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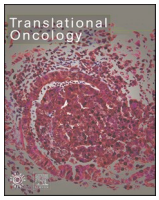

PRDM1 promotes the stemness of gastric cancer cells by enhancing the transactivation of Myc

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ABSTRACT

Objective: The roles of transcriptional factor *PR/SET domain 1 (PRDM1)* have been revealed in numerous tumor types. However, its effects on gastric cancer progression are still confusing. This study aimed to explore the roles and underlying mechanisms of *PRDM1* in gastric cancer progression.
Methods: In silico analysis was used to analyze gene expression levels, expression correlation, and the relationship with patients' survivals. Functional experiments, including Western blot, RT-qPCR, sphere-formation ability, cytometry analysis, and tumorigenic analysis, were conducted to evaluate *PRDM1*'s effects on gastric cancer stemness. ChIP, luciferase reporter, and bioinformatic analysis were performed to reveal the underlying mechanisms.
Results: we demonstrated that *PRDM1* was highly expressed in gastric cancer tissues and cells. Knockdown of *PRDM1* significantly attenuated gastric cancer stemness. Mechanistical studies indicated that PRDM1 directly bound to the promoter region of *Myc*, a critical contributor of cancer stem cells (CSCs), and thus enhanced *Myc* transcription activity.
Conclusions: Taken together, this study indicates that PRDM1 could enhance gastric cancer stemness by increasing the transcriptional activity.

Introduction

Gastric cancer is a highly prevalent malignant digestive disease, accounting for 42 % of global cases and showing a rising incidence trend [1]. The early diagnostic rate of gastric cancer remains low due to vague early symptoms and patient reluctance toward endoscopy. Most cases are diagnosed at advanced stages, where traditional treatments like surgery or chemotherapy are often ineffective, resulting in poor 5-year survival rates [2]. Therefore, exploring the pathogenesis of gastric cancer has extremely important clinical value for improving the targeted therapy and the survival rate of patients [3,4].

Convincing evidence shows that transcriptional and epigenetic mechanisms respond to external signals, modulating gene expression and ensuring coordinated cellular behaviors and fate determination [5,6]. As a master transcriptional regulator with a PR/SET domain, *PRDM1* (also known as *BLIMP1*) potentially controls gene expression by attaching to distinct locations on DNA and enlisting co-regulatory components [7]. It has been reported that PRDM1 plays a role in diffuse large B cell lymphoma [8]. Moreover, PRDM1 has been documented to be implicated in many pathways involved in tumor progression. Of note, PRDM1 might elevate USP22 transcription to reduce SPI1 protein degradation by deubiquitination, thereby increasing *PD-L1* transcription and inducing tumor cell immune evasion in hepatocellular carcinoma [7]. Additionally, the disruption of PRDM1 facilitates the proliferation of highly effective antitumor T cells, potentially benefiting various adoptive cancer immunotherapies [9]. However, the roles of PRDM1 in gastric cancer progression are never been revealed.

The concept of cancer stem cells (CSCs) or cancer stemness has been clarified as a fundamental contributor to tumor metastasis, recurrence, and drug resistance [10]. Interestingly, a prior investigation revealed that PRDM1 can inhibit the expression of genes associated with stem cells, consequently suppressing the growth of human colon tumor

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organoids [11]. Following studies demonstrated that mucosal ribosomal stress-induced *PRDM1* expression promoted chemoresistance via facilitating the stemness [12]. Consistently, PRDM1 is also involved in doxorubicin resistance by dysregulating *MDR1* expression in diffuse large B-cell lymphoma [13]. Mechanistically, PRDM1 is engaged in the modulation of the beta-catenin and Hippo pathway [14,15], both of which have been shown to be critical for CSCs expansion [16,17]. These results prompt us to speculate that PRDM1 might hold a central role in gastric cancer stemness.

Currently, we initially examined the expression of *PRDM1* in online patient samples and cells. Then functional *in vivo* and *in vitro* experiments were conducted to validate the effects of *PRDM1* on gastric cancer stemness. Furthermore, the underlying mechanisms by which PRDM1 functioned were revealed. Our findings suggested that PRDM1 enhanced gastric cancer stemness by increasing *Myc* transcriptional activity, making it a potential therapeutic target.

Materials and methods

Cell culture

Immortalized human gastric cancer cell lines (AGS, SGC7901, MKN45, BGC-823, MGC803; Procell, Wuhan, China) and the gastric epithelial cell line GES-1 (Procell, Wuhan, China) were cultured under standard conditions (37 °C, 5 % CO_2) in their respective specialized media (Procell, Wuhan, China). The cisplatin resistant AGS cells (AGS-cisR) were purchased from Procell and the resistance index was confirmed before using. However, it has been noted that the cell lines SGC-7901, BGC-823 and MGC-803 are contaminated cell lines (https://www.cellosaurus.org/CVCL_0520,https://www.cellosaurus.org/CVCL_3360,https://www.cellosaurus.org/CVCL_5334), thus, the data derived from these three cell lines were excluded from data analysis.

Real-time quantitative PCR (RT-qPCR)

Cells were initially treated with Trizol reagent (Invitrogen, Paisley Scotland, UK) to extract total RNAs, which were then reverse transcribed into complementary DNA (cDNA) using qScript cDNA SuperMix (Quantabio, Beverly, MA, USA). Subsequent amplification reactions were carried out on an ABI7500 quantitative PCR system (Applied Biosystems, Warrington, UK) utilizing Power SYBR Green Master Mix (Invitrogen). After being normalizing to *GAPDH*, the $2^{-\Delta\Delta Ct}$ method was applied for the relative expression of target genes. The primer sequences were listed as below: *Oct4*, Forward (5′- AGCGATCAAGCAGCGACTA-3′), Reverse (5′-GGAAAGGGACCGAGGAGTA-3′); *KLF4*, Forward (5′- AGCCCAGCCAGAAAGCACTACAAT-3′), Reverse (5′- AGAACCAAGACTCACCAAGCACCA-3′); *Myc*, Forward (5′- ACCCTTCGCTATCATGCCTTGGTT-3′), Reverse (5′- TTGTGTGCCTCAGCTTCCCTCTCT-3′); *GAPDH*, Forward (5′-CTTAGTTGCGTTACACCCTTTCTTG-3′), Reverse (5′- CTGTCACCTTCACCGTTCCAGTTT-3′).

Western blot

Briefly, total protein lysates were extracted from cells using ice-cold RIPA buffer (KeyGen, Nanjing, China) supplemented with protease and phosphatase inhibitors, followed by centrifugation. Proteins were separated on a 10 % SDS-PAGE gel and transferred onto PVDF membranes (Millipore, Molsheim, France). After blocking, the membranes were incubated overnight at 4 °C with primary antibodies, followed by a 2-hour incubation at 37 °C with secondary antibodies. Protein bands were visualized using an ECL reagent (Solarbio, Beijing, China) and quantified with ImageJ software.

