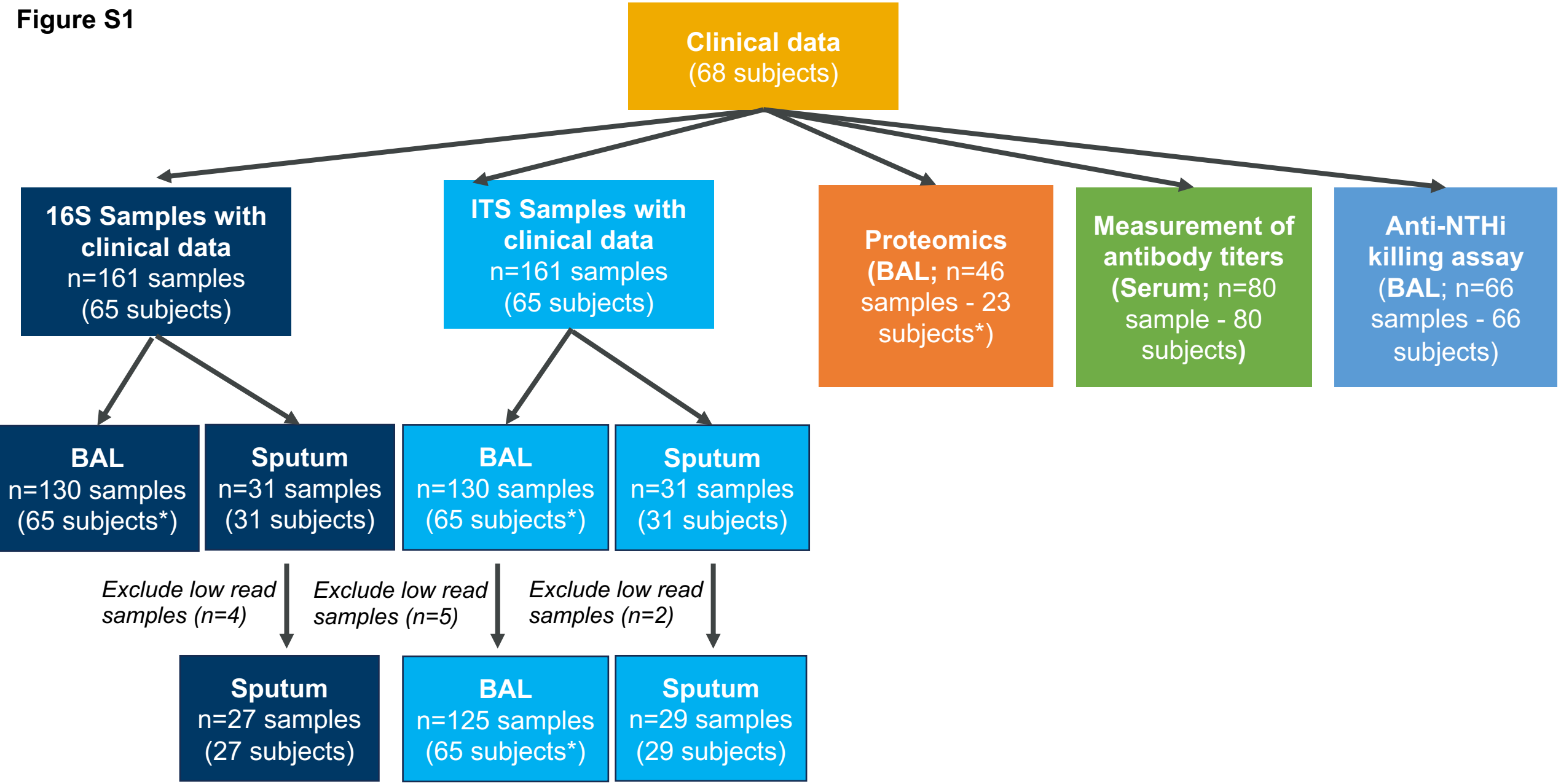
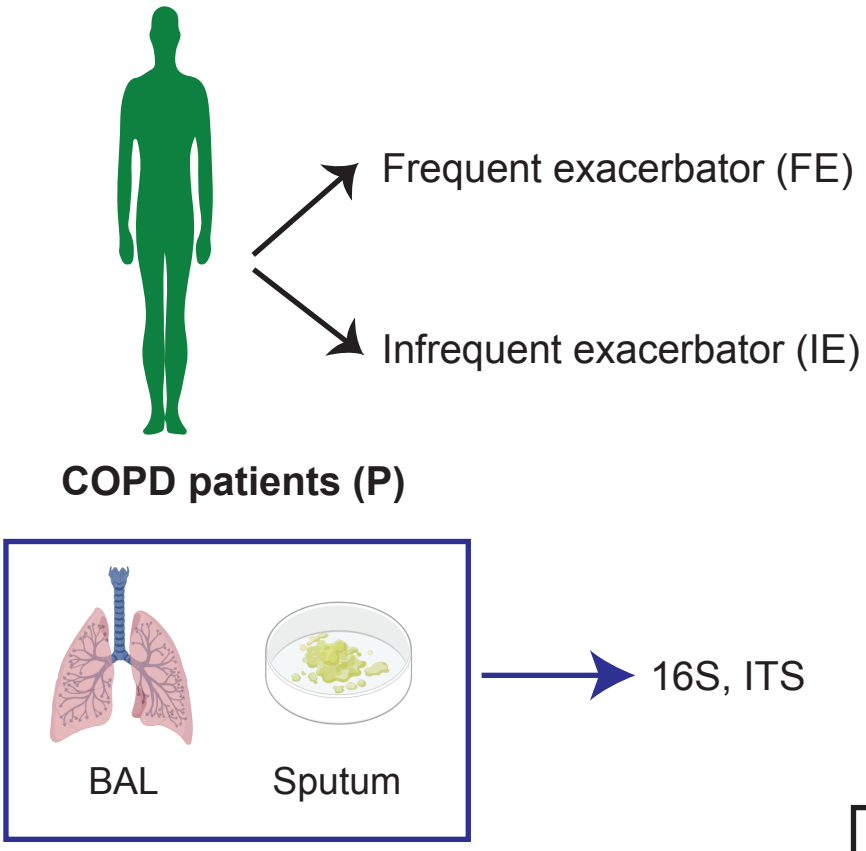
Figure S1



* 2 BAL samples per patient

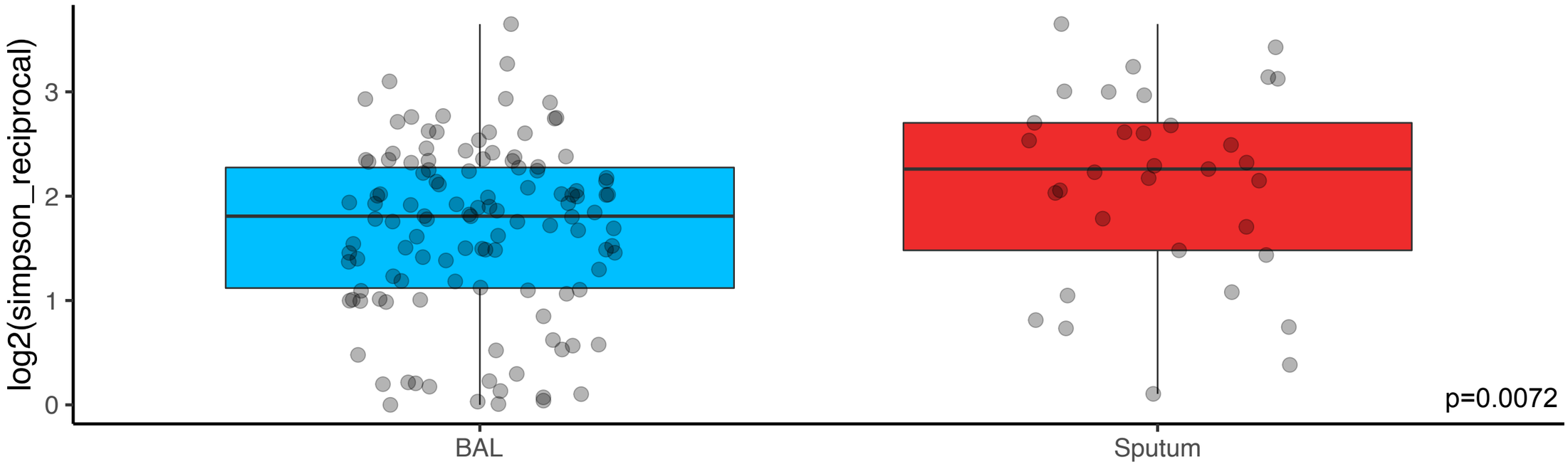
Figure S2



	P	HV
BAL	60 (30)	70 (35)
Sputum	17	10

Fig. S3

A.


log2(simpson_reciprocal)
3
2
1
0
BAL
Sputum
p=0.0072

B.

