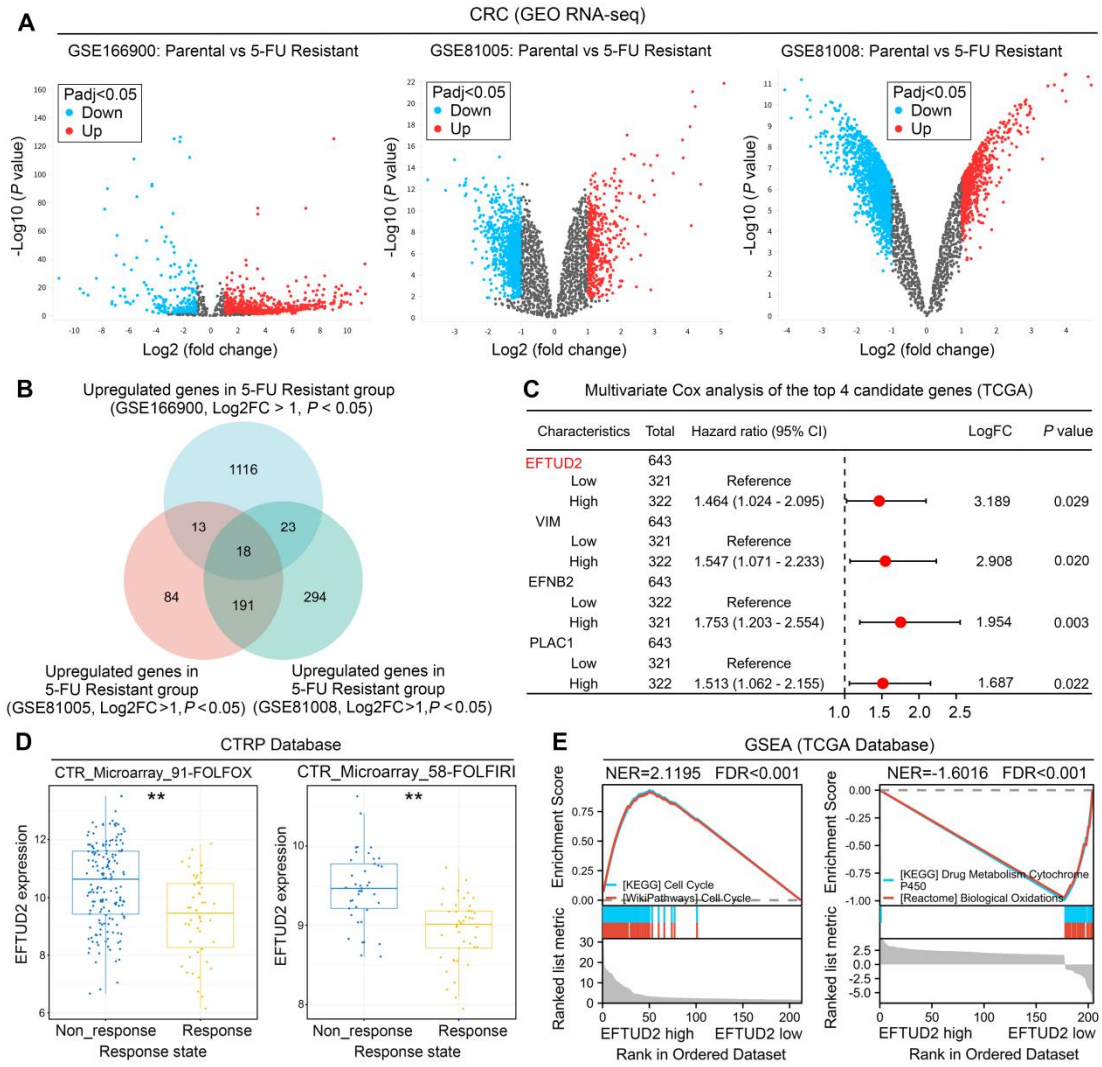

Fig.S1 EFTUD2 is significantly up-regulated in 5-FU chemotherapy-resistant cells of CRC, related to Fig.1. A The differential gene expression analysis was conducted between 5-FU resistant and parental cell lines. The volcano plot depicts the distribution of differentially expressed genes, with red and blue dots representing significantly up-regulated and down-regulated genes, respectively. **B** Venn diagram displaying the overlap of RNA-seq up-regulated gene results between 5-FU resistant and parental cell lines. The plot highlights the 18 up-regulated genes that were found to be common across all three datasets. **C** Multivariate Cox regression analysis evaluating the association between 18 candidate genes and overall survival (OS) in CRC patients. The forest plot presents the hazard ratios and 95% confidence intervals

