Combined inactivation of CTPS1 and ATR is synthetically lethal to MYC-overexpressing cancer cells

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Supplementary Materials

Supplementary Tables S1-S3

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Supplementary Tables S1-S3

Table S1. shRNA sequences.

shRNA	Sequence
Myc shRNA_1	CCTGAGACAGATCAGCAACAA
Myc shRNA _2	GGAAACGACGAGAACAGTTGA
P53 shRNA_1	CGGCGCACAGAGGAAGAGAAT
P53 shRNA_2	GTCCAGATGAAGCTCCCAGAA
ATR shRNA_1	CTCCGTGATGTTGCTTGATTT
ATR shRNA_2	CTGTGGTTGTATCTGTTCAAT
ATR shRNA_3	GATGAACACATGGGATATTTA
CTPS1 shRNA_1	CAGGAACATTCTCTCCTTATG
CTPS1 shRNA_2	CAGGCGTTAATACCTGTAGAT
CTPS2 shRNA_1	ATCTGATTGTCTGCCGAAGTT
CTPS2 shRNA_2	ATCGGTGATTCTGCAAGTAAT
Scrambled shRNA	CCTAAGGTTAAGTCGCCCTCG

Table S2. Primer sequences.

Primer	Sequence
CTPS1-F	CAGTGTGGGCACAATACTCAA
CTPS1-R	CGCTCATAGTTACCCAGGTCA
CTPS2-F	CGATGCTGGCACTTTTTCACC
CTPS2-R	ATGTGAGGGACAACTTGCACT
β-actin-F	CATGTACGTTGCTATC CAGGC
β-actin-R	CTCCTTAATGTCACGCACGAT

Table S3. sgRNA sequences.

sgRNA	Sequence
CTPS1_1	ACTTCGTGTATTTGCCCACA
CTPS1_2	GAAATAGCCAAGTTCAACAG
CTPS2_1	GGAGAAGTTGATTTAGACCT
CTPS2_2	GAAGTCTTCGTCTTAAATGA

