Supplementary data

Supplementary materials and methods

Cell culture, transfection, and viral transduction

Human HUVEC and HEK293T cells and the human gastric cancer (GC) cell lines SGC7901, BGC823, HGC-27, and MGC803 were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The human GC cell lines AGS and NCI-N87 were purchased from ATCC (MD, USA). HUVEC, SGC7901, BGC823, HGC-27, and NCI-N87 cell lines were cultured in RPMI-1640 medium. HEK293T and MGC803 cells were cultured in DMEM, and AGS cells were cultured in F12K medium. siRNA and plasmid transfections were performed using DharmaFECT4 (GE Healthcare) and Lipofectamine 3000 (Invitrogen). Virus-containing supernatant was collected 48 h after co-transfection of pCMV-VSV-G, pUMVC or pCMV-Δ8.2 and shRNA-containing, ORF-containing or pBabe-Luciferase plasmids into HEK293T cells and was then added to the target cells. 48 h later, infected cells were selected with 1 μg/ml puromycin (Gibco, for the pGLV3/H1/GFP/puromycin lentiviral vectors) or 100 μg/ml hygromycin B (Gibco, for the pBabe retroviral vector). BGC823 cells expressing luciferase (BGC823-luc) were generated as described above. All cells were cultured with 10% foetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in an incubator with 5% CO_2 at 37 °C.

siRNA, shRNA, sgRNA, and plasmid constructs

All siRNAs were designed and synthesized by RiboBio (Guangzhou, China), and the siRNA sequences are listed in supplementary table 2. METTL3, HDGF, and IGF2BP3 shRNAs designed based on the siRNA sequences and METTL3 cDNA were cloned into the pGLV3/H1/GFP and GV358 lentiviral vectors (GenePharma, Shanghai, China) or pBabe retroviral vector, respectively. To generate HGC-27 METTL3 knockout (KO-METTL3) cells, METTL3 sgRNA (sgRNA sequence: GAGCTTGGAATGGTCAGCATAGG) was designed and cloned into a plasmid co-expressing sgRNA and Cas9 (px330, YSY Biotech, Nanjing, China). Wild-type

METTL3 plasmid was purchased from addgene (catalog number: #53739, Watertown, MA, USA), the catalytic mutant (aa395-398, DPPW→APPA) METTL3 was constructed based on the wild-type METTL3, and HDGF cDNA was cloned into the pcDNA3.1 vector.

Patients and specimens

All patients in the GC cohort with gastric carcinoma treated with radical gastrectomy with or without adjuvant chemotherapy during the observation periods were included. The exclusion criteria were as follows: previous GC or active non-GC and treatment with preoperative chemotherapy or radiotherapy. The cohort included 83 patients who underwent radical gastrectomy from May 1, 1990 to June 1, 1995, which was described in our previous study [1, 2]. The median survival time was 28 months (range: 1-60 months). Gastric cancer tissues and matched normal gastric mucosal tissue were obtained from these patients. Additionally, 54 pathologically confirmed GC and the associated non-cancerous fresh-frozen gastric mucosal tissues from recent patients at the Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School (Nanjing, Jiangsu, China), were obtained for qRT-PCR, western blotting, dot blotting, immunohistochemistry (IHC) assay and GC organoid culture after signed informed consent was provided.

Tissue microarray (TMA) construction and IHC assay

The GC TMAs were created by a contract service at the National Engineering Center for Biochips in Shanghai, China, and a standard protocol was used for IHC on the TMAs, as described in a previous study [1]. Staining of METTL3 in the tissue was scored independently by two pathologists blinded to the clinical data by applying a semi-quantitative immunoreactivity score (IRS) in the cohort, as reported previously [1]. Under these conditions, samples with an IRS of 0-6 and an IRS of 8-12 were classified as having low and high expression of METTL3, respectively.

