Anaerobic bacterial fermentation products increase tuberculosis risk in antiretroviral treated HIV-patients

Supplementary Figures and Tables

Figure S1. Blood SCFA in HIV infected individuals on ART and normal blood donors. Related to Figure 1A. Shown are acetate (triangles), propionate (dots) and butyrate (squares) concentration from 193 HIV infected patients from Cape Town (red) and 50 normal blood donors (blue). Median and interquartile ranges shown by lines. HIV infected individuals have higher SCFA concentration that HIV uninfected blood donors ($p<0.001$ Mann Whitney U test). In HIV infected individuals, butyrate concentration is higher than propionate ($p<0.001$). Alternately, in HIV uninfected individuals, butyrate concentration is lower than propionate ($p<0.001$).

Figure S2. Comparison of lung, supraglotic and background microbiomes. Related to Figure 3. A) A principle component analysis demonstrates a significant difference in the β-diversity between propionate-undetectable BAL and background samples (PERMANOVA $p<0.01$). There is no significant difference in β-diversity between propionate-detectable BAL and supraglottic samples (PERMANOVA $p=0.17$). Individuals without SCFA measured (n.a.) are added to demonstrate the location of Pneumotypes with supraglottic predominate taxa and background predominate taxa. **B.** LEfSe demonstrates taxa enriched when propionate-detectable BAL samples are compared with supraglottic samples. The taxa with LDA >3.5 are shown in table S3 **C.** LEfSe demonstrates taxa enriched when propionate-undetectable BAL samples are

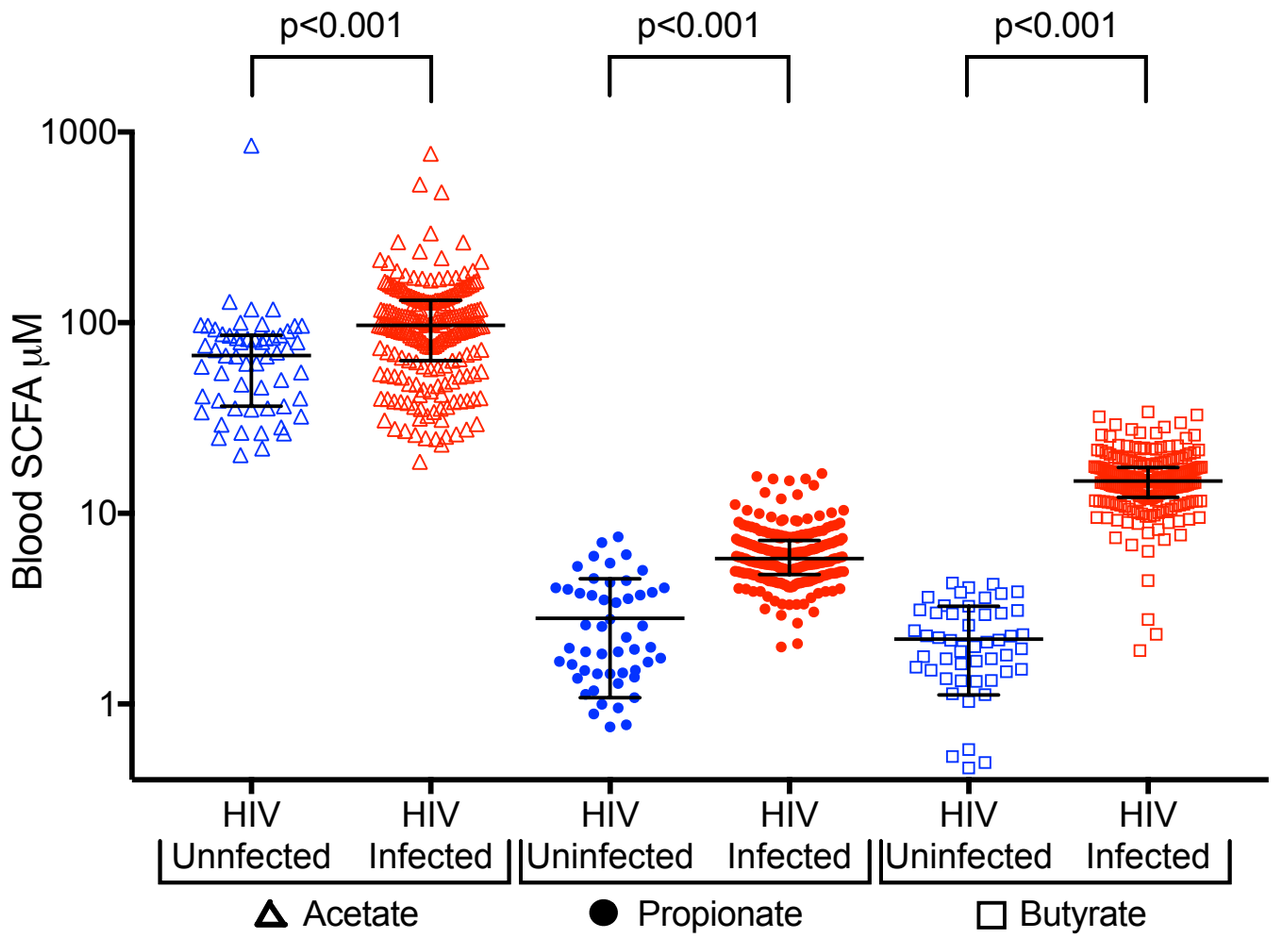
compared with background samples. The taxa with LDA >3.5 are shown in table S3.