Lentivirus package and stable cell line construction

Briefly, the lentiviral GV248 construct encoding short hairpin RNAs (shRNA) against human *PRDM1* were obtained from Genechem (Shanghai, China). Meanwhile, GV248 lentiviral empty vector was used as control (sh-NC or Vector). The siRNA sequences for *PRDM1* were: 5′-UGCCACCUUCAGUGAAGAGGdTdT-3′. The *NC* sequences were: 5′-UUCUCCGAACGUGUCACGUdTdT-3′. Subsequently, the constructs were transfected into HEK293T cells (CL-0005, Procell) with lentivirus packaging plasmids. Lentiviral particles were generated, enriched, filtered, quantified, and used to infect MKN45 and AGS cells with polybrene. Stable cell lines were selected with puromycin, and PRDM1 knockdown efficiency was confirmed by RT-qPCR.

Sphere-formation assay

The detailed procedure was referred to our previous study [18].

Flow cytometry assay

CD44+ subpopulation with stemness was detected using MKN45 and AGS cells with or without *PRDM1* knockdown through flow cytometry analysis. In summary, cells were collected at a density of 10^6 cells/mL through centrifugation at 300 × *g* and 4 °C for 10 min. Subsequently, the cells were treated with anti-CD44-APC (BD Biosciences, USA) or an isotype control, and incubated at 4 °C in darkness for 30 min. Then cells with anti-CD44 stained were washed with PBS. Finally, flow cytometer (BD, USA) was used to measure the CD44+ subpopulation.

CCK8 assay

Cell proliferation was assessed using the CCK-8 assay. Gastric cancer cells, with or without *PRDM1* knockdown, were seeded in 96-well plates (1000 cells/well) and incubated for 16 –18 h. Cells were then treated with cisplatin (500 nM) for 24, 48, and 72 h. Afterward, 10 μL of CCK-8 (Solarbio, Beijing, China) was added per well and incubated for 2 h at 37 °C in the dark. Absorbance at 450 nm was measured using a Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific). All experiments were performed in triplicate.

Transwell migration and invasion analysis

The migratory and invasive capabilities of gastric cancer cells were assessed using transwell chambers with or without Matrigel-precoated inserts (Chemicon, Temecula, *CA*, USA). In summary, cells were seeded into the upper chamber, while medium containing 10 % FBS was added to the lower chamber. After 48 h, the migrated and invaded cells were fixed, stained, and enumerated under a microscope.

Luciferase reporter assay

To identify *Myc* promoter activity, the promoter region of *Myc* possessing PRDM1-binding sites (RiboBio) were sub-cloned into pmirGLO plasmids (Promega, Madison, WI, USA). The Mut Express® II Fast Mutagenesis Kit V2 (Vazyme, Cat # C214–01, Nanjing, China) was used to construct the mutation plasmids. Then, MKN45 and AGS cells were transfected with the above-modified plasmids and sh-NC or sh-PRDM1. Cells were transfected with these plasmids and *PRDM1* or Vector. 72 h later, luciferase activities in cell lysates were monitored using Dual Luciferase Assay Kit (Promega).

Chromatin immunoprecipitation (ChIP) assay

Based on JASPAR software analysis, putative PRDM1 binding sites in the Myc promoter were predicted. Subsequently, to preliminary confirm their binding, a SimpleChIP ® Enzymatic Chromatin IP Kit (CST, Danvers, MA, USA) was used in MKN45 and AGS cells. The cells were firstly subjected to lysis on ice for a duration of 10 min. Then, isolated genomic DNA was sheared into 200 - 600 bp fragments by the sonicated. After

