Dynamic regulation of eEF1A1 acetylation affects colorectal carcinogenesis

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Supplementary material

Table S1. The relationship between eEF1A1 K439 acetylation expression and clinicopathologic characteristics in CRC patients.

	eEF1A1 K439Ac		*P*-value
Parameters	**High**	**Low**	
Total	65	15	
Gender			
Female	28	6	0.771
Male	39	7	
Age (year)	63.1±13.2	61.6±8.0	0.052
Tumor size (cm)	4.5±1.8	3.9±1.5	0.814
Tumor differentiation			
Poor	12	1	0.615
Moderate-Well	55	12	
T stage			
T1-T2	16	2	0.758
T3-T4	46	10	
N stage			
N0	36	9	0.303
N1-N2	31	4	
Distant metastasis			
M0	58	12	0.392
M1	9	0	
TNM stage			
I-II	33	9	0.100
III-IV	34	3	

$p < 0.05$ was considered statistically significant.

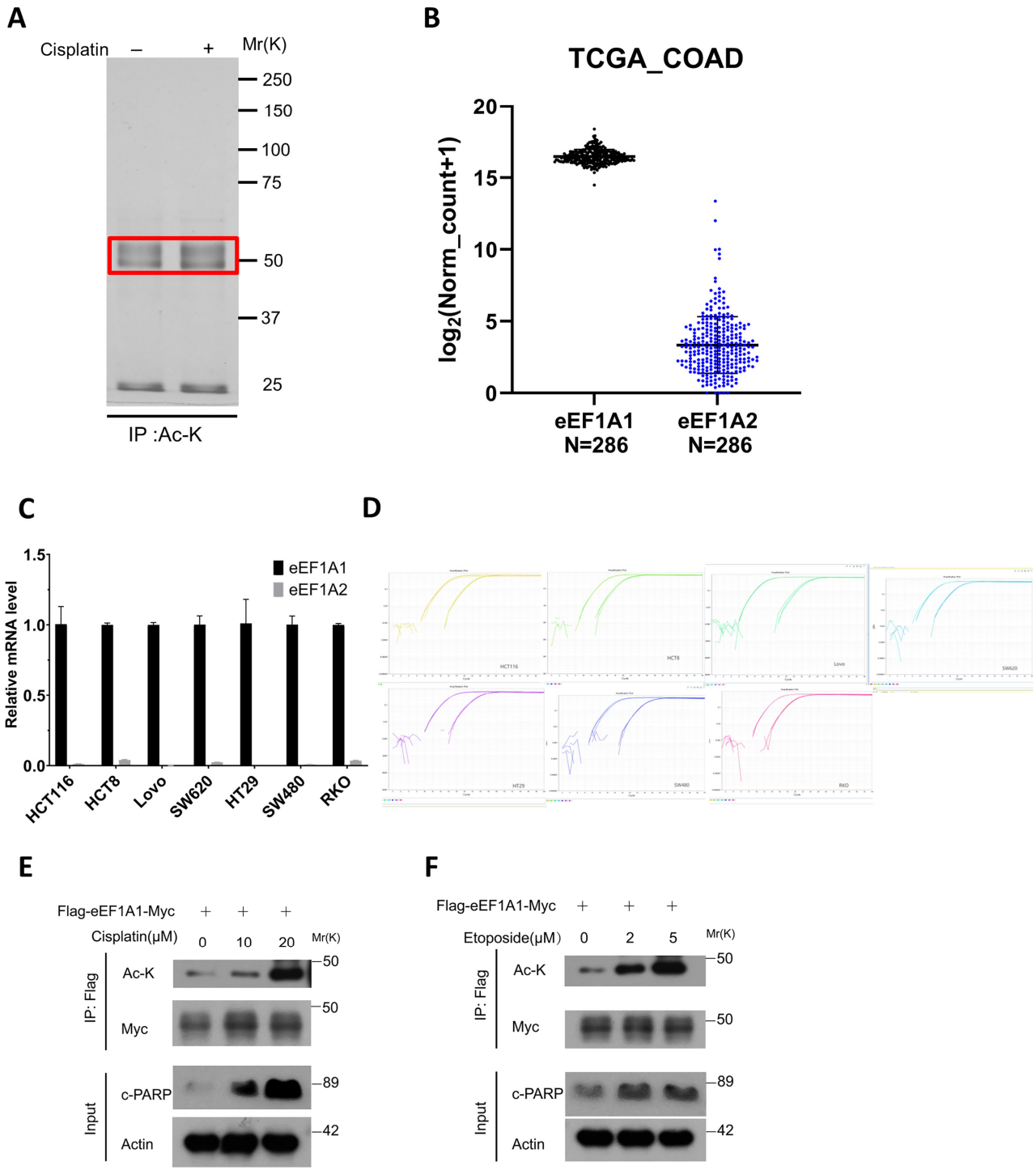

Figure S1. eEF1A1 acetylation may be a common process for CRC cell lines.

