**Supplementary Figure S1. Downregulation of GLS-AS facilitates pancreatic cancer proliferation and invasion.** (A) qPCR analyzed the knockdown efficiency of GLS-AS by the three siRNAs in BxPC-3 cells. (B) After transfected with siGLS-AS #2 or siGLS-AS #3, growth rate of the transfected BxPC-3 cells was measured by MTT assays for 5 days. (C) Colony formation assay was performed in transfected BxPC-3 cells. Relative colony number and representative images and are shown. (D) Transwell assay was conducted to observe the invasion ability of the transfected BxPC-3 cells. Histogram represents relative cell number right are the representative images. (E) Migration ability of transfected BxPC-3 cells was performed by wound healing assay. Relative wound size and representative images are shown. (F-H) BxPC-3 cells transfected with lentivirus containing sequence of siGLS-AS (LV-siGLS-AS) or empty lentivirus vector (LV-siNC) were transplanted subcutaneously into nude mice to observe tumor growth (5×106 cells per mouse). (F) A photograph of representative nude mice and tumor is presented after 3 weeks when mice were sacrificed. The tumor volumes were measured every 4 days. Two groups of tumor weights were measured individually. (G) Histogram showed number of visible liver metastases per 5 sections in each nude mouse. Representative images of livers and corresponding H&E staining section vision. (H) Histogram showed number of visible lung metastases per 5 sections in each nude mouse. The representative H&E staining section vision of lungs with metastases. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 and \*\*P< 0.01 as indicated.

**Supplementary Figure S2. Downregulation of GLS-AS apparently increased the GLS expression.** (A) BxPC-3 cells were transfected with GLS-AS siRNA (siGLS-AS) or siRNA negative control (siNC). The mRNA and protein level of GLS were analyzed by qPCR and Western blot, respectively. (B) BxPC-3 cells were transfected with GLS-AS overexpression vector (GLS-AS) or empty vector as negative control (Vector). The mRNA and protein level of GLS were analyzed by qPCR and Western blot, respectively. (C) Combined immunofluorescence of GLS protein (red) and RNA-FISH analysis of GLS-AS (green) in BxPC-3 cells transfected with siGLS-AS or ectopic GLS-AS were compared to the negative control cells individually. (D) Expression of GLS protein in the 30 paired cancer and noncancerous pancreatic (NP) tissues were analysed by Western blot. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 and \*\*P< 0.01 as indicated.

**Supplementary Figure S3. GLS-AS exert its biological function mainly through GLS.** PANC-1 and BxPC-3 cells were transfected with siNC, siGLS-AS alone or simultaneously with siGLS. (A) and (B) qPCR and Western blot analysis of GLS expression after cells were transfected as indicated for 48 hours. (C) and (D) Growth rate of the transfected PANC-1 and BxPC-3 cells was measured by MTT assays for 5 days. (E) and (F) Histogram of relative colony number and representative images of PANC-1 and BxPC-3 cells. (G) and (H) Histogram and representative images of transwell assay inPANC-1 and BxPC-3 cells. (I) and (J) Histogram and representative wound healing assay images in PANC-1 and BxPC-3 cells. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 and \*\*P< 0.01 as indicated.

**Supplementary Figure S4. The transcriptional activity of GLS promoter is not affected by GLS-AS.** (A) and (B) Luciferase activity assays were performed in BxPC-3 and PANC-1 cells transfected with pGL3 reporter vector containing GLS promoter. The luciferase density was measured when cells were co-transfected with siGLS-AS, ectopic GLS-AS, respectively.

**Supplementary Figure S5. GLS-AS inhibits GLS expression by ADAR1/Dicer-dependent RNA silencing in PANC-1 cells.** (A) The representative Western blot of the co-IP analysis with Dicer or IgG antibody validated the binding between Dicer and ADAR1 protein. (B) Relative quantification of GLS-AS and GLS pre-mRNA in RIP with Dicer or IgG antibody from cell lysis, measured by qPCR. (C) qPCR analysis of GLS-AS and GLS mRNA expression after PANC-1 cells were transfected with siDicer or negative control (siNC) for 48 hours, Western blot of GLS and Dicer protein in the transfected cells. (D) After knockdown of ADAR1 or Dicer, RIP assay was performed with Dicer antibody, relative enrichment of GLS-AS and GLS pre-mRNA were measured by qPCR. (E) After PANC-1 cells transfected with siDicer, cells were treated with α-amanitin (50uM), stability of GLS-pre-mRNA was measured by qPCR compared to time 0. (F) and (G) After knockdown or overexpression of GLS-AS, RIP assay was performed with Dicer antibody, relative enrichment of GLS-AS and GLS pre-mRNA were measured by qPCR. (H) After co-transfection with GLS-AS or siDicer, the expression of GLS and GLS-AS were examined by qPCR or Western blot, respectively. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 and \*\*P< 0.01 as indicated.

