

YMTHE, Volume 29

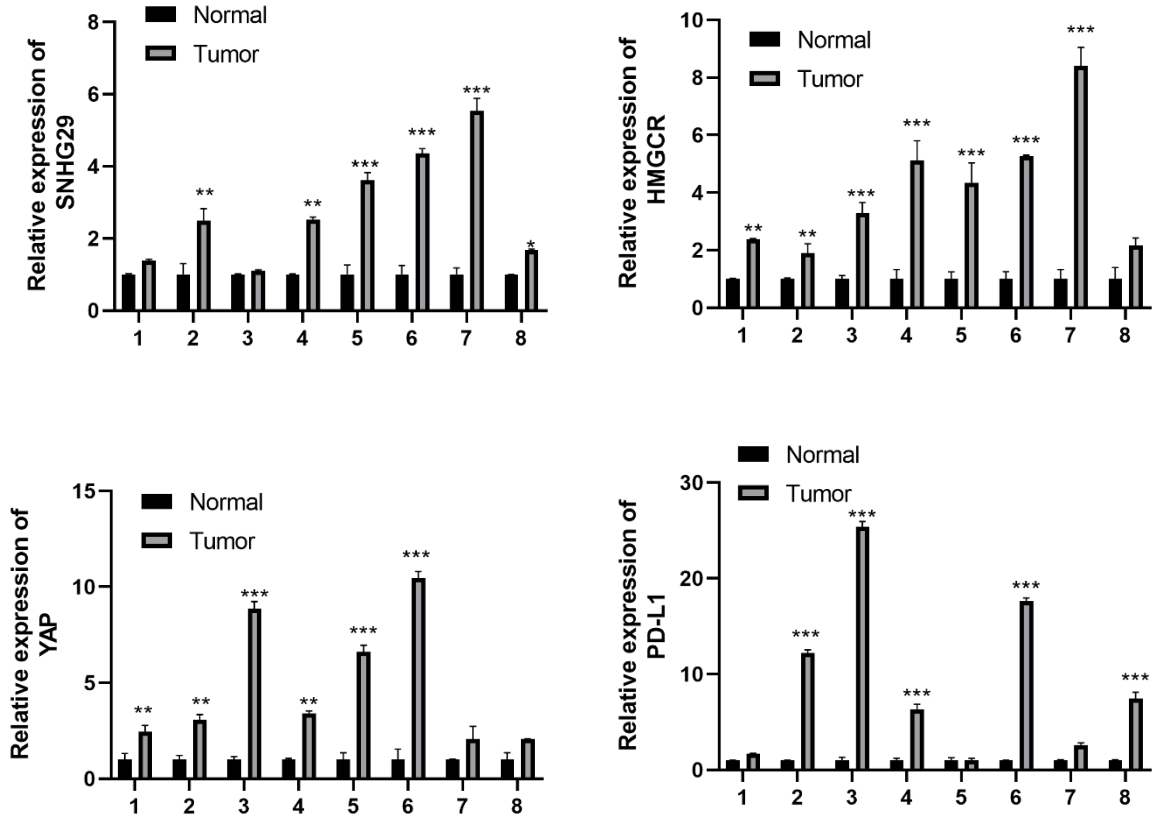
Supplemental Information

**Targeting cholesterol biosynthesis promotes
anti-tumor immunity by inhibiting long
noncoding RNA SNHG29-mediated YAP activation**

Wen Ni, Hui Mo, Yuanyuan Liu, Yuanyuan Xu, Chao Qin, Yunxia Zhou, Yuhui Li, Yuqing Li, Aijun Zhou, Su Yao, Rong Zhou, Jianping Huo, Liheng Che, and Jianming Li

