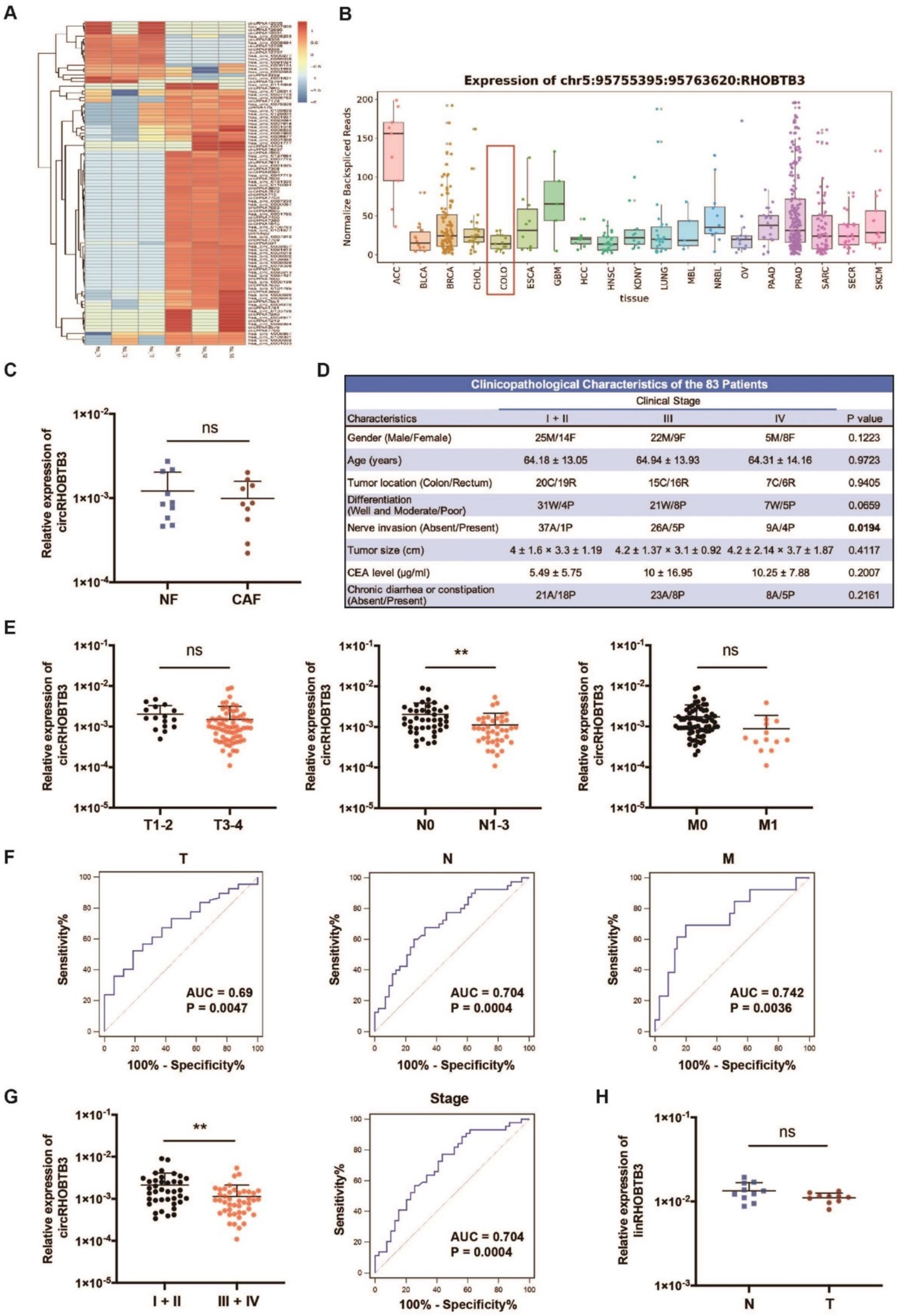
Supplementary materials:



Clinicopathological Characteristics of the 83 Patients				
	Clinical Stage			
Characteristics	I + II	III	IV	P value
Gender (Male/Female)	25M/14F	22M/9F	5M/8F	0.1223
Age (years)	64.18 ± 13.05	64.94 ± 13.93	64.31 ± 14.16	0.9723
Tumor location (Colon/Rectum)	20C/19R	15C/16R	7C/6R	0.9405
Differentiation (Well and Moderate/Poor)	31W/4P	21W/8P	7W/5P	0.0659
Nerve invasion (Absent/Present)	37A/1P	26A/5P	9A/4P	**0.0194**
Tumor size (cm)	4 ± 1.6 × 3.3 ± 1.19	4.2 ± 1.37 × 3.1 ± 0.92	4.2 ± 2.14 × 3.7 ± 1.87	0.4117
CEA level (μg/ml)	5.49 ± 5.75	10 ± 16.95	10.25 ± 7.88	0.2007
Chronic diarrhea or constipation (Absent/Present)	21A/18P	23A/8P	8A/5P	0.2161

Figure S1. Validation and expression of circRHOBTB3 and linRHOBTB3 in

different tissues. (A) Heatmap of differentially expressed circRNAs in paired CRC tissues and normal tissues (n = 6). **(B)** The overall expression of circRHOBTB3 in multiple human cancers from the MiOncoCirc database. **(C)** qRT-PCR showed the circRHOBTB3 levels in primary cultured CAFs and paired NFs from 10 CRC patients. The P values were determined by paired Student's t-tests. **(D)** Clinicopathological characteristics of the 83 CRC patients. **(E)** qRT-PCR showed the circRHOBTB3 levels in tumor tissues from 83 CRC patients with different clinical characteristics. **(F)** ROC analysis showed the diagnostic value of circRHOBTB3 in CRC patients with different clinical characteristics. **(G)** qRT-PCR and ROC analysis showed the levels and diagnostic value of circRHOBTB3 in 83 CRC patients at different clinical stages. **(H)** qRT-PCR showed the relative expression of linRHOBTB3 in CRC and adjacent normal mucosa tissues (n = 10). The P values were determined by paired Student's t-tests. The data are presented as the mean ± SD of three independent experiments, two-tailed Student's t-tests, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