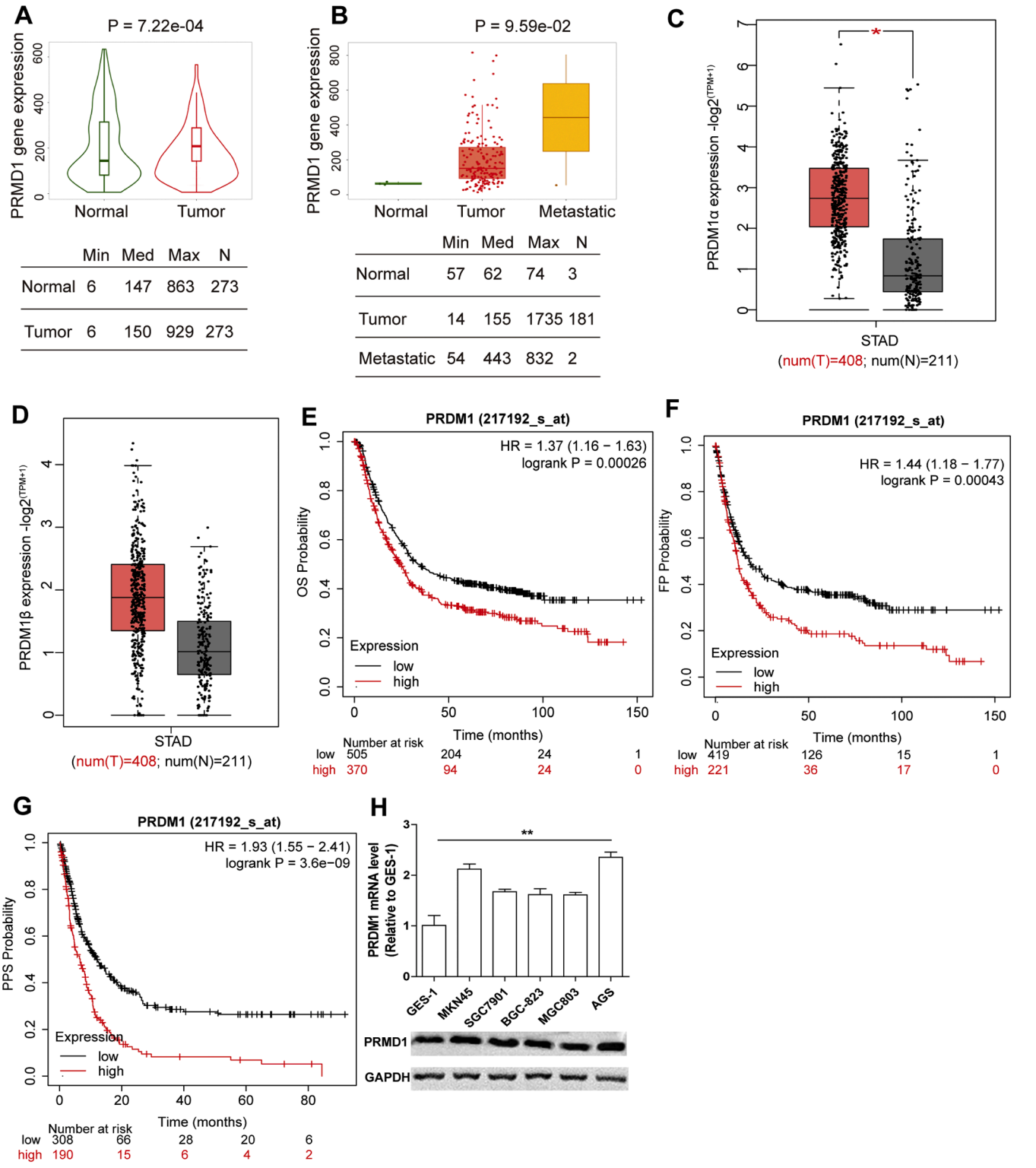

Fig. 1. *PRDM1* is highly expressed in gastric cancer tissues and cells. (A) Online databases were utilized to analyze the mRNA expression level of *PRDM1* in gastric cancer tissues and adjacent normal tissues. (B) The mRNA expression level of *PRDM1* was assessed in online gastric cancer tissues with or without metastasis, as well as in normal tissues. (C and D) GEPIA2 (Gene Expression Profiling Interactive Analysis 2) platform was used to evaluate the expression levels of *PRDM1*'s two molecular variants, *PRDM1α and PRDM1β* in gastric cancer and normal tissues. (E) Using online analytical tools, the correlation between *PRDM1* expression and the overall survival (OS) of gastric cancer patients was investigated. (F) Similarly, the correlation between *PRDM1* expression and the probability of first progression (FP) in gastric cancer patients was determined using online analytic tools. (G) Furthermore, the association between *PRDM1* expression and the probability of post-progression survival (PPS) in gastric cancer patients was evaluated using online analytic tools. (H) Finally, the mRNA and protein expression levels of *PRDM1* were examined in various types of gastric cancer patients and normal gastric epithelial cells. $N = 3$, $^{**}P < 0.01$ vs control group.

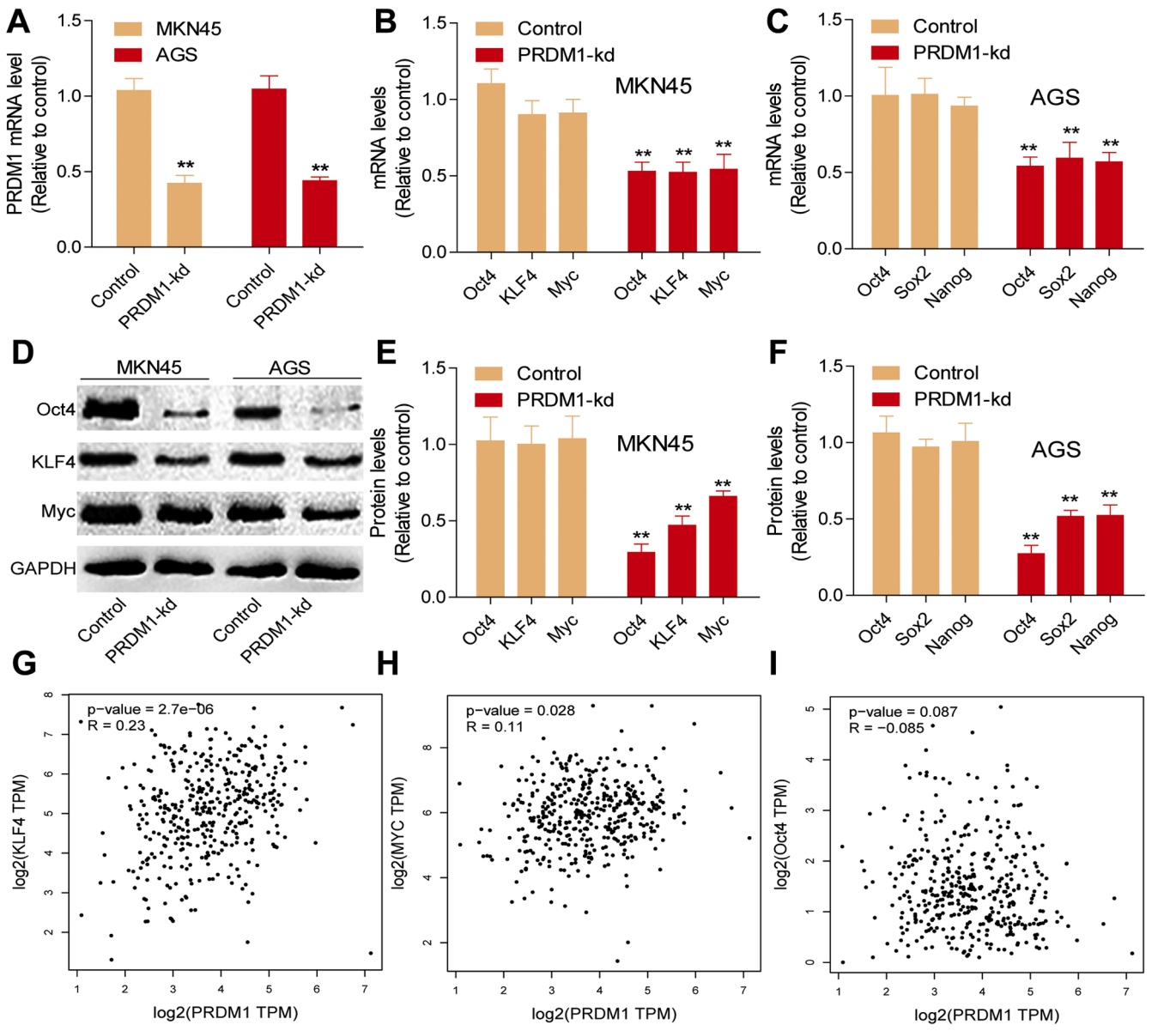

Fig. 2. Knockdown of *PRDM1* suppresses the expression of stemness markers. (A) The mRNA expression of *PRDM1* was assessed in gastric cancer cells subjected to either *PRDM1* knockdown or control conditions. (B and C) The mRNA expression levels of stemness markers were evaluated in gastric cancer cells with or without *PRDM1* knockdown. (D - F) Furthermore, the protein expression levels of stemness markers were examined in gastric cancer cells following *PRDM1* knockdown or under control conditions. (G - I) The expression correlation between *PRDM1, Oct4, KLF4*, and *Myc* was determined through GEPIA2 platform. $N = 3$, $^{**}P < 0.01$ vs control group.

centrifugation and collection, 20 μL supernatant was kept as Input group. Meanwhile, 70 μL samples and chromatin were incubated and precipitated with PRDM1 or IgG antibodies and magnetic beads for 6 h. After washing, RT-qPCR analysis for the detection of these products.