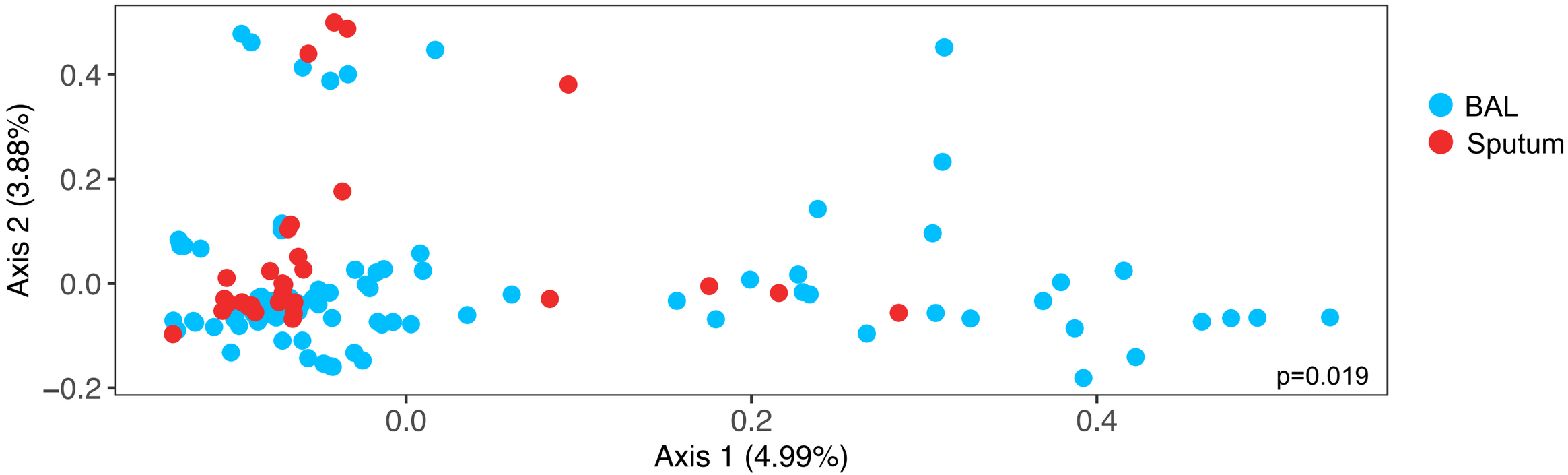

Axis 2 (3.88%)
0.4
0.2
0.0
−0.2
0.0
0.2
0.4
Axis 1 (4.99%)
p=0.019
BAL
Sputum

Figure S4

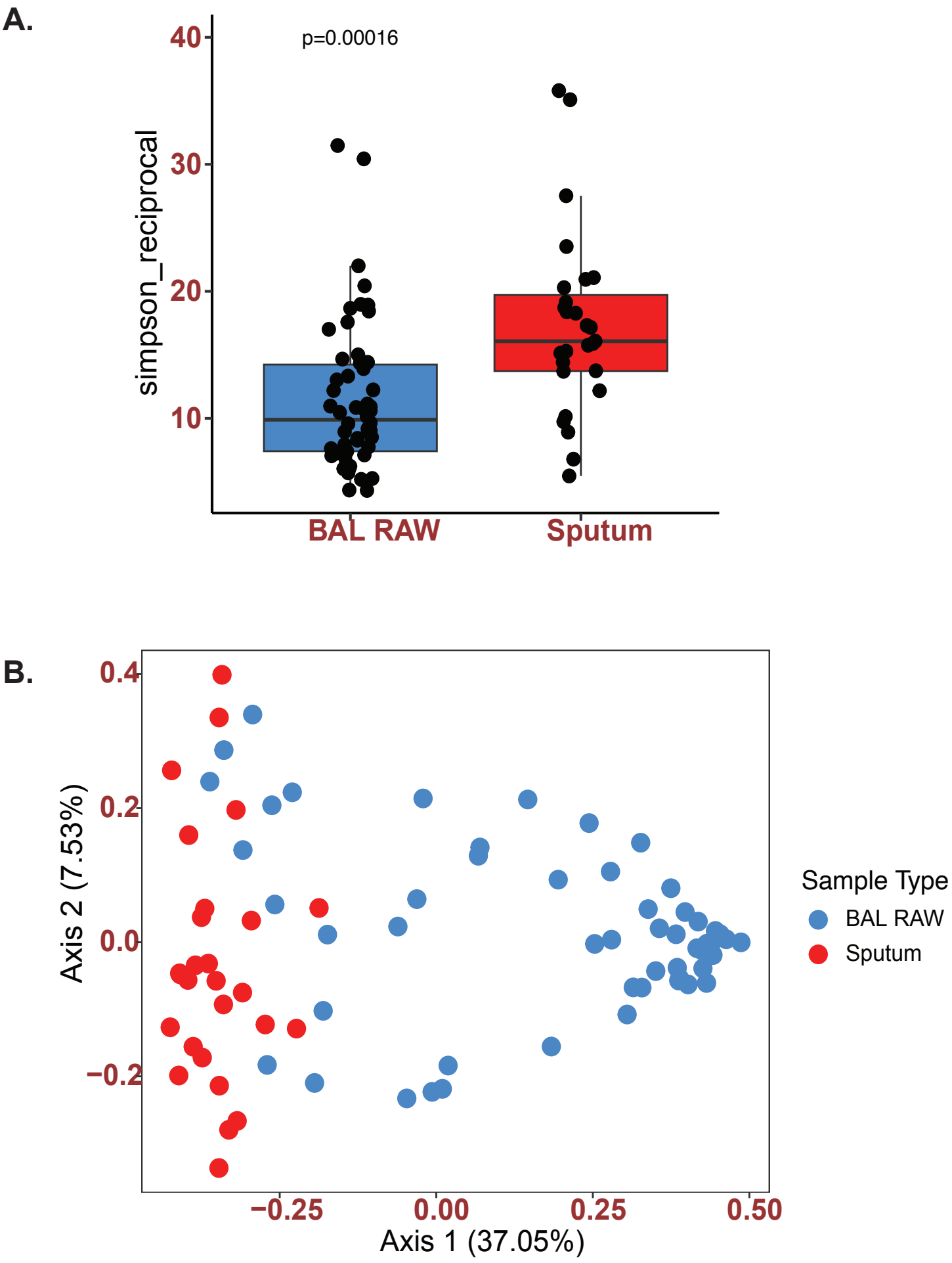
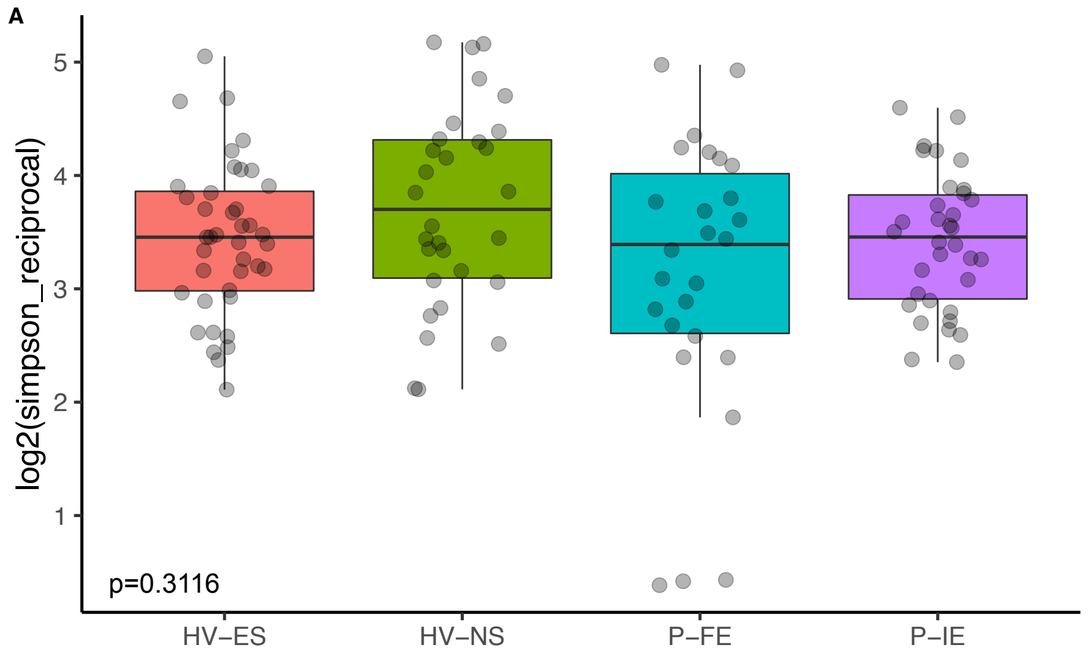
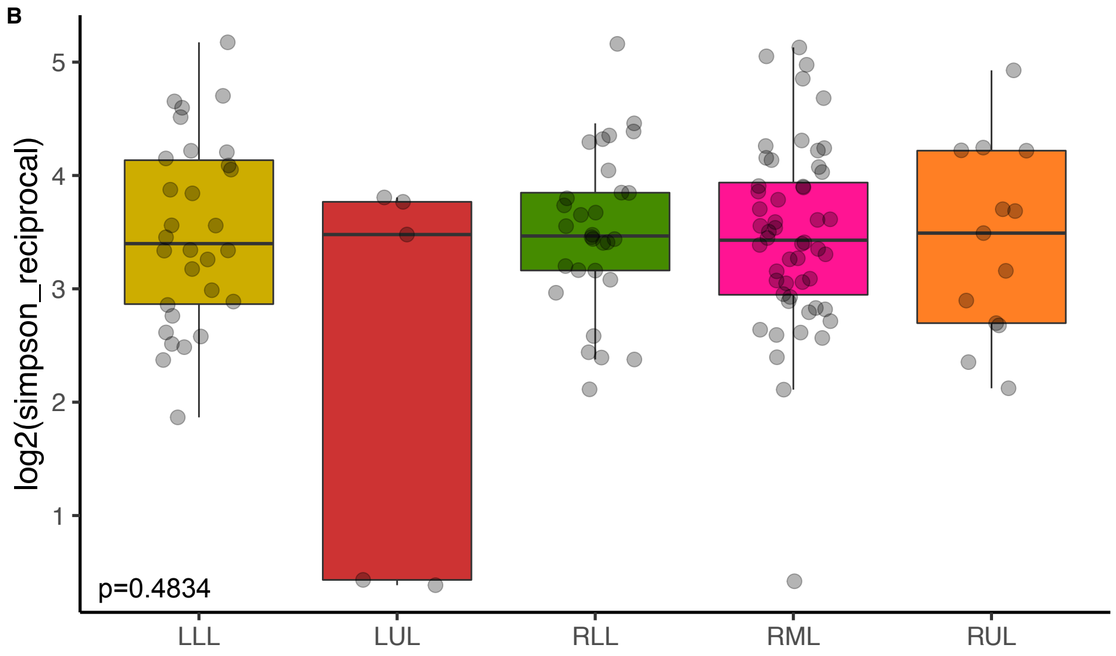
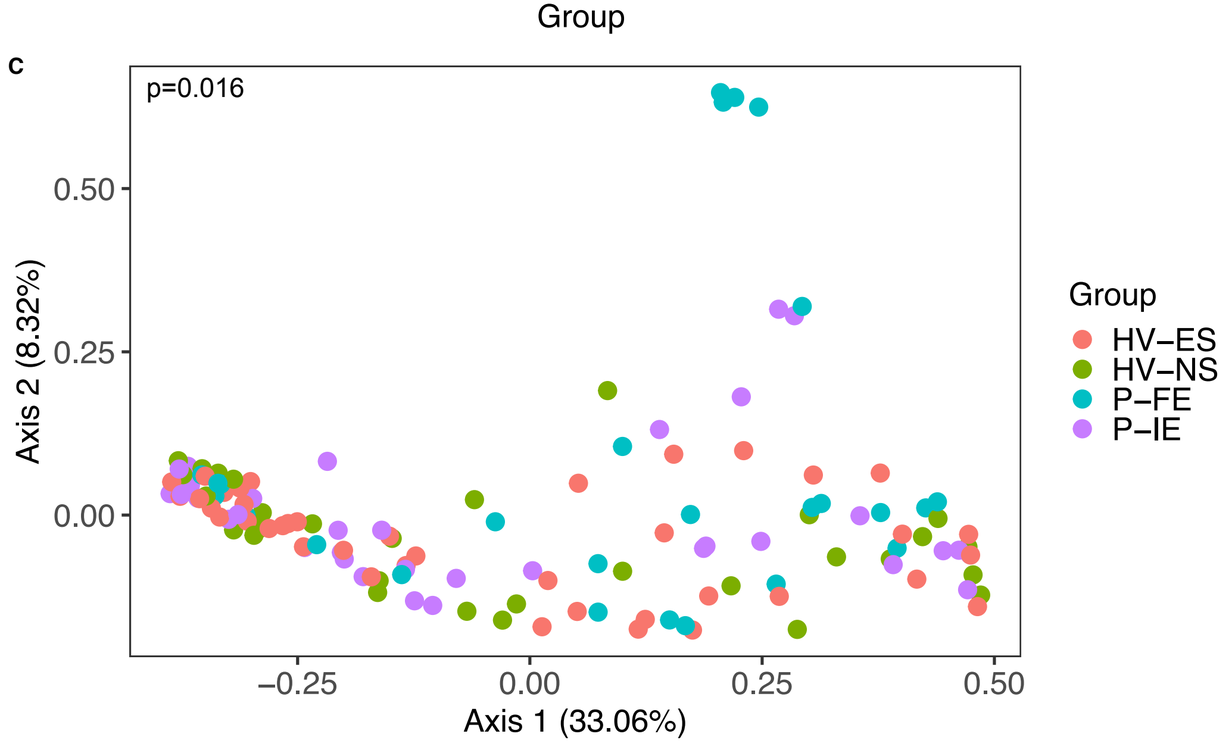

