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lncRNA PSMB8-AS1 promotes colorectal cancer progression through sponging miR-1299 to upregulate ADAMTS5

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Long non-coding RNAs (lncRNAs) have been reported to be vital participants in tumor progression. Recently, lncRNA PSMB8-AS1 has been uncovered to facilitate pancreatic cancer progression by regulating miR-382-3p/STAT1/PD-L1 network. Nonetheless, the role of PSMB8-AS1 and its underlying mechanism have not been well-explored in colorectal cancer (CRC). The expression of RNAs or proteins was detected via qRT-PCR or western blot assays. Functional assays were involved in evaluating the effects of PSMB8-AS1/miR-1299/ADAMTS5 on the malignant behaviors of CRC cells. The molecular mechanism of PSMB8-AS1 was explored via mechanism analyses in CRC cells. Based on experimental results, PSMB8-AS1 expression was notably higher in CRC cell lines than in normal cells. The downregulation of PSMB8-AS1 repressed cell viability, proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) of CRC while promoting cell apoptosis. It was also revealed that PSMB8-AS1 could sponge miR-1299 to upregulate ADAMTS5 in CRC cells. In rescue assays, we further discovered that miR-1299 inhibition or ADAMTS5 overexpression abrogated the suppressive influence of PSMB8-AS1 deficiency on CRC cell growth. In addition, PSMB8-AS1 was validated to induce M2 polarization. In conclusion, PSMB8-AS1 sponges miR-1299 to increase PSMB8-AS1 expression, thus promoting CRC cell growth.

Key words: colorectal cancer, PSMB8-AS1, miR-1299, ADAMTS5

Reportedly, colorectal cancer (CRC) is the third most predominant malignancy with the second highest death rate in the world [1–4]. In spite of improvement in the diagnosis and treatment, CRC prognosis remains unsatisfying due to high recurrence, drug resistance, and distant metastasis [5]. Consequently, it is important for researchers to investigate the molecular mechanisms of CRC and identify new biomarkers for the early diagnosis of CRC.

Long non-coding RNAs (lncRNAs) belong to a class of RNA molecules with over 200 nucleotides with limited protein-coding capabilities [6, 7]. Based on recent studies, lncRNAs have been revealed to regulate gene expressions and tumor progression through various mechanisms [8, 9], such as the competitive endogenous RNA (ceRNA) network [10]. LncRNA can be a ceRNA to compete with messenger RNA (mRNA) for microRNA (miRNA) to affect cancer development [10, 11]. Currently, accumulating evidence has elucidated that lncRNAs can be crucial participants in CRC progression [12–14]. LncRNA proteasome 20S subunit beta 8 antisense RNA 1 (PSMB8-AS1) is an opposite sequence of the "sense" RNA PSMB8. PSMB8 has been reported to be a potential biomarker for gastric cancer [15], glioma [16], rectal cancer [17], and many other cancers. PSMB8-AS1 has been proven to promote the progression of pancreatic cancer [18], glioma [19], and glioblastoma [20]. Nonetheless, the functions of PSMB8-AS1 in CRC remain unknown.

miRNAs are identified as small noncoding RNA molecules having 18–25 nucleotides in length, which can act as regulatory genes at post-transcriptional levels [21, 22]. miRNAs have been discovered to mediate cancer progression by binding with mRNAs [23, 24]. Pieces of research work have pointed out that miR-1299 inhibits cell migration and invasion in numerous malignancies [25–27]. As an mRNA, ADAM metallopeptidase with thrombospondin type 1 motif 5 (ADAMTS5) can act as a promoter in tumors such as colorectal cancer [28] and non-small cell lung cancer [29]. Nevertheless, whether miR-1299 could target ADAMTS5 in CRC cells is still unclear.

This study concentrated on investigating the biological function and specific mechanism of PSMB8-AS1 in

CRC cells. Further, the correlation between PSMB8-AS1, miR-1299, and ADAMTS5 in CRC cells was explored via a range of assays.

Materials and methods

Cell lines and cell culture. Six CRC cell lines (SW620, HT-29, DLD-1, SW480, DIFI, and HCT116) and one normal cell line (HCoEpiC), as well as THP1 cell line, were utilized in our study. SW620, HT-29, DLD-1, SW480, HCT116, and THP1 cells were obtained from ATCC (American Type Culture Collection; Manassas, USA). DIFI and HCoEpiC cells were purchased from TONGPAI Biotechnology Co., Ltd. (Shanghai, China). Cells (1.5×10^5) were incubated in RPMI 1640 medium (Thermo Fisher Scientific, USA) containing 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin with 5% CO_2 at the temperature of 37 °C. Media for THP1 cells were also supplemented with 0.05 mM β-mercaptoethanol (PB180633).

Cell transfection. The small hairpin RNAs (shRNAs) against PSMB8-AS1 (sh-PSMB8-AS1#1/#2) and their negative control containing a nonsense sequence (NC; sh-NC) were all obtained from GenePharma (Shanghai, China). NC mimics, miR-1299 mimics, pcDNA3.1/NC, and pcDNA3.1/ADAMTS5 were synthesized in GenePharma (Shanghai, China). To suppress PSMB8-AS1 expression, sh-PSMB8-AS1#1/#2 was transfected into CRC cells with sh-NC as the control group. TD-GAPDH served as an internal reference, and the knockdown efficiency was normalized to TD-GAPDH. To overexpress miR-1299, miR-1299 mimics were prepared, and NC mimics was regarded as the control group. To overexpress ADAMTS5, pcDNA3.1/ADAMTS5 was transfected into CRC cells, and pcDNA3.1/NC worked as the control group. The cell transfections were all performed using Lipofectamine 3000 reagents (Invitrogen, USA) in 0.8×10^5 cells.

Quantitative real time-polymerase chain reaction (qRT-PCR) assays. Total RNAs of 2×10^6 CRC cells and 2×10^6 normal cells were extracted with RNA extraction kits (Qiagen, Germany). The CFX96 Real-Time PCR operating instrument (Bio-Rad Laboratories, Inc., USA) was utilized for reverse transcription polymerase chain reaction (RT-PCR). Subsequently, the results were analyzed by SYBR qPCR Mix (Takara, Dalian, China). This experiment was also conducted with the use of the ABI PRISM7500 system (Applied Biosystems, USA). The PCR conditions were as followed: 30 s denaturation at 95 °C, 45 s annealing treatment at 55 °C, and 30 s amplification at 72 °C. The procedures underwent 30 cycles. NanoDrop 2000/2000c (Thermo Fisher Scientific, USA) was used for the quantification of RNA density. NanoDrop 2000/2000c was also employed for detecting the OD value at 260 nm to calculate the quantity of total RNAs. RNA purity was determined by measuring the ratio of optical density (OD) value at 260 nm/OD value at 230 nm. The RNA integrity number was found to be 6–8 which meant the extracts were of good quality. The $2^{-\Delta\Delta Ct}$ method was used for the calculation of expression levels. GAPDH functioned as an internal reference for lncRNAs and mRNAs, and U6 for miRNAs. Primer sequences are listed in Table 1.

Cell counting kit-8 (CCK-8) assay. SW480 and HCT116 cells were incubated in the indicated plates (96 wells, 1×10^4 cells/well) with added CCK-8 reagent (5 mg/ml, Yeasen Biotech Co., Ltd., Shanghai, China) at 37 °C and 5% CO_2 for 24, 48, and 72 h. Subsequently, 150 μl dimethyl sulphoxide (DMSO; Thermo Fisher, USA) was added to each well. ThermoMax Microplate Reader (Thermo Fisher Scientific, USA) was applied for the measurement of the OD values every 24 h.

Table 1. Primer sequences.

Gene name	Sequences
PSMB8-AS1	F: GATGGGTCAAGGGTCTTCCG R: TCGTGTGACGTCGACAGTTG
MMP2	F: GCTGCATCCAGACTTCCTCAG R: TCCATCGTAGCGCTCCCT
MMP9	F: AGTCCACCCTTGTGCTCTTC R: CAGGCCCCAGAGATTTCGAC
E-cadherin	F: AGGACGTTGGAAGGAAGAGC R: GCCACTGTGAAGGTGATTTCG
N-cadherin	F: AGCCGGAGAACAGTCTCCAAG R: CCGAGTGGTGCGACAAAGC
miR-1299	F: GCCGAGttctggaattctgtgt R: CTCAACTGGTGTCGTGGA
miR-8086	F: GCCGAGtgctagtctggactga R: CTCAACTGGTGTCGTGGA
miR-6880-5p	F: GCCGAGtggtggaggaagaggg R: CTCAACTGGTGTCGTGGA
miR-1273h-3p	F: GCCGAGctgcagactcgacctc R: CTCAACTGGTGTCGTGGA
miR-6881-3p	F: GCCGAGatcctctttcgtcctt R: CTCAACTGGTGTCGTGGA
miR-5683	F: GCCGAGtacagatgcagattctct R: CTCAACTGGTGTCGTGGA
miR-877-3p	F: GCCGAGtcctcttctccctcc R: CTCAACTGGTGTCGTGGA
miR-6736-3p	F: GCCGAGtcagctcctctctac R: CTCAACTGGTGTCGTGGA
ACO1	F: GTGCAGTCGGAGGAACACG R: CGAGCAGGCTTAAATGGCAC
ABCA12	F: GGACTTCGTGCAACAAGCAG R: CAGTACCCCTTTCACGGCAT
ADAMTS5	F: CACAGACACGCCGCTTCA R: GGGCCCACTTCCTTTCTTATT
CD206	F: TCATTCCGGGTGCTGTTCTC R: GATTCGGACACCCATCGGAA
Ym1	F: AAGCTCTCCAGAAGCAATCCT R: ATGCTTCATAGTCACGCAAGTC
Arg1	F: TTCTCAAAGGGACAGCCACG R: CATGGCCAGAGATGCTTCCA
GAPDH	F: GACAGTCAGCCGCATCTTCT R: TTCCCGTTCTCAGCCTTGAC
U6	F: TCCCTTCGGGGACATCCG R: AATTTTGGACCATTTCTCGATTTGT

Colony formation assays. After stable transfection, the obtained cells were inoculated in six-well plates at the 600 cells/well density and grew at 37 °C and 5% CO_2. After two-week incubation, the cells were fixed in paraformaldehyde. Then, a crystal violet solution (Gibco, USA) was applied to staining the cells, followed by close observation under the microscope.

5-ethynyl-2'-deoxyuridine (EdU) assay. CRC cell lines were cultivated in plates (96 wells, 1×10^4) for 48 h. After cells were washed with phosphate buffer saline (PBS; Gibco BRL, USA), 2 μM of EdU fluorescent reagent (Life Technologies Corporation, USA) was added to each well. Subsequently, 4',6-diamidino-2-phenylindole (DAPI; Life Technologies Corporation, USA) dye solution was added for cell staining. Cell proliferation was assessed under the fluorescence microscope (Leica Microsystems, Germany).