Supplementary Figures S1-S5

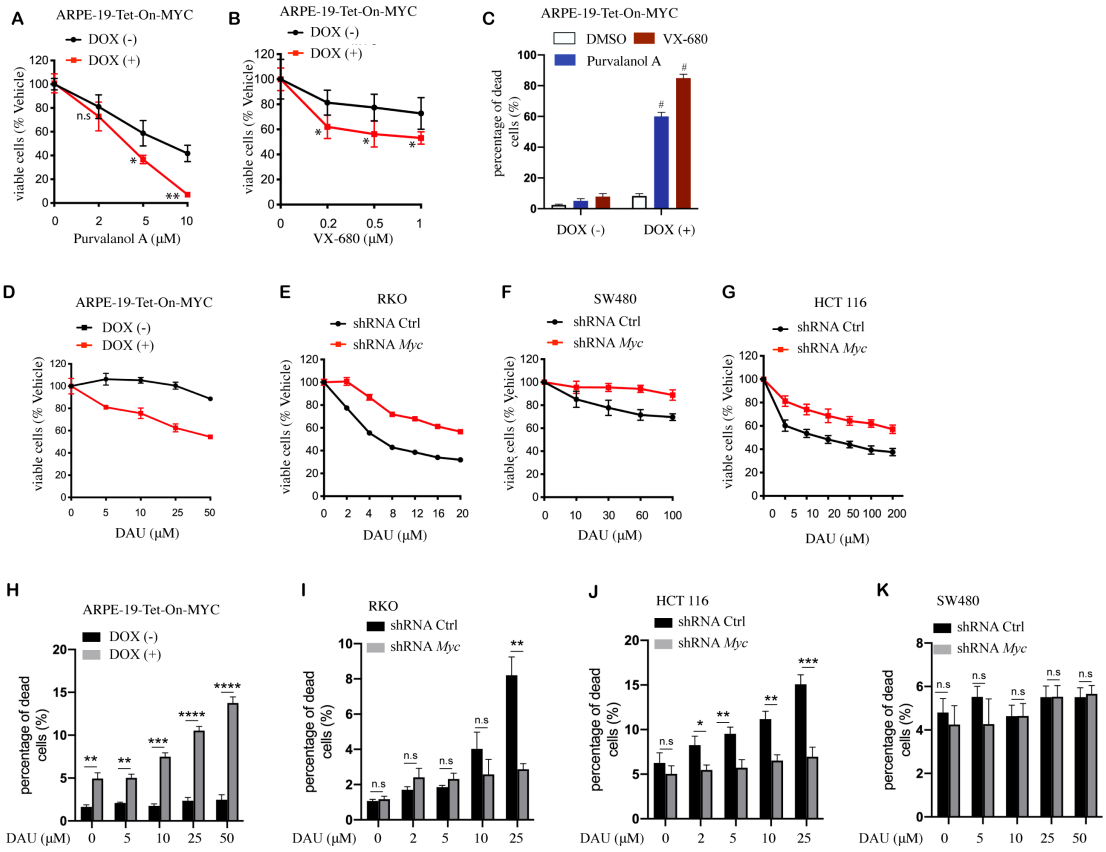

Figure S1, related to Figure 1.

(A and B) ARPE-19-Tet-On-MYC cells cultured with or without DOX (10 ng/ml) were treated with the indicated concentrations of Purvalanol A (A) or VX-680 (B) for 48 hr, and viable cells are given as a percentage of vehicle-treated cells. n = 3 biological replicates. n.s., not significant; $^{*}p < 0.05$; $^{**}p < 0.01$, significantly different from DOX (-) cells.

(C) ARPE-19-Tet-On-MYC cells cultured with or without DOX (10 ng/ml) were treated with 5 μM Purvalanol A or 0.3 μM VX-680 for 2 days. Cell death was determined by PI staining and flow cytometer assay. n = 3 biological replicates. $^{\#}p < 0.001$, significantly different from vehicle-treated group.

(D) ARPE-19-Tet-On-MYC cells cultured with or without DOX (10 ng/ml) were treated with the indicated concentrations of DAU for 2 days. Cell viability is given as percentage of vehicle-treated cells. n = 4 biological replicates.

(E-G) RKO (E), SW480 (F) and HCT116 (G) cells transduced with lentiviral vectors carrying either scrambled shRNA (shRNA Ctrl) or shRNA targeting *Myc* (shRNA *Myc*) were treated with the indicated concentrations of DAU for 2 days and viable cells are given as percentage of vehicle-treated cells. n = 6 biological

replicates.
(H-K) ARPE-19-Tet-On-MYC cells cultured with or without 10 ng/ml DOX (H), RKO (I), HCT116 (J) and SW480 (K) cells expressing control or *Myc* shRNA were treated with the indicated concentrations of DAU for one week. Cell death was determined by PI staining and flow cytometer assay. n = 3 biological replicates. n.s., not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. Graphical data are represented as mean ± SEM.

