**Transfection**

GLS-AS siRNAs(siGLS-AS), glutaminase siRNAs (siGLS), ADAR1 siRNAs (siADAR1), Dicer siRNAs (siDicer), Myc siRNAs (siMyc) and corresponding negative control were purchased from Ribobio Co. (Guangzhou, China). LncRNA- AK123493.1-001 pcDNA3.1 plasmid (GLS-AS overexpression plasmid) and the control vector were purchased from Genechem Co. (Shanghai, China). All of the small interference RNAs were transfected at a final concentration of 50nM, while and the plasmid was transfected at a final concentration with 0.2μg for 96 well plate and 1.6μg for 12 well plate. Lipofectamine 2000 (Invitrogen, USA) was used for cell transfection according to the manufacturer’s instructions. What’s more, the lentiviral vector containing GLS-AS, siGLS-AS, and negative control sequences were also bought from Genechem Co. (Shanghai, China). The lentiviral vector containing sequences were transfected into the pancreatic cancer cells according to the manufacturer’s instructions. We extracted protein and total RNA 48h post -transfection.

**Colony formation assay**

Transfected BxPC-3 and PANC-1 cells were cultured in a 6-well plate with 500 cells per well, and sustained to grow for two weeks in complete medium with 10% FBS. Then 4% paraformaldehyde was used to fix the colonies for 30 minutes, and then stained the cells with 0.1% crystal violet. Finally, we chose three visions under a microscope and counted the number of total colonies to evaluate the results.

**Cell proliferation assay**

MTT analysis was conducted to observe cells proliferation ability. Transfected cells were seeded in 96-well plates with a number of 3000/well. After cells were incubated for 1 to 5 days, 20uL of MTT solution (0.5 mg/mL) was added to the medium of per well, and the cells were incubated for another 4 hours at 37°C. Then, the media was discarded, instead of 150ul of dimethyl sulfoxide (Sigma, USA), and then we used a microplate reader to measure the absorbance of DMSO with cells was at 570nm. All the MTT assays were repeated 3 times with five samples.

**Cell invasion and migration assay**

Cells invasion was assessed using the Matrigel Invasion Chamber of pore size 8um (Corning, shanghai, china) coated with 40μl Extracellular Matrices (Sigma, USA). In brief, a total of 5×10-4 transfected cells in medium without bovine serum albumin were seeded into the upper chamber (Sigma, USA). Medium containing 30% fetal bovine serum was placed in the lower chamber. After 12 hours, the invasive cells at the bottom of the membrane were fixed with 4% paraformaldehyde solution and then stained with 0.1% crystal violet, and cells that did not pass through the filter were removed with cotton swabs, invasive cells on the lower surface of the membrane. Migration ability were done using wound healing assay. Bxpc-3 or PANC-1 cells (1×106 cells/well) were transfected with corresponding reagents, planted into 6 well plates. And wounds were made using a 10μl plastic pipette tip. The size of wound was measured after 24 hours and 48 hours and photographed.

**Western blotting analysis**

BxPC-3 and PANC-1 cells total protein was extracted by lysis buffer containing proteinase inhibitors transfected after 48h. And The protein was denatured, separated in polyacrylamide gels, and transferred to PVDF membranes (Millipore, USA) to be marked with respective antibody. Antibodies for the Western blot were as follows: rabbit anti-GLS (Proteintech, USA), rabbit anti-Myc (Cell Signaling Technology, USA) rabbit anti-GAPDH (Proteintech, USA), rabbit anti-ADAR1(Sigma, USA), mouse anti-Dicer (Abcam, UK). Rabbit and mouse secondary antibodies were purchased from CST, USA. Band signals were visualized using ECL (Pierce, USA), collected by ChemiDocTm XRS Molecular Imager system (Bio-Rad, USA).

**Real-time qRT-PCR**

Total RNA was extracted from tissues and cells by using RNAiso Plus (TaKaRa Bio, Japan). The primer sequences were listed in Supplementary Table2. For the detection of lncRNA-AK1234.1 expression, a gene specific primer was used for reverse transcription. The PrimeScript RT Master Mix Perfect Real Time (TaKaRa Bio, Japan) was used to synthesize the first strand of cDNA, followed by qRT-PCR analysis using the SYBR Premix Ex Taq II (TaKaRa Bio, Japan). The relative expression levels of GLS-AS, GLS, ADAR1, Dicer and Myc relative RNA level were measured via the 2-ΔΔCT method, which were normalized to GAPDH. All reactions were performed in triplicate.

**Immunohistochemical**

For immunohistochemical staining, tissue paraffin sections were placed in an oven at 60 ° C for 1 hour, and then dewaxed to xylene twice, each for 10 minutes. Sections were following washed by gradient alcohol: 100%, 95%, 80%, 70%, each for 5 minutes and then distilled water: 5 minutes. We then placed the sections into citrate buffer (pH = 6.0), added to the microwave box, heated to boiling in the microwave for 10 minutes, and then remove the slide from the buffer, rinsed twice with distilled water, then rinsed with PBS. Then, one drop of 3% H2O2 was added to each slice and incubate at room temperature for 10 minutes to block the activity of endogenous peroxidase.  Further, the sections were washed with PBS solution and then incubated with anti-GLS (1:100) or anti-Myc antibody overnight at 4°C.  Further, the slides were incubated with a biotinylated anti-IgG (CST, USA) as second antibody for 30 minutes at room temperature. After sections were further washed by PBS and distilled water, freshly prepared DAB solution (diaminobenzidine) were subsequently used until the tissue sections were proper to observe.