

## Supplemental Tables

**Tables S1a: Correlation analysis for clinicopathologic variables in YTHDF1 expression among 383 colorectal cancer patients**

Variable	YTHDF1 expression		<i>P</i> *
	Low, 202 (52.7%)	High, 181 (47.3%)	
Age, years			.752
≤ 60	125 (32.6)	115 (30.1)	
> 60	77 (20.1)	66 (17.2)	
Gender			.917
Male	121 (31.6)	107 (27.9)	
Female	81 (21.1)	74 (19.3)	
Clinical stage			.001
I, II	95 (24.8)	53 (13.8)	
III, IV	107 (27.8)	128 (33.4)	
Degree of differentiation			.163
Well/moderate	175 (45.7)	147 (38.4)	
Poor	27 (7.0)	34 (8.9)	
Tumor depth			.021
m/sm/mp	25 (6.5)	10 (2.6)	
ss/se/si	177 (46.2)	171 (44.6)	
Vascular invasion			.011
Absent	166 (43.3)	128 (33.4)	
Present	36 (9.4)	53 (13.8)	
Perineural invasion			.123
Absent	121 (31.6)	94 (24.5)	
Present	81 (21.1)	87 (22.7)	

\**P* values determined by Chi-square test using SPSS 20.0. All statistical tests were two-sided. m: tumor invasion of mucosa; sm: submucosa; mp: muscularis propria; ss: subserosa; se: serosa penetration; si: invasion to adjacent structures.

**Table S1b: Univariate and multivariate analyses of prognostic factors for overall survival among 383 colorectal cancer patients.**

Factors	Univariate*		Multivariate*, †, ‡	
	HR (95% CI)	<i>P</i> *	HR (95% CI)	<i>P</i> *
Age (≤60/>60)	1.650 (1.103-2.469)	.015	1.870 (1.244-2.811)	.003
Gender (male/female)	0.605 (0.391-0.936)	.025	/	/
TNM stage (I-II/III-IV)	3.487 (2.061-5.900)	<.001	2.080 (1.186-3.650)	.011
Histological grade (well, moderate/poor)	1.774 (1.101-2.857)	.019	/	/
Tumor depth (m, sm, mp/ss, se, si)	1.373 (0.636-2.965)	.420	/	/
Vascular invasion (absent/present)	3.265 (2.171-4.910)	<.001	2.192 (1.421-3.382)	<.001
Perineural invasion (absent/present)	2.185 (1.449-3.297)	<.001	1.714 (1.123-2.619)	0.013
YTHDF1 Expression (low/high)	6.431 (3.862-10.711)	<.001	6.420 (3.819-10.792)	<.001

\* Hazard ratios and *P* values were obtained from Cox proportional hazards regression. All statistical tests were two-sided. † For the multivariate model, HR and *P* values were shown for the final set of stepwise selected variables only. ‡ The parameters with *P* value less than .05 in the univariate were included in the multivariate Cox analysis using SPSS 20.0. CRC: Colorectal cancer; m: tumor invasion of mucosa; sm: submucosa; mp: muscularis propria; ss: subserosa; se: serosa penetration; si: invasion to adjacent structures. HR: hazard ratio; CI: confidence interval.