Figure S1

A



B

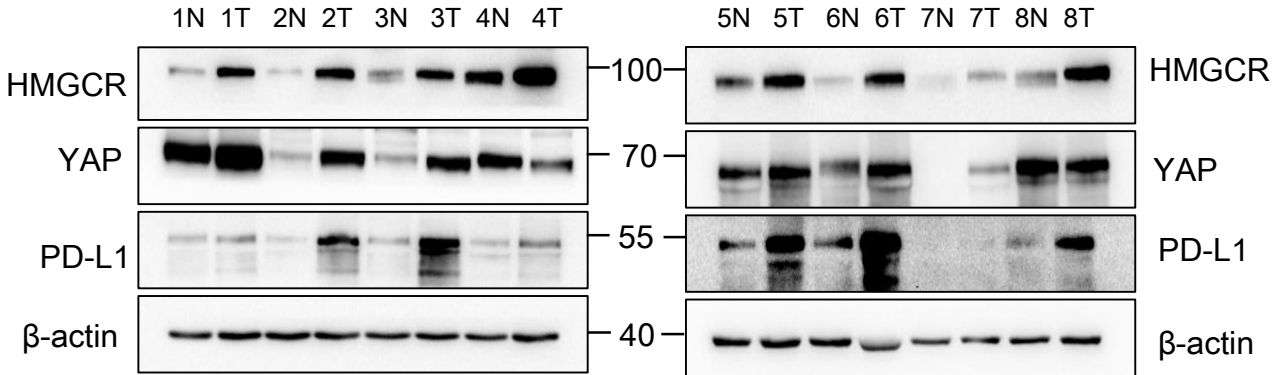
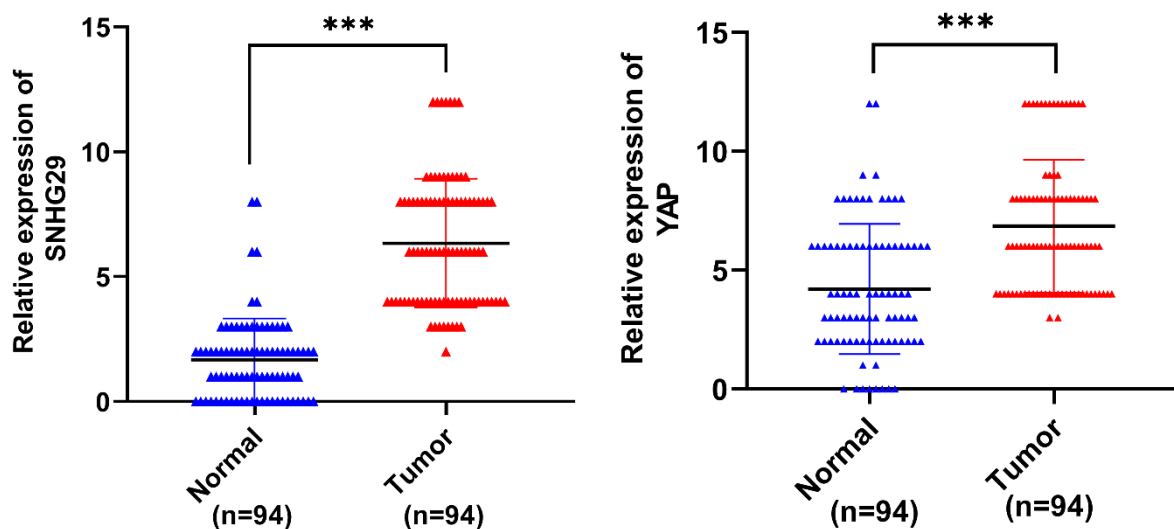


Figure S1. The expression of lncRNA SNHG29, HMGCRC, YAP and PD-L1 in CRC tumor tissues and adjacent normal counterparts. (A) qRT-PCR detection of genes expressions of SNHG29, HMGCRC, YAP and PD-L1. All experiments were performed in triplicate, and results are presented as mean \pm SD. **p < 0.01, and ***p < 0.001 (B) Western blots analysis of the indicated protein expression in CRC tumor tissues and adjacent normal counterparts.

