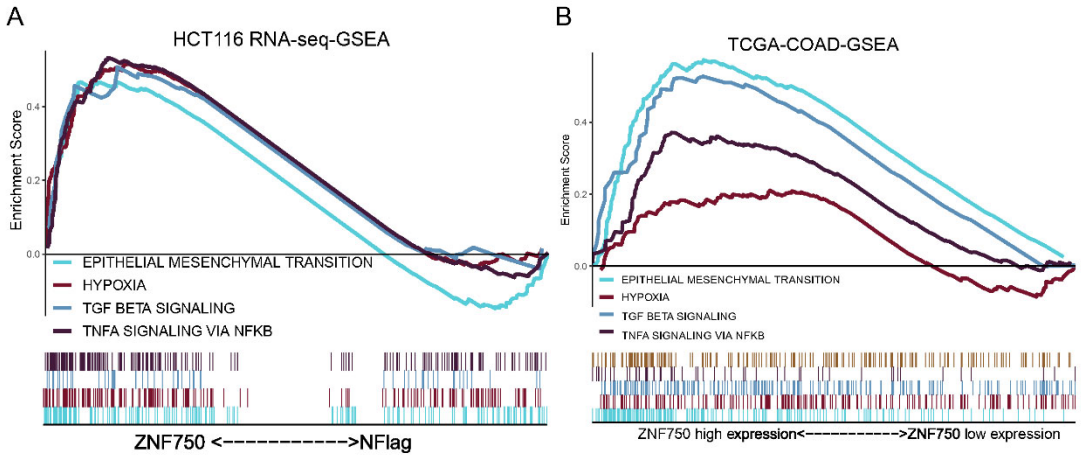

Fig. S1 GSEA analysis of RNA-seq in ZNF750-overexpressing HCT116 cells versus the control group (A), or of samples in the high and low ZNF750 groups in cohorts TCGA-COAD (B).

