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# Investigating the role of intratumoral *Streptococcus mitis* in gastric cancer progression: insights into tumor microenvironment

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**Abstract**

Growing evidence implicates that intratumoral microbiota are closely linked to cancer progression; however, research on the role of these microbiota in the development of gastric cancer remains limited. Here, using 16 S rRNA sequencing, tumor tissue proteomics and serum cytokines analysis, we identified enrichment of specific microbial communities within tumors of gastric cancer patients, possibly affecting the tumor microenvironment by immune modulation, metabolic processes, and inflammatory responses. Based on the results of in vivo experiments and intratumoral microbiota analysis, we found that *Streptococcus mitis* can inhibit gastric cancer progression via suppressing M2 macrophage polarization and infiltration, as well as altering the intratumoral microbial community. In summary, our findings suggest that the intratumoral microbiota, exemplified by *Streptococcus mites*, may be involved in regulating the progression of gastric cancer, thereby emerging as potential therapeutic targets for this disease.

**Keywords** Gastric cancer, Intratumoral Microbiota, *Streptococcus mitis*, Tumor microenvironment

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## Background

Gastric cancer is on the rise across the globe. It has been ranked 5th in the number of new cases, and 4th in causing the death of people [1]. Recent times have witnessed considerable research interest aiming at exploring the role of microorganisms in tumors, which may influence tumor progression by regulating tumor biology, immune-inflammatory response, and therapeutic efficiency [2–4]. Traditionally, symbiotic and pathogenic interactions were considered different manifestations of the bacteria-host interaction [5]. *Helicobacter pylori* (HP) has been widely acknowledged as a significant player in gastric cancer process [6]. However, only 1–2% of patients infected with HP progress to gastric cancer. Furthermore, it is of significance to highlight that a considerable proportion of gastric cancer patients consistently test negative for HP, implying the potential involvement of non-HP microorganisms in the process of gastric cancer [7–9]. Therefore, it is of great importance for the identification and analysis of symbiotic and pathogenic bacteria associated with gastric cancer.

The microbiota comprises a wide range of bacteria, viruses, bacteriophages, fungi, and protozoa, collectively forming a complex ecosystem. These microbial populations, which inhabit various sites such as the oral cavity, gastrointestinal tract, lungs, skin, and genitourinary system, have been extensively studied for their roles in preserving physiological balance and their implications in disease progression [10–12]. An increasing volume of research highlights the crucial role of gut microbiota in cancer initiation and progression, its shaping of the complex tumor microenvironment (TME), and the potential for microbiome modulation to boost anti-tumor immunity [13–15]. Nevertheless, compared to the extensively studied gut microbes, the interplay between intratumoral microbial communities and the occurrence of gastric cancer remained a mystery, necessitating further exploration [16–19].

In this study, we investigated the intratumoral mycobiome in gastric cancer and provided mechanistic insights into antitumor effects of *Streptococcus mitis*. Our findings revealed a significant decrease in microbial diversity and presence within gastric cancer tissues. After gavage with *Streptococcus mitis* cultured from the tumor tissue of gastric cancer patients, the tumor growth was significantly inhibited in mice, potentially through modulation of the tumor microenvironment. Our study offers promising insights into novel strategies for the prevention and treatment of gastric cancer by elucidating the association between gastric cancer progression and intratumoral bacteria.

## Methods

### Clinical samples collection

This study recruited 23 patients with gastric cancer confirmed by surgical procedures and pathological biopsies from the Affiliated Drum Tower Hospital of Nanjing University Medical School, as well as 11 healthy controls. A total of 23 gastric cancer tissues and 23 para-gastric cancer tissues obtained by surgery and 11 healthy controls tissues collected via endoscopic biopsy were subjected to microbiota analysis based on the American Society for Gastrointestinal Endoscopy guideline. Regarding participant selection, the inclusion criteria for healthy controls required individuals with no history of gastric disease, recent antibiotic use, or gastrointestinal surgery. For the gastric cancer group, patients were included based on histologically confirmed gastric adenocarcinoma, with no prior chemotherapy or radiotherapy, to ensure that observed microbial differences were not influenced by these treatments. The collected gastric cancer tissues were obtained through total gastrectomy or partial gastrectomy procedures. Location of tumor resection specimen includes antrum, body, and cardia. The selected adjacent tissues were from the surrounding area of the cancerous tissue, located 2 centimeters away from the cancerous tissue. Healthy group includes individuals undergoing digestive system examinations at the Digestive Department of Drum Tower Hospital, without tumors, diabetes, or other digestive system disorders. The clinical information of all enrolled patients is provided in Table S1 and Table S2.

Each study participant provided written informed consent. The Ethics Committee of the Affiliated Drum Tower Hospital of Nanjing Medical University (ID: 2021-514-02) also provided permission for the conduction of this study.

### 16 S ribosomal RNA gene sequencing

DNA from the entire genome was isolated from the specimens utilizing the CTAB technique. Amplification of the 16 S rRNA genes from the V3-V4 regions was performed using the primers 338 F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The PCR protocol commenced with an initial denaturation at 95 °C lasting 3 min, succeeded by 29 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 10 min. The PCR amplifications were then cleansed with the Qiagen Gel Extraction Kit. Following this, the sequencing libraries were prepared using the TruSeq® DNA PCR-Free Sample Preparation Kit, adhering to the instructions provided by the manufacturer, and were indexed. The library's integrity was evaluated with the Qubit@2.0 Fluorometer and the Agilent Bioanalyzer 2100 system. In the final step, the library

underwent sequencing on the Illumina Miseq system, producing paired-end reads of 300 base pairs.

Sequencing data underwent comprehensive analysis, including sequence splicing, quality control, noise removal, and classification operations. The sequences were categorized into various taxonomic classifications by the BLAST tool, using SILVA138 database as the reference standard. QIIME (v1.8.0) was utilized to generate rarefaction curves and calculate richness and diversity indices based on the ASV information. To assess the diversity and composition of microbial communities across various samples, heatmaps were constructed showcasing the 20 most abundant ASVs employing the Mothur software [20]. Utilizing the data from taxonomic identification and the proportion of species present, the R software (version 3.6.0) was employed to create bar plots for analysis. Additionally, to determine the resemblance among various samples, cluster analysis and Principal Component Analysis (PCA) were conducted in R (version 3.6.0), utilizing the ASV data from each sample [21].