**Supplementary Figure S6. GLS-AS inhibits GLS expression via ADAR1/Dicer-dependent RNA silencing in BxPC-3 cells.** (A) Co-RNA-FISH analysis of GLS-AS (green) and GLS-pre-mRNA (red) in BxPC-3 cells. (B) Biotin-labeled RNAs containing full or part length of intron-17 were subjected to RNA-RNA pulldown assay and the pulldown GLS-AS was analysed with northern blot in BxPC-3 cells. (C) and (D) After treated with α-amanitin (50uM), stability of GLS-pre-mRNA was measured by qPCR compared to time 0 in BxPC-3 cells transfected with siGLS-AS or GLS-AS plasmid. (E) The representative Western blot of the co-IP analysis with anti-ADAR1 or IgG antibody validated the binding between ADAR1and Dicer protein. (F) RIP assay detected the relative quantification of GLS-AS and GLS pre-mRNA in RIP with ADAR1 or IgG antibodies from BxPC-3 cell lysis, measured by qPCR assays. (G) After knockdown of ADAR1 with siADAR1, the expression of GLS-AS and GLS transcription was detected by qPCR in BxPC-3 cells, Western blot assay showed the expression of GLS and ADAR1 protein in treated BxPC-3 cells. (H) After knockdown of ADAR1 or Dicer in BxPC-3 cells, RIP assay was performed with ADAR1 antibody, relative enrichment of GLS-AS and GLS pre-mRNA were measured by qPCR. (I) After BxPC-3 cells were transfected with siADAR1, cells were treated with α-amanitin (50uM), stability of GLS-pre-mRNA was measured by qPCR compared to time 0. (J) and (K) After knockdown or overexpression of GLS-AS in BxPC-3 cells, RIP assay was performed with ADAR1 antibody, relative enrichment of GLS-AS and GLS pre-mRNA were measured by qPCR. (L) After co-transfection with GLS-AS or siADAR1 in BxPC-3 cells, the expression of GLS and GLS-AS were examined by qPCR or Western blot, respectively. (M) Western blot analysis of Dicer and ADAR1 protein levels in the pulldown complex. And the GLS-AS and GLS-pre-mRNA measured by northern blot. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 and \*\*P< 0.01 as indicated.

**Supplementary Figure S7. GLS-AS inhibits GLS expression by ADAR1/Dicer-dependent RNA silencing in BxPC-3 cells.** (A) The representative Western blot of the co-IP analysis with Dicer or IgG antibody validated the binding between Dicer and ADAR1 protein. (B) Relative quantification of GLS-AS and GLS pre-mRNA in RIP with Dicer or IgG antibodies from BxPC-3 cell lysis, measured by qPCR. (C) qPCR analysis of GLS-AS and GLS mRNA expression after BxPC-3 cells were transfected with Dicer siRNA (siDicer) or siRNA negative control (siNC) for 48 hours, Western blot assay showed the expression of GLS and Dicer protein in treated BxPC-3 cells. (D) After knockdown of ADAR1 or Dicer, RIP assay was performed with Dicer antibody, relative enrichment of GLS-AS and GLS pre-mRNA were measured by qPCR. (E) After PANC-1 cells transfected with siDicer, cells were treated with α-amanitin (50uM), stability of GLS-pre-mRNA was measured by qPCR compared to time 0. (F) and (G) After knockdown or overexpression of GLS-AS, RIP assay was performed with Dicer antibody, relative enrichment of GLS-AS and GLS pre-mRNA were measured by qPCR. (H) After co-transfection with GLS-AS or siDicer in BxPC-3 cells, the expression of GLS and GLS-AS were examined by qPCR or Western blot, respectively. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 and \*\*P< 0.01 as indicated.

**Supplementary Figure S8. Schematic diagram of RNA pulldown by MS2-GST.** pMS2 (a plasmid expressing MS2 RNA with hairpins), pMS2-BP-GST-NSL (a plasmid expressing a fusion protein consisting of MS2 RNA binding protein, glutathione S-transferase (GST) and a nuclear localization signal (NSL)). These plasmids were co-transfected as depicted in the schematic, and then cell lysate was used for the pulldown analysis.

**Supplementary Figure S9. ADAR1 and Dicer are not significantly varied in pancreatic cancer cells under glucose or glutamine deprivation.** (A), (B), (D) and (E) qPCR analysis of ADAR1 and Dicer mRNA levels after glutamine or glucose was deprived in PANC-1 and BxPC-3 cells. (C) and (F) Western blot analysis of ADAR1 and Dicer protein levels after glutamine or glucose was deprived in PANC-1 and BxPC-3 cells. All data were presented as means ± SD of at least three independent experiments. N.S. means “no significant”.