Figure S3. The bacterial metagenome of propionate detectable individuals have reduced relative abundance of butyrate and propionate metabolic genes. Related to Figure 3. As many different taxa can produce SCFA, we characterized bacterial metabolic potential by estimating the entire complement of bacterial genes, i.e. the metagenome. We used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to infer the genomic potential of the lower airway microbiome using taxonomic data(Langille et al., 2013) from 16S rRNA gene sequences from 10 propionate-detectable and 29 propionate-undetectable individuals. **A.** Differences in Shannon α-diversity of inferred metagenome (PICRUSt) between BAL samples from propionate-detectable and propionate-undetectable individuals (p value based on Mann Whitney). **B.** Principal component analysis based on Bray Curtis dissimilarity index of inferred metagenome (PICRUSt) data demonstrates a significant difference in the β-diversity of microbiomes of propionate-detectable and propionate-undetectable individuals (p value based on PERMANOVA). **C.** Linear Discriminant Analysis (LDA) Effect Size (LEfSe) assessed for differences in KO annotations inferred based on taxonomic composition of BAL samples (PICRUSt) comparing propionate-detectable (red) and propionate-undetectable (green) individuals. Histogram demonstrates metabolic pathways with significant enrichment (LDA >2). Compared with propionate-detectable individuals, propionate-undetectable individuals demonstrated higher relative abundance of propionate anabolic and catabolic genes.