Figure S2

A



B

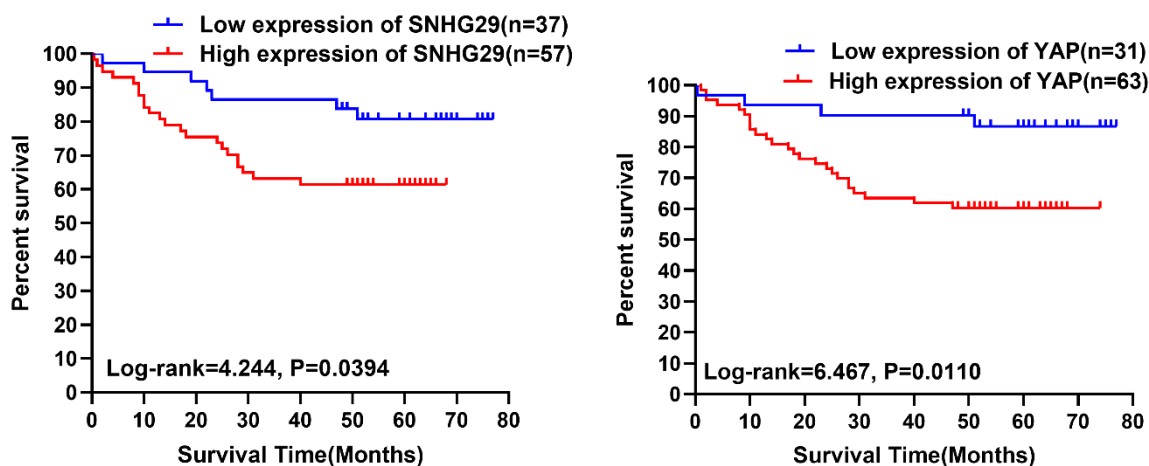


Figure S2. Survival curve of lncRNA SNHG29 and YAP in CRC patients. (A) Expressions of SNHG29 and YAP in CRC tumor tissues and adjacent normal counterparts. (B) Overall survival curve of SNHG29 and YAP expression in CRC patients. Median expression value of SNHG29 and YAP was selected as the threshold to divide CRC patients into high expression group and low expression group.