**Supplementary Figure S10. Downregulation of GLS-AS in pancreatic cancer might attributed to energy stress through Myc-depending regulation.**

 (A) Western blot analysis of Myc protein in BxPC-3 and PANC-1 cells during glucose or glutamine deprivation. (B) The binding capability between Myc and site-4 on GLS-AS promoter in BxPC-3 and PANC-1 cells were compared by ChIP analysis during glutamine or glucose deprivation. (C) Luciferase activity of BxPC-3 and PANC-1 cells containing WT or MUT was measured during normal condition glutamine or glucose starvation. (D) After treatment with siMyc during glutamine or glucose deprivation in BxPC-3 and PANC-1 cells, the expression of GLS-AS transcript and GLS protein were analyzed by qPCR and Western blot, respectively. (E) BxPC-3 or PANC-1 cells were co-transfected with GLS-AS or Myc overexpression plasmid as indicated, and then cultured in glutamine or glucose deprivation medium for 48 hours. Western blot analysis of GLS and Myc protein were conducted. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 as indicated, N.S. means “no significant”.

**Supplementary Figure S11. GLS and GLS-AS level does not affect Myc mRNA expression in pancreatic cancer cells.** (A) and (B) qPCR analysis of Myc mRNA expression after GLS knockdown or GLS-AS overexpression in BxPC-3 and PANC-1 cells. Values are significant at \*P < 0.05 as indicated, N.S. means “no significant”.

**Supplementary Figure S12. GLS-AS is conversely correlated with Myc and GLS expression in pancreatic cancer.** (A) The expression of GLS mRNA in selected samples including 30 PC and NP tissues. (B) Pearson correlation analysis of GLS-AS and GLS mRNA. (C) The expression of Myc mRNA in selected samples including 30 PC and NP tissues. (D) Pearson correlation analysis of Myc and GLS mRNA. (E) Representative images of immuno-histochemical analysis of Myc and GLS protein in selected pancreatic cancer (PC) and noncancerous pancreatic (NP) tissues. (F) and (G) Inspection of TCGA pancreatic adenocarcinoma QCMG (Nature 2016) and Pancreatic Adenocarcinoma (TCGA, Provisional) through cBioportal. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*\*P< 0.01 as indicated.

**Supplementary Figure S13. GLS-AS suppresses pancreatic cancer cell growth, invasion and migration in vitro**. BxPC-3 and PANC-1 cells were transfected with GLS-AS overexpression plasmid (GLS-AS) or the empty vector (Vector) as control. (A) Growth rate of the transfected cells was measured by MTT assays for 5 days. (B) Colony formation assay was performed in transfected cells. Relative colony number and representative images are shown. (C) Transwell assay of the transfected cells. The histogram represents relative cell number and the representative images are shown. (D) Wound healing assay of cells after transfection as indicated, statistics histogram and representative images are shown. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05 and \*\*P< 0.01 as indicated.

**Supplementary Figure S14. GLS-AS inhibits the progression of the pancreatic cancer in vivo proven with PANC-1 cells.** PANC-1 cells transfected with lentivirus containing sequence of GLS-AS overexpression (LV-GLS-AS) or empty lentivirus vector (LV-Vector) were transplanted subcutaneously into nude mice to observe tumor growth (5×106 cells per mouse). (A) A photograph of representative nude mice and tumor is presented after 3 weeks when mice were sacrificed (left panel). The tumor volumes were measured every 4 days (middle panel). Two groups of tumor weights were measured individually (right panel). (B) Left histogram shows number of visible liver metastases per 5 sections in each nude mouse while right panel are representative images of general livers and corresponding H&E staining section vision. (C) Histogram (left panel) shows number of visible lung metastases per 5 sections in each nude mouse while the photographs of H&E staining section (right panel) are the representative. (D) and (E) qPCR of GLS-AS and GLS transcripts in subcutaneously implanted tumors of each group. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05, \*\*P< 0.01 as indicated.

**Supplementary Figure S15. GLS-AS inhibits the progression of the pancreatic cancer in vivo proven with BxPC-3 cells.** (A-E) BxPC-3 cells transfected with lentivirus containing sequence of GLS-AS overexpression (LV-GLS-AS) or empty lentivirus vector (LV-Vector) were transplanted subcutaneously into nude mice to observe tumor growth (5×106 cells per mouse). (A) A photograph of representative nude mice and tumor is presented after 3 weeks when mice were sacrificed. The tumor volumes were measured every 4 days. Two groups of tumor weights were measured individually. (B) Histogram shows number of visible liver metastases per 5 sections in each nude mouse while representative images are general livers and corresponding H&E staining section vision. (C) Histogram shows number of visible lung metastases per 5 sections in each nude mouse while the photographs of H&E staining section are the representative. (D), (E) qPCR of GLS-AS and GLS transcripts in subcutaneously implanted tumors of each group. All data were presented as means ± SD of at least three independent experiments. Values are significant at \*P < 0.05, \*\*P< 0.01 as indicated.