A.
p=0.00016
simpson_reciprocal
40
30
20
10
BAL RAW
Sputum
B.
0.4
0.2
0.0
−0.2
Axis 2 (7.53%)
−0.25
0.00
0.25
0.50
Axis 1 (37.05%)
Sample Type
BAL RAW
Sputum

Figure S5


A
log2(simpson_reciprocal)
p=0.3116
HV−ES
HV−NS
P−FE
P−IE


B
log2(simpson_reciprocal)
p=0.4834
LLL
LUL
RLL
RML
RUL


C
p=0.016
Axis 2 (8.32%)
Axis 1 (33.06%)
Group
HV−ES
HV−NS
P−FE
P−IE

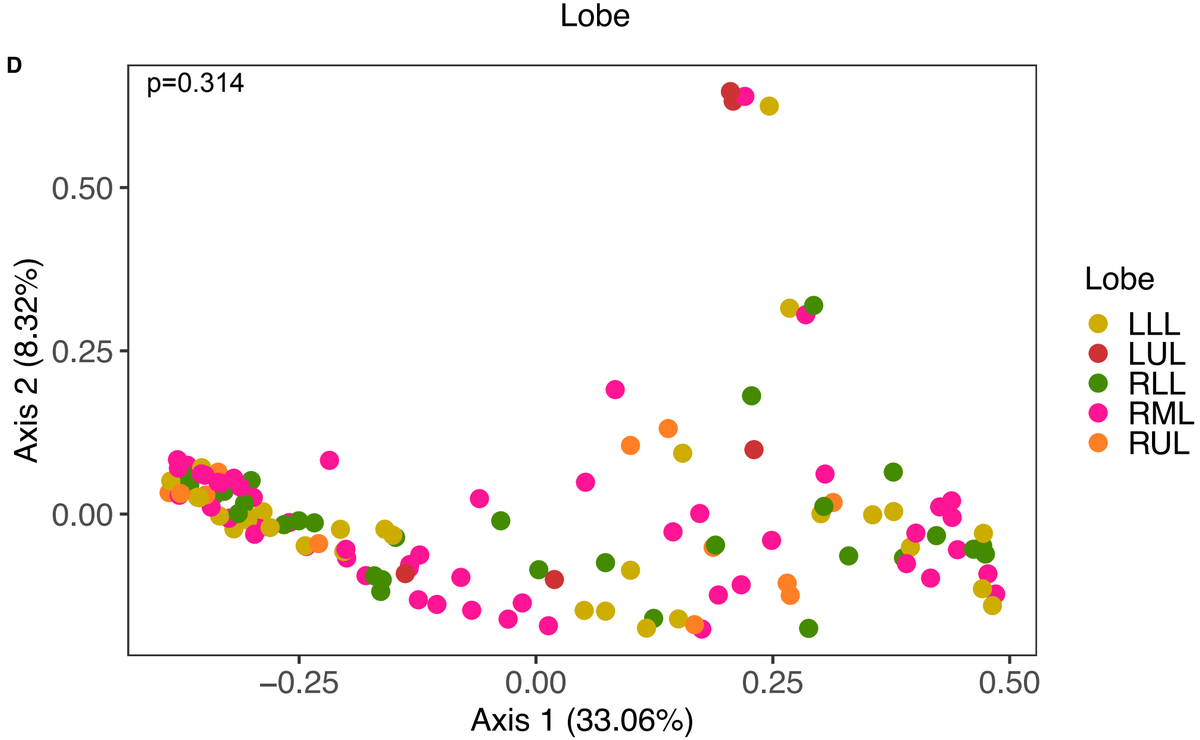
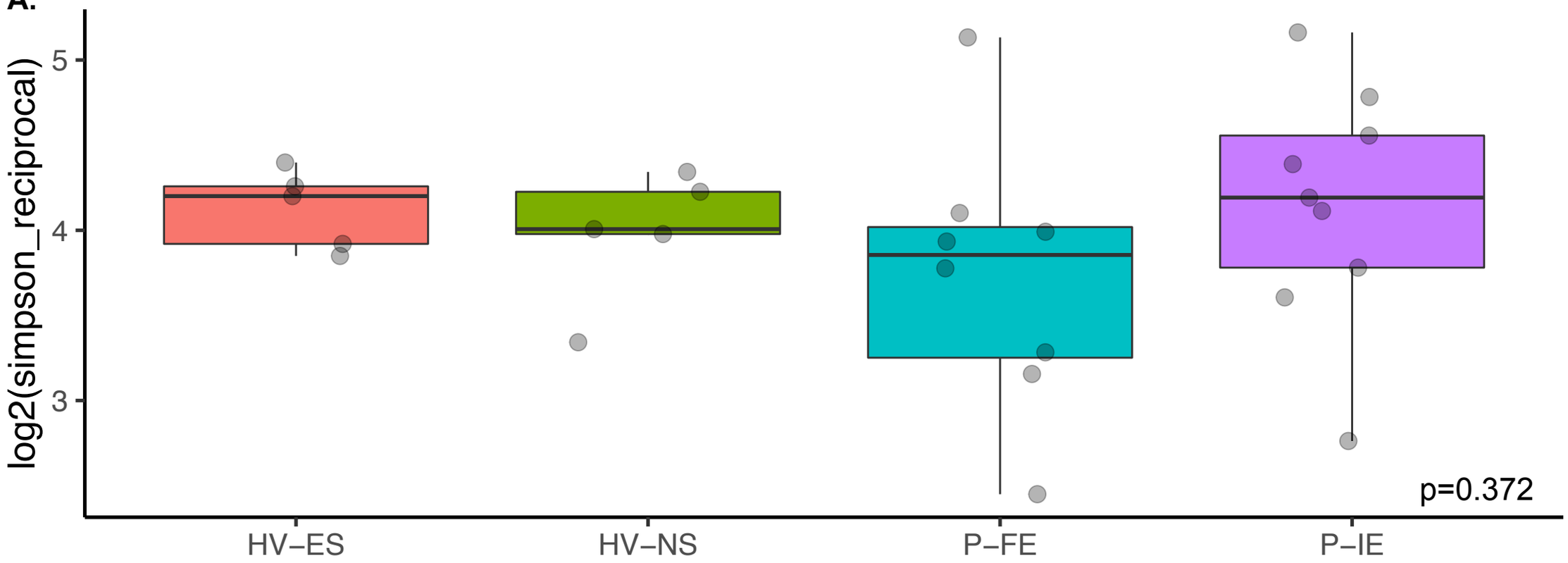

D
p=0.314
Axis 2 (8.32%)
Axis 1 (33.06%)
Lobe
LLL
LUL
RLL
RML
RUL

Fig. S6

A.


log2(simpson_reciprocal)
5
4
3
p=0.372
HV–ES
HV–NS
P–FE
P–IE

B.

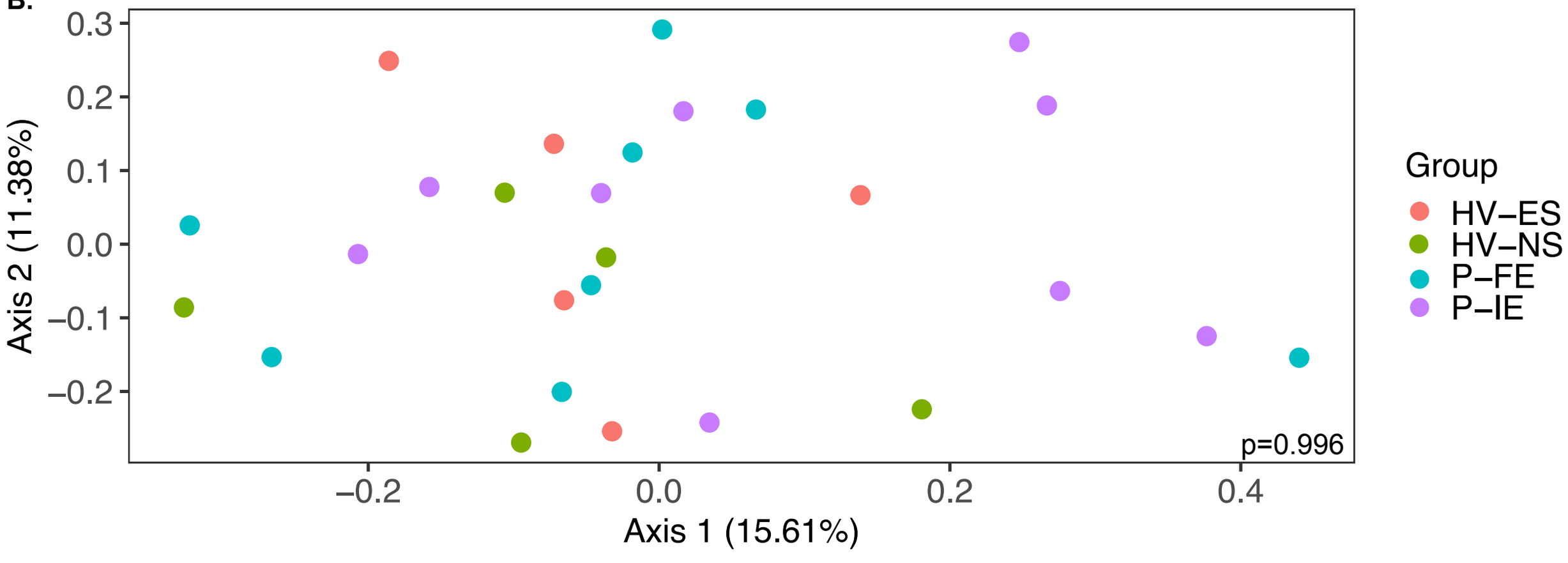

Axis 2 (11.38%)
0.3
0.2
0.1
0.0
–0.1
–0.2
p=0.996
–0.2
0.0
0.2
0.4
Axis 1 (15.61%)
Group
HV–ES
HV–NS
P–FE
P–IE

