

Figure S1. circMVP expression in CRC

(A) Heatmap plot of differentially expressed circRNAs from transcriptome analysis of colorectal cancer tissues (3) and paired adjacent normal tissues. (B) Information and molecular predictions of CircMVP (hsa_circ_0000688) included in CSCD (Cancer-Specific CircRNA Database). (C) Combining PCR with an electrophoresis assay indicated the presence of circMVP using divergent and convergent primers from cDNA or genomic DNA (gDNA) in DLD1, HCT8 and HCT116 cells. (D) The expression level of circMVP in CRC cell line (DLD1, HCT8, HCT116, RKO) and 293T were detected by separation of nucleus and cytoplasm and qRT-PCR. (E) Expression of circMVP was examined by ISH in normal epithelial tissues (n=46) and CRC tissues (n=46), (Student's t-test; log-rank test). (F) Features plot showed distribution and expression levels of circMVP and its hostgene: MVP. (G) CircMVP knockdown and overexpression cell models were constructed in DLD-1 and HCT8 cells, respectively, and transfection effect was verified by qRT-PCR. Data were presented as mean±SD. *p<0.05; **p<0.01; ***p<0.001(Student's t-test; log-rank test). CRC, colorectal cancer; ISH, *in situ* hybridization.

Figure S2. CircMVP facilitates CRC growth

(A) The effects of circMVP on cell proliferation in HCT116 and RKO. (B and C) Transwell-matrigel was used to detect the effect of circMVP on the invasion ability of cancer cells. Scale bars, 300 µm. (D and E) Transwell was used to detect the effect of circMVP on the migration ability of cancer cells. Scale bars, 300 µm. (F) The expression of circMVP in mouse cell lines (B16, BMDM, MC38, 4T1 and CT26) was detected by PCR and analyzed by gel electrophoresis. (G) The effects of circMVP on cell proliferation in MC38. (H and I) Transwell-matrigel and transwell were used to detect the effect of circMVP on the invasion and migration ability of MC38. Scale bars, 300 µm. (J) Pathological section and Spatial transcriptome MVP in the CRC samples from PRJNA942633. Each dot was a cell colored by its analyzed cell types. Data are presented as mean±SD. *p<0.05; **p<0.01; ***p<0.001(Student's t-test; log-rank test). CRC, colorectal cancer.

Figure S3. CircMVP interacts with METTL3

(A) The proteins enriched by circMVP RNA pull-down were obtained with 40kDa-72kDa specific

differential expression bands, and the METTL3 protein was found by MS analysis, peak map and proteins abundance table were displayed. **(B)** RNA-EMSA was used to detect the binding between circMVP and METTL3 protein. **(C)** CircMVP expression inhibited the ubiquitin modification of METTL1. HCT8 cells were transfected with circMVP or METTL3 and treated with MG132 (20 mmol/mL) for 3 hours, the binding ubiquitin level was detected. **(D, E, F and G)** The combination of METTL3 and circMVP was detected by RIP, and the fluorescence signal of RNA was obtained by PCR amplification and then gel electrophoresis to identify the specificity of circMVP, respectively in DLD1, 293T, HCT8 and MC38. **(H)** The protein levels of METTL1 were measured in si-circMVP DLD1 cells by Western blot. Cells were treated with CHX (50 μ g/mL) for 3 hours. MS, Mass spectrum.

Figure S4. CircMVP/METTL3/ β -catenin regulates B7-H3 expression

(A) Transcriptome analysis differentially expressed genes in HCT8-circMVP compared with HCT8-NC in scatter plot. **(B)** KEGG demonstrated the β -catenin signaling pathway (<https://www.kegg.jp/pathway/map04310>). **(C)** GSEA plot demonstrated the Wnt/ β -catenin signaling pathway in HCT8-circMVP and HCT8-NC. **(D and E)** SRAMP predicted the sites and confidence intervals of Homo sapiens (CTNNB1) and mouse (Ctnnb1) mRNA modified by m6A, respectively (<http://www.cuilab.cn/sramp>). **(F)** Transcriptome of RNAseq analysis of basic expression levels of Ctnnb1 in mouse cell lines (GEO: GSE18398) and expression of β -catenin protein in mouse cell lines (B16, BMDM, MC38, 4T1 and CT26) was detected by PCR. **(G and H)** Transcriptome and qRT-PCT were used to analyze the effect of circMVP on downstream regulatory gene expression of β -catenin signaling pathway. **(I)** The effect of circMVP/METTL3 regulation on CTNNB1 transcription level was detected by qRT-PCR. KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene Set EnrichmentAnalysis; SRAMP, sequence-based RNA adenosine methylation site predictor.

Figure S5. Single cell sequencing of CircMVP MC38 mouse tumor model

(A) UMAP plot showed the clusters of scRNA-seq in tumor tissue of MC38 cells (NC vs. circMVP). Each dot is a cell colored by its analyzed cell types. **(B)** Feature plots showed tumor Epithelial cell signatures (Grb2 and Krt14) transcript levels in MC38-NC and MC38-circMVP. **(C)**

and D) Violin plots showed tumor Immune checkpoint signatures transcript levels in MC38-NC and MC38-circMVP. **(E)** GO pathway enrichment terms of the differentially expressed genes in CD8⁺ T cells of MC38-circMVP compared with CD8⁺ T cells of MC38-NC. **(F)** scRNA sequencing analysis. The expression levels of NK cells function markers *Klrd1*, *Gzmb* and *Tigit* infiltrated by MC38-circMVP were compared with those of MC38-NC. **(G)** The expression levels of CD276 in MC38-circMVP was compared with those of MC38-NC. **(H)** scRNA sequencing analysis. The expression levels of *Mettl3* and *Tthdf1* by MC38-circMVP were compared with those of MC38-NC. UMAP, Uniform Manifold Approximation and Projection; TAM, Tumor-associated macrophages; GO, Gene ontology.