#### Animal experiments

Male nude mice, aged four weeks, were acquired from Beijing Weitong Lihua Biological Co., Ltd., and nurtured in an environment free from specific pathogens (SPF). For the purpose of orthotopic implantation, $1 \times 10^6$ MKN-45 cells suspended in 25 μL of PBS (combined with matrigel in a 2:1 volume ratio), were administered into the space between the gastric serosa and muscularis of the mice. In vivo bioluminescence imaging was performed every 7 days using the IVIS™ bioimaging system, and VivoGlo™ Luciferin (Promega) was utilized as a substrate for the imaging. 17 days after tumor formation, mice were randomly divided into two groups and gavaged with $1 \times 10^8$ CFU of *Streptococcus mitis* or saline in 200 μL once. Throughout the experiment, continuous monitoring of mice included assessments of body weight and tumor volume. Ultimately, tumor tissues were collected for 16 S rRNA sequencing, H&E staining, and immunohistochemistry.

#### Proteomic analysis

The SDT buffer (4% SDS, 100mM Tris-HCl, 1mM DTT, pH 7.6) was employed for sample lysis and protein extraction. The protein was quantified using the BCA Protein Assay Kit (Bio-Rad, USA). Protein digestion with trypsin followed the filter-aided sample preparation (FASP) procedure as outlined by Matthias Mann. After that, liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was conducted using a Q Exactive mass spectrometer (Thermo Scientific) coupled to an Easy nLC system (Proxeon Biosystems, now Thermo Fisher Scientific) with run times of 60, 120, and 240 min. The MS raw data for each sample were consolidated and subjected to identification and quantitation analysis using MaxQuant 1.5.3.17 software. Finally, bioinformatics analysis of proteomic data was conducted, encompassing functional enrichment, pathway analysis, interactome networks, and more, aiming to gain deeper insights into biological processes.

#### Flow cytometry

Cell suspensions from the spleen were filtered through Nylon cell strainers (70 μM, Falcon, USA), and red blood cells were lysed. After cells were washed with PBS containing 1% BSA, the Fc receptors were blocked with purified anti-mouse CD16/CD32 (clone 2.4G2, BD Biosciences). For surface staining, $2 \times 10^6$ of murine splenic single cell suspensions were stained with FITC-conjugated CD19 (clone 1D3, BD Biosciences), BV421-NK1.1 (clone PK136, BD Biosciences), PerCP-Cy5.5-CD11c (clone HL3, BD Biosciences), PE-CD11b (clone M1/70, BD Biosciences), PE-Cy7-F4/80 (clone BM8, ThermoFisher), FITC-CD16/32 (clone 93, ThermoFisher).

For intracellular staining, cells were stained with surface-staining antibodies followed by fixation and permeabilization with the intracellular Fixation & Permeabilization Buffer Set. Then, cells were intracellularly stained with APC-CD206 (clone MR6F3, ThermoFisher). Flow cytometry was performed on a FACSVerse device (BD Biosciences), and the data were evaluated with FlowJo v10.4 software.

#### RNA isolation and quantitative RT-PCR analyses

Total RNA was isolated from tissue using TRIzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. To detect mRNAs, total RNA was reverse transcribed to cDNA using HiScript® III RT SuperMix for qPCR (+gDNA wiper) (Vazyme, China) along with the corresponding RT primer, and incubated sequentially at 37 °C for 15 min and then at 85 °C for 5 s. Subsequently, real-time PCR was conducted using the ChamQ Universal BYBR qPCR Master Mix kit (Vazyme, China) on a Light Cycler 480 real-time PCR System (Roche, Mannheim, Germany). Relative mRNA expression in the tissue was normalized to GAPDH.

#### Statistical analysis

GraphPad Prism 9.0.0 (GraphPad Software, Inc.) was used for statistical analysis. Differences were considered statistically significant at $P < 0.05$ using Student's t-test. The results are presented as the mean ± SEM. All the experiments were performed at least three times independently, and results were reproducible.

## Results

### Intratumoral Microbiota dysregulation in patients with gastric cancer

A wealth of research has confirmed the existence of intratumoral microbiomes across a spectrum of tumors, encompassing those of the breast, pancreas, lungs, and melanoma [22, 23]. To explore the constitutive characteristics of the intratumoral microbiome in gastric cancer, we employed 16 S rRNA sequencing to assess the microbial distribution within gastric cancer tissues. It can be seen from the results that a substantial reduction has been occurred in alpha-diversity of microbes in gastric cancer tissues compared to the control groups (Fig. 1A). Beta-diversity analysis indicated differences in microbial compositions among the three groups (Fig. 1B). Apart from the shared microbial species, gastric cancer tissues contained 948 unique species, significantly fewer than the 2296 unique species found in healthy tissues (Fig. 1C). The dominant genera in gastric cancer tissues included *Streptococcus*, *Fusobacterium*,