for the expression of the top 4 genes, namely *EFTUD2*, *VIM*, *EFNB2*, and *PLAC1*, in CRC patients. **D** Differential expression analysis of EFTUD2 in the responsive and non-responsive groups to FOLFOX and FOLFIRI chemotherapy regimens. The box plot displays the distribution of EFTUD2 expression levels in the two groups. Unpaired t-test was used for statistical analysis. **E** GSEA of EFTUD2 expression in CRC. The GSEA plot illustrates the correlation of EFTUD2 expression with the Cell Cycle and Drug Metabolism Cytochrome P450 pathways.

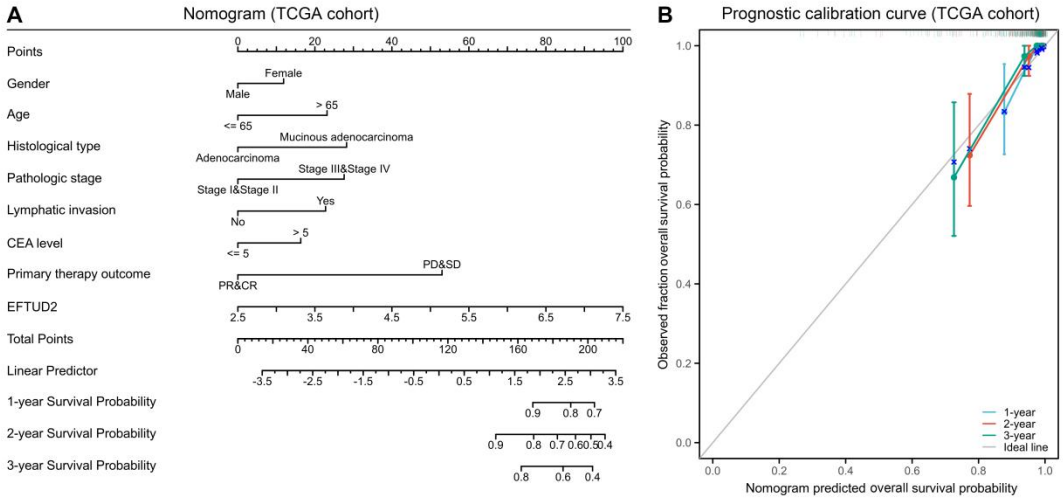

Fig.S2 High EFTUD2 expression was a potential independent predictor of poor prognosis in CRC, related to Fig.2. A Nomogram-related model for predicting survival probability in CRC patients based on Cox regression analysis. Nomogram plot showing the contribution of EFTUD2 expression to the OS probability. **B** Line graph calibration evaluation analysis was conducted to depict the differences between predicted and actual survival rates of the model at different time points (1 year, 2 years, and 3 years).

