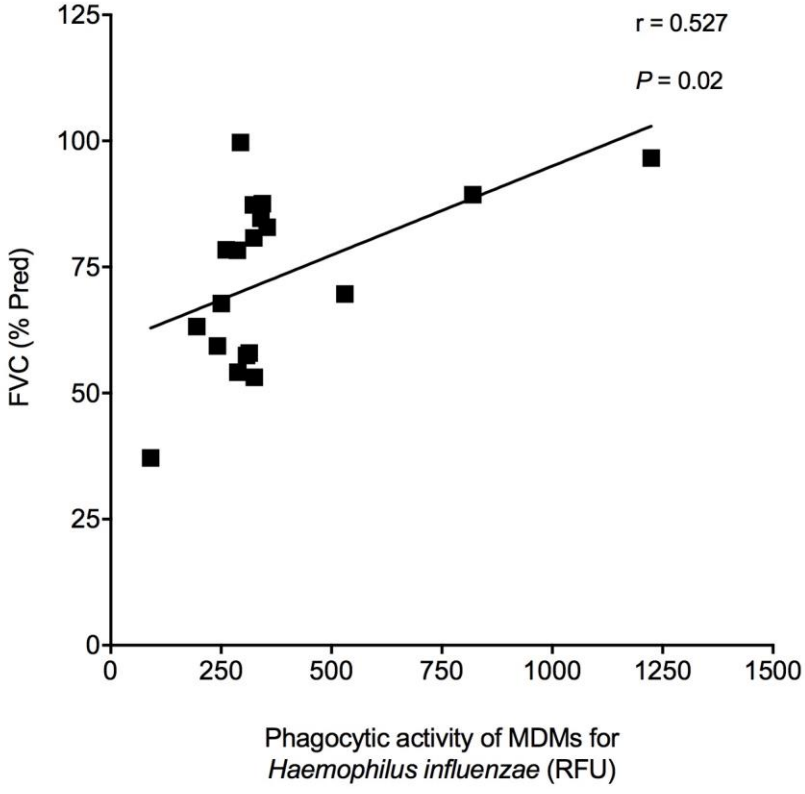
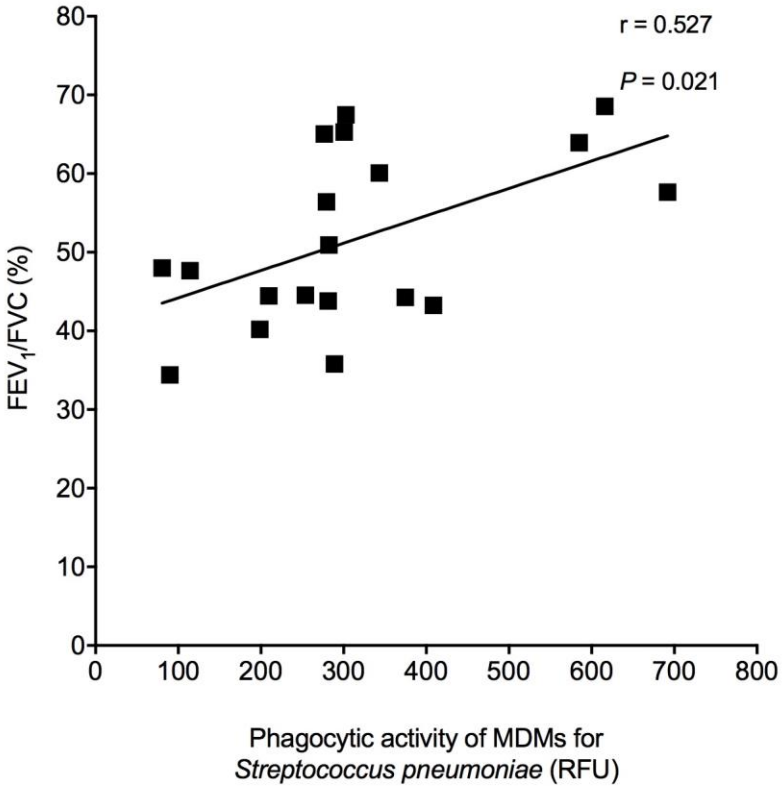

r = 0.527
P = 0.02
FVC (% Pred)
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S33: Relationship between phagocytic activity of MDMs and FVC (% Pred) in tobacco-smoke associated COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FVC (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FVC (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in S-COPD (■ n = 19). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FVC, forced vital capacity; S-COPD, tobacco-smoke associated COPD]

