Supplemental Materials and Methods

Histology and immunostaining

Gastric tissues were collected and fixed overnight in 4% paraformaldehyde (Cat# P1110; Solarbio, Beiing, China), followed by paraffin embedding as previously described[1]. Sections (4 μm) were cut, stained with hematoxylin and eosin (H&E), and histologically verified by a board-certified pathologist. For immunohistochemistry (IHC), paraffin sections were deparaffinized in xylene and rehydrated through a graded ethanol series. Antigen retrieval was performed in sodium citrate buffer (MXB Biotechnology, Beijing, China), and endogenous peroxidase activity was quenched with hydrogen peroxide (MXB Biotechnology). Sections were then blocked in phosphate-buffered saline (PBS) containing 0.2% Triton X-100 and 5% bovine serum albumin (BSA, Cat# A8010, Solarbio) for 2 h at room temperature, followed by overnight incubation at 4 °C with primary antibodies. The following day, sections were incubated with HRP-conjugated secondary antibodies (Zhongshan Biotechnology, Guangdong, China) for 30 min at room temperature. Signals were visualized using diaminobenzidine (Zhongshan Biotechnology) and counterstained with hematoxylin. The antibodies used are listed in **Supplementary Table 1**. IHC scores (range 0–9) were calculated by multiplying intensity and heterogeneity scores as previously described[2, 3], and patients were classified into low (score 0, 1, 2, or 3) and high (score 4, 6, or 9) expression groups.

Immunofluorescence (IF) assays were performed as previously described[4, 5]. Briefly, sections were blocked in 5% BSA with 0.2% Triton X-100 for 1 h, followed by overnight incubation at 4 °C with primary antibodies. After washing, sections were incubated with fluorescence-conjugated secondary antibodies for 2 h at room temperature. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Cat# C0065, Solarbio). Images were acquired using a TCS SP8 confocal microscope (Leica Microsystems, Wetzlar, Germany).

Human samples

Between 2010 and 2015, patients diagnosed with GC at Fujian Medical University Union Hospital (FMUUH) had samples of their gastric cancer (GC), metaplastic, and normal gastric tissue collected. The inclusion criteria were: (a) histologically confirmed gastric cancer (GC); (b) no other malignancies; and (c) initial or updated tumour staging according to the 7th edition of the American Joint Committee

on Cancer (AJCC) Cancer Staging Manual[6]. Tumor recurrence was defined as either biopsy-confirmed disease or imaging findings consistent with recurrence, and was classified based on anatomical location. Fresh intestinal-type GC tissue samples were also obtained from FMUUH patients for RNA sequencing (RNA-seq) and subsequent analysis of RRGs (RNA regulatory genes). This study was approved by the Ethics Committee of FMUUH and written informed consent was obtained from all enrolled patients.

Spontaneous mouse GC mode

The spontaneous gastric cancer (GC) model was established as previously described[7]. Briefly, wild-type C57BL/6 mice were administered 240 ppm N-methyl-N-nitrosourea (MNU; HY-34758, MCE, USA) in their drinking water for 6 weeks, following a schedule of 1 week of exposure alternated with 1 week of withdrawal. At 36 weeks of age, mice were euthanized, and stomach tissues were harvested for subsequent analyses.

Tamoxifen administration

Mice were given 5 mg tamoxifen by subcutaneous injection at 8 weeks of age for 3 consecutive days as previously described[3, 8]. SPEM was induced by daily intraperitoneal injections of tamoxifen dissolved in 90% corn oil and 10% ethanol (5 mg/20 g body weight; T832955, Macklin, Inc., Rochelle, IL, USA).

Cell culture, transfection, and lentivirus infection

The human gastric carcinoma (GC) cell line (AGS) was obtained from Cellcook Biotechnology Ltd (Guangzhou, China). All cell lines used in this study were confirmed to be mycoplasma-free and characterised using a short tandem repeat (STR) profile. The cells were grown in F-12K medium (Gibco, Waltham, MA, USA), supplemented with 10% fetal bovine serum (Gibco) and penicillin/streptomycin (Cat# BL505A, Biosharp). All cell lines were cultured in a 5% CO_2 incubator at 37 °C. Transient transfection of plasmids was performed using Lipofectamine 3000 reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. To generate stable cell lines, cells were infected with lentivirus for 12–24 hours at a low fusion level (20%). After 72 hours, the cells were screened with puromycin (Sigma-Aldrich) for two weeks.

Cell proliferation and cell cycle assays

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) according to the manufacturer's instructions[9]. Briefly, approximately 2×10^3 cells were seeded into 96-well plates, after which 10 μL of CCK-8 reagent was added to each well, and cells were incubated for the indicated time periods. Optical density (OD) at 450 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

For cell cycle analysis, approximately 5×10^5 cells were seeded into 6-cm dishes and cultured for 24 h. Cells were then harvested, fixed in 70% ethanol at 4 °C overnight, and washed with PBS. Fixed cells were stained with propidium iodide (PI) and filtered through a 70-μm cell strainer before analysis on a FACSVerse flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

RNA sequencing

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the standard phenol–chloroform extraction protocol. RNA samples were treated with DNase and quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). RNA integrity was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Library preparation and high-throughput sequencing were performed by Novogene (Beijing, China) and Igenebook Bioinformatics Institute (Wuhan, China) using an Illumina HiSeq platform.

Gastric cancer xenograft models

A total of 2×10^6 stably transduced AGS cells suspended in 0.1 mL PBS were subcutaneously injected into the right flank of C57BL/6 mice. After 25 days, mice were euthanized, and subcutaneous tumors were excised. Tumor volumes were measured with calipers and calculated using the formula: $V = (L \times W^2)/2$ (V, tumor volume; L, length; W, width).

Western blotting

Cells were lysed in RIPA buffer (Beyotime, Shanghai, China) supplemented with a protease and phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Protein concentrations were

determined using a BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Equal amounts of protein (30–80 μg) were separated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Burlington, MA, USA). Membranes were blocked with 5% non-fat dry milk in TBST for 2 h at room temperature, followed by overnight incubation at 4 °C with primary antibodies. After washing, membranes were incubated with appropriate HRP-conjugated secondary antibodies, and signals were visualized using an ImageQuant LAS 4000 Mini system (GE Healthcare, Chicago, IL, USA).

Quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and 800 ng of RNA was reverse transcribed into cDNA using PrimeScript RT Master Mix (Takara, Shiga, Japan) according to the manufacturer's instructions. Quantitative real-time PCR was performed using SYBR Green PCR Master Mix (Takara) on a real-time PCR system (Bio-Rad, Hercules, CA, USA). Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method[10]. The primer sequences used in this study are listed in **Supplementary Table 2**.

RNA immunoprecipitation

RIP was performed per the manufacturer's protocol using a Magna MeRIP Kit (17-10499, Merck Millipore, USA), and the immunoprecipitated RNA was analyzed using qRT-PCR.

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