Fig S7

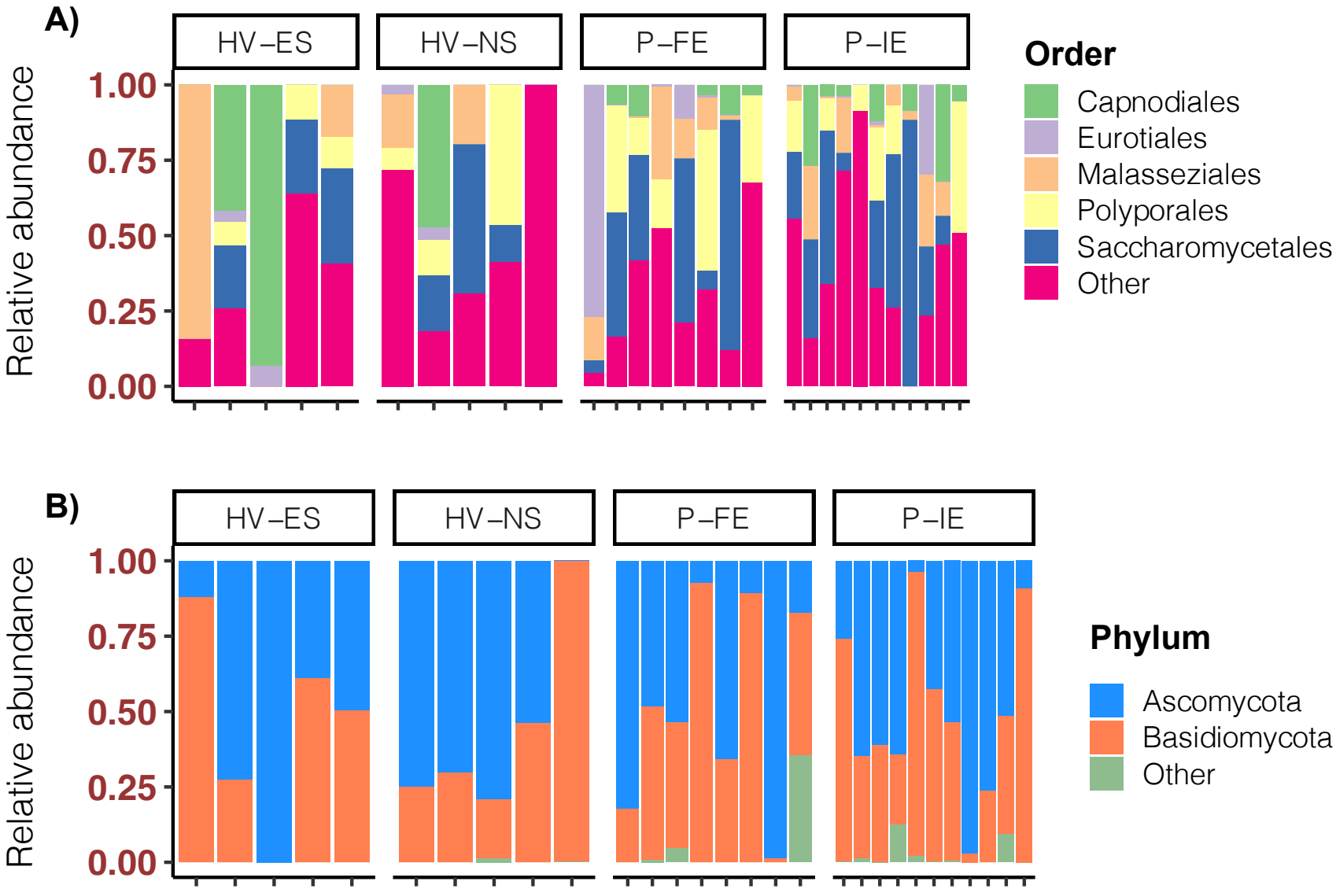

A)
HV–ES
HV–NS
P–FE
P–IE
Relative abundance
1.00
0.75
0.50
0.25
0.00
Order
Capnodiales
Eurotiales
Malasseziales
Polyporales
Saccharomycetales
Other
B)
HV–ES
HV–NS
P–FE
P–IE
Relative abundance
1.00
0.75
0.50
0.25
0.00
Phylum
Ascomycota
Basidiomycota
Other

Fig S8

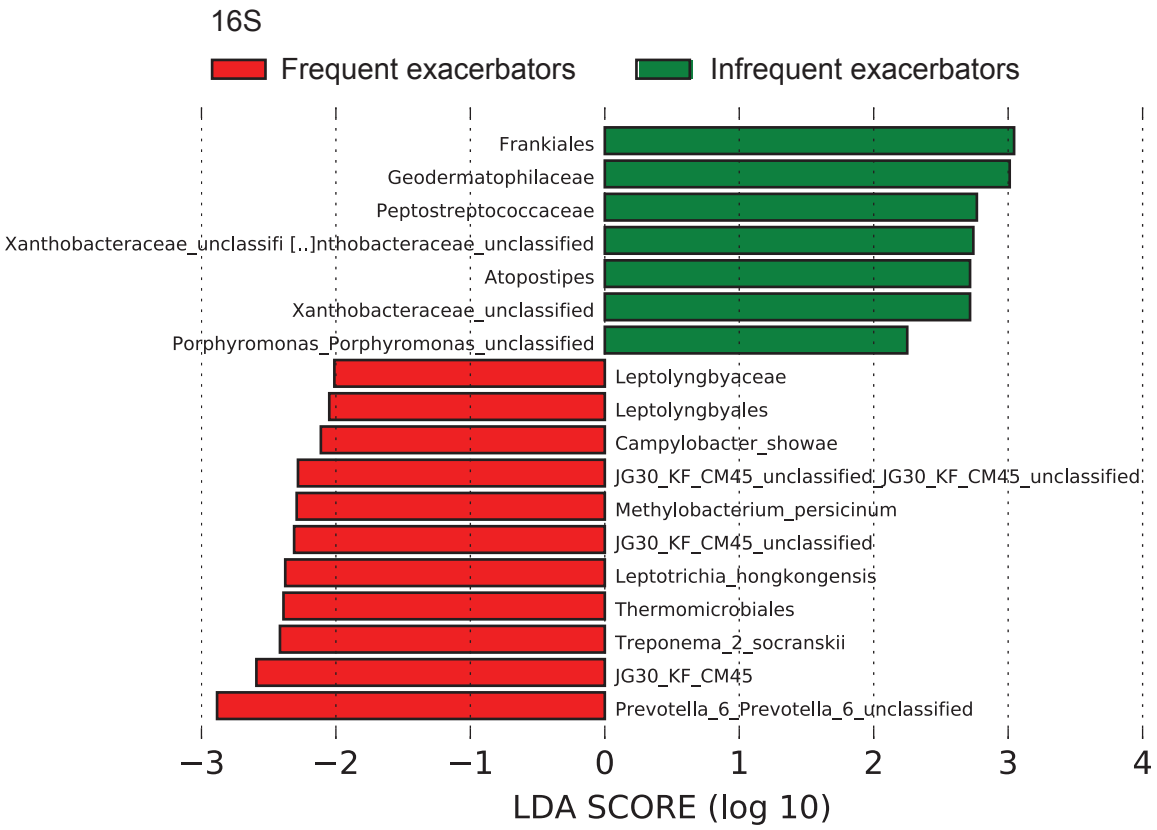

16S
Frequent exacerbators
Infrequent exacerbators
Frankiales
Geodermatophilaceae
Peptostreptococcaceae
Xanthobacteraceae_unclassifi [..]nthobacteraceae_unclassified
Atopostipes
Xanthobacteraceae_unclassified
Porphyromonas_Porphyromonas_unclassified
Leptolyngbyaceae
Leptolyngbyales
Campylobacter_showae
JG30_KF_CM45_unclassified JG30_KF_CM45_unclassified
Methylobacterium_persicinum
JG30_KF_CM45_unclassified
Leptotrichia_hongkongensis
Thermomicrobiales
Treponema_2_socranskii
JG30_KF_CM45
Prevotella_6 Prevotella_6_unclassified
−3
−2
−1
0
1
2
3
4
LDA SCORE (log 10)

Fig S9

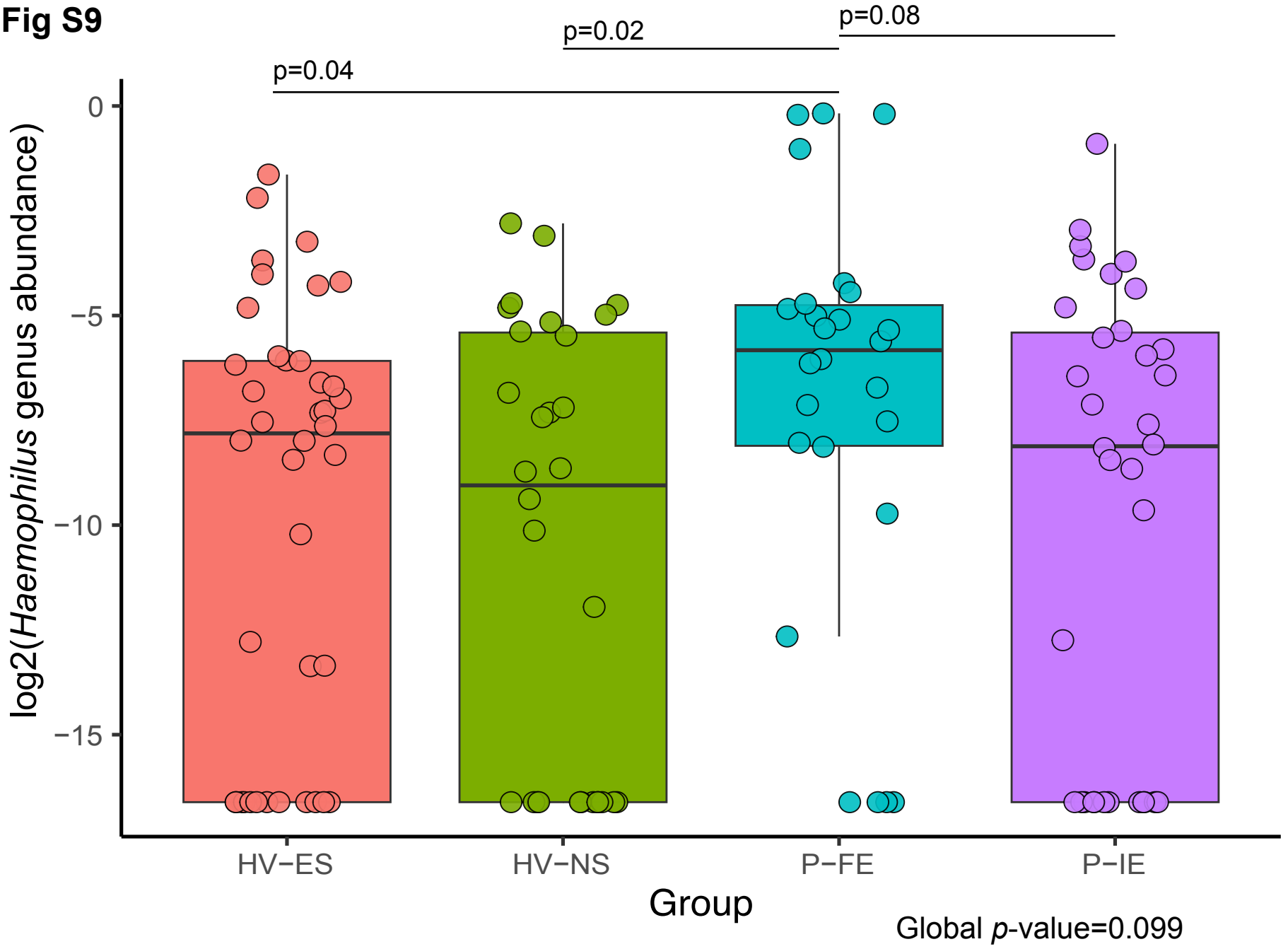

p=0.04
p=0.02
p=0.08
0
−5
−10
−15
log2(*Haemophilus* genus abundance)
HV−ES
HV−NS
P−FE
P−IE
Group
Global *p*-value=0.099

