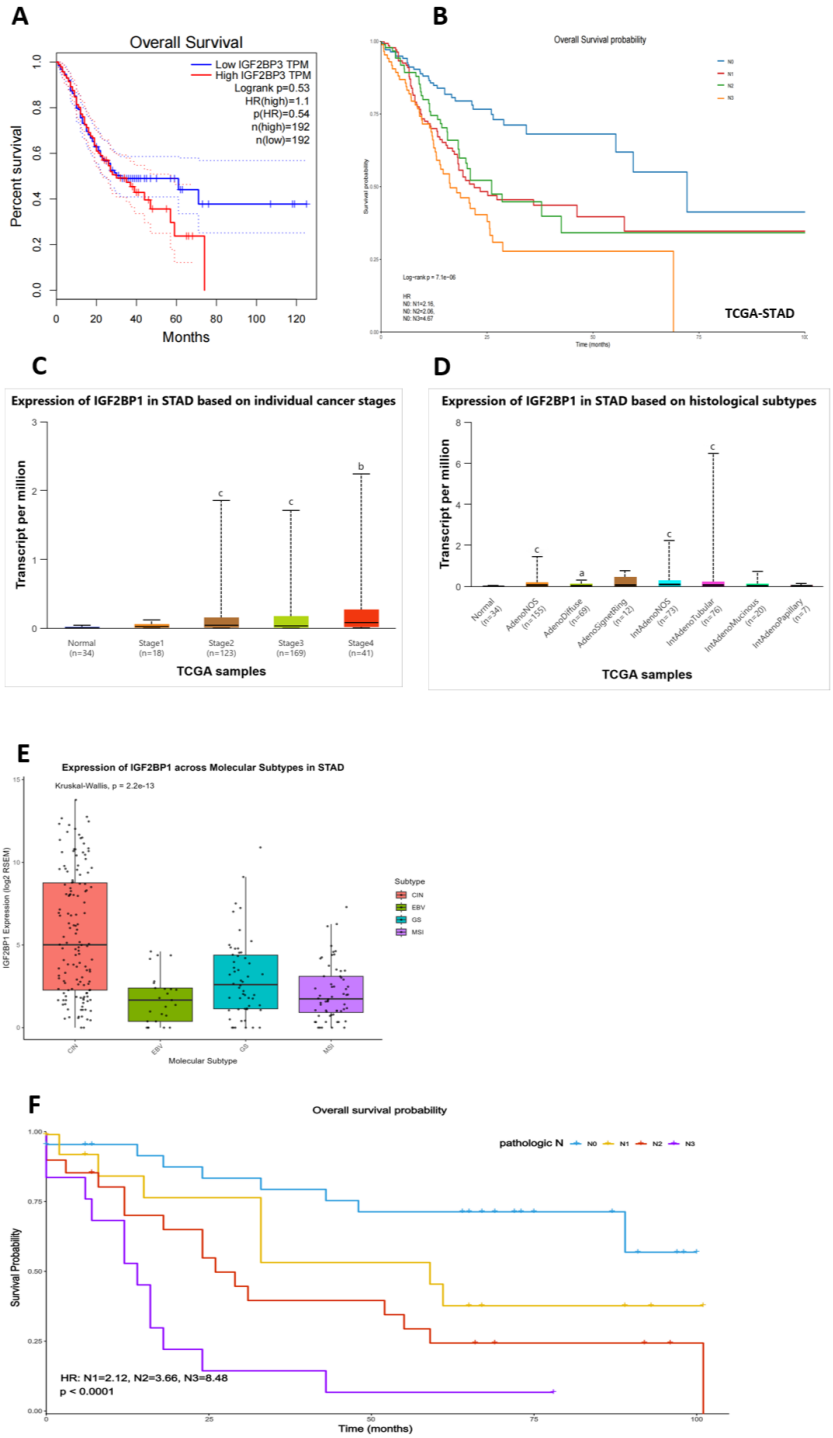