A)


r = 0.527
P = 0.021
FEV1/FVC (%)
0
10
20
30
40
50
60
70
80
0
100
200
300
400
500
600
700
800
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

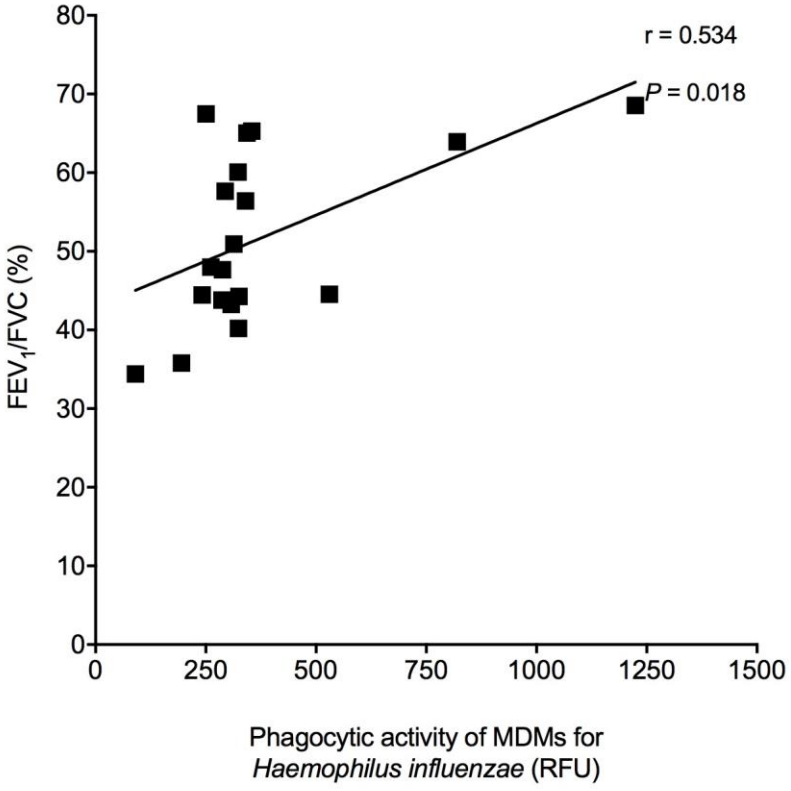
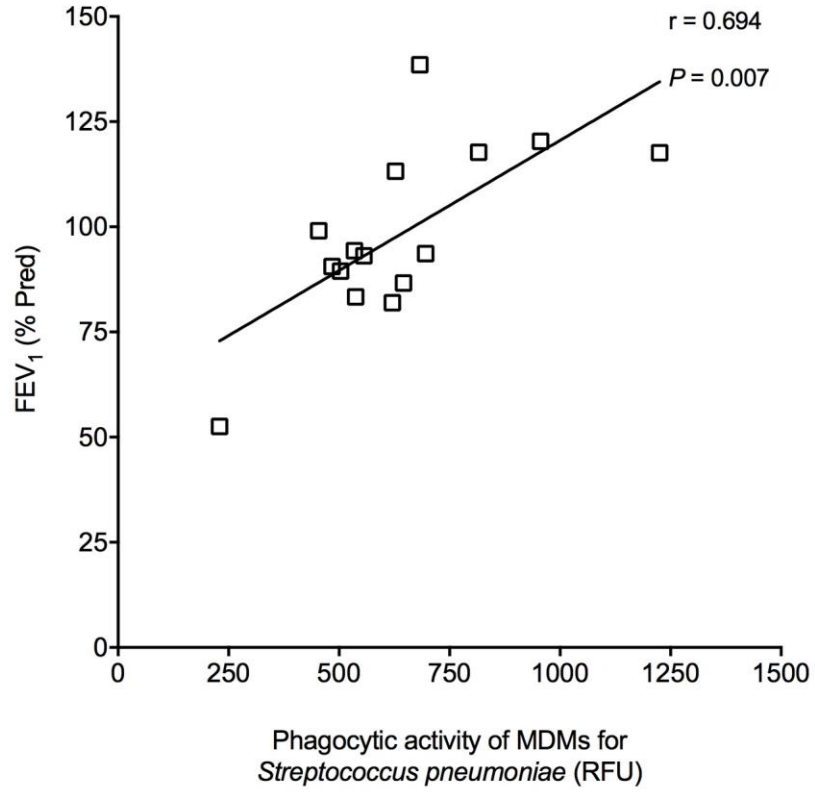