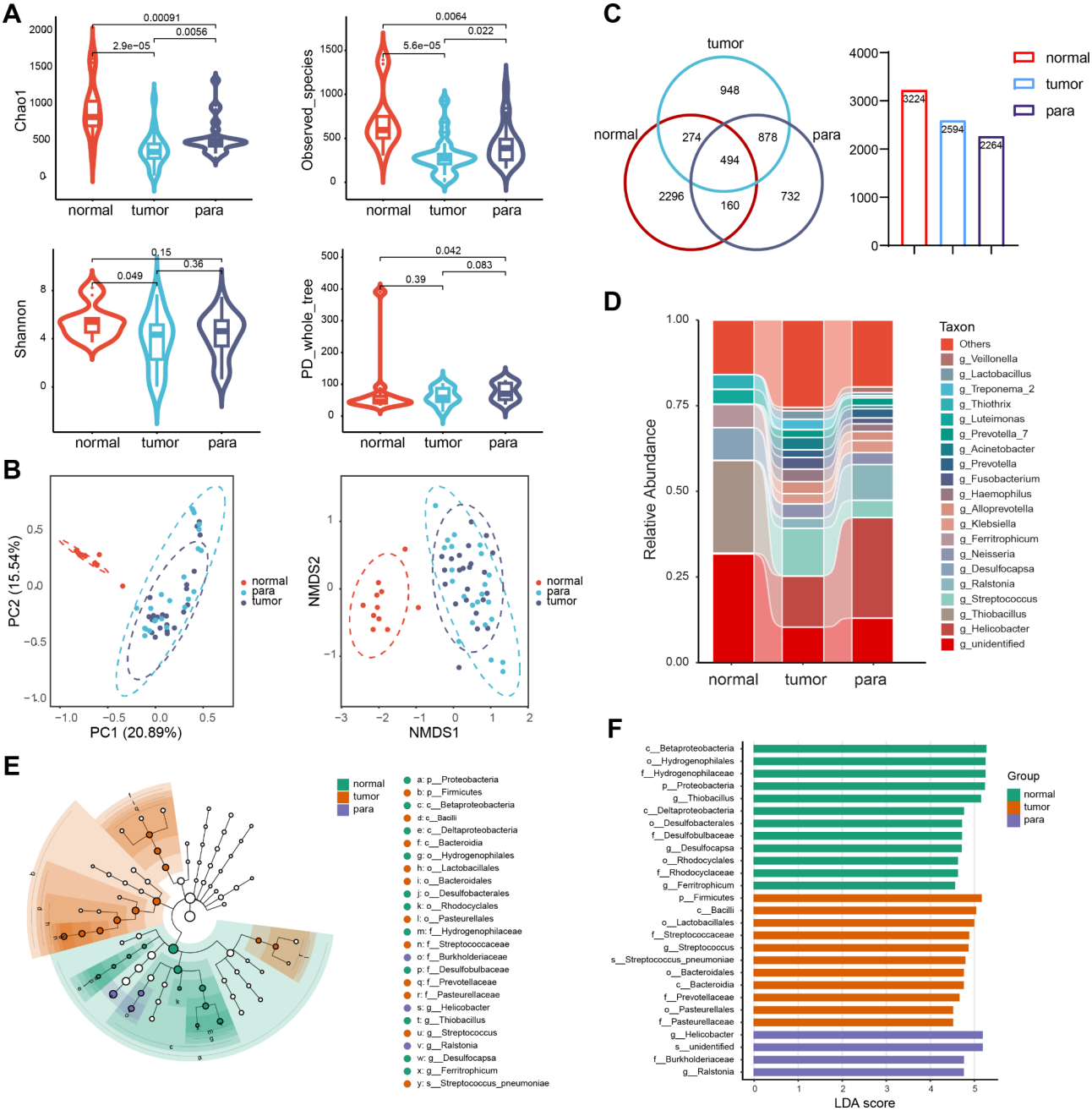


**Fig. 1** Microbial diversity analysis and species compositions in tumor tissues of gastric cancer patients. (**A**) Alpha-diversity of analysis. (**B**) Beta-diversity analysis. (**C**) Venn diagram. (**D**-**F**) Analysis of microbiota differences by LEfSe and cladogram

*Helicobacter*, and *Lactobacillus*, whereas *Thiobacillus*, *Desulfocapsa*, and *Ferritrophicum* were enriched in the healthy control group (Fig. 1D). Further, the cladogram and Linear discriminant analysis Effect Size (Fig. 1E and F) (LEfSe) showed substantial differences in the relative enrichment of bacterial communities at the genus level among the groups (LEfSe: $p < 0.05$, $q < 0.05$, LDA > 4.5). Consequently, our findings indicate that gastric cancer patients have a distinct imbalance in their intra-tumor microbiota.

### Intratumoral Microbiota is associated with the tumor microenvironment through immune modulation, metabolic processes, and inflammatory responses

The intratumoral microbiota is recognized as vital part of tumor microenvironment, garnering considerable attention for its role in regulating tumor-associated inflammatory responses and immune modulation [17, 24, 25]. To investigate the potential impact of intratumoral microbiota in gastric cancer progression, we employed proteomic techniques to analyze the expression of immune-inflammatory-related proteins in tumor tissues from gastric cancer patients (Fig. 2A-C). The analysis identified 32 upregulated proteins and 26 downregulated proteins in gastric cancer tissues compared to healthy tissues. Additionally, we employed fluorescence real-time quantitative PCR to validate the proteomic results, with a particular focus on key proteins involved in biological processes and signaling pathways. Our findings demonstrated that the expression levels of most key proteins were significantly elevated in cancer tissues compared to adjacent normal tissues, further confirming the reliability of the proteomic sequencing results (Fig. 2D). The KEGG enrichment analysis demonstrated that these proteins exhibited notable interactions with microbial metabolic pathways, including the metabolism of selenocompound, butanoate, glyoxylate and dicarboxylate, and propanoate acid (Fig. 2E). Furthermore, the GO analysis revealed that these differentially expressed proteins are predominantly involved in electron transport coupled with proton

