ENTPD1-AS1 promotes gastric cancer by activating the WNT signaling pathway through the FTO/WIF1 axis and interaction with SRSF1

Keywords
GC, WIF1, m6A, ENTPD1-AS1, SRSF1

Abstract

Introduction

Gastric cancer (GC) remains a major global health burden. Emerging evidence highlights the critical regulatory roles of long noncoding RNAs (lncRNAs) in GC pathogenesis. The purpose of this study was to explore the role and potential regulatory mechanisms of a lncRNA, ENTPD1-AS1, in GC.

Material and methods

Functional assays (CCK-8, colony formation, wound healing and Transwell assays) were performed in AGS and MKN45 cells following ENTPD1-AS1/SRSF1 knockdown or WIF1 overexpression. Targets were screened via RNA-seq and bioinformatics (UALCAN and SRAMP databases). The m6A modification levels were assessed by MeRIP-qPCR, and molecular interactions were validated by RNA immunoprecipitation (RIP) and immunofluorescence (IF) assays.

Results

Knockdown of ENTPD1-AS1 & SRSF1 and overexpression of WIF1 inhibited the proliferation, migration, and invasion of GC cells. Inhibition of ENTPD1-AS1 also increased the m6A modification levels in GC cells. Mechanistically, ENTPD1-AS1 downregulated WIF1 through FTO-mediated low levels of m6A modification to promote β-catenin expression, thereby activating the WNT signaling pathway and exacerbating GC. Additionally, ENTPD1-AS1 promoted GC progression by enhancing β-catenin expression through interaction with SRSF1 to activate the WNT signaling pathway.

Conclusions

In summary, the research highlights the significance of the ENTPD1-AS1/FTO/WIF1 axis and the ENTPD1-AS1/SRSF1 axis in collaboratively activating the WNT signaling pathway. These findings identify ENTPD1-AS1 as a potential therapeutic target for GC.

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ENTPD1-AS1 promotes gastric cancer by activating the WNT signaling pathway through the FTO/WIF1 axis and interaction with SRSF1

Running Head: ENTPD1-AS1 promotes GC via the WNT signaling pathway

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Abstract

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Keywords: ENTPD1-AS1; m6A; WIF1; SRSF1; GC

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Introduction

Gastric cancer (GC) is a global malignant disease with high incidence and mortality

rates, imposing a significant burden worldwide (1). It is a complex disease influenced by various factors, including environmental and genetic factors, and is characterized by high invasiveness and heterogeneity (2, 3). Treatment strategies for GC include systemic therapies such as chemotherapy, targeted therapy, and immunotherapy, as well as surgical interventions (4, 5). Unfortunately, due to the subtle symptoms of early-stage GC, most patients are diagnosed at advanced stages, leading to significantly reduced survival rates (6). Therefore, exploring biomarkers and therapeutic targets for GC is of great clinical significance.

In recent years, numerous studies have shown that long non-coding RNAs (lncRNAs) are widely involved in the occurrence, development, and metastasis of GC through various mechanisms, including the regulation of transcription, translation, epigenetic modifications, and signaling pathway activity. They have emerged as potential diagnostic biomarkers and therapeutic targets (7, 8). For instance, lnc_ASNRG regulates the occurrence and development of GC through the miR-519e-5p/FGFR2 axis (9), while lncRNA SNHG4 promotes GC progression by inhibiting miR-204-5p (10). Furthermore, LINC01268 activates the PI3K/AKT signaling pathway by targeting MARCKS, promoting epithelial-mesenchymal transition (EMT), and ultimately enhancing the invasion and metastasis of GC (11). The role of antisense lncRNAs in GC has also been revealeds. For instance, lncTM4SF1-AS1 can upregulate TM4SF1 and activate the PI3K/AKT signaling pathway, thereby promoting the proliferation, metastasis, and EMT of GC cells (12). Additionally, GATA6-AS1 inhibits GC cell

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proliferation and migration by upregulating PTEN through sponging miR-543, thereby inactivating the AKT signaling pathway (13). ENTPD1-AS1, as an antisense lncRNA, has been defined as a novel lncRNA that promotes GC (14). However, further exploration of its specific molecular mechanisms in GC is warranted.As biomedicine continues to advance, epigenetics has gained attention as an important field for elucidating gene expression mechanisms. N6-methyladenosine (m6A) is one of the most common genetic epigenetic modifications in eukaryotic cells, dynamically regulated by “writers” (METTL3, METTL14 and WTAP) and “erasers” (FTO and ALKBH5), and identified by “readers” (YTHDF family, YTHDF family, HNRNP family and IGF2BP family). It is present on almost all RNA types and plays a key role in a variety of physiological and pathological processes, including cancer (15, 16). This provides new perspectives for understanding the mechanisms of tumorigenesis and development. Recently, the regulatory roles of lncRNAs and RNA-binding proteins (RBPs) in tumors have garnered significant attention (17). Studies have shown that lncRNAs can interact with RBPs to regulate key signaling pathways, thereby influencing the malignant progression of tumors. For example, GAS6-AS1 activates the WNT pathway in bladder cancer by inhibiting APC expression through its interaction with PRC1 (18). In addition, silencing SCAMP1-TV2 reduces INSM1 mRNA expression by decreasing its binding to PUM2, downregulating the inhibitory activity of INSM1 on SASH1 transcription and suppressing the activity of the PI3K/AKT signaling pathway, thereby inhibiting the malignant biological behavior of breast cancer cells (19). However, it remains unclear

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whether ENTPD1-AS1 influences GC development through its interaction with RBPs. Furthermore, the WNT signaling pathway is a crucial cellular signaling pathway that regulates cellular processes to impact cell function, which contributes to the development of various cancers (20). The classical WNT signaling pathway is β-catenin-dependent (21), making β-catenin synthesis a key activating factor.

Based on the above background, this study aims to elucidate the molecular mechanism by which ENTPD1-AS1 activates the WNT signaling pathway through β-catenin synthesis, ultimately influencing GC development.

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Materials and Methods

Cell culture

Human GC cell lines AGS and MKN45 were obtained from the Cell Bank of the Chinese Academy of Sciences in Shanghai. These cell lines were cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% FBS and 1% penicillin/streptomycin. They were maintained at 37°C in a humidified incubator with 5% CO_2.

Cell transfection

In this study, short hairpin RNAs (shRNAs) specifically targeting ENTPD1-AS1(sh-ENTPD1-AS1#1/#2), WIF1(sh-WIF1#1/#2), and SRSF1(sh-SRSF1#1/#2), as well as overexpression vectors for FTO and SRSF1 and

their corresponding control vectors, were obtained from Ribobio (Guangzhou, China). Following acquisition of the vectors, they were transfected into GC cells as per experimental requirements, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Reverse-transcription quantitative PCR (RT-qPCR)

The extraction of total RNA from AGS and MKN45 cells was performed using TRIzol reagent (Invitrogen). Following reverse transcription with PrimerScript^{TM} RT Reagent (TaKaRa, Dalian, China), cDNAs were obtained and subsequently subjected to real-time PCR analysis using a SYBR Green detection system (TaKaRa) on an Applied Biosystems Prism 7900 instrument (Life Technologies, USA).

Western blot

Total proteins were extracted using RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitor. The protein concentration was determined by using BCA Kit (Beyotime). The protein samples were separated using SDS-PAGE after boiling water denaturation and then transferred to PVDF membranes (Millipore). The membranes were incubated with specific primary antibodies (anti-β-catenin, anti-SRSF1, and anti-GAPDH) at 4°C overnight after 1 h of containment using 5% non-fat milk. The next day, the primary antibodies were removed and washed three times with TBST, and the corresponding secondary antibodies were added and incubated at room

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temperature for 1 h. The protein blots were visualized by applying an ECL system (Beyotime).

Cell proliferation and colony formation assays

GC cells were seeded into 96-well plates at a density of 3,000 cells per well in triplicate. Following incubation for 24, 48, or 72 h, a 10 μL aliquot of the CCK-8 reagent (Dojindo, Kumamoto, Japan) was introduced to each well and allowed to incubate for an additional 2 h. Subsequently, the absorbance was assessed at 450 nm using a Multiskan Go microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

For colony formation assay, 1000 cells were seeded into 6-well plates containing complete, allowing for incubation over a period of 14 days. Then cells were fixed with 4% paraformaldehyde and followed by a one-hour staining process with 1% crystal violet. Finally, the resultant colonies were documented photographically and subsequently quantified using the ImageJ software application.

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Migration and invasion assay

Wound healing assay was performed to assess the migratory capacity of GC cells. GC cells transfected with various vectors were seeded into 6-well plates and cultured until reaching 90% confluence. Subsequently, scratches were generated on the cell monolayer. The cells were then incubated at 37°C in a 5% CO_2 environment, and images were captured at 0 and 24 h to measure the width of the scratches.

Transwell assay was employed to assess the invasive capacity of GC cells. GC cells in serum-free medium were seeded into the upper chamber coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The lower chamber contained medium with 10% FBS. Following a 24-hour of incubation, cells were stained with 1% crystal violet. The number of invaded cells was quantified under a light microscope (Olympus, Tokyo, Japan).

RNA immunoprecipitation (RIP)

RIP assays were carried out using a Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Cells were lysed and immunoprecipitated magnetic beads were prepared to construct immunoprecipitations of RNA-binding protein-RNA complexes according to the instructions. The immunoprecipitated RNA was then purified with RNA, and the recovered RNA was quantified by RT-qPCR.

RNA pulldown + MS analysis

To identify proteins potentially binding to ENTPD1-AS1, RNA pulldown coupled with mass spectrometry (MS) analysis was performed. The Pierce^{TM} Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher, USA) was used according to the manufacturer's instructions for the RNA pulldown assay. In brief, cell lysates were incubated with biotinylated RNA probes to form RNA-protein complexes. Subsequently, streptavidin

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magnetic beads were added to capture the complexes. The complexes were then subjected to silver staining using the Pierce Silver Stain Kit (Thermo Fisher, USA) following the operational guidelines followed by mass spectrometry identification.