r = 0.534
P = 0.018
FEV1/FVC (%)
0
10
20
30
40
50
60
70
80
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S34: Relationship between phagocytic activity of MDMs and FEV_1/FVC (%) in tobacco-smoke associated COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1/FVC (%) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1/FVC (%) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in S-COPD (■ n = 19). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; FVC, forced vital capacity; S-COPD, tobacco-smoke associated COPD]

A)


r = 0.694
P = 0.007
FEV1 (% Pred)
150
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

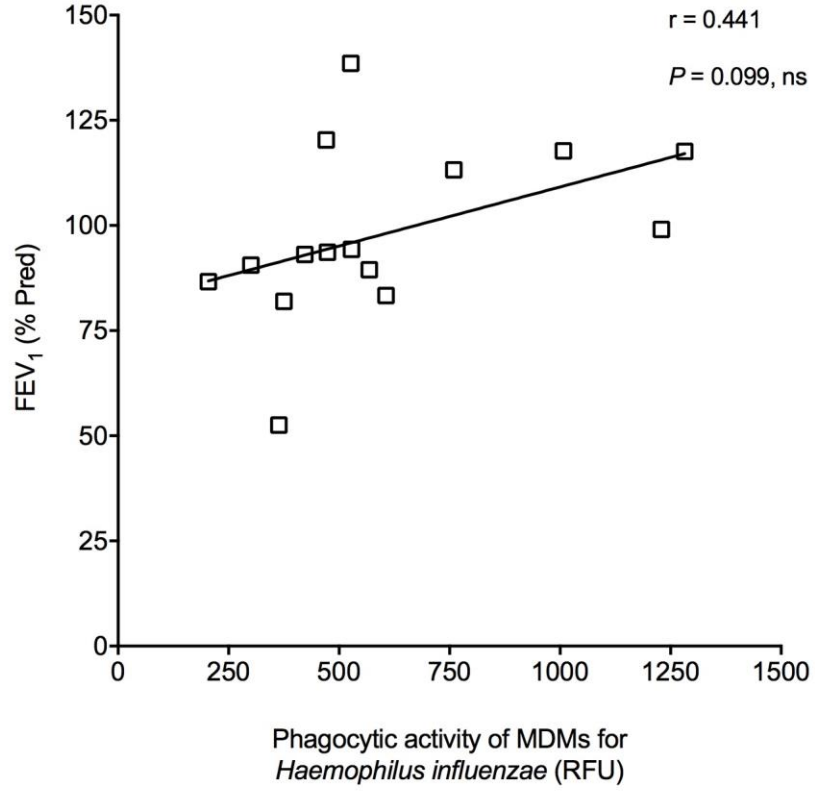
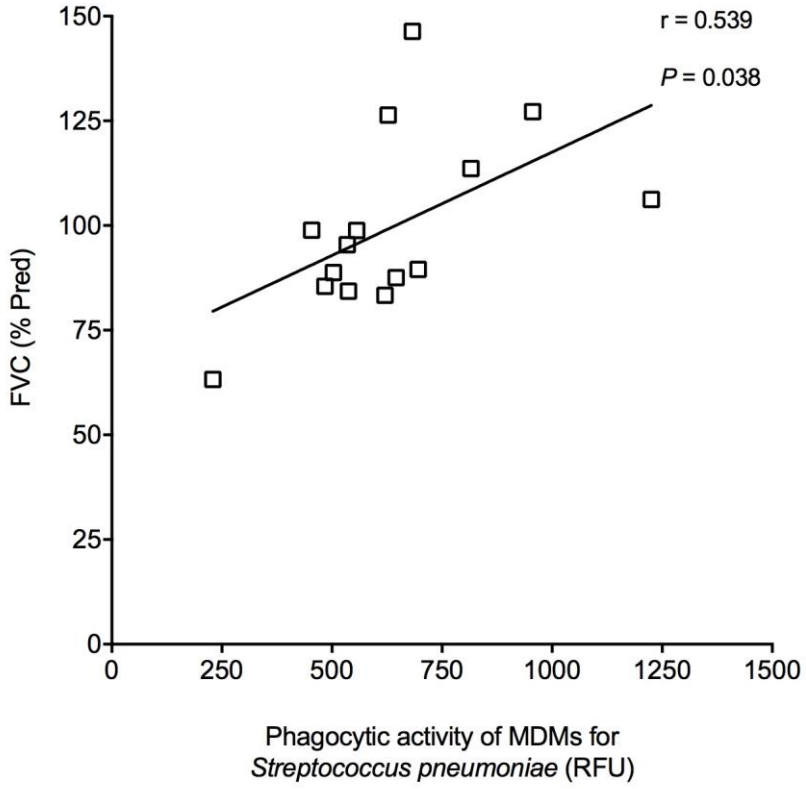