In vivo tumorigenic assay

For evaluating the effects of PRDM1 on the tumor-initiating ability of gastric cancer cells, Extreme Limiting Dilution Analysis (ELDA) (https://bioinf.wehi.edu.au/software/elda/) was performed using MKN45 cells with or without *PRDM1* stably knockdown [19]. Cells were subcutaneously injected into one side of 6-weeks old male BALB/c nude mice (18 – 22 g). All animal experiments were obtained the approval of the Ethics Committee for Animal Experimentation of Beijing Jishuitan Hospital Guizhou Hospital (2023–05–23). 1×10^7, 1×10^6, 1×10^5 cells/mice were conducted to inoculate in the right axilla of mice (6 mice per group) [20]. A total 36 mice were used for *in vivo* tumorigenic assay. Randomisation was employed to allocate the mice into control and treatment groups. A computer-generated randomization sequence was used to ensure unbiased group assignment. 21 days later, the tumor-initiating cell ratio was calculated following the protocols described in ELDA. All animals were housed in cages in a temperature-controlled (22 – 24 °C) environment with a relative humidity of 60 % – 70 % and a 12-h light and 12-h dark cycle. To minimize potential confounders, such as cage location effects, cages were randomly arranged on racks, and the individuals performing tumor size measurements were blinded to the group assignments. All animals received water and food *ad libitum* followed the 'Guide for the Care and Use of Laboratory Animals, 8th Edition' [21]. Mice were maintained on their respective diets during the treatment period. At the end of the experiment, the mice were killed by an overdose injection of pentobarbitone (200 mg/kg). And the research protocol has been approved by

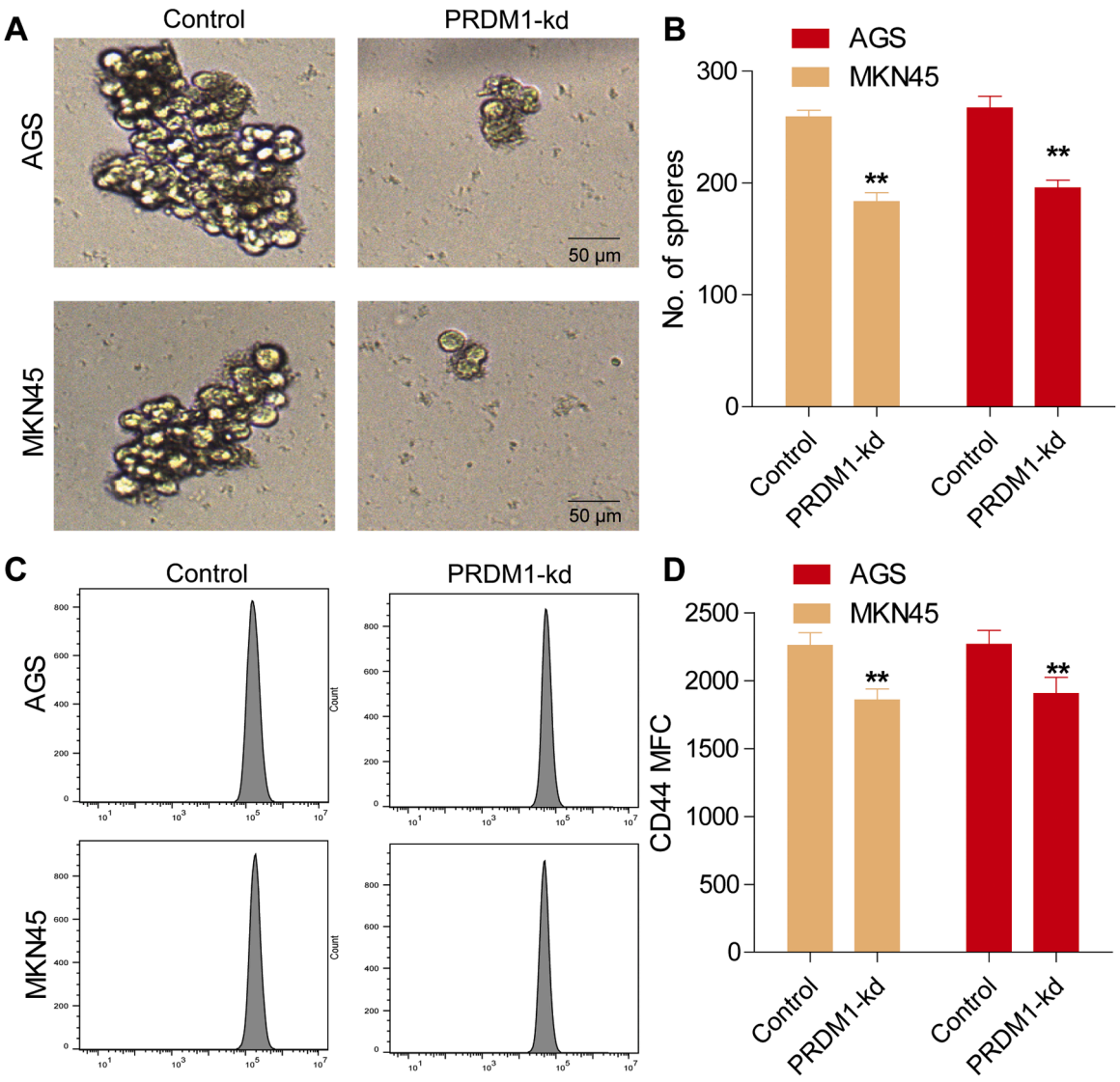

Fig. 3. Knockdown of *PRDM1* inhibits the sphere-formation ability and CD44+ subpopulation with stemness. (A) The size of spheres was evaluated in gastric cancer cells subjected to either *PRDM1* knockdown or control conditions. (B) The number of spheres was quantified in gastric cancer cells with or without *PRDM1* knockdown. (C) The CD44+ cell subpopulation was analyzed in gastric cancer cells following *PRDM1* knockdown or control conditions. (D) Subsequently, the quantification of the CD44+ cell subpopulation was performed. $N = 3$, $^{**}P < 0.01$ vs control group.

the Ethics Committee for Animal Experimentation of Beijing Jishuitan Hospital Guizhou Hospital (KT2024123101).

