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A Novel Circular RNA circPLCE1 Facilitates the Malignant Progression of Colorectal Cancer by Repressing the SRSF2-dependent PLCE1 pre-RNA Splicing

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Primary research

Keywords: Colorectal cancer, circPLCE1, SRSF2, RNA splicing

Posted Date: March 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-287316/v1

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Abstract

Background: Studies have demonstrated that circular RNAs (circRNAs) play important roles in various types of cancer; however, the mechanisms of circRNAs located in the nucleus have rarely been explored. Here, we reported a novel circular RNA circPLCE1 facilitates the malignant progression of colorectal cancer (CRC) via repression of SRSF2-dependent PLCE1 pre-RNA splicing.

Methods: qRT-PCR was used to determine the expression of circPLCE1 in CRC tissues and cells. CCK-8, transwell and flow cytometric assays were used to assess the role of circPLCE1 in CRC cell proliferation, migration and apoptosis, respectively. Animal study was carried to test the role of circPLCE1 in vivo. Further, catRAPID and RPISeq were applied to predict the possible binding protein of circPLCE1. RNA fractionation and RIP assays were used to confirm the RNA-protein interaction.

Results: In this study, we found that circPLCE1 was significantly downregulated in CRC tissues compared with adjacent normal tissues. However, circPLCE1 knockdown suppresses CRC cell proliferation, migration, invasion and increased apoptosis. Nude mice experiments showed that ectopic expression of circPLCE1 dramatically increased tumor growth in vivo. Mechanically, circPLCE1 directly binding to SRSF2 protein, repressing SRSF2-dependent PLCE1 pre-RNA splicing, resulting in the progression of CRC. Individually mutating the binding sites of circPLCE1 derepressed the production of PLCE1 mRNA.

Conclusions: Our studies revealed a novel molecular mechanism in the regulation of PLCE1 and implicated a new function of circular RNA, supporting the pursuit of circPLCE1 as a potential tool for future CRC treatment.

Full Text

This preprint is available for download as a PDF.