Figure S6. β -catenin expression, METTL3 and B7-H3 survival analysis

(A) qRT-PCR were used to analyze the effect of circMVP on downstream regulatory gene expression of CD276; ChIP-seq analysis of β -catenin acts on heat maps of CD276 chromatin Peak distribution upstream and downstream of TSS. **(B)** Expression of circMVP in in vivo tumorigenic tissue detected by ISH. **(C)** IGV tracks showing m6A peaks distribution on CD276 transcript from MeRIP-seq data in PRJNA927821. **(D)** Expression of β -catenin proteins detected by IHC in CRC tissue. Scale bar, 60 μ m. **(E)** Kaplan-Meier survival plots for the correlation between the expression of METTL3, high= 50 and low = 112 with the overall survival (OS) in patients. **(F)** Kaplan-Meier survival plots for the correlation between the expression of B7-H3, high= 82 and low = 80 with the overall survival (OS) in patients. ChIP, Chromatin immunoprecipitation; TSS, Transcription start site.

Figure S1

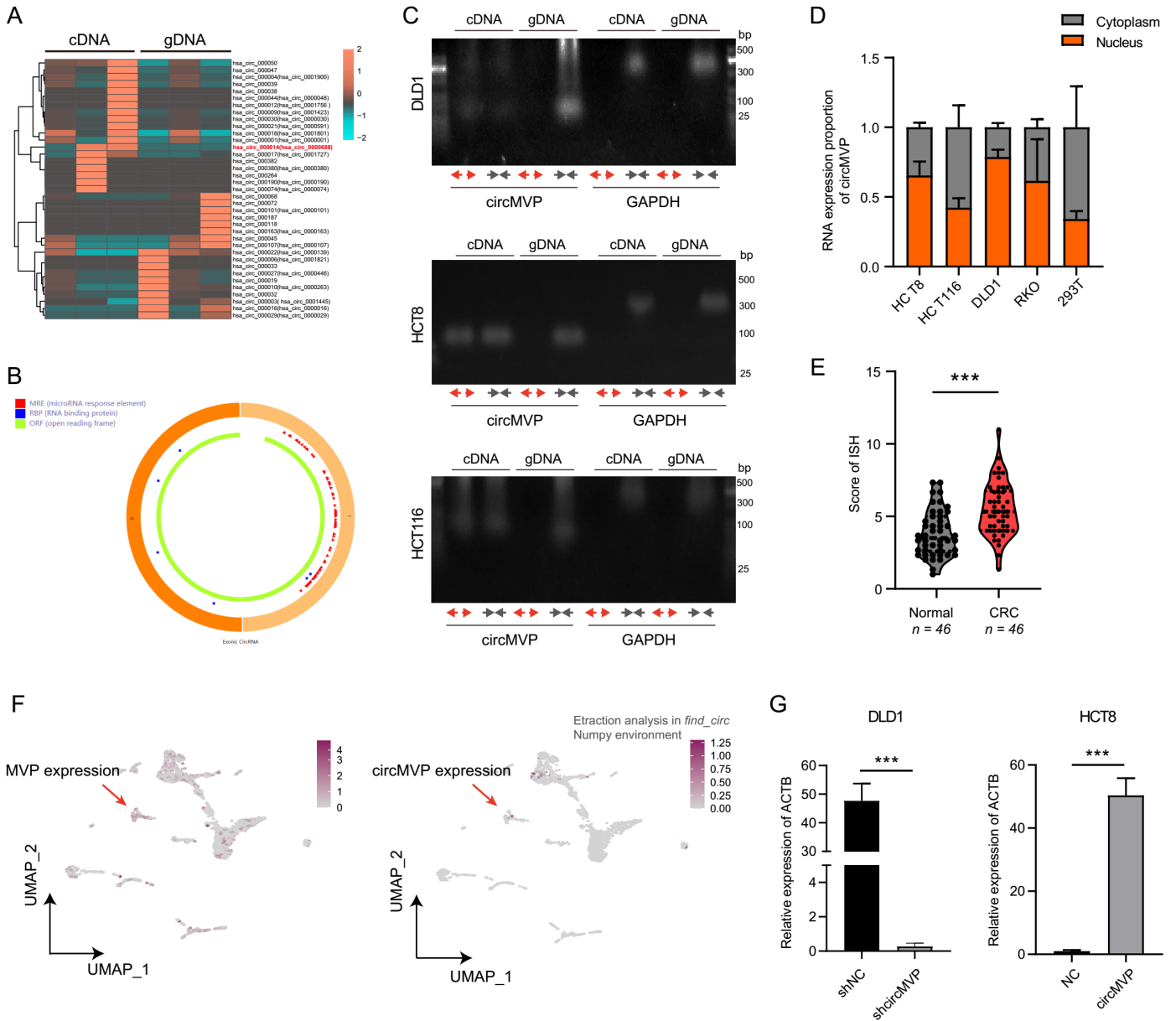


Figure S2

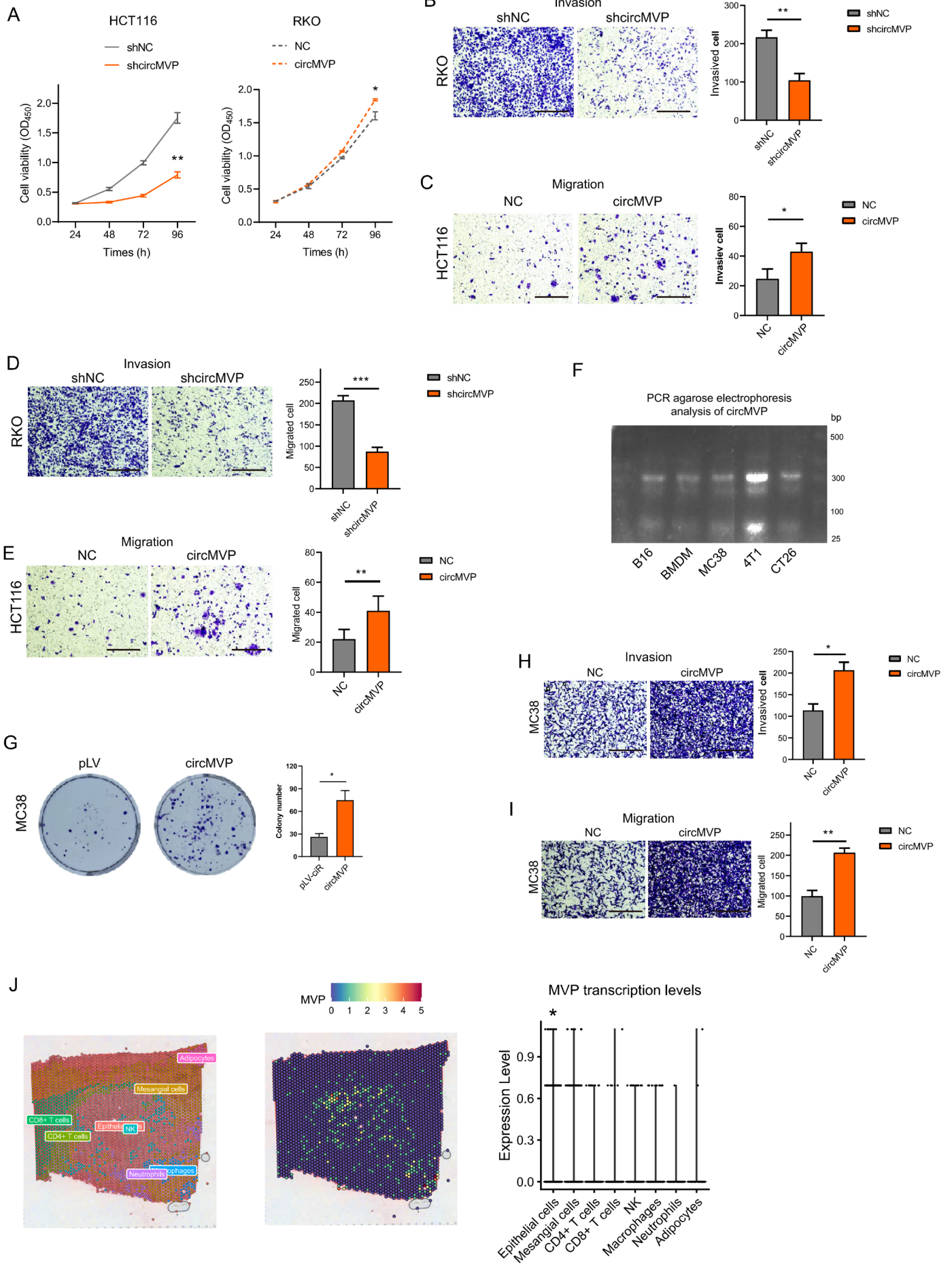
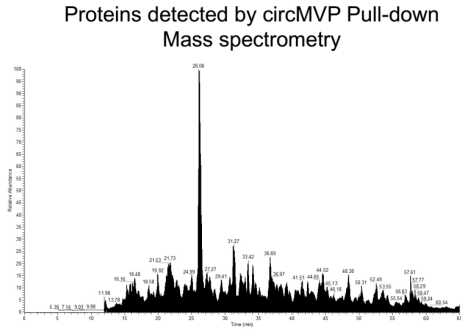


Figure S3

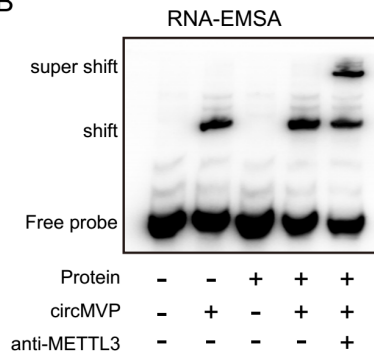
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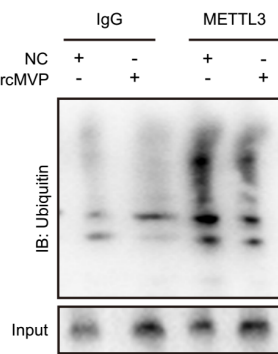
TOP15 proteins in MS