Immunohistochemistry (IHC) assay

Tissue sections were adhered to anti-release slides and baked at 60 °C for 2 h before storage at room temperature. Paraffin sections were dewaxed and hydrated with xylene and ethanol, followed by antigen retrieval in boiling citric acid solution for 10 min. The sections were then cooled at room temperature for 30 min and rinsed three times with PBS. After blocking for 1 hour at room temperature, primary antibody solution was applied and incubated overnight at 4 °C. The sections were washed twice with PBS, then incubated with biotinylated secondary antibody for 40 min. After further PBS washes, DAB solution was applied for 5 min, followed by hematoxylin re-staining for 30 s. The slides were dehydrated with gradient alcohol and xylene, sealed with neutral resin, dried, and observed under a microscope for image collection.

In silico analysis

Gastric cancer tissue samples were sourced from the online TCGA database via TNMplot (https://tnmplot.com/analysis/) [22], enabling analysis of *PRDM1* expression levels in gastric cancer tissue. Additionally, the relationship between gene expression and breast cancer patients' survivals was evaluated through an online analysis tool at KMplot (https://kmplot.com/analysis/index.php?p=background) [22,23]. Additionally, GEPIA2 (Gene Expression Profiling Interactive Analysis 2: http://gepia.cancer-pku.cn/) platform was used to evaluate the expression levels of *PRDM1*'s two molecular variants, *PRDM1α and PRDM1β* in gastric cancer and normal tissues. And the correlation among *PRDM1* expression, stemness markers expression, and *MYC* expression was also determined through GEPIA2 platform. And log2 fold change or adjusted P-value were output.

Statistical assay

In this study, all quantitative data were presented as mean ± standard deviation (SD). Statistical analyses were conducted using

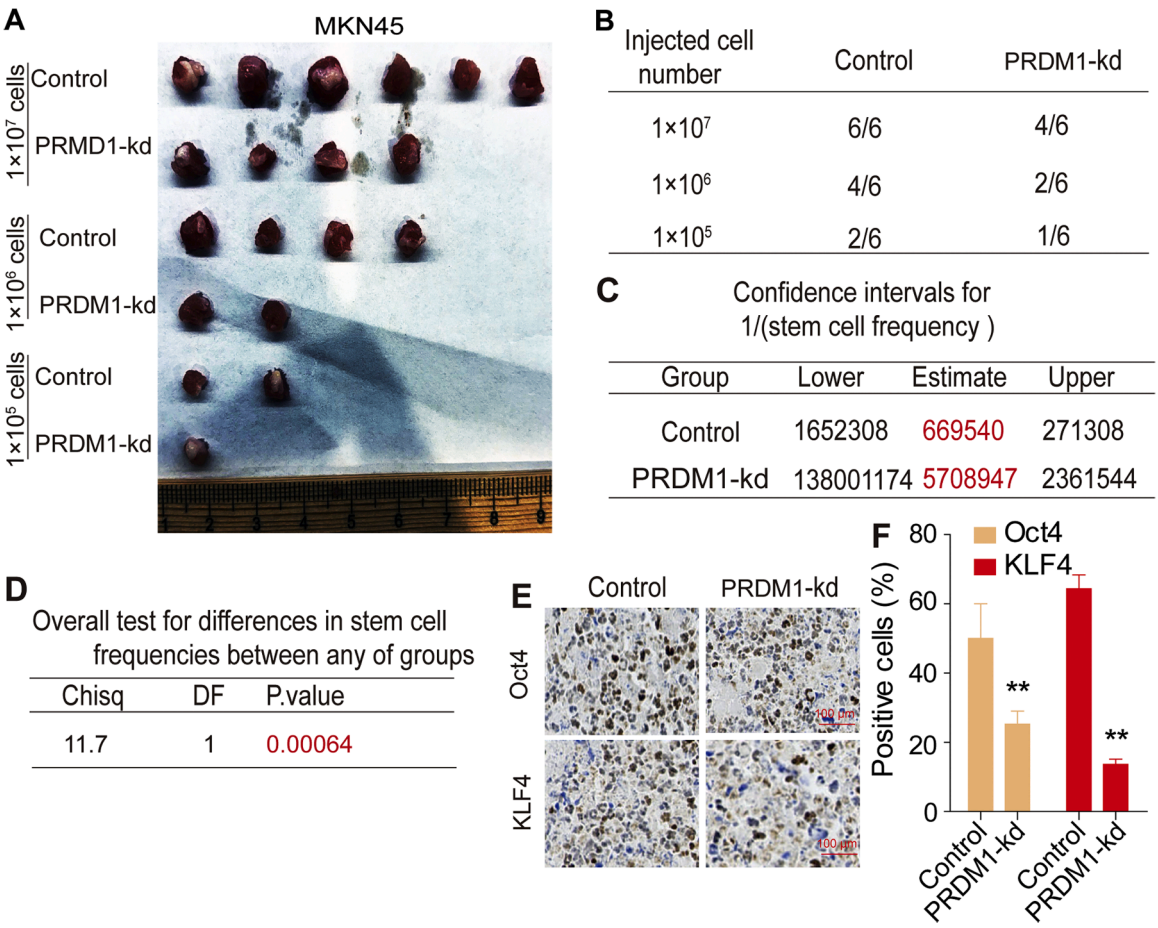

Fig. 4. Knockdown of *PRDM1* attenuates the tumorigenic ability of gastric cancer cells *in vivo*. (A) Images of tumors originating from MKN45 cells subjected to *PRDM1* knockdown or control conditions. (B) The ratio of tumor formation was determined based on the incidence of tumor formation as described in (A). (C) The frequency of stem cells was assessed utilizing ELDA, calculated from the tumor formation ratio and the number of incubated cells. (D) Differences in stem cell frequencies between the control and *PRDM1* knockdown groups were analyzed using ELDA. (E and F) Furthermore, the expression levels of *Oct4* and *KLF4* were evaluated in tumors derived from MKN45 cells with or without *PRDM1* knockdown and quantified. $N = 6$, $^{**}P < 0.01$ vs control group.

GraphPad Prism 9. Data comparisons were performed utilizing unpaired Student's *t*-test or one-way ANOVA with Tukey's post hoc tests. Statistical significance was defined as $P < 0.05$. The reporting of this study conforms to ARRIVE 2.0 guidelines [24].