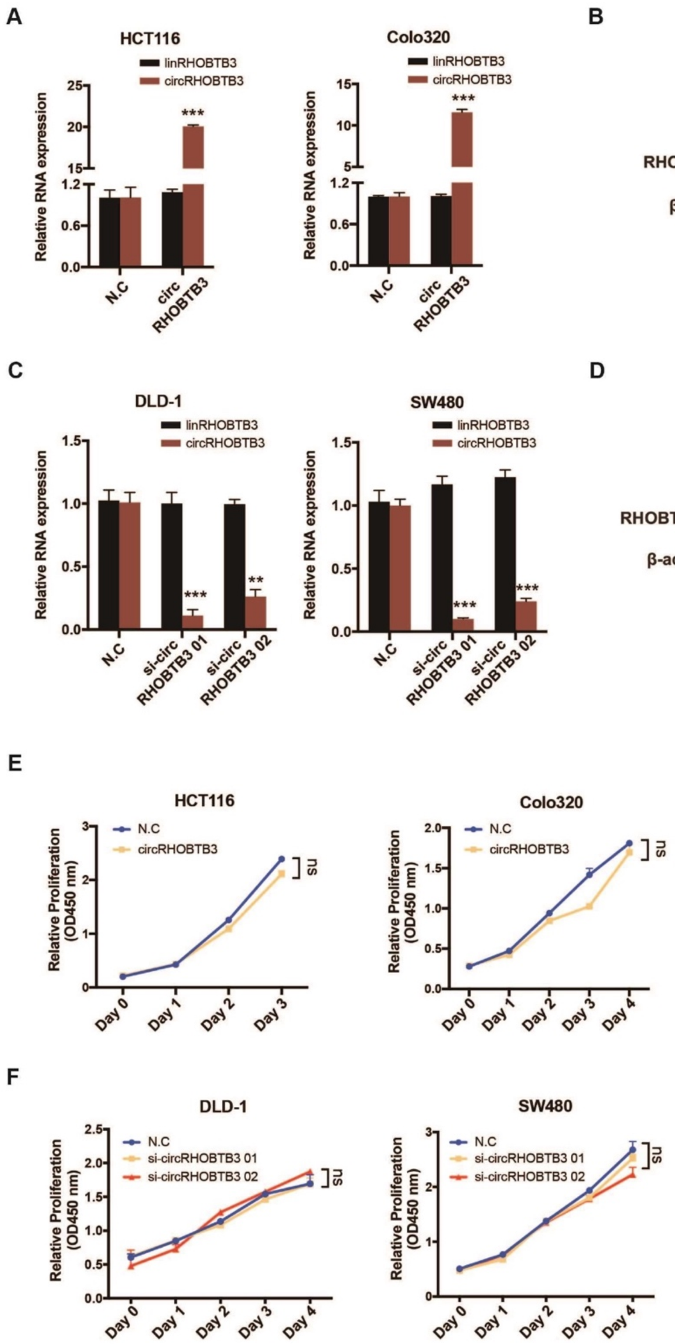
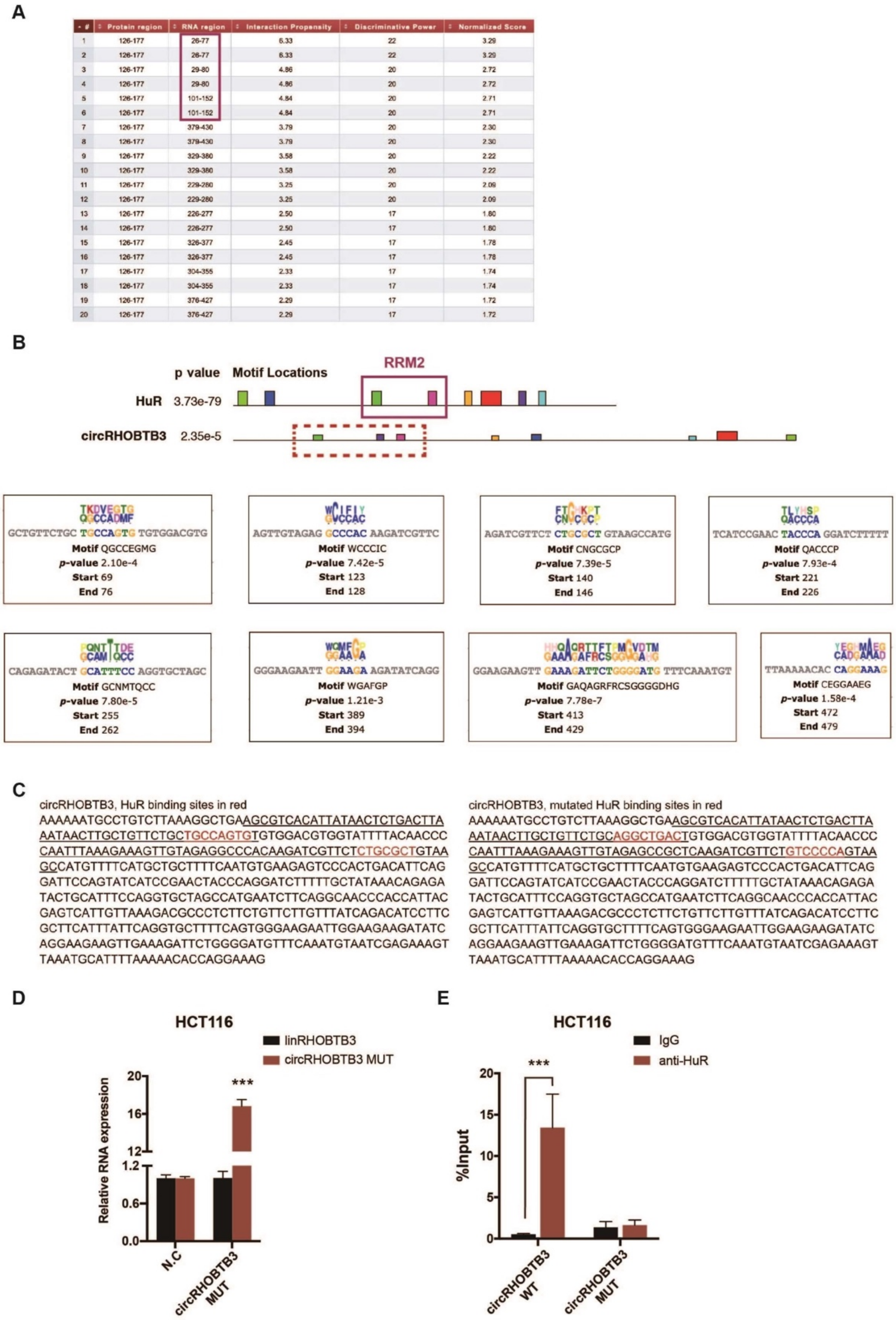