Figures

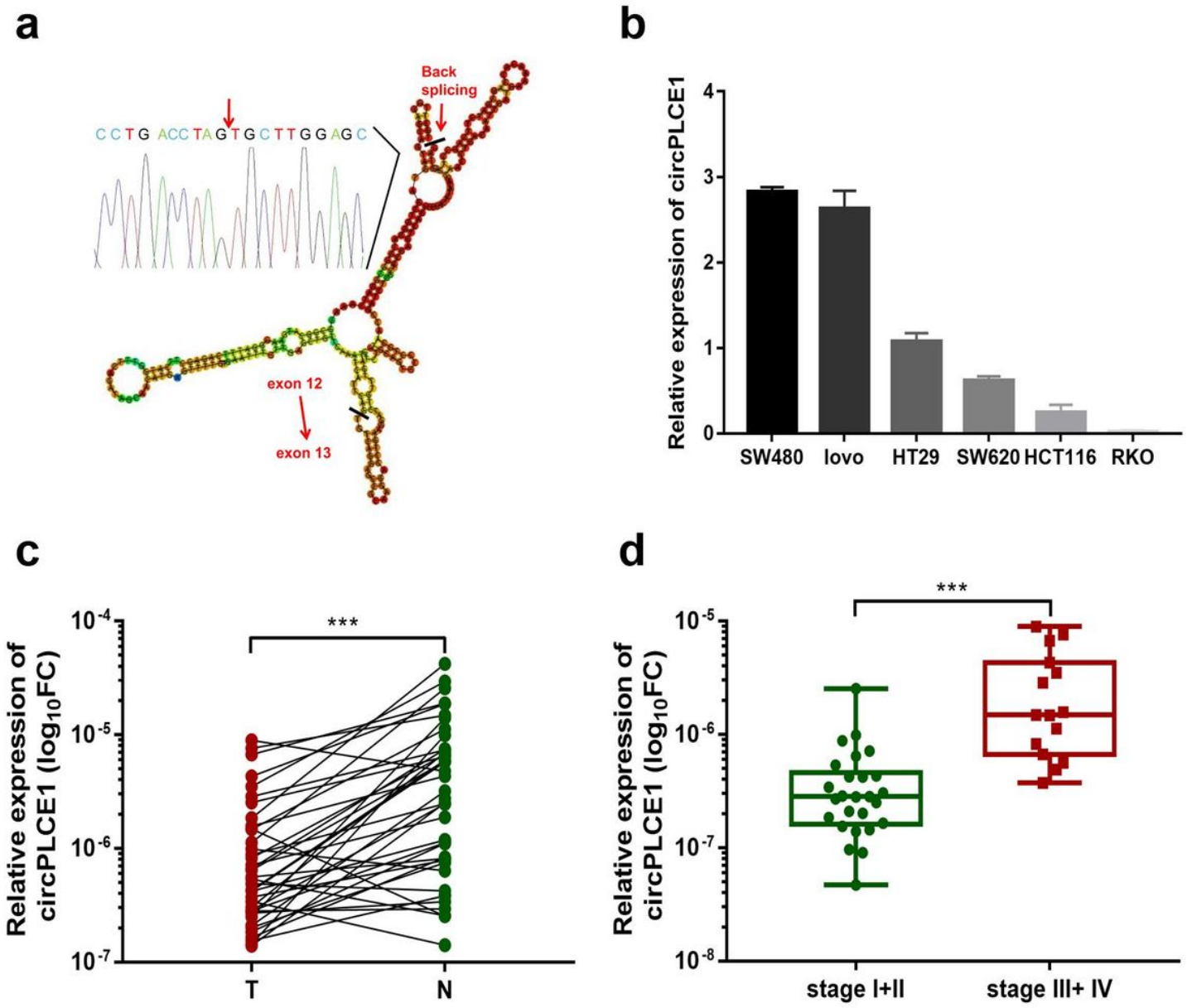

Figure 1

circPLCE1 validation and expression in colorectal cancer tissues and cells. a The secondary structure of circPLCE1 was formed by RNAfold. The presence of circPLCE1 was validated by qRT-PCR and subsequent Sanger sequencing. The red arrow represents “head-to-tail” junction of circPLCE1. b Relative expression of circPLCE1 in CRC cell lines. c circPLCE1 was markedly down-regulated in CRC tissues compared with paired adjacent mucosal tissues. d The circPLCE1 expression level in patients with different TNM stages. ***p < 0.001. T, tumor tissues; N, normal tissues.

