

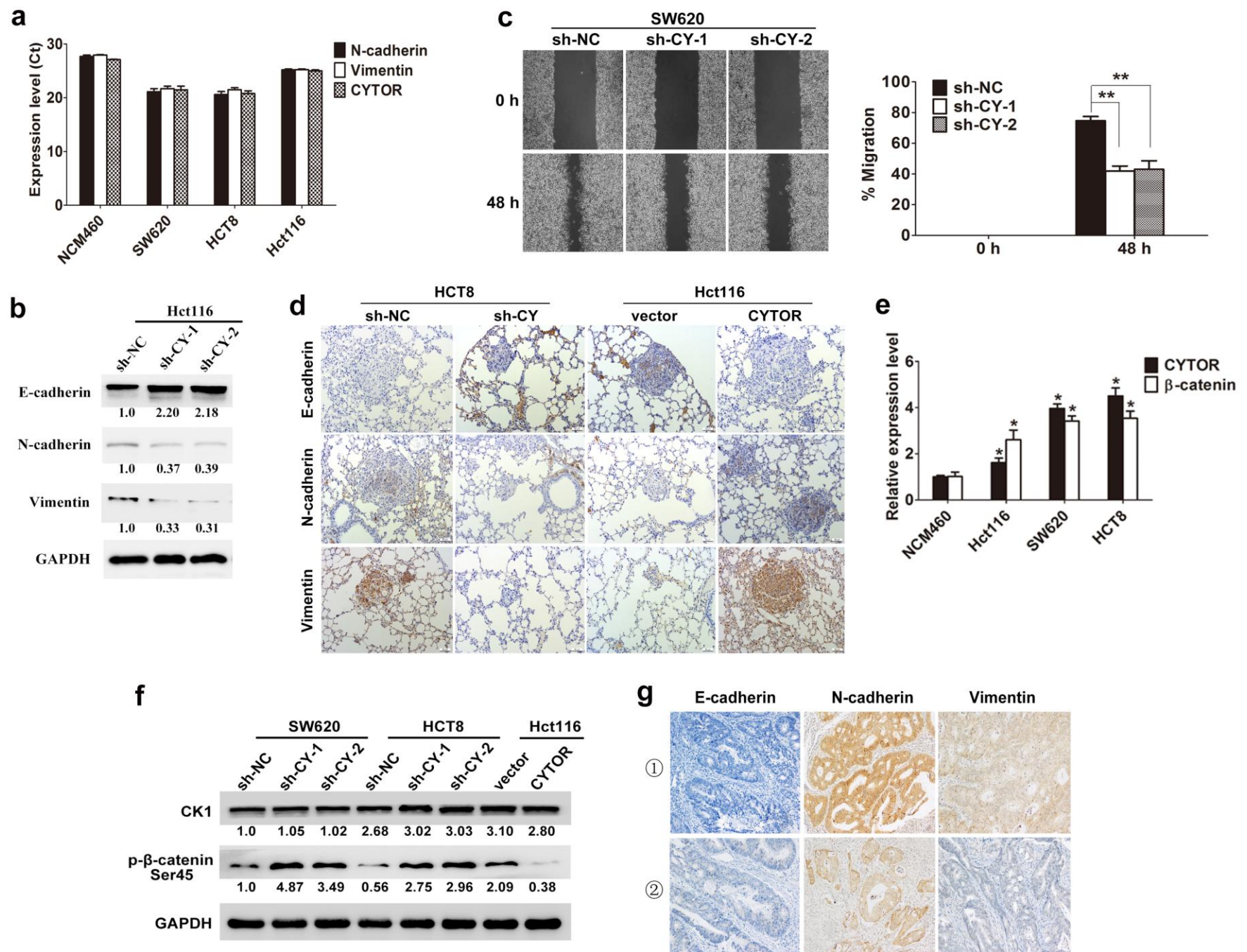
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Supplemental Information

A Positive Feed-Forward Loop between LncRNA-CYTOR and Wnt/ β -Catenin Signaling Promotes Metastasis of Colon Cancer

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Supplementary Figure S1



Supplementary Fig S1. (a) The endogenous levels of CYTOR and mesenchymal markers N-cadherin and Vimentin were comparable in human colon mucosal epithelial cell line NCM460 and colon cancer cell lines. (b) Knockdown of CYTOR resulted in a significant upregulation of E-cadherin, accompanied by a prominent downregulation of N-cadherin and Vimentin in Hct116 cells. (c) Knockdown of CYTOR significantly decreased cells' migratory ability in SW620 cell line. (d) N-cadherin and Vimentin were reduced, and E-cadherin was upregulated in the lung metastatic tumor tissues with CYTOR knockdown. N-cadherin and Vimentin were upregulated, and E-cadherin was reduced in the lung metastatic tumor tissues with CYTOR overexpression. (e) β -catenin levels are relatively higher with concurrent elevated CYTOR in colon cancer cell lines. (f) Increased phosphorylation levels of β -catenin at Ser45 was observed in cells transfected with CYTOR shRNA, whereas overexpression of CYTOR significantly decreased phospho- β -catenin^{Ser45} expression. CK1 levels showed no obvious changes in the subgroups of every cell line. (g) ① CYTOR^{high}/ β -catenin^{nuc} group, ②CYTOR^{low}/ β -catenin^{cyto} group. Representative staining of E-cadherin, N-cadherin and Vimentin in CYTOR^{high}/ β -catenin^{nuc} and CYTOR^{low}/ β -catenin^{cyto} tumor tissues. * P <0.05, ** P <0.01.