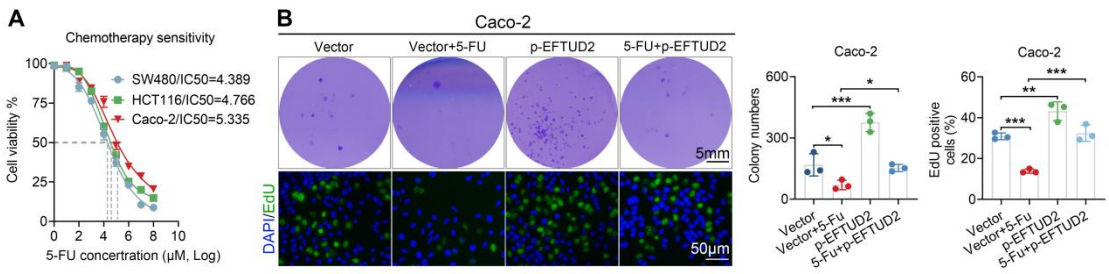

Fig.S3 EFTUD2 attenuates the chemotherapy efficacy of 5-FU *in vitro*, related to Fig.3. A Viability assay of CRC cells in response to varying concentrations of 5-FU. The inhibition curves were fitted using nonlinear regression, and the 5-FU IC50s were calculated using GraphPad Prism 8 software. **B** Effect of EFTUD2 overexpression on the proliferation of SW480 cells treated with 5-FU. The clone formation and EdU assays were conducted to examine the impact of EFTUD2 overexpression on the proliferation of SW480 cells treated with 5-FU. The results are presented as mean ± SD, n= 3, and analyzed using one-way ANOVA. Statistical significance is indicated as $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

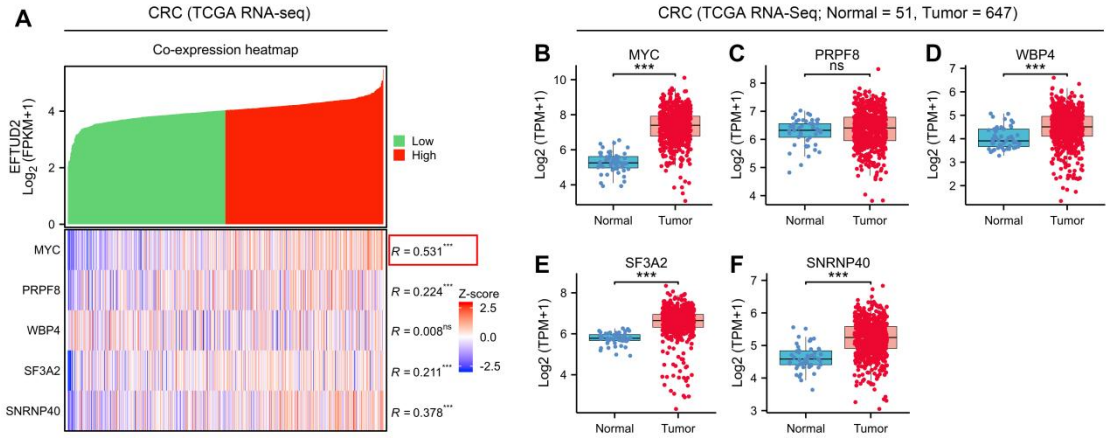

Fig.S4 Correlation analysis of interacting proteins with EFTUD2, related to Fig.5. A Heatmap depicting the co-expression between protein molecules. **B-F** mRNA expression levels of MYC, PRPF8, WBP4, SF3A2, and SNRNP40 in CRC tissues compared with normal tissues based on TCGA database analysis. The ns denotes no statistically significant difference. Statistical significance is indicated as $***P < 0.001$.