Figure S3

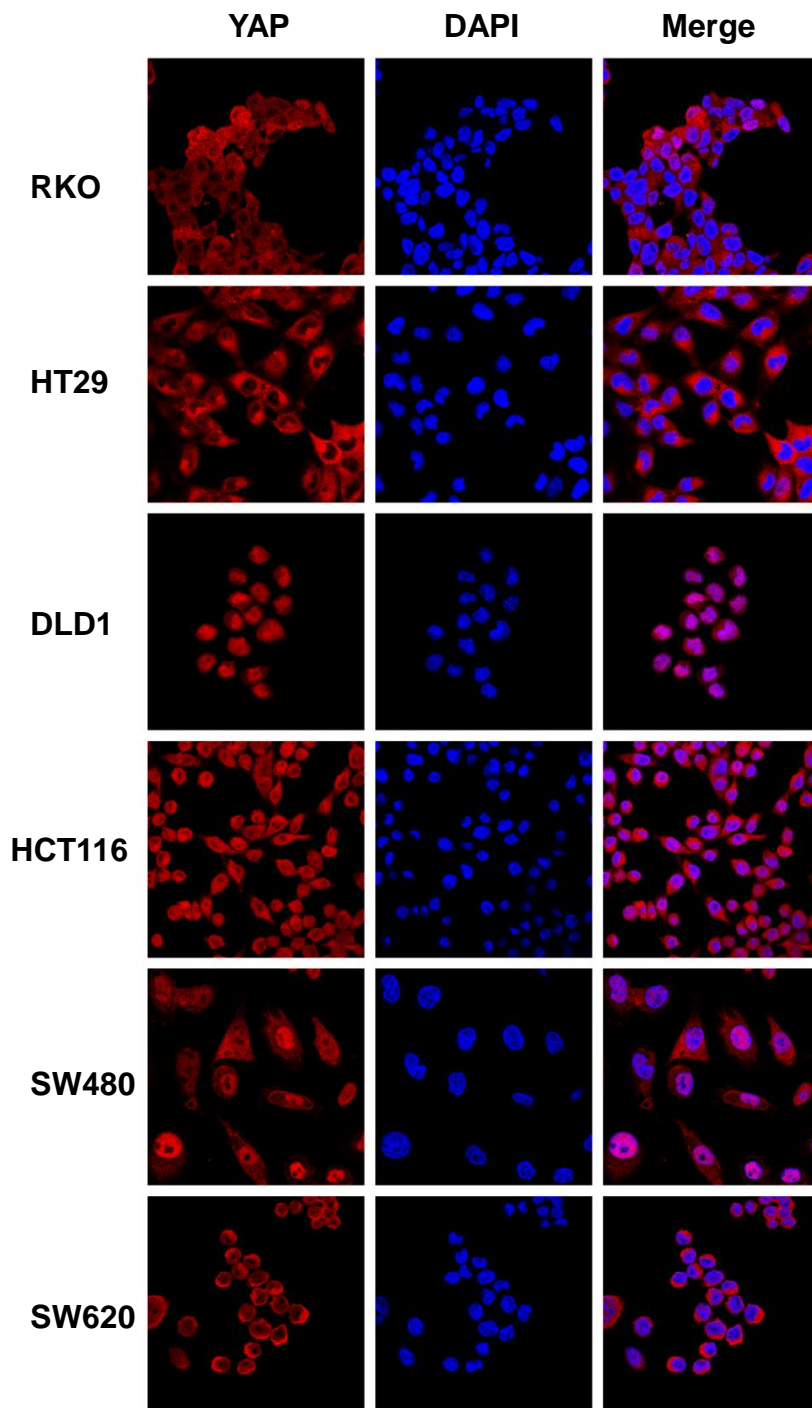


Figure S3. Immunofluorescence staining showed the localization of YAP in different CRC cells.