Protein FDR	Accession	Sum PEP Score	AAs	Molecular Function	Abundances
High	P35908	281.966	639	bone, tooth or skin structural activity;cytoskeletal activity	2571029694
High	P13645	279.866	584	bone, tooth or skin structural activity;other molecular function	4546927467
High	P68104	259.243	462	nucleic acid binding activity;other molecular function	9089267112
High	Q9UHB6	252.745	759	cytoskeletal activity;other molecular function	4591731775
High	P04264	247.527	644	bone, tooth or skin structural activity;other molecular function	6122395367
High	Q9Y5B9	235.682	1047	other molecular function	983139816.6
High	P19338	234.339	710	nucleic acid binding activity;other molecular function	15911389082
High	Q86U44	218.733	580	mRNA (2'-O-methyladenosine-N6)-methyltransferase activity; mRNA binding;other molecular function	356360533.1
High	P09429	213.081	215	signal transduction activity or receptor binding;nucleic acid binding activity;other molecular function	8005659883
High	P13639	209.514	858	translation activity;nucleic acid binding activity;other molecular function	812448307.4
High	Q9BUF5	205.62	446	cytoskeletal activity;other molecular function	101169641.3
High	P07355	197.655	339	transporter activity;enzyme regulator activity;cytoskeletal activity;other molecular function	1885017542
High	P35527	193.723	623	cytoskeletal activity	1475197347
High	P14618	191.383	531	nucleic acid binding activity;kinase activity;other molecular function	726779818.3
High	P02545	179.879	664	cytoskeletal activity;other molecular function	792568860.1

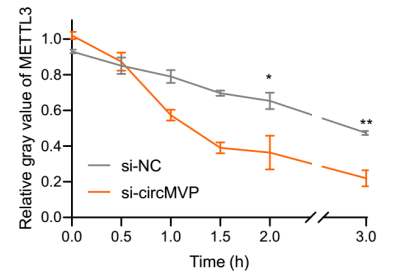
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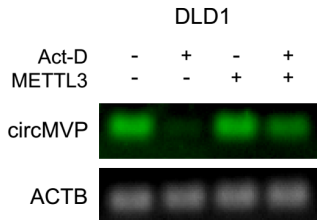
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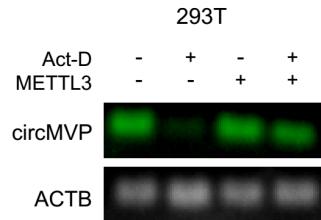
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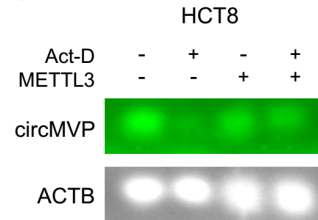
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F