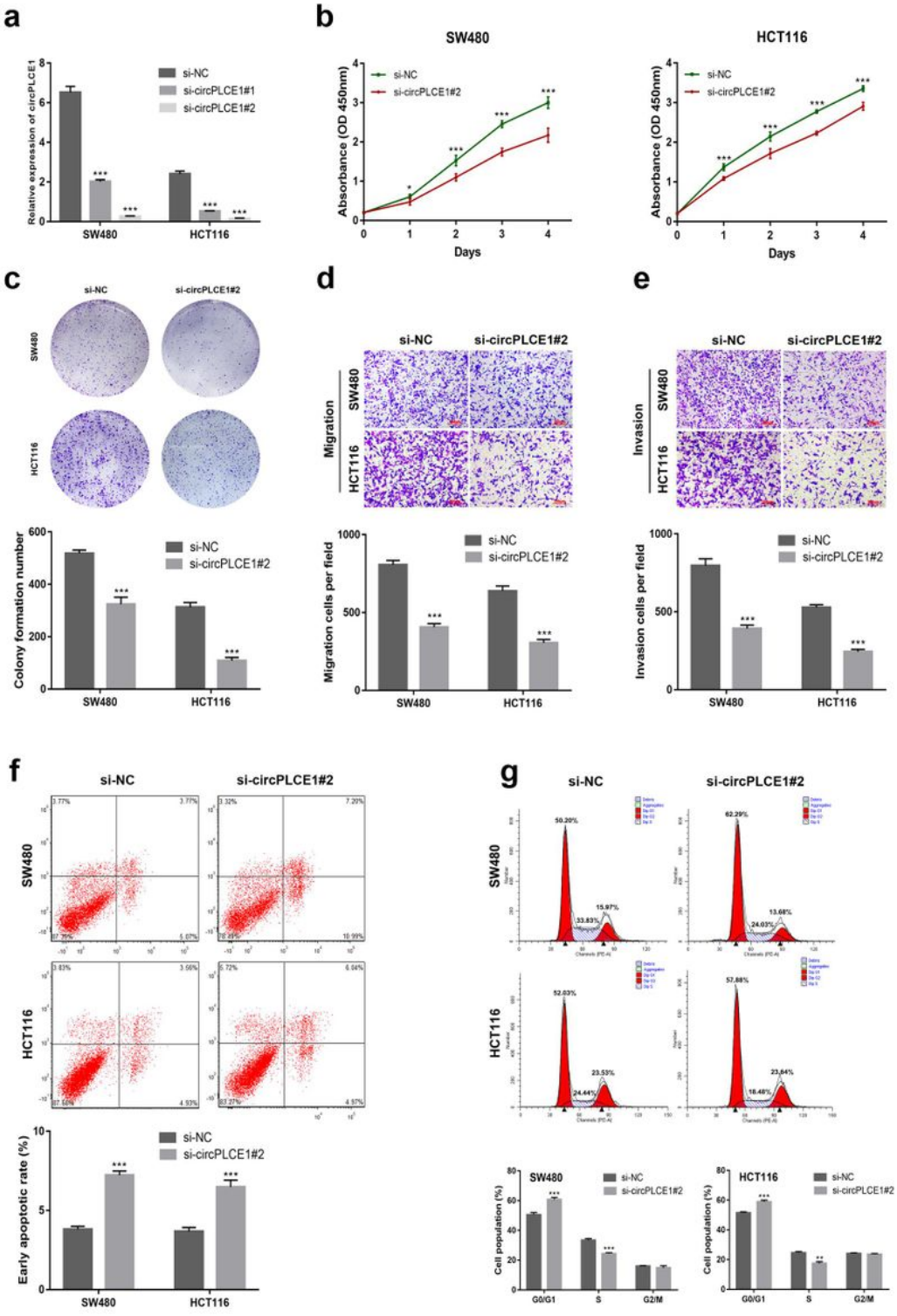

Figure 2

Knockdown of circPLCE1 inhibits cell proliferation, cell migration and cell cycle and promoted apoptosis. a Efficiency of siRNAs on circPLCE1 in SW480 and HCT116 detected by qRT-PCR. b CCK-8 assay was performed to determine cell proliferation in si-NC cells and si-circPLCE1#2 cells. c Colony formation assays were conducted in differently treated cells. d Transwell migration assay in transfected SW480 and HCT116 cells. e Transwell invasion assay in transfected SW480 and HCT116 cells. f The cell cycle of

SW480 and HCT116 cells after transfection. g Early apoptosis was assessed in cells transfected with si-NC or si-circPLCE1#2 by flow cytometry. All data are presented as the means ± SD of at least three independent experiments. *p< 0.05, **p< 0.01, ***p< 0.001. Scale bar: 200 μm.