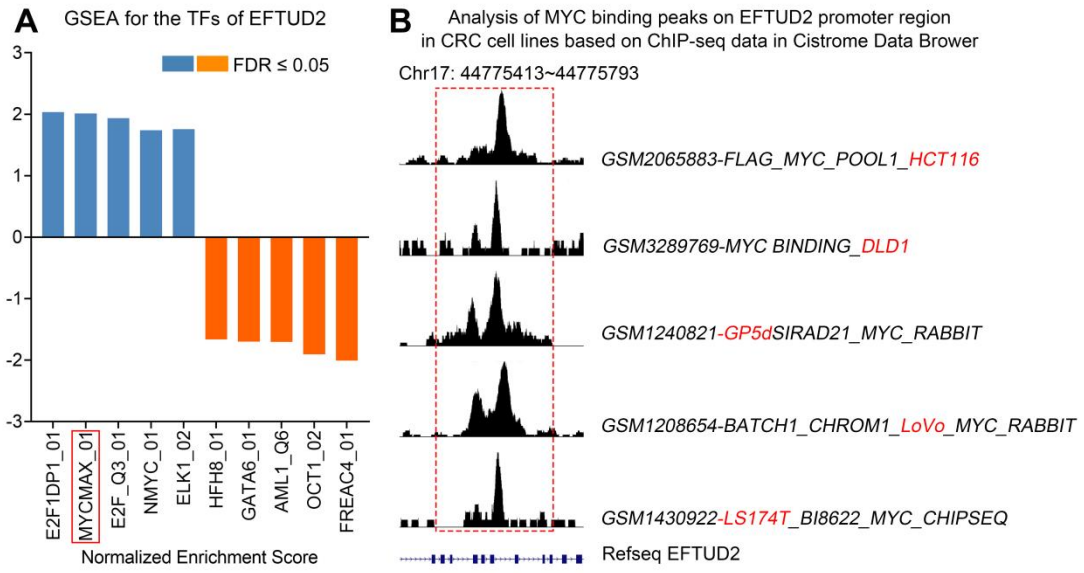

Fig.S5 c-MYC promotes the transcriptional expression of EFTUD2, related to Fig.7. A Bar chart of GSEA of TFs upstream of EFTUD2. **B** Analysis of MYC binding peaks on the EFTUD2 promoter region in CRC cell lines based on ChIP-seq data in Cistrome Data Browser.

Supplementary Table 1. Correlation Analysis of EFTUD2_RNA Molecule with Sensitivity to Common Chemotherapeutic Drugs in CRC

Compound	RNAmolecule	Omics	Correlation R	Spearman. FDR
5-Fluorouracil	EFTUD2	Expression	-0.418	$P = 0.004$
Capecitabine	EFTUD2	Expression	-0.420	$P < 0.001$
Cisplatin	EFTUD2	Expression	-0.363	$P = 0.007$
Irinotecan	EFTUD2	Expression	-0.406	$P < 0.001$
Oxaliplatin	EFTUD2	Expression	-0.283	$P < 0.001$

Supplementary Table 2. Relationship between EFTUD2 expression and clinicopathological characteristics of CRC patients (TCGA cohort)

Characteristics	Low expression of EFTUD2	High expression of EFTUD2	*P* value
n	322	322	
OS event, n (%)			**0.004**
Alive	272 (42.2%)	243 (37.7%)	
Dead	50 (7.8%)	79 (12.3%)	
Anatomic neoplasm subdivision, n (%)			0.514
Ascending Colon&Descending Colon	55 (13.1%)	53 (12.6%)	
Rectum&Sigmoid Colon&Transverse Colon	148 (35.2%)	165 (39.2%)	
Primary therapy outcome, n (%)			**0.004**
PD&SD	10 (4.4%)	28(8.9%)	
PR&CR	141 (45.2%)	133 (42.6%)	
Histological type, n (%)			**0.015**
Adenocarcinoma	266 (42%)	284 (44.9%)	
Mucinous adenocarcinoma	52 (8.2%)	31 (4.9%)	
Pathologic stage, n (%)			**0.003**
Stage I&Stage II	175 (28.1%)	138 (22.2%)	
Stage III&Stage IV	136 (21.8%)	174 (27.9%)	
Gender, n (%)			0.580
Female	147 (22.8%)	154 (23.9%)	
Male	175 (27.2%)	168 (26.1%)	
Age, n (%)			**0.017**
<= 65	123 (19.1%)	153 (23.8%)	
> 65	199 (30.9%)	169 (26.2%)	
Pathologic T stage, n (%)			0.970
T1&T2	65 (10.1%)	66 (10.3%)	
T3&T4	254 (39.6%)	256 (39.9%)	
Pathologic N stage, n (%)			0.873
N0	183 (28.6%)	185 (28.9%)	
N1&N2	137 (21.4%)	135 (21.1%)	
Pathologic M stage, n (%)			0.440
M0	240 (42.6%)	235 (41.7%)	
M1	41 (7.3%)	48 (8.5%)	
Lymphatic invasion, n (%)			0.785
No	177 (30.4%)	173 (29.7%)	
Yes	120 (20.6%)	112 (19.2%)	
CEA level, n (%)			0.456
<= 5	124 (29.9%)	137 (33%)	
> 5	79 (19%)	75 (18.1%)	