RNA sequencing (RNA-seq) and m^{6}A-modified RNA immunoprecipitation

sequencing (MeRIP-seq)

For RNA-seq, total RNA was first extracted from GC cells with stable METTL3 overexpression or knockout and their corresponding vector-transfected cells. The quality and quantity of the RNA were assessed by a NanoDrop™ ND-1000. Denaturing agarose gel electrophoresis was used to assess RNA integrity. The mRNA extraction was performed using a NEBNextR Poly(A) mRNA Magnetic Isolation Module. RNA libraries were constructed using a KAPA Stranded RNA-Seq Library Prep Kit (Illumina). Libraries were sequenced using Illumina HiSeq 4000 platforms.

For MeRIP-seq, the amount of purified total RNA was greater than 120 μg, and the integrity and quantity of each RNA sample were assessed using agarose gel electrophoresis and a NanoDrop™ instrument. Intact mRNA was first isolated from total RNA samples using an Arraystar Seq-StarTM poly(A) mRNA Isolation Kit according to the manufacturer's protocol. The isolated mRNA was chemically fragmented into 100-nucleotide-long fragments by incubation in fragmentation buffer (10 mM Zn^{2+} and 10 mM Tris-HCl, pH 7.0), and the size of the fragmented mRNA was confirmed using agarose gel electrophoresis. Then, m^6A-methylated mRNAs were immunoprecipitated with an anti-N^6-methyladenosine (m^6A) antibody (an aliquot of the fragmented mRNAs was kept as input). The major procedures included immunoprecipitation, washing, and elution. The eluted m^6A mRNA fragments were then concentrated for RNA-seq library construction. RNA-seq libraries for the m^6A antibody-enriched mRNAs and input mRNAs were prepared using a KAPA Stranded mRNA-seq Kit (Illumina). The prepared libraries were diluted to a final concentration of 8 pM, and clusters were generated on an Illumina cBot using a HiSeq 3000/4000 PE Cluster Kit (#PE-410-1001, Illumina), followed by sequencing on the Illumina HiSeq 4000.

For MeRIP-seq data analysis, the raw reads were trimmed by Trimmomatic software and aligned to Ensembl reference genome by HISAT2 software (v2.1.0). The differentially m^6A-RIP-enriched regions (peaks) between METTL3 and corresponding control group were analyzed by exomePeak software. These differential peaks are annotated using the latest Ensembl database. Sequence motifs are one of the basic

functional units of molecular evolution. The algorithms of Multiple EM for Motif Elicitation (MEME) and Discriminative Regular Expression Motif Elicitation (DREME) were used to fid motifs among the m^{6}A peak sequences.

Dot blot assay

The dot blot assay was performed according to the bio-protocol database (https://en.bio-protocol.org/e2095). Briefly, mRNA was isolated from total RNA and denatured; 100 ng of mRNA was then spotted onto a Hybond-N+ membrane and cross-linked. Then, the membrane was washed and incubated first in blocking buffer and then with an anti-m^{6}A antibody (1:1000; Abcam, USA) overnight at 4 °C with gentle shaking. Then, the membrane was washed, incubated with an anti-mouse antibody, and washed again. Finally, the membrane was exposed to Hyperfilm ECL, and images were acquired. Methylene blue (MB) was used to interact with mRNA and as the loading control.

Western blot assay

Western blot assays were performed as previously reported [1]. The antibodies used are listed in supplementary table 3.

ELISA assay

The m^{6}A levels in cancerous tissues and paired normal gastric mucosa from GC patients was detected by colorimetric ELISA-like assay via the m^{6}A RNA Methylation Quantification Kit according to the manufacturer's instructions (Epigentek, USA). HDGF level in conditioned medium from different GC cells was detected using Human HDGF ELISA Kit (elabscience, Wuhan, China).

Quantitative real-time RT-PCR (qRT-PCR)

Total RNA was extracted from cells or tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription (RT) was performed with HiScript Q RT SuperMix for qPCR (Vazyme, Jiangsu, China). RT-PCR was performed with an SYBR

Green PCR Kit (Vazyme, Jiangsu, China) on an Applied Biosystems 7900HT sequence detection system (Applied Biosystems), with triplicate reactions. The primers used are listed in supplementary table 4.

Proliferation assay

For CCK8 assay, one day before treatment, the cells were plated at a density of 8000 cells per well in 96-well plates. The cells were treated with C646 at 20 μM for 24 h. After the indicated time, cell viability was determined by using the CCK-8 assay according to the manufacturer's instructions (Dojindo, Kumamoto, Japan).