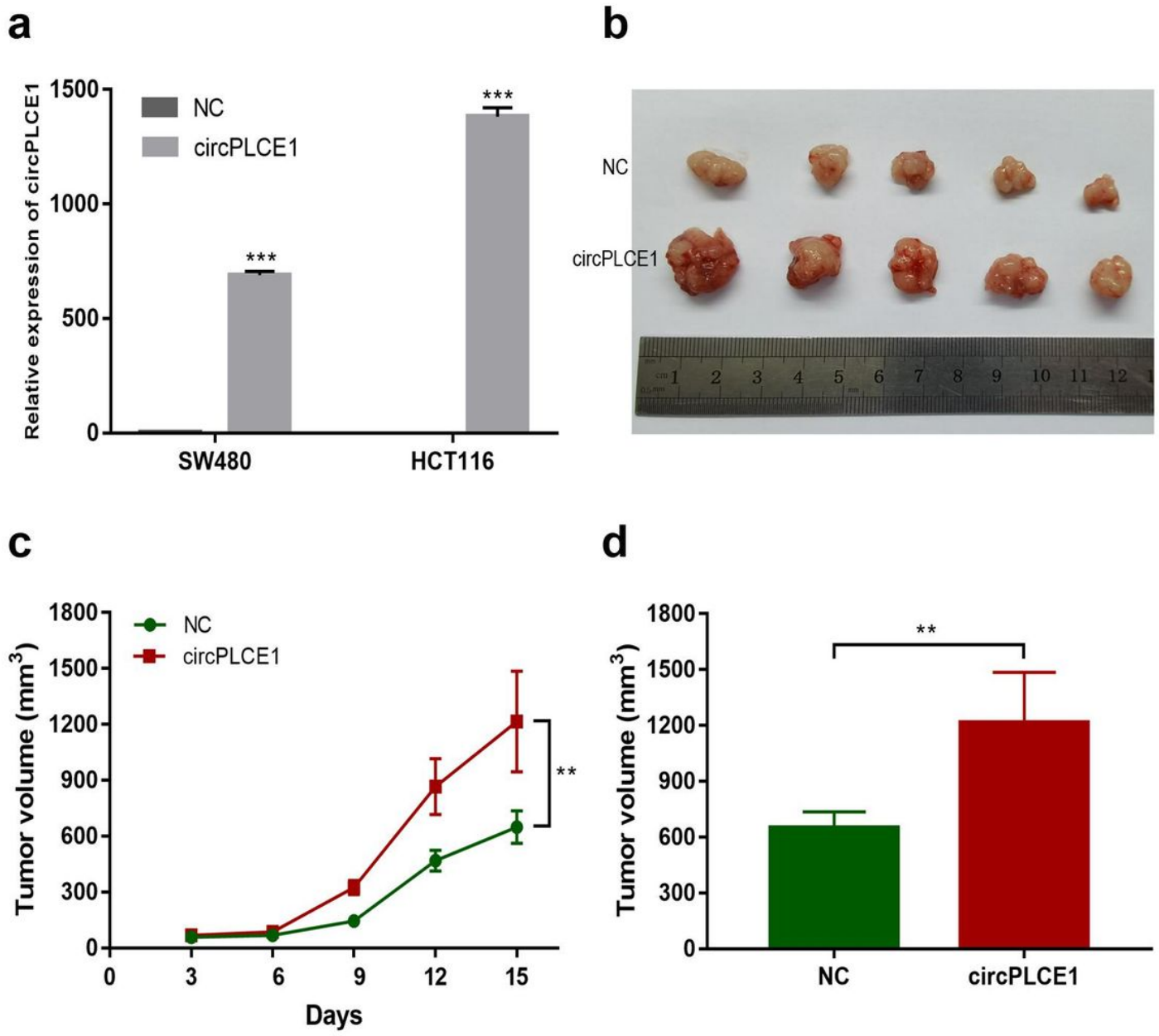

Figure 3

circPLCE1 promotes CRC growth in vivo. a Expression of circPLCE1 in SW480 and HCT116 cells after infection with circPLCE1-overexpression lentiviruses or NC lentiviruses. b Xenograft tumors of nude mice 15 days after injection of HCT116 cells (n = 5 per group). c Tumor volumes were measured every 3 days. d tumor volumes were measured after the nude mouse were executed. All data are presented as the means ± SD. **p< 0.01, ***p< 0.001.