Figure S2. Transfection efficiency and regulation of CRC cell proliferation of circRHOBTB3. **(A) and (B)** qRT-PCR and Western blotting detected the expression of circRHOBTB3, linRHOBTB3 and RHOBTB3 protein in HCT116 and Colo320 cells after stable transfection of the N.C or circRHOBTB3 plasmid. **(C) and (D)** qRT-

PCR and Western blotting detected the expression of circRHOBTB3, linRHOBTB3 and RHOBTB3 protein in DLD-1 and SW480 cells after transfection of circRHOBTB3 N.C (control siRNA) or siRNAs. **(E)** CCK-8 assay assessed the proliferation of HCT116 and Colo320 cells transfected with the N.C or circRHOBTB3 plasmid. **(F)** CCK-8 assay assessed the proliferation of DLD-1 and SW480 cells transfected with N.C or circRHOBTB3 siRNAs. The P values in **E** and **F** were determined by two-way ANOVA. The data are presented as the mean ± SD of three independent experiments, two-tailed Student's t-tests, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.



#	Protein region	RNA region	Interaction Propensity	Discriminative Power	Normalized Score
1	126-177	26-77	6.33	22	3.29
2	126-177	26-77	6.33	22	3.29
3	126-177	29-80	4.86	20	2.72
4	126-177	29-80	4.86	20	2.72
5	126-177	101-152	4.84	20	2.71
6	126-177	101-152	4.84	20	2.71
7	126-177	379-430	3.79	20	2.30
8	126-177	379-430	3.79	20	2.30
9	126-177	329-380	3.58	20	2.22
10	126-177	329-380	3.58	20	2.22
11	126-177	229-280	3.25	20	2.09
12	126-177	229-280	3.25	20	2.09
13	126-177	226-277	2.50	17	1.80
14	126-177	226-277	2.50	17	1.80
15	126-177	326-377	2.45	17	1.78
16	126-177	326-377	2.45	17	1.78
17	126-177	304-355	2.33	17	1.74
18	126-177	304-355	2.33	17	1.74
19	126-177	376-427	2.29	17	1.72
20	126-177	376-427	2.29	17	1.72

circRHOBTB3, HuR binding sites in red
AAAAAATGCCTGTCTTAAAGGCTGAAGCGTCACATTATAACTCTGACTTA
AATAACTTGCTGTTCTGCTGCCAGTGTGTGGACGTGGTATTTTACAACCC
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circRHOBTB3, mutated HuR binding sites in red
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TAAATGCATTTTAAAAACACCAGGAAAG

Figure S3. Identification of the motifs in the circRHOBTB3 sequence that interact with HuR. (A) Prediction of the binding positions of circRHOBTB3 on the HuR protein from the catRAPID database. **(B)** Prediction of the binding motifs of circRHOBTB3 with the HuR protein from the MEME database. **(C)** circRHOBTB3 sequence labeling HuR-binding sites (red) and mutated nucleotides (red). **(D)** qRT-PCR detected the expression of mutated circRHOBTB3 and linRHOBTB3 after transfection of the circRHOBTB3 MUT or N.C plasmid. **(E)** RIP experiments confirmed the combination of HuR with WT circRHOBTB3 but not MUT circRHOBTB3. The data are presented as the mean ± SD of three independent experiments, two-tailed Student's t-tests, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

