

Figure S1 Intratumoural microbiome density with different clinical characteristics. (A,B) The α - and β -diversity analysis of tumor tissues categorized by STAS. (C,D) The α - and β -diversity analysis of tumor tissues categorized by VI. (E,F) The α - and β -diversity analysis of tumor tissues categorized by VPI. (G,H) The α - and β -diversity analysis of tumor tissue as categorized by imaging pathological feature. GGO, ground-glass opacity; Hnycmb, honeycomb; partSol, part-solid; STAS, spread through air spaces; VI, vascular invasion; VPI, visceral pleural invasion.

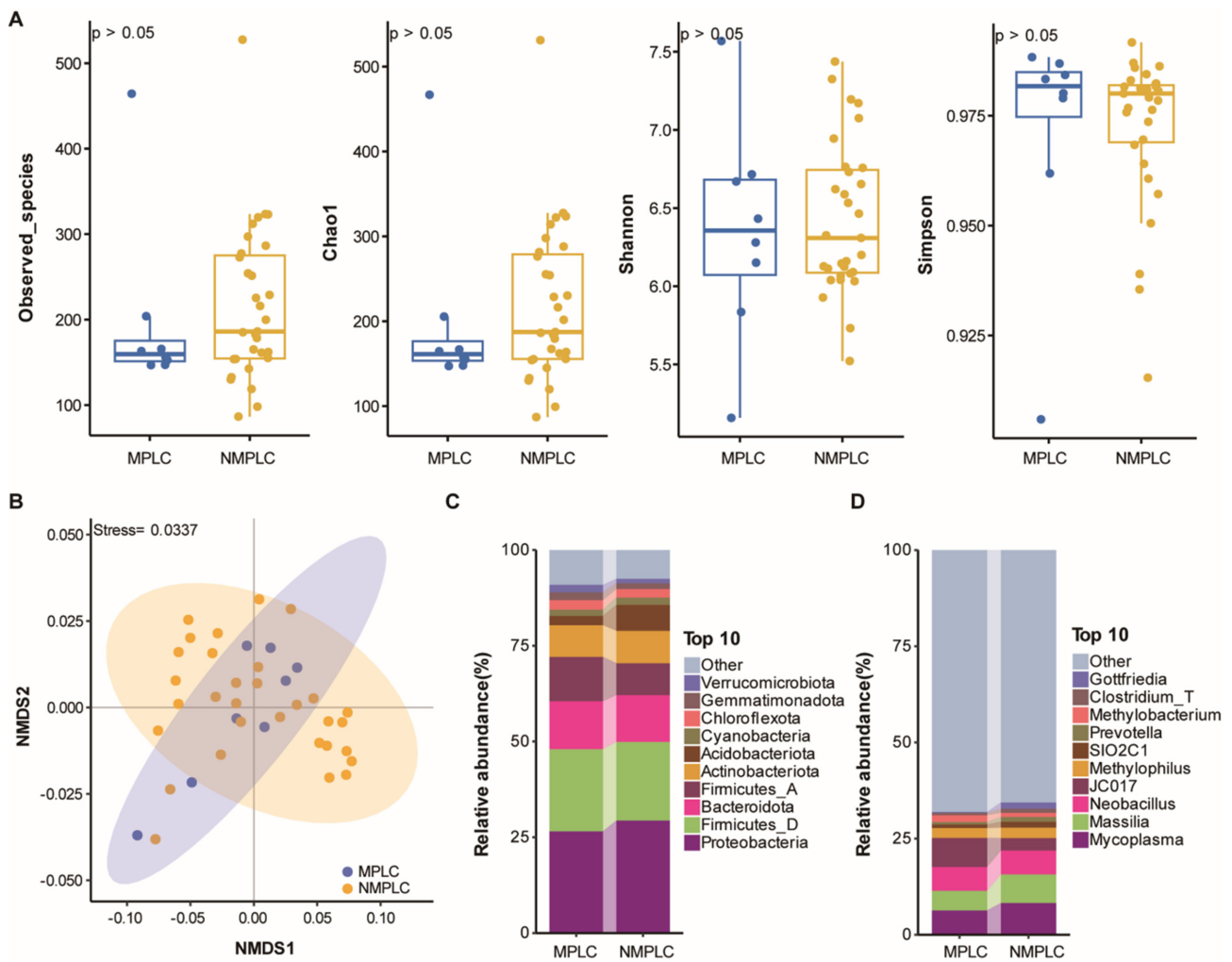


Figure S2 Microbial community differentiation from tumor-adjacent tissues between MPLC and NMPLC. (A) Alpha diversity of microbiome as indicated by observed species, Simpson, Chao1, and Shannon index. (B) NMDS analysis of microbiome in tumor adjacent tumor tissues using Bray-Curtis metric distances of beta diversity. (C) Bar plots of the phylum taxonomic levels in MPLC and NMPLC patients. Relative abundance is used. (D) Bar plots of the genus taxonomic levels in MPLC and NMPLC patients. Relative abundance is used. MPLC, multiple primary lung cancer; NMDS, non-metric multi-dimensional scaling; NMPLC, non-multiple primary lung cancer.

Table S1 Clinical characteristics of patients in 16S cohort

Clinical characteristics	MPLC (n=8)	NMPLC (n=31)	P value
Age (years)			0.418
>60	5	12	
≤60	3	19	
Gender			0.081
Female	7	14	
Male	1	17	
Smoking history			0.257
Yes	1	13	
No	7	18	
Tumor diameters (cm)	2.33±1.12	2.52±1.36	0.257
Differentiation level			>0.99
Low	3	12	
Moderate	5	19	
Pathology			0.563
LUAD	8	27	
LUSC	0	2	
Others	0	2	
TNM stage			0.542
I	6	18	
II	1	10	
III–IV	1	3	

Data are presented as number or mean ± SD. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MPLC, multiple primary lung cancer; NMPLC, non-multiple primary lung cancer; SD, standard deviation; TNM, tumor-node-metastasis.