**Table S2. Antibodies used in this study.**

Antibodies	Dilution	Company/Catalog
YTHDF1	WB (1:1000)	LSBio, LS-C660672
	IHC (1:250)	LSBio, LS-C660672
	RIP (5 µg/assay)	LSBio, LS-C660672
m <sup>6</sup> A	Dot blot (1:500)	Abcam, ab151230
	MeRIP (5 µg/assay)	SYSY, 202003
5-mC	Dot blot (1:1000)	CST, 28692S
H3	WB (1:2000)	CST, 4499T
H3K4me3	WB (1:1000)	CST, 9751T
H3K9me3	WB (1:1000)	CST, 13969T
H3K27me3	WB (1:1000)	CST, 9733T
CD8 human	IHC (1:500)	Abcam, ab 178089
	FCM (5 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 344721
CD8 mouse	IHC (1:500)	Abcam, ab217344
	FCM (2 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 100744
PD-L1 human	WB (1:1000)	Abcam, ab213524
	FCM (5 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 329707
PD-L1 mouse	WB (1:1000)	Abcam, ab213480
	FCM (1 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 124311
VISTA human	WB (1:1000)	CST, 64953
	FCM (5 µL/10 <sup>6</sup> cells in 100 µL)	Thermo Fisher, 17-1088-41
VISTA mouse	WB (1:1000)	CST, 54979
	FCM (5 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 143709
METTL3	WB (1:1000)	Abcam, ab195352
METTL14	WB (1:1000)	Abcam, ab252562
GAPDH	WB (1:20000)	Proteintech, 60004-1-Ig
CD3 human	FCM (5 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 300430
CD3 mouse	FCM (1 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 109249
CD45 human	FCM (5 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 304007
CD45 mouse	FCM (1 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 103132
Zombie Aqua	FCM (1:500)	Biolegend, 423102
IFN-γ human	FCM (5 µL /10 <sup>6</sup> cells in 100 µL)	Biolegend, 502531
IFN-γ mouse	FCM (0.5 µL /10 <sup>6</sup> cells in 100 µL)	Biolegend, 505830
GZMB	FCM (5 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 372208
Ki67 mouse	FCM (1 µL/10 <sup>6</sup> cells in 100 µL)	Biolegend, 652406

**Table S3. List of oligonucleotides and primers used in this study.**

<b>RIP qPCR primers for YTHDF1 target genes</b>	
PD-L1 human forward	5'-TGGCATTGCTGAACGCATTT-3'
PD-L1 human reverse	5'-TGCAGCCAGGTCTAATTGTTTT-3'
VISTA human forward	5'-ACGCCGTATTCCCTGTATGTC-3'
VISTA human reverse	5'-TTGTAGAAGGTCACATCGTGC-3'
EGFR human forward	5'-AGGCACGAGTAACAAGCTCAC-3'
EGFR human reverse	5'-ATGAGGACATAACCAGCCACC-3'
MDM2 human forward	5'-GAGCTTGGCTGCTTCTGGG-3'
MDM2 human reverse	5'- AAAGCAGCAGGATCTCGGTC -3'
PVR human forward	5'- CACTCAGGCATGTCCCGTAA -3'
PVR human reverse	5'- AGGCACACTGGAAGGACTCA -3'
NFE2L2 human forward	5'- TCAGCGACGGAAAGAGTATGA -3'
NFE2L2 human reverse	5'- CCACTGGTTTCTGACTGGATGT -3'
GAPDH human forward	5'- GGAGCGAGATCCCTCCAAAAT -3'
GAPDH human reverse	5'- GGCTGTTGTCATACTTCTCATGG -3'
PD-L1 mouse forward	5'- GACGCAGGCGTTTACTGCT -3'
PD-L1 mouse reverse	5'- GCGGTATGGGGCATTGACTTT -3'
VISTA mouse forward	5'- GTTGGTGAGGATGGACAGCA -3'
VISTA mouse reverse	5'- GGGAGTCAGGGAAGTGGATCT -3'
EGFR mouse forward	5'- GCCATCTGGGCCAAAGATAACC -3'
EGFR mouse reverse	5'- GTCTTCGCATGAATAGGCCAAT -3'
MDM2 mouse forward	5'- CCGTGAAGGGTCGGAAGAT -3'
MDM2 mouse reverse	5'- GCACCCTCGGTAGACACAGA -3'
PVR mouse forward	5'- ATTGCCACATTCCCCAGAGG -3'
PVR mouse reverse	5'- GTCCAGGAGGGTGACCATTG -3'
NFE2L2 mouse forward	5'- TAGATGACCATGAGTCGCTTGC -3'
NFE2L2 mouse reverse	5'- GCCAAACTTGCTCCATGTCC -3'
GAPDH mouse forward	5'- AGGTCGGTGTGAACGGATTTG -3'
GAPDH mouse reverse	5'- AGGTCGGTGTGAACGGATTTG -3'
<b>CLIP qPCR primers for YTHDF1 target genes</b>	
PD-L1-P1 forward	5'- GGTTGTGGATCCAGTCACCT -3'
PD-L1-P1 reverse	5'- TCCAGATGACTTCGGCCTTG -3'
PD-L1-P2 forward	5'- CCTCTGGCACATCCTCCAAAT -3'
PD-L1-P2 reverse	5'- ACGGAAGATGAATGTCAGTGCT -3'

