Supplemental methods

Serum sample preparation

Blood samples were collected from all participants after fasting for at least eight hours. The samples were drawn using coagulant-based tubes by trained nurses and then centrifuged to separate the serum (3500 rpm for 8 minutes). Each serum sample was assigned a unique identifier and stored at -80°C in the cancer biobanks of SYSUCC and Gastrointestinal and Anal Hospital of Guangdong Province (GIAH-GDP).

Transcriptome sequencing

At least 4 ml serums were collected from 37 GC patients and 20 healthy donors, and RiboTM Exosome Isolation Reagent (C10110-2, RiboBio) was used to extract exosomes from serums. Subsequently, RNAs were then extracted from exosomes using TRIzol™ Reagent (15596018, ThermoFisher). Transcriptome sequencing of GC serum exosomes was performed using the Illumina HiSeq X Ten platform by RiboBio Co., Ltd while Novogene Corporation (Beijing, China) conducted the RNA-seq of GC tissues and adjacent normal tissues from 20 GC patients. The counts and Fragments Per Kilobase Million (FPKM) of long non-coding RNAs (lncRNAs) and mRNAs were quantified by mapping against the GRCh37 (hg19) p13 genome and the Gencode v19 GTF file. For circRNAs, the GRCh37 (hg19) genome and circRNA database were used[1]. Variants expressed in less than 10% of all samples were excluded. The remaining markers were retained for subsequent statistical analysis. Differential expression of non-coding RNAs (ncRNAs) between tumor and normal samples was evaluated using edgeR. ncRNAs with $P < 0.05$ and log2 fold change > 1.0 were considered significant.

Isolation and characterization of exosomes

Exosome isolation was performed according to the manufacturer's instructions for Ribo™ Exosome Isolation Reagent (C10110-2, RiboBio). The serum samples were initially centrifuged at 2000 g for 20 minutes at room temperature to remove cells and debris, and the supernatant was transferred to new tubes. One-third of the volume of

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isolation reagent was added to the supernatant, and the mixture was thoroughly mixed. After incubation for 30 minutes at 4°C, the mixture was centrifuged at 15,000 g for 2 minutes. The resulting pellet, which contained exosomes, was collected after removing the supernatant. To characterize the exosomes, nanoparticle tracking analysis (NanoSight NS300, Malvern, UK) and transmission electron microscopy (FEI, Czech Republic) were conducted to assess size distribution and morphology. Additionally, western blotting was performed to confirm the presence of exosome markers using antibodies against CD9 (1:1000, Affinity Biosciences, AF5139), CD63 (1:10000, Proteintech), TSG101 (1:1000, TransGen), and laminB (1:10000, Proteintech).

Knockdown of DGCR9 and growth experiments in vitro

Short hairpin RNAs (shRNAs) targeting DGCR9 were obtained from Beijing Tsingke Biotech Co., Ltd. The effective shRNA sequences were shRNA01 (GCATCCATGCTGGGTGCTTTA) and shRNA02 (GGTCCTCACTCACTGTCTTTG). Lentiviral transductions were performed as previously described[2]. HGC27 cell line was purchased from the cell library of Shanghai Institute of Biochemistry and Cell Biology and MKN74 cell line was purchased from the cell bank of Japanese Cancer Research Resources Bank. In addition, cell proliferation was assessed using MTS assays (G3580, promega), and the absorbance of the cell culture media was measured with a Synergy™ Multi-Mode Microplate Reader (Biotek, Vermont, USA) at a wavelength of 490 nm. For the colony formation assay, the cells were seeded at 500 cells per well in six-well plates and cultured for 8 days. They were then washed with PBS, fixed with methanol and stained with 0.5% crystal violet, and colonies were counted using ImageJ software.

Gastric cancer (GC) cells, including HGC27 cells and MKN74 cells, were cultured in RPMI 1640 (C11875500BT, GIBCO) supplemented with 10% exosome depleted fetal bovine serum (C3801, Vivacell) and the culture media were collected after 36 hours. Subsequently, exosomes were isolated from culture media for RNA extraction and counting according to the manufacturer's instructions for RiboTM Exosome Isolation Reagent (C10130-1, RiboBio). After incubated with exosomes for 36h[3],

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wild-type GC cells were subjected to detect the expression of DGCR9 in cells and the growth rate via qRT-PCR assay and MTS assay.

Glycolytic Proton Efflux Rate (glycoPER)

Lactate secretion of GC cells was measured as their glycolytic proton efflux rate (glycoPER), using a Seahorse XF Glycolytic Rate Assay Kit (103344-100, Agilent) and an oxygen-controlled XFe24 extracellular flux analyzer (Seahorse Bioscience). In brief, HGC27 cells were seeded in 4 replicates in a Seahorse 24-well culture microplate at density 2×10^4 per well one day in advance while 4×10^4 per well for MKN74 cells. Seahorse base medium was supplemented with 1mM pyruvate, 2mM glutamine and 10mM glucose and added in the wells on the day of the assay. Then, the cells were incubated for 1 hour in a CO_2-free incubator at 37℃ prior to the assay. Injections of rotenone/antimycin A (Rot/AA, 0.5 mM) and 2-deoxy-D-glucose (2-DG) were loaded onto ports A and B of XFe24 sensor cartridges respectively. Specifically, Rot/AA were used to inhibit mitochondrial complex 1 and 3, respectively, while 2-DG could block glycolysis completely. Results were normalized to cell numbers.

Cell-based xenograft and PDX models

Six-week-old female NCG mice were purchased from GemPharmatech Co., Ltd. HGC27 cells (2×10^6) or MKN74 cells (5×10^6) expressing sh-ctrl or sh-DGCR9 were subcutaneously injected into the dorsal flanks of mice (six mice per group). For the construction of PDX models, fresh tumor tissues from two GC patients were immediately inoculated into flank hypodermis of NCG mice[5]. When PDXs (P1) reached approximately 500 mm^3, the PDX tumors were transplanted to other mice (P2). Finally, the mice bearing P3 grafts were utilized to examine the therapeutic effects of DGCR9 inhibitor. Twenty-one days after transplantation, scrambled or in vivo-optimized DGCR9 inhibitor (RiboBio, Guangzhou, China) were injected intratumorally every 3 days. The volume of all tumors from the cell-based xenografts or PDXs was routinely monitored every 3 days using a caliper. When the study finished, the tumor weight was measures and the tissues underwent further pathological analysis.

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Immunohistochemistry (IHC)

The IHC assays were conducted following standard protocols[5]. Briefly, paraffin-embedded tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched with 3% H_2O_2 for 10 minutes. Antigen retrieval was performed using sodium citrate buffer or EDTA at sub-boiling temperatures for 10 minutes. Following antigen retrieval, the sections were blocked with 10% fetal bovine serum (FBS) for 1 hour at room temperature. Anti-Ki67 (1:1000, ZSGB-Bio) was applied overnight at 4°C. After incubation with primary antibodies, sections were treated with a biotinylated secondary antibody for 1 hour at room temperature. Detection was performed using the Dako REAL™ EnVision™ Detection System (K5007, Copenhagen, Denmark), and Harris-modified hematoxylin was used for counterstaining. Stained sections were evaluated through the percentage of positively stained cells. In addition to IHC, hematoxylin and eosin (H&E) staining and TdT-mediated dUTP nick-end labeling (TUNEL) assays were performed on tissue sections according to established procedures[6].

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