r = 0.441
P = 0.099, ns
FEV1 (% Pred)
150
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S35: Relationship between phagocytic activity of MDMs and FEV_1 (% Pred) in smokers without COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1 (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1 (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in HS (□ n = 15). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; HS, smokers without COPD]

A)


r = 0.539
P = 0.038
FVC (% Pred)
150
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

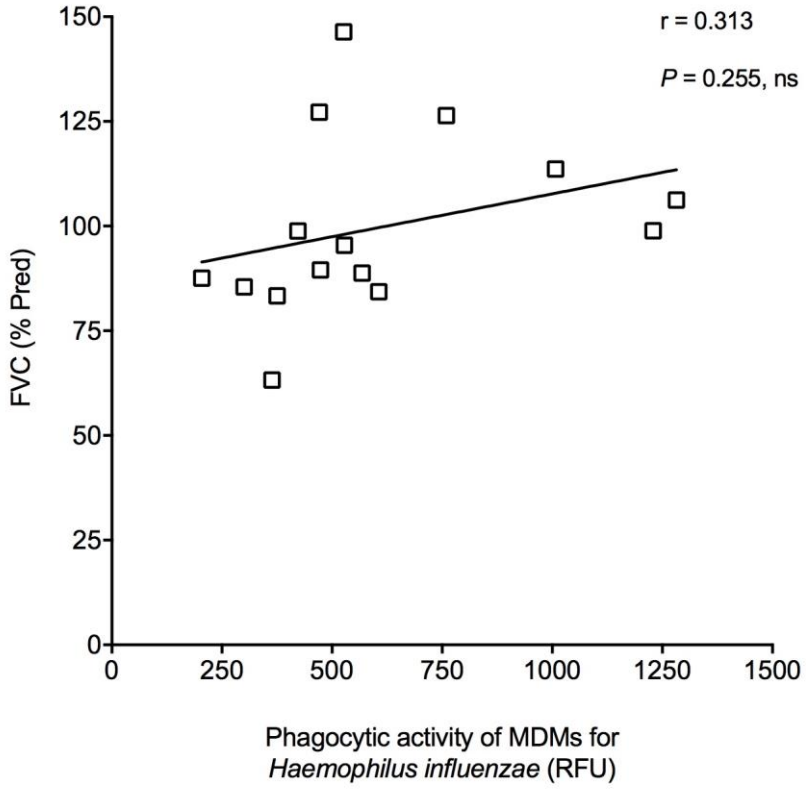
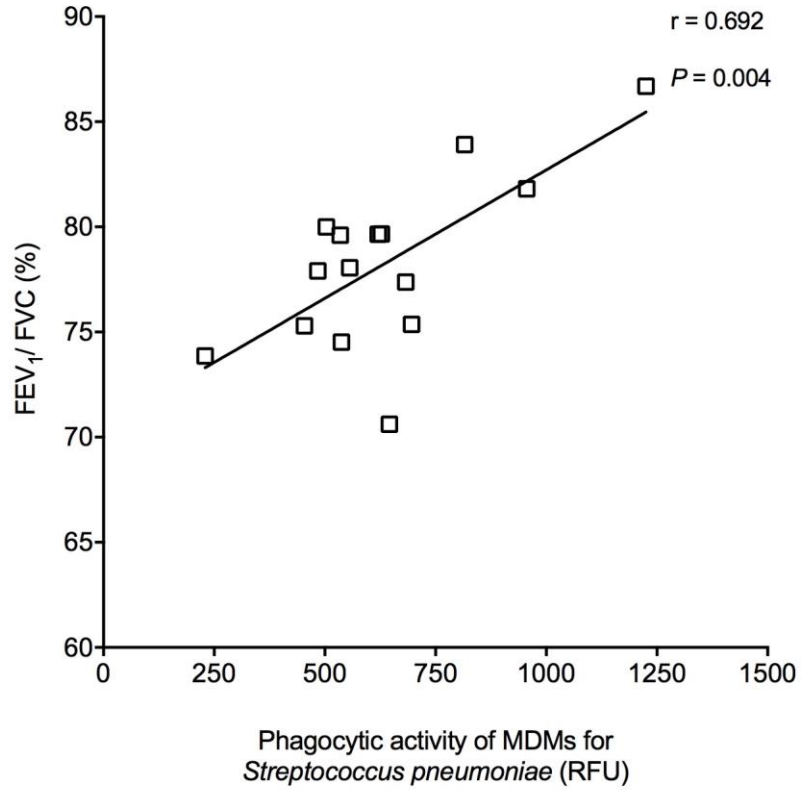