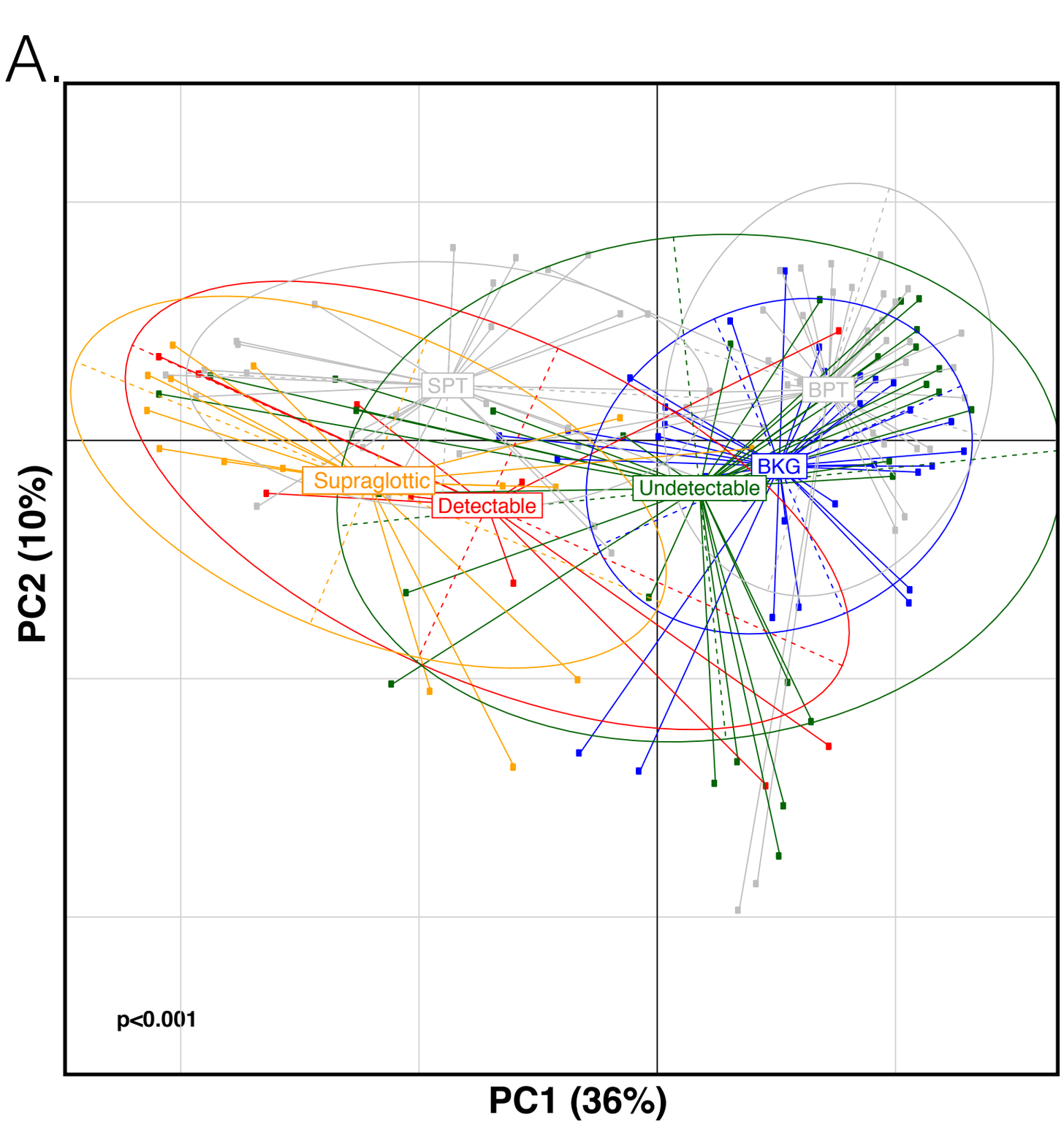
Figure S4. The bacterial shotgun metagenome of propionate-detectable and

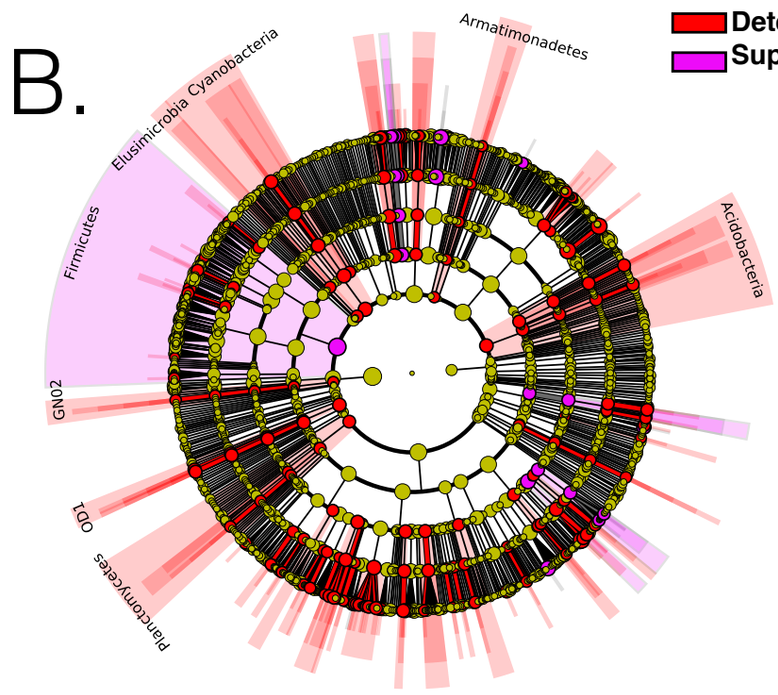
undetectable individuals. Related to Figure 3 To confirm the results of our analysis on PICRUSt-predicted metagenome, we performed shotgun metagenomic sequencing on 16 BAL samples, 8 from propionate-detectable individuals and 8 from propionate-undetectable individuals. **A.** Differences in Shannon α-diversity between the taxa in the microbiomes of 8 propionate detectable and 8 propionate undetectable individuals with shotgun sequencing of BAL to define the lower airway bacterial metagenome. Similar to PICRUSt results, there is a trend to reduced α-diversity (p=0.06) in the propionate detectable individuals when compared to the propionate undetectable individuals. **B**. Shotgun metagenome sequencing of BAL samples from 8 propionate-detectable and 8 propionate-undetectable individuals. Only propionate-undetectable individuals (green) had significant enrichment of metabolic pathways including propionate and butyrate assessed by LEfSe with LDA>2. The relative abundance of ferredoxin/flavodoxin oxidoreductase (PFOR, KEGG K03737) the major enzyme complex for SCFA synthesis, was similar in propionate-detectable and propionate-undetectable individuals (median [IQR] relative abundance %, 0.0082 [0.0022-0.034] vs. 0.012 [0.0063-0.053], Mann Whitney p=0.38).