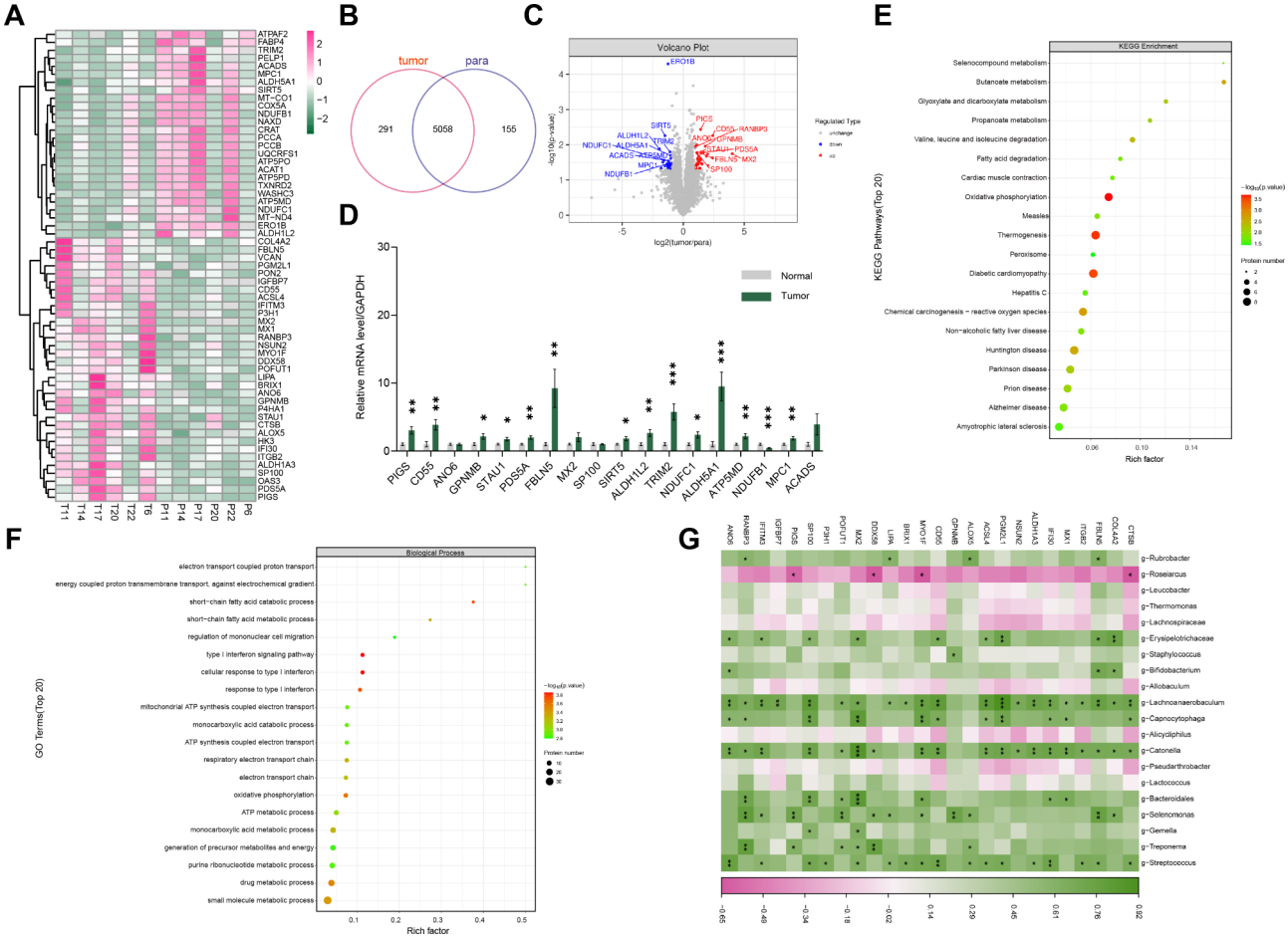


**Fig. 2** Intratumoral microbiota is associated with the tumor microenvironment through immune modulation, metabolic processes, and inflammatory responses. (**A**) Proteomic analysis of tumor tissues in gastric cancer patients. (**B**) Venn diagram. (**C**) Volcano map. (**D**) Validation of mRNA Levels of Key Proteins Identified by Proteomic Sequencing Involved in Biological Processes and Signaling Pathways. (**E**) KEGG pathway analysis. (**F**) GO analysis. (**G**) Correlation analysis between microorganisms and differentially expressed proteins within tumors

transport, as well as in short-chain fatty acid metabolism, regulation of mononuclear cell migration and type I interferon signaling pathways (Fig. 2F). Our findings also highlighted a strong correlation between these differential proteins and various intratumoral microorganisms, such as *Lachnoanaerobaculum*, *Capnocytophaga*, *Catonella*, and *Streptococcus* (Fig. 2G). Several intratumoral microbes have been reported to promote chronic inflammation, accompanied by the sustained release of inflammatory factors involved in tumor development and immune responses. We observed that the serum levels of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), interleukin-12p70 (IL-12p70), interleukin-17 (IL-17) and interferon (IFN) were significantly elevated in gastric cancer patients compared to healthy individuals, and these cytokines were also correlated with the abundance of specific microorganisms in gastric cancer tissues (Fig. 3A-B). These results suggest that intratumoral microorganisms may interact with the tumor microenvironment through immune regulation, metabolic processes, and inflammatory responses, thereby influencing tumor progression.

### Tumor-derived *Streptococcus mitis* suppresses gastric cancer progression in mice

To investigate the role of intratumoral bacteria in gastric cancer progression, we cultured microorganisms from fresh human gastric cancer tissues for in vivo experiments. Notably, we successfully isolated a viable bacterial strain on the culture plate and confirmed it as *Streptococcus mitis* through mass spectrometry analysis (Fig. 4A). We also validated the presence of *Streptococcus mitis* within gastric cancer tissues using fluorescence in situ hybridization (FISH) techniques (Fig. 4B). The receiver operating characteristic (ROC) analysis revealed a high area under curve (AUC) value of 1 for *Streptococcus*, which is significantly higher than the AUC values of other genera, such as *Acinetobacter*, *Klebsiella*, *Helicobacter*, and *Neisseria*, demonstrating its superior diagnostic performance for gastric cancer patients (Fig. 4C).

In order to determine whether *Streptococcus mitis* affects gastric cancer progression, we utilized an established murine gastric cancer model, administering *Streptococcus mitis* via gavage (approximately $1 \times 10^8$ CFU/mouse) (Fig. 4D). Following oral administration of