Results

PRDM1 is highly expressed in gastric cancer tissues and cells

Initially, we identified an elevated *PRDM1* expression in gastric cancer cells compared to normal adjacent tissues through online dataset analysis (TNMplot: differential gene expression analysis in Tumor, Normal, and Metastatic tissues, https://tnmplot.com/analysis/) [22] (Fig. 1a). As *PRDM1* gene encode for two molecular variants, *PRDM1α and PRDM1β* [25], we further evaluated these two variants expression in another analytic platform, GEPIA2 (http://gepia2.cancer-pku.cn/#index) and found that only *PRDM1α* expression exhibited a significant difference between gastric cancer and normal tissues (Fig. 1b and Fig. 1c). Thus, we focused on *PRDM1α* in this study. We demonstrated that *PRDM1* expression was positively correlated with gastric cancer progression (Fig. 1d). Furthermore, we showed that the higher *PRDM1* expression predicted poorer survival outcomes for gastric cancer patients using Kaplan-Meier Plotter analysis (http://kmplot.com/analysis/index.php?p=background) [23] (Fig. 1e, Fig. 1g). Consistently, it was observed that *PRDM1* expression was notably elevated in gastric cancer cells, particularly in MKN45 and AGS cells (Fig. 1h), which were selected for subsequent research investigations.

Knockdown of PRDM1 suppresses the expression of stemness markers

To explore the impact of PRDM1 on gastric cancer progression, our study focused on the stemness of gastric cancer cells as CSCs play a pivotal role in driving gastric cancer progression [10]. Lentiviral infection was used to generate MKN45 and AGS cells with stable *PRDM1* knockdown, which was validated by RT-qPCR (Fig. 2a). Subsequently, we indicated that *PRDM1* knockdown led to a significant reduction in the mRNA levels of stemness markers (Fig. 2b and Fig. 2c). Consistently, the protein levels of stemness markers were also diminished by *PRDM1* knockdown in gastric cancer cells (Fig. 2d, Fig. 2f). Furthermore, we used GEPIA2 platform to reveal the correlation between *PRDM1* and stemness markers expression. As shown in Fig. 2g, Fig. 2i, *PRDM1* expression exhibited a positive correlation with *KLF4* and *Myc*, but not *Oct4*. These findings collectively indicate that PRDM1 positively regulates the expression of stemness markers in gastric cancer cells.

Knockdown of PRDM1 inhibits the sphere-formation ability and CD44+ subpopulation with stemness

We next investigated the effect of PRDM1 knockdown on sphere-forming capacity and the $CD44^+$ subpopulation, both markers of gastric cancer cell stemness. *PRDM1* knockdown significantly reduced sphere-forming ability, as indicated by decreases in sphere size and

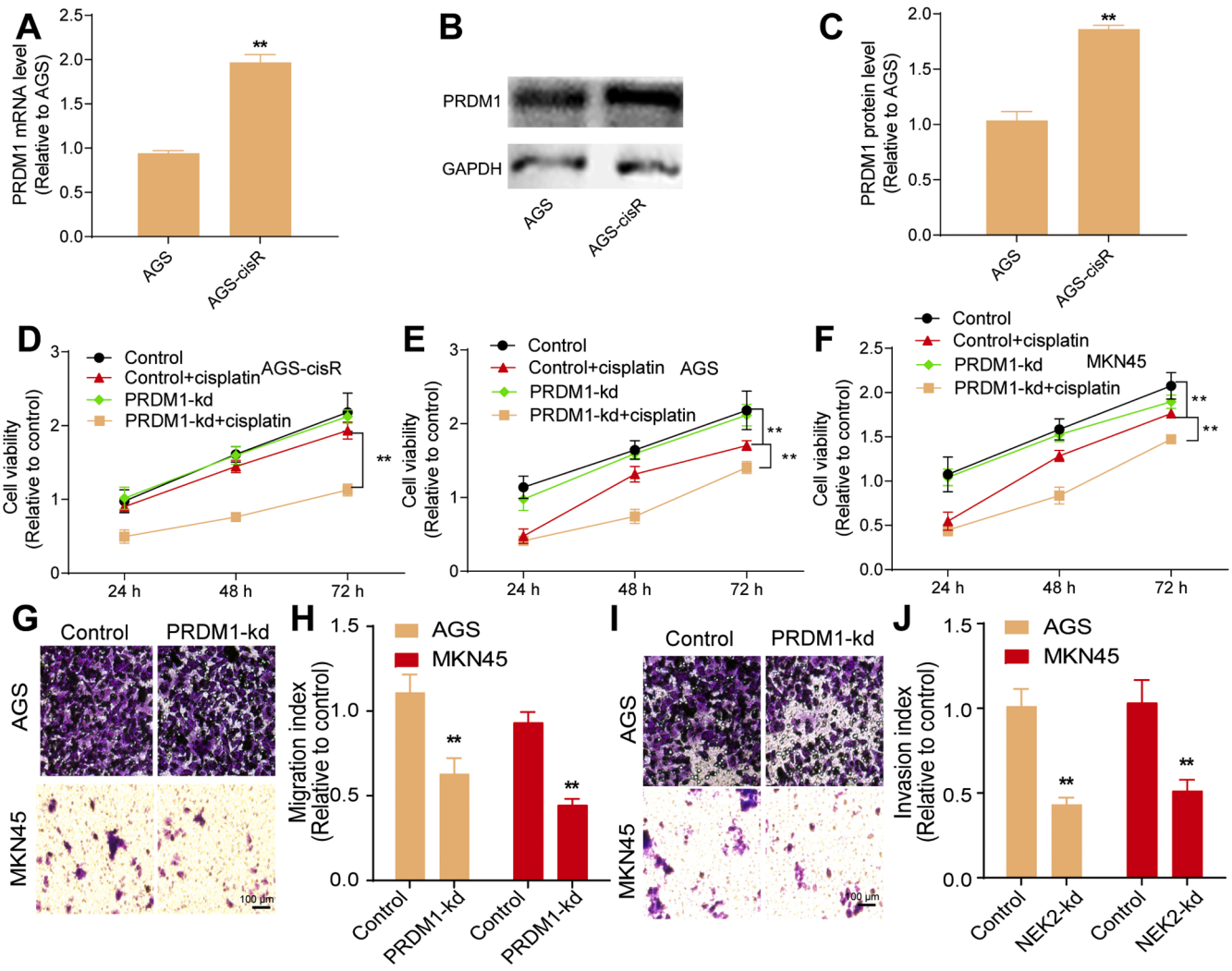

Fig. 5. Knockdown of *PRDM1* reduces the migration and invasion ability, and cisplatin resistance of gastric cancer cells. (A) The mRNA level of *PRDM1* was detected in AGS with or without cisplatin resistance. (B and C) The protein levels were assessed in AGS cells with or without cisplatin resistance. (D) Following treatment with cisplatin for 24 h, 48 h, and 72 h, the cell viability of AGS-cisR cells with or without *PRDM1* knockdown was evaluated. (E and F) Similarly, the cell viability of gastric cancer cells with or without *PRDM1* knockdown was assessed after treatment with cisplatin for 24 h, 48 h, and 72 h. (G and H) The migration ability of gastric cancer cells subjected to *PRDM1* knockdown or control conditions was determined. (I and J) Furthermore, the invasion ability of gastric cancer cells with or without *PRDM1* knockdown was measured. $N = 3$, $^{**}P < 0.01$ vs control group.

number (Fig. 3a, Fig. 3b). Flow cytometry further demonstrated a reduction in the $CD44^{+}$ subpopulation, a key indicator of stemness, following *PRDM1* knockdown (Fig. 3c, Fig. 3d). These results suggest that PRDM1 promotes gastric cancer cell stemness *in vitro*.