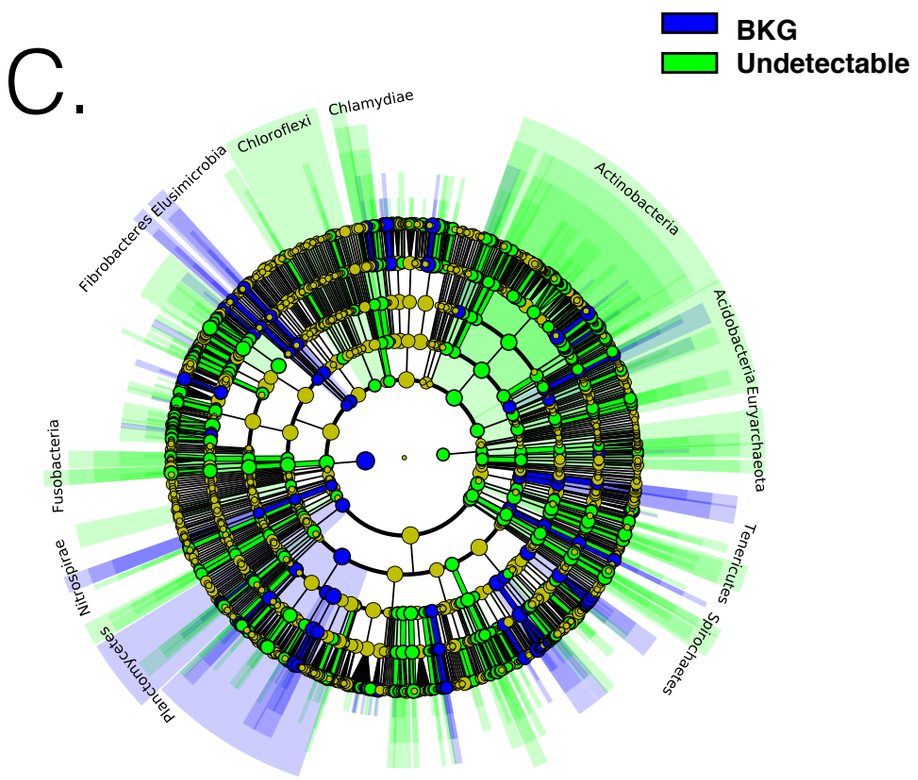
Supplementary Figure 1



Supplementary Figure 2

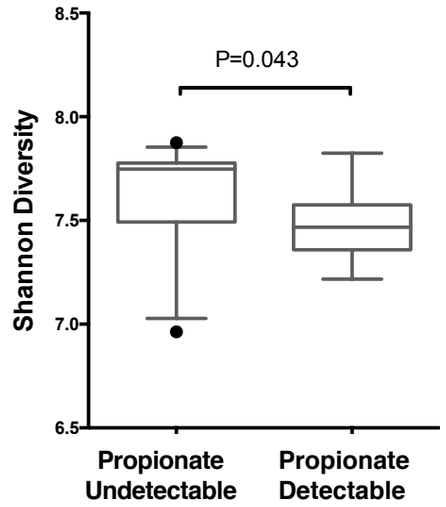