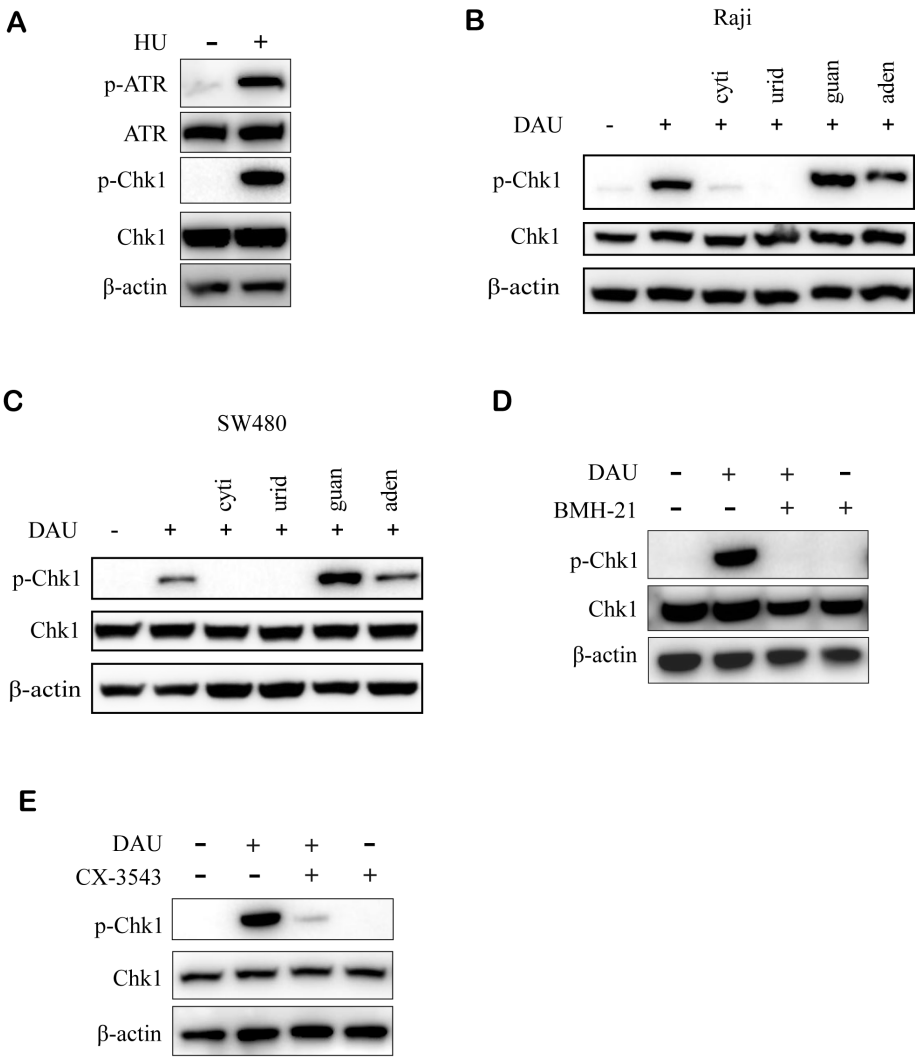

Figure S2, related to Figure 2.

(A) HCT116 cells were treated with 1mM Hydroxyurea (HU) for 6 hr. Lysates were prepared and analyzed by immunoblotting with antibodies against the total and phosphorylated Chk1 and ATR.

(B and C) Raji (B) and SW480 (C) cells were treated with vehicle or DAU (20 μM) with or without 50 μM cytidine (cyti), uridine (urid), guanosine (guan) or adenosine (aden) for 24 hr. Lysates were prepared and analyzed by immunoblotting with appropriate antibodies.

(D and E) HCT116 cells were pretreated with 1 μM BMH-21 (E) or CX-3543 (F) for 12 hr, and then treated with 5 μM DAU for another 5 hr. Lysates were prepared and analyzed by immunoblotting with the indicated antibodies.

One of three to five similar experiments is shown.

