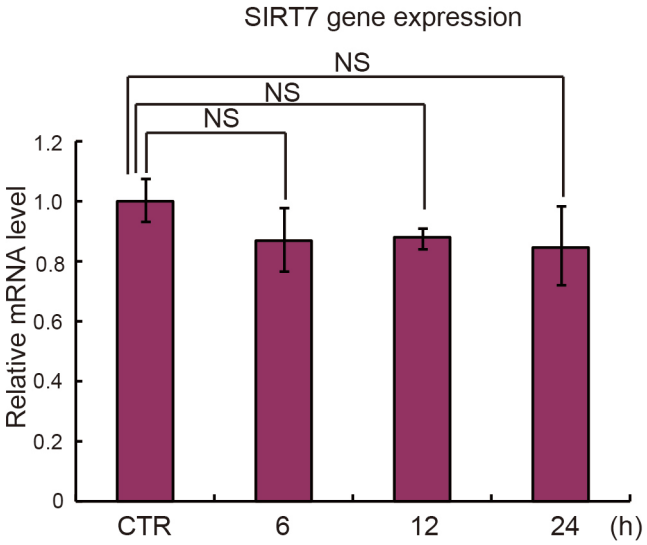
Supplementary Figure 1. *SIRT7* mRNA expression remains unchanged in 5-FU-treated HCT116 cells

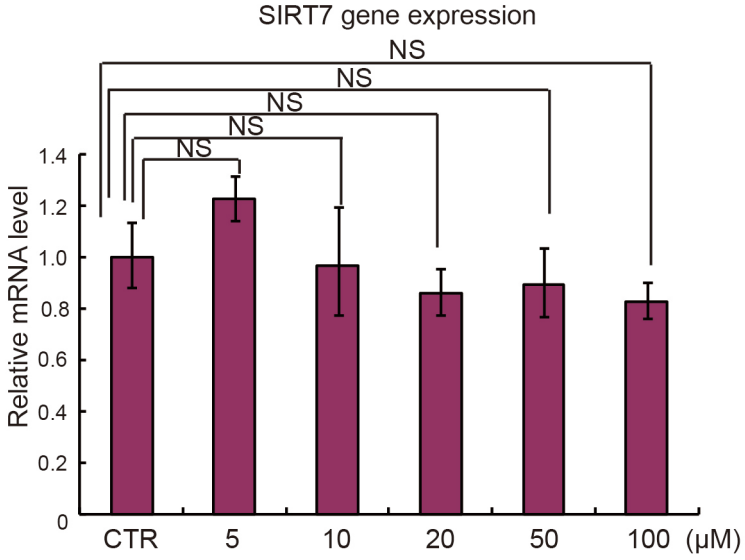
A. *SIRT7* mRNA levels in HCT116 cells following 100 μM 5-FU treatment for different periods of time (6, 12, 24 h), detected by real-time PCR.

B. *SIRT7* mRNA levels in HCT116 cells following 5-FU treatment at 5, 10, 20, 50 or 100 μM for 24 h.

A



B



Supplementary Figure 2. SIRT7 interacts with PSMC1 and PSMC2, but knockdown of PSMC1, PSMC2 does not block the 5-FU induced degradation of SIRT7.

