

Supplementary Tables

Table S1 The clinical data of 6 pairs CRC tissues from patients

	GDX	DX	CDZ	QXQ	XCZ	ZGT
Gender	Male	Female	Male	Male	Female	Male
Age	55	49	67	86	77	55
Tumor types	Rectal cancer	Rectal cancer	Rectal cancer	Colon cancer	Colon cancer	Rectal cancer
Pathological type	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma
Differentiation degree	Middle	Middle	Middle	Middle	Middle	Middle
TNM stage	T2N1M0	T2N1M0	T3N2M0	T2N2M0	T3N1M0	T2N1M0
Tumor size	4*3*1	2.5*2.5*0.5	2.5*2*0.5	4.6*3.5*1.7	1.7*1.5*1.3	3*3*1
Lymph node metastasis	Yes	Yes	Yes	Yes	Yes	Yes
Distant metastasis	No	No	No	No	No	No

Table S2 The short reverse complementary sequences in circ1662 flank sequence

Score	Expect	Identities	Gaps	Strand
21.1 bits(22)	4.4	11/11(100%)	0/11(0%)	Plus/Minus
Query 717	ATACAGGTTTC	727		
Sbjct 206	ATACAGGTTTC	196		

Score	Expect	Identities	Gaps	Strand
21.1 bits(22)	4.4	11/11(100%)	0/11(0%)	Plus/Minus
Query 1634	GTATGTTGGCA	1644		
Sbjct 1760	GTATGTTGGCA	1750		

Score	Expect	Identities	Gaps	Strand
22.9 bits(24)	1.3	12/12(100%)	0/12(0%)	Plus/Minus
Query 1758	TAGTAAATAATT	1769		
Sbjct 2091	TAGTAAATAATT	2080		

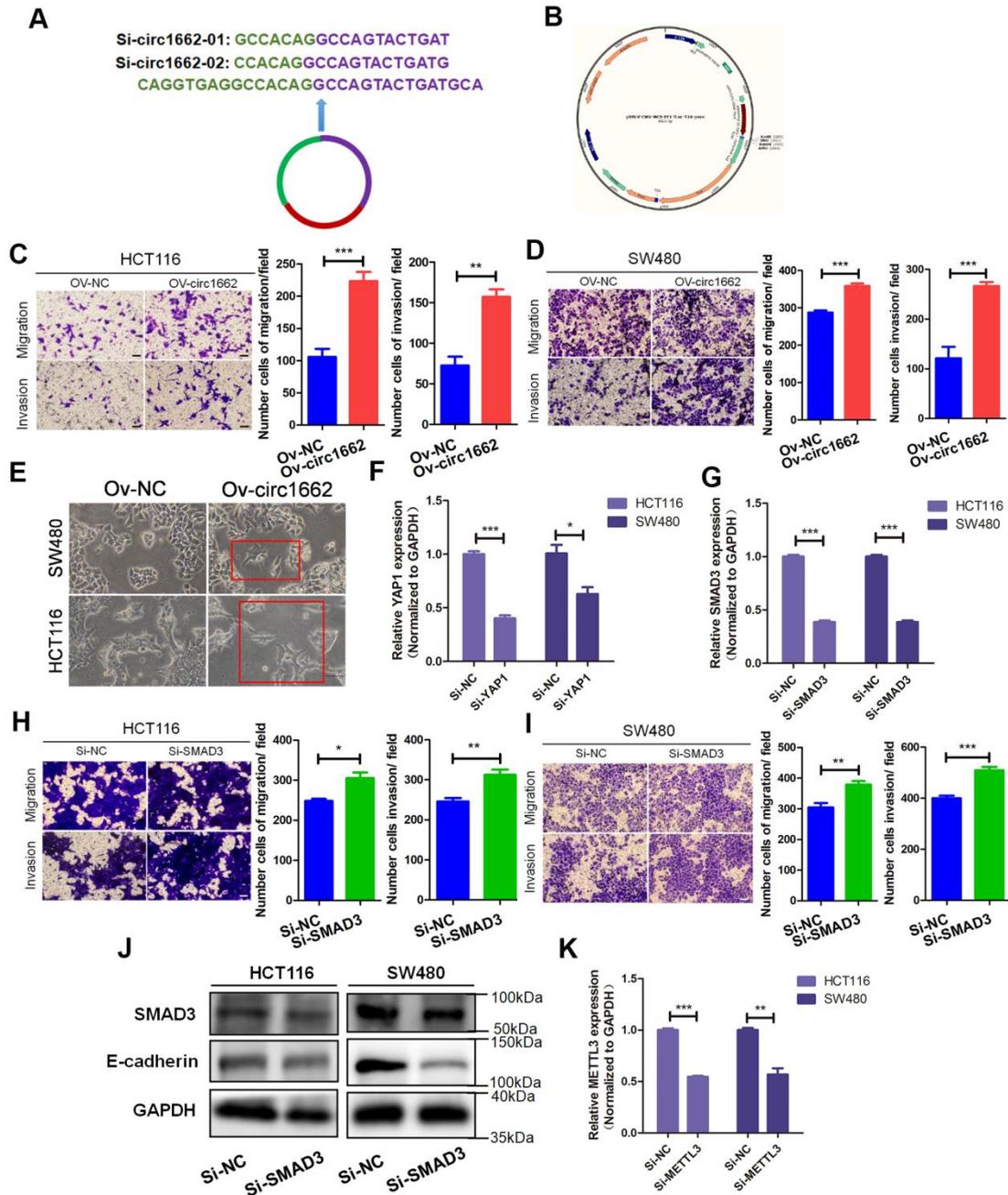
Score	Expect	Identities	Gaps	Strand
21.1 bits(22)	4.4	11/11(100%)	0/11(0%)	Plus/Minus
Query 1836	AAGTTTTTAAC	1846		
Sbjct 3246	AAGTTTTTAAC	3236		

