OMTN, Volume *12*

Supplemental Information

A Novel lncRNA, LINC00460, Affects Cell Proliferation and Apoptosis by Regulating KLF2 and CUL4A Expression in Colorectal Cancer

Yifan Lian, Changsheng Yan, Hongzhi Xu, Jiebin Yang, Yang Yu, Jing Zhou, Yongguo Shi, Jianlin Ren, Guozhong Ji, and Keming Wang

Supplementary Table 1. Correlation between clinicopathological parameters and LINC00460 expression levels in 60 CRC Patients

Characteristics	**LINC00460**		***P* Value**
	Low No. Cases (n=30)	High No. Cases (n=30)	Chi-squared test
Age(years)			0.288
≤60	14	9	
>60	16	21	
Gender			0.589
Male	18	21	
Female	12	9	
Tumor size(cm)			0.029*
≤5	15	6	
>5	15	24	
Histological grade			0.299
Well/Moderate	16	11	
Poor	14	19	
TNM Stage			0.020*
I/II	13	4	
III/IV	17	26	
Lymph node metastasis			0.047*
Positive	17	25	
Negative	13	5	

Supplementary Table 2. Univariate and Multivariate Analyses of Various Potential Prognostic Factors in 60 CRC Patients

Variables	Univariate analysis			Multivariate analysis		
	P value	HR	95% CI	P value	HR	95% CI
Age(≦60 years vs. >60 years)	0.438	0.994	0.980,1.009	0.354	0.827	0.553,1.236
Gender(male vs. femal)	0.663	0.917	0.623,1.351	0.474	0.994	0.978,1.010
TNM Stage (I+II vs. III+IV)	<0.000	2.441	1.860,3.204	<0.000	2.124	1.603,2.815
Tumor size(≦5cm vs. >5cm)	<0.000	2.112	1.502,2.970	0.014	1.593	1.100,2.308
Lymphatic metastasis (NO vs. YES)	<0.000	1.577	1.273,1.952	0.86	1.037	0.694,1.549
Grade(Well&Moderate vs. Poor)	0.507	1.144	0.769,1.703	0.248	1.292	0.836,1.995
LINC00460 expression(High vs. Low)	0.013	2.676	1.230,5.823	0.016	2.525	1.185,5.379

HR, hazard ratio; 95 % CI, 95 % confidence interval, * Overall P < 0.05.

Supplementary Table 3. Primers, siRNAs and shRNAs sequence

Primers used for qPCR Sequences (5’ to 3’)

LINC00460 (Forward)	AGAAATCCTCCAGCCCTGTT
LINC00460 (Reverse)	GGGTGACTCTTAGCCGAGAA
GAPDH (Forward)	GAAGAGAGAGACCCTCACGCTG
GAPDH (Reverse)	ACTGTGAGGAGGGGAGATTCAGT
CUL4A (Forward)	ACAACTACACGCAGGACACG
CUL4A (Reverse)	CCTGGACGTGGTCTTCACA
LSD1 (Forward)	AGCGTCATGGTCTTATCAA
LSD1 (Reverse)	GAAATGTGGCAACTCGTC
EZH2 (Forward)	TGCACATCCTGACTTCTGTG
EZH2 (Reverse)	AAGGGCATTCACCAACTCC
P21 (Forward)	AAGTCAGTTCCTTGTGGAGCC

P21 (Reverse)	GGTTCTGACGGACATCCCCA
KLF2 (Forward)	CTGCACATGAAACGGCACAT
KLF2 (Reverse)	CAGTCACAGTTTGGGAGGGG
U1 (Forward)	GGGAGATACCATGATCACGAAGGT
U1 (Reverse)	CCACAAATTATGCAGTCGAGTTTCCC

Primer used for qCHIP analysis Sequences (5’ to 3’)

KLF2(Forward)	ACGGGCTTATTGAGGTTGG
KLF2(Reverse)	GCCTGGGTGACAGAGGAGAC
P21 (Forward)	GCCTTCCTCACATCCTCC
P21 (Reverse)	CAAGAGTGCCCAGTCCAG

Interference sequences (siRNA)

si-LINC00460 1#	UUUGCUGUGAAUUUGAAGUAUGCUG
si-LINC00460 2#	CGUGGGAAAGAAGACGCAUUCUGAA
si-LINC00460 3#	CCACGCCUCUGAAAUGGUGACAAUA
si-NC	UUCUCCGAACGUGUCACGUTT
sh-LINC00460 1#	GAAAGACTGAGCGTGGGAAAG
sh-LINC004602#	GACTGAGCGTGGGAAAGAAGA