The soft agar colony formation assay was performed according to the bio-protocol database (https://en.bio-protocol.org/e2351). Briefly, the procedure included preparation of a 5% agar solution, production of the bottom layer of agar, preparation of a GC cell suspension, production of the upper layer of agar, and colony counting after 2 weeks.

For the clonogenic assay, GC cells were seeded in 6-well plates (300 cells per well) and incubated at 37 °C for 10-14 days. Then, cells were fixed with methanol for 20 min and stained with crystal violet (Beyotime, Shanghai, China) for 30 min.

Transwell assay and HUVEC tube formation assay

The cell migration and invasion capacities were evaluated by Transwell chambers, as previously reported [1, 3]. Briefly, GC cells in 100 μl of serum-free medium were seeded in the upper chambers coated with or without 50 μl of Matrigel (BD Biosciences), and 600 μl of culture medium containing 10% FBS was placed in the lower chambers. After 12 h of incubation at 37 °C, cells that migrated to the bottom of the membrane were fixed with 4% paraformaldehyde for 20 min, stained with crystal violet (Beyotime, Shanghai, China) for 30 min, and imaged.

For the HUVEC tube formation assay, GC cells were cultured in 6-well plates for 48 h, and conditioned medium was collected. HUVECs (1×10^4) were seeded in 96-well plates coated with 50 μl of Matrigel™ (BD Biosciences) and cultured in conditioned medium for 24 h. Images of tube photos were acquired under a microscope,

and the tubular structures were counted in 3 random fields.

Immunofluorescence (IF) assay

The details of the IF assay have been described previously [1]. Briefly, HGC-27 WT and KO#1 GC cells were incubated with an anti-HDGF antibody (1:300, Proteintech, Chicago, USA) at 4 °C overnight and then with corresponding Alexa Fluor-labelled secondary antibodies (Beyotime, Shanghai, China) at a 1:500 dilution for another 1 h at room temperature. Next, cells were incubated with DAPI (Beyotime, Shanghai, China) for 5 min. Images of the cells were acquired with a LEICA DMi8 system.

RNA immunoprecipitation (RIP)

The RIP assay was performed using a MagnaRIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA, USA) according to the manufacturer's instructions. Briefly, the corresponding cell lysates were incubated with beads coated with 5 μg of control IgG antibody (Beyotime, Shanghai, China), anti-m^6A antibody (Abcam, MA, USA), or anti-IGF2BP3 antibody (Abcam, MA, USA) with rotation at 4 °C overnight. Next, total RNA was extracted for the detection of HDGF expression by qRT-PCR.

Chromatin immunoprecipitation (ChIP)

The ChIP assay was performed using a ChIP assay kit (SimpleChIP® Plus Sonication Chromatin IP Kit #56383, Cell Signaling Technology, MA, USA). Briefly, GC cells were fixed with 1% formaldehyde; quenched with glycine at room temperature; and collected, washed, and resuspended in lysis buffer. The sonicated chromatin solution was immunoprecipitated with anti-P300 (Abcam, MA, USA), anti-H3K27ac (Abcam, MA, USA), and anti-HDGF (Proteintech, Chicago, USA) antibodies. Immunoprecipitated DNA was purified and analysed by qRT-PCR. The ChIP-specific primers used are shown in supplementary table 4.

Animal studies

BALB/c male nude mice (5-6 weeks old) were purchased from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, Jiangsu, China) and maintained in SPF facilities. Tumour xenograft models were established in nude mice bearing: (1) BGC823 cells stably transfected with METTL3 overexpression or lentiviral vectors (Genechem, Shanghai, China); (2) AGS cells stably transfected with METTL3 overexpression, METTL3 overexpression with HDGF knockdown, or lentiviral vectors (Genechem, Shanghai, China); (3) NCI-N87 cells stably transfected with METTL3-, HDGF-, or IGF2BP3-shRNA and the corresponding control vector. The different GC cells (5×10^6) were subcutaneously injected into the right axilla of nude mice (n=6 per group). Tumour volume was monitored every other day (volume = length × $width^2$ × 1/2). At the end of the experiment, the mice were sacrificed, and the tumours were weighed and imaged and were then fixed in 4% paraformaldehyde or frozen for further analyses.