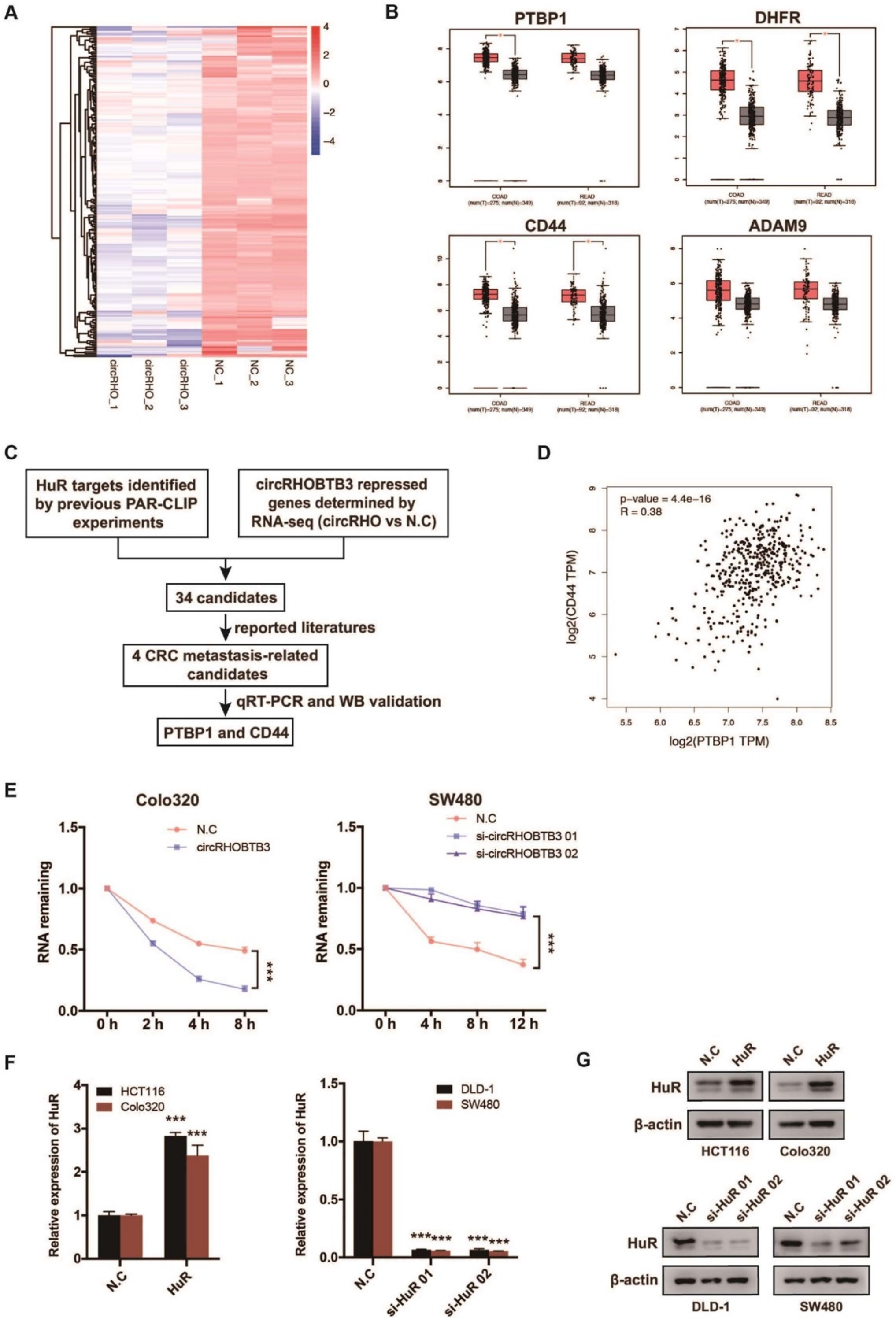

Figure S4. Identification of PTBP1 as the target of the circRHOBTB3/HuR axis. **(A)** Clustered heatmap showing significantly downregulated mRNAs in circRHOBTB3-overexpressing HCT116 cells relative to N.C cells (n = 6, Log2FC >

1). **(B)** The expression of 4 selected metastasis-related genes in CRC tissues and adjacent normal tissues from TCGA (Log2FC > 1). **(C)** Flowchart illustrating the criteria for identifying PTBP1 and CD44 as targets of circRHOBTB3. **(D)** Positive correlation between PTBP1 and CD44 mRNA levels from TCGA. **(E)** qRT-PCR estimated the influences of circRHOBTB3 on the mRNA stability of PTBP1 in Colo320 and SW480 cells treated with actinomycin D. The P values were determined by two-way ANOVA. **(F) and (G)** qRT-PCR and Western blotting detected the expression of HuR mRNA and protein levels in CRC cells after transfection of HuR plasmid or siRNAs compared with N.C groups. The data are presented as the mean ± SD of three independent experiments, two-tailed Student's t-tests, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