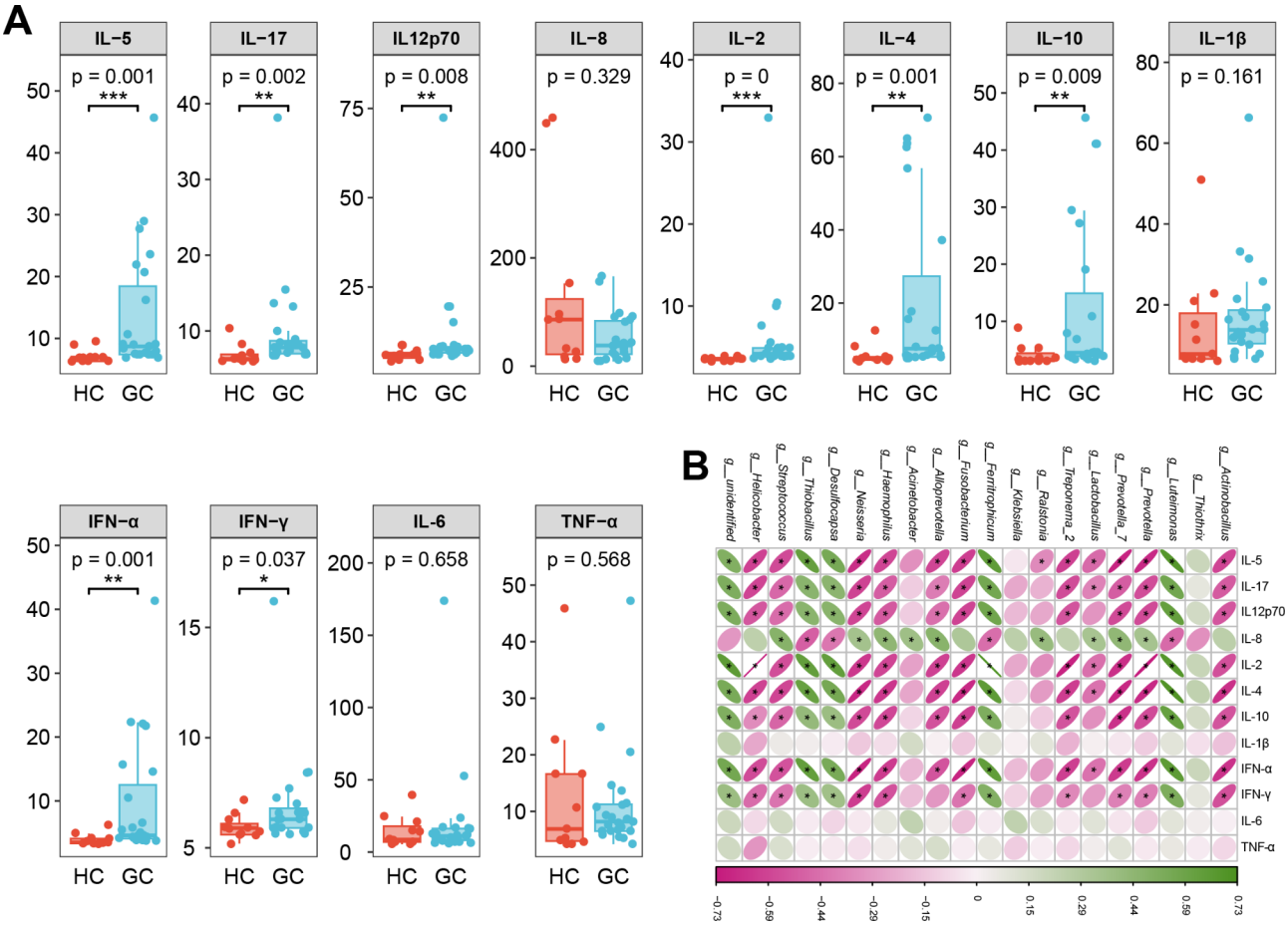


**Fig. 3** Intratumoral microbiota is associated with the serum cytokines in gastric cancer patients. (**A**) Cytokine expression in the serum of gastric cancer patients and healthy individuals. (**B**) Correlation analysis between intratumoral microorganisms and serum cytokines in gastric cancer patients