r = 0.313
P = 0.255, ns
FVC (% Pred)
150
125
100
75
50
25
0
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S36: Relationship between phagocytic activity of MDMs and FVC (% Pred) in smokers without COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FVC (% Pred) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FVC (% Pred) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in HS (□ n = 15). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FVC, forced vital capacity; HS, smokers without COPD]

A)


r = 0.692
P = 0.004
FEV1/ FVC (%)
90
85
80
75
70
65
60
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Streptococcus pneumoniae (RFU)

B)

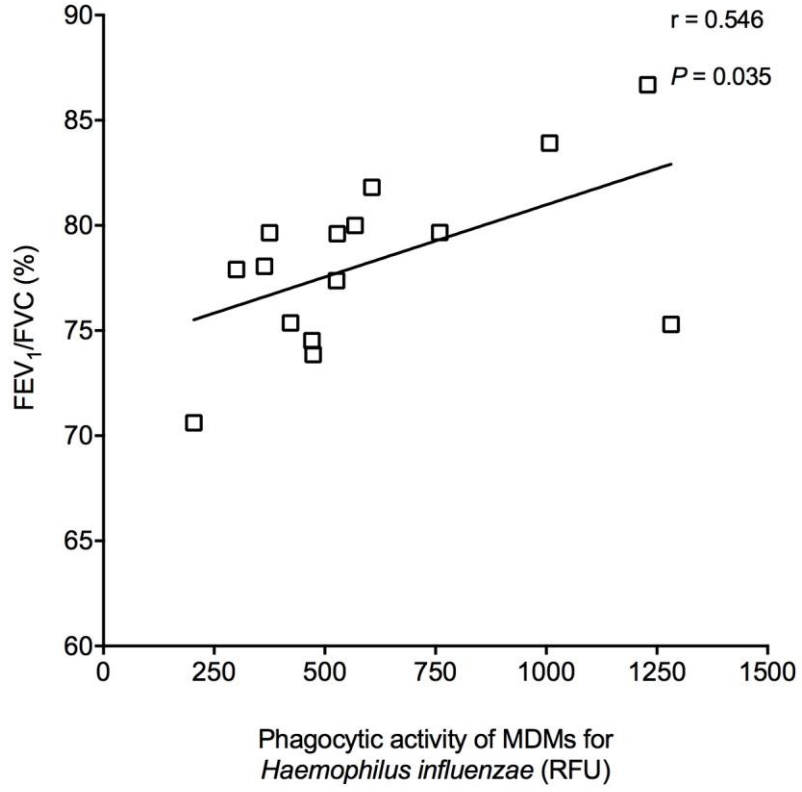

r = 0.546
P = 0.035
FEV1/FVC (%)
90
85
80
75
70
65
60
0
250
500
750
1000
1250
1500
Phagocytic activity of MDMs for
Haemophilus influenzae (RFU)

Figure S37: Relationship between phagocytic activity of MDMs and FEV_1/FVC (%) in smokers without COPD subjects.