To assess the function of METTL3 in liver metastasis, BGC823-luc cells (1×10^6) stably transfected with METTL3 overexpression or lentiviral vectors were injected into the splenic vein of nude mice (n=6 per group). Liver metastasis was examined by bioluminescence imaging weekly; the detailed experimental procedures have been described previously [4]. After 8 weeks, the mice were sacrificed, and the livers were resected, photographed, and fixed in 4% paraformaldehyde for further analyses. In addition, METTL3-deficient NCI-N87 cells (1×10^6) and the corresponding control cells were injected into the splenic vein of nude mice (n=6 per group). After 4 weeks, the mice were sacrificed, and the livers were resected, photographed, and fixed for further analyses.

Organoid culture

This study was first approved by the Institutional Review Board of Nanjing Drum Tower Hospital. The standard procedure for organoid culture has been described previously [5, 6]. Briefly, approximately 1 cm^2 of GC tissues from 3 different GC patients were minced, placed in a 10 cm Petri dish, covered with cold 1× chelating buffer (5.6 mM Na_2HPO_4, 8.0 mM KH_2PO_4, 96.2 mM NaCl, 1.6 mM KCl, 43.4 mM

sucrose, 54.9 mM D-sorbitol, and 0.5 mM DL-dithiothreitol (pH=7)), and cut into 20-50 small pieces of approximately 2-5 mm² in size. A glass microscopy slide was placed on top of the tissue pieces and pressed, and 10 ml of cold basal medium (Advanced DMEM/F12 supplemented with HEPES, Glutamax and 1× Primocin) was then added. The samples were centrifuged for 5 min at 200 × g and 4 °C. The supernatant was discarded, and approximately 100 glands per 50 μl of basement matrix were seeded in one well of a 24-well plate warmed to 37 °C. The plate was carefully transferred back to the cell culture incubator, and 500 μl of medium containing all growth factors (50 ng/ml EGF, 100 ng/ml noggin, 1 μg/ml R-spondin1, 50% Wnt-conditioned medium, 200 ng/ml FGF10, 1 nM gastrin, 2 μM TGF-beta inhibitor and 10 μM RHOKi) was carefully added to each well without disturbing the basement matrix. The medium was refreshed 3 times per week. After organoids formed, they were minced into pieces, redigested, and transfected with METTL3 overexpression or the corresponding control lentiviral vectors for 8 h. Then, the organoids were seeded into 24-well plates for 2 weeks, and organoid images were acquired with a LEICA DMi8 system.

Glycolysis assay

For the glucose uptake assay, GC cells were first seeded in 6-well plates. After 12 h, cells were refreshed with glucose-free DMEM supplemented with 10% dialyzed FBS. After 18 h, cells were treated with 2-NBDG (20 μM; Invitrogen) for 2 h, and glucose uptake was quantified using FACS analysis. Lactate production was determined using a lactic acid assay kit (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The extracellular acidification rate (ECAR) was detected by a Seahorse Bioscience XF-24 Extracellular Flux Analyzer following the manufacturer’s instructions. Briefly, GC cells were seeded and cultured in XF24-well cell culture microplates (Seahorse Bioscience) for 24 h, and glycolytic activity was assessed using a Seahorse XF glycolysis stress test kit (Agilent). Sequential injections of compounds (glucose, oligomycin A and 2-DG) were used to measure glycolytic activity.

Statistical analysis

Associations between METTL3 expression and demographic and clinicopathological features were evaluated by Pearson Chi-Square test. The differences in IRS for METTL3 staining in primary tumours and the corresponding non-tumour tissues were assessed by the Wilcoxon test (grouped). The probability of differences in the OS was ascertained by the Kaplan-Meier method with a log-rank test for significance. Univariate or multivariate Cox regression analysis was used to estimate the hazard ratios (HR) and the associated 95% confidence intervals (CI). Then, we analysed the predictive value of the parameters using time-dependent ROC curve analysis for censored data and calculated the AUC of the ROC curves as previously reported [1]. Experiments were performed at least three times. The representative data shown are means ± SEM.; $P < 0.05$ was considered statistically significant. All statistical analyses were performed with Statistical Analysis System software (version 9.1.3; SAS Institute, Cary, NC), STATA statistical software (version 10.1; Stata Corp, College Station, TX, USA), and R software (version 2.10.1; The R Foundation for Statistical Computing).

