Salivary Microbial Dysbiosis Is Associated with Systemic Inflammatory Markers and Predicted Oral Metabolites in Non-Small Cell Lung Cancer Patients

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Figure Legends

Figure S1. The rarefaction curve for each sample. For each sample, the number of observed species (Y-axis) were plotted against its number of sequences (X-axis). For each curve, reaching plateau indicates the adequacy of sequencing for this sample. LC represent non-small cell lung cancer group, and HC, healthy control.

Figure S2. Alpha diversity of saliva samples. The ACE (a), Chao1 (b), and Shannon (c) diversity indices in lung cancer patients were not statistically significant different to that in healthy subjects, while the Simpson index was significantly decreased in lung cancer group. Error bars represent standard deviation. NSCLC represent non-small cell lung cancer group, and HC, healthy control.

Figure S3. The non-metric multidimensional scaling (NMDS) plots of the samples in the two groups. NSCLC represent Lung cancer group, and HC, Healthy controls.

Figure S4. Taxonomic composition of saliva microbiota in lung cancer patients and healthy controls. Relative abundances bar plot showing the relative abundance of bacterial phyla (a) and genus (b) in each sample. NSCLC represent Lung cancer group, and HC, Healthy controls.

Figure S1.

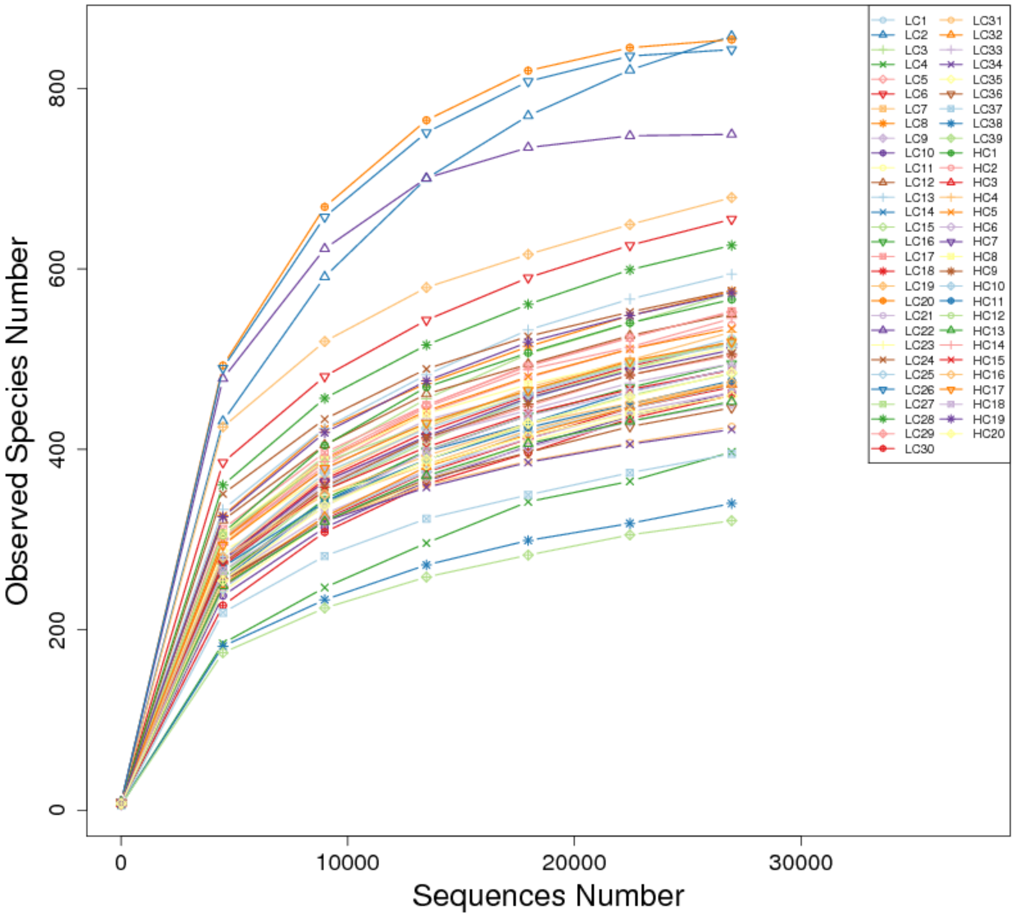
Observed Species Number
0
200
400
600
800
Sequences Number
0
10000
20000
30000
LC1
LC2
LC3
LC4
LC5
LC6
LC7
LC8
LC9
LC10
LC11
LC12
LC13
LC14
LC15
LC16
LC17
LC18
LC19
LC20
LC21
LC22
LC23
LC24
LC25
LC26
LC27
LC28
LC29
LC30
LC31
LC32
LC33
LC34
LC35
LC36
LC37
LC38
LC39
HC1
HC2
HC3
HC4
HC5
HC6
HC7
HC8
HC9
HC10
HC11
HC12
HC13
HC14
HC15
HC16
HC17
HC18
HC19
HC20

