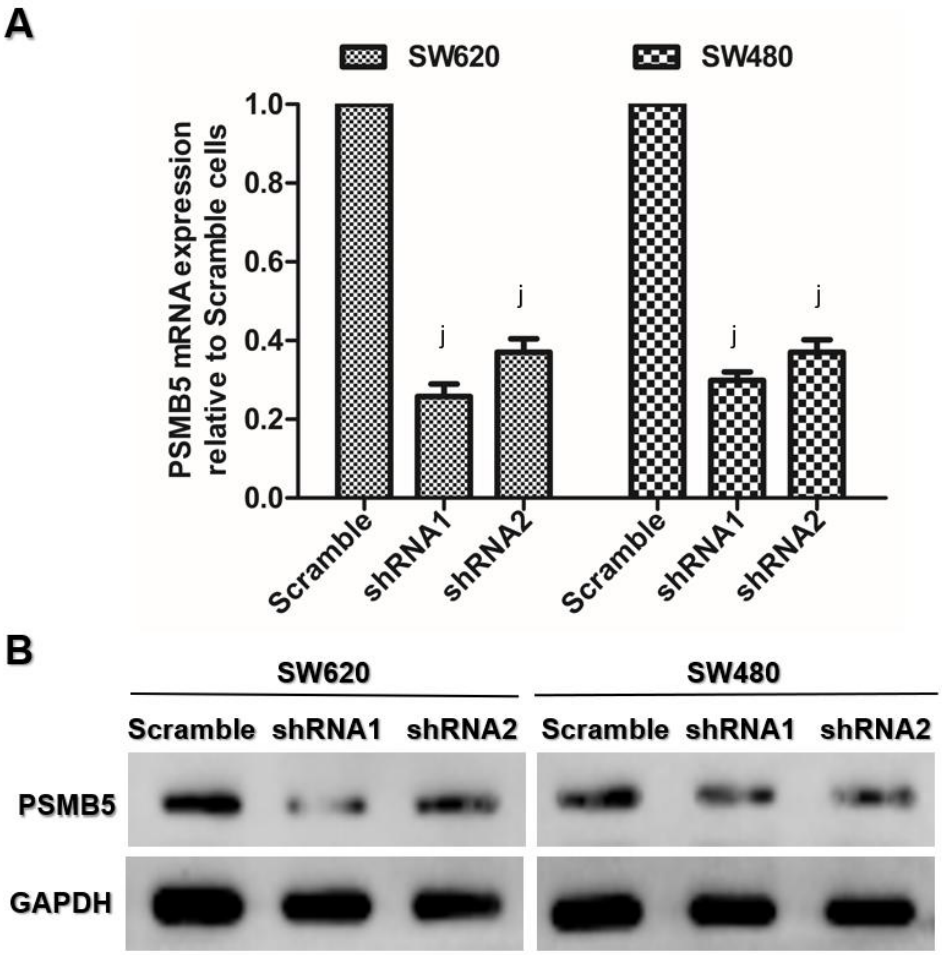
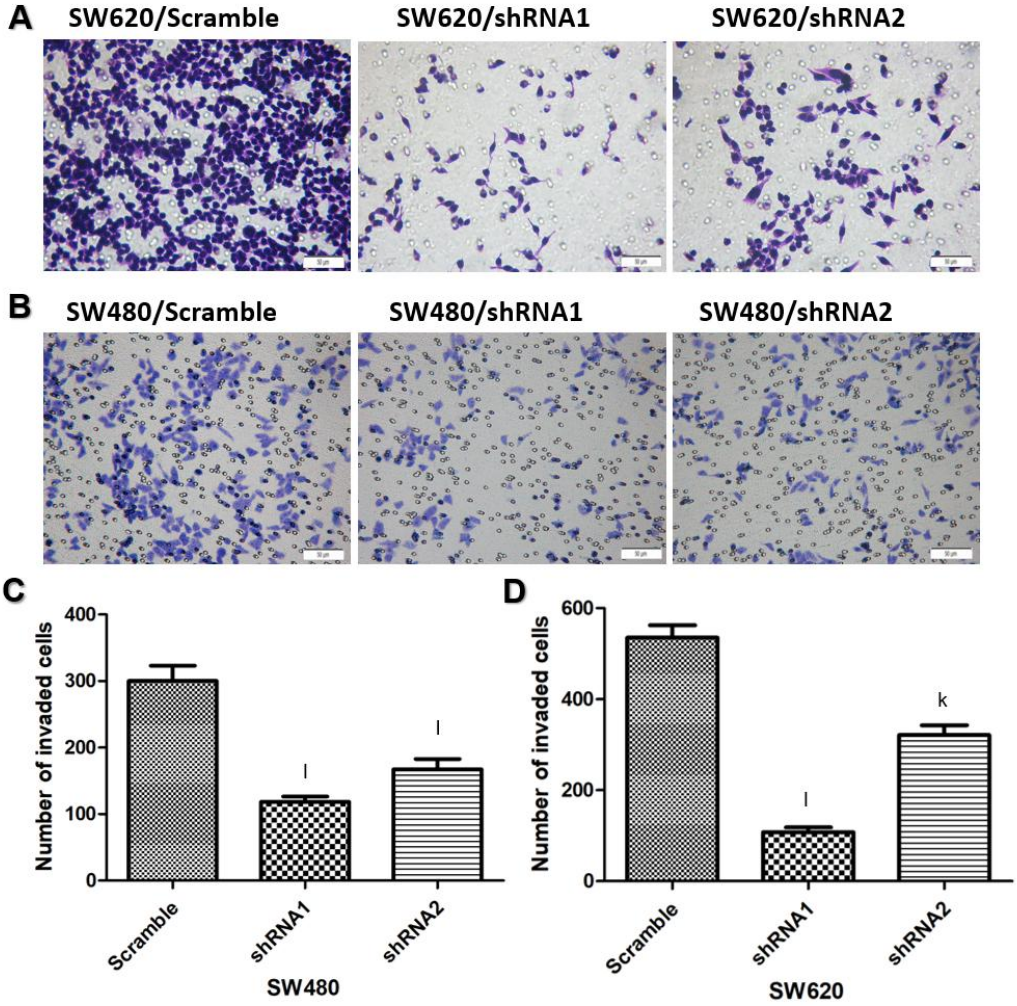
Supplementary Method