Correlation between MDM's phagocytic activity of *Streptococcus pneumoniae* and FEV_1/FVC (%) (A); Correlation between MDM's phagocytic activity of *Haemophilus influenzae* and FEV_1/FVC (%) (B); Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using Pearson correlation analysis in HS (□ n = 15). $P<0.05$ was considered statistically significant.

[*Abbreviations*: COPD, chronic obstructive pulmonary disease; MDMs, monocyte-derived macrophages; FEV_1, forced expiratory volume; FVC, forced vital capacity; HS, smokers without COPD]

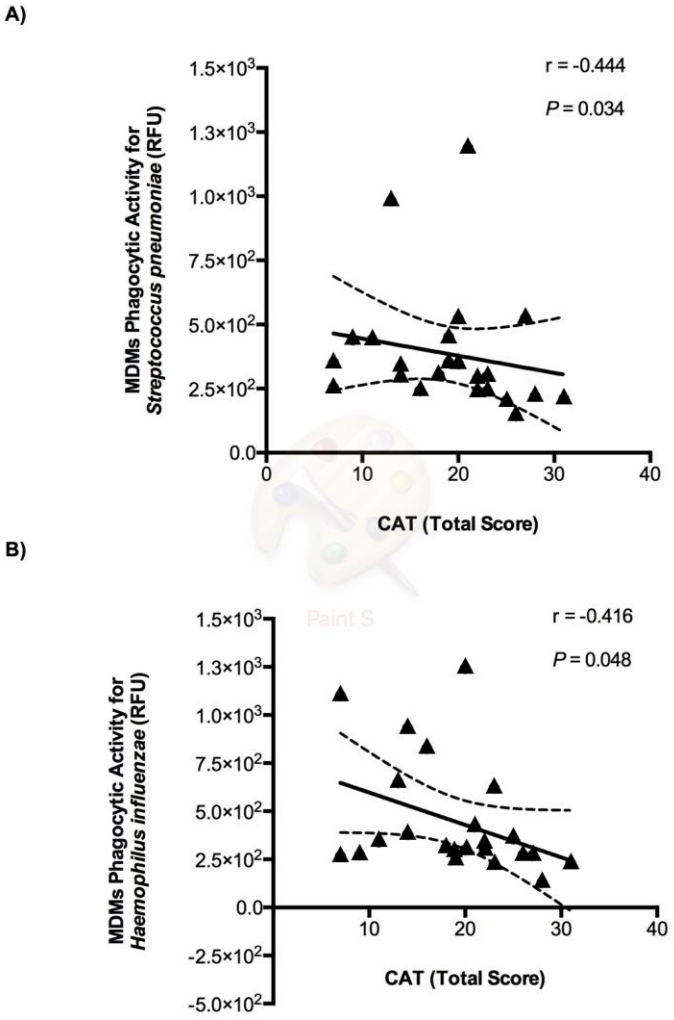


Figure S38: Relationship between bacterial phagocytosis by MDMs and total CAT score in biomass-smoke associated COPD subjects.

Correlation analysis between total CAT score and MDMs phagocytic activity *of Streptococcus pneumoniae* (A), and *Haemophilus influenzae* was determined in BMS-COPD (▲ n = 23). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation regression analysis. $P<0.05$ was considered statistically significant.