Methylated RNA Immunoprecipitation (MeRIP)

MeRIP detection was carried out using the Magna MeRIP m6A kit (Merck Millipore, Germany) following the manufacturer's instructions. Total RNA was fragmented, and anti-m6A or anti-IgG antibodies were added for overnight incubation at 4°C. The pre-treated Protein A/G magnetic beads were then incubated at 4°C for 1 h. Subsequently, the beads were collected, washed, and the RNA was extracted for RT-qPCR analysis to assess the m6A modification level of WIF1.

Topflash assay

The Topflash assay is utilized to evaluate the activity of the WNT signaling pathway. The TOPflash reporter gene vector and the Renilla luciferase vector were transfected into GC cells. After 48 h, the cells were lysed, and the Dual Luciferase Reporter Assay System (Promega) was used according to the manufacturer's instructions for measurement. Firefly luciferase signal was detected at 560 nm, Renilla luciferase signal was detected at 480 nm, and Renilla luciferase activity was used as a normalization control.

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CHX chase assay

To observe the degradation of β-catenin, the CHX chase assay was performed. 2 ml of DMEM containing 100 μg/ml cycloheximide (CHX; Sigma-Aldrich, USA) was added to each of the 4 culture dishes in each group. Total protein was extracted at 0 h, 2 h, 4 h, and 8 h time points and analyzed by western blot.

Immunofluorescence (IF) assay

GC cells were plated in 24-well plates at a density of 2×10^4 cells per well. Following a 24-hour incubation period, the cells underwent two washes with phosphate-buffered saline before fixation with 4% paraformaldehyde for 10 min. Subsequent processing included permeabilization using 0.5% Triton X-100 for 20 min and blocking with 5% bovine serum albumin for 1 h at room temperature. Then, primary antibody against SRSF1 was applied and left to incubate at 4°C overnight. After washing, cells were treated with appropriate secondary antibody for 1 h followed by nuclear counterstaining with DAPI. Fluorescence images were acquired using a confocal microscope (Leica, Wetzlar, Germany).

Fluorescence in situ hybridization (FISH) assay

FISH was carried out according to the Fluorescent In Situ Hybridization Kit protocol (RiboBio). GC cells grown on glass coverslips at 60-70% confluence were PBS-washed, fixed with 4% formaldehyde for 20 min, and permeabilized with 0.5% Triton X-100.

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Overnight hybridization at 37°C was performed using ENTPD1-AS1-specific probes (RiboBio) in a humidified chamber. Following hybridization, nuclei were counterstained with DAPI and samples were imaged using a confocal microscopy.

Bioinformatics analysis

The SRAMP website was used to predict genes potentially undergoing m6A modification. The UALCAN database was used to analyze the expression of WIF1 in stomach adenocarcinoma (STAD). The GEPIA website was employed to analyze the expression of SRSF1 in various cancers.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (IBM, Chicago, IL, USA) or GraphPad Prism 7.0 (GraphPad Prism, Inc., La Jolla, CA, USA). Each experiment was repeated at least three times, with the results expressed as the mean ± SD of three independent trials. The Student's t-test or one-way ANOVA was used to compare the means of two or more groups. A p value < 0.05 was considered statistically significant.

Results

ENTPD1-AS1 promotes proliferation, migration, and invasion of GC cells

To investigate the role of ENTPD1-AS1 in GC, functional experiments were conducted in GC cells following knockdown of ENTPD1-AS1. Cell proliferation capacity was

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observed using CCK-8 assay and colony formation assay, revealing that knockdown of ENTPD1-AS1 led to a decrease in the proliferation ability of GC cells (**Figure 1A-B**). Subsequently, changes in the migration and invasion abilities of GC cells were assessed through wound healing assay and Transwell assay, showing that knockdown of ENTPD1-AS1 also resulted in a reduction in the migration and invasion abilities of GC cells (**Figure 1C-D**). In summary, our findings indicate that ENTPD1-AS1 acts as an oncogene in GC, positively correlating with the proliferation, migration, and invasion abilities of GC cells.

ENTPD1-AS1 downregulates WIF1 through m6A modification

Next, we further explored the downstream regulatory mechanism of ENTPD1-AS1 in GC. Since m6A modification plays a crucial role in post-transcriptional gene expression regulation, we considered it while identifying the downstream target genes of ENTPD1-AS1 Using RNA-seq, we found 52 upregulated genes after knockdown of ENTPD1-AS1. Subsequently, the SRAMP database predicted 6 genes potentially undergoing m6A modification, and finally, the UALCAN database was used to screen out 4 genes (WIF1, SORBS, PHYHIPL, and CAPN13) that were downregulated in GC (**Figure 2A**). It is well-known that WNT inhibitory factor-1 (WIF1) is closely related to the WNT signaling pathway and exerts a negative regulatory role on this pathway (22). Therefore, we chose to focus on WIF1 for further investigation. Additionally, we presented the results from the UALCAN database predicting low expression of WIF1 in

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STAD (**Figure 2B**). To confirm that ENTPD1-AS1 regulates WIF1 expression through m6A modification, we conducted meRIP-qPCR and RT-qPCR (**Figure 2C-D**). The results indicated that interference with ENTPD1-AS1 upregulated the m6A modification level but increased WIF1 expression. Therefore, we can conclude that ENTPD1-AS1 downregulates WIF1 expression through m6A modification regulation.

WIF1 inhibits GC cell proliferation, migration and invasion

To determine the role of WIF1 in GC development, we overexpressed WIF1 in GC cells and performed the following experiments. The results from the CCK-8 assay and colony formation assay indicated that overexpression of WIF1 inhibited the proliferation of GC cells (**Figure 3A-B**). Additionally, results from the wound healing assay and Transwell assay demonstrated that overexpression of WIF1 inhibited the migration and invasion abilities of GC cells (**Figure 3C-D**). Therefore, we conclude that overexpression of WIF1 inhibits the proliferation, migration, and invasion of GC cells. In addition, since β-catenin is a key factor in the WNT signaling pathway, we also observed the changes in β-catenin expression under different conditions by Western blot (**Figure 3E**). Based on the experimental results, we found that knockdown of ENTPD1-AS1 inhibited β-catenin expression, while knockdown of WIF1 promoted β-catenin expression. Additionally, knockdown of WIF1 reversed the inhibitory effect of ENTPD1-AS1 knockdown on β-catenin expression. This suggests that ENTPD1-AS1 promotes β-catenin expression by down-regulating WIF1.

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ENTPD1-AS1 downregulates WIF1 through FTO

Given the results indicating that ENTPD1-AS1 downregulates WIF1 via m6A modification, we further explored the regulation of m6A. Based on RT-qPCR results, we observed that knockdown of ENTPD1-AS1 significantly decreased the expression of FTO only (**Figure 4A**). This suggests that the reduction in m6A modification is mediated by FTO. Simultaneously, we also validated the interaction between FTO and WIF1 through RIP asssay (**Figure 4B**). Subsequently, we simultaneously knocked down ENTPD1-AS1 and overexpressed FTO to observe the changes in m6A modification level and expression level of WIF1 (**Figure 4C-D**). The results indicated that FTO overexpression reversed the promoting effect of knockdown of ENTPD1-AS1 on the m6A modification level and expression level of WIF1. In conclusion, ENTPD1-AS1 downregulates WIF1 through promoting FTO-mediated reduction in m6A modification level.

Rescue experiments validate that ENTPD1-AS1 promotes GC cell proliferation, migration and invasion through WIF1

We validated the findings so far by evidence rescue experiments. After transfecting GC cells with both ENTPD1-AS1 and WIF1 knockdown vectors, cell function assays were used to observe the changes in proliferation, migration and invasion of GC cells. CCK-8 and colony formation assays showed that knockdown of WIF1 restored the proliferative

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ability inhibited by knockdown of ENTPD1-AS1 (**Figure S1A-B**). Results from the wound healing assay and Transwell assay also indicated that knockdown of WIF1 could restore the migration and invasion abilities inhibited by knockdown of ENTPD1-AS1 (**Figure S1C-D**). In summary, ENTPD1-AS1 exerts its pro-oncogenic effects on GC through WIF1.

ENTPD1-AS1 promotes β-catenin expression by binding to SRSF1

Since lncRNAs regulate downstream gene expression by binding to RNA-binding proteins (RBPs) (23), we hypothesized that ENTPD1-AS1 regulates β-catenin expression by interacting with an RBP. To validate our hypothesis, we first conducted RNA pulldown coupled with mass spectrometry analysis to identify proteins that may interact with ENTPD1-AS1 (**Figure 5A**). Among them, the RBP SRSF1 caught our attention, as it has been reported to accelerate β-catenin synthesis to activate the WNT signaling pathway, promoting tumorigenesis (24). Subsequently, we confirmed the binding of ENTPD1-AS1 and SRSF1 through RIP and IF assays (**Figure 5B-C**). Additionally, RT-qPCR results showed a positive correlation between ENTPD1-AS1 and SRSF1 expression, indicating that knockdown of ENTPD1-AS1 inhibits SRSF1 expression (**Figure 5D**). Next, we investigated the impact of SRSF1 on the WNT pathway and found that SRSF1 promotes WNT signaling (**Figure 5E**). Furthermore, we examined the effect of SRSF1 on β-catenin and observed that SRSF1 does not affect β-catenin degradation but enhances its synthesis through binding (**Figure S2A-B, 5F**).

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Therefore, based on the aforementioned experiments, it is evident that ENTPD1-AS1 promotes β-catenin expression by binding to SRSF1, thereby activating the WNT signaling pathway.

SRSF1 is highly expressed in GC tissues and promotes proliferation, migration, and invasion of GC cells

To investigate whether SRSF1 plays a pro-cancer role in GC, we conducted the following experiments. Firstly, we analyzed the expression of SRSF1 in various cancers using the GEPIA database (**Figure 6A**) and found that SRSF1 is highly expressed in GC tissues. Subsequently, we knocked down SRSF1 in GC cells and observed its effects on cell proliferation, migration, and invasion. Results from CCK-8 assay and colony formation assay showed that knockdown of SRSF1 led to inhibited proliferation of GC cells (**Figure 6B-C**). Wound healing assay and Transwell assay also indicated that knockdown of SRSF1 resulted in suppressed migration and invasion of GC cells (**Figure 6D-E**). Therefore, SRSF1 enhances the proliferation, migration, and invasion abilities of GC cells.