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Supplementary Table S1: Relationship between expression level of METTL3 and demographic and clinicopathological features of GC patients

Variables	METTL3 expression (n = 83 cases)		
	Low (%)	High (%)	*P*[a]
All patients	44 (53.0)	39 (47.0)	
Age (years)			0.948
≤65	37 (84.1)	33 (84.6)	
>65	7 (15.9)	6 (15.4)	
Gender			0.505
Males	31 (70.5)	30 (76.9)	
Females	13 (29.5)	9 (23.1)	
Depth of invasion			0.402
T1/T2	15 (34.1)	10 (25.6)	
T3/T4	29 (65.9)	29 (74.4)	
Lymph node metastasis			0.046
N0	18 (40.9)	8 (20.5)	
N1/N2/N3	26 (59.1)	31 (79.5)	
Distant metastasis			0.059
M0	38 (86.4)	27 (69.2)	
M1	6 (13.6)	12 (30.8)	
TNM stage			0.005
I	9 (20.4)	5 (12.8)	
II	19 (43.2)	5 (12.8)	
III	8 (18.2)	16 (41.0)	
IV	8 (18.2)	13 (33.4)	
Tumor diameter (cm)			0.118
≤5	31 (70.5)	21 (53.8)	
>5	13 (29.5)	18 (46.2)	
Histological type			0.171
Intestinal	16 (36.4)	20 (51.3)	
Diffuse	28 (63.6)	19 (48.7)	

[a]Pearson Chi-Square tests

Supplementary Table S2：The sequences of siRNAs

siRNAs	Sequences (5'-3')
P300 siRNA#1	CGACTTACCAGATGAATTA
P300 siRNA#2	GCACAAATGTCTAGTTCTT
METTL3 siRNA#1	CGACTACAGTAGCTGCCTT
METTL3 siRNA#2	CTGCAAGTATGTTCACTATGA
IGF2BP1 siRNA#1	GGCTCAGTATGGTACAGTA
IGF2BP1 siRNA#2	TGAAGATCCTGGCCCATAA
IGF2BP2 siRNA#1	CATGCCGCATGATTCTTGA
IGF2BP2 siRNA#2	GAACGAACTGCAGAACTTA
IGF2BP3 siRNA#1	GCTGAGAAGTCGATTACTA
IGF2BP3 siRNA#2	TAAGGAAGCTCAAGATATA
HDGF siRNA#1	GATCGAGAACAACCCTACT
HDGF siRNA#2	CAGGCATCAGAGATCATGA
HDGF siRNA#3	CTATGGAGGTGGAAAAGAA

Supplementary Table S3: Antibodies for western blot, ChIP, and IHC

Antibody name	Source	Item number
m^6A	abcam	ab208577
METTL3	proteintech	15073-1-AP
H3K27ac	abcam	ab4729
Histone-H3	proteintech	17168-1-AP
P300	abcam	ab14984
Ki-67	servicebio	GB13030-2
CD31	abcam	ab9498
HDGF	proteintech	11344-1-AP
GAPDH	beyotime	AG109
β-actin	beyotime	AA128
α-tubulin	beyotime	AT819
Rabbit IgG	beyotime	A7016
Mouse IgG	beyotime	A7028
IGF2BP3	abcam	ab177477
HRP mouse antibody	beyotime	A0216
HRP rabbit antibody	beyotime	A0208
IHC mouse antibody	servicebio	G1210-2-A
IHC rabbit antibody	servicebio	G1210-2-A
GLUT4	proteintech	21048-1-AP
ENO2	proteintech	55235-1-AP
Flag	sigma	F1804