SGK1	TTGACCCCGAGTTTACCGAA	TTGACGCTGGCTGTGACGAG
SOCS3	CCCCCAGAAGAGCCTATTACA	TCCGACAGAGATGCTGAAGAGTG
TGFBR1	CTGTGAAGCCTTGAGAGTAATG	CCTGTTGACTGAGTTGCGATA
GAPDH	AACGGATTTGGTCGTATTGG	TTGATTTTGGAGGGATCTCG
circ1662-convergent	AGCCCTGACTCCACAGCATG	GACCAGAAGATGTCTTTGCC
circ1662-intron-upstream	GTTCTTTCACCCCTGCTC	TTAGTCATCGCTTCCCAA
circ1662-intron-downstream	ACACCTGTAATCTCAACTTCTC	ACTATGCCTACTTTCCCCA
1663-up-03	GAGGTGGGGGTAGAACCG	GCGTTTCAGCCGACTGTAAA
1662-up-06	ACAAAAACCCGGGTAAAGGA	AACTGCTGGATATAAAATCTTGT
1662-up-07	ATTCATAAACTATTATAGTTTTGT	GTATTTACAACCTAGATTTG
1662-up-08	CACAACACAAACTGGTGACTG	AAGCCATCTACAATGAACAGGAT
1662-up-09	GAACATTTGGGCAGTTCAAA	TTAAGAGGTCAGTGCAGTTAT
1662-up-10	TAGTTGCCCTTACATGTCTA	CTATTAGGGAATATTAAGATG
1662-down-01	TAGAAACCAGCCTTCCACTT	GCAATCCACAATCCATGTAC
1662-down-04	AGAAGGTACTGTTGGTTTT	AAGAAACTCATAGTTATTTAGGC
1662-down-06	TAGGGCTGTATTTTAAAAGTATC	TGGAGTATCATTITCTGAGC
1662-down-07	ATGGTTCCTTGACAAACTAAG	GGTTAGTAAATAATTCTCTTTGG
1662-down-09	TATCTGACATAAAAAAGGATG	AAGAACTTTTTTTTAGTTTC
1662-down-11	CATACTAATAGACTATTTTTAG	CATGGTCTGGCTGTGTAGG