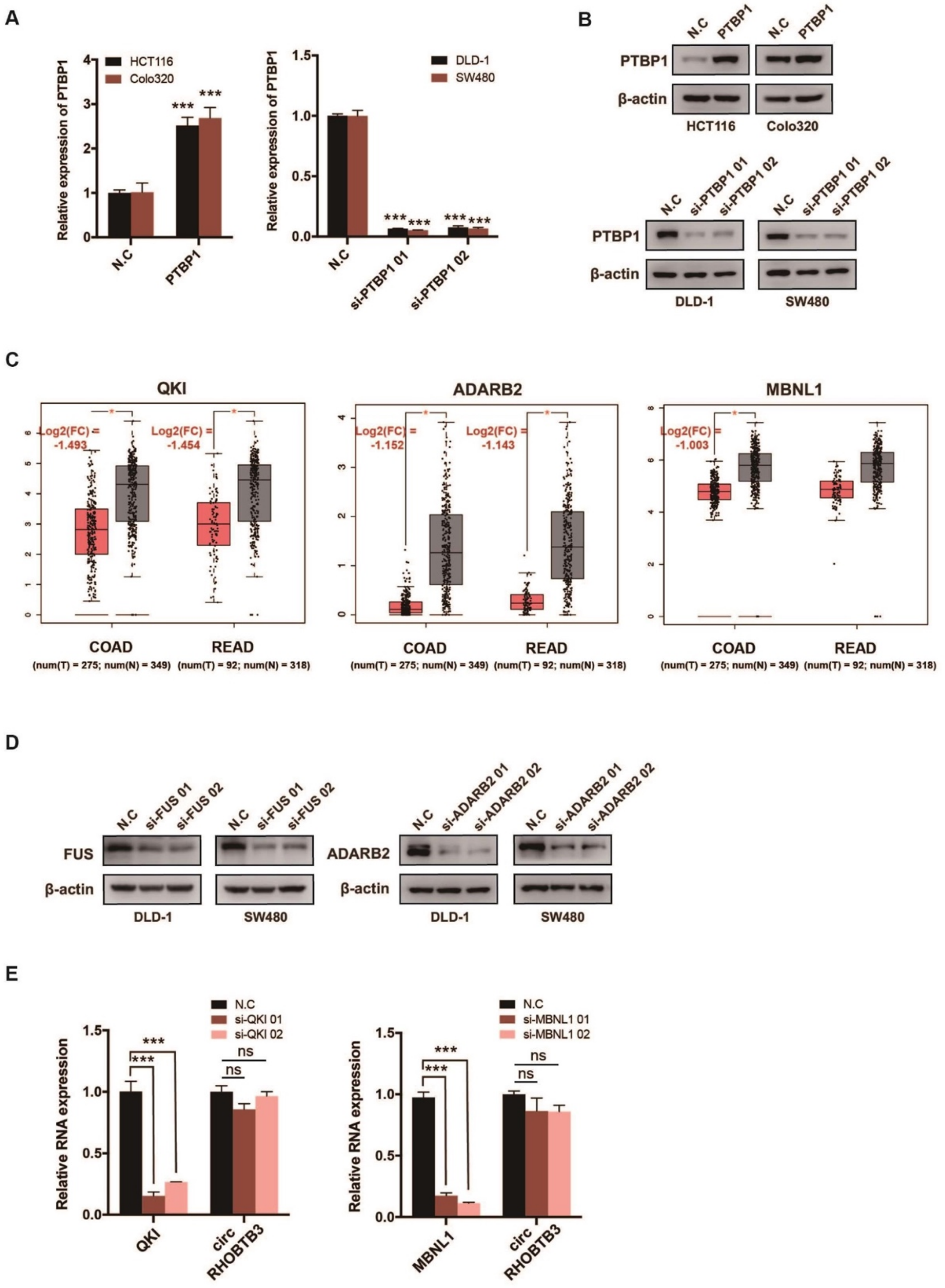

Figure S5. Downregulated RNA-editing RBPs in CRC tissues and their impact on circRHOBTB3 formation. (A) and (B) qRT-PCR and Western blotting detected the mRNA and protein expression levels of PTBP1 in CRC cells after transfection of PTBP1 plasmid or siRNAs compared with the N.C groups. **(C)** Significantly downregulated RNA-editing enzymes in CRC tissues and adjacent normal tissues from TCGA (Log2FC > 1). **(D)** Western blotting detected the protein expression of FUS and ADARB2 in DLD-1 and SW480 cells after transfection with N.C or siRNAs. **(E)** qRT-PCR analysis of the expression of circRHOBTB3 upon QKI and MBNL1 depletion using siRNAs in SW480 cells. The data are presented as the mean ± SD of three independent experiments, two-tailed Student's t-tests, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