Supplementary Table S4: The primer sequences for qRT-PCR and ChIP

Primer names	Sequences (5'-3')
For qRT-PCR	
GAPDH F	CATGTGGGCCATGAGGTCCACCAC
GAPDH R	GGGAAGCTCACTGGCATGGCCTTCC
METTL3 F	ATCCCCAAGGCTTCAACCAG
METTL3 R	AGGGTGATCCAGTTGGGTTG
METTL14 F	GACGGGGACTTCATTCATGC
METTL14 R	CCAGCCTGGTCGAATTGTAC
WTAP F	GCTTCTGCCTGGAGAGGATT
WTAP R	GTGTACTTGCCCTCCAAAGC
FTO F	AGACACCTGGTTTGGCGATA
FTO R	CCAAGGTTCCTGTTGAGCAC
ALKBH5 F	ACCCCATCCACATCTTCGAG
ALKBH5 R	CTTGATGTCCTGAGGCCGTA
P300 F	GCAGTGTGCCAAACCAGATG
P300 R	CATAGCCCATAGGCGGGTTG
HDGF R	CCCAGAAAAAGAGCTGTGTGG
HDGF F	AGGAGAGTCCTCCAGCAAGT
IGF2BP1 F	AGCTCCTTTATGCAGGCTCC
IGF2BP1 R	CCGGGAGAGCTGTTTGATGT
IGF2BP2 F	TCTTTGGGGACAGGAAGCTG
IGF2BP2 R	CCACTTTACCCGAGAGGGTC
IGF2BP3 F	ACTGCACGGGAAACCCATAG
IGF2BP3 R	CCAGCACCTCCCACTGTAAAT
CDKN2A F	CTTGGTGACCCTCCGGATTC
CDKN3A R	TCATCATGACCTGGTCTTCTAGG
C12orf45 F	CGAACTCCCTTCGTACCTCC
C12orf45 R	CAGTCGTCGAGGAACCTGTG
GLUT1 F	TTCACTGTCGTGTCGCTGTT
GLUT1 R	GGCCACGATGCTCAGATAGG
GLUT2 F	GCCACACTCACACAAGACCT
GLUT2 R	TTATTACCTGTTGAGGTGCATTGA
GLUT4 F	CGTCTCCATTGTGGCCATCT
GLUT4 R	CCCATAGCCTCCGCAACATA
GLUT10 F	CTCGCCATGGGCCACTC
GLUT10 R	CCAGGAACTCCTGCTCCAAG
GALM F	GATCACAGCCCTAGAGGTCAA
GALM R	GAGGTATCCTTCCAACTCGGC
GCK F	TGCTACTACGAAGACCATCAGT
GCK R	CCACTCGGTATTGACGCACA
GP1 F	GGAGAACCACATTCTCCCAGG
GP1 R	GCCAGGATCTGCTCTACCTTC
HK2 F	TTGACCAGGAGATTGACATGGG

