Supplementary Figure Legends

Supplementary Figure 1

(A)Growth inhibition of SW480 cells by Celastrol treatment.

(B&C)Representative macroscopic images of colon tumors in AOM/DSS mice. The numbers of colon tumors (B) and the body weights of the mice (C) were analyzed. Values are means ± S.E.M. AOM/DSS mice(n=4), AOM/DSS mice treated with Celastrol(1mg/kg)(n=5), AOM/DSS mice treated with 5-Fu(30mg/kg)(n=5).

Supplementary Figure 2

(A) Celastrol-induced β-catenin down-regulation was in a dose- and time-dependent manner in SW480 and HCT116 cells. β-actin was used as a loading control.

(B)The β-catenin transcriptional activity were detected by luciferase reporter assay. Values are means ± S.E.M.*P<0.05.

(C)Real-time PCR analysis of β-catenin target gene(c-Myc, BIRC5, CYR61 and Cyclin D1)in SW480 and HCT116 cells treated with or without Celastrol(0.75μM). Values are means ± S.E.M. (n=3).*P<0.05, **P<0.01, ***P<0.001.

(D)Western blot analysis of β-catenin and its target gene c-Myc and survivin protein in SW480 and HCT116 cells treated with or without Celastrol(0.75μM). β-actin was used as a loading control.

(E)Real-time PCR analysis of β-catenin and its target gene(BIRC5, c-Jun) in intestine tumor tissues of $APC^{Min/+}$ mice and in colon tumor tissues of AOM/DSS mice. Values are means ± S.E.M. (n=3).*P<0.05, **P<0.01, ***P<0.001.

Supplementary Figure 3

(A)Celastrol(0.5μM) and DMSO were added to T-Rex-293/β-catenin(S37A) cells in the absence and presence of Dox for 24h. β-catenin and cleaved PARP were detected. β-actin was used as a loading control.

(B)The colony formation of SW480, SW480/β-catenin(S37A) and HCT116,

HCT116/β-catenin(S37A) cells treated with or without Celastrol(0.75μM), respectively. Values are means ± S.E.M. (n=3).*P<0.05, **P<0.01, ***P<0.001.

(C)Western blot analysis of β-catenin and YAP protein in cytoplasmic and nuclear fractions from SW480 cells. β-actin was used as a loading control for cytoplasmic protein and LaminB was used as a nuclear loading control .

(D)SW480 cells transfected with plasmids expressing wide-type or mutant(S127A) YAP, control or YAP-specific siRNA for 24h,and then the cells were treated with or without Celastrol(0.75μM) for another 24h, respectively. TUNEL assay was used to detect the apoptotic phenotype and the ratio of apoptotic cells was quantified. Values are means ± S.E.M. (n=3).**P<0.01.

Supplementary Figure 4

(A)The effect of cycloheximide(CHX)(10μg/ml) alone or in combination with Celastrol(0.75μM) on LKB1 expression were evaluated in SW480 and HCT116 cells at indicated time. β-actin was used as a loading control.

(B)The effect of MDL-28170(50μM)/PS-341(100nM) alone or in combination with Celastrol(0.75μM) on LKB1 expression were evaluated in SW480 and HCT116 cells, respectively. β-actin was used as a loading control.

Supplementary Figure 5

Quantification of the Western blots in Figure 1 and Figure 2. Intensity was quantified and normalized to β-actin. For IP assay, intensity was quantified and normalized to the corresponding control IgG.

Supplementary Figure 6

Quantification of the Western blots in Figure 3 and Figure 4. Intensity was quantified and normalized to β-actin. For IP assay, intensity was quantified and normalized to the corresponding control group.

Supplementary Figure 7

Quantification of the Western blots in Figure 5 and Figure 6. Intensity was quantified and normalized to β-actin. For IP assay, intensity was quantified and normalized to the corresponding control group.