(A) SDS-PAGE and Coomassie Blue staining revealed different acetylated proteins with or without cisplatin treatment in HT29 cells. The acetylated proteins were immunoprecipitated by anti-acetylated lysine antibody agarose from HT29 cells (1×10^6) with or without cisplatin (20 μM) treatment. (B) The expression level of eEF1A1 is much higher than eEF1A2 in CRC tissues. Expression levels of eEF1A1 and eEF1A2 from TCGA-COAD database. (C) eEF1A1 was the dominant eEF1A isoform in CRC cell lines. RT-qPCR analysis was performed to evaluate the mRNA levels of eEF1A1 and eEF1A2 in seven CRC cell lines. The experiments

were repeated third, independently, with similar results. (D) Snapshot of RT-qPCR analysis showed the Ct Value of eEF1A1 and eEF1A2 in CRC cell lines. (E) Cisplatin treatment increased eEF1A1 acetylation levels in a dose-dependent manner. 80% confluent HT29 cells were transfected with eEF1A1 (5μg) in 10-cm dishes, and treated with cisplatin (0, 10, and 20 μM) for 8 h. Western blot analysis was performed to detect the acetylation levels of immunoprecipitated eEF1A1. The experiments were repeated third, independently, with similar results. (F) Etoposide treatment increased eEF1A1 acetylation levels in a dose-dependent manner. 80% confluent HT29 cells were transfected with eEF1A1 (5 μg) in 10-cm dishes, and treated with etoposide (0, 2, and 5 μM) for 8 h. Western blot analysis was performed to detect the acetylation levels of immunoprecipitated eEF1A1. The experiments were repeated third, independently, with similar results.

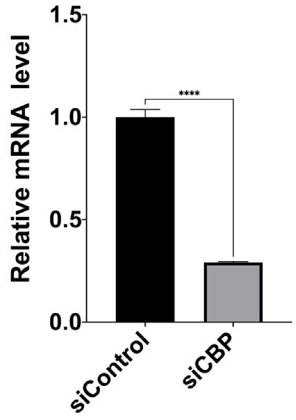

Figure S2. The knock-downing efficiency of the CBP siRNA.

Data are mean ± SD of n = 3. *p* values were calculated using unpaired, two tailed Student's t-test. ****$p < 0.0001$. The experiments were repeated third, independently, with similar results.

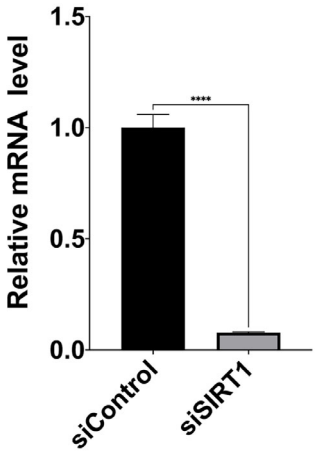

Figure S3. The knock-downing efficiency of the SIRT1 siRNA.

Data are mean ± SD of n = 3. *p* values were calculated using unpaired, two tailed Student's t-test. ****$p < 0.0001$. The experiments were repeated third, independently, with similar results.

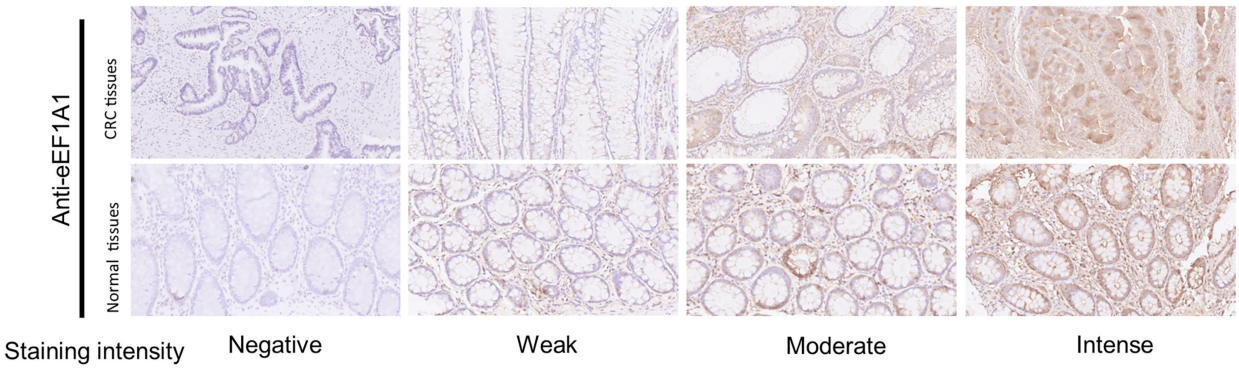

Figure S4. The representation of staining intensity of eEF1A1 antibody.

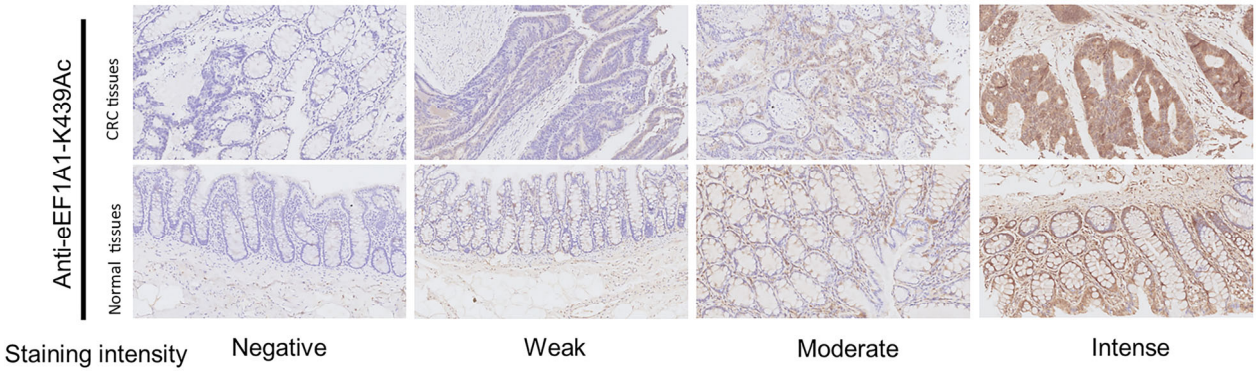

Figure S5. The representation of staining intensity of eEF1A1-K439Ac antibody.