Western blot assay. Bradford protein assay (Bio-Rad Laboratories, Inc., USA) was used to extract and quantify proteins from 2×10^6 CRC cells. SDS-PAGE was utilized for the isolation of proteins. Subsequently, the proteins were transferred to PVDF (Millipore, USA) membranes via iBlot Dry Blotting Transfer System (Life Technologies Corporation, USA). Afterward, the membranes were incubated overnight with primary antibodies, namely MMP2 (ab92536, 1:1000), MMP9 (ab38898, 1:1000), E-cadherin (ab233611, 1:1000), Bax (ab32503, 1:1000), Cleaved caspase-3 (ab2302, 1:1000), Bcl2 (CAS 10326-27-9, 1:1000), and N-cadherin (ab98952, 1:1000). Antibodies except Bcl2 were obtained from Abcam (Cambridge, USA). Bcl2 was purchased from Santa Cruz Biotechnology (Beijing, China). Next, secondary antibodies were added to the membranes for 1 h incubation. Protein bands were finally developed with an enhanced chemiluminescence detection kit (Roche, Germany), and quantified by ImageJ software. GAPDH was set as the internal reference.

Transwell migration and invasion assays. Corning Transwell chambers (NY, USA) were utilized. For migration, 1×10^6 transfected SW480 and HCT116 cells were incubated in the upper compartment containing a serum-free culture medium. The complete culture medium was added to the lower compartment. After 24 h incubation, SW480 and HCT116 cell lines in the upper chamber were scrubbed with a cotton swab. At last, 0.5% crystal violet solution was used for staining the cells in the lower chamber. For the Transwell invasion assay, the upper compartment was additionally coated with Matrigel (BD Biosciences, USA), and the following procedures were identical to the steps in the Transwell migration assay.

Flow cytometry assay. CRC cells transfected with the indicated plasmids were prepared. After cold PBS washing two times, the obtained cells were re-suspended in 1× binding buffer. Subsequently, the cell suspension was moved to a 5 ml culture tube containing 5 μl of PI and 5 μl of FITC anti-Annexin V. Following 15 min incubation at 25 °C, 400 μl of 1× binding buffer was added to each tube, and the samples were analyzed within 1 h. The fluorescence of Annexin V-FITC and PI was measured at 530 nm and 585 nm, respectively. Ultimately, FlowJo software was employed for experimental data analyses.

Nuclear and cytoplasmic fraction assay. PARIS Kit (Thermo Fisher Scientific, USA) was utilized for identifying the cellular localization of PSMB8-AS1 in 1×10^7 SW480 and HCT116 cells. U6 served as the internal reference of the nuclei, and GAPDH was the cytoplasmic reference.

Fluorescence in situ hybridization (FISH) assay. PSMB8-AS1 probe was synthesized by Sangon Biotech (Shanghai, China). A Fluorescent In Situ Hybridization Kit (RiboBio, China) was applied for detecting probe signals. CRC cells (5×10^4) were stained by DAPI (Life Technologies Corporation, USA). Ultimately, the localization of PSMB8-AS1 in CRC cells was observed and photographed by a laser scanning confocal microscope.

RNA pull-down assay. RNA pull-down assay was employed following the previous protocols [15, 22]. Eight miRNA candidates were first biotinylated by a Biotin RNA labeling mix made by Roche Diagnostics (Indianapolis, USA). Subsequently, cell lysates obtained from 2×10^7 cells and biotin-coupled RNA complex, together with streptavidin-coated magnetic beads were co-incubated. Finally, the enrichment of miRNAs pulled down was quantified through qRT-PCR.

Immunofluorescence assay. Cells (4×10^3) were placed on the culture slides to adhere. Further, they were treated with PBS, and 5% BSA was added for blocking. Then, a primary antibody of PKH67 (MX4023-100UL, MKbio, Shanghai, China) was added for incubation. After rinsing, secondary antibodies (ab150113, Abcam) were added. DAPI was utilized to stain cell nuclei. Finally, a fluorescent microscope was applied to capture fluorescent signals.

Bioinformatics analysis. DIANA database (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php) was applied to identify binding sequences of miR-1299 and PSMB8-AS1. To select out candidate mRNAs that miR-1299 might target in CRC cells, information from the RNA22 website (https://cm.jefferson.edu/), miRDB website (http://mirdb.org/miRDB/), and microT database (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index) were analyzed using Venn diagram. The potential binding sequences of miR-1299 and ADAMTS5 were obtained from the microT database.

RNA-binding protein immunoprecipitation (RIP) assay. RIP kit (Millipore, USA) was involved in this assay. HCT116 cells or SW480 cells (2×10^7) were first lysed in the RIP lysis buffer. Then, the cells were co-cultivated with magnetic beads attached with anti-Argonaute 2 (anti-Ago2; TS-10X10ML-U, Millipore) or negative control anti-IgG (MABE-253, Millipore). The enrichment of RNAs was detected through qRT-PCR.

Luciferase reporter assays. PSMB8-AS1 was mutated based on gene sequences of PSMB8-AS1 and miR-1299 using QuickChang site-directed mutagenesis kit (Agilent

Technologies, USA). Then, mutated (Mut) and wild-type (WT) binding sequences between PSMB8-AS1 and miR-1299 respectively were sub-cloned into pmirGLO vectors (Promega, Madison, USA). CRC cells (1×10^4) were then co-transfected with miR-1299 mimics or NC mimics. Forty-eight hours later, a luciferase kit (Promega, Madison, USA) was utilized for measuring the luciferase activity. Renilla luciferase activity functioned as an internal reference.

Isolation and identification of exosomes. Exosomes in 4×10^3 CRC cells were separated by Exosomes Isolation Kit (Thermo Fisher Scientific). After ultracentrifugation at 4 °C, pre-depleted of bovine exosomes were obtained. Then, cells were co-cultivated with exosome-free FBS. Then, PBS was utilized to rinse cells with 10% FBS for two days. Exosomes were subjected to isolation and purification from the cell supernatant. The morphology of exosomes was detected under transmission electron microscopy (TEM), and the images were captured with a digital camera (Olympus).

Statistical analysis. All assays were repeated in triplicate. GraphPad Prism 6.0 software (California, USA) was employed to analyze the data from the experiments above. Experimental results were demonstrated as means ± standard deviation (SD). Statistical discrepancies of two or more groups were compared with the use of the Student's t-test, one-way analysis of variance (ANOVA), or two-way ANOVA. The differences were viewed to be statistically significant only when $p<0.05$.

Results

PSMB8-AS1 promotes viability and proliferation but represses apoptosis of CRC cells. We obtained six CRC cell lines (SW620, DIFI, HT-29, SW480, DLD-1, and HCT116) cells and one normal cell line (HCoEpiC) to explore the roles of PSMB8-AS1 in CRC cells. By qRT-PCR, it was found that PSMB8-AS1 was highly expressed in the CRC cells (especially in HCT116 and SW480) compared with the normal cell line (Figure 1A, *$p<0.05$, **$p<0.01$). SW480 and HCT116 cells were thus transfected with sh-PSMB8-AS1#1/2 to silence the PSMB8-AS1 expression for conducting the following functional assays (Figure 1B, **$p<0.01$). Then the CCK-8 assay was performed, and we discovered that the cell viability was repressed by PSMB8-AS1 depletion since the OD 450 values were gradually repressed in sh-PSMB8-AS1-transfected cells (Figure 1C, **$p<0.01$). Further, it was illustrated in colony formation assays that the cell viability was restrained by the knockdown of PSMB8-AS1 as the number of cell colonies declined in HCT116 and SW480 cells transfected with sh-PSMB8-AS1#1/2 (Figure 1D, **$p<0.01$). Moreover, the EdU assay further proved that the quantity of EdU positive cells was reduced when PSMB8-AS1 was silenced in cells, verifying cell proliferation was repressed by the PSMB8-AS1 depletion (Figure 1E, **$p<0.01$). Next, western blot assay was implemented and we found the level of apoptotic proteins (Cleaved caspase-3 and Bax) was increased whereas the level of anti-apoptotic protein (Bcl2) was decreased, suggesting that the PSMB8-AS1 reduction stimulated CRC cell apoptosis (Figure 1F). Moreover, the subsequent flow cytometry analysis further confirmed the inhibiting impact of PSMB8-AS1 depletion on CRC cell apoptosis (Supplementary Figures S1A, S1B, **$p<0.01$). In summary, PSMB8-AS1 is upregulated in CRC cells and prompts CRC cell viability and proliferation while suppressing CRC cell apoptosis.

PSMB8-AS1 facilitates cell invasion, migration, and EMT of CRC cells. For the purpose of further examining if PSMB8-AS1 could affect the migratory and invasive abilities of CRC cells, the Transwell assays were carried out. Transwell experiment results showed that when sh-PSMB8-AS1#1/2 was transfected into CRC cells, cell migratory and invasive capabilities were weakened (Figures 2A, 2B, **$p<0.01$). Furthermore, knockdown of PSMB8-AS1 reduced the mRNA and protein levels of MMP2, MMP9, and N-cadherin, but elevated E-cadherin expression (Figures 2C, 2D, **$p<0.01$). The levels of MMP2 and MMP9 (proteins capable of promoting migration and invasion) lowered, indicating that PSMB8-AS1 depletion restricted CRC cell migration and invasion. The E-cadherin level increased and the level of N-cadherin decreased also demonstrating that PSMB8-AS1 knockdown hampered the EMT process of CRC cells. To sum up, PSMB8-AS1 facilitates the migration, invasion, and EMT processes of CRC cells.

PSMB8-AS1 binds to miR-1299 in CRC cells. Nuclear and cytoplasmic fraction assay was conducted and we noticed that PSMB8-AS1 was primarily amassed in the cytoplasm of CRC cells, signifying that PSMB8-AS1 might regulate gene expression at the post-transcriptional level (Figure 3A). Results of the FISH assay validated the cytoplasmic localization of PSMB8-AS1 in HCT116 and SW480 cells (Figure 3B). Thus, we suspected that PSMB8-AS1 might function as a ceRNA to sponge miRNAs. To explore the ceRNA mechanism of PSMB8-AS1 in CRC cells, miRNAs that could bind with PSMB8-AS1 were searched on DIANA (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php) website. Eight miRNAs (binding score >0.9) were screened out and involved in the RNA pull-down assay. It turned out that miR-1299 was found to be considerably pulled down by Bio-PSMB8-AS1 (Figure 3C, **$p<0.01$). Subsequently, qRT-PCR results manifested that the expression of miR-1299 was low in CRC cell lines (Figure 3D, *$p<0.05$, **$p<0.01$). Thus, we selected miR-1299 to conduct the subsequent assays. DIANA website predicted the binding sites of miR-1299 and PSMB8-AS1 (Figure 3E). RIP assay demonstrated that substantial miR-1299 and PSMB8-AS1 were detected in anti-Ago2 groups, suggesting they co-existed in RNA-induced silencing complex (RISC) (Figure 3F, **$p<0.01$). Next, qRT-PCR assay confirmed the high overexpression efficacy of miR-1299 mimics in SW480 and HCT116 cells (Figure 3G, **$p<0.01$). After the luciferase reporter assay, we noticed that miR-1299 mimics reduced the luciferase activity of PSMB8-AS1-WT in cells, which verified that