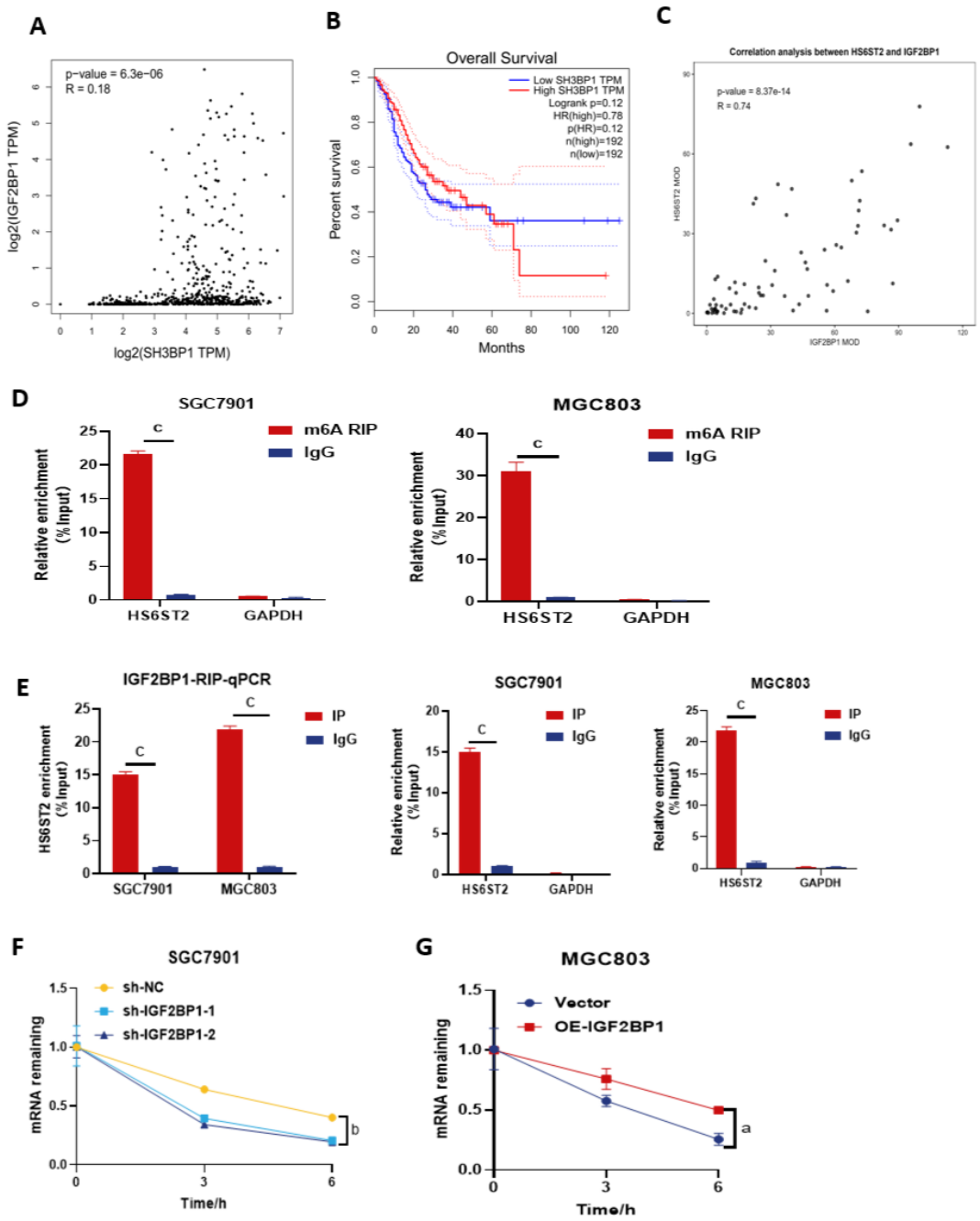