Rescue experiments validate that ENTPD1-AS1 promotes proliferation, migration, and invasion of GC cells through SRSF1

Finally, we performed a rescue experiment to verify whether ENTPD1-AS1 promotes GC development through SRSF1. CCK-8 assay and colony formation assay clearly

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demonstrated that overexpression of SRSF1 reversed the inhibitory effect of ENTPD1-AS1 knockdown on the proliferation of GC cells (**Figure S3A-B**). Wound healing assay and Transwell assay showed a similar trend, where overexpression of SRSF1 reversed the suppression of migration and invasion caused by ENTPD1-AS1 knockdown (**Figure S3C-D**). This confirms that ENTPD1-AS1 promotes the proliferation, migration, and invasion of GC cells through SRSF1.

Discussion

GC as a common malignant tumor necessitates urgent exploration of novel molecular markers for its diagnosis, treatment, and prognosis. LncRNAs, as a type of non-coding RNA, influence the occurrence and development of cancer through various molecular mechanisms. For example, lncRNA PTCSC3 inhibits the proliferation of oral cancer cells by inducing apoptosis and autophagy (25), while lncRNA PTTG3P regulates the development of lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) through a ceRNA mechanism (26). Recent studies have investigated the potential molecular mechanisms of lncRNAs in GC, primarily focusing on the ceRNA mechanism. For instance, lncRNA LIFR-AS1 upregulates MTUS1 by sponging miR-4698 to inhibit GC progression (27). Moreover, lncRNA GIHCG promotes GC development by upregulating TLE1 expression through sponging miR-1281 (28). Notably, the impact of ENTPD1-AS1 on GC has been reported to be mediated through the ceRNA network (14). However, our study indicates that ENTPD1-AS1 affects GC

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progression through m6A modification and RBP binding.

A previous study has shown that TP73-AS1 regulates the promoter methylation of WIF1 by recruiting the PRC2 complex, thereby downregulating WIF1 to activate the Wnt/β-catenin signaling pathway and ultimately promoting the development of GC (29). However, our study revealed that ENTPD1-AS1 functions as an oncogene in GC, downregulates WIF1 through low m6A modification levels mediated by FTO, leading to enhanced β-catenin synthesis to promote the malignant characteristics of GC cells. Furthermore, the positive regulation of WIF1 expression by m6A modification has been confirmed (30), which is consistent with our research findings.

Many studies have also indicated the involvement of SRSF1 in the occurrence and development of GC. For instance, CCL21 is crucial in GC progression through the MALAT1/SRSF1/mTOR axis (31), whileOnclncRNA-626 accelerates GC development by affecting the p53 signaling pathway through binding to SRSF1 (32). Additionally, SRSF1 has been found to play important roles in other diseases. For example, lncRNA HAGLR activates the WNT signaling pathway by competitively binding to miR-93-5p to enhance SRSF1, thereby promoting the development of triple-negative breast cancer (33). In our study, considering the interactions between lncRNAs and RBPs, and the reported impact of RBPs on cancer processes through the regulation of gene expression (34, 35), we further explored another molecular mechanism of ENTPD1-AS1 on the WNT signaling pathway. Mass spectrometry revealed that SRSF1 is an RBP of ENTPD1-AS1. Further experiments demonstrated

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that ENTPD1-AS1 promotes β-catenin expression by recruiting SRSF1, thereby activating the WNT signaling pathway to exacerbate GC.

This study has several limitations. First, all experimental data are derived from *in vitro* cell models, lacking validation of the oncogenic role of ENTPD1-AS1 through animal experiments and clinical sample data. Future research should establish corresponding animal models and collect a substantial number of GC clinical samples to further confirm its function. Second, although the interaction between ENTPD1-AS1 and SRSF1 has been established, the specific molecular mechanisms by which SRSF1 regulates β-catenin remain to be fully elucidated, and future studies can employ additional techniques for in-depth analysis. Furthermore, the clinical translation of ENTPD1-AS1 as a therapeutic target still faces challenges that require further exploration and breakthroughs. Despite these limitations, this study provides an important theoretical basis for developing GC treatment strategies targeting ENTPD1-AS1, and the findings warrant further validation and expansion in a more comprehensive experimental framework to facilitate the clinical translation of these results.

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Conclusions

In conclusion, this study confirms the promotive effect of ENTPD1-AS1 on GC. Mechanistically, ENTPD1-AS1 exacerbates GC through the FTO/WIF1 axis and binding with SRSF1 to activate the WNT signaling pathway. This suggests that

ENTPD1-AS1 could serve as a potential biomarker and therapeutic target for GC, increasing the possibility of cure for GC patients.

Statements

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Statement of ethics

N/A

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Availability of data

Research data can be obtained from the corresponding author if necessary.

Declarations of interest

The authors have no conflicts of interest to declare.

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Reference

1. Thrift AP, El-Serag HB. Burden of Gastric Cancer. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2020;18(3):534-42.
2. Machlowska J, Baj J, Sitarz M, et al. Gastric Cancer: Epidemiology, Risk Factors, Classification, Genomic Characteristics and Treatment Strategies. International journal of molecular sciences. 2020;21(11).
3. Petryszyn P, Chapelle N, Matysiak-Budnik T. Gastric Cancer: Where Are We Heading? Digestive diseases (Basel, Switzerland). 2020;38(4):280-5.
4. Guan WL, He Y, Xu RH. Gastric cancer treatment: recent progress and future perspectives. Journal of hematology & oncology. 2023;16(1):57.
5. Tan Z. Recent Advances in the Surgical Treatment of Advanced Gastric Cancer: A Review. Medical science monitor : international medical journal of experimental and clinical research. 2019;25:3537-41.
6. Xia JY, Aadam AA. Advances in screening and detection of gastric cancer. Journal of surgical oncology. 2022;125(7):1104-9.

7. Sexton RE, Al Hallak MN, Diab M, et al. Gastric cancer: a comprehensive review of current and future treatment strategies. Cancer metastasis reviews. 2020;39(4):1179-203.
8. Necula L, Matei L, Dragu D, et al. Recent advances in gastric cancer early diagnosis. World journal of gastroenterology. 2019;25(17):2029-44.
9. Chen Z, Li Y, Tan B, et al. Long Non-coding RNA ASNR Targeting miR-519e-5p Promotes Gastric Cancer Development by Regulating FGFR2. Frontiers in cell and developmental biology. 2021;9:679176.
10. Wang S, Zhu W, Qiu J, et al. lncRNA SNHG4 promotes cell proliferation, migration, invasion and the epithelial-mesenchymal transition process via sponging miR-204-5p in gastric cancer. Molecular medicine reports. 2021;23(1).
11. Tang LH, Ye PC, Yao L, et al. LINC01268 promotes epithelial-mesenchymal transition, invasion and metastasis of gastric cancer via the PI3K/Akt signaling pathway and targeting MARCKS. World journal of gastrointestinal oncology. 2023;15(8):1366-83.
12. He C, Qi W, Wang Z. Effect and mechanism of downregulating the long-chain noncoding RNA TM4SF1-AS1 on the proliferation, apoptosis and invasion of gastric cancer cells. World journal of surgical oncology. 2021;19(1):226.
13. Jin Y, Jiang D. GATA6-AS1 via Sponging miR-543 to Regulate PTEN/AKT Signaling Axis Suppresses Cell Proliferation and Migration in Gastric Cancer. Mediators of inflammation. 2023;2023:9340499.
14. Yuan HM, Pu XF, Wu H, et al. ENTPD1-AS1-miR-144-3p-mediated high expression of COL5A2 correlates with poor prognosis and macrophage infiltration in gastric cancer. World journal of gastrointestinal oncology. 2023;15(7):1182-99.
15. Sun T, Wu R, Ming L. The role of m6A RNA methylation in cancer. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2019;112:108613.
16. Huang H, Weng H, Chen J. m(6)A Modification in Coding and Non-coding RNAs: Roles and Therapeutic Implications in Cancer. Cancer cell. 2020;37(3):270-88.
17. Zhang N, Wen K. The role of lncRNA binding to RNA-binding proteins to regulate mRNA stability in cancer progression and drug resistance mechanisms (Review). Oncology reports. 2024;52(5).
18. Zhou X, Xiao L, Meng F, et al. GAS6-AS1 drives bladder cancer progression by increasing MMP7 expression in a ceRNA- and RBP-dependent manner. Translational oncology. 2024;48:102065.
19. Tao W, Ma J, Zheng J, et al. Silencing SCAMP1-TV2 Inhibited the Malignant Biological Behaviors of Breast Cancer Cells by Interaction With PUM2 to Facilitate INSM1 mRNA Degradation. Frontiers in oncology. 2020;10:613.
20. Koni M, Pinnarò V, Brizzi MF. The Wnt Signalling Pathway: A Tailored Target in Cancer. International journal of molecular sciences. 2020;21(20).
21. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene. 2017;36(11):1461-73.
22. Kawano Y, Kypta R. Secreted antagonists of the Wnt signalling pathway. Journal of cell science. 2003;116(Pt 13):2627-34.
23. Catana CS, Crişan CA, Opre D, et al. Implications of Long Non-Coding RNAs in Age-Altered Proteostasis. Aging and disease. 2020;11(3):692-704.