G



H

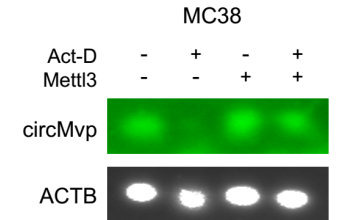


Figure S4

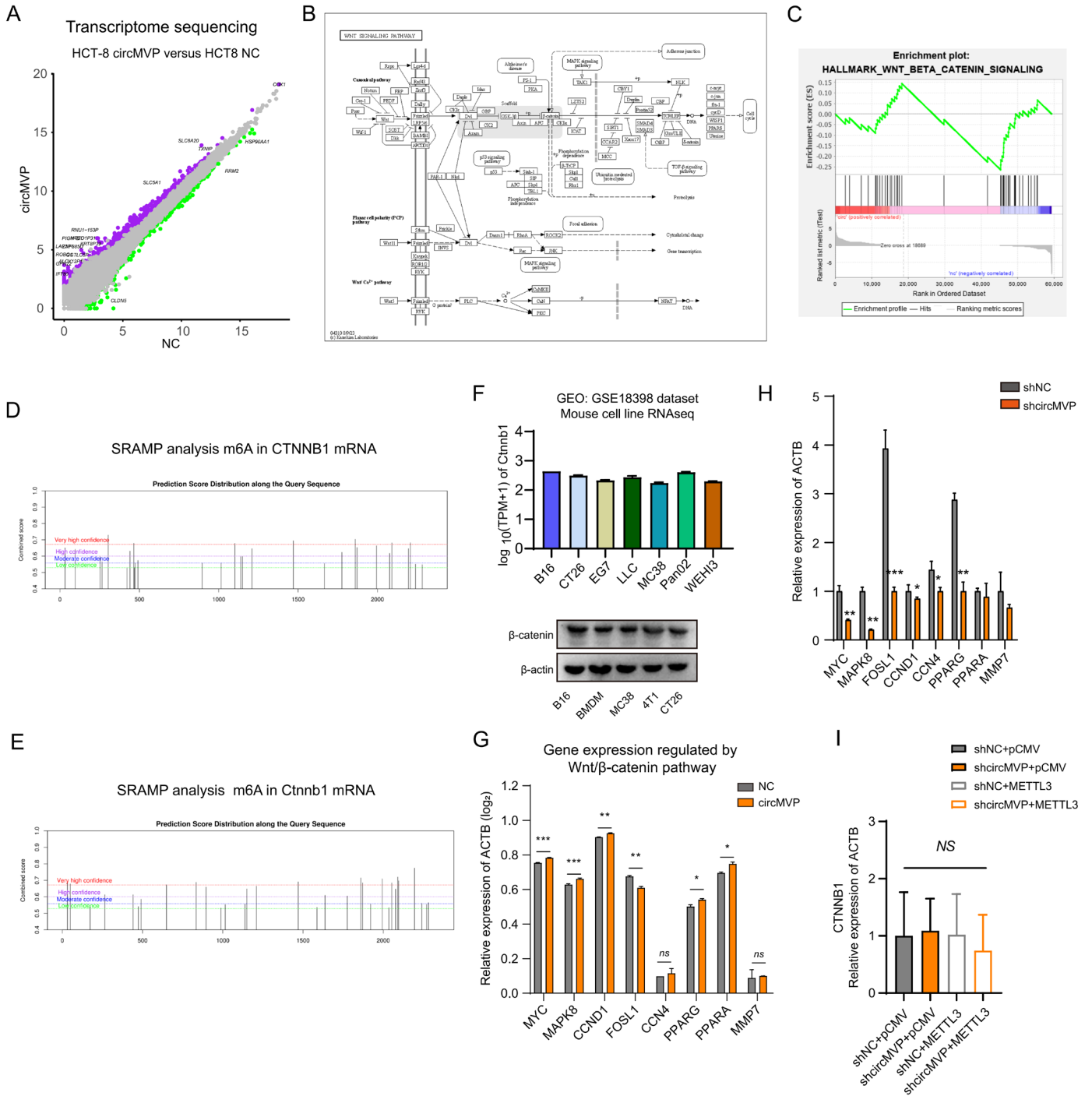


Figure S5

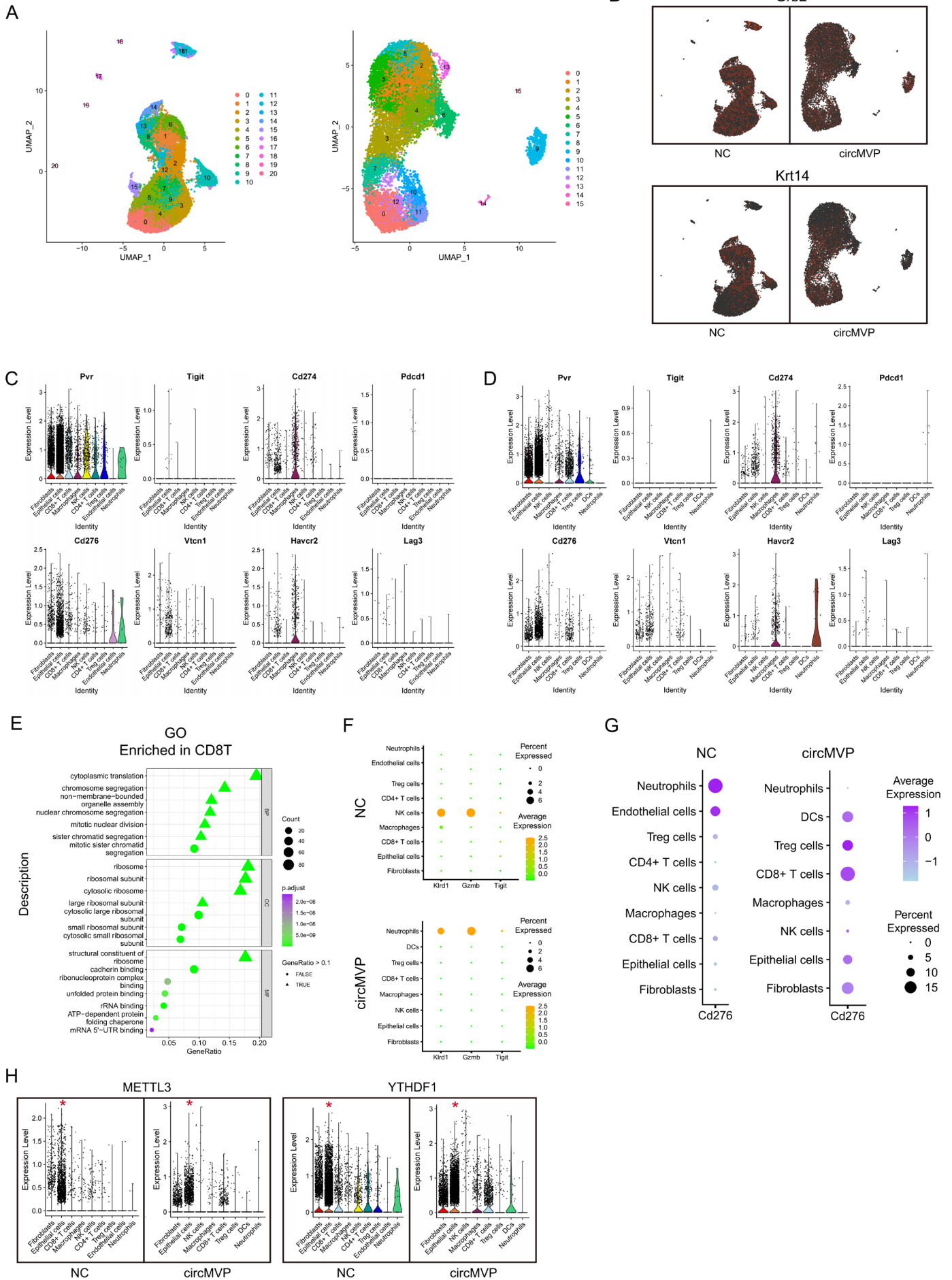


Figure S6

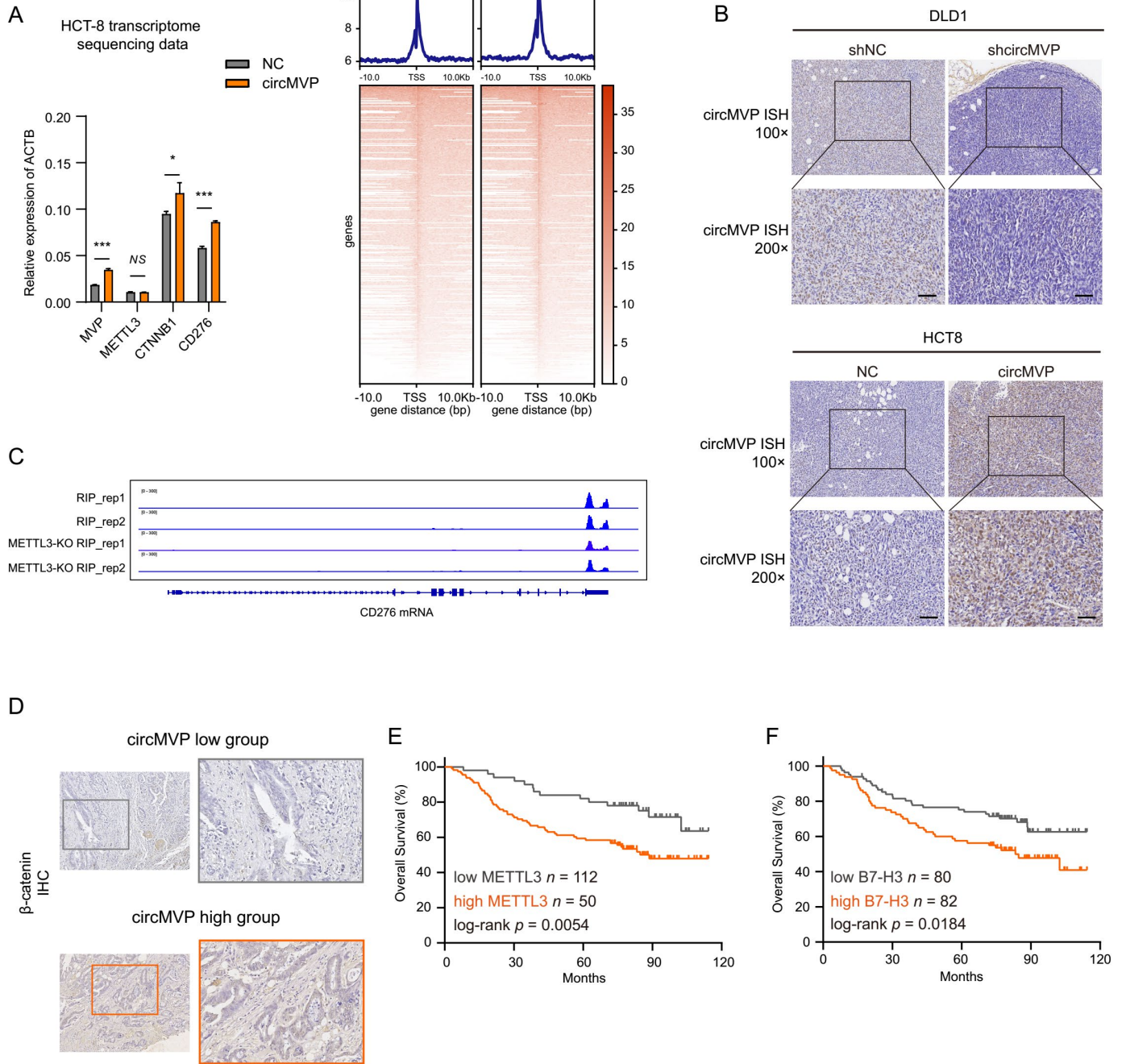


Table S1. Colorectal cancer pathological characteristics table of circMVP in Cohort of affiliated hospital of Jiangnan University.

Clinical parameters	ISH of circMVP		χ^2	<i>P</i> value
	Low (n=92)	High (n=70)		
Gender			0.096	0.756
Female	43(46.7%)	31(44.3%)		
Male	49(53.3%)	39(55.7%)		
Age			2.127	0.145
<60	42(45.7%)	24(34.3%)		
≥60	50(54.3%)	46(65.7%)		
Location			5.535	0.019*
Colon	28(30.4%)	34(48.6%)		
Rectum	64(69.6%)	36(51.4%)		
T stage			4.017	0.045*
T1-T2	30(32.6%)	13(18.6%)		
T3-T4	62(67.4%)	57(81.4%)		
N stage			7.685	0.006**
N0	57(62.0%)	28(40.0%)		
N1-N4	35(38.0%)	42(60.0%)		
M stage			13.285	<0.001***
M0	89(96.7%)	55(78.6%)		
M1	3(3.3%)	15(21.4%)		
METTL3			3.704	0.054
IHC score ≤3	34(37.0%)	16(22.9%)		
IHC score >3	58(63.0%)	54(77.1%)		
B7-H3			8.953	0.004**
IHC score ≤4	56(60.9%)	26(37.1%)		
IHC score >4	36(39.1%)	44(62.9%)		

Pearson χ^2 test or Fisher exact test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table S2. Primer and sequences.