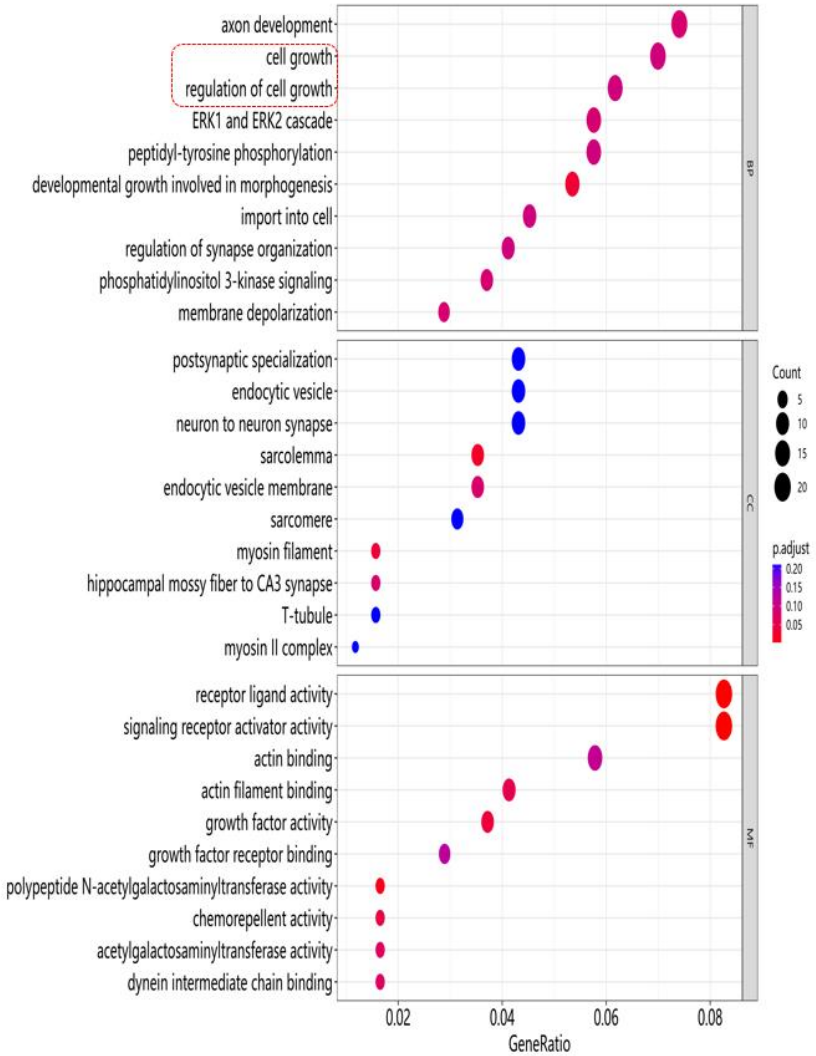
Supplementary Figure 1 IGF2BP1 is highly expressed in GC tissues and correlates with lymph node metastasis. A: Kaplan-Meier survival analysis (TCGA-STAD) shows no significant correlation between IGF2BP3 expression levels and overall survival in gastric cancer patients;B: Kaplan-Meier survival analysis (TCGA-STAD) shows that lymph node status(N stage)is significantly associated with patient overall survival; C**:** Box plot showing the transcript