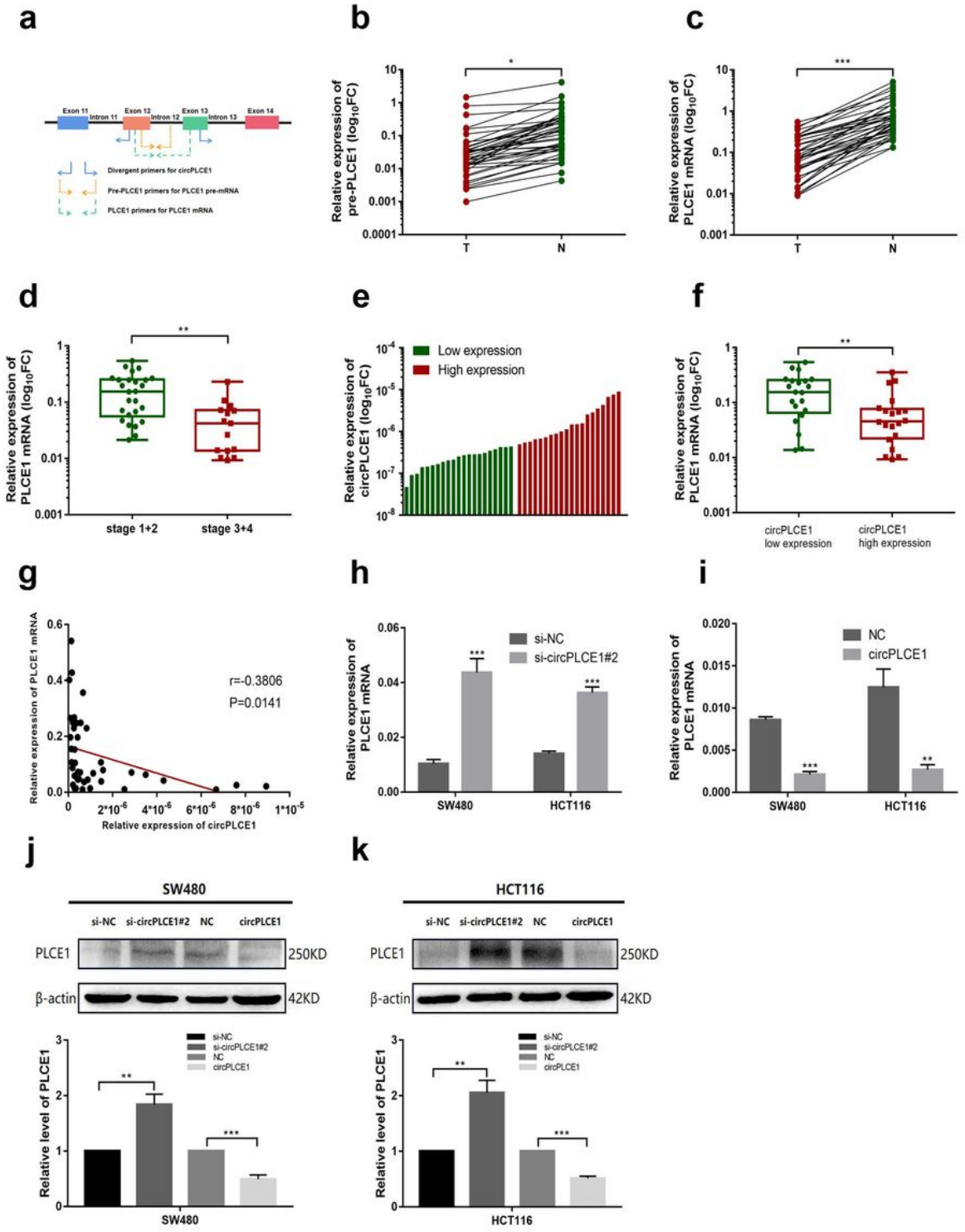

Figure 4

PLCE1 mRNA expression was antagonized by circPLCE1 in CRC tissues and cell lines. a Primers to distinguish between circPLCE1, pre-PLCE1, and PLCE1 mRNA. b, c Pre-PLCE1 and PLCE1 mRNA expressions were significantly decreased in CRC tissues compared with normal tissues. d The PLCE1 mRNA expression in patients with different TNM stages. e Using the median expression level of circPLCE1 as a cutoff value, the 41 CRC tissues were divided into low- and high-expression groups. f The

PLCE1 mRNA expression in low- and high-expression groups. g Correlation between circPLCE1 expression and PLCE1 mRNA expression in CRC samples. h, i The PLCE1 mRNA expression was detected through qRT-PCR after overexpression or silencing of circPLCE1 in CRC cells. j, k The protein levels of PLCE1 were detected through Western blotting after overexpression or silencing of circPLCE1 in CRC cells. Data are listed as means ± SD. *p< 0.05, **p< 0.01, ***p< 0.001.