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24. Fu Y, Huang B, Shi Z, et al. SRSF1 and SRSF9 RNA binding proteins promote Wnt signalling-mediated tumorigenesis by enhancing β-catenin biosynthesis. EMBO molecular medicine. 2013;5(5):737-50.
25. Zhang H, Wang J, Xun W, et al. Long non-coding RNA PTCSC3 inhibits human oral cancer cell proliferation by inducing apoptosis and autophagy. Archives of medical science : AMS. 2021;17(2):492-9.
26. Huang HT, Xu YM, Ding SG, et al. The novel lncRNA PTTG3P is downregulated and predicts poor prognosis in non-small cell lung cancer. Archives of medical science : AMS. 2020;16(4):931-40.
27. Zhao J, Li X, Fu L, et al. lncRNA LIFR-AS1 inhibits gastric carcinoma cell proliferation, migration and invasion by sponging miR-4698. Molecular medicine reports. 2021;23(2).
28. Liu G, Jiang Z, Qiao M, et al. Lnc-GIHCG promotes cell proliferation and migration in gastric cancer through miR- 1281 adsorption. Molecular genetics & genomic medicine. 2019;7(6):e711.
29. He ZC, Yang F, Guo LL, et al. LncRNA TP73-AS1 promotes the development of Epstein-Barr virus associated gastric cancer by recruiting PRC2 complex to regulate WIF1 methylation. Cellular signalling. 2021:110094.
30. Zhang QC. METTL3 is aberrantly expressed in endometriosis and suppresses proliferation, invasion, and migration of endometrial stromal cells. The Kaohsiung journal of medical sciences. 2023;39(3):266-77.
31. Fu Q, Tan X, Tang H, et al. CCL21 activation of the MALAT1/SRSF1/mTOR axis underpins the development of gastric carcinoma. Journal of translational medicine. 2021;19(1):210.
32. Wu ZH, Liu CC, Zhou YQ, et al. OnclncRNA-626 promotes malignancy of gastric cancer via inactivated the p53 pathway through interacting with SRSF1. American journal of cancer research. 2019;9(10):2249-63.
33. Zhang C, Yang Y, Yi L, et al. HOXD Antisense Growth-Associated Long Noncoding RNA Promotes Triple-Negative Breast Cancer Progression by Activating Wnt Signaling Pathway. Journal of breast cancer. 2021;24(3):315-29.
34. Wang S, Sun Z, Lei Z, et al. RNA-binding proteins and cancer metastasis. Seminars in cancer biology. 2022;86(Pt 2):748-68.
35. Yao ZT, Yang YM, Sun MM, et al. New insights into the interplay between long non-coding RNAs and RNA-binding proteins in cancer. Cancer communications (London, England). 2022;42(2):117-40.

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Figure legends

Figure 1. ENTPD1-AS1 promotes proliferation, migration, and invasion of GC

cells

A-B. The effect of ENTPD1-AS1 knockdown on GC cell proliferation was assessed using CCK8 and colony formation assays, revealing that silencing ENTPD1-AS1 reduced GC cell proliferation. **C.** Wound healing assay was used to analyze the effect of ENTPD1-AS1 knockdown on GC cell migration, revealing that knockdown of ENTPD1-AS1 inhibited GC cell migration. **D.** Transwell assay was used to analyze the effect of ENTPD1-AS1 knockdown on GC cell invasion, indicating that knockdown of ENTPD1-AS1 suppressed GC cell invasion. $^{**}p < 0.01$.

Figure 2. ENTPD1-AS1 downregulates WIF1 through m6A modification

A. WIF1, SORBS2, PHYHIPL and CAPN13 were screened using RNA-seq, SRAMP and UALCAN databases. **B.** The UALCAN database was analyzed for the expression level of WIF1 in gastric cancer tissues. **C.** MeRIP-qPCR was utilized to assess the effect of ENTPD1-AS1 knockdown on the m6A modification of WIF1, showing that knockdown of ENTPD1-AS1 significantly upregulated the m6A levels of WIF1. **D.** RT-qPCR demonstrated that ENTPD1-AS1 knockdown significantly increased WIF1 expression. $^{**}p < 0.01$.

Figure 3. WIF1 inhibits GC cell proliferation, migration and invasion

A-B. The effect of WIF1 overexpression on GC cell proliferation were evaluated using CCK8 and colony formation assays, revealing that WIF1 overexpression inhibited GC

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cell proliferation. **C.** Wound healing assay was conducted to analyze the effect of WIF1 overexpression on GC cell migration, indicating that WIF1 overexpression reduced GC cell migration. **D.** Transwell assay was performed to evaluate the effect of WIF1 overexpression on GC cell invasion, demonstrating that WIF1 overexpression inhibited GC cell invasion. **E.** Western blot analysis of the effects of ENTPD1-AS1 and WIF1 knockdown on β-catenin expression revealed that ENTPD1-AS1 knockdown suppressed β-catenin expression, while WIF1 knockdown increased β-catenin levels, and WIF1 overexpression reversed the decrease in β-catenin expression induced by ENTPD1-AS1 knockdown. *$p < 0.05$, **$p < 0.01$.

Figure 4. ENTPD1-AS1 downregulates WIF1 through FTO

A. RT-qPCR analysis of the effect of ENTPD1-AS1 knockdown on the expression of METTL3, WTAP, METTL14, and FTO, showing that ENTPD1-AS1 knockdown significantly downregulated FTO mRNA levels. **B.** RIP assay results indicated the binding of FTO to WIF1. **C**. MeRIP-qPCR analysis was performed to evaluate the effect of ENTPD1-AS1 knockdown and FTO overexpression on the m6A level of WIF1 mRNA, demonstrating that FTO overexpression reversed the increase in m6A modification levels induced by ENTPD1-AS1 knockdown. **D.** RT-qPCR was utilized to analyze the effect of ENTPD1-AS1 knockdown and FTO overexpression on WIF1 mRNA expression, showing that FTO overexpression reversed the increase in WIF1 mRNA levels mediated by ENTPD1-AS1 knockdown. **$p < 0.01$, ***$p < 0.001$.

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Figure 5. ENTPD1-AS1 promotes β-catenin expression by binding to SRSF1

A. RNA pull-down combined with mass spectrometry analysis was performed to identify potential ENTPD1-AS1 binding proteins. **B-C.** RIP and IF assays were conducted to confirm the binding of ENTPD1-AS1 to SRSF1. **D.** RT-qPCR analysis was carried out to evaluate the effect of ENTPD1-AS1 knockdown on SRSF1 expression, indicating that SRSF1 expression was inhibited by ENTPD1-AS1 knockdown. **E.** Topflash assay was employed to analyze the effect of SRSF1 on the WNT signaling pathway, showing that SRSF1 promoted WNT signaling activation. **F.** Western blot analysis was performed to assess the effect of SRSF1 overexpression on β-catenin protein expression, revealing that SRSF1 overexpression increased β-catenin protein levels. $^{**}p < 0.01$.

Figure 6. RSF1 is highly expressed in GC tissues and promotes proliferation, migration, and invasion of GC cells

A. GEPIA analysis of SRSF1 expression in pan-cancers. **B-C.** CCK8 and colony formation assays were utilized to analyze the effect of SRSF1 knockdown on the proliferation of GC cells, demonstrating that SRSF1 knockdown inhibited GC cell proliferation. **D.** Wound healing assay was performed to assess the effect of SRSF1 knockdown on the migration of GC cells, indicating that SRSF1 knockdown hindered GC cell migration. **E**. Transwell assay was conducted to evaluate the effect of SRSF1

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knockdown on the invasion of GC cells, showing that SRSF1 knockdown obstructed GC cell invasion. *$p < 0.05$, **$p < 0.01$.

Figure S1. Rescue experiments validate that ENTPD1-AS1 promotes GC cell proliferation, migration and invasion through WIF1

A-B. CCK8 assay and colony formation assay were used to analyze the effect of simultaneous knockdown of ENTPD1-AS1 and WIF1 on the proliferation of GC cells, revealing that WIF1 knockdown restored the reduced proliferation of GC cells caused by ENTPD1-AS1 knockdown. **C.** Wound healing assay was performed to assess the effect of simultaneous knockdown of ENTPD1-AS1 and WIF1 on the migration of GC cells, indicating that WIF1 knockdown restored the decreased migration of GC cells induced by ENTPD1-AS1 knockdown. **D.** Transwell assay was conducted to evaluate the effect of simultaneous knockdown of ENTPD1-AS1 and WIF1 on the invasion of GC cells, demonstrating that WIF1 knockdown restored the reduced invasion of GC cells caused by ENTPD1-AS1 knockdown. *$p < 0.05$, **$p < 0.01$.

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Figure S2. SRSF1 does not affect β-catenin degradation but binds to β-catenin.

A. CHX chase assay was performed to analyze the effect of SRSF1 on β-catenin protein degradation, showing that changes in SRSF1 expression did not affect β-catenin degradation. **B.** RIP analysis indicated the binding of SRSF1 to β-catenin mRNA.

Figure S3. Rescue experiments validate that ENTPD1-AS1 promotes proliferation, migration, and invasion of GC cells through SRSF1

A-B. CCK8 assay and colony formation assay were utilized to analyze the effect of ENTPD1-AS1 knockdown and SRSF1 overexpression on the proliferation of GC cells, demonstrating that SRSF1 overexpression restored the decreased proliferation of GC cells caused by ENTPD1-AS1 knockdown. **C.** Wound healing assay was performed to assess the effect of ENTPD1-AS1 knockdown and SRSF1 overexpression on the migration of GC cells, indicating that SRSF1 overexpression restored the decreased migration of GC cells induced by ENTPD1-AS1 knockdown. **D.** Transwell assay was conducted to evaluate the effect of ENTPD1-AS1 knockdown and SRSF1 overexpression on the invasion of GC cells, showing that SRSF1 overexpression restored the reduced invasion of GC cells caused by ENTPD1-AS1 knockdown.

$^{**}p < 0.01$.

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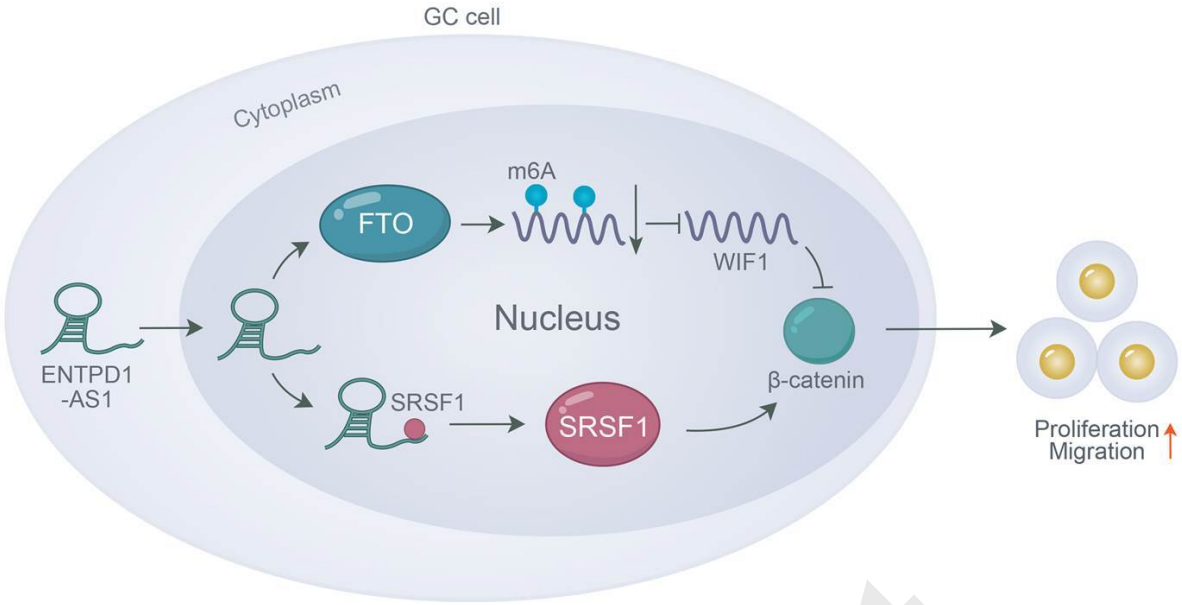

ENTPD1-AS1 promotes gastric cancer progression by downregulating WIF1 through FTO-mediated reduction of m6A modification, leading to increased β-catenin expression and activation of the WNT signaling pathway. Additionally, ENTPD1-AS1 enhances β-catenin expression by interacting with SRSF1, further activating the WNT signaling pathway. This dual mechanism exacerbates the progression of GC.