levels of IGF2BP1 in gastric cancer tissues at different clinical stages (I - IV) and normal tissues, based on TCGA - STAD; D: Box plot analysis of IGF2BP1 transcript levels across different Lauren classifications of GC; E: Box plot showing the expression levels of IGF2BP1 across the four major molecular subtypes (CIN, EBV, GS, MSI) in the TCGA STAD cohort; C: Kaplan-Meier survival analysis based on tissue microarray shows significant differences in overall survival among patients with different pathological N stages. Statistical significance was determined by the Kruskal - Wallis test. Data are expressed as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.



Supplementary Figure 2 IGF2BP1 enhances HS6ST2 expression via an m6A-dependent mechanism to promote poor prognosis. A: Correlation analysis of the TCGA-STAD dataset shows a significant positive correlation between IGF2BP1 and SH3BP1 mRNA expression levels; B: Kaplan-Meier survival analysis(TCGA-STAD)shows no significant correlation between SH3BP1 expression levels and overall survival in gastric cancer patients; G: Scatter plot of Pearson correlation analysis between IGF2BP1 and HS6ST2 protein expression levels in clinical gastric cancer samples; C: MeRIP -qPCR

assay showing the enrichment of m6A modification on HS6ST2 mRNA in SGC7901 and MGC803 cells; D: IGF2BP1 RIP- qPCR assay confirming the direct binding between IGF2BP1 protein and HS6ST2 mRNA; E and F: Effect of IGF2BP1 knockdown (E) or overexpression (F) on the stability of HS6ST2 mRNA, assessed by Actinomycin D assay in SGC7901 and MGC803 cells. Statistical significance was determined by the Kruskal - Wallis test. Data are expressed as mean ± SD. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.



Supplementary Figure 3 KEGG pathway enrichment analysis of differentially expressed genes upon HS6ST2 knockdown.

Some detail methods:

Database Analysis

The data analyzed in this study were sourced from multiple public databases.Clinical and transcriptomic profiles of stomach adenocarcinoma(STAD)patients were obtained from The Cancer Genome Atlas(TCGA)and the Gene Expression Omnibus(GEO)under accession numbers GSE163310 and GSE90639.Expression data of IGF2BP1 mRNA in GC cell lines came from the DepMap database(Public 22Q4).Similarity-based RNA Adenosine Methylation Prediction(SRAMP),a tool based on a random forest machine learning framework,was used to predict potential m6A modification sites based on sequence features.

Survival Analysis

STAD patients were divided into groups with high and low expression based on the median expression value of IGF2BP1,HS6ST2,and SH3BP1.Survival differences between the two groups were initially compared using the"survminer"package in R,based on gene expression values,survival time,and survival status.The significance of differences in survival rates was validated through Kaplan-Meier analysis and the log-rank test.

qRT-PCR

RIBOBIO(Guangzhou,China)made the primer sequences used for qRT-PCR.They are listed below:

GAPDH:Forward5'-GAACGGGAAGCTCACTGG-3',

Reverse 5'-GCCTGCTTCACCACCTTCT-3'

IGF2BP1:Forward5'-GGGCCATCGAGAATTGTTGC-3',

Reverse5'-CGGGAGCCTGCATAAAGGAG-3'

HS6ST2:Forward5'-TGCTCTTCCTATTTGCCGTGA-3',

Reverse 5'-ATTGTAGCGGGGCACGAAC-3'

RNA Sequencing(RNA-seq)

We used Trizol reagent(Ambion,Invitrogen,USA)to get total RNA from stable cells.RNA concentration and purity were measured spectrophotometrically.RNA sequencing and subsequent bio-informatics analysis were performed by Cosmos Wisdom(Hangzhou,China).

MeRIP-qPCR

Sonication broke down 50-100 *g* of total RNA into about 100 *nt* pieces for each reaction.The fragmented RNA was dissolved in 400μL of IP buffer containing an RNase inhibitor.Immunoprecipitation was carried out by incubating the RNA fragments with an m6A-specific antibody(1 mg/ml)or an equivalent amount of normal IgG(1 mg/ml)as a control at 4°C overnight.The bead-antibody-RNA complexes were washed three times with IP buffer.To get rid of bound RNA,we utilized elution buffer with Proteinase K for 30 minutes at 55°C.Then,we purified it and made cDNA from it.qRT-PCR was applied to measure the enrichment of certain RNA fragments and compare them to the input control.qRT-PCR was applied to measure the enrichment of certain RNA fragments and compare them to the input control.

Western blot analysis

The primary antibodies used as follow: β-actin (AB0035; Abways, Shanghai, China), GAPDH (AB0037; Abways), m6A (ab151230; Abcam), IGF2BP1 (ab184305; Abcam), HS6ST2 (ab122220; Abcam), phosphoinositide 3-kinase (PI3K) (YM8045; Immunoway, Plano, TX, United States), phospho-PI3K (YP0765; Immunoway), Akt (YT0185; Immunoway), phospho-Akt (YP0006; Immunoway), mTOR (YT2913; Immunoway), and phospho-mTOR (YP0176; Immunoway).