Fig S10

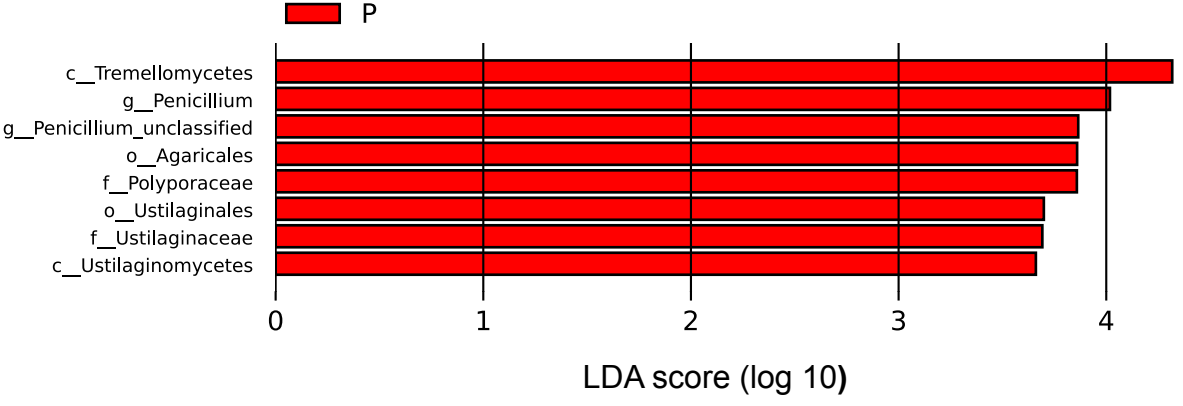

P
c__Tremellomycetes
g__Penicillium
g__Penicillium_unclassified
o__Agaricales
f__Polyporaceae
o__Ustilaginales
f__Ustilaginaceae
c__Ustilaginomycetes
0
1
2
3
4
LDA score (log 10)

Figure S11

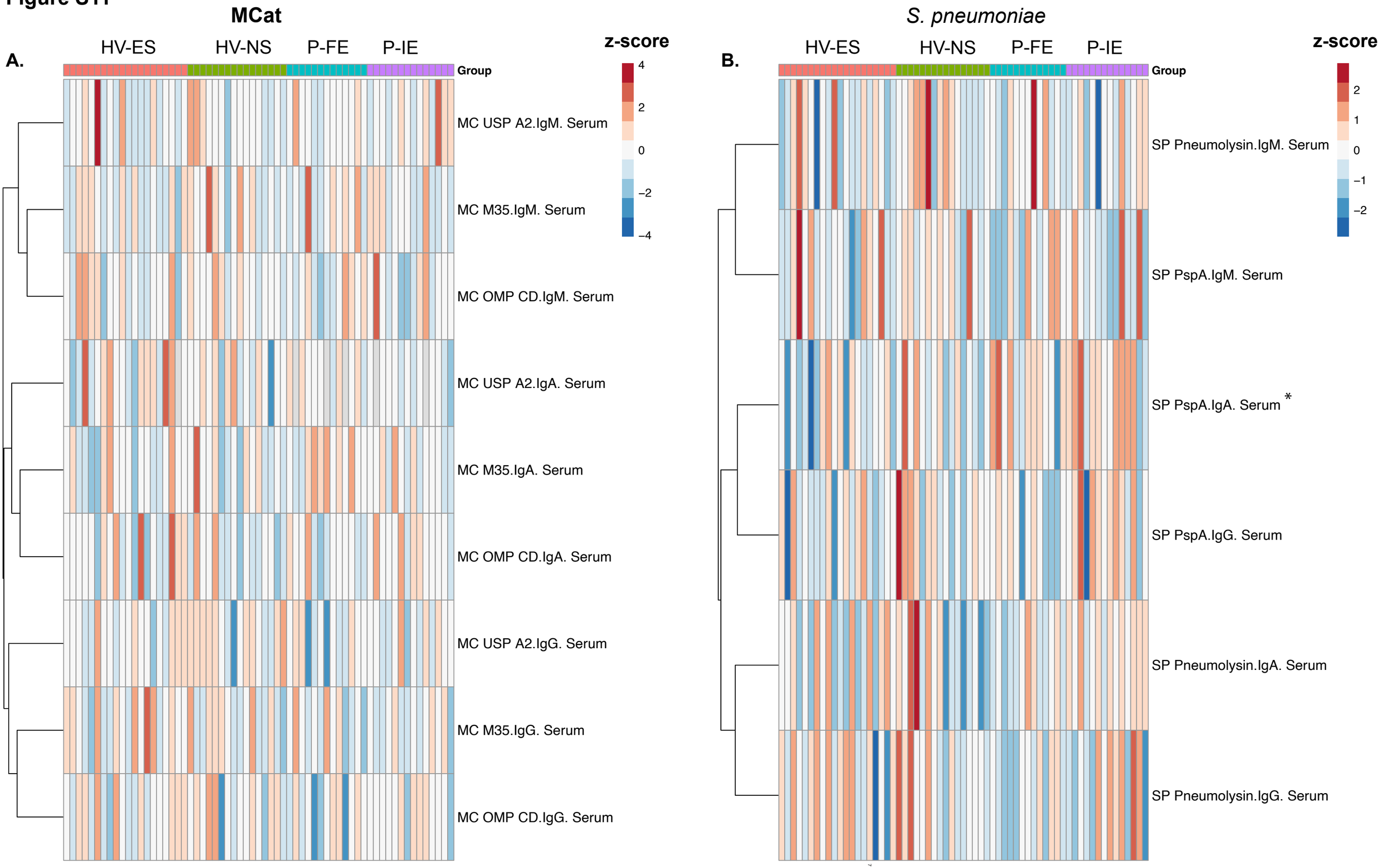

MCat
A.
HV-ES
HV-NS
P-FE
P-IE
Group
z-score
4
2
0
−2
−4
MC USP A2.IgM. Serum
MC M35.IgM. Serum
MC OMP CD.IgM. Serum
MC USP A2.IgA. Serum
MC M35.IgA. Serum
MC OMP CD.IgA. Serum
MC USP A2.IgG. Serum
MC M35.IgG. Serum
MC OMP CD.IgG. Serum
S. pneumoniae
B.
HV-ES
HV-NS
P-FE
P-IE
Group
z-score
2
1
0
−1
−2
SP Pneumolysin.IgM. Serum
SP PspA.IgM. Serum
SP PspA.IgA. Serum *
SP PspA.IgG. Serum
SP Pneumolysin.IgA. Serum
SP Pneumolysin.IgG. Serum

Fig. S12

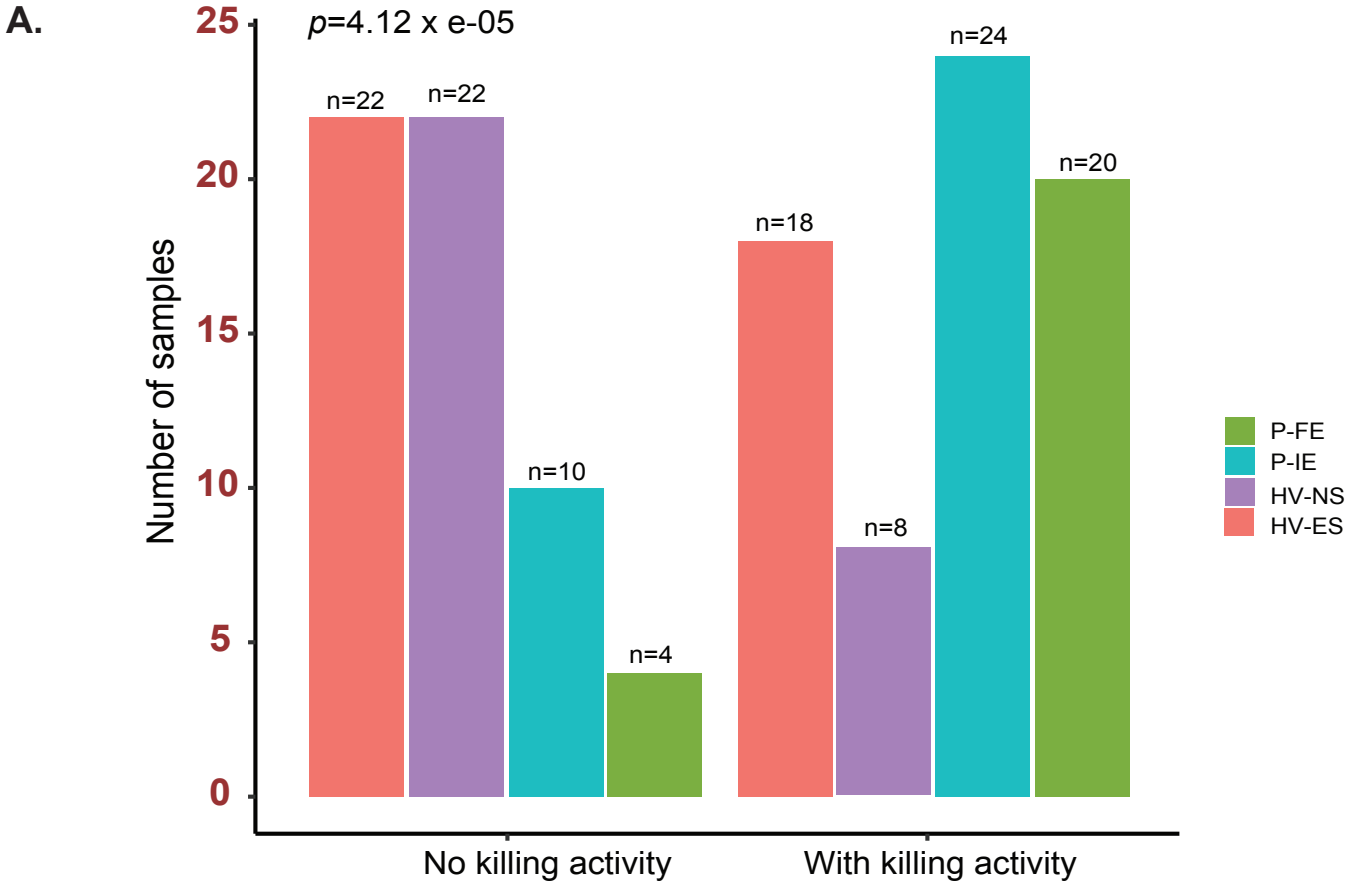

A.
p=4.12 x e-05
Number of samples
25
20
15
10
5
0
n=22
n=22
n=10
n=4
n=18
n=8
n=24
n=20
No killing activity
With killing activity
P-FE
P-IE
HV-NS
HV-ES