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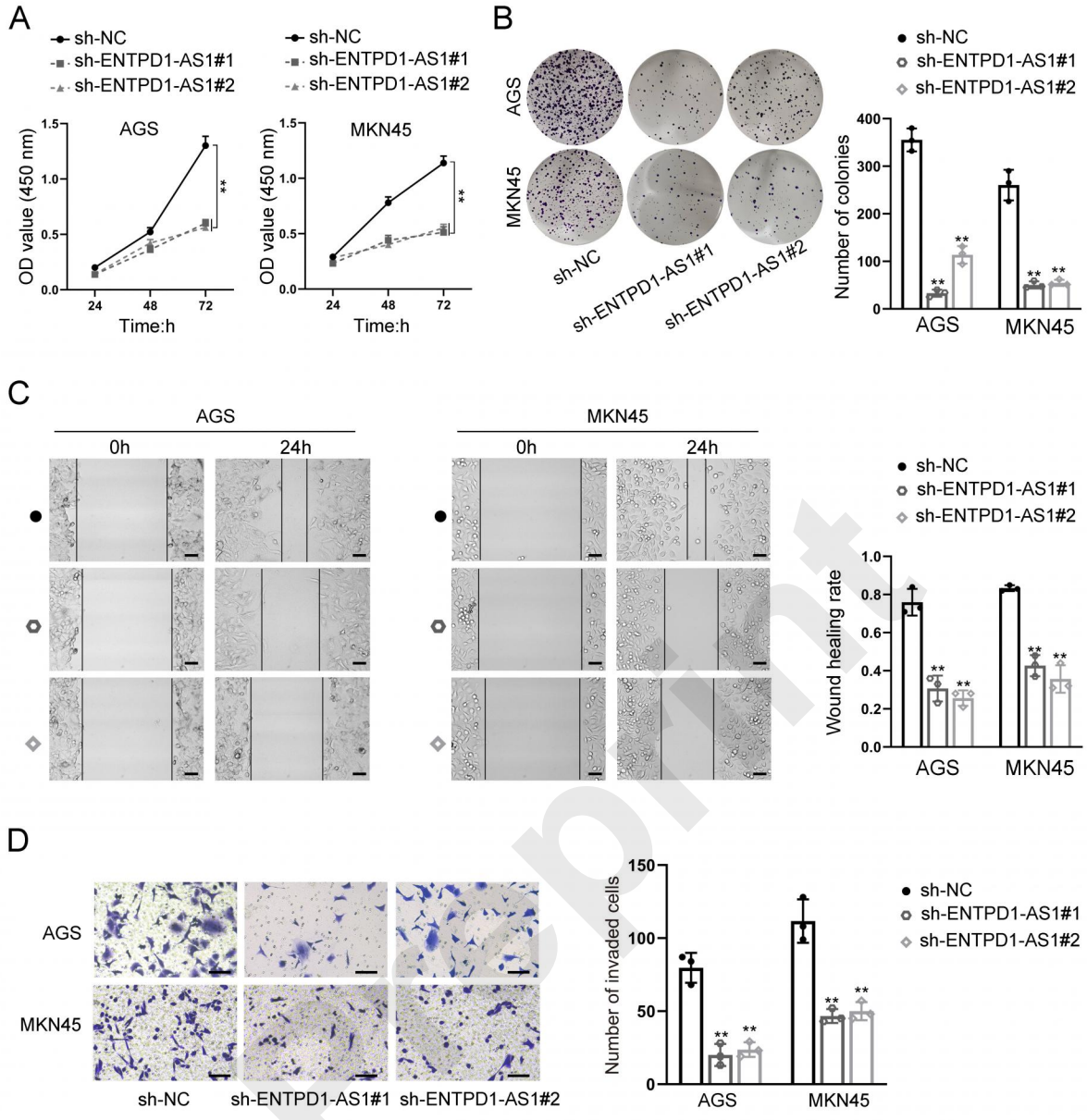
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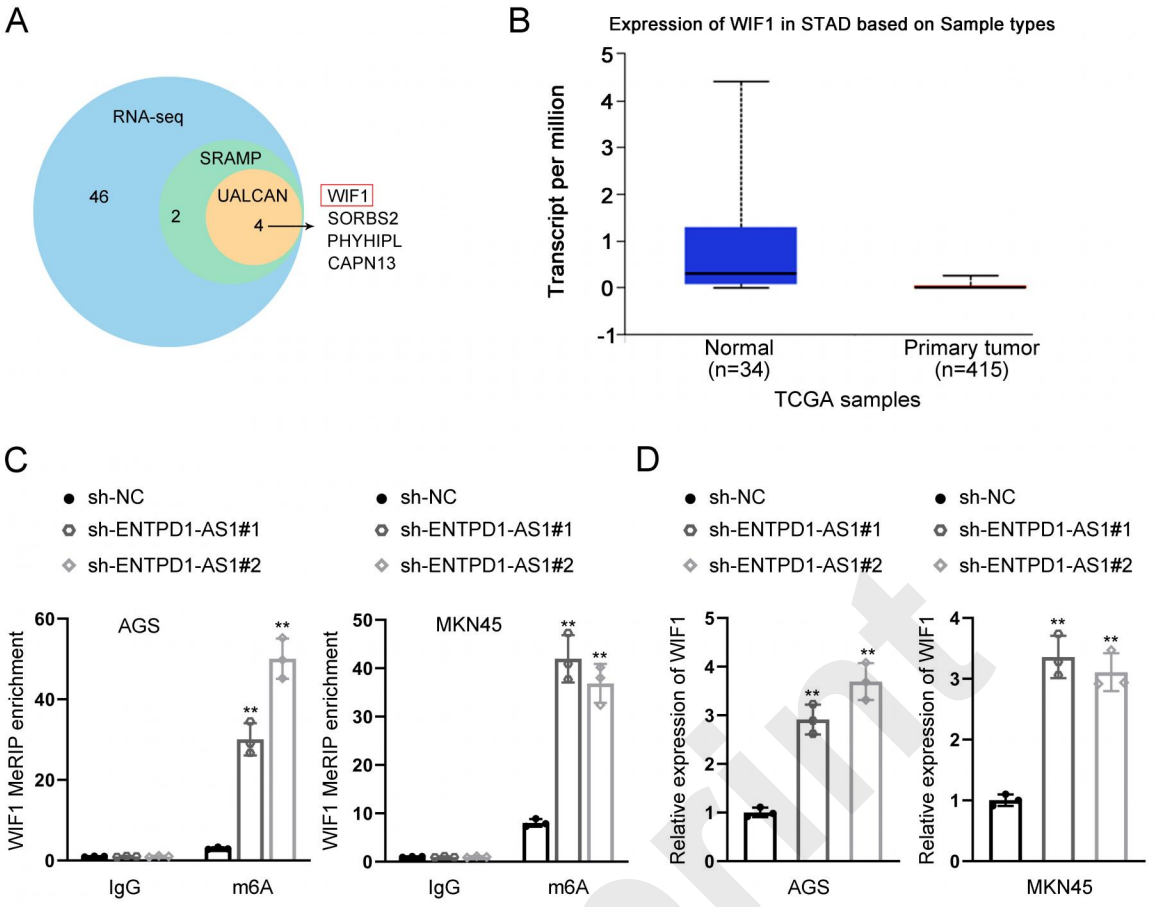
sh-ENTPD1-AS1#2
AGS
MKN45
OD value (450 nm)
Time:h
B
AGS
MKN45
sh-NC
sh-ENTPD1-AS1#1
sh-ENTPD1-AS1#2
Number of colonies
C
0h
24h
Wound healing rate
D
Number of invaded cells