Supplementary Table S1. Association between clinicopathologic features and CYTOR expression

Variable	n=100	Expression of CYTOR		P value
		High (n=50)	Low (n=50)	
Age (years)				0.829
<65	31	16	15	
≥65	69	34	35	
Gender				0.841
Male	47	24	23	
Female	53	26	27	
Location				0.216
Right	38	16	22	
Others	62	34	28	
pT stage				0.033*
T1	3	1	2	
T2	11	2	9	
T3	40	18	22	
T4	46	29	17	
pN stage				<0.001*
N0	32	7	25	
N1	40	23	17	
N2	28	20	8	
pM stage				0.006*
M0	92	42	50	
M1	8	8	0	
TNM stage				<0.001*
I / II	32	7	25	
III/IV	68	43	25	
Differentiation				0.111
Well	47	21	26	
Moderate	40	19	21	
Poor	13	10	3	
Vessel invasion				0.317
No	90	43	47	
Yes	10	7	3	

* $p < 0.05$ indicates a significant association among the variables.

Supplementary Table S2. Univariate and multivariate analysis of overall survival and disease-free survival after surgery

Variable	Overall survival				Disease-free survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
Age	1.09 (0.54, 2.21)	0.810			1.01 (0.51, 1.99)	0.982		
Gender	1.12 (0.59, 2.14)	0.732			1.09 (0.58, 2.05)	0.788		
Location	0.97 (0.77, 1.23)	0.800			1.02 (0.81, 1.28)	0.878		
T stage	1.73 (1.06, 2.82)	0.028*			1.89 (1.16, 3.08)	0.011*		
N stage	3.69 (2.23, 6.11)	<0.001*	2.93 (1.71, 5.04)	<0.001*	3.57 (2.20, 5.79)	<0.001*	2.73 (1.61, 4.64)	<0.001*
M stage	10.60 (4.31, 26.01)	<0.001*	5.34 (2.07, 13.77)	0.001*	7.62 (3.33, 17.42)	<0.001*	3.47 (1.44, 8.33)	0.005*
TNM stage	6.24 (3.07, 12.67)	<0.001*			4.65 (2.51, 8.64)	<0.001*		
Differentiation	1.28 (0.81, 2.03)	0.286			1.40 (0.90, 2.17)	0.131		
Vessel invasion	2.93 (1.28, 6.71)	0.011*			3.52 (1.61, 7.72)	0.002*	2.45 (1.10, 5.47)	0.029*
CYTOR	3.89 (1.88, 8.05)	<0.001*	2.32 (1.08, 4.98)	0.031*	3.86 (1.88, 7.94)	<0.001*	2.22 (1.04, 4.73)	0.039*

**P* < 0.05 indicated that 95% CI of HR was not including. HR, hazard ratio; 95% CI, 95% confidence interval

Supplementary Table S3. The correlation between expression levels of CYTOR and β -catenin in 100 cases of colon cancer tissues

	Expression of CYTOR		<i>p</i> -value
	High	Low	
β -catenin			0.006*
+	46	34	
-	4	16	
β -catenin			0.008*
Nuclear	36	17	
Cytoplasmic	10	17	

**p*<0.05 indicates a significant association among the variables.

Supplementary Table S4. Detailed sequence (5'-3') information

CYTOR	Forward	TGGGAATGGAGGGAAATAAA
	Reverse	CCAGGAACTGTGCTGTGAAG
E-cadherin	Forward	GCCCC ATCAG GCCTC CGTTT
	Reverse	ACCTT GCCTT CTTTG TCTTT GTTGG A
GAPDH	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCAGG
N-cadherin	Forward	TGGACCATCACTCGGCTTA
	Reverse	AACTGGCAAACCTTCACG
Vimentin	Forward	CGAAACTTCTCAGCATCACG
	Reverse	GCAGAAAGGCACTTGAAAGC
β -catenin	Forward	TCATGCACCTTTGCGTGAGC
	Reverse	GGCGCTGGGTATCCTGATGT
c-myc	Forward	TTCGGGTAGTGGAAAACCAG
	Reverse	CAGCAGCTCGAATTTCTTCC
cyclin D1	Forward	GTGCTGCGAAGTGGAAACC
	Reverse	ATCCAGGTGGCGACGATCT
sh-CYTOR-1	sense	TGTCTGCATCCCTCGAATAACTTTCAAGAGAAGTTATTCGAGGGATGCAGACTTTTTTC
	anti-sense	TCGAGAAAAAAGTCTGCATCCCTCGAATAACTTCTCTTGAAAGTTATTCGAGGGATGCAGACA
sh-CYTOR-2	sense	TGACTCTGAGGCCTCTGCATTTCAAGAGAATGCAGAGGCCTCAGAGTCTTTTTTC
	anti-sense	TCGAGAAAAAAGACTCTGAGGCCTCTGCATTCTCTTGAAATGCAGAGGCCTCAGAGTCA
LV-CYTOR	Forward	CGCAAATGGGCGGTAGGCGTG
	Reverse	CATAGCGTAAAAGGAGCAACA

Supplementary Table S5. Detailed sequence of Primers used for ChIP-PCR

TBE1	Forward	TTCTAGTGGGGCTGCCACA
	Reverse	AGCCTGCTGGTTTTCCAGAA
TBE2	Forward	AGGCTCCAAGGGCACCATTG
	Reverse	GCCTCCCACAGCTTCAAGCA
Neg	Forward	AATGATCCAGAAGGCTCCTA
	Reverse	GCCAGCCTTTACCTTCTTTA