Fig S13

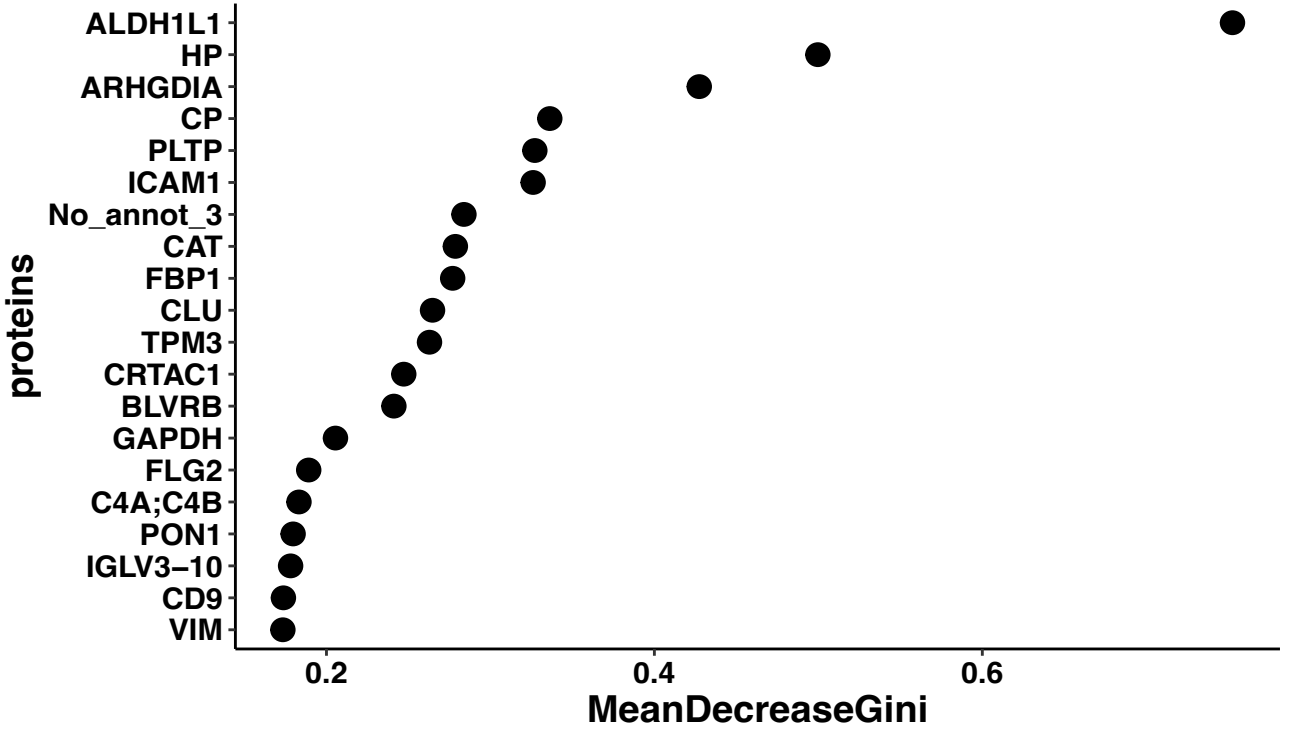
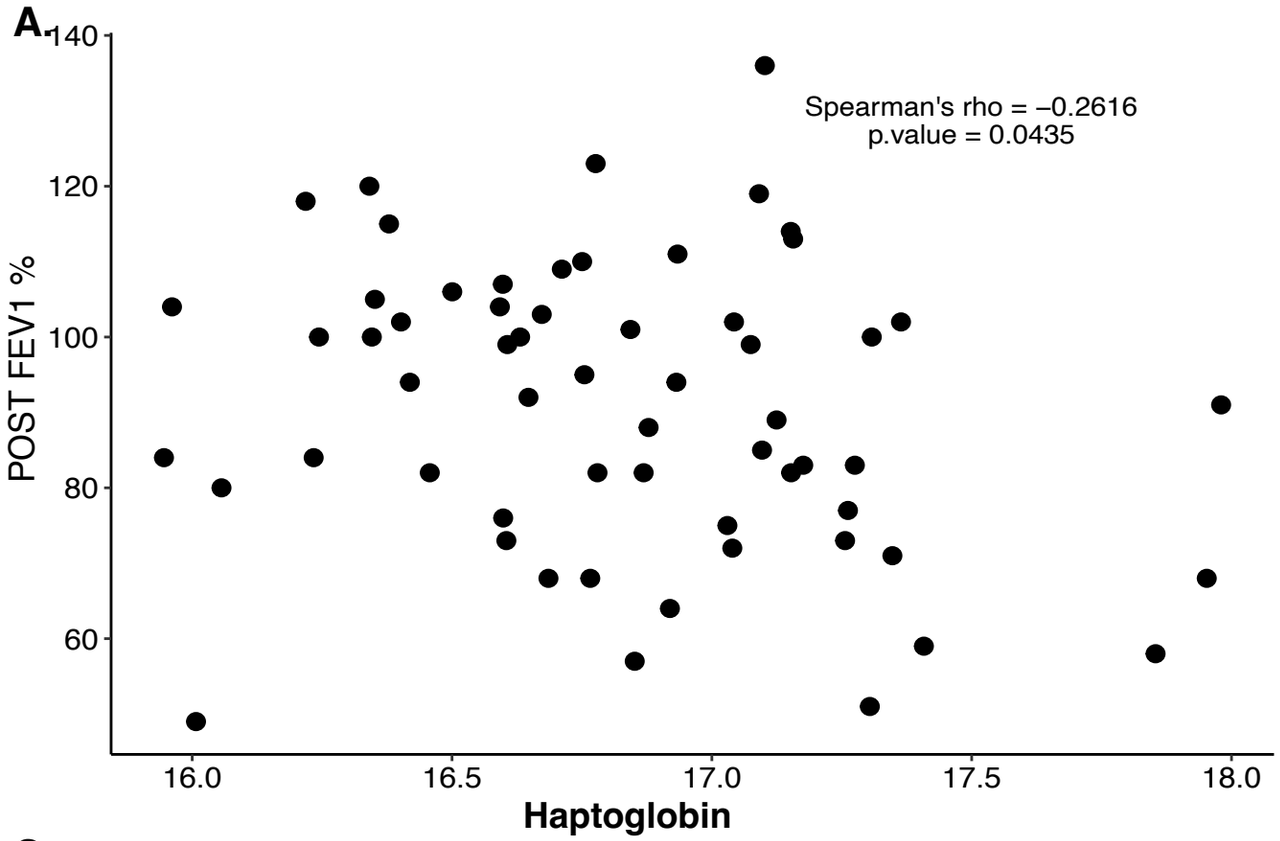