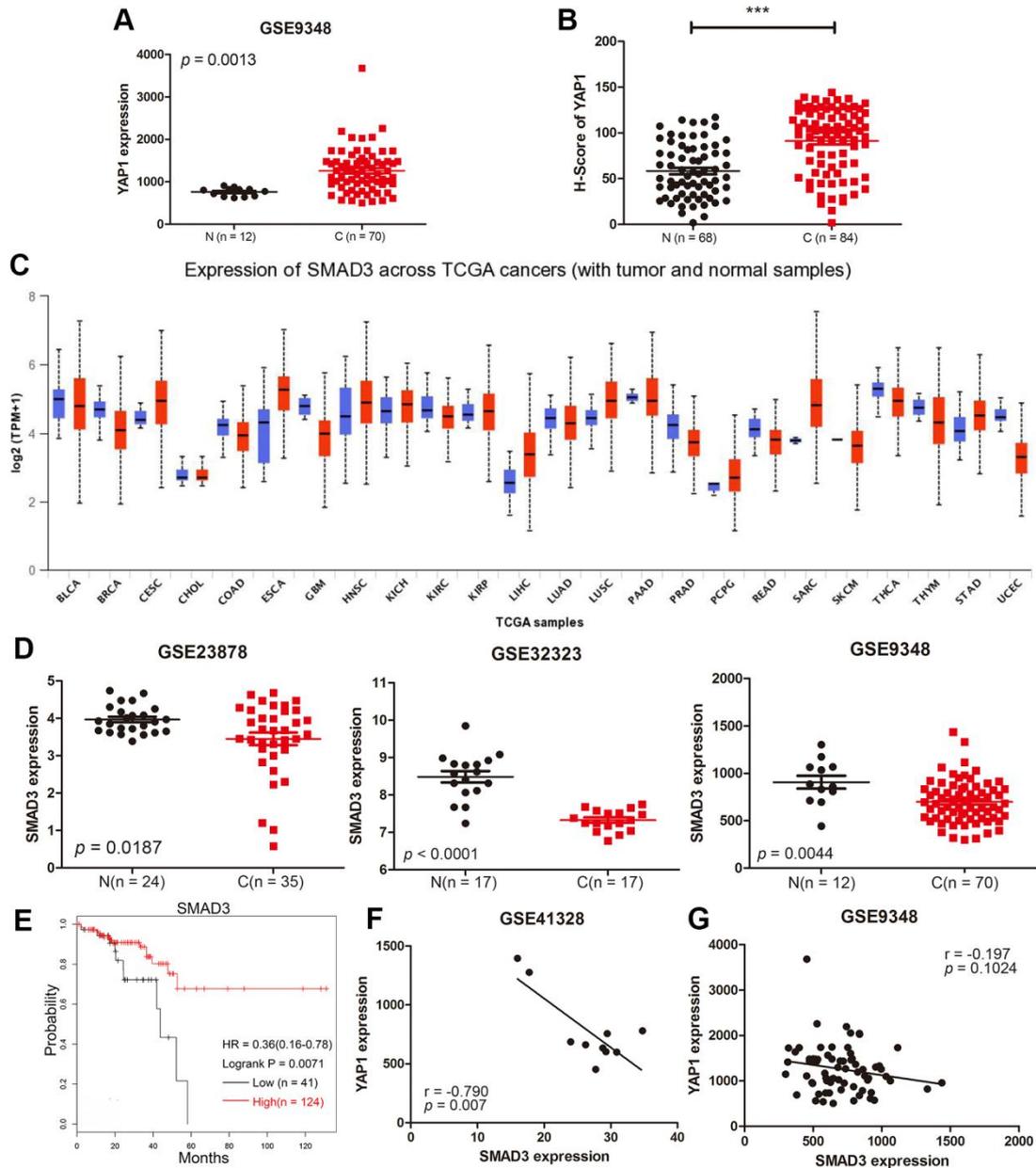
siRNA	Target sequence
si-circ1662-01	GCCACAGGCCAGTACTGAT
si-circ1662-02	CCACAGGCCAGTACTGATG
si-YAP1	CAGTGGCACCTATCACTCT
si-METTL3	GCTGCACTTCAGACGAATT
si-SMAD3	GAGGCTGTCTACCAGTTGA