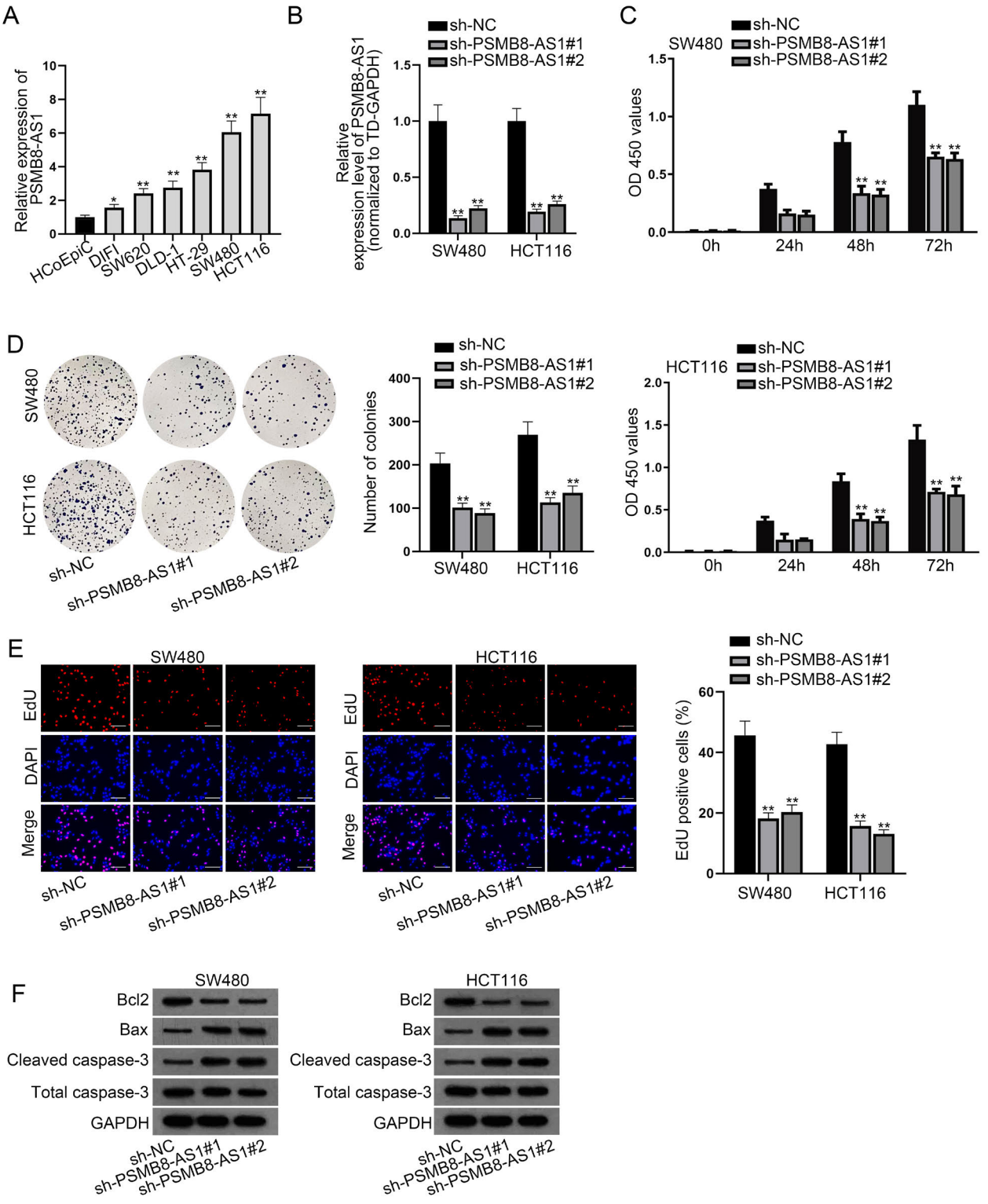

Figure 1. PSMB8-AS1 displays high expression and acts as an oncogene in CRC cells. A) qRT-PCR was performed to measure PSMB8-AS1 expression in CRC cells (HT-29, DIFI, SW480, SW620, DLD-1, and HCT116) and normal human colonic epithelial cells (HCoEpiC). B) The knockdown efficacy of sh-PSMB8-AS in SW480 and HCT116 cells was tested with the help of qRT-PCR. C–E) CCK-8, colony formation as well as EdU experiments were done to assess the viability and proliferation of CRC cells upon the PSMB8-AS1 knockdown. Scale bar 100 μm. F) Western blot was conducted to test the levels of apoptosis-associated proteins. *p<0.05, **p<0.01

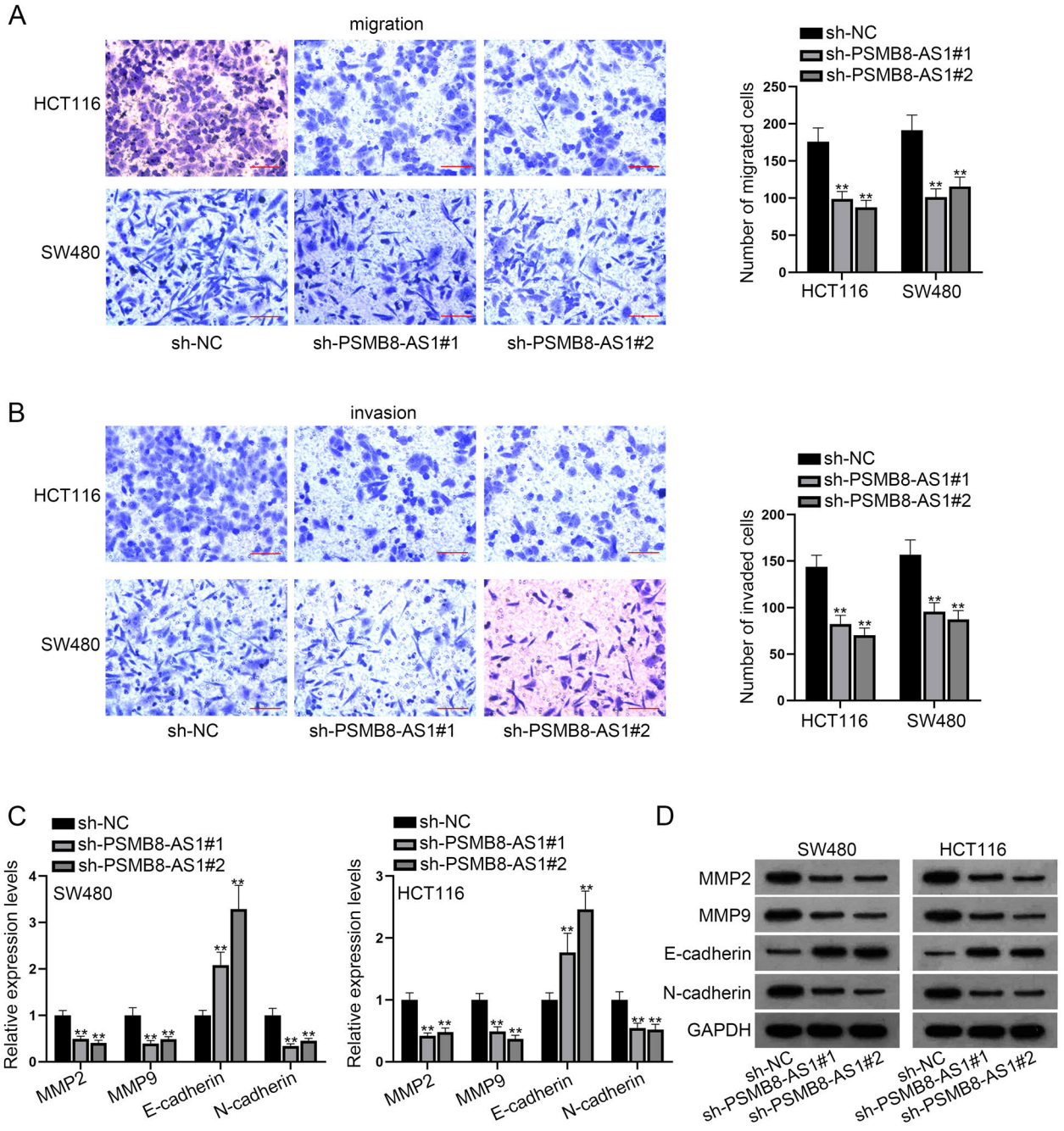

Figure 2. PSMB8-AS1 knockdown represses CRC cell invasion, migration, and EMT. A, B) Transwell experiments were done for assessing the migratory and invasive properties of transfected CRC cells upon the PSMB8-AS1 downregulation. Scale bar 100 μm. C, D) Western blot along with qRT-PCR was implemented to examine the levels of proteins connected with migration, invasion, and EMT processes in CRC cells with the PSMB8-AS1 depletion. **p<0.01

PSMB8-AS1 could bind to miR-1299 (Figure 3H, **p<0.01). To summarize, PSMB8-AS1 combines with miR-1299 in CRC cells.

PSMB8-AS1 facilitates CRC cell viability and proliferation but restrains cell apoptosis by sequestering miR-1299. In this part, the simultaneous function of PSMB8-AS1 and miR-1299 in the viability, proliferation, and apoptosis of CRC cells was explored. First of all, HCT116 and SW480 cells were co-transfected with miR-1299 inhibitor and sh-PSMB8-AS1#1 for the subsequent experiments. Data from CCK-8, colony formation assay along with EdU experiments showed that the repressive influence of PSMB8-AS1 depletion on