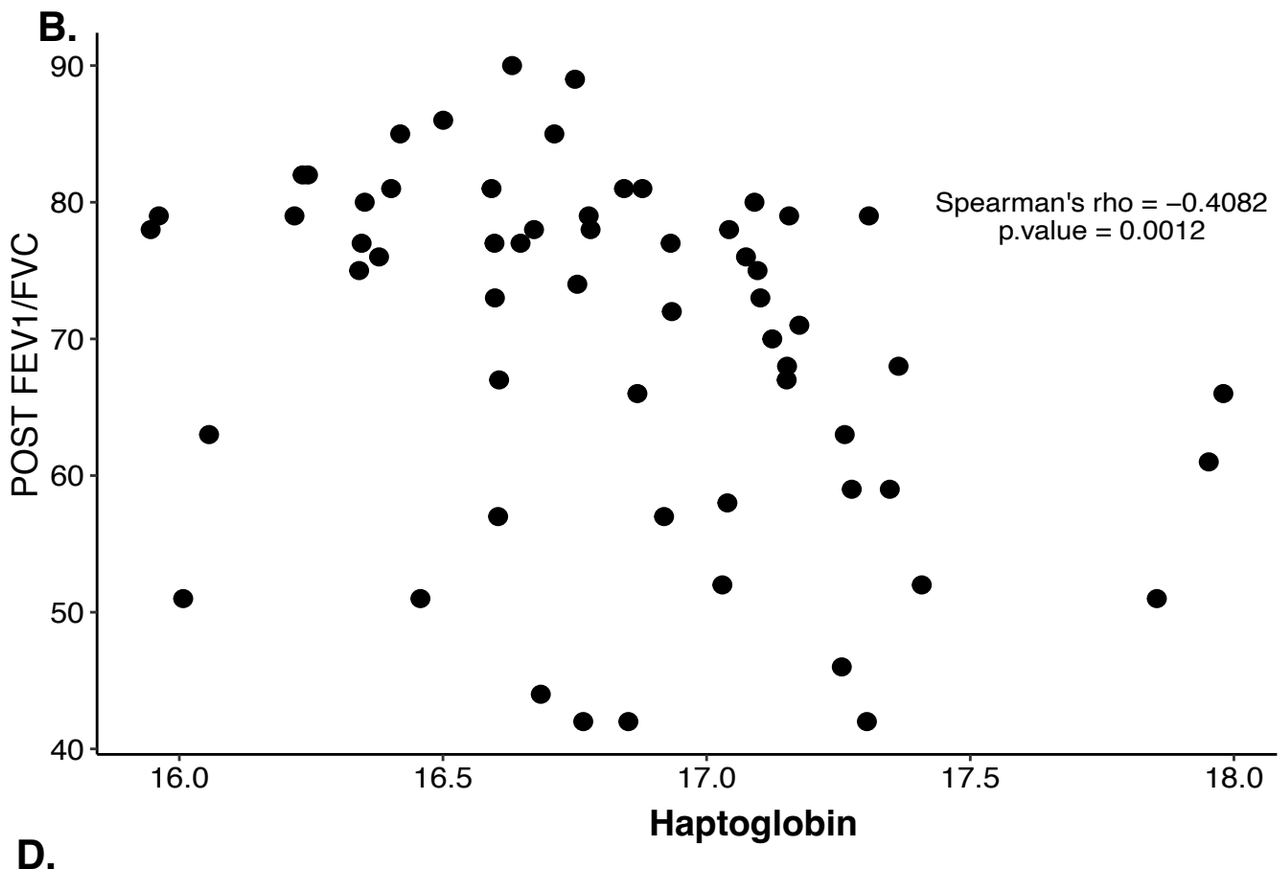
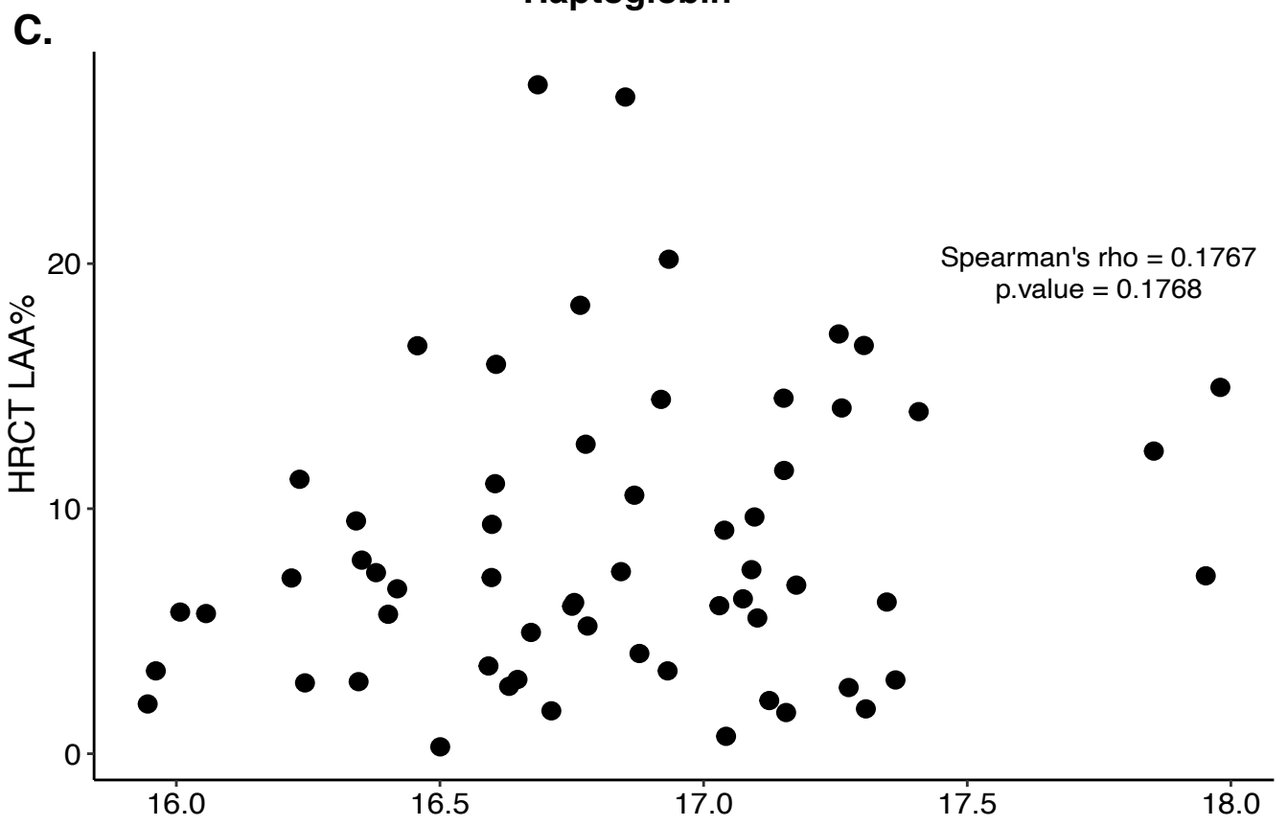
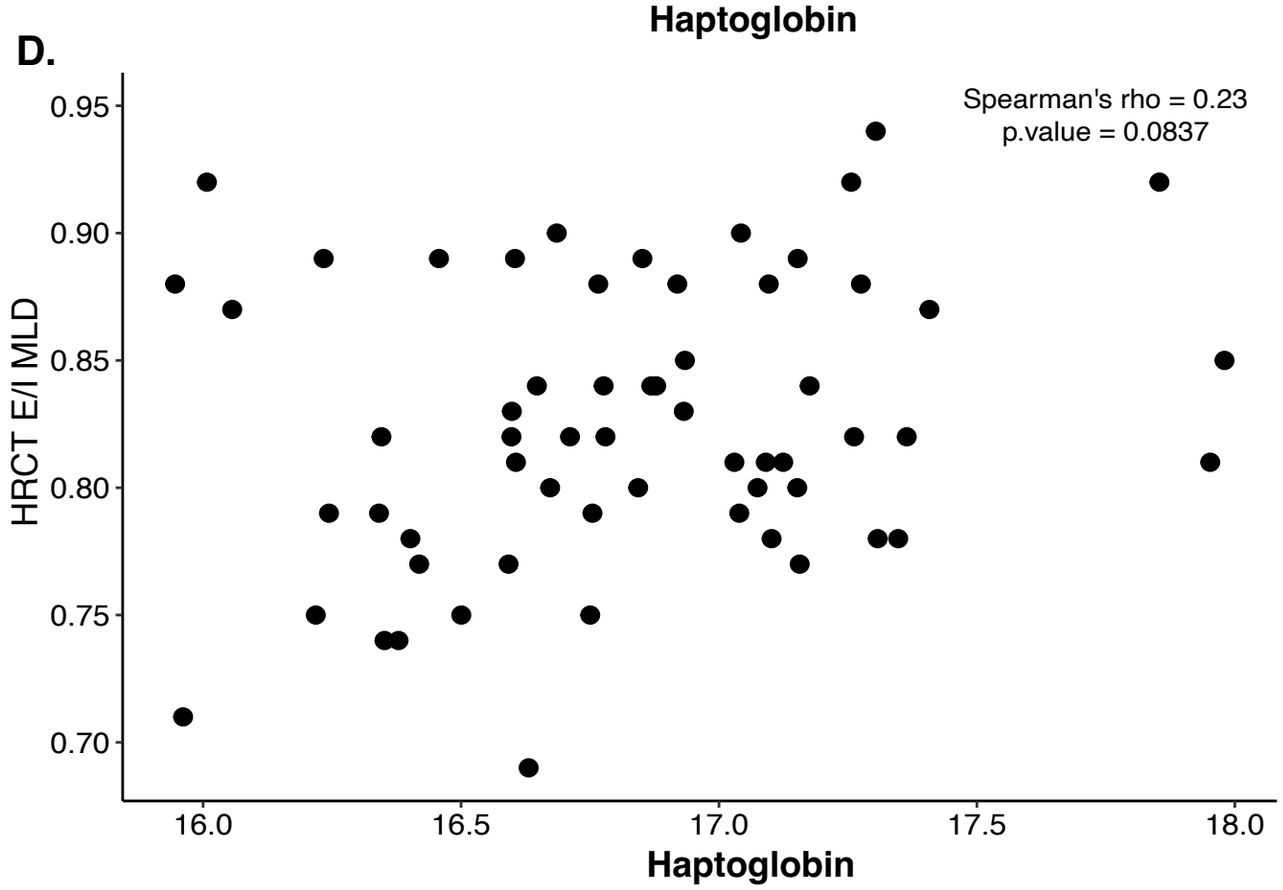