Figure S4

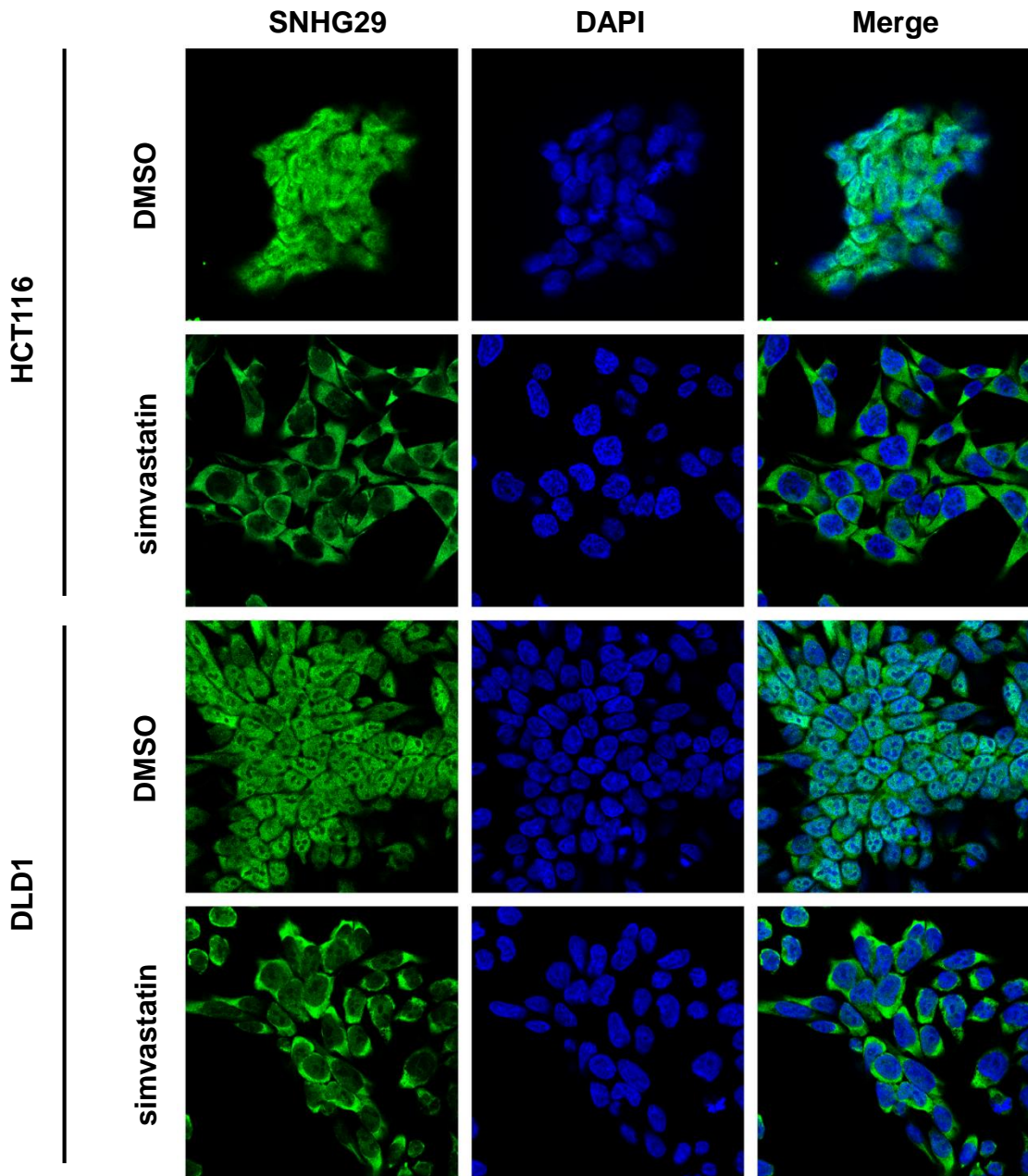


Figure S4. RNA FISH analysis show the subcellular location of lncRNA SNHG29 affected by simvastatin treatment in CRC cells.

Figure S5

HCT116

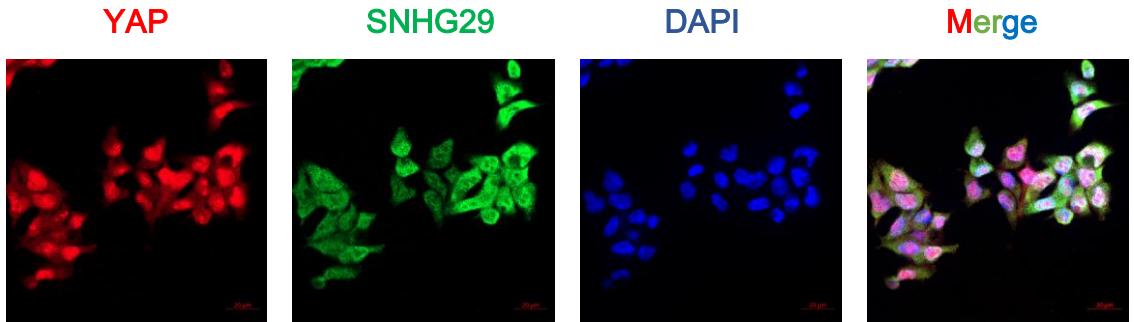


Figure S5. RNA FISH and immunofluorescence co-staining showed colocalization of SNHG29 and YAP in CRC cells.