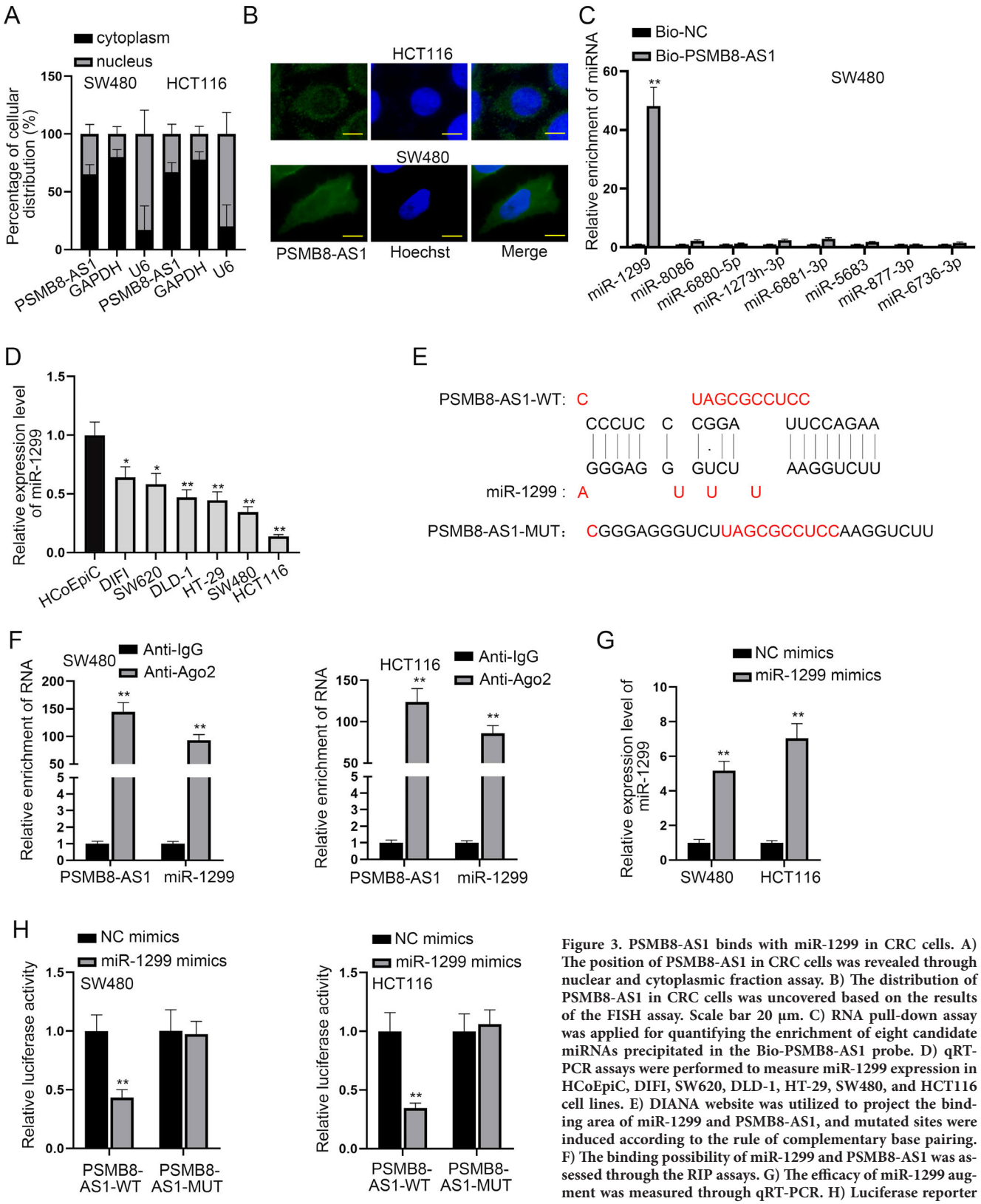

Figure 3. PSMB8-AS1 binds with miR-1299 in CRC cells. A) The position of PSMB8-AS1 in CRC cells was revealed through nuclear and cytoplasmic fraction assay. B) The distribution of PSMB8-AS1 in CRC cells was uncovered based on the results of the FISH assay. Scale bar 20 μm. C) RNA pull-down assay was applied for quantifying the enrichment of eight candidate miRNAs precipitated in the Bio-PSMB8-AS1 probe. D) qRT-PCR assays were performed to measure miR-1299 expression in HCoEpiC, DIFI, SW620, DLD-1, HT-29, SW480, and HCT116 cell lines. E) DIANA website was utilized to project the binding area of miR-1299 and PSMB8-AS1, and mutated sites were induced according to the rule of complementary base pairing. F) The binding possibility of miR-1299 and PSMB8-AS1 was assessed through the RIP assays. G) The efficacy of miR-1299 augment was measured through qRT-PCR. H) Luciferase reporter assay was done for detecting the luciferase activity of pmirGLO-PSMB8-AS1-WT/MUT in CRC cells under the condition of miR-1299 elevation. *p<0.05, **p<0.01

CRC cell viability and proliferation could be offset by the transfection of the sh-PSMB8-AS1#1+miR-1299 inhibitor (Figures 4A–4C, **p<0.01). Western blot analysis suggested that cell apoptosis promoted by PSMB8-AS1 depletion could be repressed by the addition of miR-1299 inhibitor (Figure 4D), which was also supported by the results of flow cytometry analysis (Supplementary Figures S1C, S1D, **p<0.01). To sum up, PSMB8-AS1 contributes to CRC cell viability and proliferation while impeding cell apoptosis through binding with miR-1299.

PSMB8-AS1 facilitates CRC cell migration, invasion, and EMT via binding to miR-1299. Based on the Transwell assays, CRC cell migration and invasion repressed by PSMB8-AS1 knockdown could be recovered by miR-1299 inhibition (Figures 5A, 5B, **p<0.01). After western blot and qRT-PCR assays, we discovered that miR-1299 inhibition could countervail the inhibitory function of silencing PSMB8-AS1 on cell migration, invasion, and EMT processes (Figures 5C, 5D, **p<0.01). In conclusion, PSMB8-AS1 facilitates CRC migratory and invasive capabilities as well as the EMT process by binding to miR-1299.

miR-1299 targets ADAMTS5 in CRC cells. We further searched for the target genes of miR-1299 in CRC cells. The intersection of RNA22, miRDB, and microT databases predicted three qualified mRNAs, namely ACO1, ABCA12, and ADAMTS5 (Figure 6A). Expression of the three mRNAs in CRC and normal cells was quantified using qRT-PCR assay, and ADAMTS5 displayed the highest expression in CRC cells (Figure 6B, *p<0.05, **p<0.01). Thus, ADAMTS5 was involved in the following assays. The binding area of ADAMTS5 and miR-1299 from the microT database is shown in Figure 6C. In the luciferase reporter assay, miR-1299 mimics reduced the luciferase activity of ADAMTS5-WT in cells, indicating that miR-1299 targeted ADAMTS5 in CRC cells (Figure 6D, **p<0.01). Then, qRT-PCR and western blot assays proved that ADAMTS5 mRNA level and protein level could be diminished by miR-1299 mimics (Figures 6E–F, **p<0.01). Further, results from the RIP experiment manifested that ADAMTS5, PSMB8-AS1, and miR-1299 co-existed in the RISC complex (Figure 6G, **p<0.01). To conclude, ADAMTS5 acts as the target gene of miR-1299 in CRC cells.

PSMB8-AS1 promotes CRC cell viability and proliferation but hampers cell apoptosis via upregulating ADAMTS5. To figure out whether PSMB8-AS1 affected CRC cell viability, proliferation, and apoptosis through regulating ADAMTS5 expression, the following rescue assays were performed. Above all, qRT-PCR experiments were conducted and we noticed that ADAMTS5 expression could be reduced by the PSMB8-AS1 downregulation (Figure 7A, **p<0.01). Later, we overexpressed ADAMTS5 by transfecting pcDNA3.1-ADAMTS5 in CRC cells (Figure 7B, **p<0.01). Subsequently, CRC cells were transfected with sh-PSMB8-AS1#1 and pcDNA3.1/ADAMTS5 for further study. Outcomes of CCK-8, colony formation assay along with EdU experiments suggested that transfection of sh-PSMB8-AS1#1+pcDNA3.1/ADAMTS5 restored the suppressed CRC cell viability and proliferation induced by PSMB8-AS1 depletion (Figures 7C–7E, **p<0.01). Results of western blot and flow cytometry analysis indicated that cell apoptosis promoted by PSMB8-AS1 depletion could be reversed by ADAMTS5 overexpression (Figure 7F, Supplementary Figures S1E, S1F, **p<0.01). Taken together, PSMB8-AS1 prompts CRC cell viability and proliferation while hindering cell apoptosis via upregulating ADAMTS5.

PSMB8-AS1 pushes CRC cell migration, invasion, and EMT via enhancing ADAMTS5 expression. Results from the Transwell assays revealed that CRC cell migration and invasion repressed by silencing PSMB8-AS1 could be reversed by the upregulation of ADAMTS5 (Figures 8A, 8B, **p<0.01). Based on qRT-PCR and western blot analysis, the repressed migration, invasion, and EMT processes caused by PSMB8-AS1 depletion could be recovered by the ADAMTS5 overexpression (Figures 8C, 8D, **p<0.01). Collectively, these results clearly elucidate that PSMB8-AS1 facilitates CRC migratory, invasive abilities along with the EMT process through upregulating ADAMTS5.

CRC cell-derived exosomal PSMB8-AS1 stimulates M2 macrophage polarization. Through bioinformatics prediction, we discovered that PSMB8-AS1 was highly expressed in CRC-derived exosomes (Figure 9A). Thus, we detected the exosomes released by CRC cells. Exosomes isolated from SW480 and HCT116 cells were named SW480/exo and HCT116/exo. Through TEM, we observed the shape and size of exosomes (Figure 9B). Next, PKH67 was utilized to carry out an immunofluorescence assay, which further proved the existence of exosomes (Figure 9C). Furthermore, we observed the exosomal markers (CD63, CD9, TSG101, and HSP90) by western blot. According to the results, these markers could all be detected (Figure 9D). These assays manifested that we succeeded in isolating CRC cell exosomes. Exosomes can exert a crucial function in intercellular communication by transferring lncRNAs, and tumor-associated macrophages are found to be the most abundant cells in the tumor microenvironment [30]. Thus, we further investigated the interaction of exosomal PSMB8-AS1 and macrophages. We co-cultured THP1 cells with the culture medium (CM) of SW480 and HCT116 cells and discovered that the PSMB8-AS1 level was increased in THP1 cells treated with SW480-CM or HCT116-CM (Figure 9E, **p<0.01). Then, we utilized qRT-PCR to prove the existence of PSMB8-AS1 in exosomes. From the results, we observed that PSMB8-AS1 was highly expressed in THP1 cells treated with SW480/exo or HCT116/exo (Figure 9F, **p<0.01). Additionally, it was illustrated from qRT-PCR analysis and western blot assay that the treatment of IL3, SW480/exo, or HCT116/exo increased the level of M2 polarization markers (CD206, Ym1, and Arg1) in THP1 cells, while the further transfection of sh-PSMB8-AS1#1/2 fully restored their levels (Figures 9G, 9H, **p<0.01). These results prove that CRC cell-secreted exosomal PSMB8-AS1 facilitates M2 macrophage polarization.