ALDH1L1
HP
ARHGDIA
CP
PLTP
ICAM1
No_annot_3
CAT
FBP1
CLU
TPM3
CRTAC1
BLVRB
GAPDH
FLG2
C4A;C4B
PON1
IGLV3−10
CD9
VIM
proteins
0.2
0.4
0.6
MeanDecreaseGini

Figure S14


A.
Spearman's rho = −0.2616
p.value = 0.0435
POST FEV1 %
Haptoglobin


B.
Spearman's rho = −0.4082
p.value = 0.0012
POST FEV1/FVC
Haptoglobin


C.
Spearman's rho = 0.1767
p.value = 0.1768
HRCT LAA%
Haptoglobin


D.
Spearman's rho = 0.23
p.value = 0.0837
HRCT E/I MLD
Haptoglobin

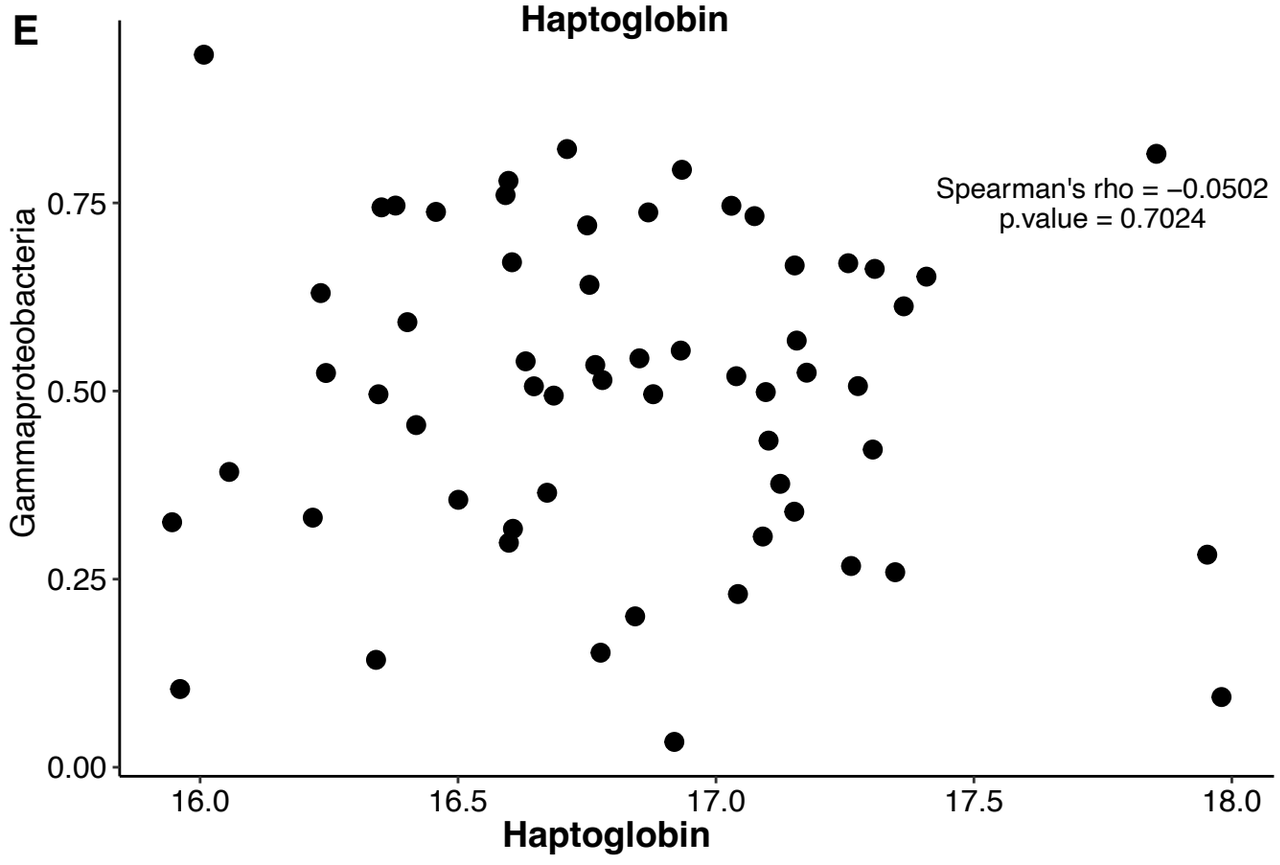

E
Spearman's rho = −0.0502
p.value = 0.7024
Gammaproteobacteria
Haptoglobin

Fig. S15

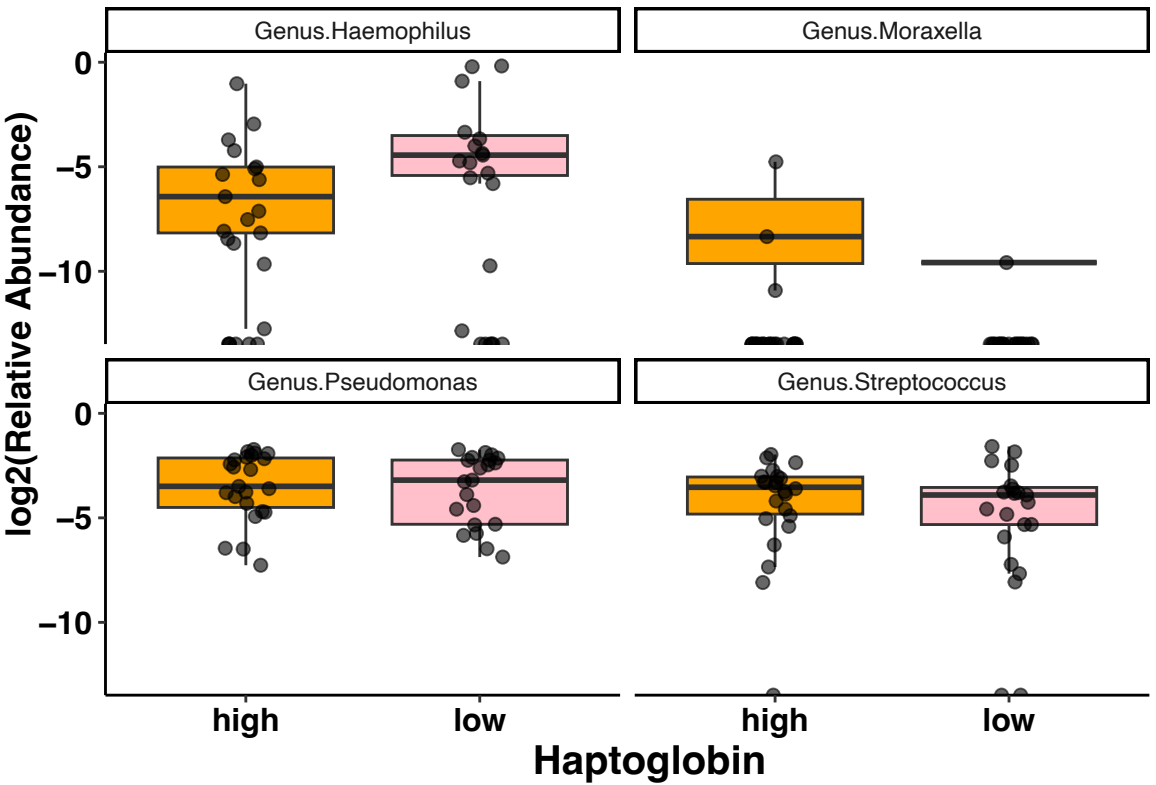

Genus.Haemophilus
Genus.Moraxella
Genus.Pseudomonas
Genus.Streptococcus
log2(Relative Abundance)
0
−5
−10
high
low
Haptoglobin

Table S1: Demographics and clinical table

Group	**HV-ES**	**HV-NS**	**P-FE**	**P-IE**	*p-value*
Subjects, n	20	15	13	17	
Male/female, n	11/9	9/6	12/1	13/4	0.0998*
Age, median	67.5	63	72	70	0.0153
Smoking Pack Years, median	25	0.16	29	51	0.0000
BMI (kg/m2), median	27.69	27.95	30.71	27.81	0.7988
POST FEV1 %, median	100.5	103	71	73	0.0000
POST FEV1/FVC, median	77.5	80	58	57	0.0000
HRCT LAA%, median	5.855	4.95	9.12	14.46	0.0001
HRCT E/I MLD, median	0.8	0.8	0.84	0.88	0.0002
ICS users, n	0	0	12	7	

* *p-value by Fisher's exact test. All others by Kruskal-Wallis test*

Table S2: Summary of medications

Medication	HV.ES (n)	HV.ES (%)	HV.NS (n)	HV.NS (%)	P.FE (n)	P.FE (%)	P.IE (n)	P.IE (%)
5 alpha-reductase inhibitor					1	1.89		
ace inhibitor	3	5.66	3	5.66	3	5.66	1	1.89
alpha 1-adrenergic blocker			2	3.77	2	3.77	1	1.89
anti-platelet					2	3.77		
anticholinergic, antimuscarinic	1	1.89			12	22.64	10	18.87
antihistaminic	2	3.77			3	5.66	1	1.89
antimitotic alkaloid	1	1.89					1	1.89
beta-2-agonist	1	1.89			11	20.75	12	22.64
beta-blocker					2	3.77		
biguanide antidiabetic			1	1.89	3	5.66	2	3.77
calcium channel blocker	2	3.77	3	5.66	2	3.77	1	1.89
calcium, vitamin			1	1.89				
carbonic anhydrase inhibitor							1	1.89
cholesterol absorption inhibitor					1	1.89		
corticosteroid	1	1.89					2	3.77
corticosteroid, beta-2-agonist					12	22.64	8	15.09
corticosteroid, vitamine d derivative			1	1.89				
dietary supplement	2	3.77					1	1.89
dipeptidyl peptidase-4 (dpp-4) inhibitor¬†			1	1.89				
estrogen steroid hormone	1	1.89						
globular tetradecapeptide; agonist of cell surface receptor of guanylate cyclase 2c (gc-c)					1	1.89		
glucocorticoid							1	1.89
hmg-coa reductase inhibitor	5	9.43	1	1.89	6	11.32	4	7.55

hormone replacement therapy	2	3.77	1	1.89				
hydroxy acids			1	1.89				
laxative	1	1.89			2	3.77		
lipase					1	1.89		
loop diuretic	1	1.89			1	1.89		
mast cell stabilizer							1	1.89
mucolytic					2	3.77		
neurotoxin							1	1.89
nitrate					2	3.77		
non-steroidal anti-inflammatory	2	3.77	1	1.89	4	7.55	1	1.89
noradrenergic and specific serotonergic antidepressant	1	1.89					1	1.89
occlusive emollient			1	1.89				
opioid pain killer			1	1.89	1	1.89	1	1.89
peripheral opioid receptor agonist (anti-diarrhea)	1	1.89						
peripheral pain killer							1	1.89
peripheral pain killer, opioid pain killer	1	1.89			1	1.89	2	3.77
phenothiazine (neuroleptic)					1	1.89		
proton-pump inhibitor	7	13.21	1	1.89	4	7.55	5	9.43
ras (rening angiotensin system) inhibitor/angiotensin receptor blocker	2	3.77			2	3.77	2	3.77
selective¬†agonist¬†at the¬†prostaglandin f receptor							1	1.89
selective¬†prostaglandin f receptor agonist	1	1.89						
selective serotonin reuptake inhibitor¬†SSRI antidepressant	2	3.77	1	1.89	1	1.89	2	3.77
serotonin-norepinephrine reuptake inhibitor¬†(SNRI)			1	1.89				

sulfonylurea			1	1.89				
terpene	1	1.89						
thiazide diuretic					2	3.77	2	3.77
thioxanthene antipsychotic							1	1.89
thyroid hormone	6	11.32					1	1.89
tricyclic antidepressant	2	3.77	1	1.89			3	5.66
vitamin	1	1.89					1	1.89
vitamin a, vitamin d	1	1.89	1	1.89			1	1.89
vitamin d derivative			1	1.89				
xanthine					1	1.89		
xanthine oxidase enzyme inhibitor	1	1.89					1	1.89

Table S3: Distribution of microbiome samples by Group and Gender (for 16S analysis)

Sample type: BAL					
Group		**HV-NS**	**HV-ES**	**P-IE**	**P-FE**
Gender	**F**	12	18	8	2
	M	18	22	26	24
Sample type: Sputum					
Group		**HV-NS**	**HV-ES**	**P-IE**	**P-FE**
Gender	**F**	2	2	3	0
	M	3	3	8	10

Supplemental figure legends

Fig S1: Sample availability and analyses flowchart.

Fig. S2: Schema for patient groups and biospecimen collection

Fig S3: Comparison of fungal diversity by sample type. A) Comparison of alpha-diversity (measured using the Inverse Simpson index) in sputum (n=33) versus BALF (n=120 samples; 60 subjects). *p*-value by Wilcoxon ranksum test. **B)** Ordination of beta-diversity Bray-Curtis distances by principal coordinate analysis (*p*=0.019). *p*-value by ANOSIM 999 permutations.

Fig S4: Comparison of bacterial diversity by sample type in subjects who provided both sample types. A) Alpha-diversity (measured using the Inverse Simpson index) in sputum (n=27) versus BALF (n=54 samples; 27 subjects). *p*-value by Wilcoxon ranksum test. **B)** Ordination of beta-diversity Bray-Curtis distances by principal coordinate analysis (*p*=0.001). *p*-value by ANOSIM 999 permutations.

Fig S5: Comparison of bacterial diversity in BALF by lobe of lung and patient subgroup. A) Comparison of alpha-diversity (measured using the Inverse Simpson index by A) patient subgroup (HV-NS: n=30 samples–15 patients; HV-ES: n=40 samples–20 patients; P-IE: n=34 samples–17 patients; P-FE: n=26 samples–13 patients) and **B)** lobe of lung (LLL n=30; LUL n=5; RLL n=30; RML n=52; RUL n=13). *P*-value by Kruskal Wallis test. Ordination of beta-diversity (Bray-curtis) distances by principal coordinate analysis by **C)** patient subgroup and D) lobe of lung. *P*-value by ANOSIM 999 permutations. *LLL: Left lower lobe; LUL: Left upper lobe; RLL: Right lower lobe; RRML: Right middle lobe; RUL: Right upper lobe*.

Fig S6: Comparison of bacterial diversity in sputum. A) Comparison of alpha-diversity (measured using the Inverse Simpson index) in sputum by patient subgroup (HV-NS n=5; HV-ES n=5; P-IE n=9; P-FE n=8). *P*-value by Kruskal Wallis test. **B)** Ordination of beta-diversity distances (Bray-Curtis) by principal coordinate analysis (*p*=0.996). *p*-value by ANOSIM (999 permutations).

Fig S7: Characterization of fungal diversity in sputum samples. Stacked bar plot by patient subgroup at the **A)** order and **B)** phylum levels. (HV-ES: n=5; HV-NS: n=5; P-FE: n=8; P-IE: n=11)

Fig. S8: Differential abundance comparisons in BALF – 16S rRNA sequencing. LEfSe LDA score plot for *bacteria* by anti-NTHi killing activity in frequent (n=26 samples, 13 patients) vs infrequent exacerbators (n=34 samples, 17 patients). (LDA score minimum=2.0, p=0.05).

Fig. S9: Comparison of *Haemophilus* abundance in BAL between study groups . Box plot showing median, interquartile range, and range of *Haemophilus* abundance in study groups. Global *p*-value by Kruskal- Wallis test. Pairwise *p*-values are from the Wilcoxon ranksum test. (HV-ES: n=40 samples–20 patients; HV-NS: n=30 samples–15 patients; P-FE: n=26 samples–13 patients; P-IE: n=34 samples–17 patients)

Fig. S10: Differential abundance comparisons in BALF – ITS. LefSe LDA score plot for *fungi* in COPD patients (n=55 samples, 30 patients) versus healthy volunteers (n=64 samples, 35 patients). No differentially enriched fungi were identified in HV. (LDA score minimum=2.0, p=0.05).

Fig S11: Serum immunological response to *Moraxella catarrhalis* and *Streptococcus pneumoniae* antigens. Unsupervised hierarchical clustering heatmap of antibody titers to **A)** *M. catarrhalis* and **B)** *S. pneumoniae* antigens. (HV-ES: n=20; HV-NS: n=16; P-FE: n=13; P-IE: n=14)

Fig S12: Associations of anti-NTHi killing activity by patient subgroup. *P-value* by Fishers exact test

Fig S13: Random forest classification of proteomics by NTHi killing activity - 500 trees, 18 features per split, Out-of-bag error rate = 19.57%)

Fig S14: Association of Haptoglobin expression with **A) to D)** lung function parameters **E)** Gammaproteobacteria abundance

Fig S15: Distribution of relative abundances of candidate genera by haptoglobin categories: top left – Haemophilus (p=0.29), **top right** – Moraxella (p=0.35), **bottom left –** Pseudomonas (p=0.74), **bottom right** - Streptococcus (p=0.27).