[*Abbreviations*: MDMs, Monocyte-derived macrophages; COPD, chronic obstructive pulmonary disease; BMS-COPD, biomass-smoke associated COPD; CAT, COPD assessment test]

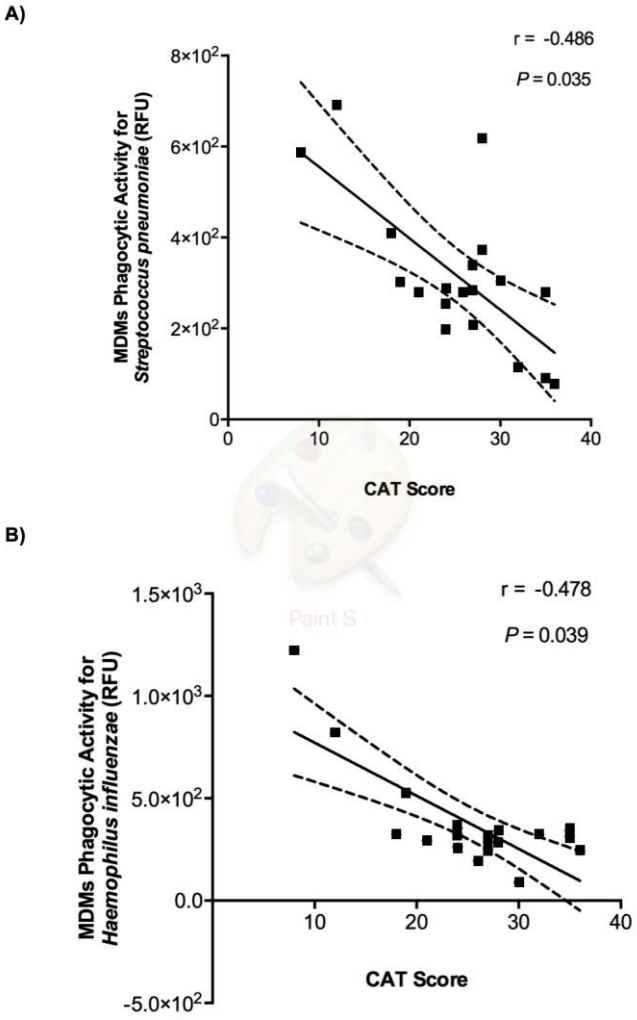


Figure S39: Relationship between bacterial phagocytosis by MDMs and total CAT score in tobacco-smoke associated COPD subjects.

Correlation analysis between total CAT score and MDMs phagocytic activity of *Streptococcus pneumoniae* (A), and *Haemophilus influenzae* was determined in S-COPD (■ n = 19). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation regression analysis. $P<0.05$ was considered statistically significant.

[*Abbreviations*: MDMs, Monocyte-derived macrophages; COPD, chronic obstructive pulmonary disease; S-COPD, tobacco-smoke associated COPD; CAT, COPD assessment test]