Preprint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RNA-seq
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UALCAN
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WIF1
SORBS2
PHYHIPL
CAPN13
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Expression of WIF1 in STAD based on Sample types
Transcript per million
5
4
3
2
1
0
-1
Normal
(n=34)
Primary tumor
(n=415)
TCGA samples
C
sh-NC
sh-ENTPD1-AS1#1
sh-ENTPD1-AS1#2
AGS
MKN45
WIF1 MeRIP enrichment
60
40
20
0
50
40
30
20
10
0
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IgG
m6A
D
Relative expression of WIF1
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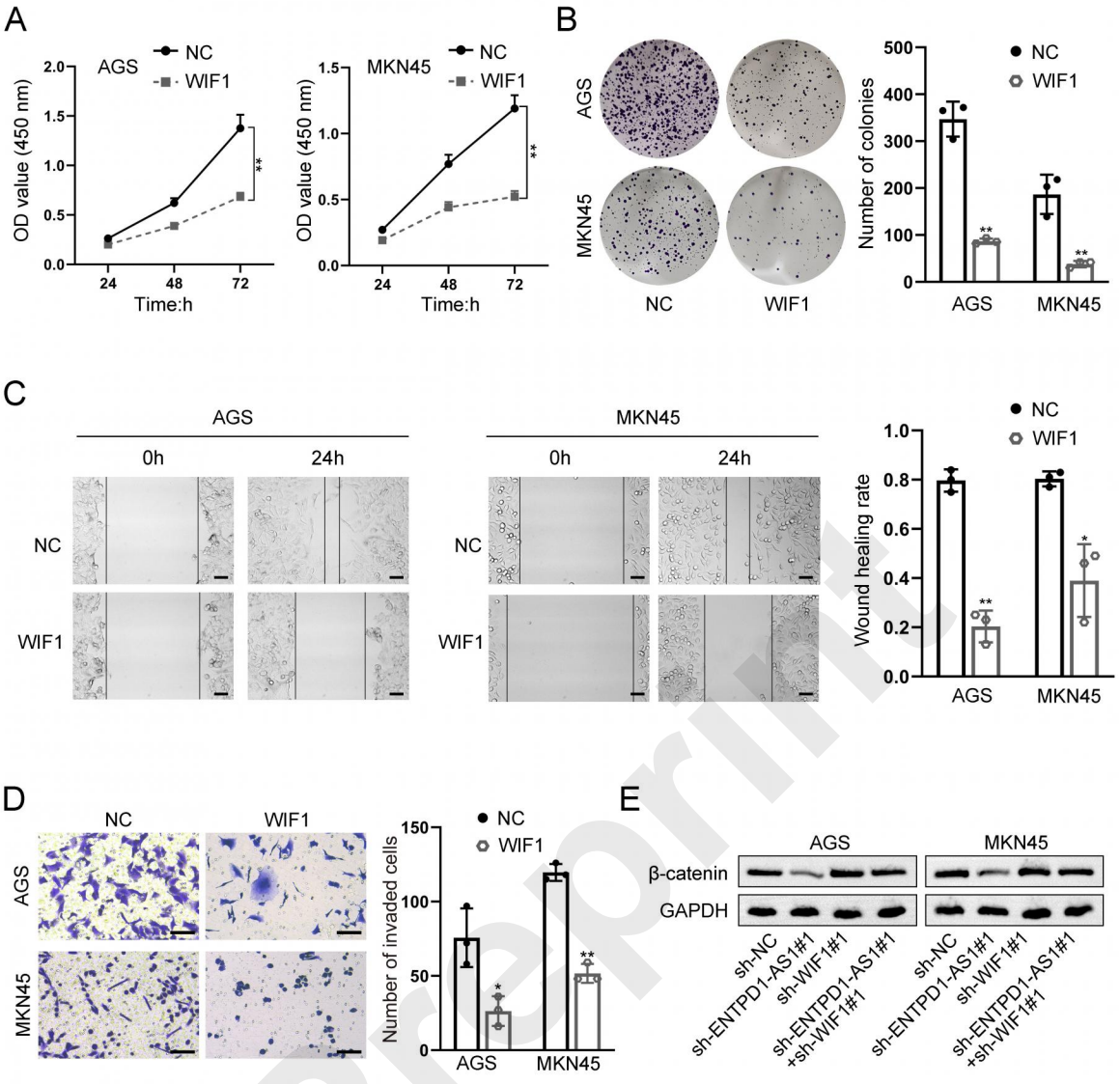
4
3
2
1
0
AGS
MKN45

Preprint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Time:h
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B
Number of colonies
C
0h
24h
Wound healing rate
D
Number of invaded cells
E
β-catenin
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sh-WIF1#1
sh-ENTPD1-AS1#1 +sh-WIF1#1

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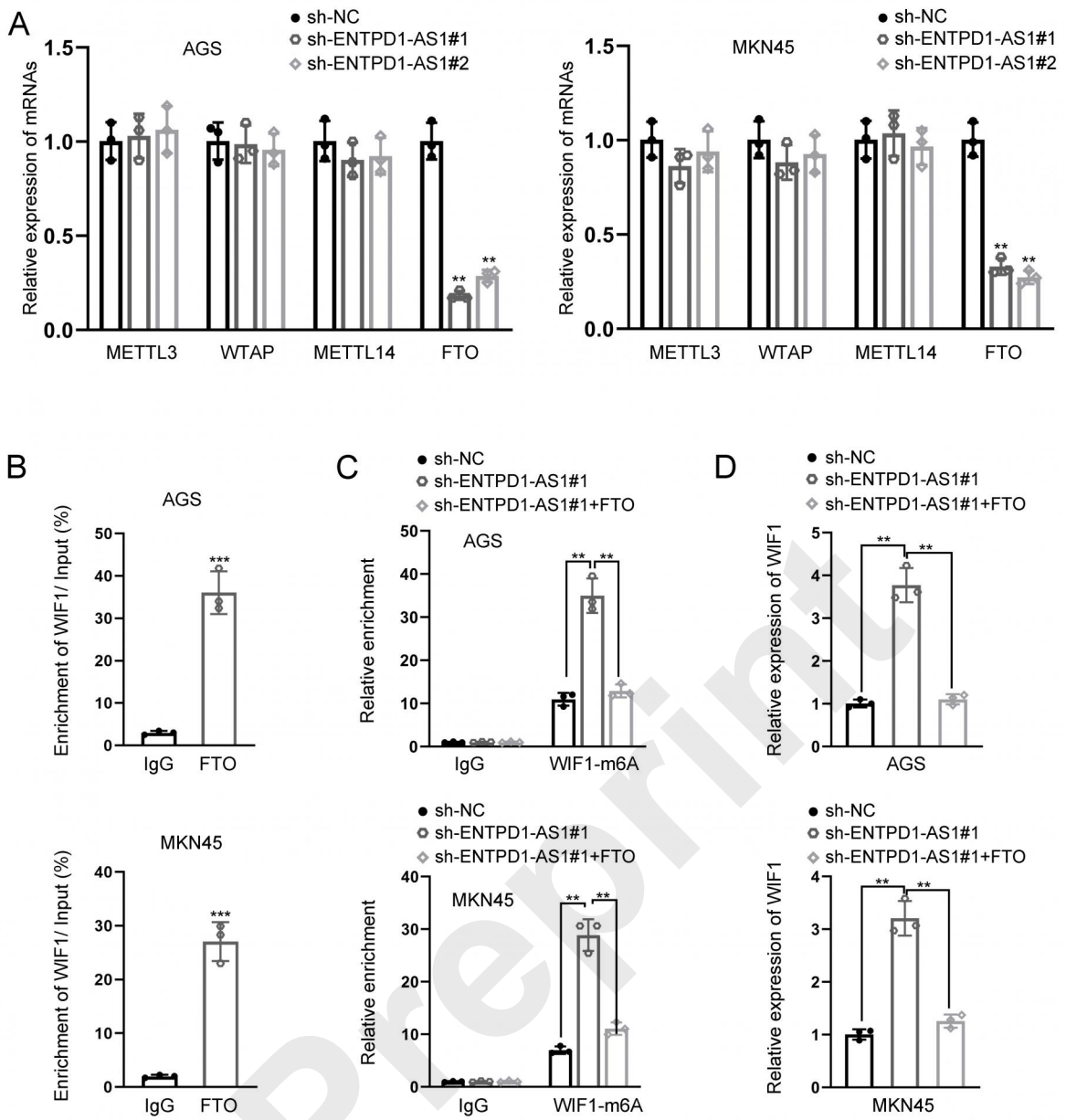
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sh-ENTPD1-AS1#2
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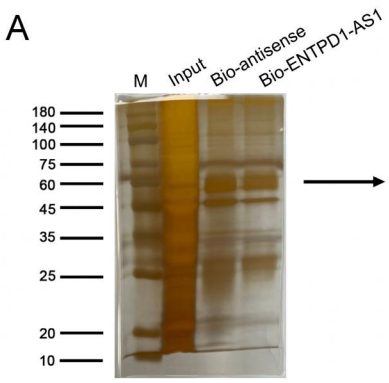
1.0
0.5
0.0
METTL3
WTAP
METTL14
FTO
**
B
AGS
MKN45
Enrichment of WIF1/ Input (%)
***
IgG
FTO
C
sh-ENTPD1-AS1#1+FTO
Relative enrichment
WIF1-m6A
D
Relative expression of WIF1

Preprint

A



Protein FDI	Accession	Description	Sum PEP Sc	Coverage [%	# Peptides	# PSMs	# Unique Pe	# AAs	MW [kDa]	calc. pI	Score Sequ	Gene Symb
High	Q9UQ35	Serine/argi	543.37	36	87	154	87	2752	299.4	12.06	467.73	SRRM2
High	P11387	DNA topois	271.192	51	48	101	48	765	90.7	9.31	269.5	TOP1
High	Q07955	Serine/argi	123.655	63	25	78	24	248	27.7	10.36	192.43	SRSF1
High	P11940	Polyadenyl	227.962	50	35	64	24	636	70.6	9.5	161.77	PABPC1
High	P16401	Histone H1.	136.54	31	15	45	11	226	22.6	10.92	151.71	H1-5
High	Q13247	Serine/argi	120.459	42	20	53	15	344	39.6	11.43	133.51	SRSF6
High	P84103	Serine/argi	98.787	49	13	43	12	164	19.3	11.65	131.53	SRSF3
High	Q13310	Polyadenyl	162.337	45	32	50	21	644	70.7	9.26	128.88	PABPC4
High	P42766	60S riboson	92.024	52	13	46	12	123	14.5	11.05	126.72	RPL35
High	P49756	RNA-bindir	166.39	32	28	49	28	843	100.1	6.32	126.25	RBM25
High	P84098	60S riboson	104.819	42	17	46	16	196	23.5	11.47	126.22	RPL19
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High	P08670	Vimentin C	152.422	59	29	43	28	466	53.6	5.12	117.56	VIM
High	P16402	Histone H1.	122.085	32	15	34	7	221	22.3	11.02	114.75	H1-3
High	P52272	Heterogene	128.67	46	31	45	31	730	77.5	8.7	111.53	HNRNPM
High	P18583	Protein SOI	133.71	18	33	44	33	2426	263.7	5.64	110.86	SON
High	P38919	Eukaryotic i	130.331	41	26	39	26	411	46.8	6.73	109.12	EIF4A3
High	P04264	Keratin, typ	129.326	53	29	36	25	644	66	8.12	109.02	KRT1
High	P62995	Transforme	78.176	42	18	44	14	288	33.6	11.25	107.67	TRA2B
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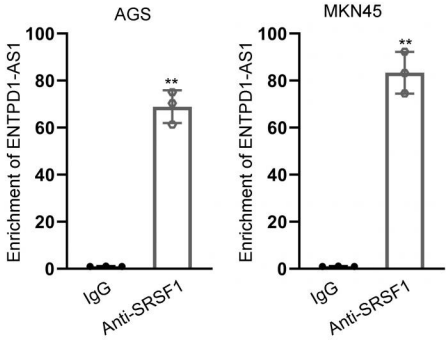