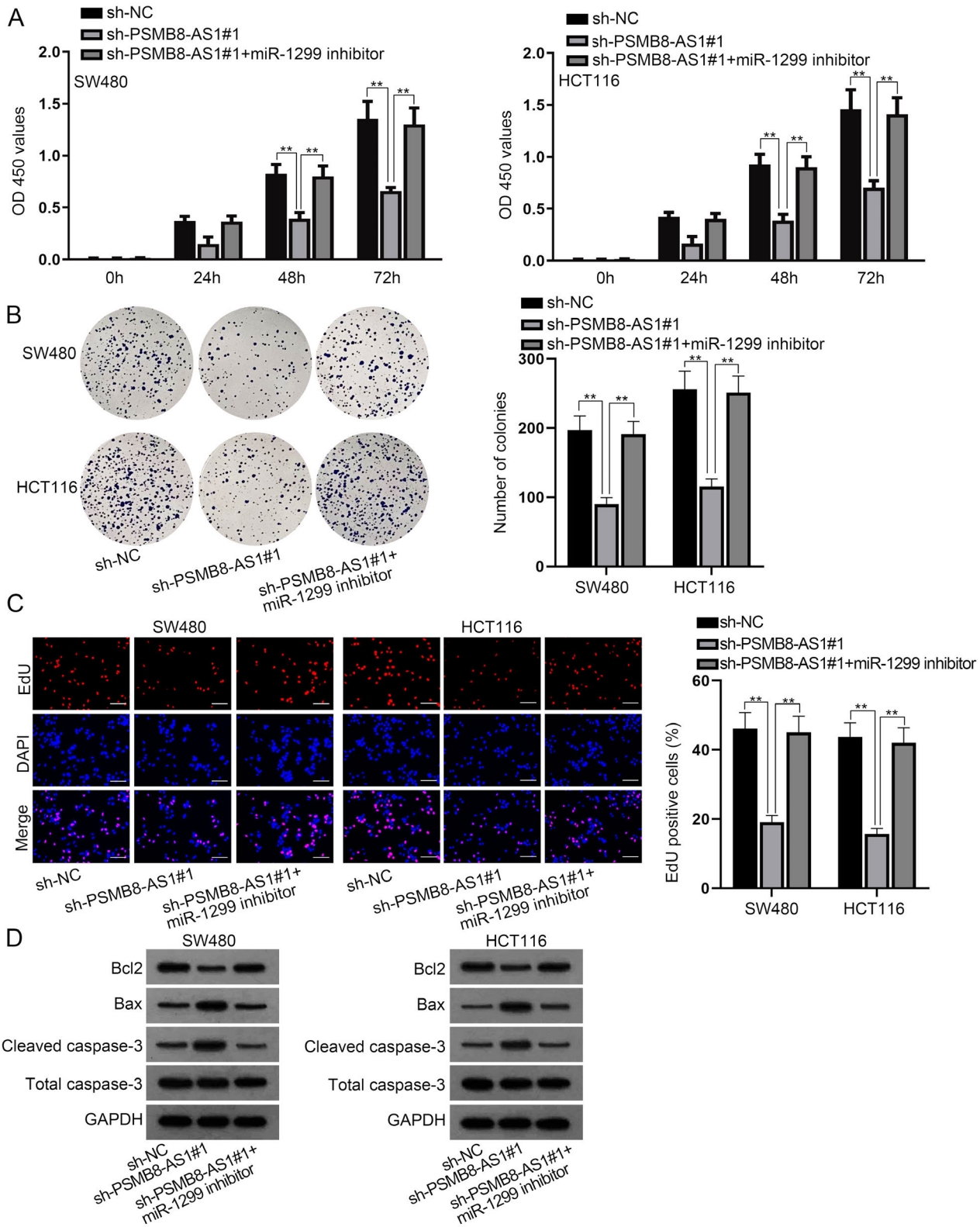

Figure 4. PSMB8-AS1 facilitates CRC cell viability and proliferation while inhibiting cell apoptosis by serving as a miR-1299 sponge. A–C) CCK-8 and colony formation assays together with EdU assays were performed to reveal viability and proliferation changes of indicated CRC cells in several groups: sh-NC, sh-PSMB8-AS1#1, and sh-PSMB8-AS1#1+miR-1299 inhibitor. Scale bar 100 μm. D) The levels of proteins that were connected with cell apoptosis were measured in SW480 and HCT116 cells with different conditions via western blot analysis. **p<0.01

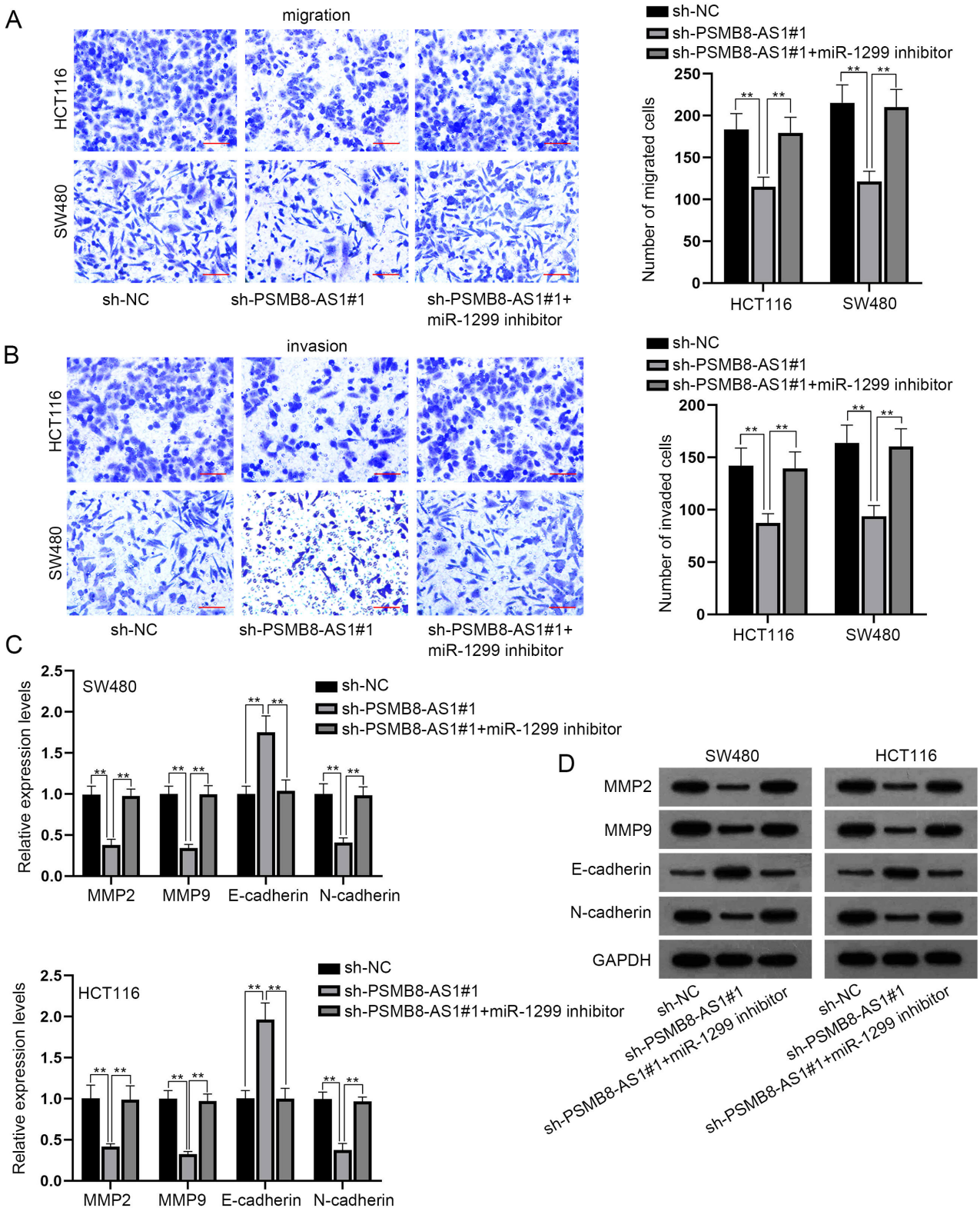

Figure 5. Migration, invasion, and EMT of CRC cells can be regulated through the PSMB8-AS1/miR-1299 axis. A, B) Transwell assay was done to evaluate the migratory and invasive properties of SW480 and HCT116 cells in multiple groups (sh-NC, sh-PSMB8-AS1#1, and sh-PSMB8-AS1#1+miR-1299 inhibitor). Scale bar 100 μm. C, D) qRT-PCR and western blot were performed to analyze the protein levels of MMP2, MMP9, E-cadherin, and N-cadherin in differently transfected CRC cells. **p<0.01

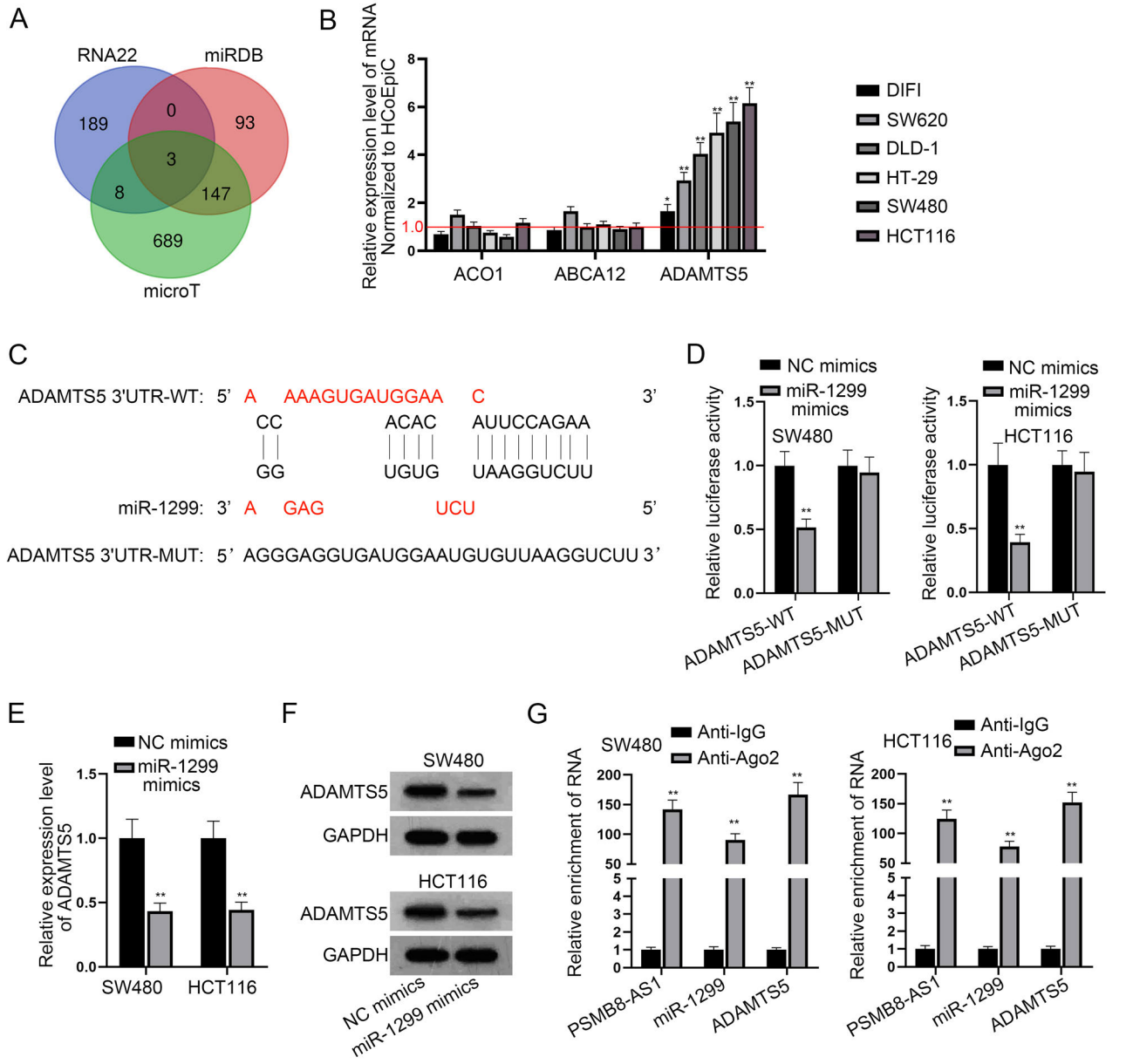

Figure 6. miR-1299 targets ADAMTS5 in CRC cells. A) Venn diagram showed the candidate mRNAs projected by several databases (RNA22, miRDB, and microT). B) The expression levels of ACO1, ABCA12, and ADAMTS5 in CRC cells and HCoEpiC cells were quantified using qRT-PCR. C) The binding sequence of ADAMTS5 and miR-1299 was obtained from the microT database. D) Luciferase activity of ADAMTS5-WT/MUT in CRC cells upon miR-1299 overexpression was detected by luciferase reporter assays. E, F) ADAMTS5 level in CRC cells with miR-1299 overexpression was tested with the help of qRT-PCR and western blot. G) RIP assay evaluated the enrichment of PSMB8-AS1, miR-1299, and ADAMTS5 in anti-Ago2. *p<0.05, **p<0.01

Discussion

LncRNAs are found to function as a promoter or a suppressor in multiple malignancies. For example, lncRNA SATB2-AS1 suppresses CRC progression by regulating SATB2 [31]. Ni et al. have found that lncRNA GAS5 inhibits CRC progression through YAP activation [32]. Bao et al. have illustrated that lncRNA GLCC1 promotes CRC carcinogenesis by stabilizing c-Myc [33]. Wu et al. have elucidated that lncRNA FTX functions as a CRC promoter by sponging miR-215 and inhibiting phosphorylation of vimentin [34]. Du et al. have pointed out that lncRNA NEAT1 interacts with DDX5 and activates the Wnt/β-catenin pathway to promote CRC progression [35]. The present study examined the functions of PSMB8-AS1 in CRC cells.