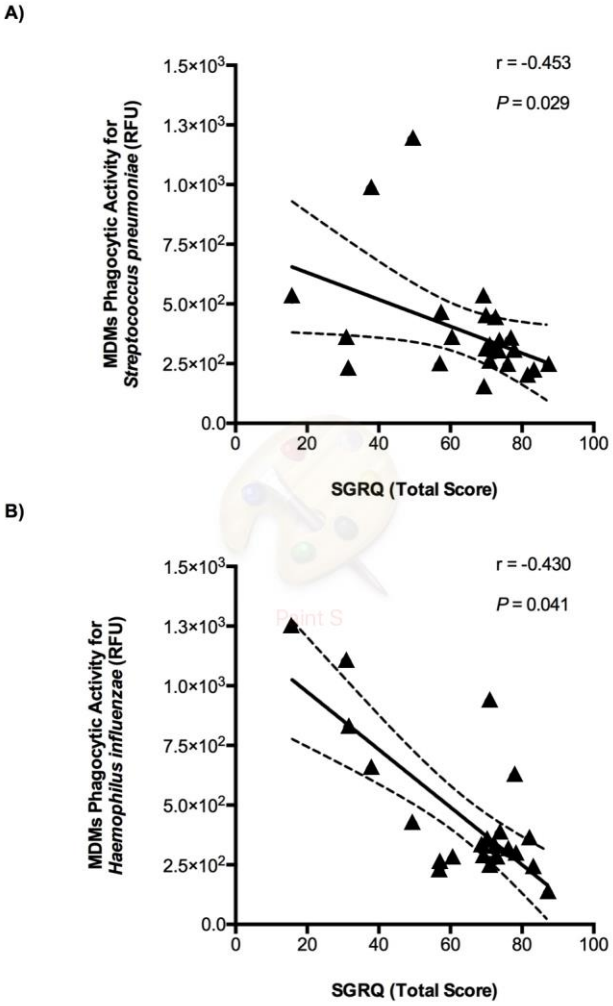


Figure S40: Relationship between bacterial phagocytosis by MDMs and total SGRQ score in BMS-COPD subjects.

Correlation analysis between total SGRQ score and MDMs phagocytic activity of *Streptococcus pneumoniae* (A), and *Haemophilus influenzae* was determined in BMS-COPD (▲ n = 23). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation regression analysis. $P<0.05$ was considered statistically significant.

[*Abbreviations*: MDMs, Monocyte-derived macrophages; COPD, chronic obstructive pulmonary disease; BMS-COPD, biomass-smoke associated COPD; SGRQ, St. George's Respiratory Questionnaire]

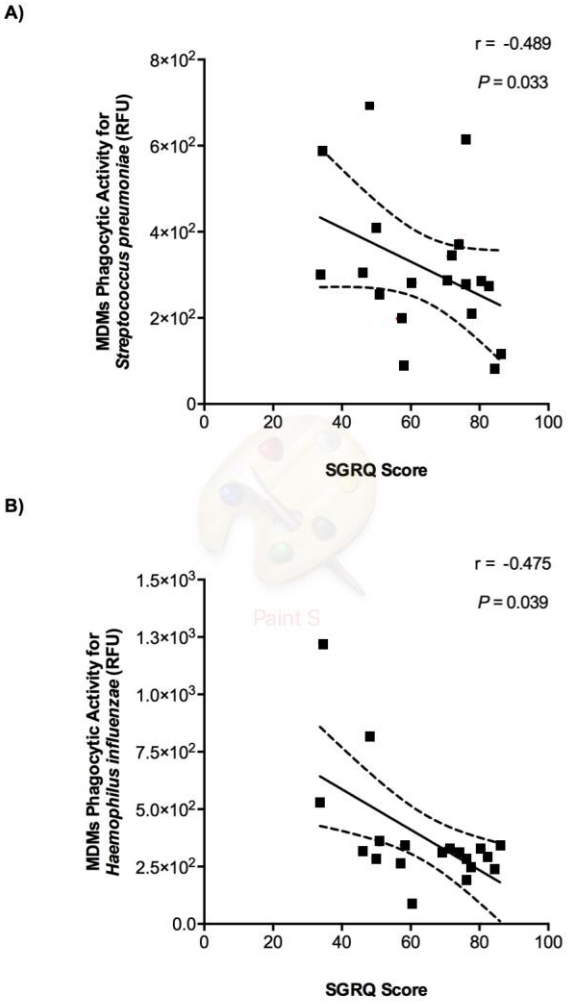


Figure S41: Relationship between bacterial phagocytosis by MDMs and total SGRQ score in tobacco-smoke associated COPD subjects.

Correlation analysis between total CAT score and MDMs phagocytic activity of *Streptococcus pneumoniae* (A), and *Haemophilus influenzae* was determined in S-COPD (■ n = 19). Shapiro-Wilk normality test was performed. Correlation coefficients were calculated using non-parametric Spearman correlation regression analysis. $P<0.05$ was considered statistically significant.

[*Abbreviations*: MDMs, Monocyte-derived macrophages; COPD, chronic obstructive pulmonary disease; S-COPD, tobacco-smoke associated COPD; SGRQ, St. George's Respiratory Questionnaire]