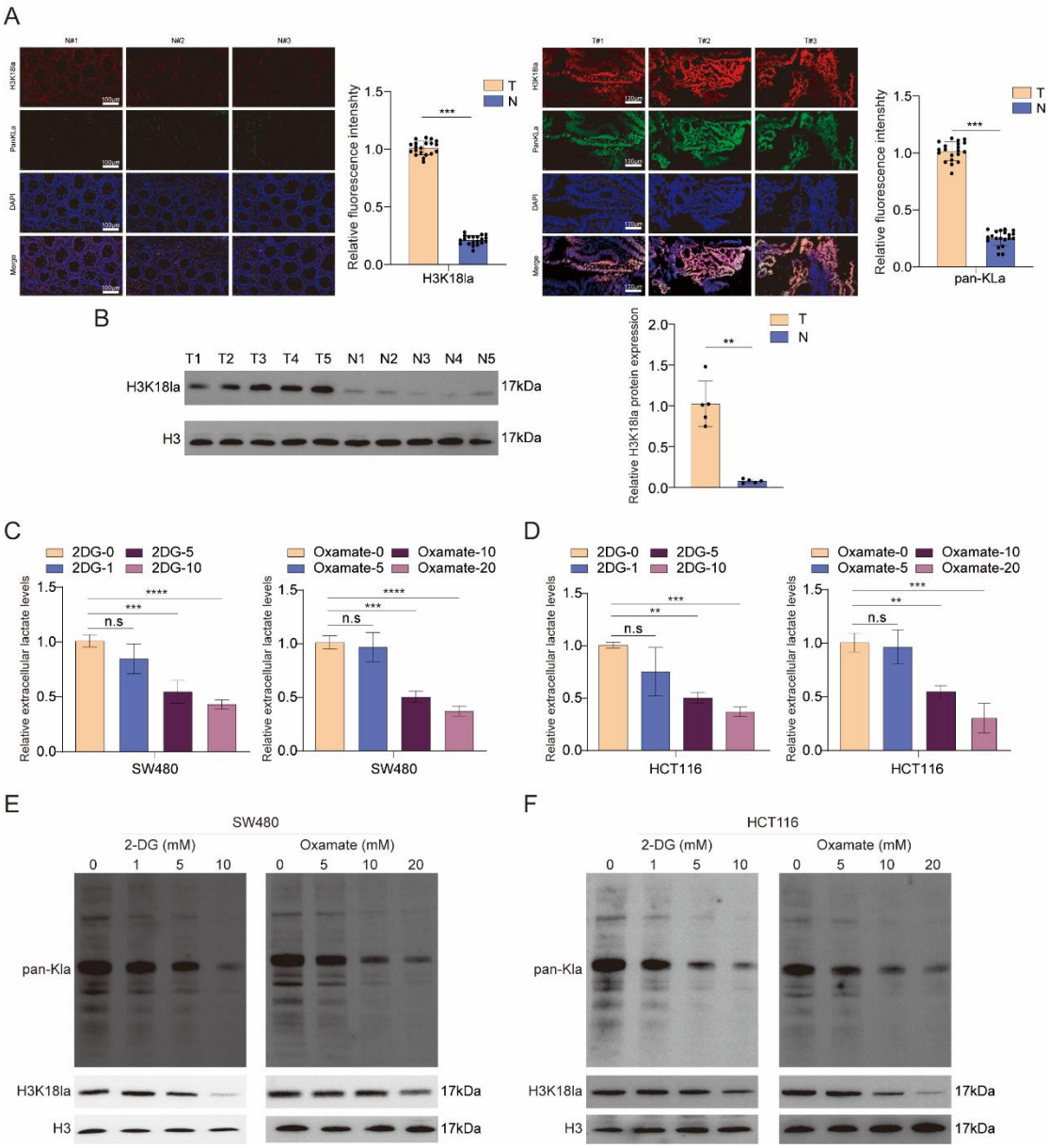