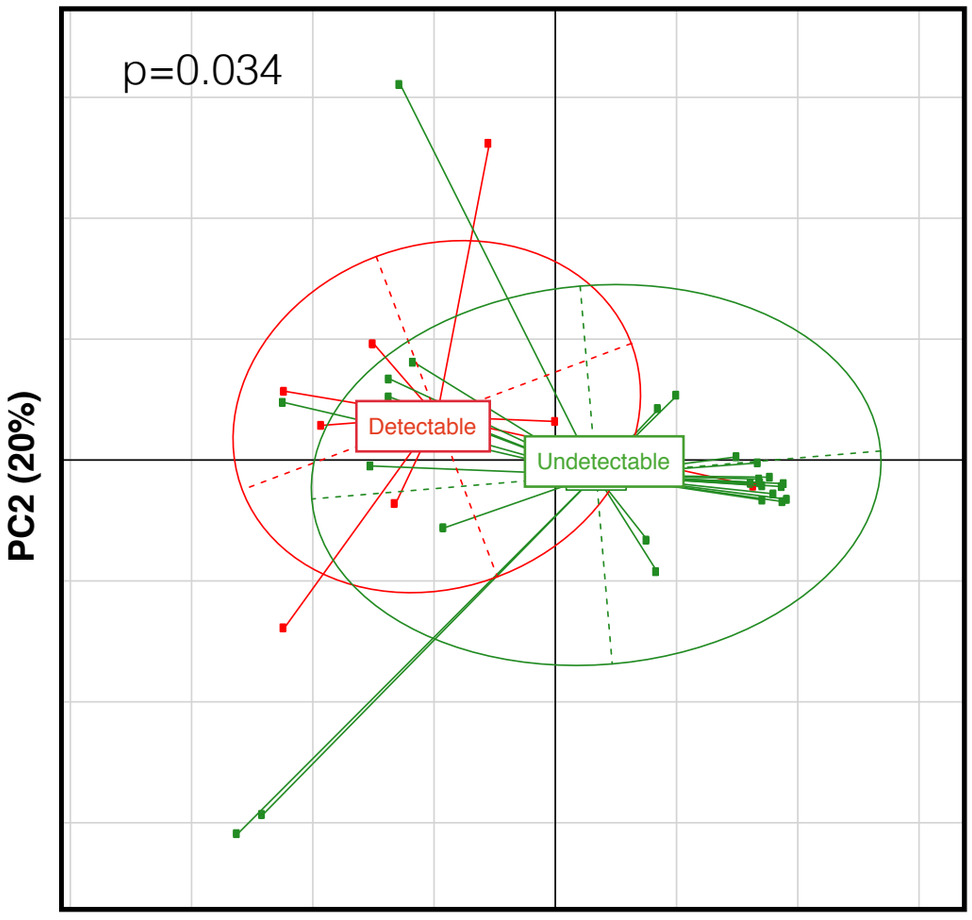


Supplementary Figure 3

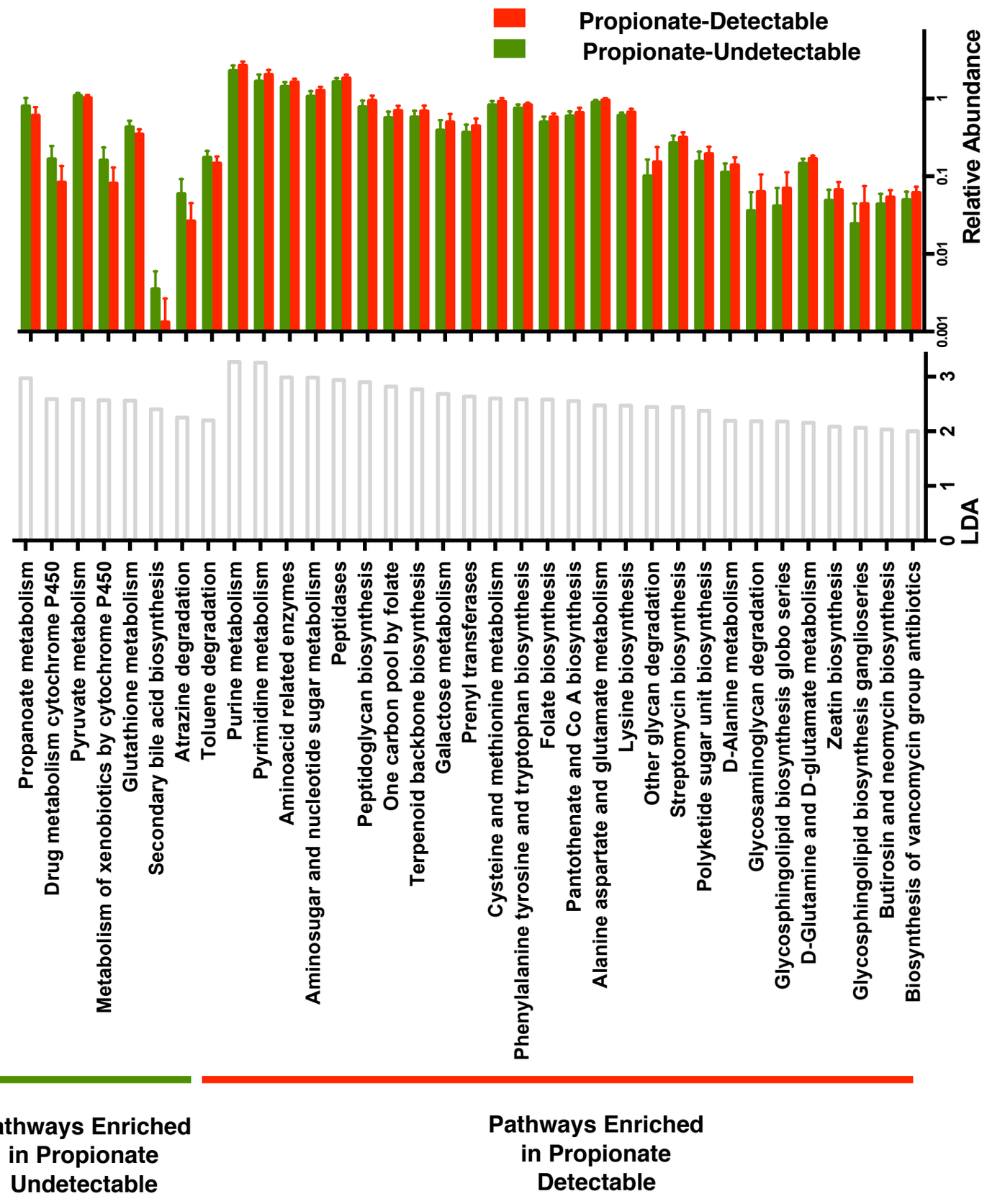
A.



