Estrogen receptor beta (ERβ) mediated-CyclinD1 degradation via autophagy plays an anti-proliferation role in colon cells

Supplementary Materials and Methods

Cell culture and treatments

The normal human colon mucosal epithelial cell line (NCM460), human colon adenocarcinoma cell line (HCT116) and mouse embryonic fibroblasts (MEFs) were cultivated in DMEM medium supplemented with 10% (v/v) Fetal Bovine Serum (FBS, Biological Industries) and 1% penicillin/streptomycin (Gibco, USA). All cells were routinely cultured in a humidified atmosphere of 5% CO_2 at 37°C incubator.

Western blotting

Cellular protein was extracted with RIPA buffer (Beyotime, China) supplemented with complete protease inhibitor mixture (Roche, Mannheim). Subsequently, western blot analysis was performed as the previous description [1]. The primary antibodies used include: ERβ (SAB2702145), LC3B (L7543), and β-actin (A1978) from Sigma-Aldrich; mTOR (#2972), Phospho-mTOR (#5536), ATG7 (#8558), PTEN (#9552), AKT (#9272), Phospho-Akt (#4060), Beclin1 (#3738), Phospho-Beclin1 (#84966), ULK1 (#4776) and Phospho-ULK1 (#14202) from Cell Signaling technology (San Diego, USA), CyclinD1 (ab134175) from Abcam Biotechnology (Cambridge, MA, USA); Caspase-3 (19677-1-AP), CyclinD1 (60186-1-Ig), p62/SQSTM1 (18420-1-AP) and ERβ (14007-1-AP) from Proteintech (Wuhan, China); LAMP2 (A14017) and BNIP3 (A5683) from Abclonal (Wuhan, China);

peroxidase-conjugated immunopure goat anti-rabbit and anti-mouse IgG (HL) from Abclonal. Enhanced chemiluminescence (ECL) horseradish peroxidase (HRP) was used to visualize protein bands. NIH Image J software was used to measure the intensity of the bands.

References

[1] N. Zheng, P. Yuan, C. Li, et al., Luteolin Reduces BACE1 Expression through NF-κB and through Estrogen Receptor Mediated Pathways in HEK293 and SH-SY5Y Cells, J Alzheimers Dis 45 (2015) 659-671.