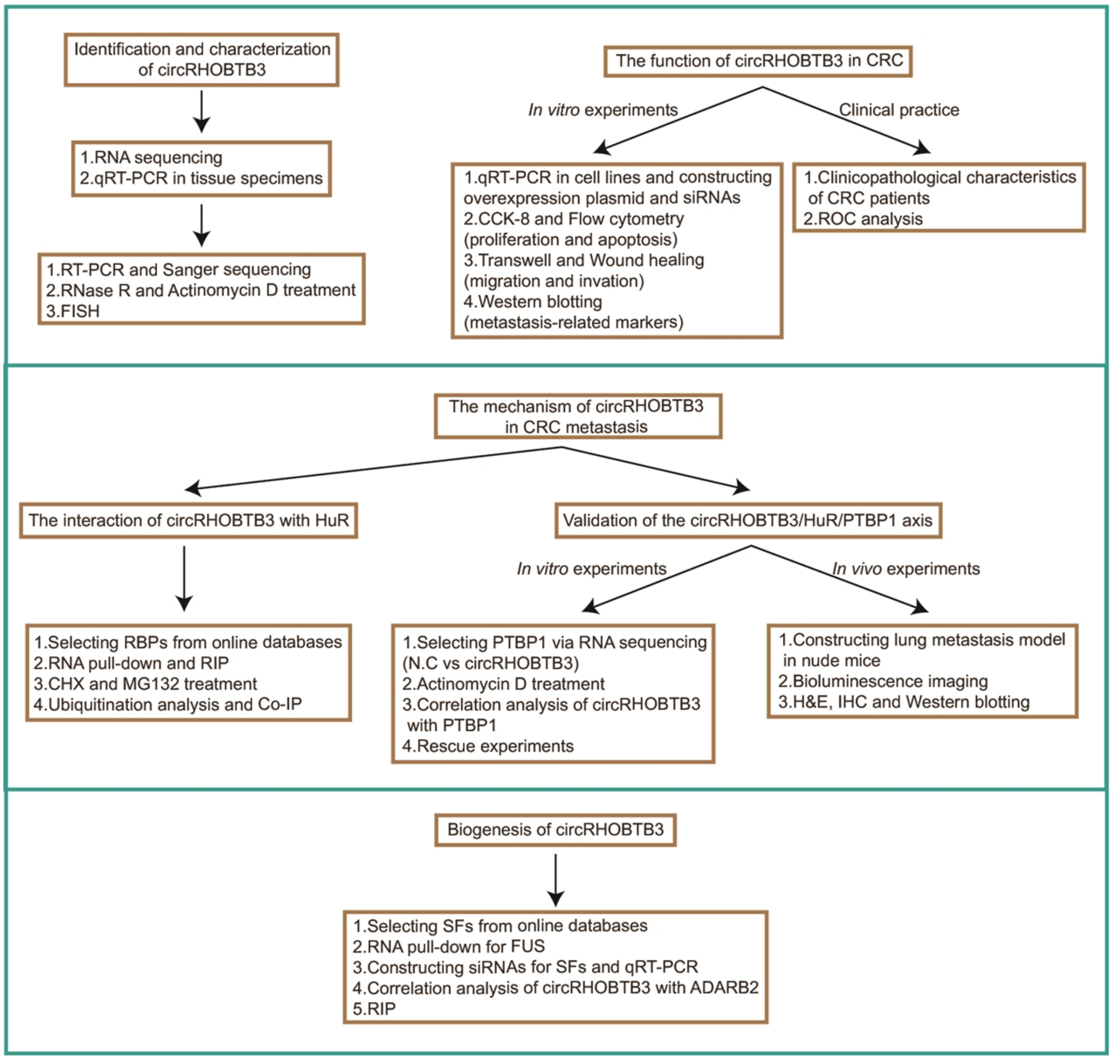

Figure S6. Flowchart illustrating our experimental procedure.

Table S1. The sequences of all primers and oligonucleotides used in the study.

Gene	Forward primer (5’ to 3’)	Reverse primer (5’ to 3’)
circ_0006867	TCCAGTGTTTCCTTGGGTCA	TTCCATGCAGCCCTTCTGTA
circ_0000690	CGCGAGCGAGTTTGTGAAAA	GGAACACCTCTGTCCCTCAA
circ_0008832	TAATTTCACAGCCCCAGGTG	AGTCATGCTAGGAGAAGCTGTG
circ_0004524	GGCTTGCCACCAACAGAATAAA	AGTCTGTCTGATGGTTGACTGG
circ_0001546	TGTCATCGAGAAGGCAGAGA	GAATTCTTGGCTGGCTCACA
circ_0007444	TTCAGTGGGAAGAATTGGAAGAA	TGGCAGCAGAACAGCAAGTTA
circ_0004368	CACCAGCAGAAGAACCAAATATGA	TTCTCATATTTGGTTCTTCTGCTGG
circ_0009043	AGCATCCGCAAACATTCAGAC	TGCTTCAGCTCTTCCATTGCTA
circ_0002490	AGCTTGTGTTCAGGTGTGTCA	ATACACTTGGTGGGATCTGCT
circ_0001535	GAAGAAAATACCCAGCACCCA	TCAAAAAGGTGCTGTTCCACA
linRHOBTB3	GCCCTTGGATTTGAGATCATGT	GTCAGCGCCCCCAATTAGAT
β-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
HuR	CAGCATTGGTGAAGTTGAATCTG	CCTTAATGGTTTTTGACTGGAGC
PTBP1	ATGGACGGCATTGTCCCAGATATAG	CTAGATGGTGGACTTGGAGAAGGAGA
DHFR	TTCCAGAGAATGACCACAACCT	TGCCACCAACTATCCAGACC
CD44	CTGCCGCTTTGCAGGTGTA	CATTGTGGGCAAGGTGCTATT
ADAM9	TCTTGCCACAGACCCGGTAT	ATCTCCAGTCCAACTAGCACA
FUS	TTATGGCCAGAGCCAGAACAC	GCTACCGTAACTTCCCGAGG
QKI	AAGCCCACCCCAGATTACCT	ACTCTGCTAATTTCTTCGTCCAG
ADARB2	CCTAAACACCAAGGCGAGAT	AAAACTAACGGGGCACACTG
MBNL1	GCTGTTAGTGTCACACCAATTCG	AGGCGATTACTCGTCCATTTTC
pre-RHOBTB3	CTGCTGTGAGTAGGAAGGACA	ACTTTCCCTTATCACACCATATCC
circRHOBTB3 MUT	TTCAGTGGGAAGAATTGGAAGAA	AGCCTGCAGAACAGCAAGTTA
Oligonucleotide	Sense (5’ to 3’)	Antisense (5’ to 3’)
si-circRHOBTB3 01	AACACCAGGAAAGAAAAAA	
si-circRHOBTB3 02	AAAGAAAAAATGCCTGTCT	
si-HuR 01	GGUUUGGGCGGAUCAUCAATT	UUGAUGAUCCGCCCAAACCTT
si-HuR 02	GAACGAAUUUGAUCGUCAATT	UUGACGAUCAAAUUCGUUCTT
si-PTBP1 01	CCCUCAUUGACCUGCACAATT	UUGUGCAGGUCAAUGAGGGTT
si-PTBP1 02	GCCUCAACGUCAAGUACAATT	UUGUACUUGACGUUGAGGCTT
si-FUS 01	CGUGGUGGCUUCAAUAAAUTT	AUUUAUUGAAGCCACCACGTT
si-FUS 02	GAGCAGCUAUUCUUCUUAUTT	AUAAGAAGAAUAGCUGCUCTT
si-QKI 01	GCUGCUCCAAGGAUCAUUATT	UAAUGAUCCUUGGAGCAGCTT
si-QKI 02	GGAGCAUCUAAAUGAAGAUTT	AUCUUCAUUUAGAUGCUCCTT
si-ADARB2 01	GCUGAGCAGUCAACUCAAATT	UUUGAGUUGACUGCUCAGCTT
si-ADARB2 02	CGGACCUGGAGAUUAUCAATT	UUGAUAAUCUCCAGGUCCGTT
si-MBNL1 01	CCAGACACGGAAUGUAAAUTT	AUUUACAUUCCGUGUCUGGTT
si-MBNL1 02	GCCUGGUCCCGGCAGAGAUTT	AUCUCUGCCGGGACCAGGCTT