B.

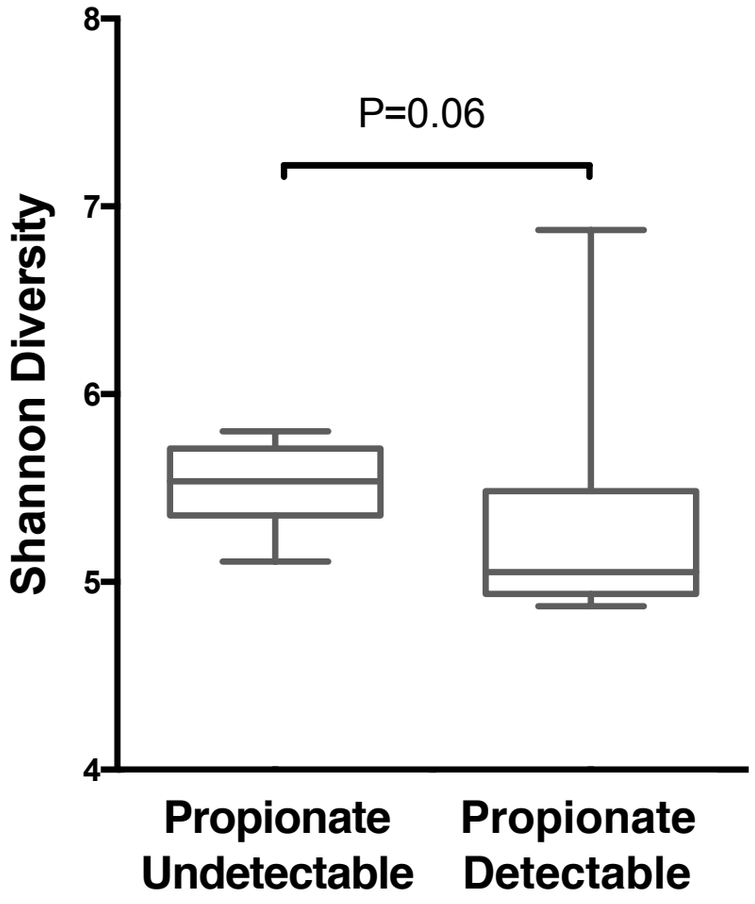


C.