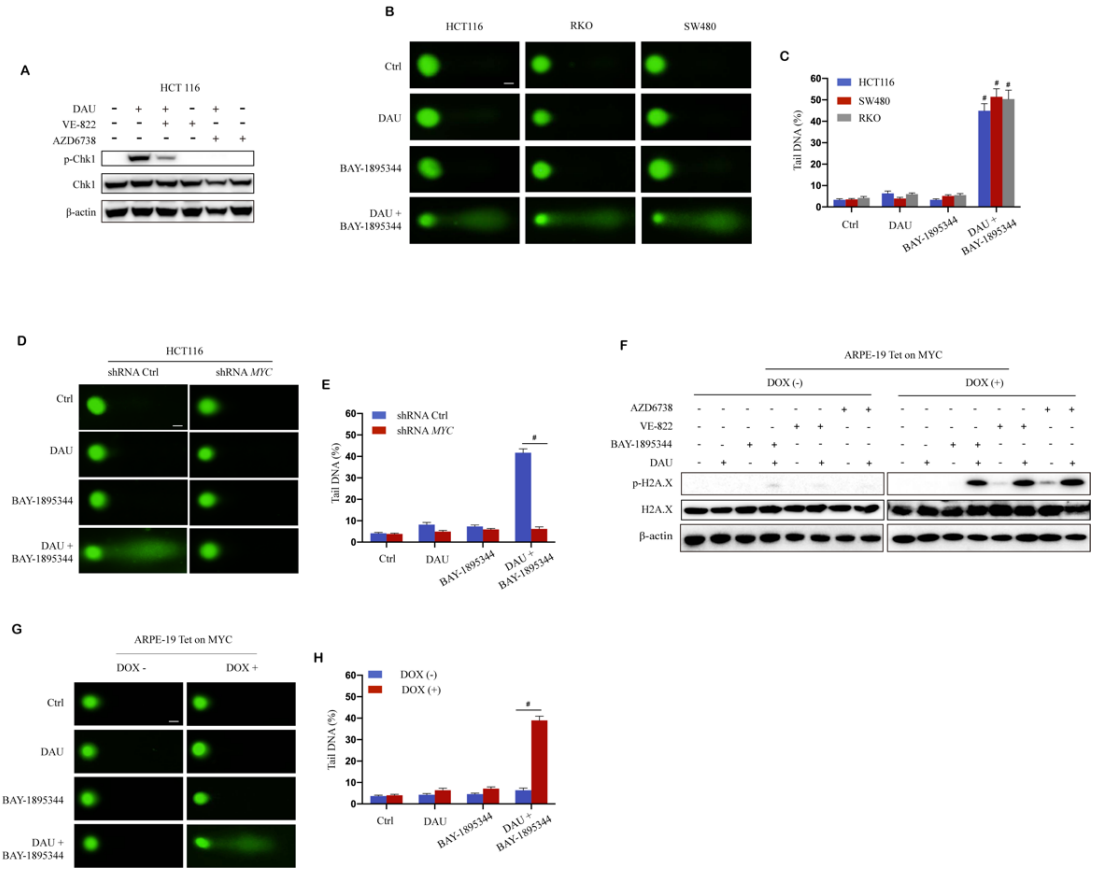

Figure S3, related to Figure 3.

(A) Immunoblots of total and phosphorylated Chk1 in HCT116 cells treated for 24 hr with vehicle, or 5 μM DAU with or without 200 nM VE-822 or 5 μM AZD6738.

(B and C) HCT116, RKO, and SW480 cells were treated with 20 μM (SW480) or 5 μM (HCT116 and RKO) DAU and 50 nM BAY-1895344 alone or together for 24 hr. DNA damage was assessed using the comet assay. Representative images are shown in panel (B). Quantification of the percentage of comet tail DNA after comet assay is shown in panel (C). Data are presented as means ± SEM. $^{\#}p < 0.0001$, significantly different from vehicle-treated group. Scale bar, 10 μm.

(D and E) HCT116 cells expressing control or *Myc* shRNA were treated with 5 μM DAU and 50 nM BAY-1895344 alone or together for 24 hr. DNA damage was assessed by the comet assay. Representative images are shown (D) and the percentage of comet tail DNA was quantified (E). Data are presented as means ± SEM. $^{\#}p < 0.0001$. Scale bar, 10 μm.

(F) ARPE-19-Tet-On-MYC cells cultured with or without DOX (10 ng/ml) were treated for 16 hr with vehicle, DAU (20 μM), AZD6738 (5 μM), VE-822 (200 nM) or BAY-1895344 (50 nM) and analyzed by immunoblotting with the indicated antibodies.