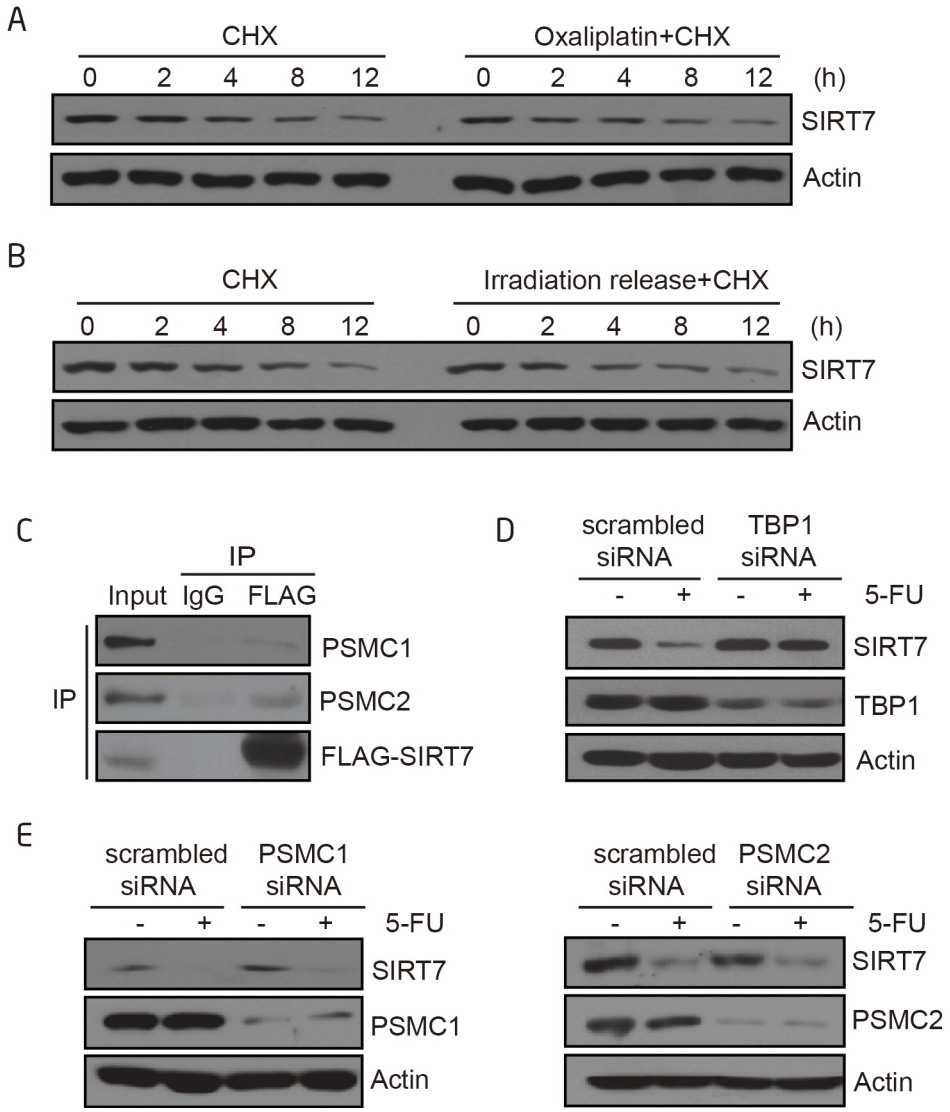
A. Immunoblots showing SIRT7 levels in HCT116 cells treated by 100 μg/ml cycloheximide (CHX) with or without 10 μM oxaliplatin for the indicated time.

B. Immunoblots showing SIRT7 levels in HCT116 cells treated by 100 μg/ml cycloheximide (CHX) with or without 10 Gy irradiation for the indicated time.

C. Exogenous interaction between SIRT7 and PSMC1 or PSMC2. HCT116 cells were transfected with a FLAG-tagged SIRT7 as indicated, and cells lysates were subjected to immunoprecipitation with anti-FLAG antibody and probed with anti-PSMC1, PSMC2 antibody.

D. Immunoblots to detect changes of SIRT7 levels treated with 100 μM 5-FU for 24 h following TBP1 knockdown (TBP1 siRNA) in LoVo cell line.

E. Immunoblots to detect changes of SIRT7 levels treated with 100 μM 5-FU for 24 h following PSMC1 or PSMC2 knockdown (PSMC1 siRNA or PSMC2 siRNA) in HCT116 cell line.


A
CHX
Oxaliplatin+CHX
0 2 4 8 12 0 2 4 8 12 (h)
SIRT7
Actin
B
CHX
Irradiation release+CHX
0 2 4 8 12 0 2 4 8 12 (h)
SIRT7
Actin
C
IP
Input IgG FLAG
IP
PSMC1
PSMC2
FLAG-SIRT7
D
scrambled siRNA
TBP1 siRNA
- + - + 5-FU
SIRT7
TBP1
Actin
E
scrambled siRNA
PSMC1 siRNA
- + - + 5-FU
SIRT7
PSMC1
Actin
scrambled siRNA
PSMC2 siRNA
- + - + 5-FU
SIRT7
PSMC2
Actin

Supplementary Figure 3. 5-FU induces TBP1 Tyrosine 381 dephosphorylation but not the mRNA or protein level of TBP1.