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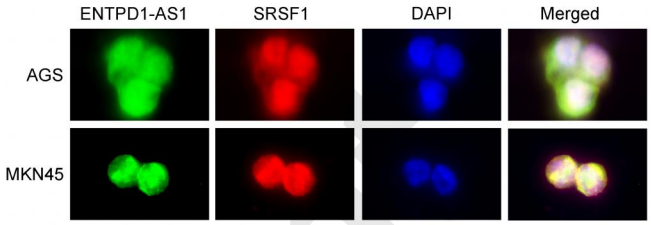

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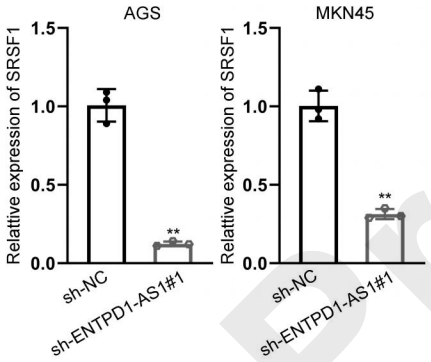

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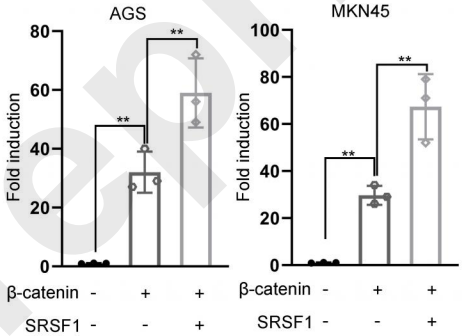

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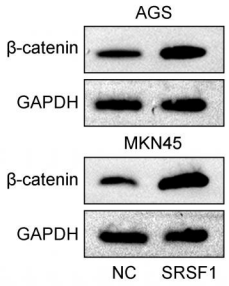

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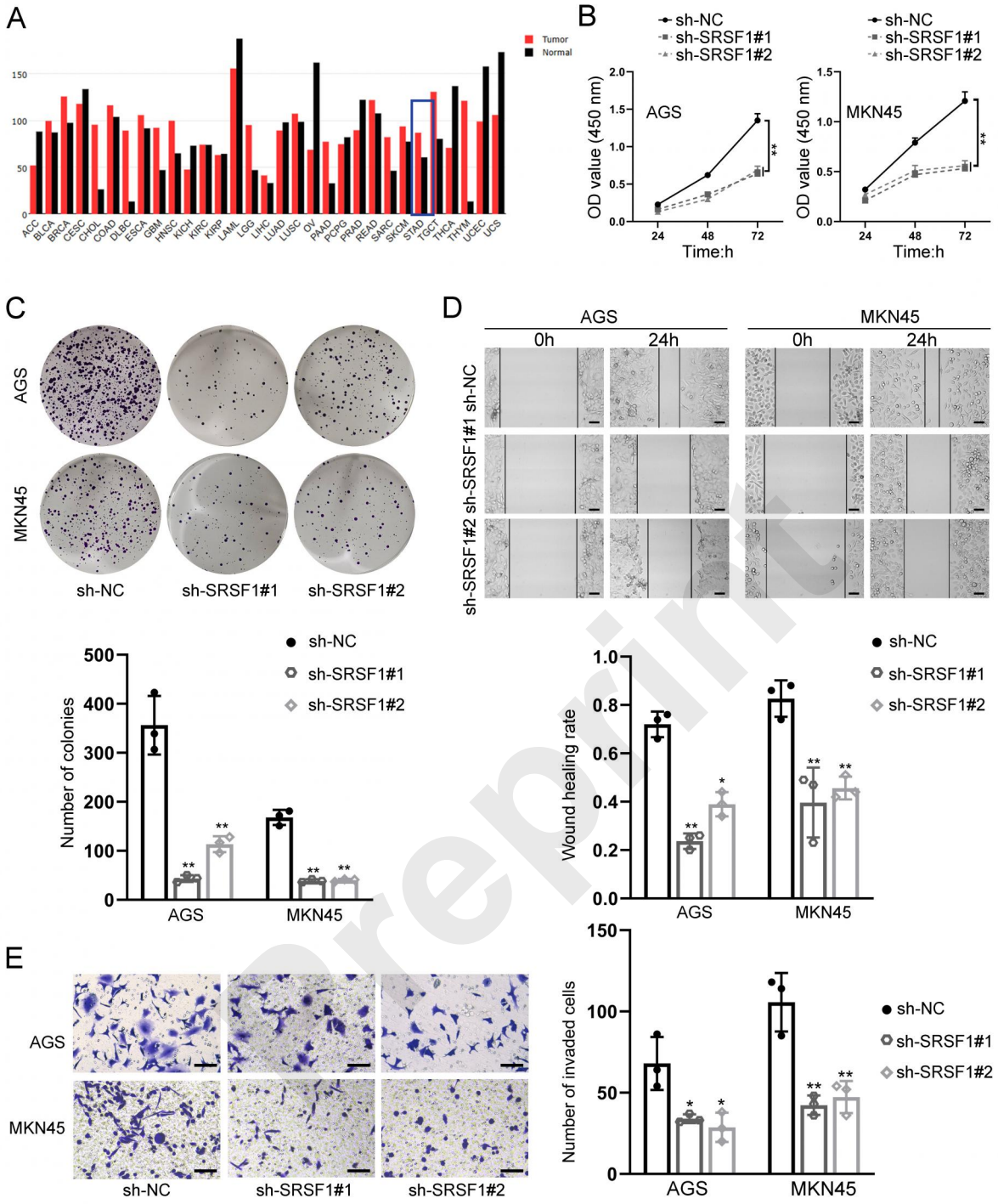
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Tumor
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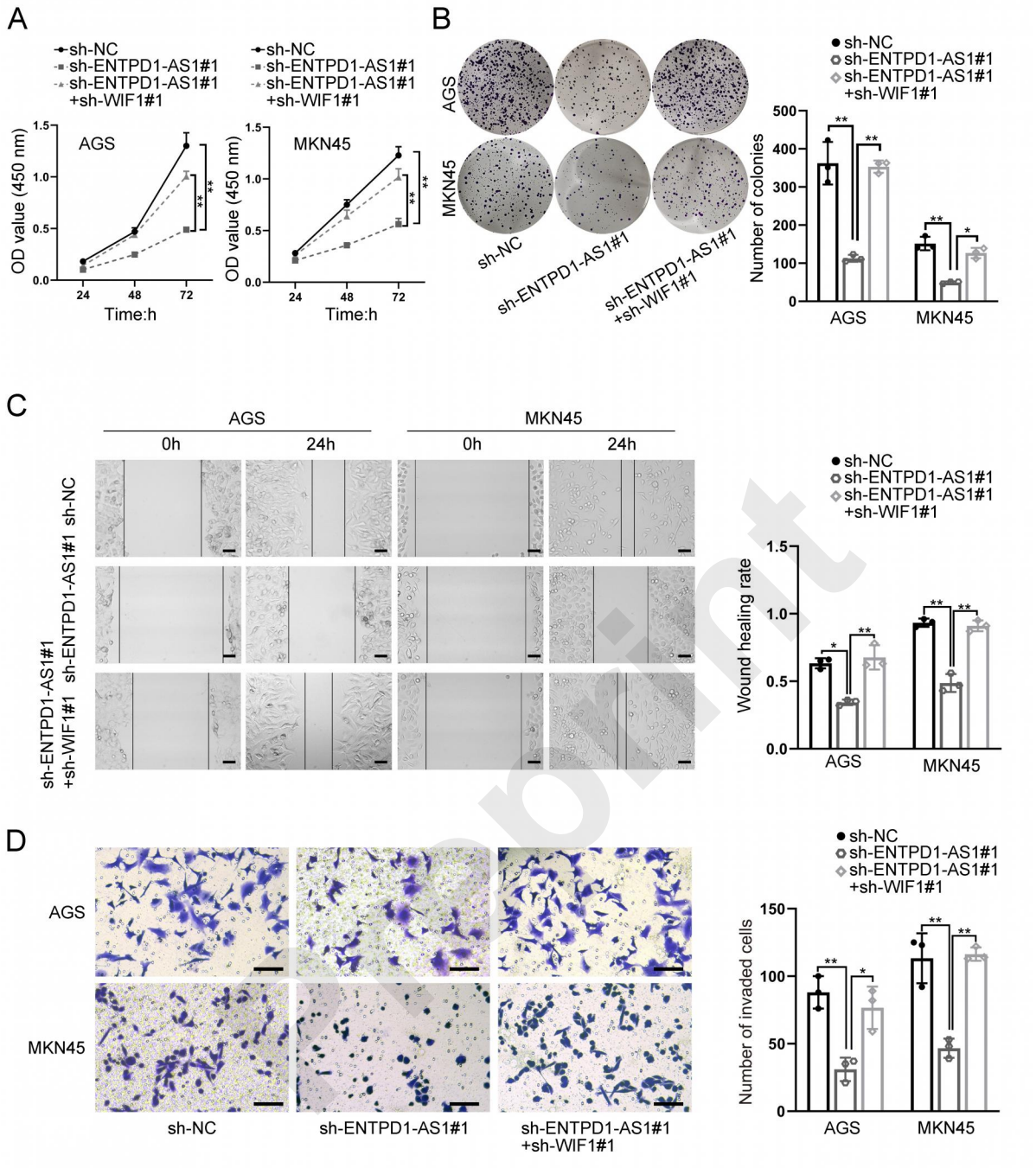
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS
B
sh-NC
sh-SRSF1#1
sh-SRSF1#2
AGS
MKN45
OD value (450 nm)
Time:h
C
AGS
MKN45
sh-NC
sh-SRSF1#1
sh-SRSF1#2
Number of colonies
D
AGS
MKN45
0h
24h
sh-NC
sh-SRSF1#1
sh-SRSF1#2
Wound healing rate
E
Number of invaded cells