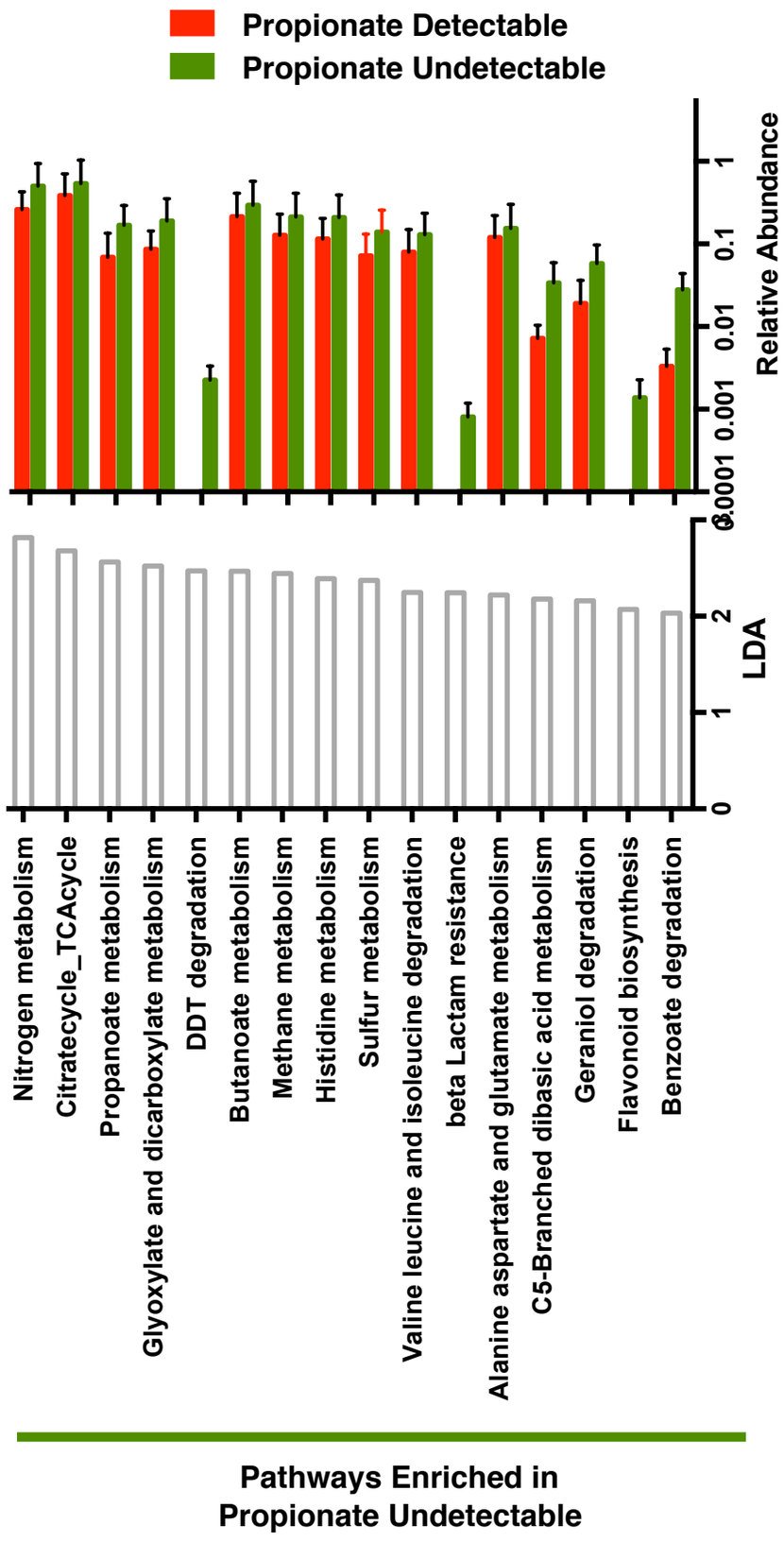


Supplementary Figure 4

A.



B. *Shotgun Metagenome*



Supplementary Tables

Table S1 related to Table 1: Demographic, Clinical Characteristics and Serum Analytes of Longitudinal Cohort , No-TB controls and TB cases