A. Immunoblots of TBP1 levels following 5-FU treatment at 5, 10, 20, 50 or 100 μM for 24 h in HCT116 cells.

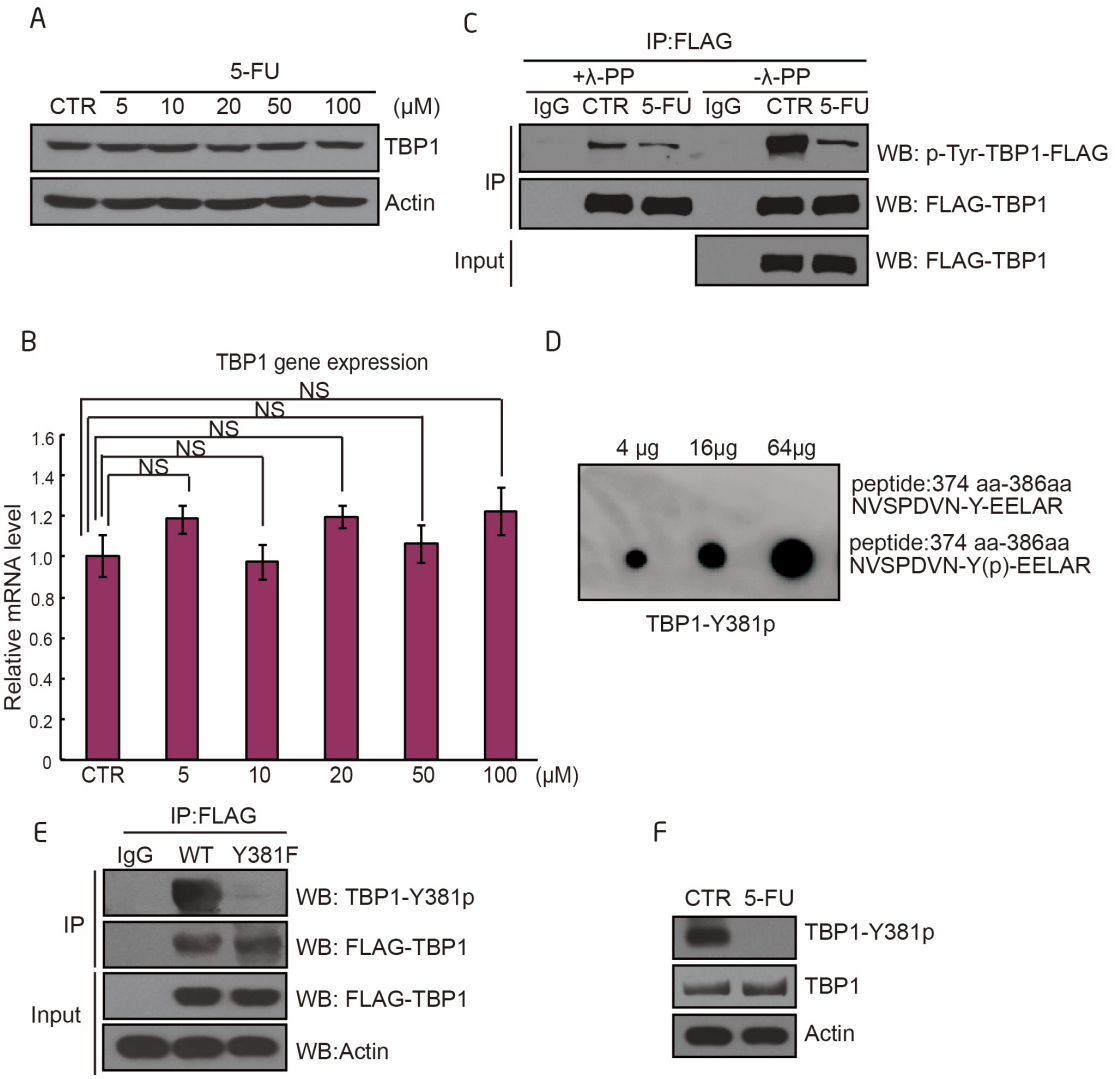
B. *TBP1* mRNA levels in HCT116 cells following 5-FU treatment at 5, 10, 20, 50 or 100 μM for 24 h by real-time PCR.