(G and H) ARPE-19-Tet-On-MYC cells cultured with or without DOX (10 ng/ml) were treated with 20 μM DAU and 50 nM BAY-1895344 alone or together for 16 hr. DNA damage was assessed by the comet assay. Representative images are shown (G) and the percentage of comet tail DNA was quantified (H). Data are presented as means ± SEM. $^{\#}p < 0.0001$. Scale bar, 10 μm.
For western blotting, one of three to five similar experiments is shown.

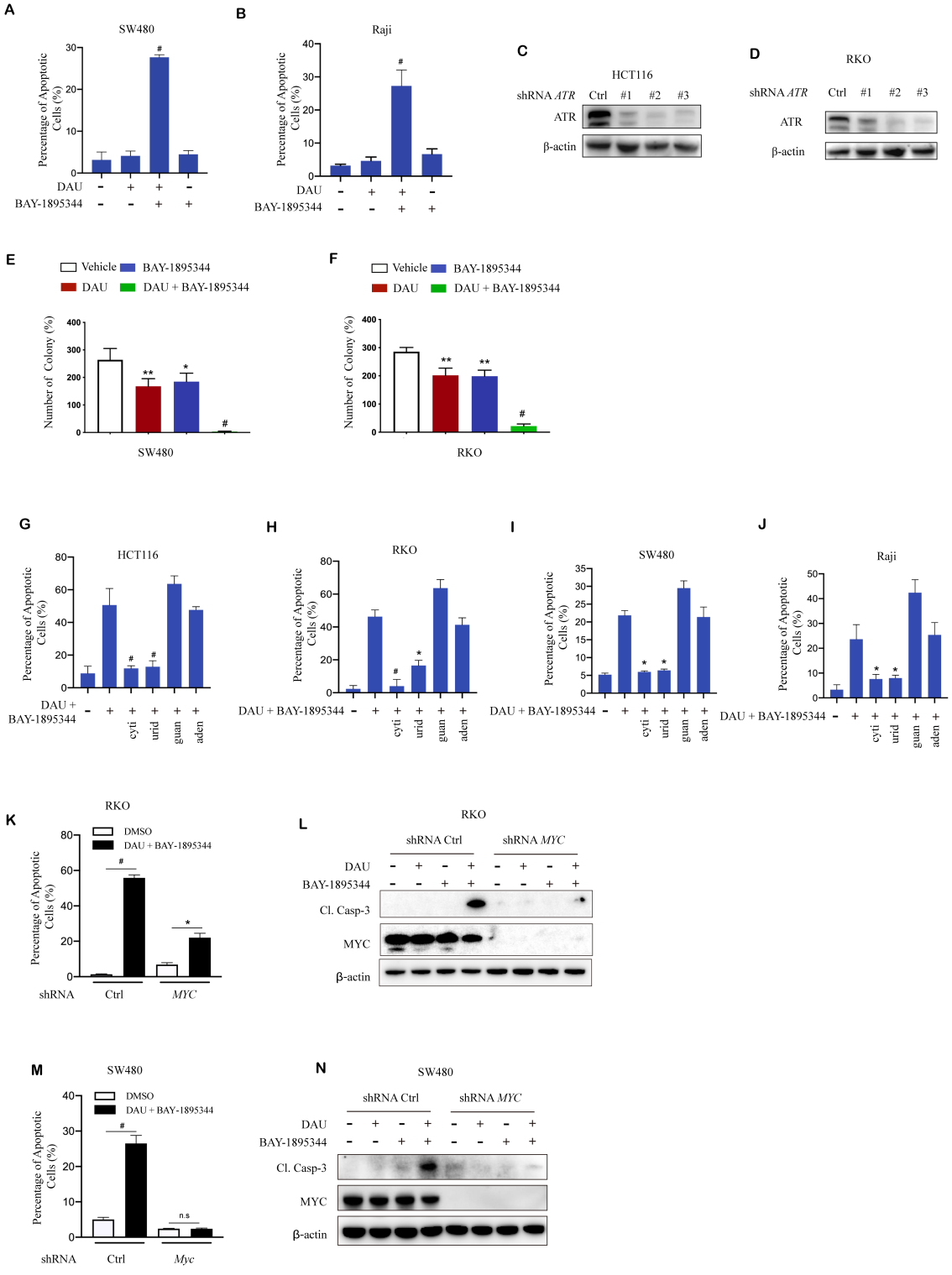

Figure S4. Related to Figure 4.