Word S1. The sequence of Alu elements of circRHOBTB3 analysis

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Note:

1. **5kb intron upstream of RHOBTB3 Exon6 have 3 Alu elements and the sequences are marked with yellow background.**
2. **5kb intron downstream of RHOBTB3 Exon7 have 3 Alu element and the sequence is marked with gray background.**
3. **circRHOBTB3 sequence region (Exon6-Exon7) with green background.**
4. **FUS binding sites are marked with red background.**

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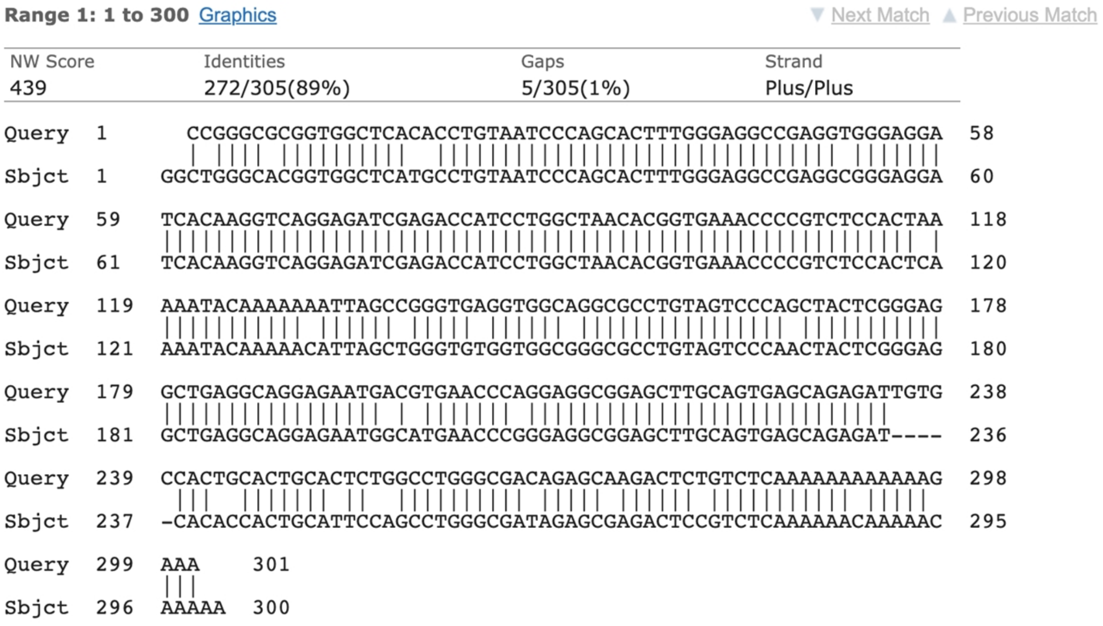

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Range 1: 1 to 300 Graphics                        ▼ Next Match ▲ Previous Match

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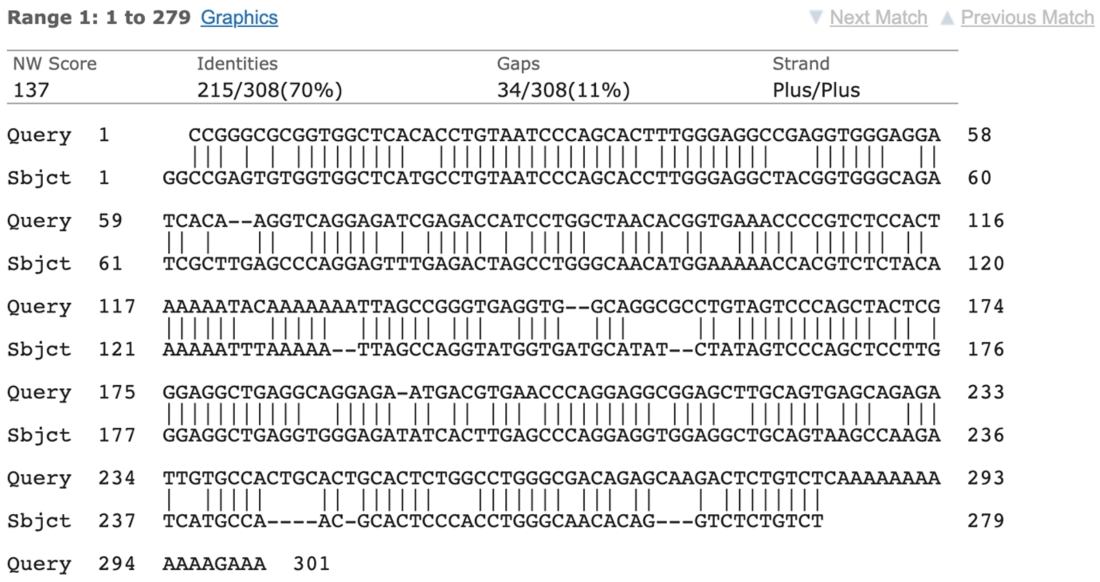