C. Lambda Protein Phosphatase (λ-PP) to confirm the tyrosine phosphorylation of TBP1. Cell lysates were subjected to immunoprecipitation with anti-FLAG antibody, and eluted with 3X FLAG peptide. Eluted TBP1 were incubated in the reaction buffer with λ-PP or without λ-PP at 30° C for 30 min, followed by immunoblotting with anti-pan-Tyrosine antibody.

D. Dot blot to detect the specificity of a TBP1-Y381p antibody. Increased amounts of TBP1 peptides (374-386 aa: NVSPDVN-Y (p)-EELAR) exhibiting tyrosine phosphorylation at Y381 were dropped onto nitrocellulose membranes and detected with an anti-TBP1-Y381p antibody.

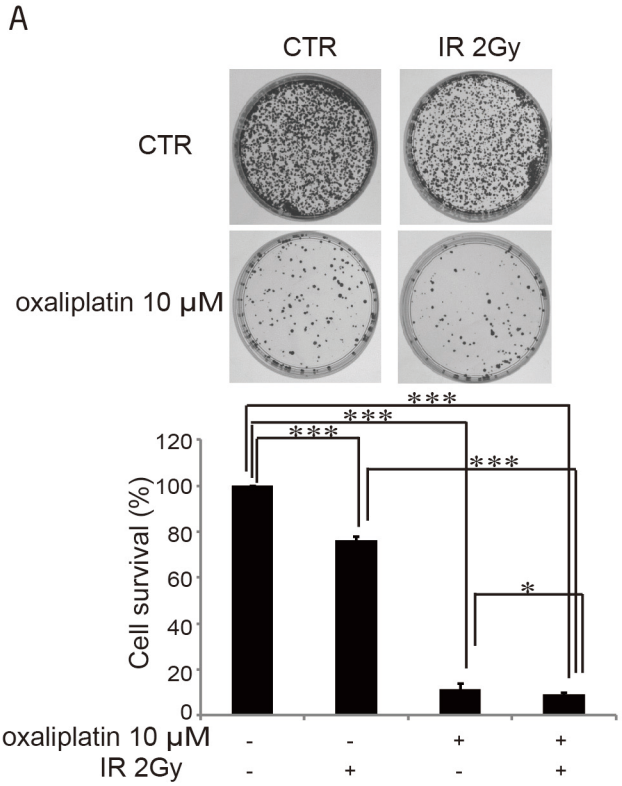
E. Immunoblots were performed with a specific anti-TBP1-Y381p antibody to detect changes in the phosphorylation status of TBP1-WT and TBP1-Y381F in LoVo cells.

F. Immunoblots were performed with a specific anti-TBP1-Y381p antibody to detect changes in endogenous phosphorylation in untreated LoVo cells or treated with 100 μM 5-FU for 24 h.


A
5-FU
CTR 5 10 20 50 100 (μM)
TBP1
Actin
C
IP:FLAG
+λ-PP
-λ-PP
IgG CTR 5-FU IgG CTR 5-FU
IP
Input
WB: p-Tyr-TBP1-FLAG
WB: FLAG-TBP1
WB: FLAG-TBP1
B
TBP1 gene expression
NS
Relative mRNA level
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
CTR 5 10 20 50 100 (μM)
D
4 μg 16μg 64μg
peptide:374 aa-386aa
NVSPDVN-Y-EELAR
peptide:374 aa-386aa
NVSPDVN-Y(p)-EELAR
TBP1-Y381p
E
IP:FLAG
IgG WT Y381F
IP
Input
WB: TBP1-Y381p
WB: FLAG-TBP1
WB: FLAG-TBP1
WB:Actin
F
CTR 5-FU
TBP1-Y381p
TBP1
Actin

Supplementary Figure 4. Oxaliplatin with radiation did not show synergistic effect

A. A colony formation assay was performed with HCT116 cells treated with 10 μM oxaliplatin alone, 2 Gy IR alone or both treatments combined, as indicated. All samples were seeded with 20,000 cells. Colonies containing >50 cells were counted. Error bars represent standard deviations. *** represents $p<0.001$, * represents $p<0.05$.



Supplementary Figure 5. Schematic flow for patients' sample preparation procedure

A. A schematic flow showing the treatment process for each patient and the sample preparation procedure.

A