Construction and Transfection of PSMB5-Targeting shRNAs

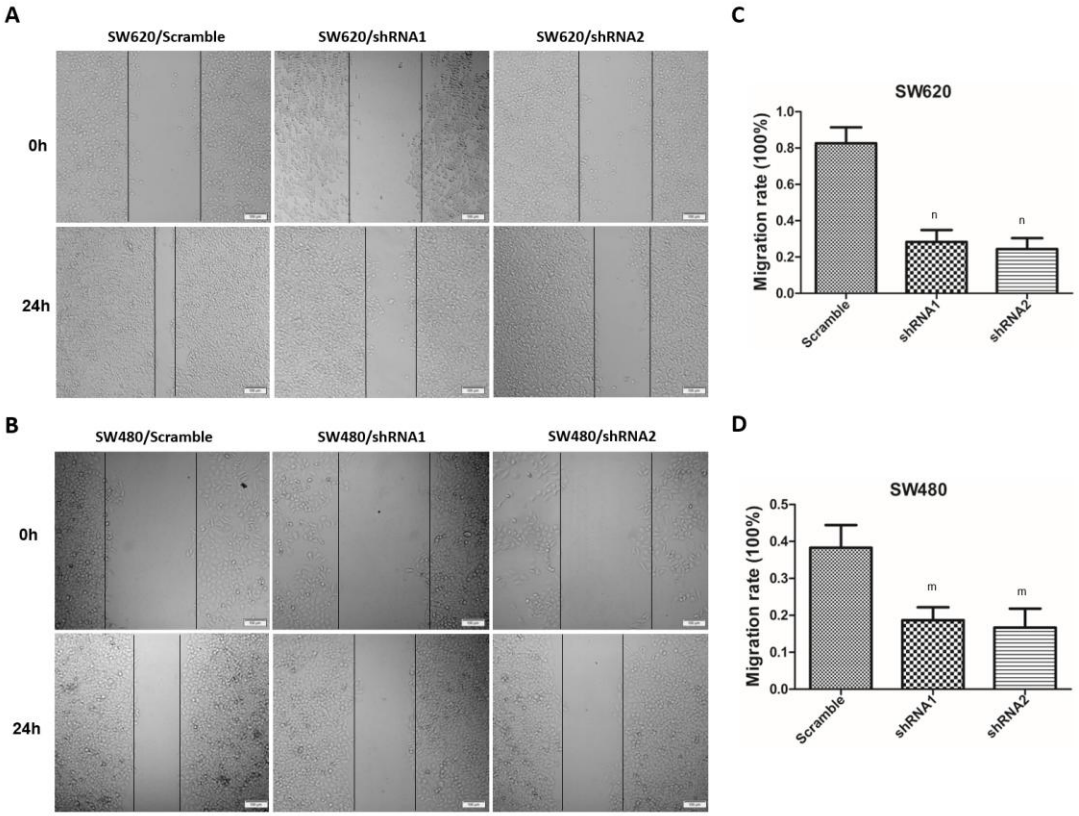
Two short hairpin RNA (shRNA) sequences targeting the human PSMB5 gene were designed based on a prior study [19] (Table S1). The targeting sequence for shRNA1 was 5′-CCCATCCTCCATCCTATTTAT-3′. Oligonucleotides encoding these shRNAs were synthesized, annealed, and cloned into the pGC-U6 vector (Genechem Biotechnology, Shanghai, China), generating the plasmids pGC-U6-shRNA1 and pGC-U6-shRNA2. A non-targeting scramble shRNA vector (pGC-U6-Scramble) served as the control. For transfection, SW620 and SW480 cells were seeded in six-well plates at a density of 1×10^6 cells per well. Upon reaching approximately 90% confluence, cells were transiently transfected with 3 μg of plasmid DNA using Lipofectamine™ 3000 (Invitrogen) according to the manufacturer's instructions. Cells were harvested 48 hours post-transfection for subsequent analysis.