Figure S2.

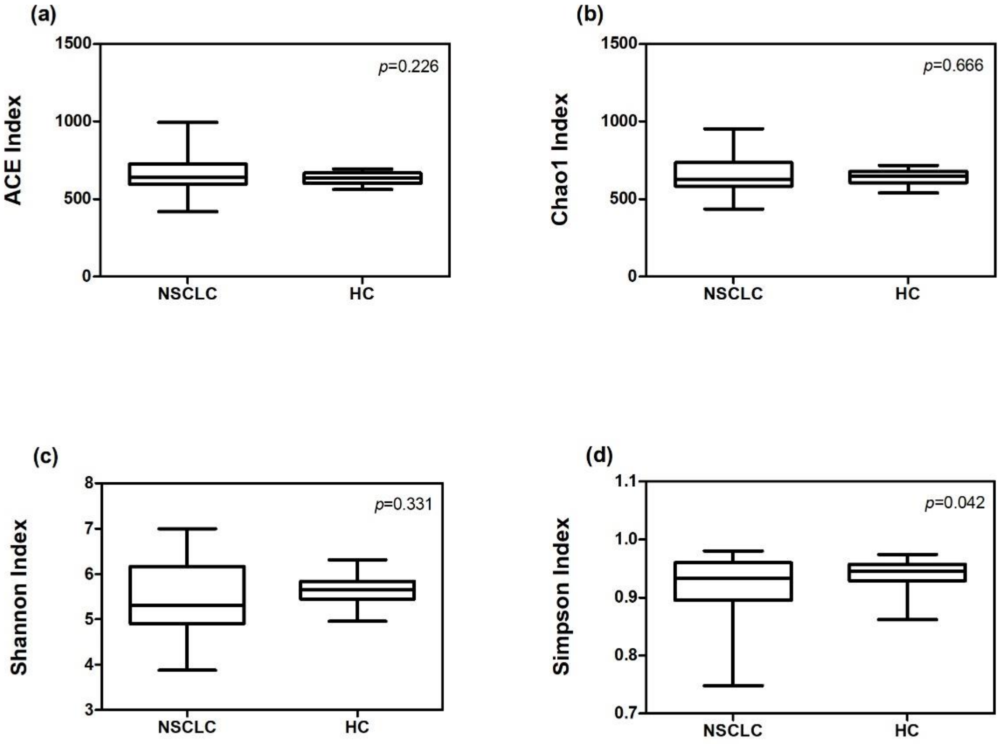
(a)
ACE Index
1500
1000
500
0
p=0.226
NSCLC
HC
(b)
Chao1 Index
1500
1000
500
0
p=0.666
NSCLC
HC
(c)
Shannon Index
8
7
6
5
4
3
p=0.331
NSCLC
HC
(d)
Simpson Index
1.1
1.0
0.9
0.8
0.7
p=0.042
NSCLC
HC

Figure S3.