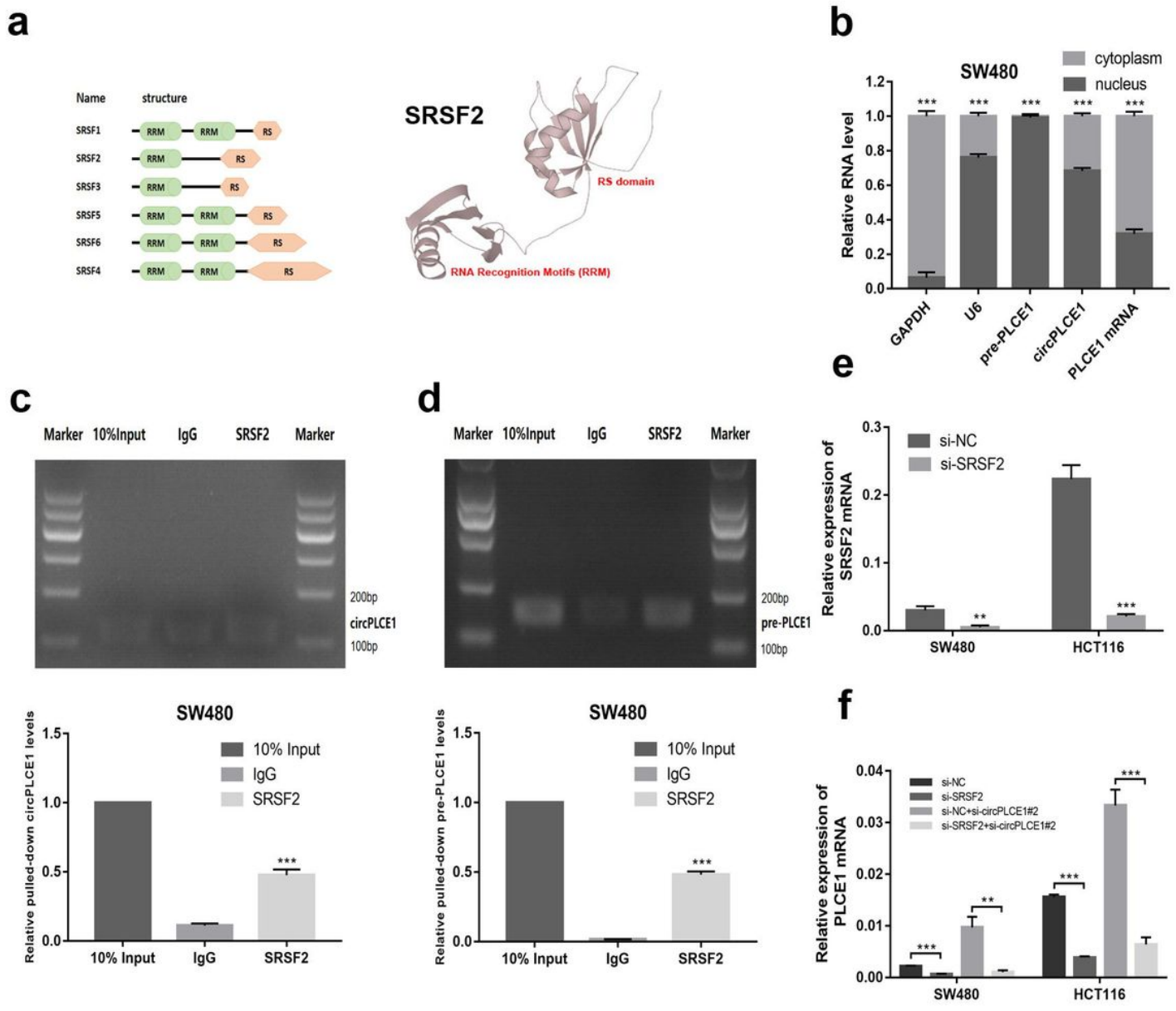

Figure 5

Binding of circPLCE1 with SRSF2 protein. a Structure of classic SR proteins, which are characterized by one or two RRM at the N-terminus and the signature RS domain at the C-terminus. b Subcellular location of circPLCE1, pre-PLCE1 and PLCE1 mRNA in SW480 cells. c, d RNA immunoprecipitation experiments were performed using anti-SRSF2 antibody in SW480 cells by qRT-PCR. Nucleic acid electrophoresis was performed to detection the PCR products. e Expression of SRSF2 mRNA in SW480 and HCT116 cells

treated with SRSF2 siRNA. f Expression of PLCE1 mRNA in SW480 and HCT116 cells treated with SRSF2 siRNA or circPLCE1 siRNA. **p< 0.01, ***p< 0.001.