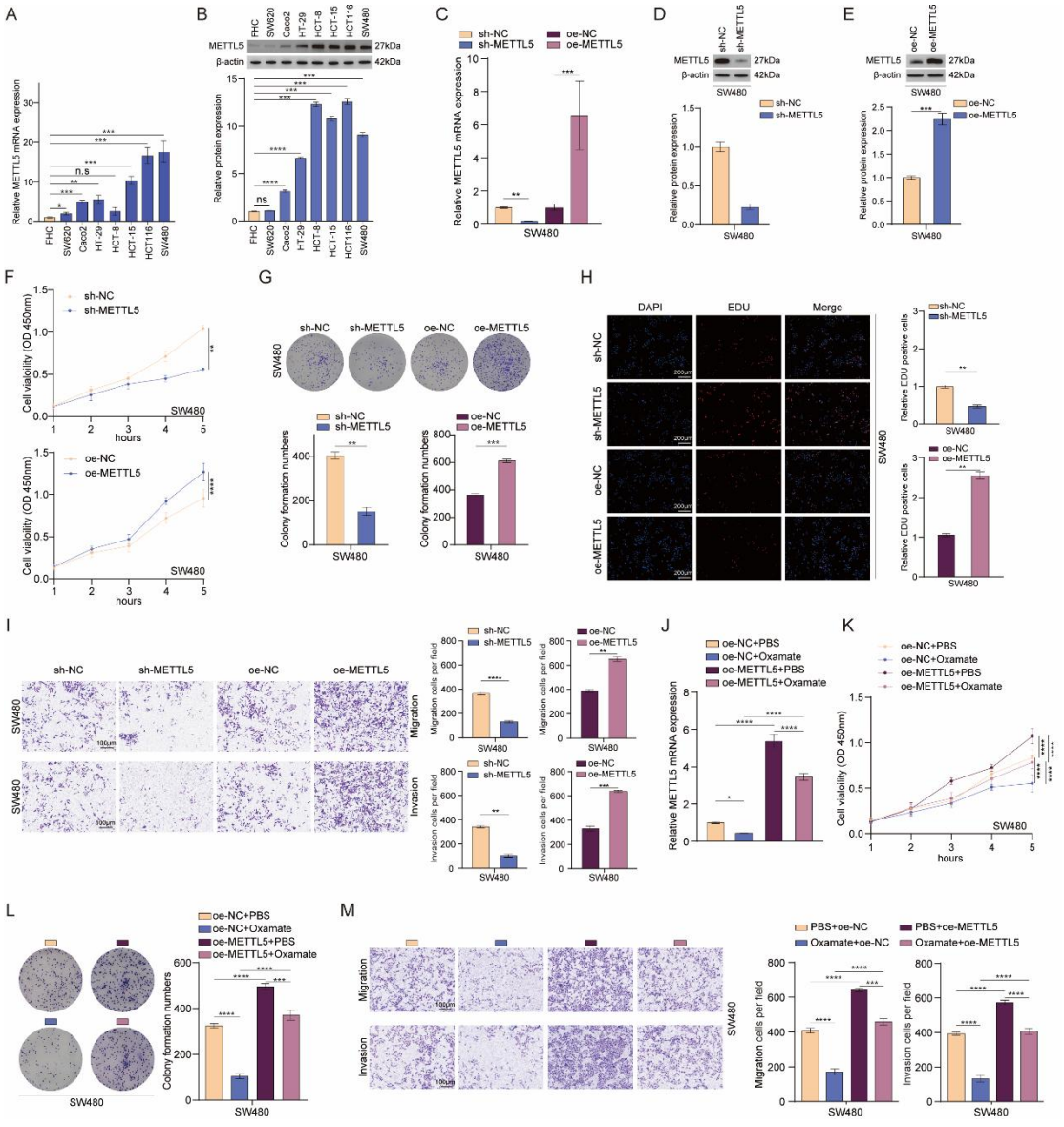