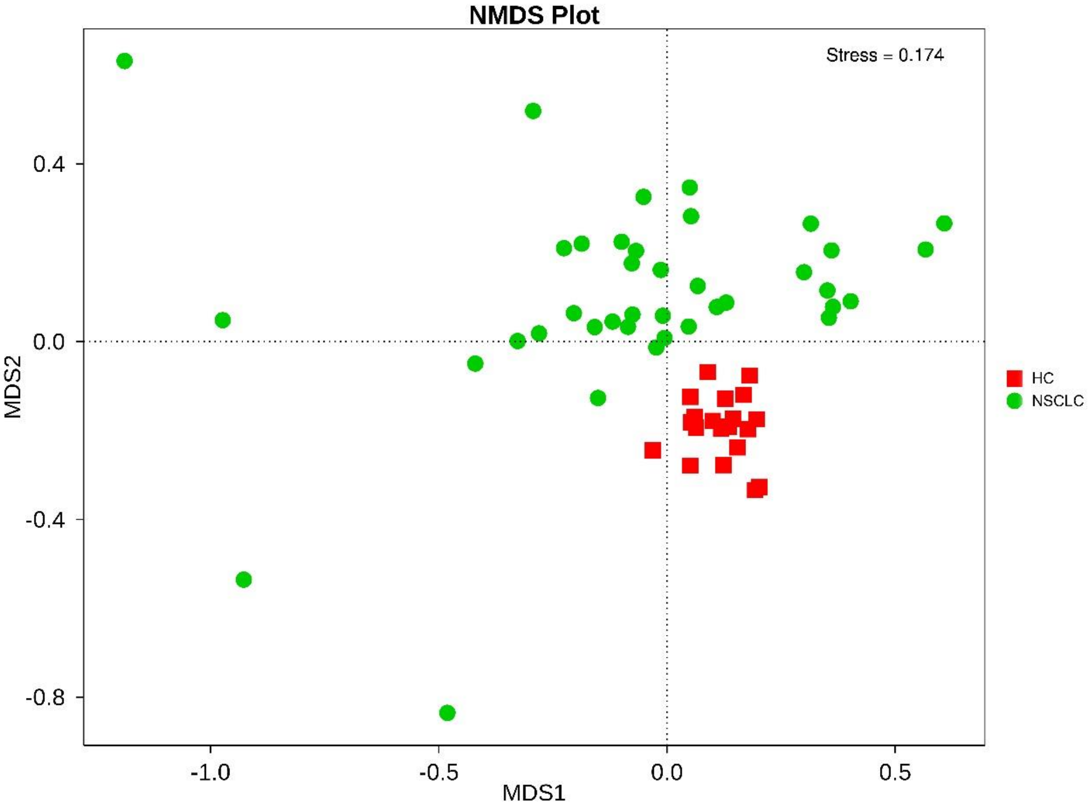

Figure S4.