Gene	Probe sequence
Circ1662(ISH)	5'-DIG-ATCAGTACTGGCCTGTGGCCTCACCTG-DIG-3'
Circ1662(FISH)	5' Cy3-AGTACTGGCCTGTGGCCTCACCT
Circ1662(Pull down)	5'-ATCAGTAC+TGGCCTGTGGCC+TCACCTGC-3'
NC (Pull down)	5'-GCTACGCAGCCGTCTCTCGTATGTACCG-3'

Supplementary figures



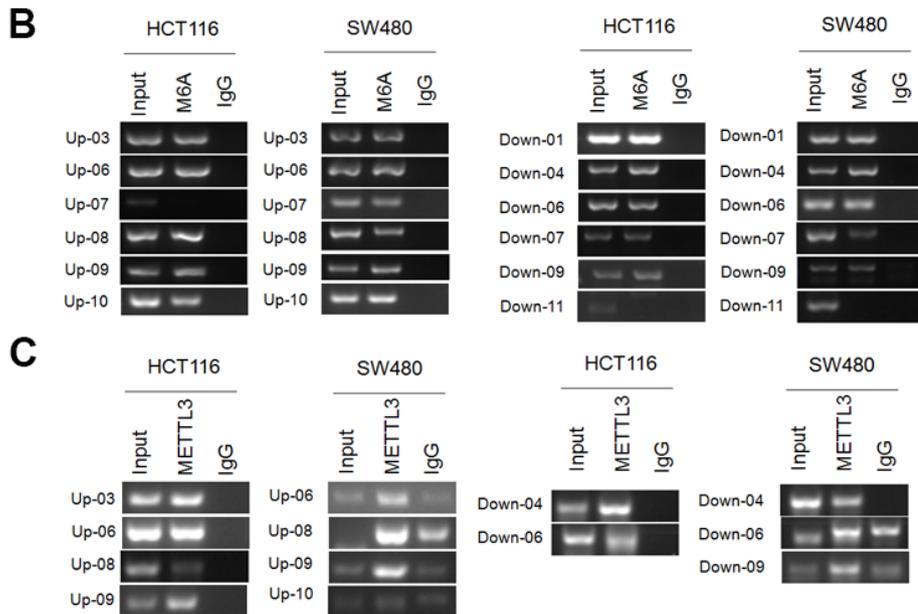
Supplementary Figure 1. (A) The design of si-circ1662 which was across the junction site. (B) Overexpressed vector of circ1662. (C, D) Transwell assay of HCT116 and SW480 transfected circ1662 vector for evaluating migration and invasion. The number of migration and invasion was counted in HCT116 and SW480. (E) The cell morphology of HCT116 and SW480 transfected circ1662 overexpression plasmids after 72h. The effect of YAP1 (F) and SMAD3 (G) siRNA in HCT116 and SW480 using qPCR analysis. (H, I) Transwell assay of HCT116 and SW480 transfected SMAD3 siRNA. The number of migration and invasion was counted in HCT116 and SW480. (J) Western blot analysis of SMAD3 and E-cadherin in HCT116 and SW480 transfected SMAD3 siRNA. (K) The effect of METTL3 siRNA in HCT116 and SW480 using qPCR analysis. GAPDH is the negative control. Student's t test was used to determine the *P* value (**P* < 0.05, 0.001 < ***P* < 0.01, ****P* < 0.001), Mean ± SEM.