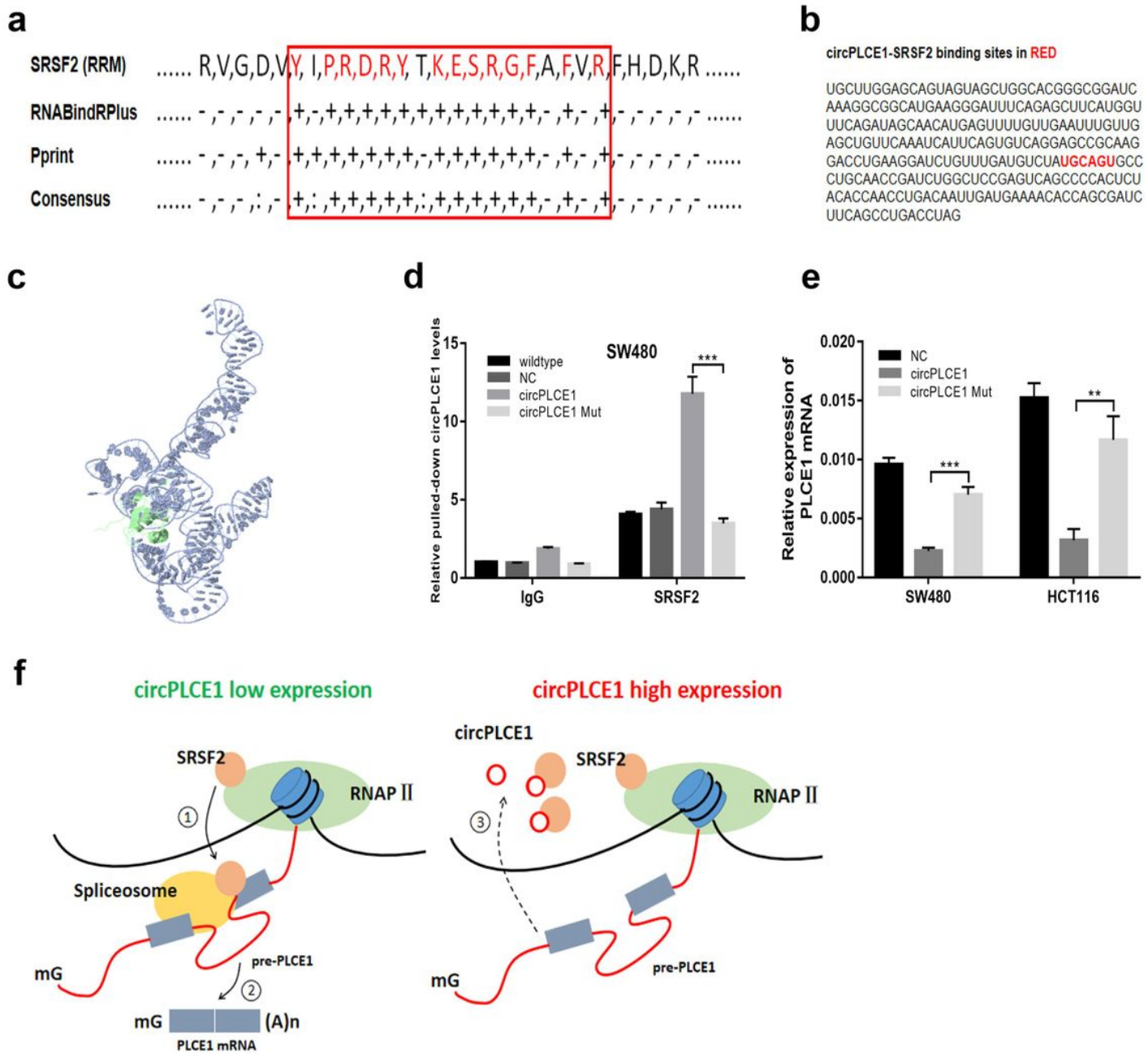

Figure 6

Identify the binding sites of circPLCE1 with SRSF2. a Prediction of probable RNA-binding residues of SRSF2 was carried out by submitting the SRSF2 sequence to Pprint and RNABindRPlus servers. “+” indicates the predicted RNA-binding residues. b The interactive sites of circPLCE1 with SRSF2 was highlighted in red. c Graphical representation of three-dimensional structures of the docking models of circPLCE1 with the binding fragment of SRSF2 RRM domain by NPDock. d In SW480 cells, SRSF2 antibody precipitated higher levels of circPLCE1 in the circPLCE1-overexpression cells relative to the wildtype, NC and circPLCE1-Mut-overexpression cells. e PLCE1 mRNA expression in CRC cells transfected

with NC vector, circPLCE1, circPLCE1 Mut were examined by qRT-PCR. f Model depicting the proposed mechanism of suppression of PLCE1 pre-RNA splicing by circPLCE1. In the circPLCE1 low expression CRC cells, SRSF2 is responsible for initiating spliceosome assembly on pre-PLCE1 , which promote the production of PLCE1 mRNA . Conversely, in the circPLCE1 high expression CRC cells, circPLCE1 is directly binding to SRSF2 and suppress the process of PLCE1 pre-RNA splicing . **p< 0.01, ***p< 0.001.

Supplementary Files

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- Supplementary.pdf