Primers for PCR and real time PCR		
Primers	Sequences-F	Sequences-R
circMVP#1	5'-CCCGAAAGATCCATGGAGAGA-3'	5'-GGCTGGGTGGTGAATGTAGA-3'
circMVP#2	5'-AAGTGGAGGTCGTGGAGATC-3'	5'-CCACCACCTTGTCTCCATCT-3'
circMVP-co nvergent	5'-CTGTCACCCTCTCCTTGCC-3'	5'-ATGAGTGGCTTTTCGAGGGA-3'
MVP	5'-ATCGAAACGGCGGATCATGCCA-3'	5'-GGCATCACCTACAAAGTCTGGC-3'
β -actin (ACTB) for human	5'-AACTCCATCATGAAGTGTGACG-3'	5'-GATCCACATCTGCTGGAAGG-3'
β -actin (ACTB) for mouse	5'-CATTGCTGACAGGATGCAGAAGG- 3'	5'-TGCTGGAAGGTGGACAGTGAGG- 3'
GAPDH for human	5'-GTCTCCTCTGACTTCAACAGCG-3'	5'-ACCACCCTGTTGCTGTAGCCAA-3'
Gapdh for mouse	5'-CATCACTGCCACCCAGAAGACTG- 3'	5'-ATGCCAGTGAGCTTCCCGTTCAG- 3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTACGAATTTGCGT-3'
CTNNB1 for human	5'-CACAAGCAGAGTGCTGAAGGTG-3'	5'-GATTCCTGAGAGTCCAAAGACAG -3'
Ctnnb1 for mouse	5'-GTTTCGCCTTCATTATGGACTGCC-3'	5'-ATAGCACCTGTTCCCGCAAAG-3'
CD276 for human	5'-CTGGCTTTCGTGTGCTGGAGAA-3'	5'-GCTGTCAGAGTGTTTCAGAGGC-3 ,
Cd276 for mouse	5'-AGCATCCAGGACTTTGACAGCG-3'	5'-CGTGATGGTCACCATGTTCCCT-3'

CD276 for ChIP	5'-CTGGGAGCACTGTGGTTCT-3'	5'-TTGGTATCTGTCAGCTGCCA-3'
METTL3 for human	5'-CTATCTCCTGGCACTCGCAAGA-3'	5'-GCTTGAACCGTGCAACCACATC-3'
Mettl3 for mouse	5'-CAGTGCTACAGGATGACGGCTT-3'	5'-CCGTCCTAATGATGCGCTGCAG-3'
CTNNB1 for RIP	5'-CTCCTAGTGTTTGGCCTGGA-3'	5'-CGGTTCCATGCATACACAGG-3'
MYC	5'-CCTGGTGCTCCATGAGGAGAC-3'	5'-CAGACTCTGACCTTTTGCCAGG-3'
MAPK8	5'-GACGCCTTATGTAGTGA CT CGC-3'	5'-TCCTGGAAAGAGGATTTTGTGGC-3'
FOSL1	5'-GGAGGAAGGAACTGACCGACTT-3'	5'-CTCTAGGCGCTCCTTCTGCTTC-3'
CCND1	5'-TCTACACCGACA ACTCCATCCG-3'	5'-TCTGGCATTTTGGAGAGGAAGTG-3'
CCN4	5'-AAGAGAGCCGCCTCTGCAACTT-3'	5'-TCATGGATGCCTCTGGCTGGTA-3'
PPARG	5'-AGCCTGCGAAAGCCTTTTGGTG-3'	5'-GGCTTCACATTCAGCAAACCTGG-3'
PPARA	5'-TCGGCGAGGATAGTTCTGGAAG-3'	5'-GACCACAGGATAAGTCACCGAG-3'
MMP7	5'-TCGGAGGAGATGCTCACTTCGA-3'	5'-GGATCAGAGGAATGTCCCATACC-3'
Sequences for gene knockdown		
Si-circMVP #1	GGAUAAAGAUGGAGACAAG	CUUGUCUCCAUCUUUAUCC
Si-circMVP #2	GGAGAGGGUGACAGGACAU	AUGUCCUGUCACCCUCUCC
sh-circMVP #1	GGATAAAGATGGAGACAAG	CTTGTCTCCATCTTTATCC
sh-circMVP	GGAGAGGGTGACAGGACAT	ATGTCCTGTCACCCTCTCC

#2		
Si-METTL3	CAGUGGAUCUGUUGUGAUAUC	UAUCACAACAGAUCCACUGAG
Gene sequences		
circMVP	GACATCACACCCCTGCAGGTGGTTCTGCCCAACACTGCCCTCCATCTAAAGG CGCTGCTTGATTTTGAGGATAAAGATGGAGACAAGGTGGTGGCAGGAGATGA GTGGCTTTTCGAGGGACCTGGCACGTACATCCCCGGAAGGAAGTGGAGGTC GTGGAGATCATT CAGGCCACCATCATCAGGCAGAACCAGGCTCTGCGGCTCA GGGCCCCGAAGGAGTGCTGGGACCGGGACGGCAAGGAGAGGGTGACAG	
circMVP Probe	5'-Cy3/Biotin-TGTGATGTCCTGTCACCCTCT-3'	

Table S3. The detailed antibody information used in this study.