Supplementary Figure 1. Validation of PSMB5 knockdown efficiency by shRNAs in CRC cells. (A) Relative PSMB5 mRNA levels in SW620 and SW480 cells transfected with two independent PSMB5-targeting shRNAs (shRNA1, shRNA2) or a non-targeting scramble control (shCtrl), as determined by RT-qPCR. Data are normalized to GAPDH. (B-C) Representative Western blot images (B) and corresponding quantitative analysis (C) of PSMB5 protein expression in the same set of cells. GAPDH served as the loading control. Data are presented as mean ± SD of three independent experiments. ^{j} $p < 0.001$ vs. scramble group.



Supplementary Figure 2 PSMB5 knockdown inhibits CRC cell invasion. (A and B) Representative images and quantitative analysis (C and D) of Transwell invasion assays for SW620 and SW480 cells transfected with shRNA1, shRNA2, or Scramble. Invaded cells were stained and counted. Data are presented as mean ± SD.^{k} $P < 0.01$, ^{1} $P < 0.001$ vs. scramble group.



Supplementary Figure 3 PSMB5 knockdown impairs CRC cell migration. (A-B) Representative images (A) and quantitative analysis (B) of scratch wound healing assays for SW620 and SW480 cells transfected with shRNA1, shRNA2, or Scramble. Wound closure was monitored at 0 and 24 hours. The migration rate is expressed as the percentage of wound closure. Scale bar, 200 μm. Data are presented as mean ± SD. ^{m} $p < 0.01$, ^{n} $p < 0.001$ vs. scramble group.

Supplementary Table 1 Sequences of the two PSMB5-targeting shRNAs

siRNA name/ID	Target sequence (DNA, 5′ → 3′)	Cloning oligonucleotides (5′ → 3′)
shPSMB5-#1	CCCATCCTCCATCCTATTTAT[19]	Top Strand: GATCCCC**CCCATCCTCCATCCTATTTAT**TTCAAGAGA**ATAAATAGGATGGAGGATGGG**TTTTTGGAAA
(shRNA1)		Bottom Strand: AGCTTTTCCAAAAA**CCCATCCTCCATCCTATTTAT**TCTCTTGAA**ATAAATAGGATGGAGGATGGG**GGG
shPSMB5-#2	CCATCCTCCATCCTATTTATT	Top Strand: GATCCCC**CCATCCTCCATCCTATTTATT**TTCAAGAGA**AATAAATAGGATGGAGGATGG**TTTTTGGAAA
(shRNA2)		Bottom Strand: AGCTTTTCCAAAAA**CCATCCTCCATCCTATTTATT**TCTCTTGAA **AATAAATAGGATGGAGGATGG**GGG
shCtrl (Scramble)	TTCTCCGAACGTGTCACGTTT	Top Strand: GATCCCC**TTCTCCGAACGTGTCACGT**TTCAAGAGA**ACGTGACACGTTCGGAGAA**TTTTTGGAAA
		Bottom Strand: AGCTTTTCCAAAAA**TTCTCCGAACGTGTCACGT**TCTCTTGAA**ACGTGACACGTTCGGAGAA**GGG

Notes: shPSMB5, PSMB5-targeting short hairpin RNA; Scramble, non-targeting control shRNA. The targeting sequence (for shPSMB5) or the scrambled sequence (for Scramble) is shown in bold within the cloning oligonucleotides. The loop sequence (TTCAAGAGA) is underlined. Restriction sites for *BamHI* and *HindIII* are included for cloning.