HK2 R	CAACCGCATCAGGACCTCA
HK3 F	GTGAGGTTGGGCTAGTTGTAGA
HK3 R	GTCCAGGGTATGGTCGAAGGT
KHK F	CTAAGGAGGACTCGGAGATAAGG
KHK R	CATTGAGCCCATGAAGGCAC
PFKFB3 F	ATTGCGGTTTTCGATGCCAC
PFKFB3 R	GCCACAACTGTAGGGTCGT
PFKL F	GGCTTCGACACCCGTGTAA
PFKL R	CGTCAAACCTCTTGTCATCCA
PDK1 F	CTGTGATACGGATCAGAAACCG
PDK1 R	TCCACCAAACAATAAAGAGTGCT
PDK2 F	ATGAAAGAGATCAACCTGCTTCC
PDK2 R	GGCTCTGGACATACCAGCTC
PDK3 F	CGCTCTCCATCAAACAATTCCT
PDK3 R	CCACTGAAGGGCGGTTAAGTA
PDK4 F	GGAAGCATTGATCCTAACTGTGA
PDK4 R	GGTGAGAAGGAACATACACGATG
ENO1 F	TGGTGTCTATCGAAGATCCCTT
ENO1 R	CCTTGGCGATCCTCTTTGG
ENO2 F	CCGGGAACTCAGACCTCATC
ENO2 R	CTCTGCACCTAGTCGCATGG
ENO3 F	TATCGCAATGGGAAGTACGATCT
ENO3 R	AAGCTCTTATACAGCTCTCCGA
BPGM F	TGCTTGGAATAAGGAGAACCGT
BPGM R	CCACAGTTCCGAGCTTCCTC
ALDOA F	CAGGGACAAATGGCGAGACTA
ALDOA R	GGGGTGTGTTCCCCAATCTT
ALDOB F	TCCATCAACCAGAGCATCGG
ALDOB R	TCAAGCCCTTGAATGGTGGTT
ALDOC F	ATGCCTCACTCGTACCCAG
ALDOC R	TTTCCACCCCAATTTGGCTCA
PGK1 F	GAACAAGGTTAAAGCCGAGCC
PGK1 R	GTGGCAGATTGACTCCTACCA
PKM2 F	AAGGGTGTGAACCTTCCTGG
PKM2 R	GCTCGACCCCAAACTTCAGA
For ChIP	
METTL3 F	AACCCCGTCTCTACTAAA
METTL3 R	TCATACCCGCTCTCTCAT
ENO2 F	CGTGGGTGAGAGCCAAGACC
ENO2 R	ACTGCCAGTGAAGCCCAAGA
GLUT4 F	ACTCTCCACGCTCTGGGTAT
GLUT4 R	CGAGGGACAAGTGGTCACAA
HK2 F	GTGATCCTGTGGCCGAAGTT
HK2 R	GCCCAAACTTTTTCCGCTCC

Abbreviations: F, Forward; R, Reverse.

Supplementary Figure Legends:

Supplementary figure S1 METTL3 expression is elevated in GC. (A-D) The levels of METTL14 (A), WTAP (B), ALKBH5 (C), and FTO (D) expression were detected in GC and paired normal gastric mucosa by qRT-PCR (n=28). (E) METTL3 expression was positively correlated with m^6A level in GC (linear regression, n=28). (F) METTL3 protein levels were detected in human gastric mucosa tissue (hGT) and different GC cell lines by western blot. (G) Univariate analyses were performed in the GC cohort. All the bars correspond to 95% confidence intervals (CI); HR: hazard rate.

Supplementary figure S2 P300-mediated H3K27 acetylation promotes METTL3 transcriptional levels in GC. (A) The levels of P300 expression were analysed in GC (n=413) and normal gastric mucosa (n=32) using TCGA data. (B) The cell viability of HGC-27 cells treated with C646 at 20 μM for 24 h was determined by CCK-8 assay. (C) The mRNA levels of METTL3 in HGC-27 treated with C646 at the indicated doses for 24 h were determined by qRT-PCR. (D-E) The cell viability of NCI-N87 (D) and SGC7901cells (E) treated with C646 at 20 μM for 24 h was determined by CCK-8 assay. (F) The P300 knockdown efficiency was verified at the mRNA levels in NCI-N87 cells by qRT-PCR. The data are the means ± SEM of three independent experiments, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary figure S3 Knockdown of METTL3 suppresses cell growth in GC. (A-B) The overexpression (A) and knockdown (B) efficiencies were verified at the mRNA levels in different GC cells by qRT-PCR. (C) The protein levels of METTL3 in HGC-27 and NCI-N87 cells with METTL3 knockdown by western blotting (upper panel). The mRNAs isolated from METTL3 knockdown or corresponding control GC cells were used in dot blot analyses with m^6A antibody (bottom panel). MB (Methylene blue) staining served as a loading control. (D) Knockdown of METTL3 reduced GC cell anchorage-independent growth in soft agar (scale bars=200 μm, left panel); quantification results of soft agar colony formation (right panel). (E) Knockdown of METTL3 impaired colony-formation ability in GC cells (left panel); quantification