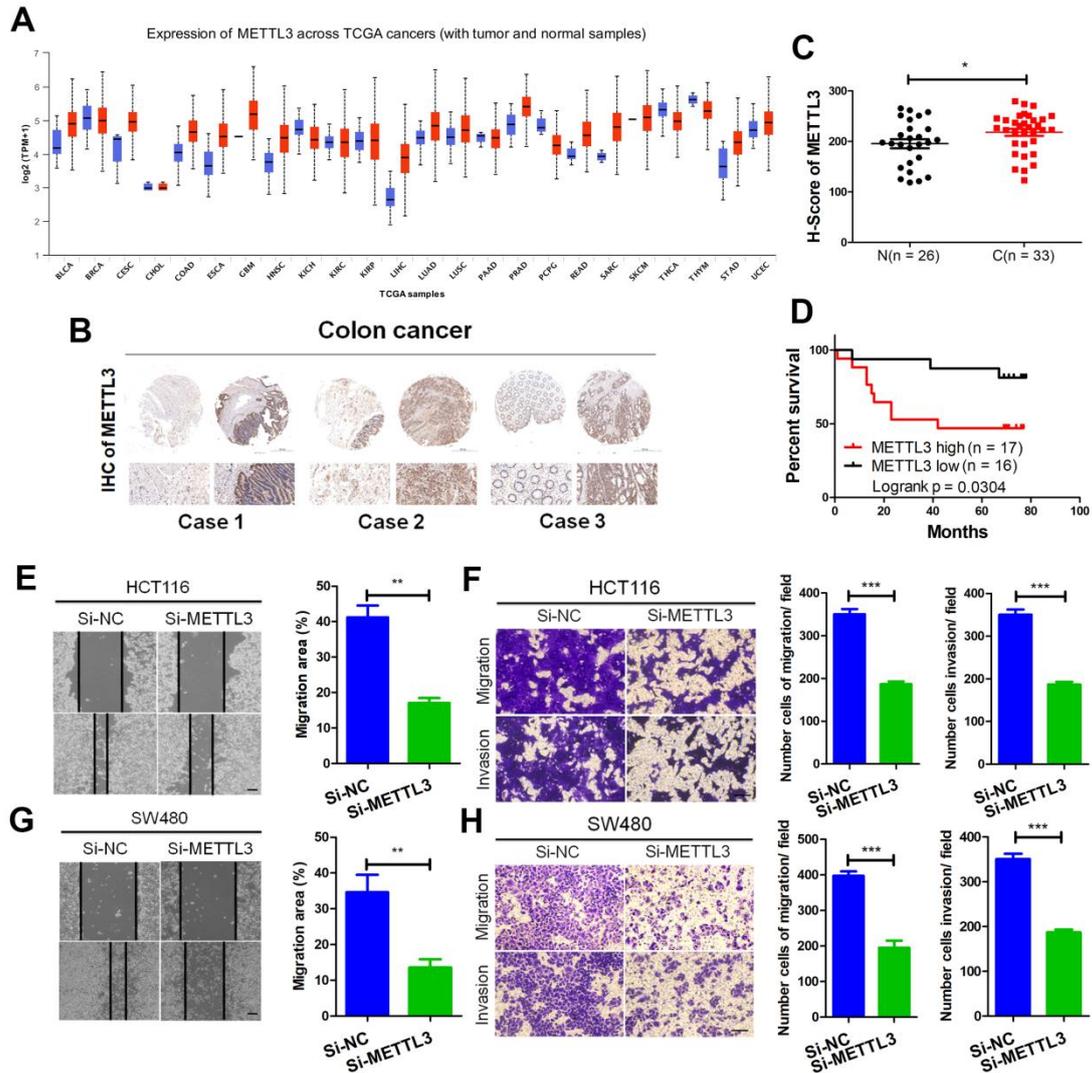
Supplementary Figure 2. (A) GEO data (GSE9348) analyzing SMAD3 expression in CRC tissues (n = 12) compared normal tissues (n = 70). (B) H-score of YAP1 in 54 pairs CRC tissuea compared to normal tissues from tissue chip. (C) UALCAN showing the pan-cancer of SMAD3 expression which data from TCGA. (D) GEO data (GSE23878) analyzing SMAD3 expression in CRC tissues (n = 35) compared normal tissues (n = 24). GEO data (GSE32323) analyzing SMAD3 expression in CRC tissues (n = 17) compared normal tissues (n = 17). GEO data (GSE9348) analyzing SMAD3 expression in CRC tissues (n = 12) compared normal tissues (n = 70). (E) Survival analysis of high SMAD3 expression compared to low SMAD3 expression in rectal cancer via KM-plotter online tool. Then it was assessed by log-rank test. (F, G) GEO data (GSE41328, GSE9348) analyzing the correlation between YAP1 and SMAD3 in CRC tissues. Student's t test was use to determined the *P* value (**P* < 0.05, 0.001 < ** *P* < 0.01, *** *P* < 0.001), Mean ± SEM.