Supplementary Figure 1 Lactylation Is Closely Associated with the Prognosis of Patients with CRC. (A) Twenty pairs of samples were randomly selected from 80 pairs of CRC tissues (T) and adjacent non-tumor tissues (N). Immunofluorescence was used to detect the expression of H3K18la and pan-Kla, and fluorescence signals were statistically and quantitatively analyzed. (B) Five pairs of samples were randomly selected from 80 pairs of matched CRC and adjacent non-tumor tissues. Western blotting was performed to detect the expression level of H3K18la, with histone H3 used as the internal reference. Image Lab software was used to calculate the grayscale values of H3K18la and H3 bands, and the relative expression level of H3K18la was quantitatively

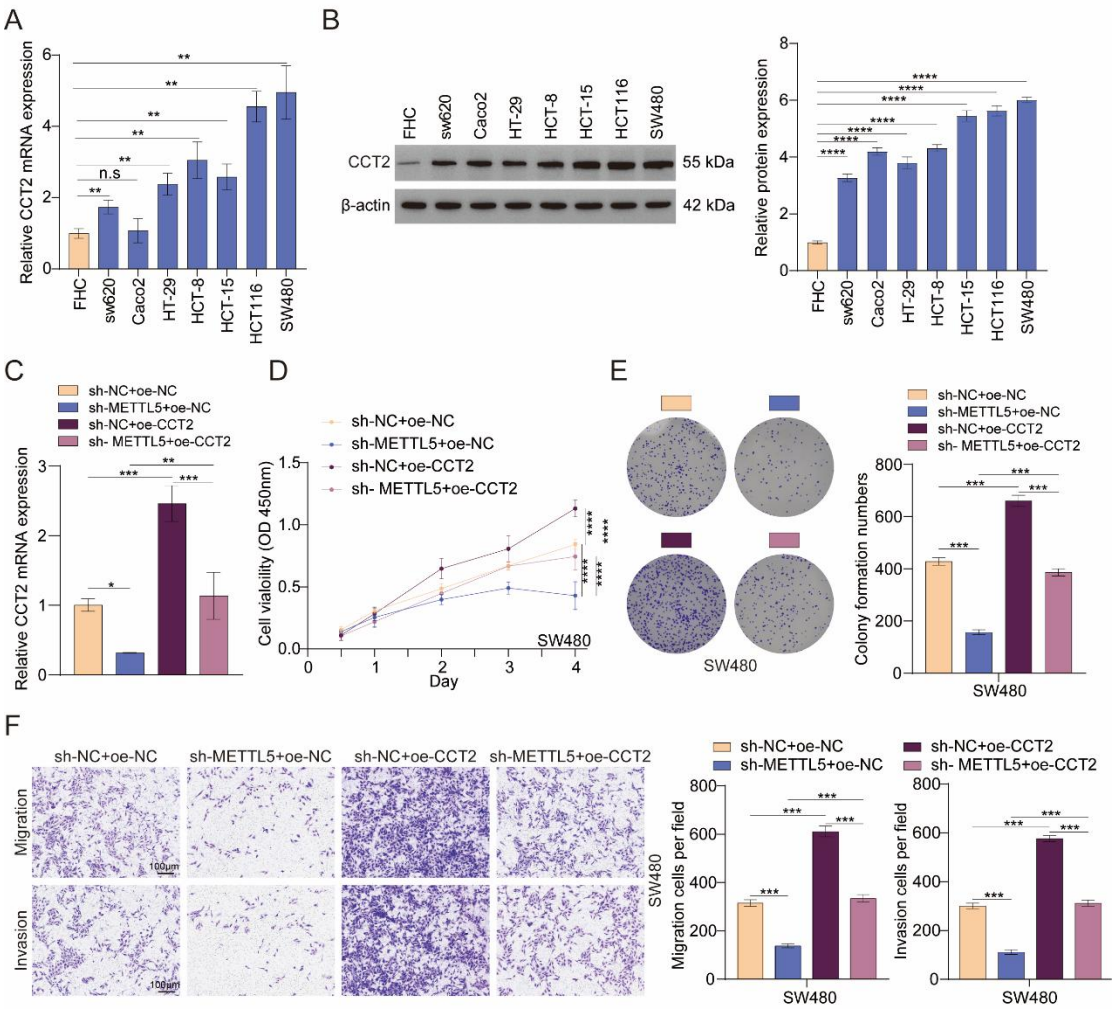
analyzed. (C and D) SW480 (C) and HCT116 (D) cells, at a density of 1 × 10^5 cells/well, treated with different concentrations of 2-DG or oxamate for 24 h. Lactate content in the culture medium was determined using a lactate detection kit. (E and F) SW480 (E) and HCT116 (F) cells treated with different concentrations of 2-DG or oxamate for 24 h. Western blotting was used to detect the protein expression levels of H3K18la and pan-Kla in total protein extracts.