results of colony formation (right panel). (F) Tube formation assay in HUVECs cultured with medium collected from wild-type or catalytic mutant METTL3-overexpressing AGS cells or corresponding control cells. Tubes were imaged (scale bars=200 μm, left panel) and quantified for tube formation and cell growth (right panel). (G) Tube formation assay in HUVECs cultured with medium collected from HGC-27 cells with METTL3 deficiency or corresponding control cells. Tubes were imaged (scale bars=200 μm, left panel) and quantified for tube formation and cell growth (right panel). The data are the means ± SEM of three independent experiments, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary figure S4 Knockdown of METTL3 suppresses GC cell migration and invasion. (A) The migration and invasion of AGS cells with wild-type or catalytic mutant METTL3 overexpression were determined. Representative images (scale bars=200 μm, left panel) and quantification (right panel). (B-C) Representative images (scale bars=200 μm, upper panel) and quantification (bottom panel) of the cell migration and invasion assay results in METTL3-deficient HGC-27 cells (B) and NCI-87 cells (C) are shown. The data are the means ± SEMs of three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary figure S5 METTL3-mediated m^6A modification promotes HDGF expression. (A) The mRNA levels of HDGF in METTL3 knockdown HGC-27 and NCI-N87 cells were measured by qRT-PCR. (B-D) The mRNA levels of CDKN2A in METTL3-overexpressing (B), METTL3 knockdown (C), and METTL3 knockout (D) GC cells were measured by qRT-PCR. (E-G) The mRNA levels of C12orf45 in METTL3-overexpressing (E), METTL3 knockdown (F), and METTL3 knockout (G) GC cells were measured by qRT-PCR. (H) The mRNA levels of HDGF in wild-type or catalytic mutant METTL3-overexpressing AGS cells were measured by qRT-PCR. (I) The HDGF levels in conditional medium from METTL3-knockout HGC-27 cells were measured via ELISA assay. (J) An IHC assay was used to investigate METTL3 and HDGF expression in liver metastatic lesions of mice (scale bars=100 μm). (K-M) The

knockdown efficiencies of IGF2BP1 (K), IGF2BP2 (L), and IGF2BP3 (M) were verified at the mRNA level in HGC-27 cells by qRT-PCR. (N-O) The mRNA levels of HDGF in IGF2BP1 knockdown (N) and IGF2BP2 knockdown (O) GC cells were measured by qRT-PCR. The data are the means ± SEMs of three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary figure S6 METTL3 promotes GC malignant progression by upregulating HDGF expression. (A) The knockdown efficiency of HDGF using specific siRNAs in METTL3-overexpressing AGS cells was detected by western blotting. (B) Representative images (scale bars=200 μm, left panel) and quantification (right panel) of the cell migration and invasion assay results in METTL3 knockout HGC-27 cells incubated with rHDGF or PBS. (C) HDGF antibody blocked tube formation and HUVEC cell growth in METTL3-overexpressing GC cells. Tubes were imaged (scale bars=200 μm, left panel), and tube formation and cell growth were quantified in HUVECs cultured with medium from METTL3-overexpressing cells incubated with anti-IgG or anti-HDGF (right panel). (D) rHDGF restored angiogenesis in METTL3 knockout GC cells. Tubes were imaged (scale bars=200 μm, left panel), and tube formation and cell growth were quantified in HUVECs cultured with medium from METTL3 knockout cells incubated with PBS or rHDGF (right panel). The data are the means ± SEMs of three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary figure S7 The METTL3/HDGF axis accelerates glycolysis by up-regulating GLUT4 and ENO2 expression. (A) Pathway analysis indicated different metabolism-related pathways involved in GC progression upon METTL3 overexpression, especially glycolysis/gluconeogenesis. DE gene: Differential gene. (B-C) Knockout of METTL3 reduced glucose uptake (B) and lactate production (C) in HGC-27 cells. (D) HDGF expression at the mRNA level was verified by qRT-PCR in HGC-27 cells transfected with HDGF expression plasmids for 48 h. (E) GLUT4 and ENO2 were identified as METTL3/HDGF-regulated genes. The expression of a panel

of glucose metabolism-related genes was assessed by qRT-PCR in METTL3 knockout cells and the corresponding control cells. (F) Kaplan-Meier survival curves of OS based on GLUT4 and ENO2 expression were generated using the online bioinformatics tool Kaplan-Meier plotter. The data are the means ± SEMs of three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.