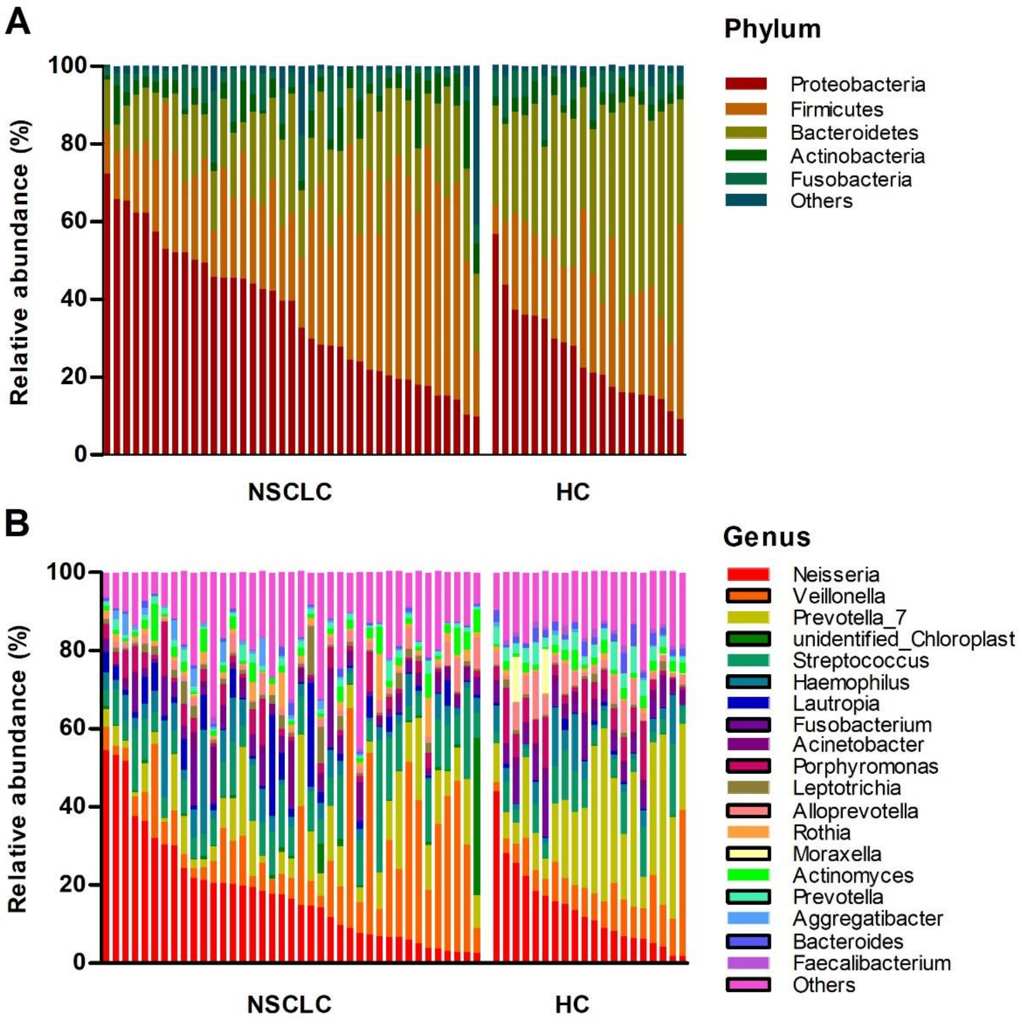
A
Relative abundance (%)
100
80
60
40
20
0
NSCLC
HC
Phylum
Proteobacteria
Firmicutes
Bacteroidetes
Actinobacteria
Fusobacteria
Others
B
Relative abundance (%)
100
80
60
40
20
0
NSCLC
HC
Genus
Neisseria
Veillonella
Prevotella_7
unidentified_Chloroplast
Streptococcus
Haemophilus
Lautropia
Fusobacterium
Acinetobacter
Porphyromonas
Leptotrichia
Alloprevotella
Rothia
Moraxella
Actinomyces
Prevotella
Aggregatibacter
Bacteroides
Faecalibacterium
Others

Table S1. Summary of the significant correlations between bacteria. Spearman correlation coefficients (r) with *p* <0.001.