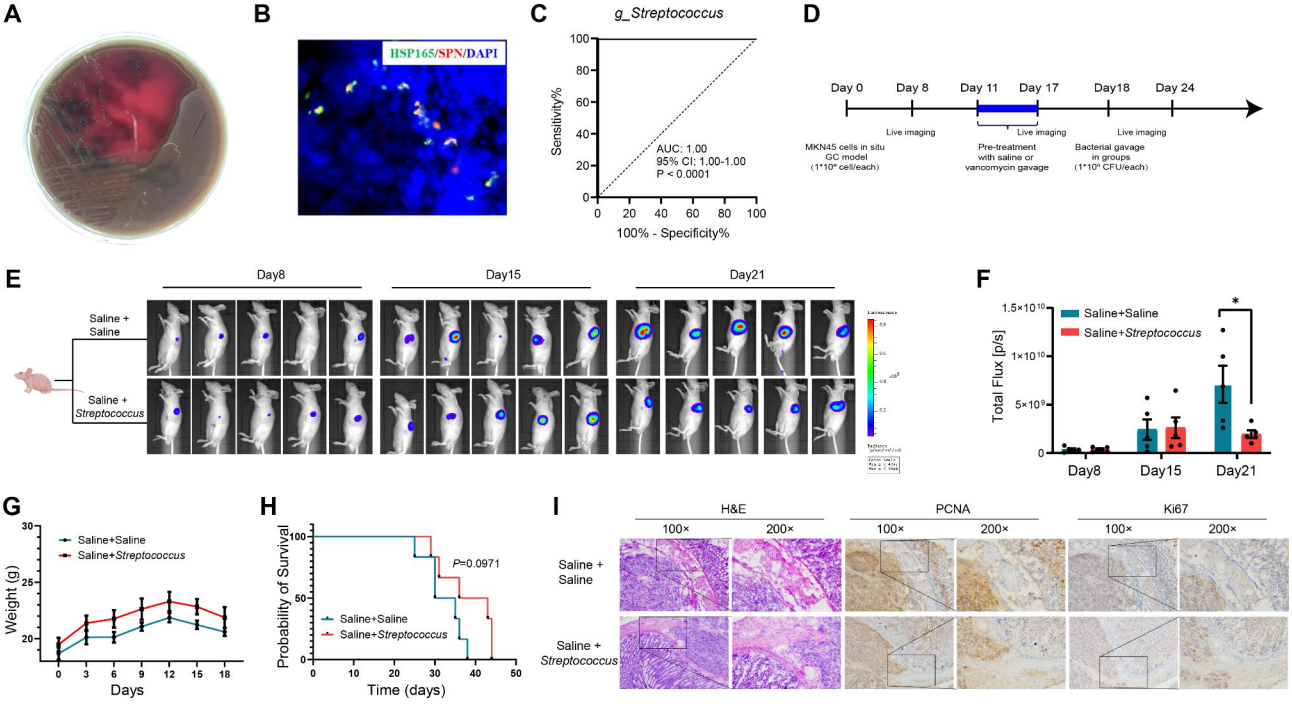


**Fig. 4** Administration of *Streptococcus mitis* influences gastric cancer progression in tumor bearing mice. (**A**) Identification of *Streptococcus* from gastric cancer tissues by bacterial culture plate. (**B**) *Streptococcus* in tumor tissues by FISH. (**C**) ROC analysis for *Streptococcus* in gastric cancer. (**D**) Flow chart of the in vivo experiment. (**E**-**F**) In vivo imaging of tumor bearing mice. (**G**) Body weight of tumor bearing mice. (**H**) Survival curve of tumor bearing mice. (**I**) HE and immunohistochemical staining of tumor tissues in mice

*Streptococcus mitis* to tumor-bearing mice, the treated group exhibited a significant reduction in tumor volume compared to the control group (Fig. 4E-F). Despite the lack of substantial variance in body weight alterations between the two groups, those receiving *Streptococcus mitis* demonstrated a markedly extended survival period (Fig. 4G-H). Additionally, the treated group exhibited decreased expression levels of proliferating cell nuclear antigen (PCNA) and the proliferation marker protein Ki-67 (Ki67) in gastric cancer tissues contrasted to the control group, indicating a significant inhibition of tumor cell proliferation following oral administration of *Streptococcus mitis* (Fig. 4I).

### *Streptococcus mitis* suppresses gastric cancer progression by inhibiting M2 macrophage polarization and infiltration

In response to pathogenic factors, an organism's immune system serves as a crucial component of its defense mechanism. To further investigate the potential mechanism underlying the anti-tumor systemic effects triggered by *Streptococcus mitis*, we evaluated immune cells in the spleen following the oral administration of *Streptococcus mitis* in a gastric cancer model. We observed no significant changes in the spleen/body weight ratios of mice treated with *Streptococcus mitis* compared to those treated with saline (Fig. 5A). Flow cytometry analysis demonstrated no significant differences in the proportions and numbers of splenic B cells, NK cells, DCs and macrophages between the two groups (Fig. 5B-E). However, gavage with *Streptococcus mitis* significantly reduced the proportion of M2 phenotype (CD206+) in the spleen (Fig. 5F) and M2 infiltration in tumor tissues (Fig. 5H), despite the expression of M1 phenotype marker (CD16/32+) remained unaltered after oral administration of *Streptococcus mitis* (Fig. 5G). These findings conclusively demonstrate that the tumor-inhibiting effect of *Streptococcus mitis*-induced immunoregulation is mediated through the reduction of M2 macrophage polarization and infiltration in the tumor microenvironment.

### *Streptococcus mitis* influences gastric cancer progression by reshaping the intratumoral microbiota

The gastrointestinal microbiota has been reported to modulate the intratumoral microbiota [17, 26]. In order to investigate the impact of orally administered *Streptococcus mitis* on the microbial communities within tumors, we conducted 16 S rRNA sequencing of gastric cancer tissues from mice. Remarkably, notable changes were observed in the microbial composition within mouse tumors subsequent to oral administration of *Streptococcus mitis*, despite the absence of substantial differences in the alpha-diversity of the intratumoral microbiota between the two cohorts (Fig. 6A).