PSMB8-AS1 is an antisense RNA of PSMB8, which can promote the progression of pancreatic cancer [15] and glioma

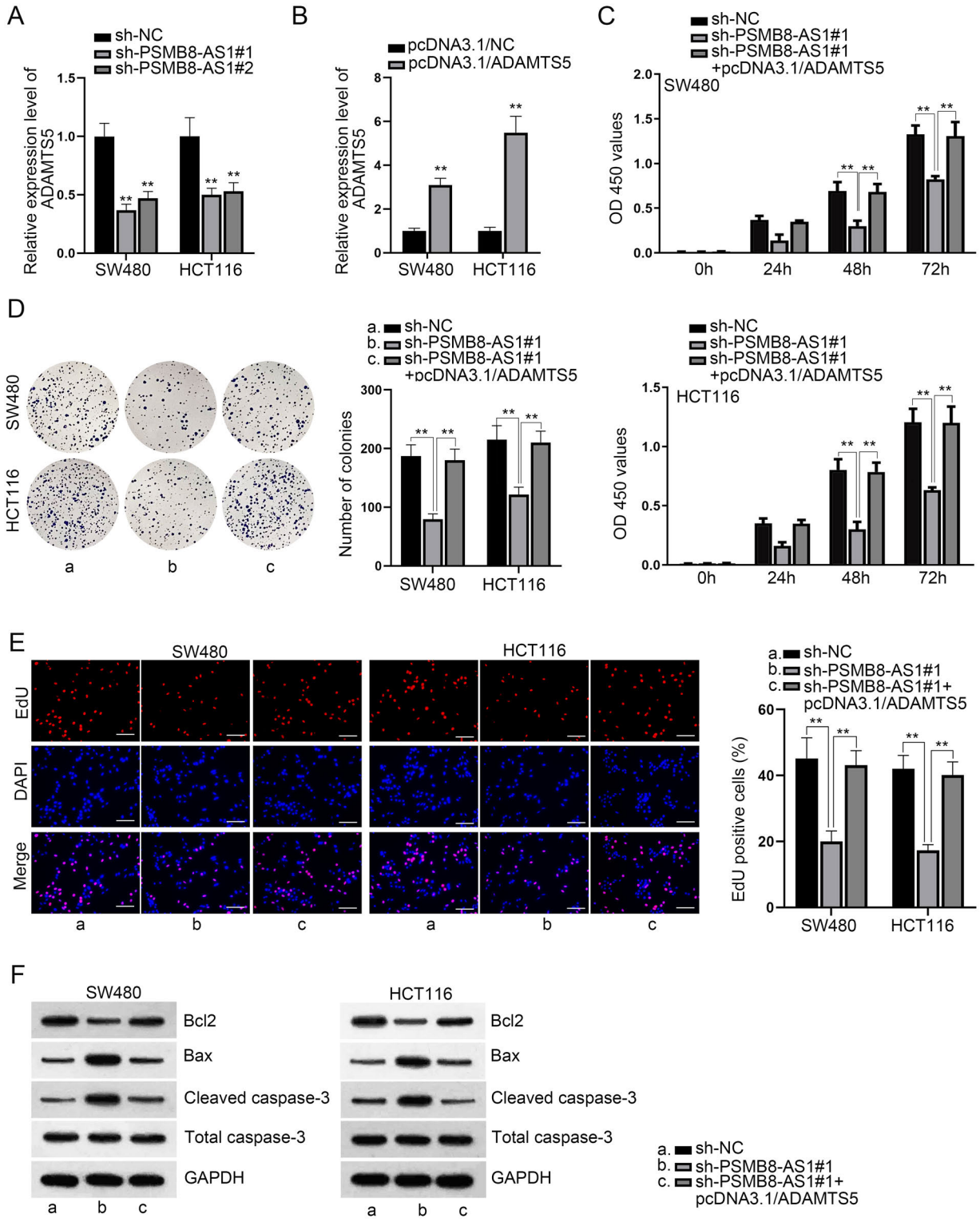

Figure 7. PSMB8-AS1 prompts CRC cell viability and proliferation but restrains cell apoptosis via upregulating ADAMTS5. A) The level of ADAMTS5 was quantified via qRT-PCR when PSMB8-AS1 was reduced in SW480 and HCT116 cells. B) The efficacy of ADAMTS5 overexpression was measured by qRT-PCR. Subsequently, CRC cells were divided into three groups (sh-NC, sh-PSMB8-AS1#1, and sh-PSMB8-AS1#1+pcDNA3.1/ADAMTS5). C–E) CCK-8, colony formation assays, as well as EdU assays, were implemented to assess the viability and proliferation of SW480 and HCT116 cells in different groups. Scale bar 100 μm. F) Western blot was done to measure the levels of apoptosis-associated proteins in different groups of CRC cells. **p<0.01

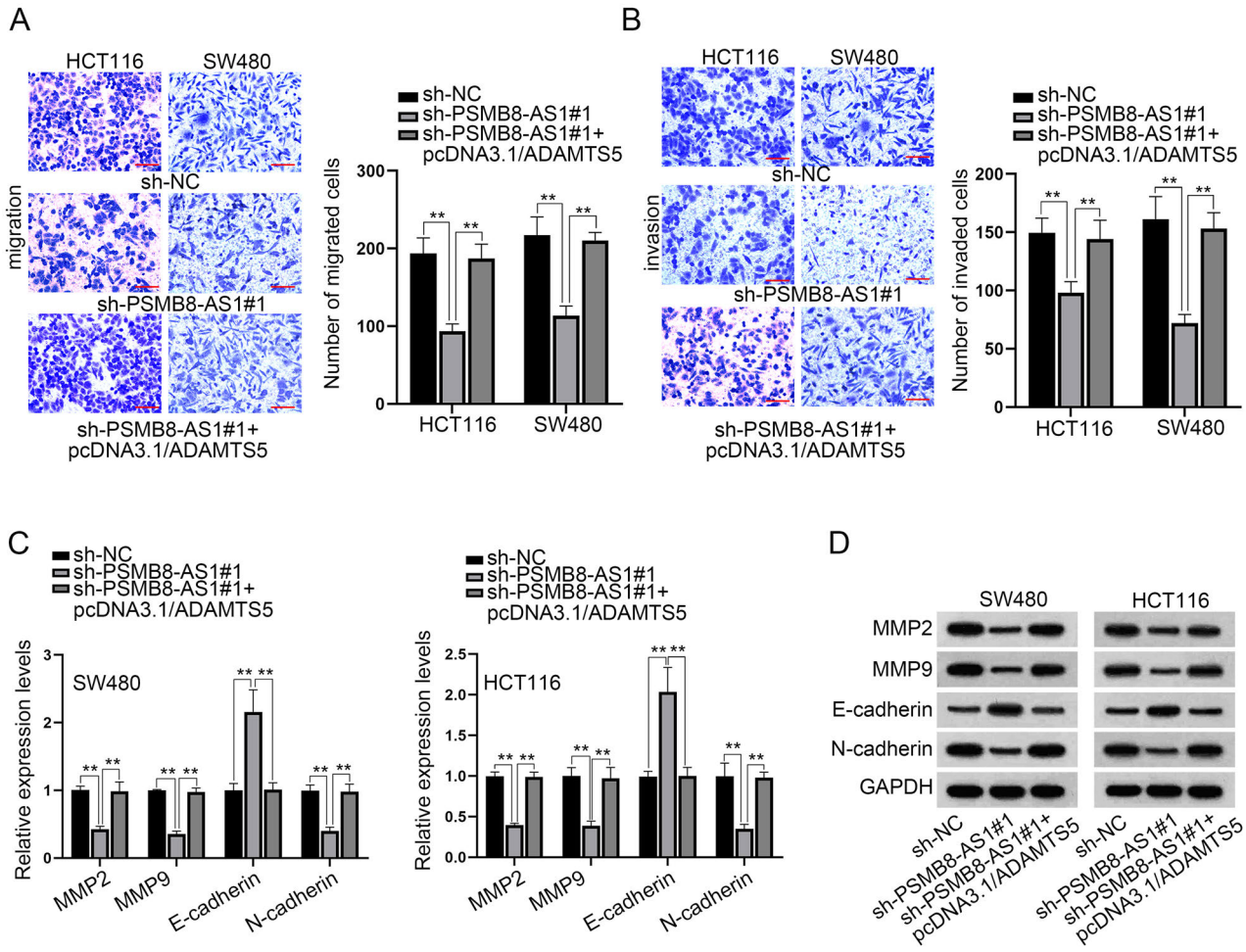

Figure 8. PSMB8-AS1 enhances migratory and invasive properties of CRC cells by elevating ADAMTS5 expression. CRC cells were involved in three groups (sh-NC, sh-PSMB8-AS1#1, and sh-PSMB8-AS1#1+pcDNA3.1/ADAMTS5). A, B) Transwell assays were operated to uncover the changes in migration and invasion capabilities of CRC cells under different conditions. Scale bar 100 μm. C, D) qRT-PCR and western blot were employed for detecting the expression of migration/invasion/EMT-associated proteins in different groups of CRC cells. **p<0.01

[36]. However, the molecular mechanism of PSMB8-AS1 in CRC has not been well studied. In the current research, we discovered that PSMB8-AS1 was overexpressed in CRC cells. CCK-8, colony formation along with EdU assays showed that the PSMB8-AS1 knockdown restricted CRC cell viability and proliferation. Data from western blot and flow cytometry analysis indicated that PSMB8-AS1 knockdown stimulated CRC cell apoptosis. Further, cell migration, invasion, and EMT processes could be hampered by PSMB8-AS1 depletion. These results jointly elucidated that PSMB8-AS1 acted as an oncogene in CRC cells.