From	To	r	*p*
Veillonella	Neisseria	-0.638106371	5.44E-08
Veillonella	Megasphaera	0.69261008	1.22E-09
Fusobacterium	Porphyromonas	0.633853033	7.09E-08
Fusobacterium	unidentified_SR1_(Absconditabacteria)	0.666467268	8.32E-09
Acinetobacter	Brevundimonas	0.627404553	1.05E-07
Acinetobacter	Pseudomonas	0.630941944	8.48E-08
Acinetobacter	Massilia	0.635805756	6.28E-08
Acinetobacter	Chryseobacterium	0.831226791	4.44E-16
Acinetobacter	Acidovorax	0.612772533	2.49E-07
Brevundimonas	Pseudomonas	0.822261856	1.33E-15
Brevundimonas	Stenotrophomonas	0.830019276	4.44E-16
Brevundimonas	Chryseobacterium	0.719121748	1.40E-10
Brevundimonas	Acidovorax	0.754043237	5.42E-12
Brevundimonas	Blastomonas	0.684345923	2.29E-09
Bacteroides	Faecalibacterium	0.702438041	5.63E-10
Pseudomonas	Brevundimonas	0.822261856	1.33E-15
Pseudomonas	Empedobacter	0.637184392	5.76E-08
Pseudomonas	Stenotrophomonas	0.838170062	0
Pseudomonas	Massilia	0.678868735	3.43E-09
Pseudomonas	Chryseobacterium	0.677808724	3.71E-09
Pseudomonas	Acidovorax	0.697057077	8.64E-10
Pseudomonas	Blastomonas	0.828967216	4.44E-16
Empedobacter	Blastomonas	0.610430134	2.85E-07
Stenotrophomonas	Chryseobacterium	0.755335826	4.75E-12
Stenotrophomonas	Acidovorax	0.834582816	2.22E-16
Stenotrophomonas	Blastomonas	0.735646151	3.21E-11
Massilia	Chryseobacterium	0.642078177	4.23E-08
Chryseobacterium	Acidovorax	0.739625846	2.21E-11
Acidovorax	Blastomonas	0.679343191	3.31E-09

Table S2. Summary of the significant correlations between systemic inflammatory markers and bacteria. Spearman correlation coefficients (r) with p <0.05

From	To	r	*p*
NLR	Veillonella	0.34951	0.006661
NLR	unidentified_Chloroplast	0.311075	0.01648
NLR	Fusobacterium	-0.40782	0.001345
NLR	unidentified_SR1_(Absconditabacteria)	-0.31121	0.016432
PLR	Pseudomonas	0.306381	0.018271
PLR	Megasphaera	0.331667	0.010283
LMR	unidentified_Chloroplast	-0.37108	0.003811
LMR	Streptococcus	-0.33991	0.008439
LMR	Fusobacterium	0.392168	0.002127
LMR	Moraxella	0.325692	0.011829
LMR	Prevotella	0.325038	0.01201
LMR	Brevundimonas	-0.32309	0.012563
LMR	unidentified_SR1_(Absconditabacteria)	0.335816	0.009316
LMR	Blastomonas	-0.30329	0.019541
AMC	unidentified_Chloroplast	0.361266	0.004936
AMC	Lautropia	0.381515	0.002869
AMC	Abiotrophia	0.307516	0.017824
AMC	Moraxella	-0.31791	0.014141
AMC	Faecalibacterium	-0.32369	0.012389
IL-6	Acidovorax	0.326147	0.011704
IL-1β	Bacteroides	0.303192	0.019582

Table S3 Functional orthologues of gut microbiota enriched in different disease status