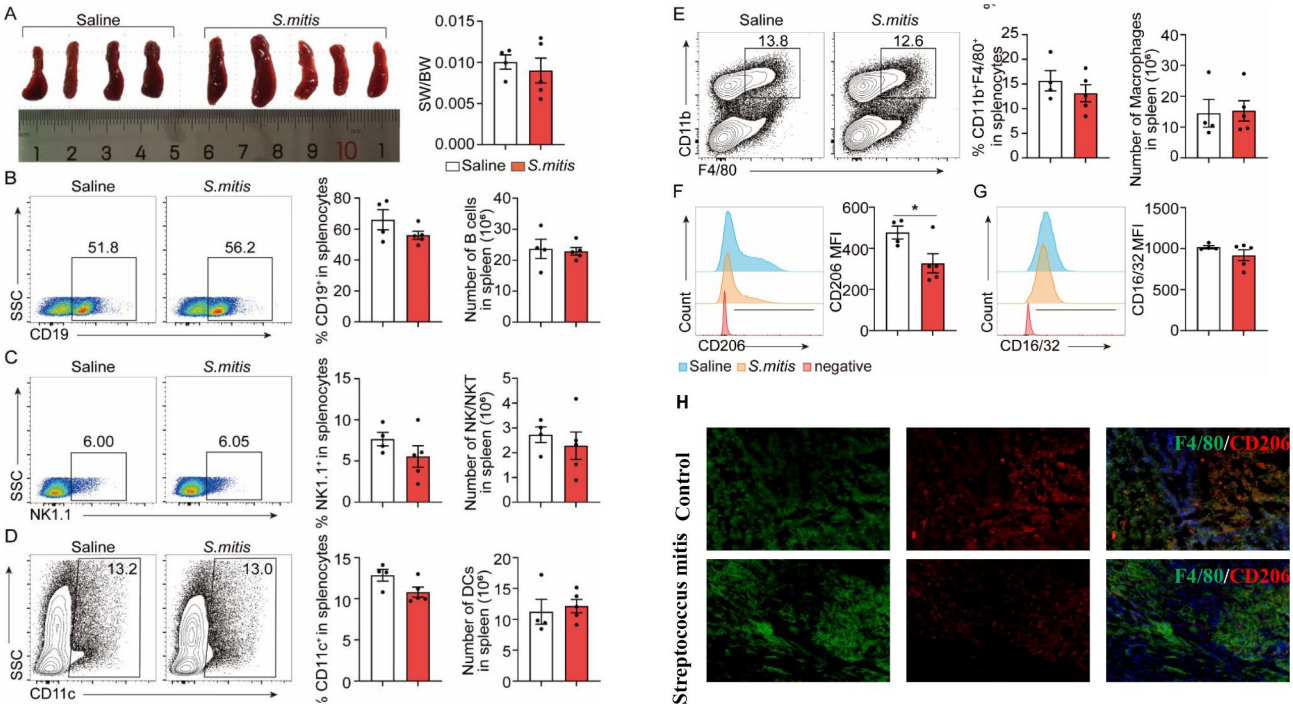


**Fig. 5** Administration of *Streptococcus mitis* influences distinct immune cell populations in the spleens and tumors of tumor bearing mice. (**A**) Gross view and mean SW/BW of spleens of saline and *Streptococcus mitis*-treated mice. (**B**) Proportion and absolute number of $CD19^+$ (lymphocytes and singlets pre-gates) in spleen. (**C**) Percentage and absolute number of the $NK1.1^+$ subset in spleen. (**D**) The frequency and total number of $CD11c^+$ cells within the total splenocytes. (**E**) Percentage and absolute number of macrophages ($CD11b^+F4/80^+$) in the spleen of Saline and *Streptococcus mitis*-treated mice. (**F**) Mean fluorescence intensity (MFI) of CD206 in $CD11b^+F4/80^+$ macrophages. Histogram plots depict representative CD206 expression for *Streptococcus mitis*-treated (orange) and control mouse (blue). (**G**) Representative flow cytometry histograms, and summary MFI data for CD16/32 expression in macrophages in the spleen. (**H**) M2 phenotype infiltration in tumor tissues by immunofluorescence

Both Principal Coordinates Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS) analyses demonstrated differences in the microbial composition between the two sample sets (Fig. 6B). Specifically, the dominant bacteria genera in tumor tissues treated with *Streptococcus mitis* were *Lactobacillus*, *Escherichia shigella*, *Morganella* and *Bacteroides*, while the saline-treated group primarily consisted of *Enterococcus*, *Mycoplasma* and *Proteus* (Fig. 6C). The Venn diagram illustrated 92 microbial species shared between the two groups, with 15 and 28 unique species found in the *Streptococcus mitis* treated group and control group, respectively (Fig. 6D). The abundance of *Streptococcus mitis* in tumor tissues decreased after oral administration of *Streptococcus mitis* (Fig. 6E). These results suggest that the introduction of *Streptococcus mitis* may alter the composition and overall ecological balance of the microbiota in mouse tumor tissues, thereby modifying the tumor microenvironment and subsequently influencing tumor progression.

## Discussion

The exact etiology and pathogenesis of gastric cancer remain unclear at present. Emerging research on tumor-related microbiota has provided us with new insights into the progression of gastric cancer. Several studies have reported the existence of intratumoral microbiota, which possess tumor-specific characteristics in variety of cancers [4, 23, 27, 28]. The abundance of *Porphyromonas gingivalis* and oral microbiota such as *Clostridium* and *Streptococcus* in esophageal and gastric cancer tissues has been reported to be significantly higher than in paired adjacent non-cancerous tissues. *Fusobacterium nucleatum* is enriched in colorectal cancer tissues but not in the adjacent normal tissues [4]. In our study, we observed a distinctive intratumoural microbiota in gastric cancer patients, with the predominant presence of *Streptococcus*, *Fusobacterium*, *Helicobacter*, and *Lactobacillus*. There may be three potential sources of intratumoral microorganisms [29, 30]: (1) Microorganisms colonizing the mucosa could invade the tumor through the damaged mucosal barrier. (2) The intratumoral microbiota may spread from normal adjacent tissues. (3) The intratumoral microbiota originates from hematogenous dissemination. These provide routes for the source of microorganisms in gastric tissue, resulting in an elevated risk of gastric cancer, although the underlying mechanism requires further investigation.

Recent evidence has elucidated various mechanisms potentially explain the association between intratumoral