PD-L1-P3 forward	5'- TGAGCGTGACAAGAGGAAGG -3'
PD-L1-P3 reverse	5'- GAGCCCCTCAGGCATTTGAA -3'
VISTA-V1 forward	5'- TGGATGCTACATGGGGATGC -3'
VISTA-V1 reverse	5'- TGTCTCAGAACGAGAGCTGC -3'
VISTA-V2 forward	5'- AGACATGAGCCTTGGGATGTG -3'
VISTA-V2 reverse	5'- AGAAGTCTCTCCACCGTGCC -3'
VISTA-V3 forward	5'- CTTGGGCAATCTGAGGCCAG -3'
VISTA-V3 reverse	5'- AGCATCCCGCTTCATACTCAG -3'
<b>Sequences of siRNAs/shRNA</b>	
METTL3 human #1	5'- GCACTTGGATCTACGGAAT -3'
METTL3 human #2	5'- CGACTACAGTAGCTGCCTT -3'
METTL14 human #1	5'- CAACTACAATGCAGAAACA -3'
METTL14 human #2	5'- GAAGACGCCTTCATCTATT -3'
YTHDF1 human #1	5'- CCTACGGACAGCTCAGTAA -3'
YTHDF1 human #2	5'- CCTGCTCTTCAGCGTCAAT -3'
METTL3 mouse #1	5'- CAAGGAAGAGTGCATGAAA -3'
METTL3 mouse #2	5'- GAAAGGTCTTGGAGAGGTA -3'
METTL14 mouse #1	5'- GCATTGGTGCTGTGTTAAA -3'
METTL14 mouse #2	5'- TTGAAGAATACCCTAAACT -3'
YTHDF1 mouse #1	5'- GCACTGACTGGTGTCTTTT -3'
YTHDF1 mouse #2	5'- GCACACAACCTCTATCTTT -3'
<b>RNA probe sequence</b>	
PD-L1	P1-m <sup>6</sup> A 5'biotin-CCUCUG <b>A(m<sup>6</sup>A)</b> CAUG <b>A(m<sup>6</sup>A)</b> CUGACAU-3'
	P1-A 5'biotin-CCUCUG <b>A</b> CAUG <b>A</b> CUGACAU-3'
	P1-T 5'biotin-CCUCUG <b>T</b> CAUG <b>T</b> CUGACAU-3'
	P3-m <sup>6</sup> A 5'biotin-GCAAUGUGGG <b>A(m<sup>6</sup>A)</b> CUUAAAAGGC-3'
	P3-A 5'biotin-GCAAUGUGGG <b>A</b> CUUAAAAGGC-3'
	P3-T 5'biotin-GCAAUGUGGG <b>T</b> CUUAAAAGGC-3'
VISTA	V2-m <sup>6</sup> A 5'biotin-GUGGG <b>A(m<sup>6</sup>A)</b> CAAGAUGG <b>A(m<sup>6</sup>A)</b> CACUGG-3'
	V2--A 5'biotin-GUGGG <b>A</b> CAAGAUGG <b>A</b> CACUGG-3'
	V2--T 5'biotin-GUGGG <b>T</b> CAAGAUGG <b>T</b> CACUGG-3'
	V3-m <sup>6</sup> A 5'biotin-CACCCUGGG <b>A(m<sup>6</sup>A)</b> CACUUCUGAG-3'
	V3-A 5'biotin-CACCCUGGG <b>A</b> CACUUCUGAG-3'
	V3-T 5'biotin-CACCCUGGG <b>T</b> CACUUCUGAG-3'