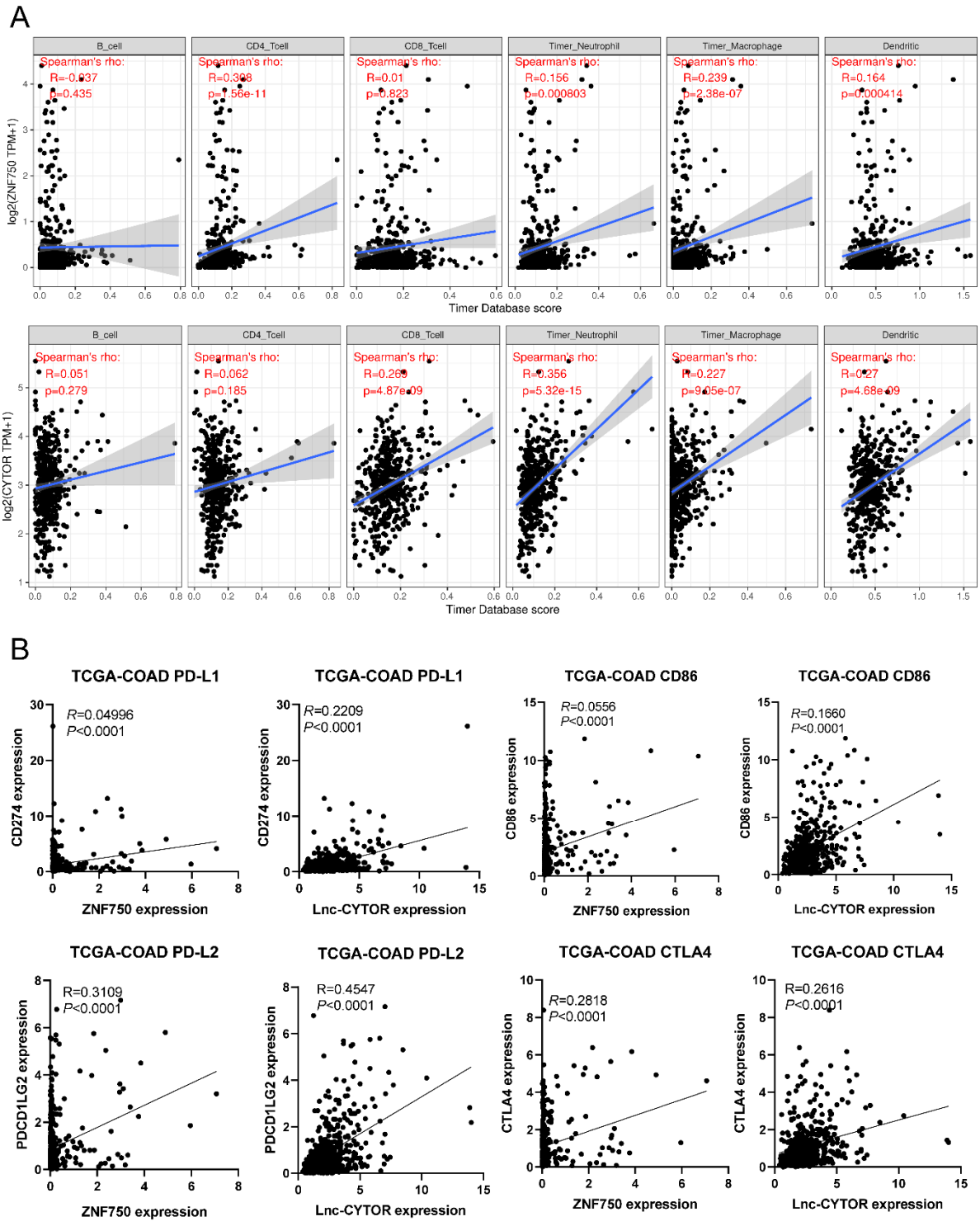

Fig. S2 Immunotherapeutic response related-factor analysis in the cohort TCGA-COAD. (A)Spearman correlation of ZNF750 or *CYTOR* with the abundance of TILs in COAD (TIMER). (B) Spearman correlation between *PD-L1*, *CD86*, *PD-L2*, *CTLA4* and ZNF750 or *CYTOR* expression in COAD.

Materials and methods

1.1 Cell culture

SW620 cells were purchased from Chinese Academy of Sciences (CAS, China) and were cultured in 1640 medium at 37 ℃ without CO2. 293T cells (CAS, China) and were maintained in DMEM medium at 37 ℃ with 5% CO2. Mediums for conventional culture were supplemented with 10% fetal bovine serum (FBS).2.1.2 Resolution

1.2 Western blotting

Proteins were extracted from SW620 cells via lysis buffer（RIPA Beyotime, China）supplementing with 1% protease & phosphatase inhibitor (Roche, USA). Protein samples were then qualified, separated, transferred onto the PVDF membranes, blocking and incubated with primary antibody and corresponding secondary antibody as mentioned previously(Xia et al., 2021). The primary antibodies were: ZNF750 (1:1,000; Sigma-Aldrich, USA), β-actin (1:1000; Abcam, UK). The secondary antibody is anti-rabbit-HRP conjugated (1:10000) and anti-rabbit-HRP conjugated (1:10000). The protein bands were visualized using chemiluminescence system.

1.3 Cell proliferation assay

Cells at a density of 4000 cells per well were plated onto a CellCarrier 96-well plate (PerkinElmer, USA). Imaging and analysis were conducted as previously reported(Lian et al., 2019).

1.4 Cloning formation assay

200 cells were seeded in 6-well plates, when the clones are well formed (which takes 14 days or longer time), fixed with 4% paraformaldehyde for 5 min, and stained with 0.1% crystal violet for 15 min. Excess crystal violet were moved by rinsing with running water.

1.5 Transwell assay

Transwell chambers (8 μm pore size, Corning) were placed into 24-well plates with or without BD Matrigel Matrix (BD Biosciences, USA). The cell density was adjusted to 1×105 cells/mL, and 200 μL of cell suspension in serum-free medium was added to the upper well. About 600 μL of medium containing 10% FBS was added to the lower chamber and incubated at 37 °C for 24 h. The remaining cells on the upper surface of the membrane were wiped off with a wet cotton swab. The cells on the lower surface of the membrane were immobilized with 4% paraformaldehyde for 30 min, stained with 0.5% crystal violet for 15 min. The fields of were photographed and counted under a microscope with 200 time magnification.

1.6 Xenograft models in immunodeficient mouse

Seven weeks after injection of cells (transfected with empty vector or pLV-CS2.0-NFLAG-ZNF750 respectively), the mice were sacrificed and the tumors were measured. These procedures had been approved by the Institutional Animal Care and Use Committee of Xiamen University.

1.7 ChIP-qPCR

Cells were fixed with 1% formaldehyde and subsequently quenched with glycine. After washing with PBS, the fixed samples were suspended in cell lysis buffer (1%SDS, 12.5mM EDTA pH 8.0, 50mM Tris-Cl pH 7.8, and 1X proteinase inhibitor cocktail). For ZNF750 ChIP, lysates were sonicated with Sonifier (Bioruptor, Belgium) to obtain chromatin fragments of 150-300 bp, and 10 folds diluted with ChIP dilution buffer (20mM Tris-Cl pH 7.8, 2mM EDTA pH 8.0, 1.0% TritonX100, and 1X proteinase inhibitor cocktail). The diluted samples were incubated with ZNF750 and IgG antibodies overnight. Immunoprecipitated complexes were collected using protein 60μl protein A/G magnet beads (MCE, USA) at 4°C for 6h. Subsequently, beads were washed