LncRNAs have been proved to act as ceRNAs to prompt or restrain tumor development [10]. The ceRNA network refers to that lncRNA could sponge miRNAs to regulate mRNA expression at the post-transcriptional level [37]. Jia et al. have selected 15 lncRNAs to explore their roles in CRC. They concluded that the 15 lncRNAs could act as ceRNAs to modulate cancer-related pathways [38]. In our study, it was confirmed that PSMB8-AS1 bound to miR-1299 in CRC cells. Rescue assays also uncovered that miR-1299 inhibition can promote CRC cell viability, proliferation, migration, invasion, and EMT suppressed by PSMB8-AS1 depletion, suggesting that PSMB8-AS1 could bind with miR-1299 to promote malignant behaviors of CRC cells. Previously, miR-1299 has been reported to repress the development of lung cancer through the EGFR/PI3K/AKT pathway [27]. Moreover, miR-1299 could suppress metastasis of prostate cancer by targeting NEK2 [25]. Furthermore, miR-1299 has been proved to restrain cell growth in CRC [39]. The above-mentioned evidence further supported our finding that miR-1299 played a suppressive role in the malignant phenotypes of CRC cells. Subsequently, ADAMTS5 was verified to be targeted by miR-1299. In addition, ADAMTS5 expression was negatively regulated by miR-1299 and positively

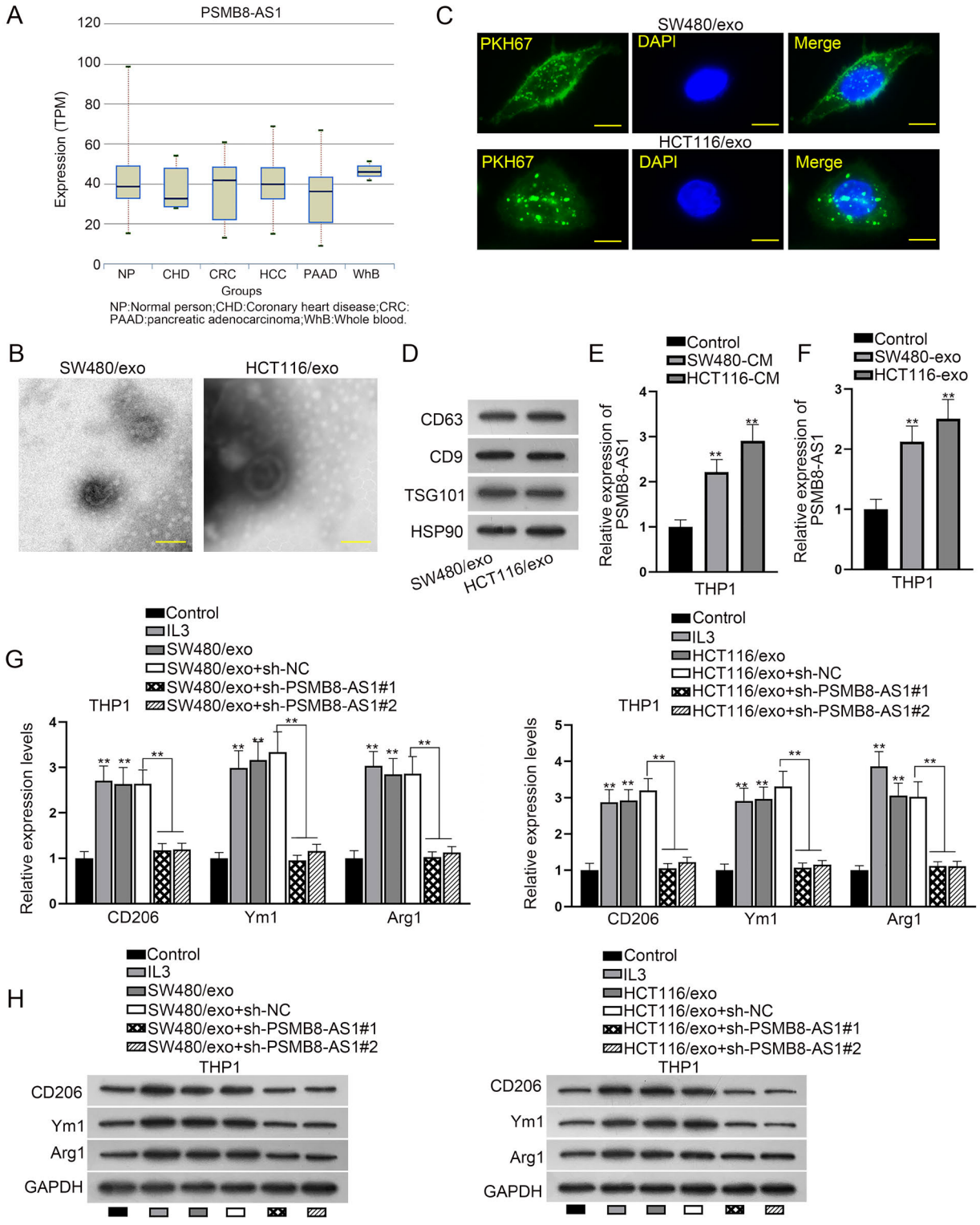

Figure 9. CRC cell-derived exosomal PSMB8-AS1 stimulates M2 macrophage polarization. A) PSMB8-AS1 was highly expressed in CRC cell-derived exosomes. B, C) TEM observation (scale bar 100 nm) and PKH67-immunofluorescence analysis (scale bar 20 μm) were applied to identify exosomes released by CRC cells. D) Western blot assay measured the expression levels of exosome markers. E) PSMB8-AS1 level was quantified via qRT-PCR in THP1 cells treated with SW480-CM or HCT116-CM. F) PSMB8-AS1 level was quantified through qRT-PCR in THP1 cells treated with SW480/exo and HCT116/exo. G, H) qRT-PCR and western blot were carried out to analyze the mRNA and protein levels of CD206, Ym1, and Arg1 in different groups of THP1 cells. **p<0.01

modulated by PSMB8-AS1. ADAMTS5 has been reported to regulate cancer progression [36, 40]. Our experiments also showed that ADAMTS5 could promote CRC cell malignant phenotypes. To conclude, PSMB8-AS1 promoted the malignant processes of CRC cells via the modulation of the miR-1299/ADAMTS5 axis.

Exosomes are the microvesicles from multivesicular bodies and their diameter is between 70 to 120 nm [41]. Exosomes play a pivotal part in intercellular communication via transferring lncRNAs, and tumor-linked macrophages are identified to be the most amassed cells in the tumor microenvironment [30]. M2 macrophage polarization is closely associated with tumorigenesis and immunosuppression [42]. In our research, we discovered that CRC-derived exosomes could transport PSMB8-AS1 to activate M2 polarization, eventually accelerating CRC cell malignant processes.

In conclusion, this study first finds that PSMB8-AS1 is upregulated in CRC cells and facilitates malignant phenotypes of CRC cells. Further, miR-1299 binds with PSMB8-AS1 and targets ADAMTS5 in CRC cells. Additionally, CRC-derived exosomes could transport PSMB8-AS1 to activate M2 polarization. The overall results show that PSMB8-AS1 competitively binds with miR-1299 to upregulate ADAMTS5, which contributes to CRC cell malignant processes.

Supplementary information is available in the online version of the paper.

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References

[1] FERLAY J, SHIN HR, BRAY F, FORMAN D, MATHERS C et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893–2917. https://doi.org/10.1002/ijc.25516

[2] JEMAL A, BRAY F, CENTER MM, FERLAY J, WARD E et al. Global cancer statistics. CA Cancer J Clin 2011; 61: 69–90. https://doi.org/10.3322/caac.20107

[3] BRENNER H, KLOOR M, POX CP. Colorectal cancer. Lancet 2014; 383: 1490–1502. https://doi.org/10.1016/s0140-6736(13)61649-9

[4] SIEGEL RL, MILLER KD, JEMAL A. Cancer statistics, 2019. Cancer J Clin 2019; 69: 7–34. https://doi.org/10.3322/caac.21551

[5] NISHIHARA R, WU K, LOCHHEAD P, MORIKAWA T, LIAO X et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. N Engl J Med 2013; 369: 1095–1105. https://doi.org/10.1056/NEJMoa1301969

[6] WANG W, GE L, XU XJ, YANG T, YUAN Y et al. LncRNA NEAT1 promotes endometrial cancer cell proliferation, migration and invasion by regulating the miR-144-3p/EZH2 axis. Radiol Oncol 2019; 53: 434–442. https://doi.org/10.2478/raon-2019-0051

[7] CABILI MN, TRAPNELL C, GOFF L, KOZIOL M, TAZON-VEGA B et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev 2011; 25: 1915–1927. https://doi.org/10.1101/gad.17446611

[8] ESTELLER M. Non-coding RNAs in human disease. Nature Rev Genet 2011; 12: 861–874. https://doi.org/10.1038/nrg3074

[9] QUINN JJ, CHANG HY. Unique features of long non-coding RNA biogenesis and function. Nature Rev Genet 2016; 17: 47–62. https://doi.org/10.1038/nrg.2015.10

[10] QI X, ZHANG DH, WU N, XIAO JH, WANG X et al. ceRNA in cancer: possible functions and clinical implications. J Med Genet 2015; 52: 710–718. https://doi.org/10.1136/jmedgenet-2015-103334

[11] LIZ J, ESTELLER M. lncRNAs and microRNAs with a role in cancer development. Biochim Biophys Acta 2016; 1859: 169–176. https://doi.org/10.1016/j.bbagrm.2015.06.015

[12] KAZEMZADEH M, SAFARALIZADEH R, FEIZI MA, RAVANBAKHSH R, SOMI MH et al. LOC100287225, novel long intergenic non-coding RNA, misregulates in colorectal cancer. Cancer Biomark 2016; 16: 499–505. https://doi.org/10.3233/cbm-160589

[13] WANG F, NI H, SUN F, LI M, CHEN L. Overexpression of lncRNA AFAP1-AS1 correlates with poor prognosis and promotes tumorigenesis in colorectal cancer. Biomed Pharmacother 2016; 81: 152–159. https://doi.org/10.1016/j.biopha.2016.04.009

[14] SUN Z, OU C, REN W, XIE X, LI X et al. Downregulation of long non-coding RNA ANRIL suppresses lymphangiogenesis and lymphatic metastasis in colorectal cancer. Oncotarget 2016; 7: 47536–47555. https://doi.org/10.18632/oncotarget.9868

[15] KWON CH, PARK HJ, CHOI YR, KIM A, KIM HW et al. PSMB8 and PBK as potential gastric cancer subtype-specific biomarkers associated with prognosis. Oncotarget 2016; 7: 21454–21468. https://doi.org/10.18632/oncotarget.7411

[16] CHEN H, LIU T, LIU J, FENG Y, WANG B et al. Circ-ANAPC7 is Upregulated in Acute Myeloid Leukemia and Appears to Target the MiR-181 Family. Cell Physiol Biochem 2018; 47: 1998–2007. https://doi.org/10.1159/000491468

[17] HA YJ, TAK KH, KIM CW, ROH SA, CHOI EK et al. PSMB8 as a Candidate Marker of Responsiveness to Preoperative Radiation Therapy in Rectal Cancer Patients. Int J Radiat Oncol Biol Phys 2017; 98: 1164–1173. https://doi.org/10.1016/j.ijrobp.2017.03.023

[18] ZHANG H, ZHU C, HE Z, CHEN S, LI L et al. LncRNA PSMB8-AS1 contributes to pancreatic cancer progression via modulating miR-382-3p/STAT1/PD-L1 axis. J Exp Clin Cancer Res 2020; 39: 179. https://doi.org/10.1186/s13046-020-01687-8