(A and B) SW480 (A) and Raji (B) cells were treated with 20 μM DAU and 50 nM BAY-1895344 alone or together for 48 hr, followed by Annexin V / PI staining, and analyzed by flow cytometry. n = 4 biological replicates. $^{\#}p < 0.001$,

significantly different from vehicle-treated group.
(C and D) Western blot assay to verify the knockdown efficiency of shRNA targeting ATR. One of two similar experiments is shown.
(E and F) Quantitative data for colony numbers of SW480 and RKO shown in Figure 4 H. n.s., not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{\#}p < 0.0001$, significantly different from vehicle-treated group.
(G-J) HCT116 (G), RKO (H), SW480 (I) and Raji (J) were treated with 5 μM (HCT116 and RKO) or 20 μM (SW480 and Raji) DAU and BAY-1895344 (50 nM) with or without 50 μM cytidine (cyti), uridine (urid), guanosine (guan) or adenosine (aden) for 48 hr, followed by Annexin V / PI staining, and analyzed by flow cytometry. n = 4 biological replicates. $^{*}p < 0.01$, $^{\#}p < 0.001$, significantly different from DAU/BAY-1895344-treated group.
(K and M) RKO (K) and SW480 (M) cells expressing control or *Myc* shRNA were treated with 5 μM (RKO) or 20 μM (SW480) DAU together with 50 nM BAY-1895344 for 48 hr. Cell death was determined by FACS buffer after staining with Annexin V / PI. n = 4 biological replicates. $^{*}p < 0.05$, $^{\#}p < 0.001$.
(L and N) RKO and SW480 cells from (K) and (M) were treated with 5 μM (RKO) or 20 μM (SW480) DAU alone or together with 50 nM BAY-1895344 for 48 hr, and analyzed by immunoblotting with appropriate antibodies. One of three similar experiments is shown.
Graphical data are represented as means ± SEM.