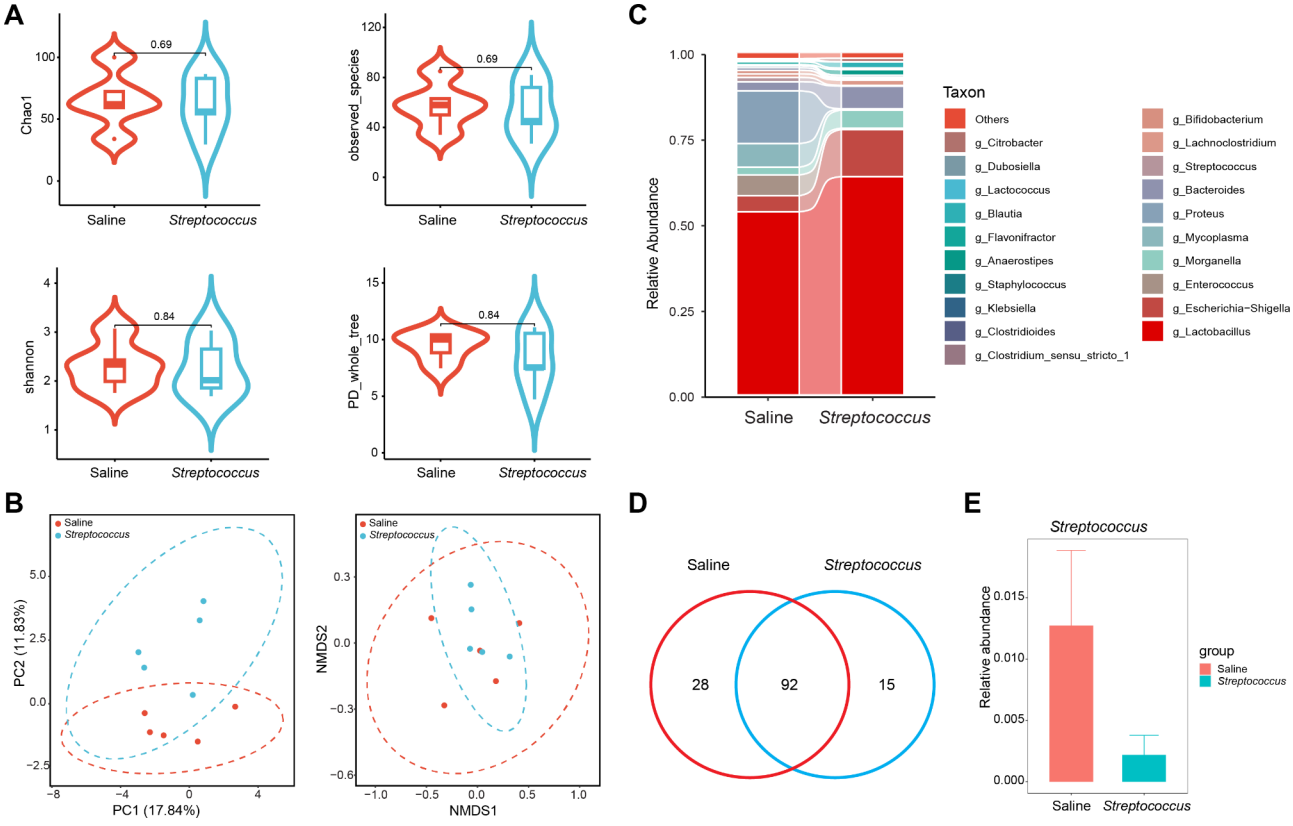


**Fig. 6** Microbial diversity analysis and species compositions of tumor tissues in mice. (**A**) Alpha-diversity of analysis. (**B**) Beta-diversity analysis. (**C**) Analysis of microbiota differences by LEfSe. (**D**) Venn diagram. (**E**) Abundance of Streptococcus in tumor tissue

microbiota and cancers, including host genomic alterations, inflammation-related signaling pathways, immune regulation, interaction with the tumor microenvironment [4, 26, 31]. Pathogenic *Escherichia coli* can secrete the genotoxin colibactin, which causes DNA damage in colonic epithelial cells, thereby leading to colorectal cancer [32]. Jun yu et al. demonstrated that *Streptococcus anginosus* enriched in the gastric mucosa contributes to gastric tumorigenesis through direct interaction with gastric epithelial cells in the TMPC-ANXA2-MAPK axis [33]. Our results indicated that intratumoral microbiota in gastric cancer can influence the expression of various proteins and inflammatory factors. These findings reveal the potential of intratumoral microorganisms to interact with the tumor microenvironment by affecting intratumoral immune regulation, microbial metabolic pathways, and the inflammatory reaction, thereby influencing tumor progression.

In several studies, microbiota trigger the activation and polarization of various immune cells including DC, NK, and monocytes via STING signaling, reprogramming of the tumor microenvironment [34, 35]. To further investigate the potential mechanism underlying the anti-tumor effects triggered by *Streptococcus mitis*, we evaluated the changes in multiple immune cell subtypes following the oral administration of *Streptococcus mitis* in a gastric cancer model. We found that gavage with *Streptococcus mitis* inhibited the growth of gastric cancer in mice, probably by reducing M2 macrophage polarization and infiltration, with minimal impact on other immune cell subtypes. Quorum sensing-mediated microbial interactions [36] and competitive exclusion dynamics among microbial communities [37] have attracted the attention of researchers. In our study, orally administered *Streptococcus mitis* significantly altered the composition of the intratumoral microbiota in mice, providing another potential mechanism by which it may influence gastric cancer progression. Furthermore, we precleared the microbiota in the gastrointestinal tract of gastric cancer model mice by administering oral vancomycin before gavaging with *Streptococcus mitis*. We found that *Streptococcus mitis* had no impact on tumor growth in tumor-bearing mice but extended their survival time (Supplementary Fig. 1). We propose two potential explanations for this observation: (1) the residual vancomycin in the mice may retain sufficient antibacterial activity to eliminate the administered *Streptococcus mitis* shortly after its introduction into the gastrointestinal tract; (2) *Streptococcus mitis* alone may not exert an inhibitory effect on tumor growth, instead requiring synergistic

interactions with one or more specific bacterial species present in the mouse microbiota to achieve this effect. These findings imply the potential to modify the intratumoral microbiota and modulate the tumor microenvironment by regulating the gastrointestinal microbiota.

Our study also has several limitations. Although our findings imply a potential relationship between the *Streptococcus mitis* and gastric cancer progression, the precise interactions between *Streptococcus mitis* and immune cells within the tumor microenvironment has not been fully elucidated. Consideration should also be given to elucidating the mechanisms underlying the reshaping of the intratumoral microbiota through oral administration of *Streptococcus* mites in mice. Additionally, interactions between the *Streptococcus mitis* and other microorganisms, such as potential interactions with H. pylori, should be investigated further to determine whether it serves as an independent pathogenic factor for gastric cancer. Finally, we did not include negative controls for tissue samples, which may have led to DNA contamination affecting the composition and distribution of the tissue microbiota. We will prioritize addressing this factor in future studies.

In conclusion, we present evidence of *Streptococcus mitis* obtained from gastric cancer patients, marking the first instance of *Streptococcus mitis* can inhibit gastric cancer progression by reducing M2 macrophage infiltration and reshaping the intratumoral bacterial composition. In-depth understanding of intratumoral microbiota in gastric cancer can provide novel insights into pathogenesis of gastric cancer, potentially paving the way for novel avenues in gastric cancer therapy.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06142-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

### Author contributions

ZX, HS, FJ and ML: conceptualization, methodology, formal analysis, PY, GL and YN: methodology and data validation. PY: formal analysis. ZX, YW, ML, and HS guided the study. AK: methodology. PY and ML writing the original draft. ZX, HS and YW: revised the manuscript, XZ, XC helped in the collection of samples and clinical information. All authors have reviewed and approved the final manuscript.

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### Data availability

The data that support the findings of this study are openly available in National Center for Biotechnology Information (NCBI) [SUB14343890] at [https://submit.ncbi.nlm.nih.gov/subs/], Accession number [PRJNA1093016].

## Declarations

### Ethics approval and consent to participation

We adhered to the guidelines of the Declaration of Helsinki, and our research was endorsed by the ethics committee of Nanjing Drum Tower Hospital (Ethics code 2021-514-02).

### Consent for publication

Not applicable.

### Conflict of interest

Authors unanimously state the absence of any conflicts of interest in this study.

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