Supplementary Figure 2 Lactate and H3K18la Promote *METTL5* Transcription. (A and B) qPCR (A) and Western blotting (B) were used to detect the mRNA and protein expression levels of METTL5 in FHC and multiple CRC

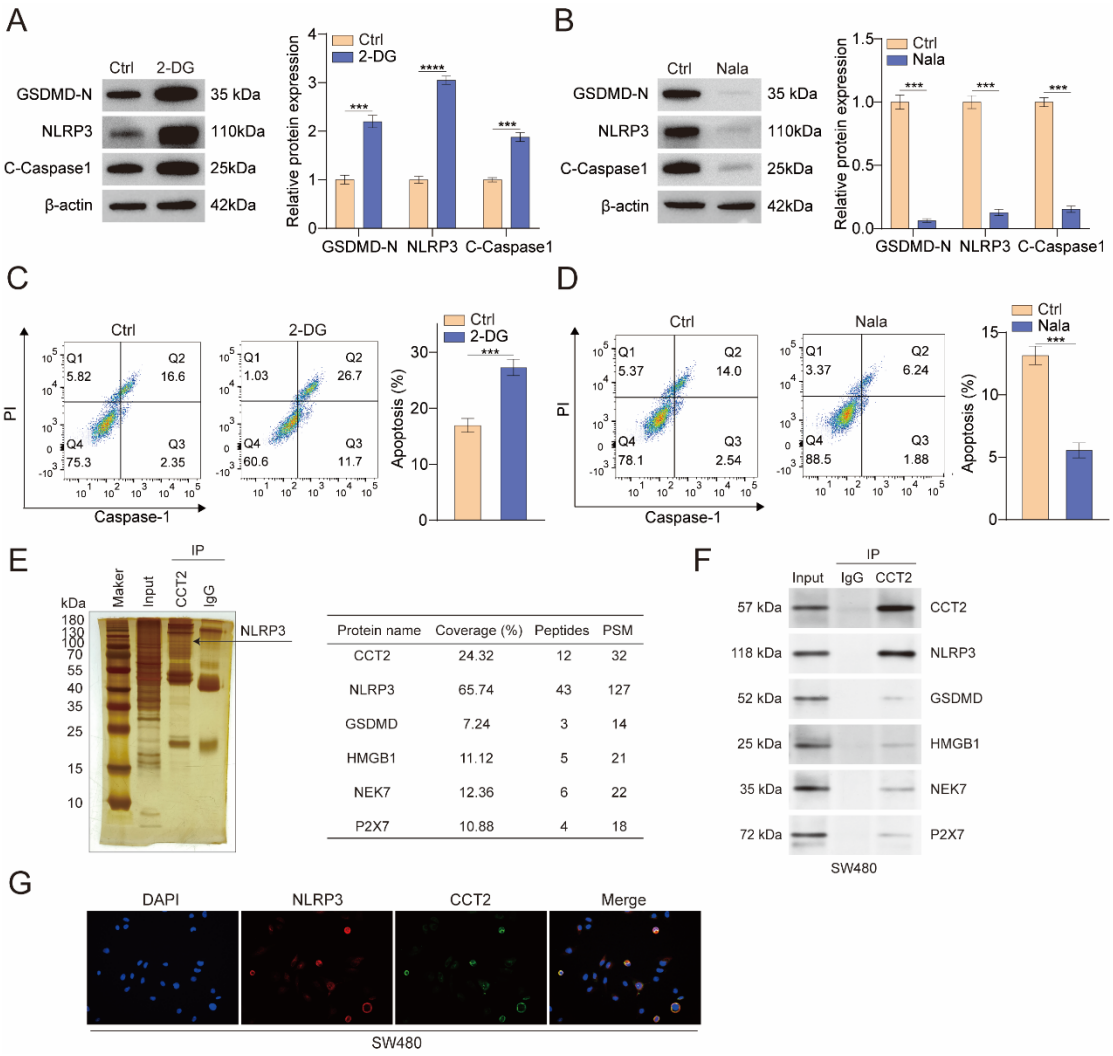
cell lines (SW620, Caco-2, HT-29, HCT-8, HCT-15, HCT116, and SW480), respectively, with subsequent quantitative comparison. (C–E) SW480 cells were infected with lentiviruses carrying sh-NC, sh-METTL5, oe-NC, or oe-METTL5 and selected with puromycin (5 μg/mL) for 24 h. qPCR was used to verify METTL5 mRNA expression (C). Western blotting was used to evaluate METTL5 knockdown (D) and overexpression (E) efficiency. (F) SW480 cells with stable METTL5 knockdown or overexpression (1×10^4 cells/well) were cultured daily for 5 days. The CCK-8 kit was used to detect cell growth activity, and growth curves were plotted. (G) Cells (from F; 5×10^2 cells/well) were cultured for 14 days. Colony formation was observed via crystal violet staining; the number of colonies was counted and subjected to quantitative analysis. (H) Cells (from G; 5×10^3 cells/well) were cultured for 24 h, followed by incubation with Edu for an additional 2 h. Fluorescence microscopy was used to detect the number of Edu-positive cells, with subsequent quantification. (I) Cells (from H; 1.5×10^4 cells/well) were seeded in the upper chamber of a Transwell insert or a Matrigel-coated Transwell insert. After 48 h of culture, crystal violet staining was performed to observe cell migration and invasion (scale bar: 100 μm); the number of penetrating cells was counted and subjected to quantitative comparison. (J) SW480 cells stably overexpressing empty vector (oe-NC) or oe-METTL5 were treated with culture medium containing PBS or 10 mM oxamate for 24 h. qPCR was used to detect METTL5 mRNA expression. (K) SW480 cells with stable METTL5 overexpression (from J; 1×10^4 cells/well) were cultured in the presence of PBS or 10 mM oxamate. Cell growth was detected by the CCK-8 method for five consecutive days, and growth curves were plotted. (L) Cells (from K; 5×10^2 cells/well) were cultured for 14 days in the presence of PBS or 10 mM oxamate. Crystal violet staining was performed to observe colony formation; the number of colonies was counted and subjected to quantitative analysis. (M) Cells (from L; 1.5×10^4 cells/well) were seeded in the upper chamber of a Transwell insert or a Matrigel-coated Transwell insert. PBS or 10 mM oxamate was added to both the upper and lower chambers. After 48