Knockdown of PRDM1 attenuates the tumorigenic ability of gastric cancer cells in vivo

Since CSCs contribute to the tumorigenesis, we conducted *in vivo* tumorigenic assays through MKN45 cells with or without *PRDM1* knockdown to validate the *in vitro* results. 1×10^7, 1×10^6, 1×10^5 MKN45 cells with or without *PRDM1* knockdown were subcutaneously seeded into nude mice, individually. After 21 days, the tumor formation ratio and tumor size were recorded. As shown in Fig. 4a and Fig. 4b, MKN45 cells with *PRDM1* knockdown displayed a lower tumor formation ratio and size. Additionally, employing ELDA, it was observed that the frequency of cancer stem cells (CSCs) was notably diminished in MKN45 cells following *PRDM1* knockdown (Fig. 4c and Fig. 4d). Furthermore, IHC analysis revealed reduced expression levels of stemness markers (*Oct4, KLF4*) in tumors originating from MKN45 cells with *PRDM1* knockdown (Fig. 4e and Fig. 4f). Thus, these findings underscore the role of PRDM1 in enhancing the tumor-initiating potential of gastric cancer cells.

Knockdown of PRDM1 reduces the migration and invasion ability, and cisplatin resistance of gastric cancer cells

Considering that tumor cell stemness significantly contributes to tumor metastasis and drug resistance [16], we delved deeper into the influence of PRDM1 on the migratory and invasive capacities of gastric cancer cells, along with cisplatin resistance. Initially, we observed an upregulation of *PRDM1* expression in AGS-cisR cells compared to parental AGS cells (Fig. 5a, Fig. 5c). Subsequently, CCK8 analysis unveiled that *PRDM1* knockdown mitigated cisplatin resistance in AGS cells (Fig. 5d) while enhancing cisplatin sensitivity in gastric cancer cells (Fig. 5e and Fig. 5f). Remarkably, PRDM1 exhibited minimal effect on gastric cancer cell viability (Fig. 5d, Fig. 5e). Additionally, transwell migration assays illustrated a significant suppression of gastric cancer cell migration following *PRDM1* knockdown (Fig. 5*g* and Fig. 5h). Furthermore, consistent outcomes were obtained in transwell invasion assays (Fig. 5i and Fig. 5j). Collectively, these findings underscore the role of PRDM1 in promoting the stemness of gastric cancer cells.

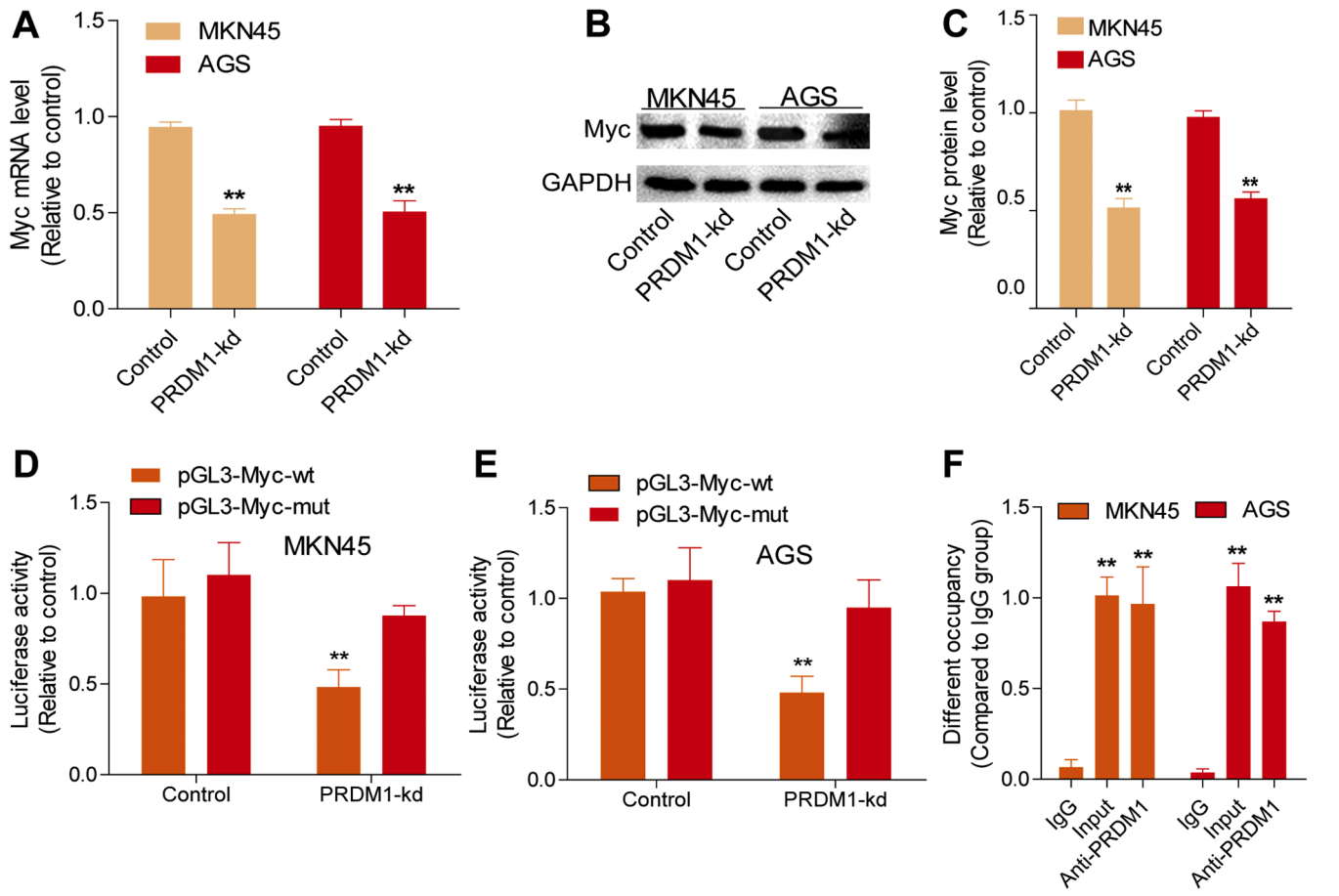

Fig. 6. PRDM1 directly binds to the promoter of *Myc* and enhance the transcription activity of *Myc*. (A - C) *Myc* expression levels were assessed in MKN45 cells subjected to either *PRDM1* knockdown or control conditions. (D and E) Luciferase reporter assays were conducted to investigate the impact of *PRDM1* knockdown on the promoter activity of *Myc*. (F) ChIP analysis was conducted to examine the abundance of the *Myc* promoter in DNA pulled down by anti-PRDM1 antibodies. $N = 3$, $^{**}P < 0.01$ vs control group.