sequentially with low-salt buffer(0.1%SDS, 1.0% TritonX100, 2mM EDTA pH 8.0, 20mM Tris-Cl pH 7.8, 150mM NaCl and 1X proteinase inhibitor cocktail), high-salt buffer(0.1%SDS, 1.0% TritonX100, 2mM EDTA pH 8.0, 20mM Tris-Cl pH 7.8, 400mM NaCl and 1X proteinase inhibitor cocktail), and twice with TE buffer (20mM Tris-Cl pH 7.4, 1mM EDTA pH 8.0), and then eluted in 300 μl of TE/1% SDS buffer. The eluates and input sample (1/30 lysates) were incubated in Eppendorf Thermomixer at 65°C, 1400rpm overnight to reverse the cross-linking. Next, all samples were extracted and purified. Subsequently, quantitative-PCR was conducted with AriaMx Real-Time PCR System (Agilent, USA) according to the recommendations of the manufacturer of the SYBR Green PCR Mix (Takara, Japan), the primers used are listed in Table S1.

1.8 RNA isolation and reverse transcription

TRIzol (Invitrogen, USA) was used to extract RNA from cells stably transfected with pLV-CS2.0-NFlag-ZNF750 or pLV-CS2.0-NFlag control vector following the manufacturer's procedure. The cDNA libraries was constructed using a reverse transcriptase kit (Takara, Japan) along with random primers.

1.9 Quantitative real-time PCR and RNA sequencing

Quantitative real-time PCR was performed in a Applied Biosystems ViiA™ 7 Real-Time PCR System (Life, USA) using the SYBR Green PCR Mix. The relative expression of ZNF750 and *CYTOR* were quantified using the $2^{-\triangle\triangle CT}$ method, normalizing to GAPDH levels. The primers used are listed in Table S1. RNA sequencing and data analysis were conducted as previously described(Xia, et al., 2021).

1.10 TCGA and GEO dataset analysis

RNA sequencing data and clinicopathological data for 473 colon adenocarcinoma patients were downloaded from the TCGA data portal (portal.gdc.cancer.gov/) as the cohort TCGA-COAD. GSE39582 containing RNA sequencing and the clinicopathological datasets for 585 colon adenocarcinoma (COAD) patients were downloaded from GEO (www.ncbi.nlm.nih.gov/geo/). Gene set enrichment analysis (GSEA) of the TCGA-COAD dataset and RNA-seq data were performed using GSEA Desktop v3.0 software. The TCGA-COAD dataset was divided into high and low ZNF750 expression groups based on quartile values. In the TCGA-COAD dataset, screen the significant differential long non-coding RNAs (lncRNAs) between ZNF750 high expression and low expression groups($|FC|>1$, $P<0.05$), and screen the lncRNAs that are significantly related to ZNF750 through Pearson correlation analysis ($P<0.05$). TIMER was applied to evaluate the relevance between ZNF750 expression and tumor-infiltrating immune cells in COAD (timer.cistrome.org/). The X-tile was used to calculate the optimal cut-off values of survival analysis for ZNF750 and *CYTOR*.

1.11 Statistical analysis

Two-tailed student's t-test was used to verify the significance of difference between groups. Chi-square test was used to examine the association between ZNF750 or *CYTOR* expression levels and clinical pathological factors. Overall survival curves were calculated according to the Kaplan-Meier method using the log-rank test. $P<0.05$ was considered to be statistically significant. The statistical analysis was performed using SPSS 20.0 unless otherwise mentioned.

Table S1 Primers of different genes

For ChIP-qPCR, related to Figs. 4c and 4d	
CHIP-*CYTOR*-1-F:	TTACGTCCTTTCCTTTTCCTTAACG
CHIP-*CYTOR*-1-R:	AATACCTATCAAGATCAAGTCAGGC
CHIP-*CYTOR*-2-F:	AAAACTCTGTTGATGTGGAATCAGA
CHIP-*CYTOR*-2-R:	GGGTGTCTAAGTAATGATGATGACT
CHIP-*CYTOR*-3-F:	TCAACTTTTCAATAAACACACAGGC
CHIP-*CYTOR*-3-R:	TCAAATATTGGCAAGCAACAAACAT
For RT-qPCR, related to Fig. 4e	
ZNF750-F:	GCCTTCAGGCCTGTTAAGAA
ZNF750-R:	GGCTGCCAGGTTTATCTCTG
CYTOR-F:	ATGCCCAAAGTTACGGAGGA
CYTOR-R:	TTGGAATGTGGATGGAGGCT
GAPDH-F:	CGGAGTCAACGGATTTGGTCGTAT
GAPDH-R:	AGCCTTCTCCATGGTGGTGAAGAC