h of culture, crystal violet staining was performed to observe cell migration and invasion (scale bar: 100 μm); the number of transmembrane cells was counted and subjected to quantitative comparison.



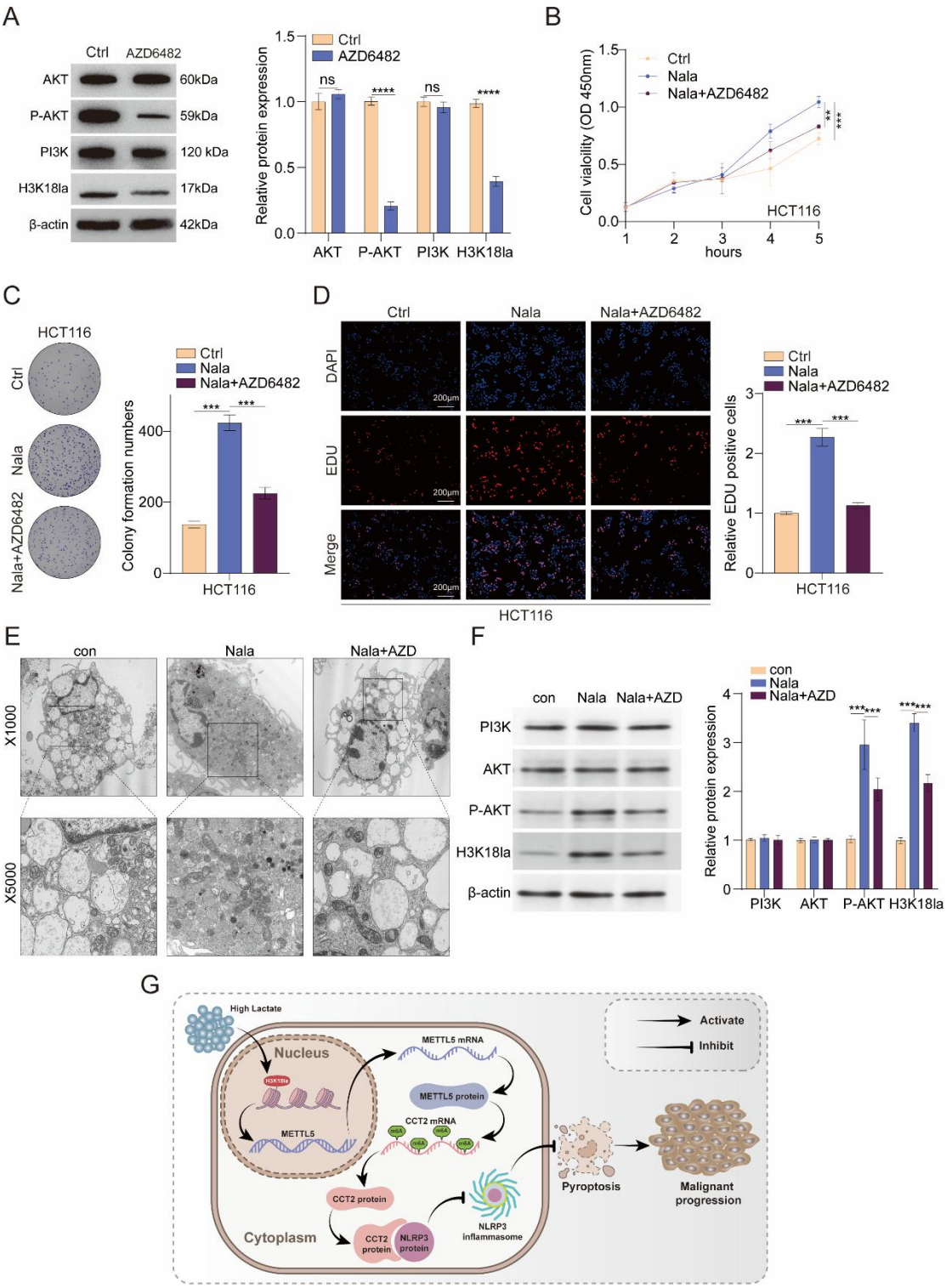
Supplementary Figure 3 METTL5 Promotes CCT2 Expression in an m^{6}A-dependent Manner. (A and B) qPCR (A) and Western blotting (B) were used to detect the mRNA and protein expression levels of CCT2 in FHC and multiple CRC cell lines (SW620, Caco-2, HT-29, HCT-8, HCT-15, HCT116, and SW480), with subsequent quantitative comparison. (C) SW480 cells with sh-NC or sh-METTL5 were stably transfected with oe-NC or oe-CCT2. qPCR was used to detect CCT2 mRNA expression. (D) SW480 cells from different treatment groups (as in C) were seeded at a density of 1 × 10^4 cells/well and cultured

continuously for 5 days. The CCK-8 kit was used to detect cell viability, and growth curves were plotted. (E) Cells (from D; 1 × 10^3 cells/well) were cultured for 14 days. Crystal violet staining was performed to observe colony formation; the number of colonies was counted and subjected to quantitative analysis. (F) Cells (from E; 1.5 × 10^4 cells/well) were seeded in the upper chamber of a Transwell insert or a Matrigel-coated Transwell insert. After 48 h of culture, crystal violet staining was performed to observe cell migration and invasion (scale bar: 100 μm); the number of transmembrane cells was counted and subjected to quantitative comparison.



Supplementary Figure 4 H3K18la–METTL5–CCT2 Signaling Axis Promotes Tumor Progression by Inhibiting Pyroptosis in CRC Cells. (A and B) SW480