Antibodies	Source	Cat#
Anti- β -actin	Cell Signaling Technology	Cat#3700
Anti-METTL3 (Rabbit)	Cell Signaling Technology	Cat#86132
Anti-METTL3 (Mouse)	Proteintech	Cat#67733-1-Ig
Anti-YTHDF1	Cell Signaling Technology	Cat#57530
Anti-B7-H3	Cell Signaling Technology	Cat#14058
Anti- β -catenin (Rabbit)	Cell Signaling Technology	Cat#8480
Anti- β -catenin (Mouse)	Proteintech	Cat#66379-1-Ig
Anti-m6A	Cell Signaling Technology	Cat#56593
Anti-Histone 3	Cell Signaling Technology	Cat#4499
Methylene blue	Sigma-Aldrich	Cat#M9140
Phalloidin	Beyotime	Cat#25682-1-AP
Omburtama	Selleck	Cat#1895083-75-6
Mouse IgG1	Selleck	Cat#MOPC-21
Anti-Mouse CD16/CD32, APC	MultiScience	Cat#F210163203
FoxP3/Transcription Factor Staining Buffer Kit	MultiScience	Cat#IC001
Anti-CD326 (EpCAM) Monoclonal, PE, eBioscience™	Invitrogen	Cat#12-9326-42
Anti-Mouse CD45, FITC	MultiScience	Cat#F2104501
Anti-Mouse CD3 ϵ , PE	MultiScience	Cat#F2100302
Anti-Mouse CD4, APC	MultiScience	Cat#F2100403
Anti-Mouse CD8 α , FITC	MultiScience	Cat#F2100801
Anti-Mouse NK1.1(CD161), PE-Cy7	MultiScience	Cat#F2116105
Anti-Human/Mouse CD11b, PerCP-Cy5.5	MultiScience	Cat#F41011b04
Anti-Mouse F4/80, APC	MultiScience	Cat#F21480A03
Anti-CD206/MRC1 Rabbit mAb (Alexa Fluor® 594)	MultiScience	Cat#59414

eBioscience™ Fixable Viability Dye	Invitrogen	Cat#65-2860-40
Anti-mouse-IgG-HRP for WB	Promega	Cat#W4021
Anti-rabbit-IgG-HRP for WB	Promega	Cat#W4011
Cy3-labeled Goat Anti-Mouse IgG (H+L)	Beyotime	Cat#A0521
Cy3-labeled Goat Anti-Rabbit IgG (H+L)	Beyotime	Cat#A0516
FITC-labeled Goat Anti-Mouse IgG (H+L)	Beyotime	Cat#A0568
FITC-labeled Goat Anti-Rabbit IgG (H+L)	Beyotime	Cat#A0562
Anti-mouse-IgG-HRP for IHC	Beyotime	Cat#A0216
Anti-rabbit-IgG-HRP for IHC	Beyotime	Cat#A0208
Multiplex Fluorescence IF Staining Kit	ABSIN	Cat#abs50013
DAB detection kit	ABSIN	Cat#abs9210
ChIP or CUT&RUN Assay Kit	Vazyme	Cat#HD101

Table S4. Differentially expressed circRNAs in Colorectal cancer.

circBASE ID	circBANK ID	logFC	P.Value	Regulation
hsa_circ_000014		1.271174896	0.038990425	UP
hsa_circ_000050		0.754344802	0.045015071	UP
hsa_circ_000017		1.123316537	0.089556881	UP
hsa_circ_000018		0.526715161	0.129154697	UP
hsa_circ_000047		0.74216951	0.137060076	UP
hsa_circ_000030	hsa_circ_0000030	1.135330787	0.209592288	UP
hsa_circ_000004		0.602451641	0.219659551	UP
hsa_circ_000021		0.744220252	0.222333938	UP
hsa_circ_000009		0.528320834	0.226712002	UP
hsa_circ_000380	hsa_circ_0000380	1.092661582	0.236663442	UP
hsa_circ_000044		0.754344802	0.241032831	UP
hsa_circ_000001	hsa_circ_0000001	0.62917509	0.242771623	UP
hsa_circ_000012		0.654491375	0.24374094	UP
hsa_circ_000039		0.356796443	0.244314542	UP

hsa_circ_000038		0.528320834	0.24951087	UP
hsa_circ_000264		0.459503874	0.254739529	UP
hsa_circ_000382		0.356796443	0.268399785	UP
hsa_circ_000190	hsa_circ_0000190	0.255178249	0.298624688	UP
hsa_circ_000074	hsa_circ_0000074	0.255178249	0.298624688	UP
hsa_circ_000022		-0.635630199	0.060452328	DOWN
hsa_circ_000016	hsa_circ_0000016	-0.868023775	0.061351332	DOWN
hsa_circ_000029	hsa_circ_0000029	-1.101016695	0.066824723	DOWN
hsa_circ_000010	hsa_circ_0000263	-0.816300317	0.087858824	DOWN
hsa_circ_000003		-0.489828428	0.095842268	DOWN
hsa_circ_000032		-1.137593842	0.099286803	DOWN
hsa_circ_000068		-0.836987306	0.21413584	DOWN
hsa_circ_000027		-0.615998969	0.218970453	DOWN
hsa_circ_000045		-0.512017633	0.232109455	DOWN
hsa_circ_000019		-0.40054462	0.237044391	DOWN
hsa_circ_000006	hsa_circ_0001821	-0.712501175	0.242032814	DOWN
hsa_circ_000033		-0.477653136	0.253149432	DOWN
hsa_circ_000072		-0.379167841	0.264530021	DOWN
hsa_circ_000107	hsa_circ_0000107	-0.421011469	0.266238623	DOWN
hsa_circ_000118		-0.308666473	0.27944487	DOWN
hsa_circ_000163	hsa_circ_0000163	-0.308666473	0.27944487	DOWN
hsa_circ_000187		-0.282665636	0.287588805	DOWN
hsa_circ_000101	hsa_circ_0000101	-0.282665636	0.287588805	DOWN