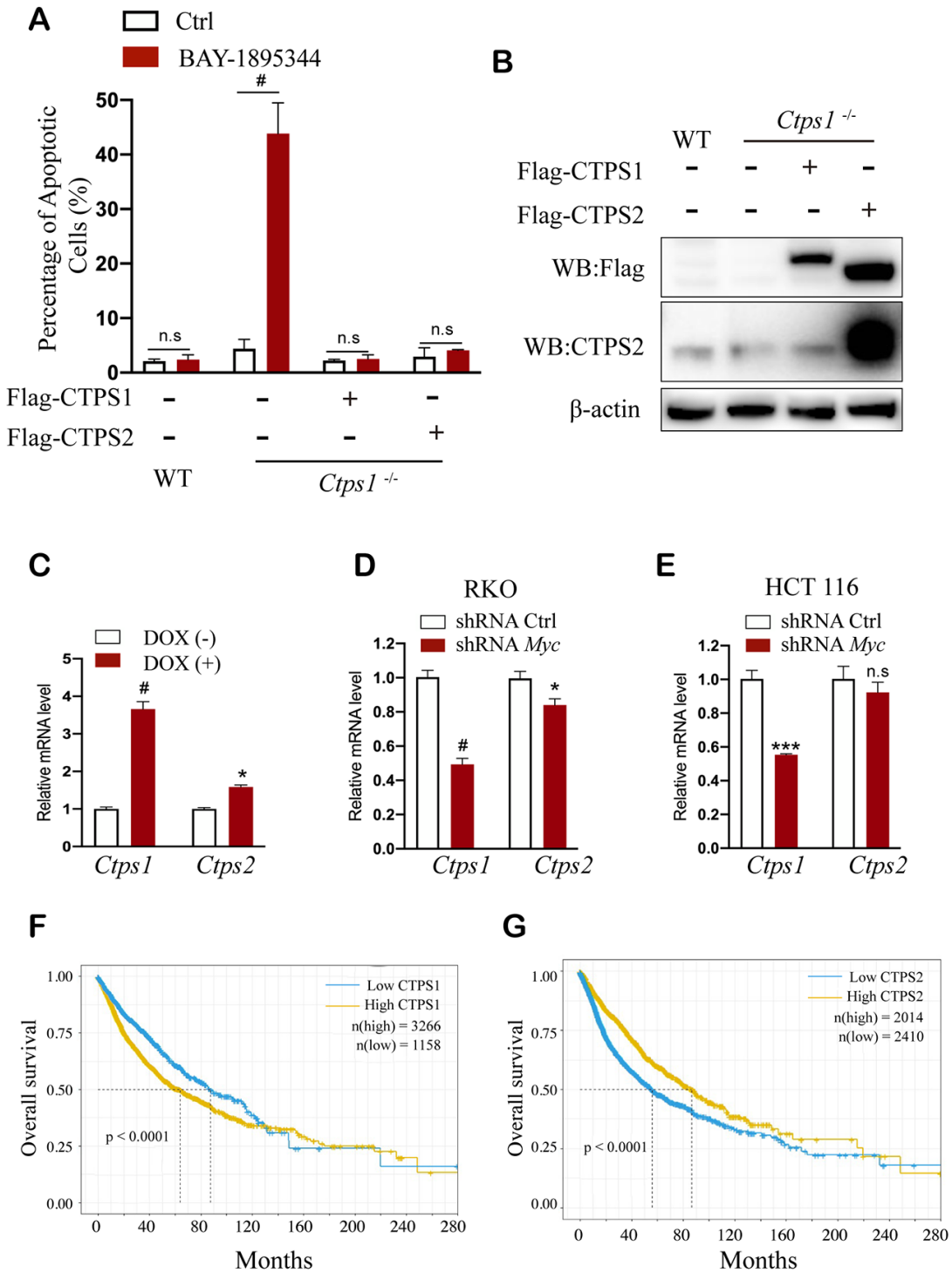

Figure S5, related to Figure 6.

(A and B) WT and *Ctps1*$^{-/-}$ cells, and *Ctps1*$^{-/-}$ cells stably expressing Flag-CTPS1 or Flag-CTPS2 were treated with BAY-1895344 for 48 hr, followed by flow cytometry analysis after staining with Annexin V / PI (A). The protein expression of Flag-CTPS1 and Flag-CTPS2 is shown in the immunoblots (B). n = 3 biological replicates. n.s., not significant; $^{\#}p < 0.0001$.

(C) ARPE-19-Tet-On-MYC cells were cultured with or without DOX (10 ng/ml) for 48 hr. The *Ctps1* and *Ctps2* mRNA levels were analyzed by quantitative reverse transcription PCR (qRT-PCR). n = 3 biological replicates. n.s., not significant; $^{*}p < 0.05$; $^{\#}p < 0.001$.

(D and E) RKO (D) and HCT116 (E) cells expressing scrambled or *MYC* shRNA were cultured for two days. The *Ctps1* and *Ctps2* mRNA levels were analyzed by quantitative reverse transcription PCR (qRT-PCR). n = 3 biological replicates. n.s., not significant; $^{*}p < 0.05$; $^{\#}p < 0.001$.

(F and G) Correlation between *Ctps1* or *Ctps2* expression levels and *Myc* amplified patients' survival. Based on the median expression of *Ctps1* and *Ctps2*, patients were separated into high (yellow) and low (blue) *Ctps1* or *Ctps2* expression.

Graphic data are presented as means ± SEM.