cells treated with 5 mM 2-DG (A) or 5 mM Nala (B) for 24 h. Western blotting was performed to detect the protein expression levels of GSDMD-N, NLRP3, and C-caspase1, and β-actin served as the internal reference protein. (C and D) SW480 cells were treated with 5 mM 2-DG (C) or 5 mM Nala (D) for 24 h. Flow cytometry was used to detect the level of cell pyroptosis, followed by quantitative statistics. Propidium iodide (PI) staining was used to evaluate cell membrane integrity, and Caspase-1 activity was used as an indicator of inflammatory caspase activation. (E, F) SW480 cells were cultured with 5 mM sodium lactate (Nala) for 24 h. Co-immunoprecipitation was performed using an anti-CCT2 antibody. CCT2 and its potential interacting proteins were separated and purified by SDS-PAGE, followed by LC-MS/MS analysis (left panel of E); enrichment of CCT2 and its interacting proteins in the mass spectrometry data is shown (right panel of E). The interactions between CCT2 and NLRP3, GSDMD, HMGB1, NEK7, and P2X7 were examined by Western blotting (F). (G) SW480 cells were treated under the same conditions as described in (E, F). Co-localization of CCT2 and NLRP3 was observed by immunofluorescence staining.



Supplementary Figure 5 AZD6482 Enhances the Cytotoxic Sensitivity of Oxaliplatin to CRC Cells in a High-Lactate Microenvironment. (A) SW480 cells were treated with medium containing DMSO (Ctrl) or 20 μM AZD6482 for 24 h. Western blotting was used to detect the protein expression levels of AKT, phosphorylated AKT (S473), and PI3Kβ, and grayscale quantitative analysis of

the bands was performed. (B) SW480 cells (1×10^4 cells/well) were cultured under the following treatment conditions: PBS (Ctrl), 5 mM Nala, or 5 mM Nala + 20 μM AZD6482. After continuous culture for 5 days, cell viability was detected using the CCK-8 kit every day, and growth curves were plotted. (C) SW480 cells (1×10^3 cells/well) were cultured under the same treatment conditions as in (B) for 14 days. Crystal violet staining was performed to observe cell colony formation; the number of monoclonal cell clusters was counted and subjected to quantitative comparison. (D) SW480 cells (5×10^3 cells/well) were treated with PBS (Ctrl), 5 mM Nala, or 5 mM Nala + 20 μM AZD6482 for 24 h and incubated with Edu for an additional 2 h. The number of Edu-positive cells was detected by fluorescence microscopy, and quantitative analysis was conducted. (E) SW480 cells were treated with medium supplemented with PBS (Ctrl), 5 mM Nala, or 5 mM Nala + 20 μM AZD6482 for 48 h. Before harvesting, cells were incubated with 5 μM Nigericin sodium salt for 4 h, and pyroptosis-related characteristics were observed by transmission electron microscopy. (F) SW480 cells were treated for 24 h in medium containing 5 mM Nala or 5 mM Nala plus 20 μM AZD6482. The protein expression levels of AKT, phosphorylated AKT (S473), PI3Kβ, and H3K18la were determined by Western blotting, followed by quantitative analysis of band gray intensity. (G) Schematic of lactic acid-driven H3K18la-METTL5-CCT2 axis in colorectal cancer malignant progression.

Supplementary Table 1 Antibodies used for Western blotting

Antibody	Catalog #	Source
METTL5	HA720002	HUABIO
Pan-Kla	A23004	ABclonal
H3K18la	PTM-1427RM	PTM BIO
H3	HA601335	HUABIO
β-actin	20536-1-AP	Proteintech
NLRP3	30109-1-AP	Proteintech
cleaved caspase 1	22915-1-AP	Proteintech
IL-1β	16806-1-AP	Proteintech
GSDMD-N	20770-1-AP	Proteintech
RUBCNL	13798-1-AP	Proteintech
CCT2	24896-1-AP	Proteintech
AKT	#9272	Cell Signaling Technology
P-AKT-S473	#4060	Cell Signaling Technology
m^6A	ab208577	Abcam
PI3K	20584-1-AP	Proteintech
GSDMD	20770-1-AP	Proteintech
Ki67	27309-1-AP	Proteintech
LDHA	19987-1-AP	Proteintech
HMGB1	10829-1-AP	Proteintech
NEK7	31422-1-AP	Proteintech
P2X7	28207	Proteintech
Anti-Rabbit IgG (Alexa Fluor® 488)	ab150077	Abcam
Anti-Mouse IgG (Alexa Fluor® 594)	ab150116	Abcam