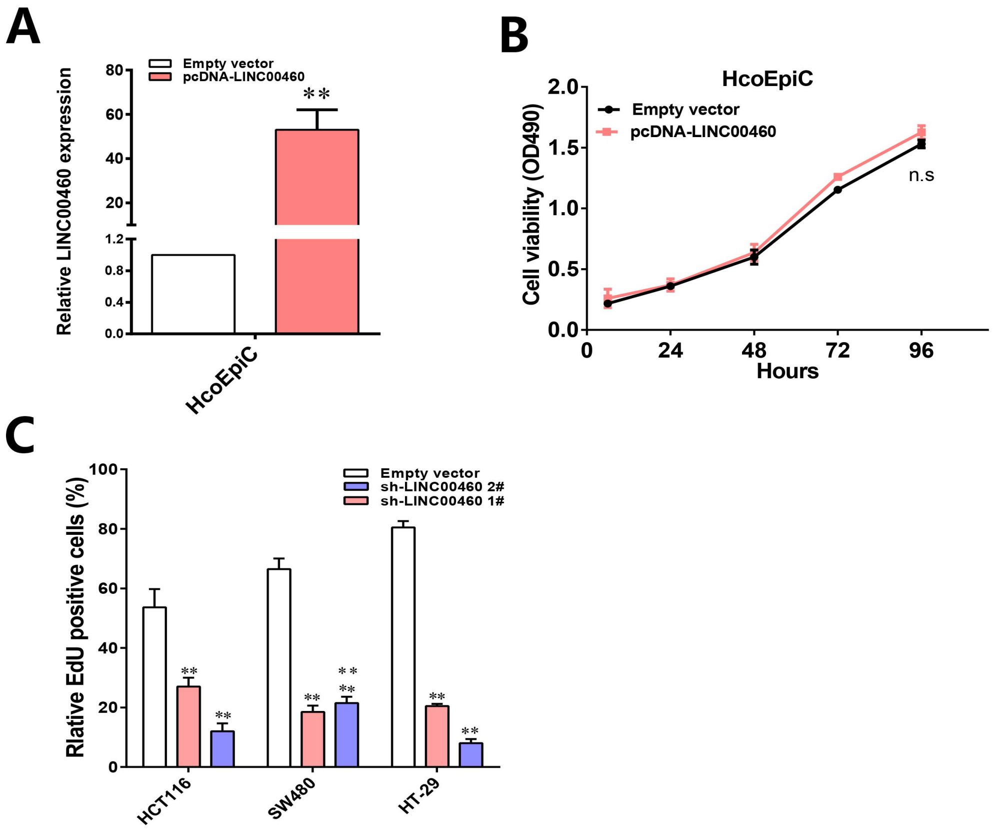


Figure S1. (A) qRT-PCR analysis of LINC00460 expression in HcoEpiC cells after transfection with Empty vector or pcDNA-LINC00460. **(B)** MTT assays were performed to determine the cell viability for pcDNA-LINC00460-transfected HcoEpiC. **(C)** Cell proliferation of HCT116, SW480 and HT-29 was evaluated 48h after transfection with sh-LINC00460 or Empty vector using EdU-incorporation assays. Red: EdU staining of proliferating cells; blue: DAPI staining of the cell nuclei. Quantitative result of TUNEL assay was analyzed. Representative images and data based on three independent experiments. Bars: s.d, $*P<0.05$,$**P<0.01$. n.s., not significant.

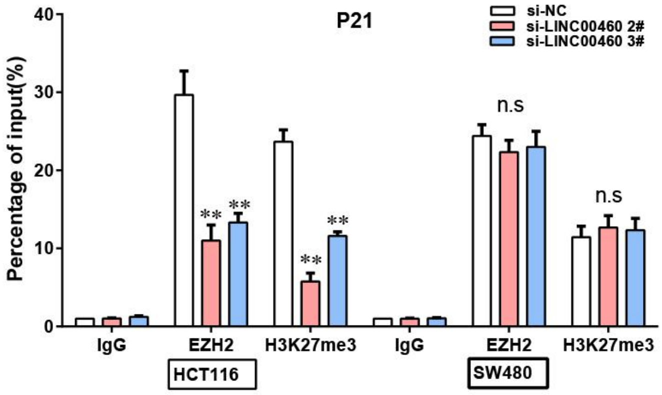


Figure S2. ChIP-qPCR of H3K27me3 and EZH2 of the promoter region of the p21 locus after siRNA treatment targeting si-NC or si-LINC00460 in HCT116 and SW480 cells. Antibody enrichment was quantified relative to the amount of input DNA. Antibody directed against IgG was used as a negative control.*P < 0.05, **P < 0.01. n.s., not significant.