KEGG Level2	KEGG Level 3	Enriched class	*p*-value
Cell Growth and Death			
Cell Growth and Death	Cell_cycle___Caulobacter	HC	p<0.001
Cell Motility			
Cell Motility	Bacterial_chemotaxis	LC	0.016
Cell Motility	Bacterial_motility_proteins	LC	p<0.001
Cell Motility	Cytoskeleton_proteins	HC	0.001
Cell Motility	Flagellar_assembly	LC	p<0.001
Membrane Transport			
Membrane Transport	ABC_transporters	LC	p<0.001
Membrane Transport	Bacterial_secretion_system	LC	p<0.001
Membrane Transport	Secretion_system	LC	p<0.001
Membrane Transport	Transporters	LC	0.003
Signal Transduction			
Signal Transduction	Two_component_system	LC	p<0.001
Signaling Molecules and Interaction			
Signaling Molecules and Interaction	Bacterial_toxins	HC	0.009
Sorting and Degradation			
Sorting and Degradation	Chaperones_and_folding_catalysts	HC	p<0.001
Sorting and Degradation	RNA_degradation	HC	p<0.001
Sorting and Degradation	Sulfur_relay_system	LC	p<0.001
Replication and Repair			
Replication and Repair	Chromosome	HC	p<0.001
Replication and Repair	DNA_repair_and_recombination_proteins	HC	p<0.001
Replication and Repair	DNA_replication	HC	p<0.001
Replication and Repair	DNA_replication_proteins	HC	p<0.001
Replication and Repair	Homologous_recombination	HC	p<0.001
Replication and Repair	Mismatch_repair	HC	p<0.001
Replication and Repair	Nucleotide_excision_repair	HC	p<0.001
Transcription			
Transcription	Transcription_factors	LC	0.007
Transcription	Transcription_machinery	HC	p<0.001
Translation			
Translation	Aminoacyl_tRNA_biosynthesis	HC	0.001
Translation	Ribosome	HC	p<0.001
Translation	Ribosome_Biogenesis	HC	0.033
Translation	Translation_factors	HC	p<0.001

Infectious Diseases			
Infectious Diseases	Tuberculosis	HC	0.003
Neurodegenerative Diseases			
Neurodegenerative Diseases	Huntingtons_disease	LC	p<0.001
Amino Acid Metabolism			
Amino Acid Metabolism	Alanine,_aspartate_and_glutamate_metabolism	HC	p<0.001
Amino Acid Metabolism	Amino_acid_related_enzymes	HC	p<0.001
Amino Acid Metabolism	Arginine_and_proline_metabolism	LC	p<0.001
Amino Acid Metabolism	Cysteine_and_methionine_metabolism	LC	0.007
Amino Acid Metabolism	Glycine,_serine_and_threonine_metabolism	HC	p<0.001
Amino Acid Metabolism	Lysine_degradation	LC	0.008
Amino Acid Metabolism	Phenylalanine_metabolism	LC	p<0.001
Amino Acid Metabolism	Tryptophan_metabolism	LC	p<0.001
Amino Acid Metabolism	Valine,_leucine_and_isoleucine_biosynthesis	LC	p<0.001
Amino Acid Metabolism	Valine,_leucine_and_isoleucine_degradation	LC	0.032
Biosynthesis of Other Secondary Metabolites			
Biosynthesis of Other Secondary Metabolites	Streptomycin_biosynthesis	HC	0.009
Carbohydrate Metabolism			
Carbohydrate Metabolism	Amino_sugar_and_nucleotide_sugar_metabolism	HC	p<0.001
Carbohydrate Metabolism	Butanoate_metabolism	LC	0.012
Carbohydrate Metabolism	C5_Branched_dibasic_acid_metabolism	LC	p<0.001
Carbohydrate Metabolism	Citrate_cycle_(TCA_cycle)	HC	0.006
Carbohydrate Metabolism	Fructose_and_mannose_metabolism	HC	p<0.001
Carbohydrate Metabolism	Galactose_metabolism	HC	0.008
Carbohydrate Metabolism	Glyoxylate_and_dicarboxylate_metabolism	LC	p<0.001
Carbohydrate Metabolism	Propanoate_metabolism	LC	p<0.001
Carbohydrate Metabolism	Pyruvate_metabolism	LC	0.007
Carbohydrate Metabolism	Starch_and_sucrose_metabolism	HC	p<0.001
Energy Metabolism			