Supplementary Table 2 Primer set for real-time quantitative PCR

Gene	Primer sequence (5′→3′)
METTL5	F: AAGGAACTAGAGAGTCGCCTG
	R: GCGGCCTGGTAGGATACTG
CCT2	F：GTGATGGCACTACCTCTGTTAC
	R：GCTATGATGGTCTGTGGATGAA
PSAT1	F: TGCCGCACTCAGTGTTGTTAG
	R: GCAATTCCCGCACAAGATTCT
RANBP1	F: AATACAGACGAGTCCAACCATGA
	R: GAACAGTTTTGCCCGCATTTTA
ANXA3	F: GCGGGAACAAACGAAGATGC
	R: AGTCACCAGATGTTTCGGAACTA
EMC1	F: CTACGAAGACCAAGTGGGCA
	R: TGATCACATCCTGTCCGTGC
GAPDH	F: GGAGCGAGATCCCTCCAAAAT
	R: GGCTGTTGTCATACTTCTCATGG

Supplementary Table 3 Primer for ChIP-real-time quantitative PCR

Gene	Primer sequence (5′→3′)
METTL5	F: GAGAGCGGGTATGAGGACTAT
	R: GGAGCTGAGCAAGACAGAAA

Supplementary Table 4 In this study, 80 colon cancer patients were recruited, all of whom had complete clinical information. The clinicopathological information of these 80 patients was organized and recorded, mainly for the classification of the patient's age, gender, tumor diameter, T stage and N stage and other characteristics

Characteristics	N(%)
Age (years)	
≤69	48(60.00)
>69	32(40.00)
Gender	
Male	41(51.25)
Female	39(48.75)
Tumor Diameter（cm）	
≤5	29(36.25)
>5	51(63.75)
T Stage	
I–II	18(22.50)
Ⅲ-IV	62(77.50)
N Stage	
N0-1	58(85.35)
N2–3	22(27.50)

Supplementary Table 5 Cox regression analysis of prognostic factors for colon cancer

Factors		Univariate analysis HR (95% CI)	*P*	Mulvariate analysis HR (95% CI)	*P*
Gender	Female	1 (Reference)		1 (Reference)	
	Male	0.948 (0.452-1.989)	0.888	0.843 (0.393-1.807)	0.660
Age (years)	≤69	1 (Reference)		1 (Reference)	
	> 69	0.928 (0.435-1.981)	0.847	0.846 (0.387-1.853)	0.677
Pathological grading	I-II	1 (Reference)			
	III	2.114(0.802-5.571)	0.130		
T	I-II	1 (Reference)			
	III-IV	1.902(0.889-4.073)	0.098		
N	N0	1 (Reference)		1 (Reference)	
	N1-3	3.176 (1.505-6.701)	0.002	2.663 (1.223-5.795)	0.014
H3K18la	Low	1 (Reference)		1 (Reference)	
	High	3.150 (1.472-6.742)	0.003	2.649 (1.208-5.810)	0.015

Supplementary Table 6 Univariate Cox Regression Analysis

Factors	***B***	***Wald***	***HR***	***95% CI***	***P***
Gender (Female=0/Male=1)	-0.053	0.020	0.948	(0.452-1.989)	0.888
Age (years) (≤69=0/>69=1)	-0.075	0.037	0.928	(0.435-1.981)	0.847
Pathological grading (I-II=0/III=1)	0.749	2.292	2.114	(0.802-5.571)	0.130
T (I-II=0/III-IV=1)	0.643	2.742	1.902	(0.889-4.073)	0.098
N (N0=0/N1-3=1)	1.155	9.195	3.176	(1.505-6.701)	0.002
H3K18la (Low=0/High=1)	1.147	8.737	3.150	(1.472-6.742)	0.003

Supplementary Table 7 Multivariate Cox Regression Analysis

Factors	***B***	***Wald***	***HR***	***95% CI***	***P***
Gender (Female=0/Male=1)	-0.171	0.193	0.843	(0.393-1.807)	0.660
Age (years)(≤69=0/>69=1)	-0.167	0.174	0.846	(0.387-1.853)	0.677
N (N0=0/N1-3=1)	0.979	6.092	2.663	(1.223-5.795)	0.014
H3K18la (Low=0/High=1)	0.974	5.907	2.649	(1.208-5.810)	0.015