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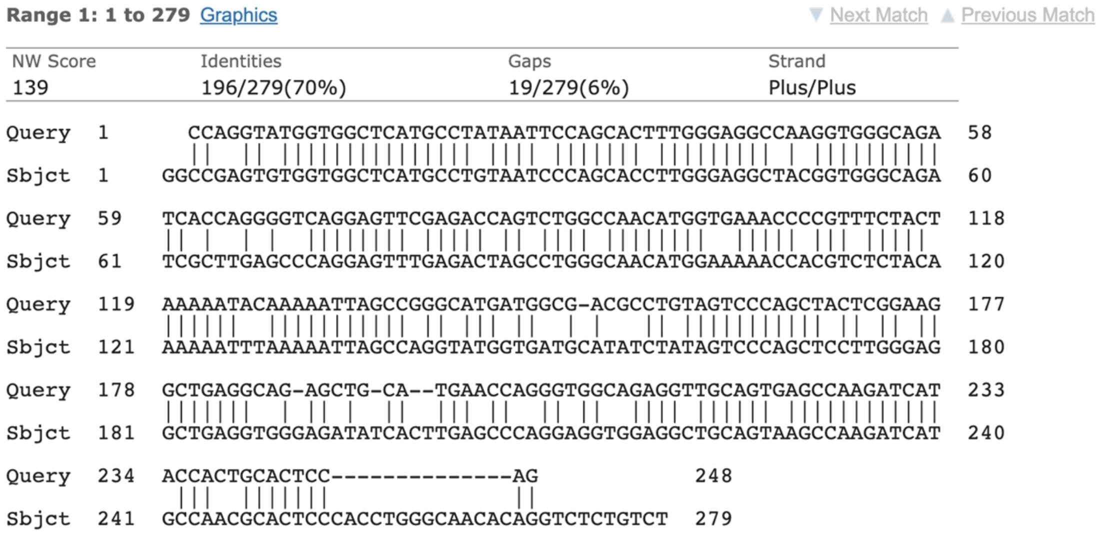

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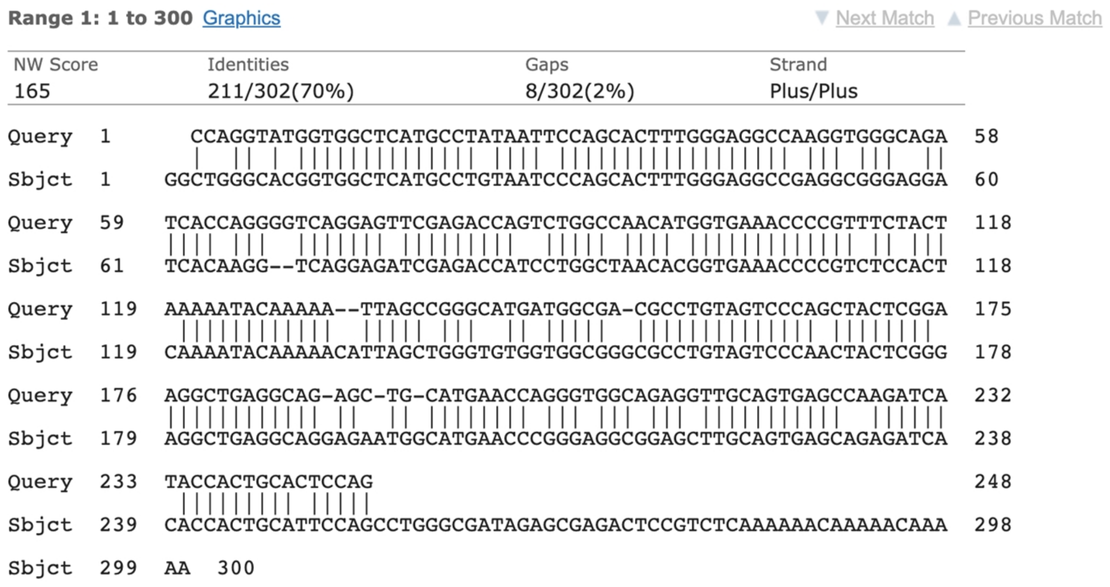
AluSx Matches AluY:



Range 1: 1 to 300

NW Score	Identities	Gaps	Strand
165	211/302(70%)	8/302(2%)	Plus/Plus

```
Query  1       CCAGGTATGGTGGCTCATGCCTATAATTCCAGCACTTTGGGAGGCCAAGGTGGGCAGA  58
                 |  || | |||||||||||||| |||| |||||||||||||||||| ||| |||  ||
Sbjct  1     GGCTGGGCACGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGAGGA  60

Query  59    TCACCAGGGGTCAGGAGTTCGAGACCAGTCTGGCCAACATGGTGAAACCCCGTTTCTACT  118
             |||| |||  ||||||| |||||||||  ||||| |||| ||||||||||||| || |||
Sbjct  61    TCACAAGG--TCAGGAGATCGAGACCATCCTGGCTAACACGGTGAAACCCCGTCTCCACT  118

Query  119   AAAAATACAAAAA--TTAGCCGGGCATGATGGCGA-CGCCTGTAGTCCCAGCTACTCGGA  175
              ||||||||||||  ||||| |||  ||  |||||  |||||||||||||| |||||||| 
Sbjct  119   CAAAATACAAAAACATTAGCTGGGTGTGGTGGCGGGCGCCTGTAGTCCCAACTACTCGGG  178

Query  176   AGGCTGAGGCAG-AGC-TG-CATGAACCAGGGTGGCAGAGGTTGCAGTGAGCCAAGATCA  232
             |||||||||||| ||  || |||||||| ||| ||| ||| |||||||||||  ||||||
Sbjct  179   AGGCTGAGGCAGGAGAATGGCATGAACCCGGGAGGCGGAGCTTGCAGTGAGCAGAGATCA  238

Query  233   TACCACTGCACTCCAG                                            248
              ||||||||| |||||
Sbjct  239   CACCACTGCATTCCAGCCTGGGCGATAGAGCGAGACTCCGTCTCAAAAAACAAAAACAAA  298

Sbjct  299   AA  300
```