Figure S6

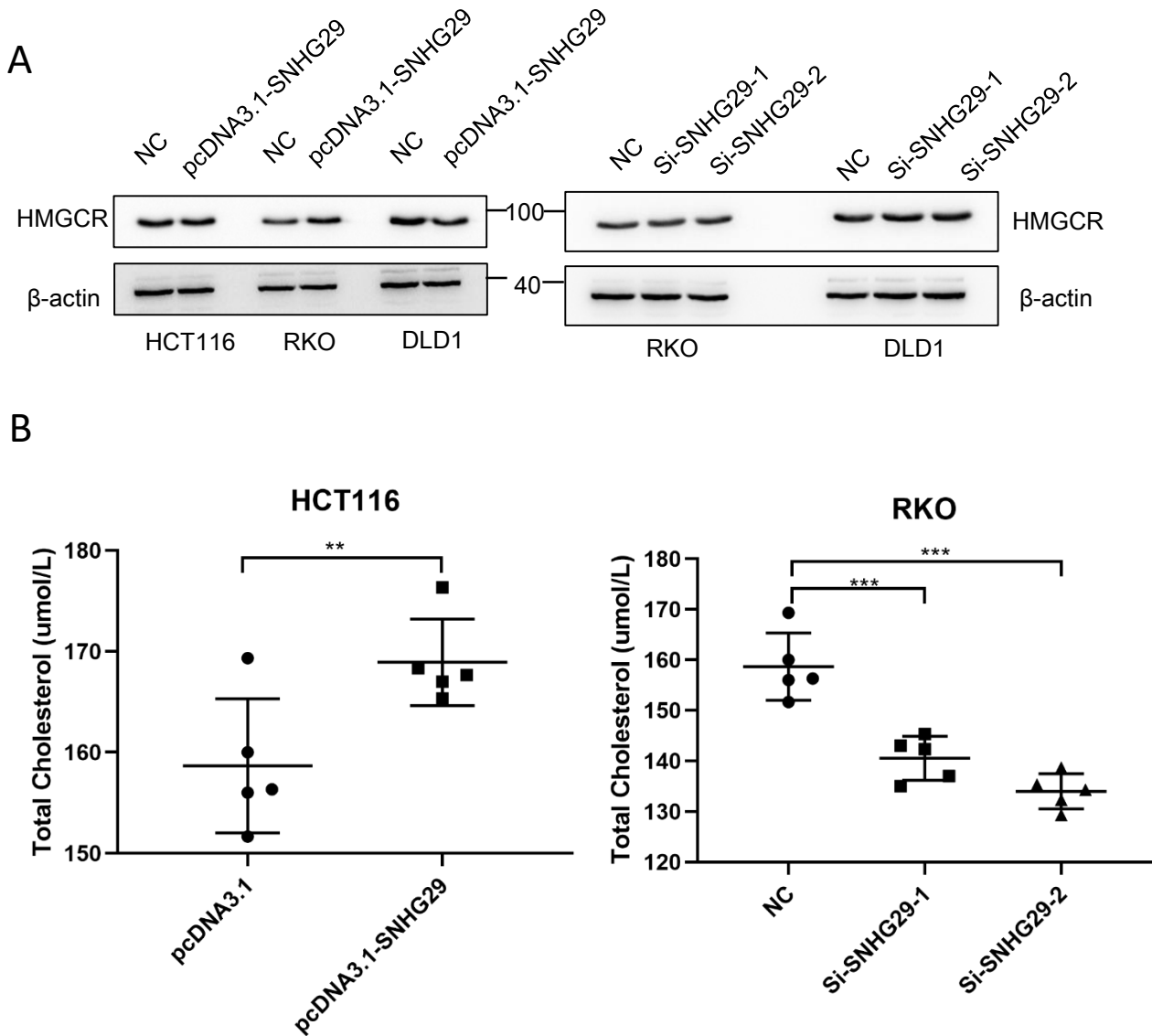


Figure S6. The HMGR levels (A) and cholesterol production (B) are examined in SNHG29 overexpression or knockdown CRC cells.

Table S5: Correlations between lncRNA SNHG29 express levels and clinicopathological features in 163 CRC cases

Clinical character	Clinical group	All cases	lncRNA SNHG29		χ^2	P value
			High	Low		
Age(Year)	< 50	26	14	12	0.106	0.745
	\geq 50	137	91	46		
Gender	Female	62	33	29	0.213	0.645
	Male	101	50	51		
Pathological Grade	II	121	61	60	0.048	0.826
AJCC stage	I - II	59	32	27	0.407	0.523
	III-IV	104	51	53		
LN involvement	Yes	86	39	47	2.261	0.133
	No	77	44	33		
Metastasis	M0	106	58	48	1.748	0.186
	M1	57	25	32		

P value <0.05 was considered to indicate statistical significance. The P values were calculated in SPSS 19.0 using Pearson's chi-square test.