A

RepeatMasker Information	RepeatMasker Information	RepeatMasker Information
Name: MIRc (link requires registration) Family: MIR Class: SINE SW Score: 288 Divergence: 33.3% Deletions: 10.7% Insertions: 0.0% Begin in repeat: 17 End in repeat: 147 Left in repeat: 121 Position: chr11:102113332-102113448 Band: 11q22.1 Genomic Size: 117 Strand: -	Name: AluSz6 (link requires registration) Family: Alu Class: SINE SW Score: 2057 Divergence: 13.8% Deletions: 1.4% Insertions: 0.0% Begin in repeat: 1 End in repeat: 293 Left in repeat: 19 Position: chr11:102206564-102206852 Band: 11q22.1 Genomic Size: 289 Strand: +	Name: FLAM_C (link requires registration) Family: Alu Class: SINE SW Score: 1029 Divergence: 10.7% Deletions: 0.0% Insertions: 0.0% Begin in repeat: 1 End in repeat: 131 Left in repeat: 12 Position: chr11:102207410-102207540 Band: 11q22.1 Genomic Size: 131 Strand: +



Supplementary Figure 3. The sequence alignment and qPCR production identified by agarose gel electrophoresis. (A) The up-and down flank sequences of circ1662 were aligned in the UCSC Genome Browser. MIRc element, MIR family; AluSz6 and FLAM C elements, Alu family. (b) Circ1662-up-03, 06, 07, 08, 09, 10 and circ1662-down-01, 04, 06, 07, 09, 11 in HCT116 and SW480 were detected by MeRIP-qPCR. (c) Circ1662-up-03, 06, 08, 09 and circ1662-down-04, 06 were pulled down by METTL3 antibody using RIP-qPCR in HCT116. Circ1662-up-06, 08, 09, 10 and circ1662-down-04, 06, 09 were pulled down by METTL3 antibody using RIP-qPCR in SW480. Input group was the positive control and IgG group was the negative control.



Supplementary Figure 4. The role of METTL3 in CRC (A) UALCAN showing the pan-cancer of METTL3 expression which data from TCGA. (B) IHC image of METTL3 expression in CRC tissue chip. (C) H-score of METTL3 in CRC tissues (n = 26) compared to normal tissues (n = 33) from tissue chip. (D) Survival analysis of high METTL3 expression compared to low METTL3 expression in CRC tissues from CRC tissue chip. Then it was assessed by log-rank (Mantel-Cox) test. (E, F) The migrated and invasive ability of HCT116 transfected METTL3 siRNA using wound healing assays and transwell assays. The migration area was counted by image J. (G, H) The migrated and invasive ability of SW480 transfected METTL3 siRNA using wound healing assays and transwell assays. The migration area was counted by image J. Statistical significance was calculated by Student's t test, * $P < 0.05$, $0.001 < ** P < 0.01$, *** $P < 0.001$, Mean \pm SEM.