Energy Metabolism	Carbon_fixation_in_photosynthetic_organisms	HC	p<0.001
Energy Metabolism	Carbon_fixation_pathways_in_prokaryotes	HC	p<0.001
Energy Metabolism	Methane_metabolism	HC	p<0.001
Energy Metabolism	Sulfur_metabolism	LC	p<0.001
Enzyme Families			
Enzyme Families	Peptidases	HC	p<0.001
Enzyme Families	Protein_kinases	LC	p<0.001
Glycan Biosynthesis and Metabolism			
Glycan Biosynthesis and Metabolism	Glycosyltransferases	HC	0.030
Glycan Biosynthesis and Metabolism	Other_glycan_degradation	HC	p<0.001
Glycan Biosynthesis and Metabolism	Peptidoglycan_biosynthesis	HC	p<0.001
Lipid Metabolism			
Lipid Metabolism	Biosynthesis_of_unsaturated_fatty_acids	LC	0.010
Lipid Metabolism	Fatty_acid_metabolism	LC	0.015
Lipid Metabolism	Glycerolipid_metabolism	LC	p<0.001
Lipid Metabolism	Sphingolipid_metabolism	HC	p<0.001
Metabolism of Cofactors and Vitamins			
Metabolism of Cofactors and Vitamins	**Folate_biosynthesis**	**HC**	**0.002**
Metabolism of Cofactors and Vitamins	Nicotinate_and_nicotinamide_metabolism	HC	p<0.001
Metabolism of Cofactors and Vitamins	**One_carbon_pool_by_folate**	**HC**	**p<0.001**
Metabolism of Cofactors and Vitamins	Porphyrin_and_chlorophyll_metabolism	LC	0.006
Metabolism of Cofactors and Vitamins	Riboflavin_metabolism	HC	p<0.001
Metabolism of Cofactors and Vitamins	Vitamin_B6_metabolism	HC	p<0.001
Metabolism of Other Amino Acids			
Metabolism of Other Amino Acids	D_Glutamine_and_D_glutamate_metabolism	HC	p<0.001
Metabolism of Other Amino Acids	Glutathione_metabolism	LC	p<0.001

Metabolism of Terpenoids and Polyketides			
Metabolism of Terpenoids and Polyketides	Polyketide_sugar_unit_biosynthesis	HC	p<0.001
Metabolism of Terpenoids and Polyketides	Prenyltransferases	HC	p<0.001
Metabolism of Terpenoids and Polyketides	Terpenoid_backbone_biosynthesis	HC	p<0.001
Metabolism of Terpenoids and Polyketides	Tetracycline_biosynthesis	LC	0.007
Nucleotide Metabolism			
Nucleotide Metabolism	Purine_metabolism	HC	p<0.001
Nucleotide Metabolism	Pyrimidine_metabolism	HC	p<0.001
Xenobiotics Biodegradation and Metabolism			
Xenobiotics Biodegradation and Metabolism	Aminobenzoate_degradation	LC	0.038
Xenobiotics Biodegradation and Metabolism	Benzoate_degradation	LC	0.043
Xenobiotics Biodegradation and Metabolism	Chloroalkane_and_chloroalkene_degradation	LC	p<0.001
Xenobiotics Biodegradation and Metabolism	Drug_metabolism___cytochrome_P450	LC	p<0.001
Xenobiotics Biodegradation and Metabolism	Drug_metabolism___other_enzymes	HC	p<0.001
Xenobiotics Biodegradation and Metabolism	Metabolism_of_xenobiotics_by_cytochrome_P450	LC	p<0.001
Cellular Processes and Signaling			
Cellular Processes and Signaling	Cell_motility_and_secretion	LC	0.026
Cellular Processes and Signaling	Inorganic_ion_transport_and_metabolism	LC	0.035
Cellular Processes and Signaling	Signal_transduction_mechanisms	LC	p<0.001
Genetic Information Processing	Restriction_enzyme	HC	p<0.001
Genetic Information Processing	Translation_proteins	HC	0.027
Metabolism			
Metabolism	Energy_metabolism	HC	p<0.001
Metabolism	Metabolism_of_cofactors_and_vitamins	LC	p<0.001
Metabolism	Others	LC	0.026
Poorly Characterized			

Poorly Characterized	Function_unknown	LC	0.001
Poorly Characterized	General_function_prediction_only	HC	0.013