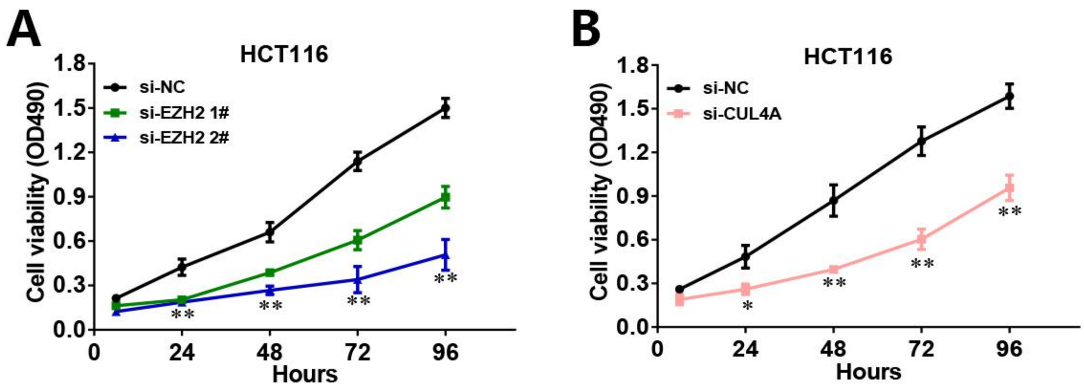
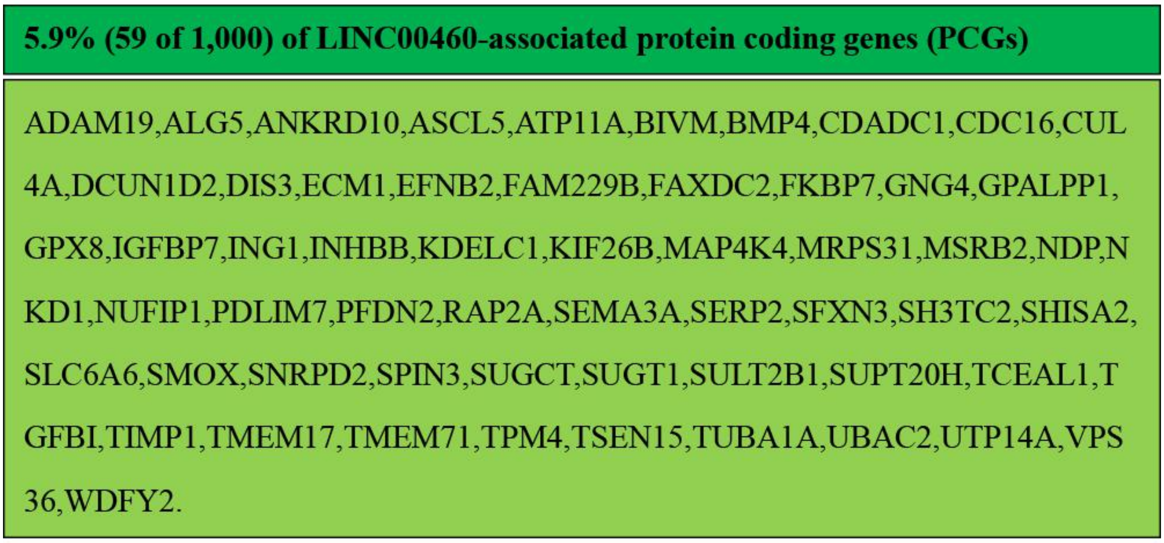


Figure S3. (A) MTT assays were used to determine the cell viability when HCT116 cells were transfected with si-NC, si-EZH2. **(B)** MTT assays were used to determine the cell viability when HCT116 cells were transfected with si-NC, si-CUL4A.



5.9% (59 of 1,000) of LINC00460-associated protein coding genes (PCGs)

ADAM19,ALG5,ANKRD10,ASCL5,ATP11A,BIVM,BMP4,CDADC1,CDC16,CUL4A,DCUN1D2,DIS3,ECM1,EFNB2,FAM229B,FAXDC2,FKBP7,GNG4,GPALPP1,GPX8,IGFBP7,ING1,INHBB,KDELC1,KIF26B,MAP4K4,MRPS31,MSRB2,NDP,NKD1,NUFIP1,PDLIM7,PFDN2,RAP2A,SEMA3A,SERP2,SFXN3,SH3TC2,SHISA2,SLC6A6,SMOX,SNRPD2,SPIN3,SUGCT,SUGT1,SULT2B1,SUPT20H,TCEAL1,TGFBI,TIMP1,TMEM17,TMEM71,TPM4,TSEN15,TUBA1A,UBAC2,UTP14A,VPS36,WDFY2.

Figure S4. 5.9% (59 of 1,000) of LINC00460-associated PCGs were shared by all three microarray profiles (TCGA-CRC, GSE33113, and GSE41328).