[19] SHEN G, MAO Y, SU Z, DU J, YU Y et al. PSMB8-AS1 activated by ELK1 promotes cell proliferation in glioma via regulating miR-574-5p/RAB10. Biomed Pharmacother 2020; 122: 109658. https://doi.org/10.1016/j.biopha.2019.109658

[20] HU T, WANG F, HAN G. LncRNA PSMB8-AS1 acts as ceRNA of miR-22-3p to regulate DDIT4 expression in glioblastoma. Neurosci Lett 2020; 728: 134896. https://doi.org/https://doi.org/10.1016/j.neulet.2020.134896

[21] D'ANGELO B, BENEDETTI E, CIMINI A, GIORDANO A. MicroRNAs: A Puzzling Tool in Cancer Diagnostics and Therapy. Anticancer Res 2016; 36: 5571–5575. https://doi.org/10.21873/anticanres.11142

[22] KIM VN, NAM JW. Genomics of microRNA. Trends Genet 2006; 22: 165–173. https://doi.org/10.1016/j.tig.2006.01.003

[23] MURPHY MFR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics 2010; 11: 537–561. https://doi.org/10.2174/138920210793175895

[24] FARAZI TA, HOELL JI, MOROZOV P, TUSCHL T. MicroRNAs in human cancer. Adv Exp Med Biol 2013; 774: 1–20. https://doi.org/10.1007/978-94-007-5590-1_1

[25] ZHANG FB, DU Y, TIAN Y, JI ZG, YANG PQ. MiR-1299 functions as a tumor suppressor to inhibit the proliferation and metastasis of prostate cancer by targeting NEK2. Eur Rev Med Pharmacol Sci 2019; 23: 530–538. https://doi.org/10.26355/eurrev_201901_16865

[26] ZHU H, WANG G, ZHOU X, SONG X, GAO H et al. miR-1299 suppresses cell proliferation of hepatocellular carcinoma (HCC) by targeting CDK6. Biomed Pharmacother 2016; 83: 792–797. https://doi.org/10.1016/j.biopha.2016.07.037

[27] CAO S, LI L, LI J, ZHAO H. MiR-1299 Impedes the Progression of Non-Small-Cell Lung Cancer Through EGFR/PI3K/AKT Signaling Pathway. Onco Targets Ther 2020; 13: 7493–7502. https://doi.org/10.2147/ott.S250396

[28] HARAGUCHI N, OHARA N, KOSEKI J, TAKAHASHI H, NISHIMURA J et al. High expression of ADAMTS5 is a potent marker for lymphatic invasion and lymph node metastasis in colorectal cancer. Mol Clin Oncol 2017; 6: 130–134. https://doi.org/10.3892/mco.2016.1088

[29] GU J, CHEN J, FENG J, LIU Y, XUE Q et al. Overexpression of ADAMTS5 can regulate the migration and invasion of non-small cell lung cancer. Tumour Biol 2016; 37: 8681–8689. https://doi.org/10.1007/s13277-015-4573-x

[30] ALLAVENA P, SICA A, SOLINAS G, PORTA C, MANTOVANI A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol 2008; 66: 1–9. https://doi.org/10.1016/j.critrevonc.2007.07.004

[31] XU M, XU X, PAN B, CHEN X, LIN K et al. LncRNA SATB2-AS1 inhibits tumor metastasis and affects the tumor immune cell microenvironment in colorectal cancer by regulating SATB2. Mol Cancer 2019; 18: 135. https://doi.org/10.1186/s12943-019-1063-6

[32] NI W, YAO S, ZHOU Y, LIU Y, HUANG P et al. Long noncoding RNA GAS5 inhibits progression of colorectal cancer by interacting with and triggering YAP phosphorylation and degradation and is negatively regulated by the m(6)A reader YTHDF3. Mol Cancer 2019; 18: 143. https://doi.org/10.1186/s12943-019-1079-y

[33] TANG J, YAN T, BAO Y, SHEN C, YU C et al. LncRNA GLCC1 promotes colorectal carcinogenesis and glucose metabolism by stabilizing c-Myc. Nat Commun 2019; 10: 3499. https://doi.org/10.1038/s41467-019-11447-8

[34] YANG Y, ZHANG J, CHEN X, XU X, CAO G et al. LncRNA FTX sponges miR-215 and inhibits phosphorylation of vimentin for promoting colorectal cancer progression. Gene Ther 2018; 25: 321–330. https://doi.org/10.1038/s41434-018-0026-7

[35] ZHANG M, WENG W, ZHANG Q, WU Y, NI S et al. The lncRNA NEAT1 activates Wnt/β-catenin signaling and promotes colorectal cancer progression via interacting with DDX5. J Hematol Oncol 2018; 11: 113. https://doi.org/10.1186/s13045-018-0656-7

[36] LI C, XIONG Y, YANG X, WANG L, ZHANG S et al. Lost expression of ADAMTS5 protein associates with progression and poor prognosis of hepatocellular carcinoma. Drug Des Devel Ther 2015; 9: 1773–1783. https://doi.org/10.2147/dddt.S77069

[37] TAY Y, RINN J, PANDOLFI PP. The multilayered complexity of ceRNA crosstalk and competition. Nature 2014; 505: 344–352. https://doi.org/10.1038/nature12986

[38] WANG X, ZHOU J, XU M, YAN Y, HUANG L et al. A 15-lncRNA signature predicts survival and functions as a ceRNA in patients with colorectal cancer. Cancer Manag Res 2018; 10: 5799–5806. https://doi.org/10.2147/cmar.S178732

[39] WANG Y, LU Z, WANG N, ZHANG M, ZENG X et al. MicroRNA-1299 is a negative regulator of STAT3 in colon cancer. Oncol Rep 2017; 37: 3227–3234. https://doi.org/10.3892/or.2017.5605

[40] YU L, LU Y, HAN X, ZHAO W, LI J et al. microRNA -140-5p inhibits colorectal cancer invasion and metastasis by targeting ADAMTS5 and IGFBP5. Stem Cell Res Ther 2016; 7: 180. https://doi.org/10.1186/s13287-016-0438-5

[41] THÉRY C. Exosomes: secreted vesicles and intercellular communications. F1000 Biol Rep 2011; 3: 15. https://doi.org/10.3410/b3-15

[42] ESSANDOH K, LI Y, HUO J, FAN GC. MiRNA-Mediated Macrophage Polarization and its Potential Role in the Regulation of Inflammatory Response. Shock 2016; 46: 122–131. https://doi.org/10.1097/shk.0000000000000604

https://doi.org/10.4149/neo_2022_220111N42

lncRNA PSMB8-AS1 promotes colorectal cancer progression through sponging miR-1299 to upregulate ADAMTS5

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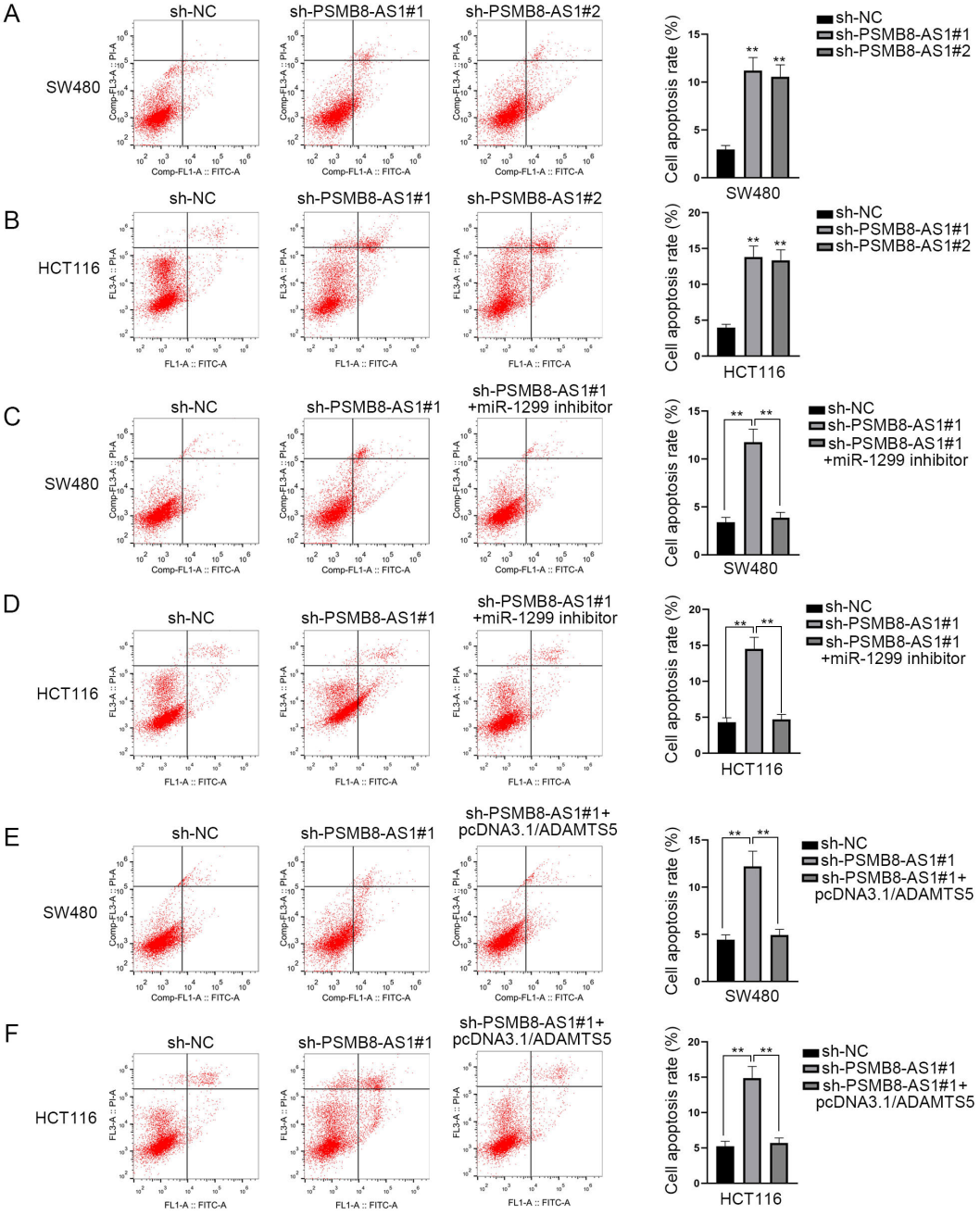
Supplementary Information



Supplementary Figure S1. A, B) Flow cytometry analysis was conducted to reveal the impact of PSMB8-AS1 depletion on the apoptosis of CRC cells. C, D) The apoptosis of cells in several groups (sh-NC, sh-PSMB8-AS1#1 and sh-PSMB8-AS1#1+miR-1299 inhibitor) was examined by flow cytometry analysis. E, F) Flow cytometry analysis was done to assess apoptosis of CRC cells in several groups (sh-NC, sh-PSMB8-AS1#1 and sh-PSMB8-AS1#1+pcDNA3.1/ADAMTS5). **p<0.01