Variable	Cohort N=193	No-TB N=183	TB N=10	p-value
Age years ± SD	38±8.1	38.4±8.1	36.6±7.7	> 0.1 *
Female	56%	55%	70%	> 0.1 ¶
Ever Smoker	29%	31%	20%	> 0.1 ¶
BMI ± SD	25.7±5.6	25.7±5.7	25.8±3.9	> 0.1*
ART years± SD	2.89±1.94	2.92±1.94	2.44±2.02	> 0.1*
CD4+ Lymph./ml ± SD	383±203	389±202	271±218	> 0.1*
Prior TB	46%	46%	40%	> 0.1 ¶
Quantiferon positive	47%	46%	70%	> 0.1 ¶
Isoniazid Prophylaxis	15%	16%	10%	> 0.1 ¶
Vital Capacity % predicted	98.3±15.5	98.5±18	94±18	> 0.1 *
FEV1/VC ratio	82±7	82±7	82±6	> 0.1 *
Acetate mM Median (IQR)	97.3 (63.3-130.9)	97.4 (65.7-131.2)	72.7 (38.6-133.8)	> 0.1§
Propionate mM Median (IQR)	5.8 (4.8-7.2)	5.8 (4.8-7.2)	6.1 (4.9-7.7)	> 0.1§
Butyrate mM Median (IQR)	14.8 (12.3-17.4)	14.7 (12.1-17.4)	16.4 (15.2-19.8)	0.063§
IFN-g pg/ml Median (IQR)	10 (6.9-12.1)	10 (7-12.1)	6.1 (5.4-10.0)	0.031§
IL-17A pg/ml Median (IQR)	3.5 (2.2-6.1)	3.6 (2.2-6.1)	1.6 (0.65-4.7)	0.056§

*t test; ¶ Chi Squared; § Mann-Whitney

Table S2 related to Figure 3: Demographic and Clinical Characteristics of Bronchoscopy Cohort

	HIV Negative		HIV Positive			
Variable	**Propionate negative N=12**	**Propionate n.a N=8**	**Propionate negative N=17**	**Propionate positive N=10**	**Propionate n.a N=35**	**p-value**
Age years	51±17	55±11	42±8	44±8	42±9	ns
Female (%)	42%	17%	47%	30%	19%	ns
Ever smoker	66%	74%	53%	60%	85%	ns
CD4+ Lymphocytes/ml	n.a	n.a.	353±260	374±261	466±227	ns
BMI kg/m2	28±4	27±5	27±5	25±9	28±6	ns
FVC % predicted	102±13	104±18	97±19	98±18	96±14	ns
FEV1/VC ratio	73±11	72±5	79±9	75±7	76±11	ns
Epithelial Lining Fluid dilution	70±68	n.a.	77±56	77±56	n.a.	ns
Log10 16S rDNA genes/ml BAL	5.0±0.53	n.a.	5.2±0.38	5.3±0.44	n.a.	ns

*All values are mean± SD unless otherwise stated, *p by ANOVA

Table S3 related to Figure 3: Taxa enriched with LDA of 3.5 or greater

Background vs. Propionate Undetectable	Background		Undetectable		
	Mean	SD	Mean	SD	LDA
Enriched in Propionate Undetectable					
Prevotella	0.005	0.017	0.0576	0.0967	4.43
Actinomycetales(u.g.)	0.003	0.0108	0.0371	0.1422	4.28
Psychrobacter	0.0032	0.0095	0.0427	0.1316	4.26
Treponema	0	0	0.0134	0.0406	3.85
Porphyromonas	0.0006	0.0025	0.0129	0.0427	3.79
Leptotrichia	0	0	0.0094	0.0322	3.73
Brevibacterium	0	0	0.008	0.0397	3.66
Staphylococcus	0.0161	0.0436	0.0224	0.0325	3.62
Moraxella	0	0	0.0065	0.0326	3.51
Micrococcaceae(u.g.)	0.0018	0.006	0.0085	0.0179	3.5
Enriched in background					
S24_7(u.g.)	0.0572	0.0981	0.0006	0.0021	4.46
Akkermansia	0.0467	0.0858	0.0002	0.0012	4.41
Enterobacteriaceae(u.g.)	0.0411	0.0747	0.0016	0.0024	4.37
Acidocella	0.0754	0.0583	0.0355	0.0406	4.31
Ralstonia	0.0116	0.0321	0.0002	0.001	3.76
Ignatzschineria	0.008	0.0289	0	0	3.6
Corynebacterium	0.0201	0.0534	0.0193	0.031	3.56

Supraglottic vs Propionate Detectable	Supraglottic		Detectable		
	Mean	SD	Mean	SD	LDA
Enriched in Propionate Detectable					
Corynebacterium	0.0058	0.0154	0.0775	0.1993	4.54
Haemophilus	0.0207	0.0307	0.0909	0.147	4.48
Comamonadaceae(u.g.)	0.0006	0.0008	0.0065	0.01	3.54
Enriched in Supraglottic					
S24_7(u.g.)	0.0393	0.0958	0.0002	0.0008	4.29
Enterobacteriaceae(u.g.)	0.0229	0.0669	0.0003	0.001	4.05
Akkermansia	0.0105	0.0376	0	0	3.69