Table S6: Correlations between YAP express levels and clinicopathological features in 163 CRC cases

Clinical character	Clinical group	All cases	YAP		χ^2	P value
			High	Low		
Age(Year)	< 50	26	13	13	0.142	0.707
	\geq 50	137	60	77		
Gender	Female	62	36	26	0.885	0.347
	Male	101	51	50		
Pathological Grade	II	121	63	58	0.323	0.570
AJCC stage	I - II	59	29	30	0.662	0.416
	III-IV	104	58	46		
LN involvement	Yes	86	46	40	0.001	0.975
	No	77	41	36		
Metastasis	M0	106	59	47	0.637	0.425
	M1	57	28	29		

P value <0.05 was considered to indicate statistical significance. The P values were calculated in SPSS 19.0 using Pearson's chi-square test.

Table S7: Correlations between PD-L1 express levels and clinicopathological features in 163 CRC cases

Clinical character	Clinical group	All cases	PD-L1		χ^2	P value
			High	Low		
Age(Year)	< 50	26	17	9	0.645	0.212
	≥ 50	137	83	54		
Gender	Female	62	39	23	0.102	0.750
	Male	101	61	40		
Pathological Grade	II	121	75	46	0.080	0.778
	III	42	25	17		
AJCC stage	I - II	59	42	17	3.773	0.052
	III-IV	104	58	46		
LN involvement	Yes	86	48	38	2.353	0.1625
	No	77	52	25		
Metastasis	M0	106	69	37	1.793	0.181
	M1	57	31	26		

P value <0.05 was considered to indicate statistical significance. The P values were calculated in SPSS 19.0 using Pearson's chi-square test.

Table S8. Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
YAP (D8H1X) XP® rabbit mAb	Cell Signaling Technology	Cat# 14074
Rabbit monoclonal Anti-YAP1 antibody [EPR19812] (Alexa Fluor® 647)	abcam	Cat# ab225440
PD-L1 (E1L3N®) XP® Rabbit mAb	Cell Signaling Technology	Cat#13684
Mouse PD-L1/B7-H1 Antibody (Mouse Specific)	R&D	Cat# AF1019
LATS1 (C66B5) rabbit mAb	Cell Signaling Technology	Cat#3477
Anti-HMGCR antibody	abcam	Cat# ab174830
Phospho-YAP (Ser127) (D9W2I) rabbit mAb	Cell Signaling Technology	Cat#13008
CTGF (D8Z8U) rabbit mAb	Cell Signaling Technology	Cat#86641
Ubiquitin (P4D1) mouse mAb	Cell Signaling Technology	Cat#3936
Beta Actin Monoclonal Antibody	Proteintech	Cat# 66009-1-Ig
Rabbit polyclonal anti-GAPDH	Proteintech	Cat#10494-1-AP
Mouse monoclonal anti-Lamin B1	Proteintech	Cat#66095-1-Ig
Mouse polyclonal anti-Ki67	ZSGB-BIO	Cat#ZM-0166
CD8 α (C8/144B) Mouse mAb	Cell Signaling Technology	Cat# 38553
CD3 ϵ (D7A6E™) XP® Rabbit mAb	Cell Signaling Technology	Cat# 95194
CD4 (D7D2Z) Rabbit mAb	Cell Signaling Technology	Cat# 25229
CD8- α (D4W2Z) XP® Rabbit mAb (Mouse Specific)	Cell Signaling Technology	Cat# 98941
Granzyme B (D6E9W) Rabbit mAb	Cell Signaling Technology	Cat# 46890
Granzyme B antibody	ZSGB-BIO	Cat# ZA-0599-3.0
PerCP/Cyanine5.5 anti-human CD86	Biolegend	Cat# 305419
FITC anti-human CD69	Biolegend	Cat# 310904
Ms CD25 BV786	BD	Cat# 564368
Hu HLA-DR BV605	BD	Cat# 562845
ANTI-HU CD14 61D3 FITC	eBioscience	Cat# 11-0149-42
Bacterial and Virus Strains		
DH5alpha Competent E. coli	TIANGEN	Cat#CB101
Chemicals, Peptides, and Recombinant Proteins		
Cycloheximide	MedChemExpress	Cat#HY-12320
MG-132	Selleck	Cat#133407-82-6
Actinomycin D	MedChemExpress	Cat#HY-17559
Critical Commercial Assays		
Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit	Millipore	Cat# 17-701
PARIS™ Kit	Invitrogen	Cat# AM1921

SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads)	Cell Signaling Technology	Cat#9003
Dual-Glo® Luciferase Assay System	Promega	Cat#E2920
Deposited Data		
lncRNA-seq Raw data	This paper	SRP313172
Experimental Models: Cell Lines		
Human: DLD1	Cellcook Biotech Co.,Ltd.	Cat#CC0507
Human: SW480	Cellcook Biotech Co.,Ltd.	Cat#CC0505
Human: SW620	Cellcook Biotech Co.,Ltd.	Cat#CC0503
Human: HCT116	Cellcook Biotech Co.,Ltd.	Cat#CC0506
Human: RKO	Cellcook Biotech Co.,Ltd.	Cat#CC0501
Human: HT29	Cellcook Biotech Co.,Ltd.	Cat#CC0504
Experimental Models: Organisms/Strains		
Mice: B-NDG nude	BIOCYTOGEN	N/A
Mice: C57BL/6	Guangdong Medical Laboratory Animal Center	N/A
Mice: BALB/C nude	Guangdong Medical Laboratory Animal Center	N/A
Oligonucleotides		
LNA™ ISH probe: SNHG29 (/5DigN/TGATGCTGATTCTTCACGAAT /3Dig_N/)	Exiqon	328067375
YAP-F: TAGCCCTGCGTAGCCAGTTA	The Beijing Genomics Institute	N/A
YAP-R: TCATGCTTAGTCCACTGTCTGT	The Beijing Genomics Institute	N/A
SNHG29-F: CAGTCCCAAGGACCAGTAGC	The Beijing Genomics Institute	N/A
SNHG29-R: CTGCTGAGCATGTCCTCTGA	The Beijing Genomics Institute	N/A
CTGF-F: CAGCATGGACGTTCTGCTG	The Beijing Genomics Institute	N/A
CTGF-R: AACCACGGTTTGGTCCTTGG	The Beijing Genomics Institute	N/A
CYR61-F: CTCGCCTTAGTCGTCACCC	The Beijing Genomics Institute	N/A
CYR61-R: CGCCGAAGTTGCATTCCAG	The Beijing Genomics Institute	N/A
GAPDH-F: AGCTGAACGGGAAGCTCACT	The Beijing Genomics Institute	N/A
GAPDH-R: TGCTTAGCCAAATTCGTTG	The Beijing Genomics Institute	N/A
esiRNA target: YAP-1;F:GGUCAGAGAUACUUCUAAA	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A

R:UUAAGAAGUAUCUCUGACCAG	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
esiRNA target: YAP-2;F:GGUGAUACUAUCAACCAAAGC	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
R:UUUGGUUGAUAGUAUCACCUG	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
esiRNA target: SNHG29-1;F:CGAGAGAACUGGGUUGCAAU	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
R: UUGCAACCCAGUUCUCUCGGG	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
esiRNA target: SNHG29-2;F:GUUGCAAUUCGUGAAGAAUU	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
R: UUCUUCACGAAUUUGCAACUU	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
siRNA(NC), F:UUCUCCGAACGUGUCACGUTT	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
R:ACGUGACACGUUCGGAGAATT	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
Recombinant DNA		
pcDNA3.1 (+) -YAP1-6xhis	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
pCDH-MSCV-MCS-EF1-GFP-puro-YAP1	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
pcDNA3.1 (+) -SNHG29	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A
pCDH-MSCV-MCS-EF1-GFP-puro-SNHG29	Shanghai Integrated Biotech Solutions Co.,Ltd	N/A