Preprint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MKN45
OD value (450 nm)
Time:h
B
AGS
MKN45
sh-NC
sh-ENTPD1-AS1#1
sh-ENTPD1-AS1#1 +sh-WIF1#1
Number of colonies
C
0h
24h
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sh-ENTPD1-AS1#1
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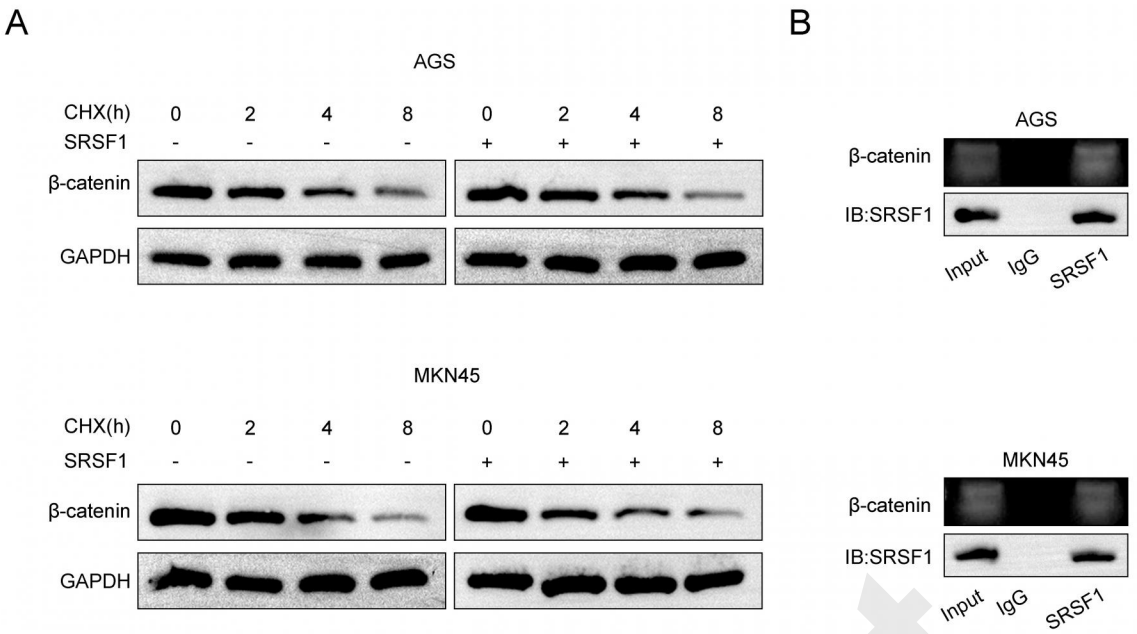
Wound healing rate
D
Number of invaded cells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CHX(h) 0 2 4 8 0 2 4 8
SRSF1 - - - - + + + +
β-catenin
GAPDH
MKN45
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SRSF1 - - - - + + + +
β-catenin
GAPDH
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Input IgG SRSF1
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β-catenin
IB:SRSF1
Input IgG SRSF1

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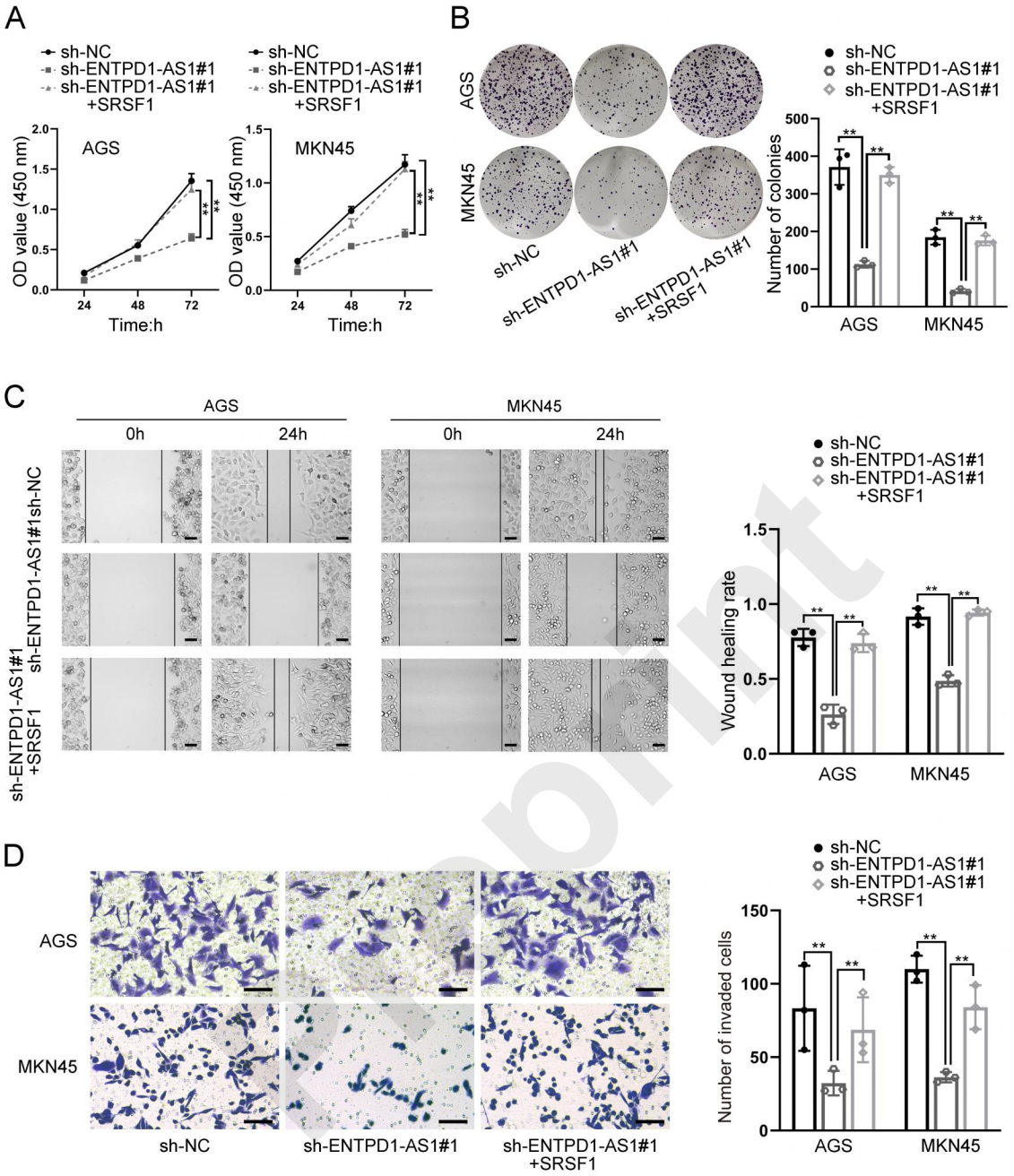
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AGS
MKN45
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Time:h
B
AGS
MKN45
sh-NC
sh-ENTPD1-AS1#1
sh-ENTPD1-AS1#1 +SRSF1
Number of colonies
C
0h
24h
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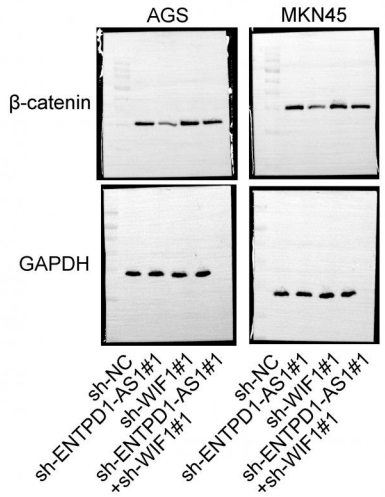
sh-ENTPD1-AS1#1 +SRSF1
Wound healing rate
D
Number of invaded cells

3E



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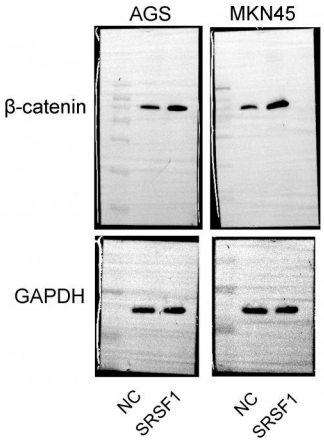

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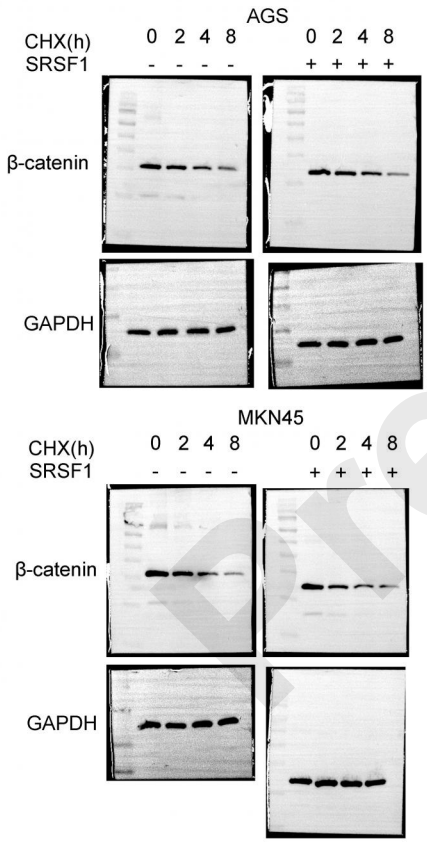

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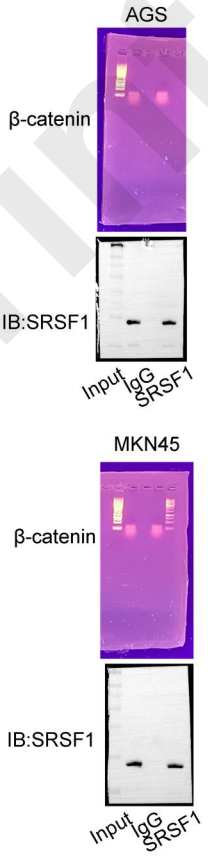

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