PRDM1 directly binds to the promoter of myc and enhances the transcription activity of myc

Finally, we investigated the mechanisms through which PRDM1 enhances the stemness of gastric cancer cells. Considering PRDM1′s classification as a transcription factor and its positive regulation of stemness marker expression, we hypothesized its potential direct binding to the promoters of these markers. The JASPAR database predicted that PRDM1 could act as a transcriptional factor of *Myc*, but not *KLF4* and *Oct4*. Thus, we speculated that *Myc* was a critical downstream effector of PRDM1 responsible for PRDM1's roles in gastric cancer stemness. As expected, *PRDM1* knockdown decreased the mRNA levels of *Myc* in gastric cancer cells (Fig. 6a). Consistently, gastric cancer cells exhibited reduced Myc protein expression upon *PRDM1* knockdown (Fig. 6b and Fig. 6c). Additionally, luciferase reporter analysis showed that the promoter activity of *Myc* exhibited a lower activity in MKN45 cells with *PRDM1* knockdown, while the activity was unaffected upon the binding sites were mutated (Fig. 6d and Fig. 6e). Furthermore, ChIP analysis revealed a significant enrichment of the *Myc* promoter in DNA pulled down by the PRDM1 antibody (Fig. 6f). Therefore, our findings suggest that PRDM1 enhances the stemness of gastric cancer cells by functioning as a transcription factor for *Myc*.

Discussion

Gastric cancer poses a major global health challenge due to its high prevalence, late diagnosis, and poor prognosis with limited treatment options [1]. Elucidating the molecular mechanisms of gastric cancer progression, particularly the role of transcriptional regulators, is essential for advancing targeted therapies [26]. In this study, we investigated the role of PRDM1, a transcriptional factor, in gastric cancer stemness, focusing on its interaction with the oncogenic transactivation of *Myc*.

Our findings reveal that *PRDM1* is highly expressed in gastric cancer tissues and cells, particularly in metastatic tissues, suggesting its potential as a prognostic marker for gastric cancer patients. Consistent with its role in other tumor types [11,12], PRDM1 appears to promote gastric cancer progression by enhancing cancer stemness. This is supported by our functional experiments, which demonstrated that knockdown of PRDM1 suppressed the expression of stemness markers and reduced the sphere-forming ability and CD44+ subpopulation in gastric cancer cells. It must be noted that *PRDM1* expression only exhibited a positive correlation with *KLF4* and *Myc*, but not *Oct4* through in silico analysis (Fig. 2g, Fig. 2i), this might be due to the limited sample size and further research should be conducted to further confirm the correlation between *PRDM1* and *Oct4* expression in gastric cancer tissues.

Importantly, our study uncovered a novel mechanism by which PRDM1 regulates gastric cancer stemness through its interaction with *Myc transactivation*. We demonstrated that PRDM1 directly bound to the promoter of *Myc*, leading to increased transcriptional activity and upregulation of *Myc* expression. Given the well-established role of Myc in promoting cancer stemness [27–29], our findings provide mechanistic insight into how PRDM1 contributes to gastric cancer progression.

Notably, previous studies have shown that both of Oct4 and KLF4, another two critical CSC master regulators, act as the downstream effectors of Myc [30–32], thus we speculate that PRDM1 regulates the expression of *Oct4* and *KLF4* through Myc in gastric cancer cells, which should be explored in the future.

Our findings highlight the role of PRDM1 in promoting gastric cancer stemness by enhancing *Myc* transcriptional activity, adding to the growing body of literature that connects PRDM1 and Myc transactivation interactions in various cancers. Previous studies have revealed context-dependent functions of PRDM1 as both a tumor suppressor and a regulator of Myc activity. In diffuse large B-cell lymphoma (DLBCL), PRDM1 is frequently inactivated, and this loss correlates with *Myc* overexpression, leading to decreased p53 pathway activity and a poor prognosis in ABC-DLBCL patients [33,34]. Specifically, PRDM1 inactivation upregulates *Myc* expression at both the gene and protein levels, as demonstrated in cell lines and patient samples [34]. This contrasts with our observation in gastric cancer, where PRDM1 appears to act as a promoter of Myc transcription. Such functional divergence underscores the importance of cellular context in defining PRDM1′s role in tumorigenesis. Moreover, in the context of co-infection models, it has been reported that PRDM1 interacts with the retinoblastoma (pRb) pathway to regulate *Myc* expression and cell cycle progression. In Epstein-Barr virus (EBV)-positive Burkitt lymphoma, pRb knockdown resulted in decreased PRDM1 expression and increased *Myc* levels, further suggesting a nuanced regulatory network between PRDM1 and *Myc* that varies by cellular and pathological context [35]. This finding aligns with the notion that PRDM1 can function as a transcriptional regulator of *Myc*, but it also raises intriguing questions about the dualistic roles of PRDM1 across cancer types. Further comparative studies are warranted to elucidate the molecular determinants of PRDM1′s functional switch between tumor suppression and oncogenic promotion. In addition, these results expand our understanding of the regulatory networks underlying gastric cancer stemness and highlight PRDM1 as a potential therapeutic target for inhibiting tumor growth and metastasis. Targeting PRDM1 or its downstream effectors, such as *Myc*, could represent a promising strategy for developing novel anti-cancer therapies aimed at eradicating gastric CSCs and improving patient outcomes.

Conclusions

In conclusion, our study identifies PRDM1 as a critical regulator of gastric cancer stemness and elucidates its functional interaction with *Myc* promoter. Further investigation into the therapeutic potential of targeting the PRDM1-*Myc* axis in gastric cancer is warranted and may ultimately lead to the development of more effective treatment strategies for this devastating disease.

CRediT authorship contribution statement

Hai Qin: Writing – review & editing, Writing – original draft, Funding acquisition. **Manqin Yuan:** Data curation, Conceptualization. **Yaqin Yuan:** Visualization, Validation. **Xue Liu:** Methodology, Investigation. **Siye Yi:** Software, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests, personal relationships, or professional affiliations that could influence or appear to influence the work reported in this manuscript. All authors confirm that this research was conducted in the absence of any commercial, financial, or institutional relationships that could be construed as potential conflicts of interest.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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