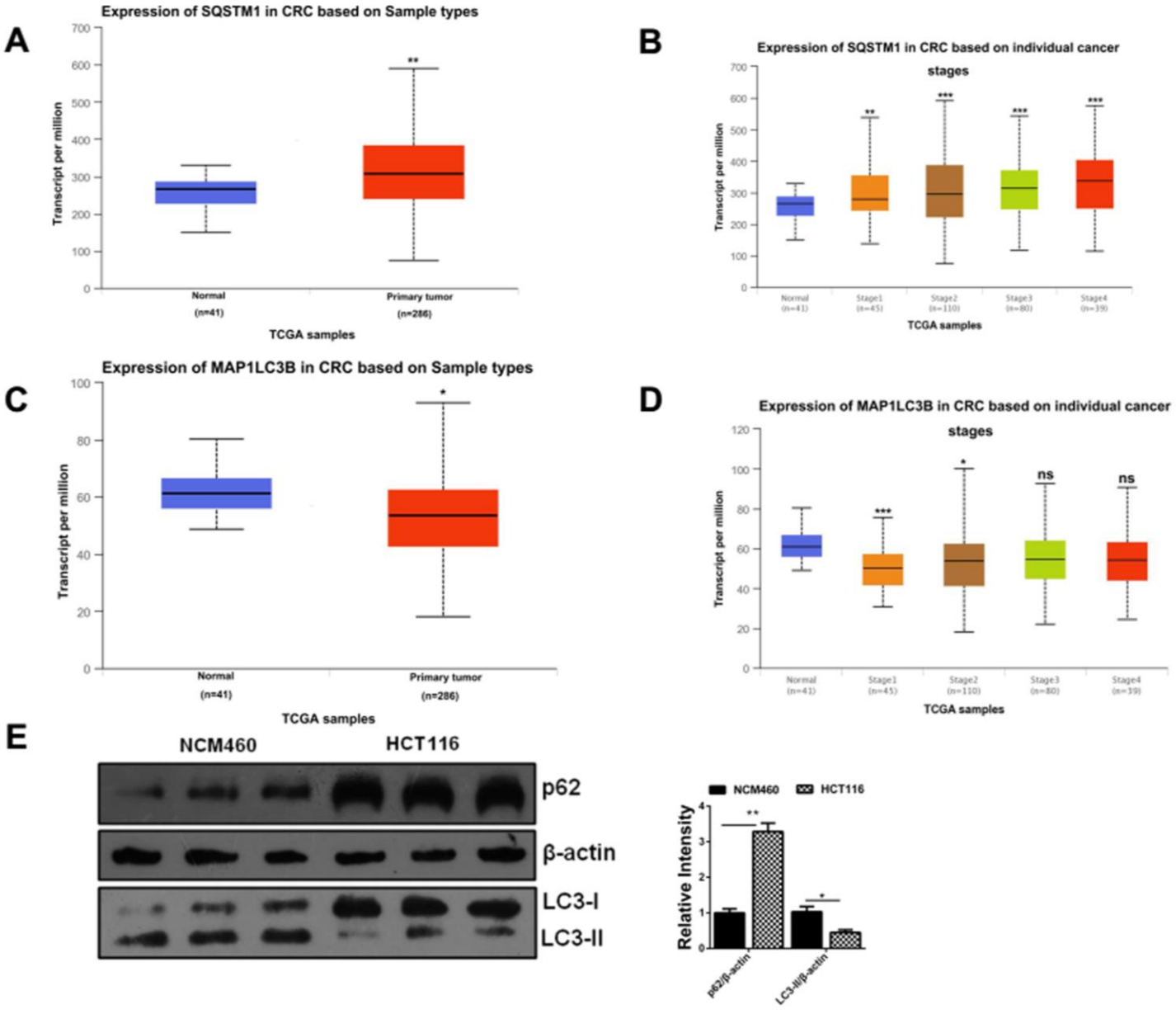
Supplementary Figure Legends

Figure S1. Autophagy level in normal and colorectal cancer. (A) Boxplot showing relative expression of SQSTM1 in TCGA samples (normal vs primary tumor). **(B)** Boxplot showing relative expression of SQSTM1 in TCGA samples. (normal vs stage 1-4 colorectal cancer patients). **(C)** Boxplot showing relative expression of MAP1LC3B in TCGA samples (normal vs primary tumor). **(D)** Boxplot showing relative expression of MAP1LC3B in TCGA samples. (normal vs stage 1-4 colorectal cancer patients). **(E)** Western blot analysis of p62 and LC3-II expression in NCM460 and HCT116. Bar graph (right) indicates the relative ratio of LC3-II and p62 to β-actin of triplicate experiments in NCM460 and HCT116. Western blot was quantified using ImageJ software. Data shown are mean ± S.D. of three independent experiments. (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

Figure S2. mTOR-related signals in HCT116 cells. (**A)** Western blot analysis of mTOR and p-mTOR expression in NCM460 and HCT116. Bar graph (right)

indicates the relative ratio of mTOR and p-mTOR to β-actin of triplicate experiments in NCM460 and HCT116. (**B)** Overexpression of ERβ and ATG7 in HCT116 cells for 48 h reduced the level of p-mTOR and p-AKT and increased the expression of LC3-II, PTEN, p-Beclin1, and p-ULK1. Bar graph (right) indicates the relative ratio of p-mTOR/m-TOR, p-ULK1/ULK1, p-Beclin1/Beclin1, p-AKT/AKT and PTEN/β-actin of triplicate experiments in HCT116. Western blot was quantified using ImageJ software. Data shown are mean ± S.D. of three independent experiments. (*, $P<0.05$; **, $P<0.01$).

Supplementary Figure 1



Supplementary Figure 2